Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials

Birgit Sawitzki, Paul N Harden, Petra Reinke, Aurélie Moreau, James A Hutchinson, David S Game, Qizhi Tang, Eva C Guinan, Manuela Battaglia, William J Burlingham, Ian S D Roberts, Mathias Streitz, Régis Josien, Carsten A Böger, Cristiano Scottà, James F Markmann, Joanna L Hester, Karsten Juerchott, Cecile Braudeau, Ben James, Laura Contreras-Ruiz, Jeroen B van der Net, Tobias Bergler, Rossana Caldara, William Petchey, Matthias Edinger, Nathalie Dupas, Michael Kapinsky, Ingrid Mutzbauer, Natalie M Otto, Robert Öllinger, Maria P Hernandez-Fuentes, Fadi Issa, Norbert Ahrens, Christoph Meyenberg, Sandra Karitzky, Ulrich Kunzendorf, Stuart J Knechtle, Josep Grinyó, Peter J Morris, Leslie Brent, Andrew Bushell, Laurence A Turka, Jeffrey A Bluestone, Robert I Lechler, Hans J Schlitt, Maria C Cuturi, Stephan Schlickeiser, Peter J Friend, Tewfik Miloud, Alexander Scheffold, Antonio Secchi, Kerry Crisalli, Sang-Mo Kang, Rachel Hilton, Bernhard Banas, Gilles Blancho, Hans-Dieter Volk, Giovanna Lombardi, Kathryn J Wood, Edward K Geissler

Summary

Background Use of cell-based medicinal products (CBMPs) represents a state-of-the-art approach for reducing general immunosuppression in organ transplantation. We tested multiple regulatory CBMPs in kidney transplant trials to establish the safety of regulatory CBMPs when combined with reduced immunosuppressive treatment.

Methods The ONE Study consisted of seven investigator-led, single-arm trials done internationally at eight hospitals in France, Germany, Italy, the UK, and the USA (60 week follow-up). Included patients were living-donor kidney transplant recipients aged 18 years and older. The reference group trial (RGT) was a standard-of-care group given basiliximab, tapered steroids, mycophenolate mofetil, and tacrolimus. Six non-randomised phase 1/2A cell therapy group (CTG) trials were pooled and analysed, in which patients received one of six CBMPs containing regulatory T cells, dendritic cells, or macrophages; patient selection and immunosuppression mirrored the RGT, except basiliximab induction was substituted with CBMPs and mycophenolate mofetil tapering was allowed. None of the trials were randomised and none of the individuals involved were masked. The primary endpoint was biopsy-confirmed acute rejection (BCAR) within 60 weeks after transplantation; adverse event coding was centralised. The RTG and CTG trials are registered with ClinicalTrials.gov, NCT01656135, NCT02252055, NCT02085629, NCT02244801, NCT02371434, NCT02129881, and NCT02091232.

Findings The seven trials took place between Dec 11, 2012, and Nov 14, 2018. Of 782 patients assessed for eligibility, 130 (17%) patients were enrolled and 104 were treated and included in the analysis. The 66 patients who were treated in the RGT were 73% male and had a median age of 47 years. The 38 patients who were treated across six CTG trials were 71% male and had a median age of 45 years. Standard-of-care immunosuppression in the recipients in the RGT resulted in a 12% BCAR rate (expected range 3·2–18·0). The overall BCAR rate for the six parallel CTG trials was 16%. 15 (40%) patients given CBMPs were successfully weaned from mycophenolate mofetil and maintained on tacrolimus monotherapy. Combined adverse event data and BCAR episodes from all six CTG trials revealed no safety concerns when compared with the RGT. Fewer episodes of infections were registered in CTG trials versus the RGT.

Interpretation Regulatory cell therapy is achievable and safe in living-donor kidney transplant recipients, and is associated with fewer infectious complications, but similar rejection rates in the first year. Therefore, immune cell therapy is a potentially useful therapeutic approach in recipients of kidney transplant to minimise the burden of general immunosuppression.

Funding The 7th EU Framework Programme.

Introduction

Combinations of general immunosuppressive drugs have enabled the widespread application of life-saving organ transplantation; however, transplant survival is shortened by chronic rejection and immunosuppression side-effects, and has plateaued over the past decade.[1] Organ rejection can mean that secondary transplantations are needed when there is already an inadequate number of organs available for first-time transplantation, while the morbidity and economic costs associated with life-long general immunosuppression accrue. To address this problem, the organ transplantation community urgently needs new strategies to decrease our dependency on immunosuppressive drugs to prevent allograft rejection.[2]

Lancet 2020; 395: 1627–39

See Comment page 1589

Institute of Medical Immunology (Prof B Sawitzki PhD, M Streitz Diplom, S Schlickeiser PhD, Prof H-D Volk MD) **and BIH Center for Regenerative Therapies, Charité and Berlin Institute of Health** (M Streitz, K Juerchott PhD, S Schlickeiser, Prof H-D Volk), **Charité, Universitätsmedizin Berlin, Berlin, Germany; Oxford Transplantation Centre, Oxford University Hospitals NHS Foundation Trust, University of Oxford, Oxford, UK** (P N Harden FRCS, J B van der Net PhD, W Petchey PhD); **BeCAT, BCRT, and Department of Nephrology & Intensive Care, Charité Universitätsmedizin Berlin, and Berlin Institute of Health, Berlin, Germany** (Prof P Reinke MD, N M Otto MD); **Centre de Recherche en Transplantation et Immunologie, Nantes Université, Inserm, Nantes, France** (A Moreau PhD, Prof R Josien MD, C Braudeau PhD, Prof M C Cuturi MD, Prof G Blancho MD), **Institute of Transplantation Urology Nephrology** (A Moreau, R Josien, C Braudeau, Prof M C Cuturi, Prof G Blancho) **and Laboratoire d'Immunologie, Cimna, Centre Hospitalier Universitaire** (R Josien, C Braudeau), **Nantes, France; Department of Surgery,** (J A Hutchinson MD, B James BSc, I Mutzbauer Diplom, Prof H J Schlitt MD, Prof E K Geissler PhD), **Department of Nephrology** (C A Böger MD, T Bergler MD, Prof B Banas MD), **Institute for**

Clinical Chemistry and Laboratory Medicine, Transfusion Medicine (N Ahrens MD) **and Department of Internal Medicine III** (Prof M Edinger MD), **University of Regensburg, University Hospital Regensburg, Regensburg, Germany; Guy's & St Thomas' NHS Foundation Trust, Guy's Hospital, London, UK** (D S Game FRCP, R Hilton MD); **Division of Transplantation, Department of Surgery** (Prof Q Tang PhD, Prof S-M Kang MD), **and UCSF Diabetes Center** (Prof J A Bluestone PhD), **University of California, San Francisco, San Francisco, CA, USA; Department of Radiation Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston MA, USA** (Prof E C Guinan MD); **Diabetes Research Institute** (M Battaglia PhD), **Vita-Salute San Raffaele University Milan** (A Secchi MD), **and Transplant Medicine** (R Caldara MD), **Istituto di Ricovero e Cura a Carattere Scientifico, San Raffaele Scientific Institute, Milan, Italy; Division of Transplantation, Department of Surgery, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA** (Prof W J Burlingham PhD); **Department of Cellular Pathology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK** (Prof I S D Roberts FRCPath); **MRC Centre for Transplantation, Peter Gorer Department of Immunobiology, School of Immunology & Microbial Sciences, King's College London, London, UK** (C Scottà PhD, M P Hernandez-Fuentes PhD, Prof R I Lechler PhD, Prof G Lombardi PhD); **Center for Transplantation Sciences, Mass General Hospital, Boston, MA, USA** (Prof J F Markmann MD, Prof L A Turka MD, K Crisalli BSN); **Transplantation Research and Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK** (J L Hester PhD, F Issa FRCS, A Bushell DPhil, Prof K J Wood DPhil);

Research in context

Evidence before this study

New therapies that limit exposure to general immunosuppression in recipients of kidney transplants are needed to advance the field. With this aim, we united a group of European and American investigators to test the hypothesis that immunoregulation induced through cellular immunotherapy is safe and could benefit recipients of kidney transplants. The types of cell therapies tested in six parallel trials included different T regulatory (Treg) and monocyte-derived (dendritic cell, Mreg) cell products; each trial was done with only one cell product, but in the same living-donor patient population using identical baseline immunosuppression and with the option to minimise immunosuppression to tacrolimus monotherapy. For comparison, a multicentre reference trial was also done in the same patient population by all centres involved, using standard immunosuppression without minimisation. To establish the current evidence base in PubMed, we referred to a recent publication summarising Treg and monocyte-derived cell therapy kidney transplant trials. We also searched Clinicaltrials.gov by combining the terms "Treg", "dendritic cell", or "Mreg" with "kidney transplantation". These sources revealed five clinical trials, with one being of an unknown status, three currently recruiting, and one completed. None of these trials are being run comparatively, nor do they include a multicentre or other comparator group to assess outcomes, changes in immune parameters, or adverse event differences. Therefore, no immune cell therapy trial to date has, to our knowledge, been able to comparatively evaluate outcomes, safety and benefits, and immunological effects of multiple regulatory immune cell therapies in kidney transplantation.

Added value of this study

We showed that immune regulatory cell therapies as a whole are safe and, importantly, we provided the first data that recipients of kidney transplants receiving immune cell therapy have fewer episodes of common viral infections, which often cause clinically significant comorbidities in organ transplantation. We also provide the first evidence that nearly all of the patients on cell therapy, in whom minimisation of immunosuppression was attempted, could be successfully weaned within the first year post-transplantation to monotherapy. Furthermore, immune monitoring data from the cell therapy trials, in comparison to patients on standard immunosuppression, show no loss of Treg-specific demethylated region demethylation as an indicator of stable Tregs, no increase of $CD8^+$ terminally differentiated effector memory cells, and a healthy control-like restoration of immune cell composition (eg, marginal zone-like B cells), providing the first evidence that cell therapy has positive systemic immunological effects. Our study not only provides guidance for clinical trials introducing different immune cell therapies in organ transplantation, it is also relevant for similar immune cell-based therapy trials outside the field of transplantation, including those involving autoimmune diseases.

Implications of all the available evidence

Our study suggests that immune cell therapy is a potentially useful therapeutic approach in recipients of living-donor kidney transplants to minimise the burden of general immunosuppression. Furthermore, our results provide evidence that cell therapy can lead to a restoration of the immune system towards more normal, non-inflammatory levels, thereby decreasing adverse side-effects of conventional immunosuppressive drugs, such as reactivation of harmful persistent infections. Therefore, immune cell therapy in transplantation warrants further study in larger clinical trials.

Indeed, international networks have been established with this explicit purpose, notably including a series of EU-funded programmes and, in North America, the Immune Tolerance Network. Research from these expert networks, and from numerous research laboratories worldwide, consistently call for novel therapies that will reduce our reliance on full immunosuppression to prevent organ rejection. At least two general strategies have been considered, including a deletional approach based on establishment of donor bone-marrow chimerism to reduce donor-reactive immune cells, and an immune regulation-based approach that takes advantage of regulatory cells or pathways that control immunity and restrain immune responses to autologous antigens.[3] Although protocols to create chimerism in recipients of organ transplants have been trialled for more than a decade, finding conditioning regimens with acceptable toxicity and avoiding the problem of graft-versus-host disease has been a persistent obstacle. Regarding the second strategy of building immune regulation, a therapeutic means to augment these cellular networks has only recently come of age for clinical testing.[3]

Regulatory cell therapy has emerged as one attractive therapeutic approach to establish immune regulation aimed at protecting organ allografts.[4–6] The overall principle of this approach is to expand specific regulatory immune cell populations ex vivo in the form of cell-based medicinal products (CBMPs), which can then be infused into transplant recipients. Toward this aim, an EU-funded consortium called The ONE Study was initiated to develop a range of CBMPs and to test the cell products in early-phase clinical trials. The six CBMPs developed and tested in six parallel cell therapy group (CTG) trials in The ONE Study included two polyclonal T regulatory (pTreg-1 and pTreg-2), two donor-antigen reactive Treg (darTreg-CSB and darTreg-sBC), one tolerogenic dendritic cell (autologous tolerogenic dendritic cell [ATDC]), and one regulatory macrophage (Mreg) cell product. Central to the concept of the study was that all CBMPs be tested with the equivalent patient population

of recipients of living-donor kidney transplants, who received identical background immunosuppressive treatment, placing testing of the six CBMPs on a directly comparable basis. Also fundamental to this study was that a larger reference group trial (RGT) be done with an equivalent patient population using standard-of-care immunosuppression. Although the RGT is not strictly a true control group because of inclusion of basiliximab in place of cell therapy, it serves two purposes. First, since we have applied our CBMPs under similar, but reduced, immunosuppression, the RGT provides a recognised standard-of-care benchmark to assess whether currently expected outcomes are generally attainable with regulatory cell therapy and less immunosuppression. Second, with a standard-of-care RGT, performance of centralised immune monitoring allows for reliable detection of potential immunological changes caused by cell therapy. Here, we present the novel study design, clinical data, safety results, and immune monitoring data for The ONE Study RGT and combined CTG trials, which is intended as a foundation for further regulatory cell therapy trials in organ transplantation.

We aimed to explore the safety and immunological effects of regulatory cell-based therapy as an adjunct immunosuppressive treatment in recipients of a living-donor kidney transplant through a series of clinical trials sharing the same general design.

Methods

Study design and participants

We did seven single-arm trials and a harmonised analysis to compare the CTG trials with standard-of-care treatment. The first trial was a single-arm multicentre RGT done at all clinical sites that were also planning to do an individual cell therapy trial. The RGT formed the basis for the other six individual trials testing CBMPs (the CTG trials). Enrolment for the RGT was completed before any of the CTG trials commenced. The RGT was initiated while regulatory approvals for the CTG trials and cell manufacturing procedures were being obtained.

The multicentre RGT was done at eight international hospitals, including the University Hospital Regensburg (Regensburg, Germany), Charité (Berlin, Germany), Centre Hospitalier Universitaire Nantes (Nantes, France), Ospedale San Raffaele (Milan, Italy), Oxford University Hospitals NHS Foundation Trust (Oxford, UK), Guy's Hospital (London, UK), Massachusetts General Hospital (Boston, MA, USA) and University of California, San Francisco Medical Center (San Francisco, CA, USA; figure 1). After completing enrolment for the RGT, seven centres did a separate CTG trial with one of the six regulatory cell products: pTreg-1 (Oxford and London), pTreg-2 (Berlin), darTreg-CSB (Boston, MA), darTreg-sBC (San Francisco, CA), ATDCs (Nantes), or Mreg (Regensburg). Unlike the five centres that recruited patients into their respective single-centre CTG trials, the Oxford and London sites joined forces to recruit patients into one CTG trial (pTreg-1). Notably, the Milan site participated only in the RGT, because their cell product was not approved for clinical trial testing during The ONE Study. None of the trials were randomised and none of the individuals involved in the study were masked.

Recipients of living-donor kidney transplants were selected for inclusion into all seven trials. Living donors were chosen for these trials to allow for maximal planning logistics regarding obtaining informed consent, having a medically stable recipient population, coordinating regulatory cell manufacturing from donor or recipient cells (in the CTG trials), and obtaining pre-transplant immune monitoring samples. The core inclusion and exclusion criteria that were common to all trials for both the donors and recipients are listed in the appendix (p 1). The main exclusion criteria were patients transplanted previously, recipients with panel-reactive antibody more than 40%, and HLA identical donor-recipient mismatches (0-0-0 mismatches); all patients were aged 18 years or older. Ethical approval was given for all trials by the local ethics committee or institutional review board, and written informed consent was obtained from all enrolled trial participants.

Procedures

In the course of The ONE Study project, six regulatory cell products were approved for manufacture and therapeutic testing in the CTG trials by the national competent authority in each participating country. Two of the six cell products consisted of pTreg cells approved in the UK (pTreg-1)[7] and Berlin (pTreg-2).[8] The third and fourth cell products consisted of Treg, but were generated in the presence of donor antigen during manufacturing; one product was exposed under conditions of costimulatory blockade in the presence of donor peripheral blood mononuclear cells in Boston (MA, USA)[9] referred to as costimulatory blockade darTreg-CSB, and the other product was developed in San Francisco (CA, USA), where Tregs sorted from peripheral blood mononuclear cells were stimulated with donor B cells that had been activated with K562 cells expressing human CD40L (referred to as donor alloantigen-reactive darTreg-sBC).[10] The fifth and sixth cell products were derived from peripheral blood monocytes, in which monocytes were stimulated in Nantes, France with granulocyte macrophage colony-stimulating factor to produce ATDCs,[11] or in Regensburg with macrophage colony-stimulating factor and IFNγ to produce regulatory macrophages (Mreg-UKR).[12] All six regulatory cell products were derived from recipient leucocytes (blood or leucopheresates), with the exception that Mreg-UKR were donor-derived. An overview of the overall characteristics of the CBMPs, including a reference to cell production methods is provided in the appendix (p 2).

The ONE Study group of clinicians developed the RGT immunosuppression design based on their own local standard-of-care protocols, which included some features

Department of Experimental Medicine, DFCI, Boston, MA, USA (L Contreras-Ruiz PhD); **Beckman Coulter Life Sciences, Immunotech, Marseille, France** (N Dupas MS, T Miloud PhD); **Beckman Coulter, Krefeld, Germany** (M Kapinsky PhD); **Department of Surgery, Charité Campus Mitte, Campus Virchow Klinikum, Charité Universitätsmedizin, Berlin, Germany** (R Öllinger MD); **KOEHLER eClinical, Freiburg, Germany** (C Meyenberg MSc); **Miltenyi Biotec, Bergisch Gladbach, Germany** (S Karitzky PhD); **Clinic for Nephrology and Hypertension** (Prof U Kunzendorf MD), **and Institute for Immunology** (Prof A Scheffold PhD), **Christian Albrechts University, University Clinic Schleswig-Holstein, Kiel, Germany; Department of Surgery, Duke Transplant Center, Duke University Medical Center, Durham, NC, USA** (Prof S J Knechtle MD); **Kidney Transplant Unit, Nephrology Department, Bellvitge University Hospital, IDIBELL, Barcelona University, Barcelona, Spain** (Prof J Grinyó MD); **Centre for Evidence in Transplantation, Clinical Effectiveness Unit, Royal College of Surgeons of England, London, UK** (Prof P J Morris FRCS); **St Mary's Hospital Transplant Unit, Paddington, London, UK** (Prof L Brent PhD); **Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK** (Prof P J Friend MD, Prof P J Morris); **Division of Personalized Tumor Therapy, Fraunhofer Institute for Experimental Medicine and Toxicology, Regensburg, Germany** (Prof E K Geissler, B James, I Mutzbauer); **and Regensburg Center for Interventional Immunology, University of Regensburg, Regensburg, Germany** (Prof E K Geissler, Prof M Edinger)

Correspondence to:
Prof Edward K Geissler, Department of Surgery, Division of Experimental Surgery, University of Regensburg, University Hospital Regensburg, 93053 Regensburg, Germany
edward.geissler@klinik.uni-regensburg.de

For the **Immune Tolerance Network** see https://www.immunetolerance.org/

See Online for appendix

of the ELITE-Symphony study,[13] for the selected non-high risk kidney transplant patient population. The study protocol (NCT01656135) consisted of: basiliximab administration 2 h or less before transplant surgery and on day 4 after surgery (20 mg intravenously); prednisolone starting on day 0 (day of kidney transplant) and gradually tapered away by week 15; mycophenolate mofetil at 2 g per day from day –1 to day +14 and 1·5 g per day thereafter; and tacrolimus starting on day –4 at 3–12 ng/mL and gradually reduced over 9 months to 3–6 ng/mL. A diagram showing the exact dosing scheme is shown in the appendix (p 9). Patient follow-up was continued for 60 weeks. The target recruitment number for the RGT was 60 patients.

The clinical protocol for the six CTG trials closely followed that of the RGT (appendix p 9). All cell products were delivered once intravenously between day –7 and day +10 relative to the day of kidney transplant; within this timeframe, monocyte-derived cell products were administered before kidney transplant and T-cell derived products were given after kidney transplant. The exact cell numbers infused will be provided in the individual CTG trial descriptions to be reported elsewhere, but ranged from 0·5 to 10×10⁶ cells per kg bodyweight for all cell products except darTreg-CSB, in which a range between 2×10³ to 2×10⁶ cells per kg bodyweight was targeted. Pharmacological immunosuppression and dosing were the same as with the RGT, except that basiliximab induction therapy was omitted, and at 9 months post-kidney transplant an option was included to completely taper away mycophenolate mofetil by 1 year post-kidney transplant; with mycophenolate mofetil cessation, tacrolimus was continued as a monotherapy. Tapering of mycophenolate mofetil was not allowed if a biopsy at 9 months post-transplant showed signs of subclinical rejection or there was evidence of declining renal function. Patient follow-up continued for approximately 60 weeks, after which time immunosuppressive treatment was decided by the local transplant physician. The number of patients given cell therapy did not exceed 12 in any individual CTG trial.

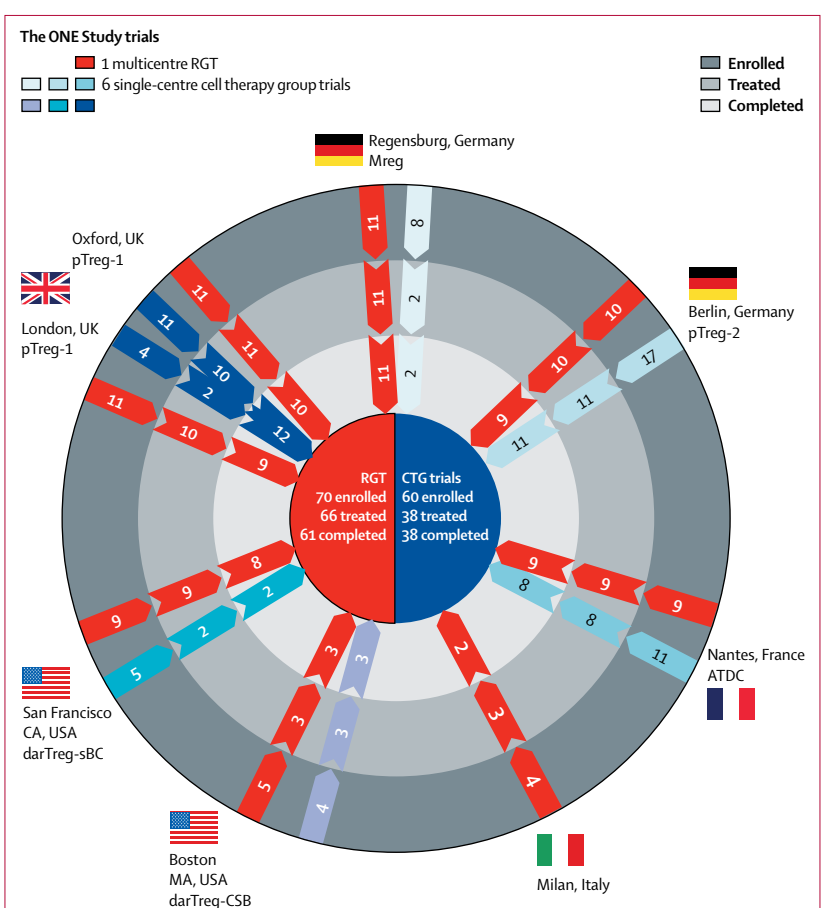


Figure 1: **Trial profile**
RGT=reference group trial. CTG=cell therapy group. pTreg=polyclonal T regulatory cells. ATDCs=autologous tolerogenic dendritic cells. darTreg=donor-antigen reactive Treg. Mreg=regulatory macrophages.

We used a mixed model of locally and centrally performed assays to compare pre-transplant and post-transplant immune status of patients in the RGT and CTG trials.[14] The following analyses were done as detailed in the appendix (p 13): immune cell composition by whole blood flow cytometry, Treg-specific demethylated region demethylation and gene expression (appendix p 13), and anti-donor as well as anti-cytomegalovirus IFNγ EliSpot. To reveal differences in peripheral blood immune cell composition between patients with end-stage renal disease (RGT and CTG before transplantation) and healthy individuals, we did comparative analyses with age-matched and sex-matched healthy controls from our published cohort dataset.[15]

Outcomes

Biopsy-confirmed acute rejection (BCAR) was the primary endpoint. Histopathological grading of kidney transplant biopsies was done by a central pathologist (ISDR, Oxford University) for all trials within The ONE Study, with the standard assessment done according to the Banff criteria.[16] Notably, a case of borderline histological change in a for-cause biopsy with clinical evidence of acute rejection was considered as BCAR. However, histological changes consistent with acute rejection that were not accompanied by clinical evidence of rejection were not recorded as BCAR, but were logged as a secondary endpoint. Estimated glomerular filtration rate (eGFR using the Modification of Diet in Renal Disease method) was recorded as a secondary endpoint.

Statistical analysis

For the RGT, we estimated a BCAR rate of approximately 10% after 60 weeks under standard immunosuppressive therapy in our study population. With this assumption, a two-sided 95% CI for a single proportion of 0·106 predicts a rejection rate ranging from 3·2–18·0% with a sample size of 66 patients; a BCAR rate falling

outside this interval would suggest that the rejection rate is atypical.

Clinical data from all trials were entered into a web-based data capture platform consisting of electronic case report forms custom-made for The ONE Study (Koehler eClinical, Freiburg, Germany). A core set of clinical data were collected from all trials to ensure that these parameters could be directly compared. Selected data items for evaluation of the study endpoints were verified for accuracy against source documents during on-site monitoring visits. Additionally, data were reviewed, queried, and cleaned remotely by a central team of data managers using both automatic and manual data validation checks. All adverse events and serious adverse events were coded centrally using version 20.1 of the Medical Dictionary for Regulatory Activities and quality-controlled to ensure consistency of coding across all trials and study sites. To compare safety events reported from cohorts of different sizes, serious adverse event and adverse event frequencies were normalised using a cohort-specific patient study-years denominator. Patient study-years are the cumulative amount of time spent by trial participants in study follow-up and were calculated and applied for the RGT and CTGs separately. A safety advisory board received serious adverse event reports for all CTG trials as they occurred and reviewed all safety data twice per year. To be sure of open communication within the trial series, safety alerts or conclusions from the safety advisory board were shared with all centres doing CTG trials.

A statistical analysis plan defined the conventions and analyses, and emphasised the exploratory nature of the study; accordingly the proposed statistical examination of clinical data was descriptive. The reported comparative analyses of changes in immune cell composition and functionality between patients in the RGT and CTG were done as post-hoc analyses.

For clinical data, results for baseline characteristics, safety, and transplant function or rejection endpoints were summarised descriptively. No formal testing was done. In addition to crude rejection rates, time to first BCAR was analysed using Kaplan-Meier methods. The primary BCAR endpoint is reported descriptively for the intention-to-treat population (66 for RGT, 38 for CTG); the time-to-event Kaplan-Meier BCAR analysis is presented for both the intention-to-treat (66 for RGT, 38 for CTG) and per-protocol (47 for RGT, 32 for CTG) populations. All other variables (donor-specific antibody [DSA], eGFR, tacrolimus levels) are summarised for the number of patients who were tested at the relevant study timepoints. Incidence of adverse events normalised per 100 patient study-years was calculated and based on the intention-to-treat population.

Differences in immune monitoring results between patients in the RGT before transplantation and healthy controls were analysed applying Kruskal Wallis tests followed by Dunn-Bonferroni tests. Changes between pre-transplant and post-transplant timepoints of the same patient were analysed applying Wilcoxon matched-pairs signed rank test. To reveal differences in immune cell composition or Treg-specific demethylated region changes after transplantation between patients in the RGT and CTG trials, we used a Kruskal Wallis and a post-hoc Dunn's multiple comparison test. p values less than 0·05 were considered significant. The RTG and CTG trials are registered with ClinicalTrials.gov, NCT01656135, NCT02252055, NCT02085629, NCT02244801, NCT02371434, NCT02129881, and NCT02091232.

Role of the funding source

The funders of the study had no role in data collection, data analysis, data interpretation, or writing of the report. EKG, as The ONE Study Consortium FP7 project coordinator, had full access to all the data in the study; BS and BJ had full access to all the data in the study. As a group, members of this FP7 consortium discussed the publication plans, and therefore were involved in the decision to submit the manuscript; EKG and BS had final responsibility for the decision to submit for publication.

Results

Of 782 patients assessed for eligibility, 130 (17%) patients were enrolled and 104 were treated and included in the analysis. Recruitment to the RGT began on Dec 11, 2012, with the last patient's last visit on Dec 29, 2015. Figure 1 shows that 70 patients were enrolled in the RGT, with 66 (94%) receiving a kidney transplant. Of the four pre-kidney transplant withdrawals, two patients had their transplant postponed, one patient needed treatment for DSA that did not allow further inclusion into the study protocol, and one patient withdrew consent. 61 patients in the RGT completed the study: of the five who were non-completers, one patient withdrew consent (at 8 days), one patient was lost to follow-up (at 33 weeks), one patient had a major vascular complication and graft loss (at 8 days), one patient received anti-thymocyte globulins instead of basiliximab induction therapy (discovered on day 11), and one patient violated the eligibility criteria (noted at 24 weeks). None of these five patients registered a primary endpoint. In the RGT, median follow-up time was 60·1 weeks (IQR 1·3). Figure 1 summarises patient recruitment into the six individual CTG trials (non-red arrow bars), in which 60 patients were recruited into the various trials, with the first patient's first visit done on May 13, 2014, and the last patient's last visit done on Nov 14, 2018. Of the 60 enrolled patients, 38 received a kidney transplant and the designated cell therapy. All of these patients completed the 60-week follow-up planned in the study. The causes for withdrawal of 22 patients were: cell manufacturing failures (n=14), early development of acute rejection before the planned cell infusion (n=5), discovery of ineligibility criteria after enrolment (n=2), or requirement for a second abdominal surgery

shortly after kidney transplant (n=1). Cell manufacturing failures were because of not meeting release criteria (n=9), cancellation (n=2), microbiology testing positive (n=2), and leucapheresis side-effects (n=1). No trial was stopped due to lack of manufacturing feasibility. In the CTG, median follow-up time was 60·0 weeks (IQR 0·6). A summary of the recipient and donor demographic data for the RGT and CTG trials is provided in the appendix (pp 3–4). Data for recipient and donor age, sex, ethnicity, renal replacement therapy, relationship of donor and recipient, and underlying diagnosis show that the RGT and combined CTG trials were well balanced when compared with each other. Median age of recipients in the RGT was 47 years, compared with 45 years in the CTG trials; median donor age was 53 years in the RGT, versus 51 years in the CTG trials. Notably, 73% of recipients in the RGT were male, with a similar over-representation of male recipients (71%) in the CTG trials. Because sex-related effects are known in transplantation, the greater number of male recipients should be taken into consideration when interpreting the results.

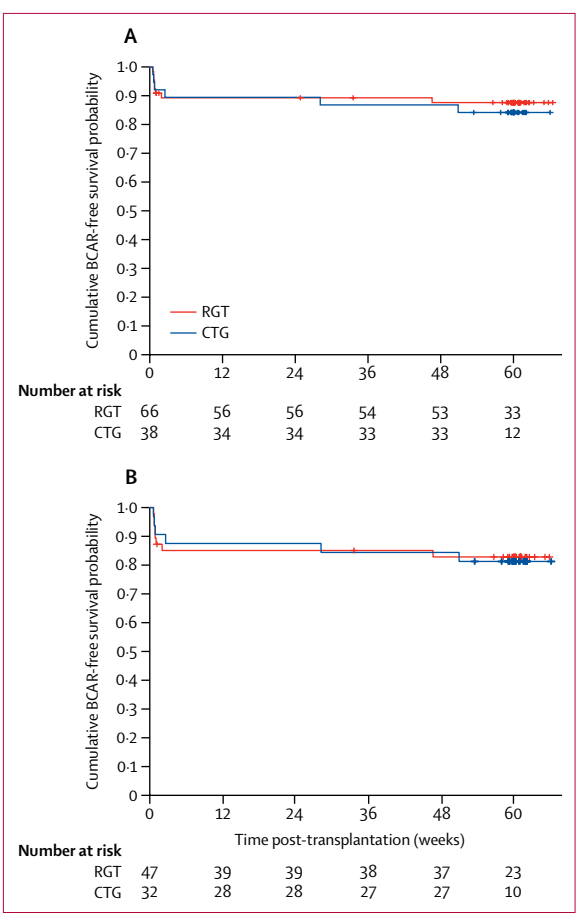


Figure 2: **Primary endpoint (BCAR) data**
(A) Kaplan-Meier estimates of the cumulative BCAR-free survival probability in the RGT (n=66) and CTG (n=38) intention-to-treat analysis sets (88% *vs* 84% at 60 weeks). (B) Kaplan-Meier estimates of the cumulative BCAR-free survival probability in the RGT (n=47) and CTG (n=32) per-protocol analysis sets (83% *vs* 81% at 60 weeks). Censored patients are indicated with ticks. RGT=reference group trial. CTG=cell therapy group. BCAR=biopsy-confirmed acute rejection.

A set of per-protocol criteria were defined based mostly on overall adherence to the planned immunosuppression regime in both the RGT and CTG trials (appendix p 5). In the RGT, 47 (71%) of 66 kidney transplant patients received treatment that closely followed the clinical protocol, whereas 32 (84%) of the 38 patients in the CTG trials were treated with close adherence to the protocol. Reasons for non-adherence varied widely among the trials, but were mostly related to adjustments or switching of immunosuppression that the treating physician deemed necessary. Furthermore, ONE Study physicians doing the CTG trials tapered immunosuppression to tacrolimus monotherapy (optional) in 17 (45%) of 38 patients. The immunosuppression was successfully tapered in all but two cases, in which triple therapy was later reinstated due to a BCAR and detection of recurrent IgA nephropathy.

BCAR rate in the RGT was 12% (eight of 66), which is within the expected range of 3·2–18·0%. BCAR occurred in six (16%) of 38 of the patients receiving cell therapy within the combined CTG trials, which was within the expected range calculated for the RGT. The Kaplan-Meier curves in figure 2A highlight the early incidence of BCARs in all trials. The severity of the first BCAR by Banff scoring was distributed similarly between the RGT and the group of CTG trials (appendix p 6); one patient in the RGT had a second BCAR episode, but other BCARs in all trials were single episodes and were successfully treated. Only one of eight first BCAR episodes in the RGT occurred more than 2 weeks after kidney transplant; similarly, four of six episodes of BCAR in the CTG trials occurred within 3 weeks after kidney transplant. Specific BCAR data from individual sites will be published separately for each CTG trial. We also did a Kaplan-Meier analysis for the per-protocol patients in the RGT and CTG trials (figure 2B), which shows that the rate and timing of the BCAR episodes were essentially the same.

A set of tests was done at study end (60 weeks) to further assess outcomes in the trials, including DSA detection, eGFR, and tacrolimus blood concentrations. At study end, DSA testing revealed that seven (14%) of 51 of the RGT recipients who were tested had a DSA, with five (15%) of 33 of those tested showing DSA in the combined CTG trials. Of the patients in the CTG trials tapered to monotherapy, two (13%) of the 15 tested had a new DSA. Regarding kidney function (appendix p 10), eGFR measurements in the RGT and CTG trials showed a similar increase over the study period (20% in the RGT and 21% in the CTG) when comparing median eGFR at 60 weeks post-kidney transplant to median eGFR at 1 week post-kidney transplant. As a reflection of immunosuppressive load at study end, tacrolimus trough concentrations were similar in the RGT and combined CTG trials, at a mean of 6·1 ng/mL (SD 2·1;

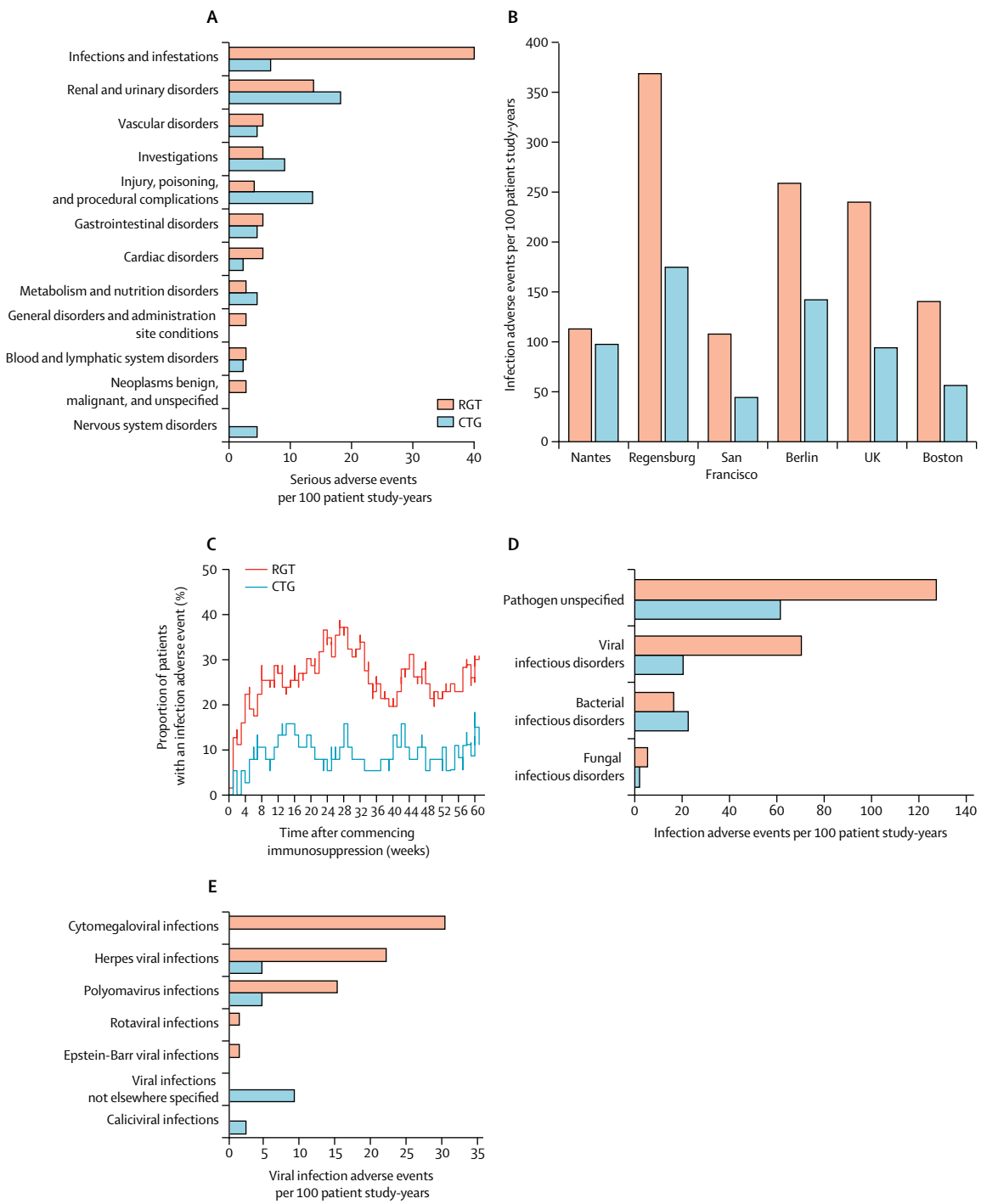


Figure 3: **Normalised safety data**

(A) Incidence of treatment-emergent serious adverse events by MedDRA primary system organ class. (B) Incidence of treatment-emergent infections (all adverse events) by study site. (C) Incidence proportion of treatment-emergent infections (all adverse events) over time. (D) Incidence of treatment-emergent infections (all adverse events) by MedDRA high-level group term. (E) Incidence of treatment-emergent viral infections (all adverse events) by MedDRA high-level term. All adverse events coded using MedDRA version 20.1. Treatment-emergent events are events with onset date equal to or after first dose of any study drug. All events coded to the MedDRA preferred term transplant rejection were excluded as rejection was measured as the primary efficacy endpoint. RGT=reference group trial. CTG=cell therapy group. MedDRA=Medical Dictionary for Regulatory Activities.

n=44 tested) in the RGT and a mean of 6·6 ng/mL (1·6; n=32 tested) in the combined CTG trials. Furthermore, immunosuppressive burden with tacrolimus (trough concentration) and mycophenolate mofetil (dose) was similar or even tended to be lower throughout the study period in the patients in the CTG trials versus the RGT

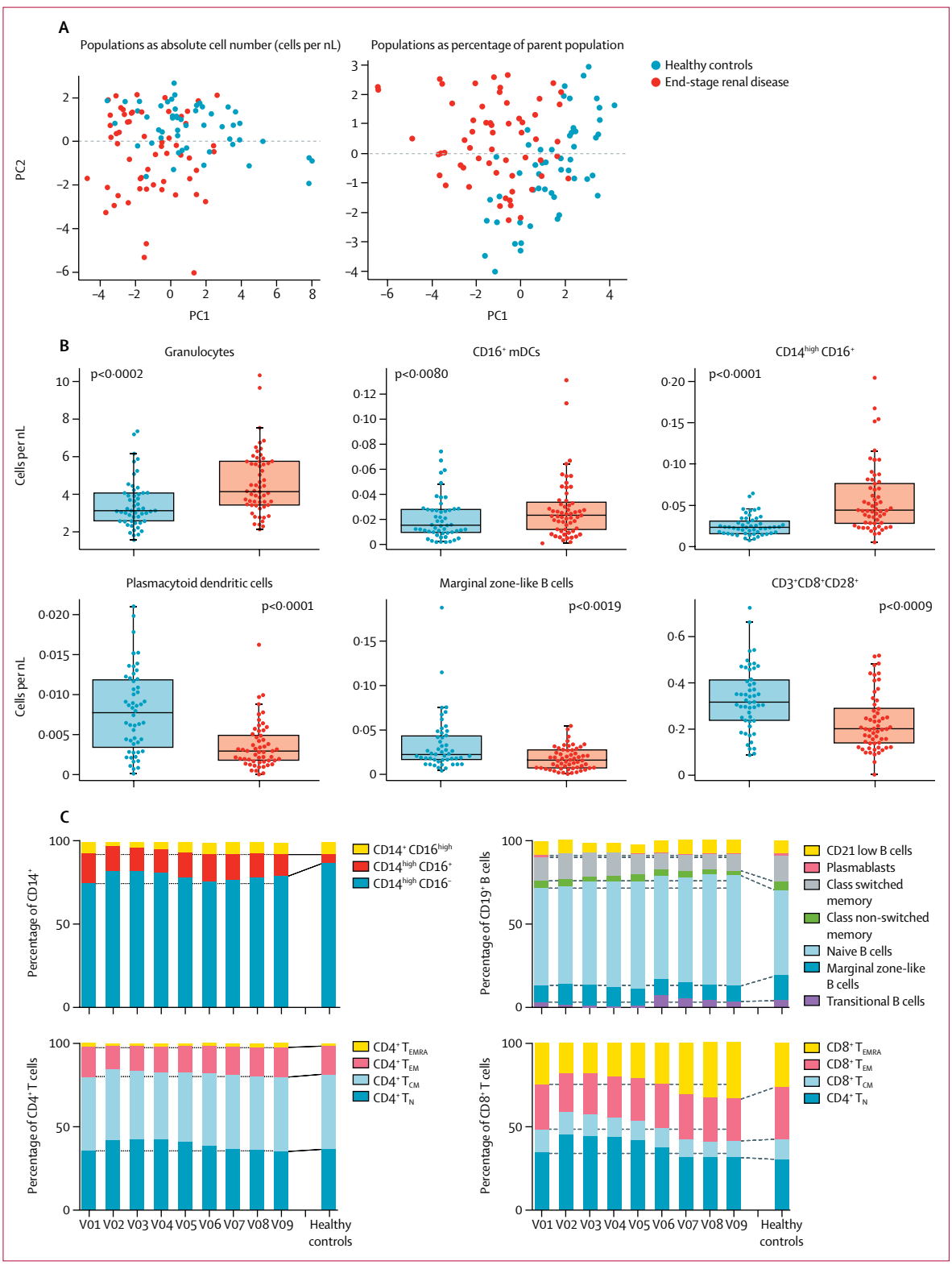


Figure 4: **Leucocyte subset alterations in patients with end-stage renal disease and time-dependent changes after kidney transplantation**
(A) PCA revealing the differences in leucocyte subsets between whole blood samples from end-stage renal disease (n=70) and healthy controls (n=98). (B) Box-and-whiskers plots of absolute numbers from leucocyte subpopulations with highest influence at the PCA shown in A. (C) Time-dependent changes from visit 1 before transplantation (V01) to visit 10 at 60 weeks post-transplant (V10) of monocyte, B cell, $CD4^+$, and $CD8^+$ T cell subset composition (stacked bars of mean proportions) in whole blood samples of patients in the RGT (n=59). Statistical analysis by Kruskal-Wallis-Test. PCA=principal component analysis. PC=principal component. mDCs=myeloid dendritic cells.

(appendix p 11). Together, these data should be considered with the understanding that 15 (40%) of 38 patients in the CTG trials were on tacrolimus monotherapy at study end, whereas 60 (98%) of 61 patients in the RGT continued on at least dual immunosuppression.

The normalised incidence in the RGT (n=66) for treatment-emergent serious adverse events was 91·2 per 100 patient study-years and for adverse events was 1614·6 per 100 patient study-years. The normalised incidence for the CTG trials (n=38) for serious adverse events was 70·7 per 100 patient study-years and for adverse events was 1452·0 per 100 patient study-years. These results indicated no increase in adverse events with cell therapy (appendix p 7). In the CTG trials, there was special attention given to identifying serious adverse events and adverse events related to cell therapy infusion. Overall, there were 12 adverse events reported with a possible relationship to the cell infusion, only one of which was a serious adverse event (increased creatinine; appendix p 8). All potentially related adverse events only occurred once, so no specific pattern was exposed in the 38 patients given CBMPs. No deaths were reported in any of the trials.

A descriptive analysis of normalised data comparing Medical Dictionary for Regulatory Activities-coded serious adverse events in the RGT versus the combined data from the CTG trials revealed that most serious medical problems were similar in frequency (figure 3A). However, there was one substantial difference that emerged, which is worth considering in detail. The incidence of serious adverse events in the RGT related to infections and infestations was nearly six-times higher than the combined CTG trials. After examining all infection-related adverse events recorded in the trials, this pattern of decreased infections in the CTG trials was consistently observed across each of the CTG trials (figure 3B) and was evident during the entire post-kidney transplant observation period (figure 3C). Also, we found that the main difference was a reduced number of viral infections in the CTG trials (figure 3D). Notably, there was also an appreciable difference in the number of infections recorded without specifying the pathogen, but numbers of bacterial and fungal infections were essentially the same. Breaking the data down even further regarding adverse events, the main decreases in viral infections in the CTG trials were for cytomegalovirus, herpes (including herpes simplex, herpes-zoster, oral herpes, nasal herpes, and varicella-zoster), and polyoma virus (figure 3E). The decreased rate of viral infection in the CTG was not due to more preventive measures, since 43 (65%) of 66 patients in the RGT and 20 (53%) of 38 patients in the CTG trials received antiviral prophylaxis in the first 3 months after kidney transplant. Also, notably, the percentage of cytomegalovirus-positive to cytomegalovirus-negative donor to recipient transplants was 18% (12 of 66) in the RGT and 21% (eight of 38) in the CTG trials. Therefore, patients in the CTG trials in general developed fewer viral infections than patients in the RGT.

Identical standardised immune monitoring testing of peripheral blood cells was done in all patients in the seven trials. In general, principal component analyses showed that before kidney transplant, patients in the RGT had major alterations in absolute and relative blood immune cell population composition compared with age-matched and sex-matched healthy controls (figure 4A). Populations contributing most to those alterations were granulocytes, $CD16^{+}$ myeloid dendritic cells, and $CD14^{high}CD16^{+}$ intermediate monocytes, which were increased in RGT patient samples, but also plasmacytoid dendritic cells, marginal zone-like B cells, and $CD8^{+}CD28^{+}$ T cells, which were higher in samples of healthy controls (figure 4B). Post-kidney transplant longitudinal analysis revealed only moderate normalisation of CD16-expressing monocytes and no normalisation of marginal zone-like B cells (figure 4C). Furthermore, although composition of conventional $CD4^{+}$ T-cell subsets remained normal and similar to healthy controls, $CD8^{+}$ T-cell subset composition showed major alterations over the post-kidney transplant course. Although naive T cells increased early after transplantation, we observed a skewing towards terminal differentiation of $CD8^{+}$ T cells in the long-term, starting at 24 weeks post-kidney transplant (figure 4C).

Examining immunophenotyping results from the RGT and combined CTG trials, we did not observe significant differences in numbers or proportions of $CD4^{+}CD25^{high}CD127^{low}$ Tregs between the groups at 60 weeks post-kidney transplant (figure 5A). A significant reduction in Treg-specific demethylated region demethylation occurred in patients in the RGT, but not in patients in the CTG trials. Furthermore, only patients in the RGT showed a significant increase in $CD8^{+}$ T_{EMRA} cells and $CD8^{+}CD57^{+}$ chronically activated T cells (figure 5B), whereas in samples from patients in the CTG trials, we observed more $CD8^{+}CD28^{+}$ T cells. Both patient groups showed a reduction of donor-specific IFNγ producing memory T cells after kidney transplant (appendix p 12). However, patients in the RGT, in contrast to patients in the CTG trials, showed higher anti-cytomegalovirus T-cell responses (appendix p 12), which correlated with absolute $CD8^{+}$ T_{EMRA} numbers (appendix p 12). This increase is well known in patients with a kidney transplant and is probably related to inflammation triggered reactivation of cytomegalovirus, which we also only observed in patients in the RGT but not the CTG trials (figure 3E). Although both patient groups had more plasmacytoid dendritic cells 60 weeks post-kidney transplant, we only observed a normalisation of marginal zone-like B-cell numbers and a significant reduction of $CD14^{high}CD16^{+}$ monocytes in patients in the CTG trials (figure 5C). In addition, patients in the CTG trials showed increased mRNA expression of genes described to be high in

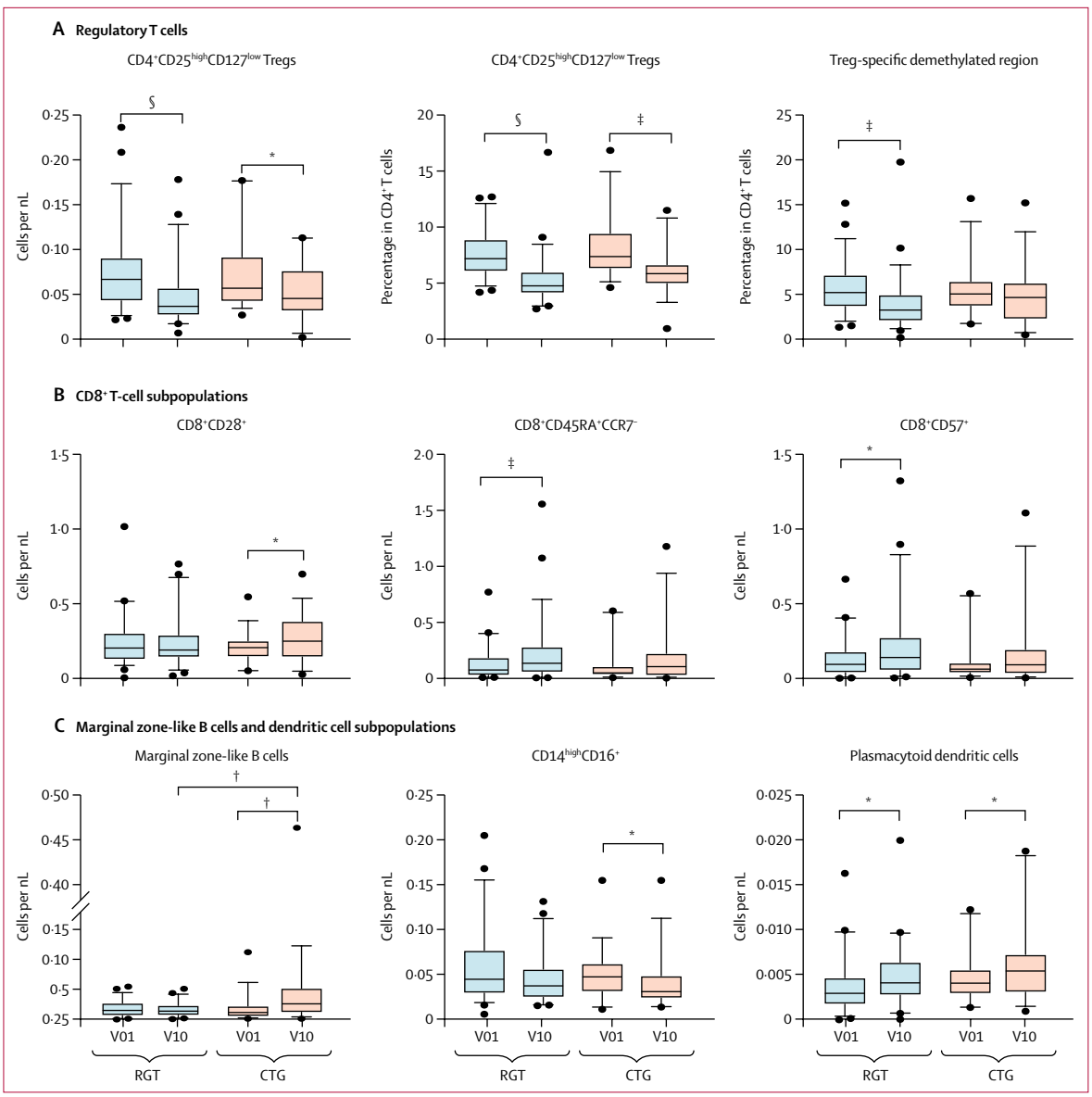


Figure 5: **Differences in post-transplant changes between patients in the RGT and CTG trials**
(A) Differences in post-transplant changes in regulatory T cells. Box and whisker plots of absolute numbers and proportions of $CD4^+CD25^{high}CD127^{low}$ Tregs as well as the percentage of $CD4^+$ T cells with demethylated Treg-specific demethylated region in whole blood samples collected pre-transplant (V01) and at the end of the observation period (60 weeks post-transplant, V10) from patients in the RGT (n=59) and CTG trials (n=38). (B) Differences in post-transplant changes in $CD8^+$ T cell subpopulations. Box and whisker plots of absolute numbers of $CD8^+CD28^+$, $CD8^+CD45RA^+CCR7^-$–T_{EMRA} and $CD8^+CD57^+$ chronically activated cells in whole blood samples collected pre-transplant (V01) and at the end of the observation period (60 weeks post-transplant, V10) from patients in the RGT (n=59) and CTG trials (n=38). (C) Differences in post-transplant changes in marginal zone-like B cells and dendritic cell subpopulations. Box and whisker plots of absolute numbers and proportions of marginal zone-like B cells, $CD16^+$ myeloid dendritic cells, and plasmacytoid dendritic cells in whole blood samples collected pre-transplant (V01) and at the end of the observation period (60 weeks post-transplant, V10) from patients in the RGT (n=59) and CTG trials (n=38). Statistical analysis by Wilcoxon matched-pairs signed rank and Dunn's multiple comparison test. RGT=reference group trial. CTG=cell therapy group. *p<0·05. †p<0·01. ‡p<0·001. §p<0·0001.

immunosuppression-free operationally tolerant patients with a kidney transplant (eg, *Ms4A1*) and co-inhibitory molecules (*CD200*), but reduced expression of rejection-associated genes (*HMMR*, appendix p 12). Together, these data suggest that our CTG trial patients show a healthy control-like restoration of immune cell composition.

Discussion

The ONE Study consortium has taken the unique approach of side-by-side trialling of different T cell, dendritic cell, and macrophage regulatory cell products in recipients of kidney transplants of low to medium risk for early graft loss. In this coordinated group of six international early phase clinical trials (the CTG trials), we showed that CBMP

application in this patient population is feasible for multiple regulatory cell types, and their categorical application near the time of kidney transplant revealed no apparent safety concerns. Furthermore, 15 (40%) of the 38 patients given CBMPs were successfully weaned to tacrolimus monotherapy during the 60-week observation period. The reference trial (the RGT) by the same clinical sites collecting matching clinical information and immune monitoring data provided a standard-of-care benchmark to confidently assess essential safety and immunological parameters, and also to evaluate whether reduction of immunosuppression through CBMP application could have potential benefits to patients. Remarkably, the rate of infections was considerably lower in patients given regulatory cell products than in standard-of-care treatment, particularly for viral infections. Furthermore, centralised immune monitoring of peripheral blood leucocyte populations suggested a return of CBMP-treated (CTG), but not conventionally treated (RGT) recipients towards a state of immune homoeostasis. Therefore, our results have established a fundamental basis for further testing of regulatory CBMP therapy in organ transplantation, and provide initial evidence that reducing general immunosuppressive burden through cell therapy could potentially decrease serious side-effects in recipients of kidney transplants.

This initial report focuses only on the CTG trials as a combined group, and not on results from the individual CTG trials. While each of the six individual CTG trials followed the same clinical treatment protocol regarding background immunosuppression, thus allowing for a comprehensive analysis of the CTG trials as a whole group, there are important details from each of those trials that deserve in-depth reporting and explanations in additional follow-up publications. Forthcoming details from the individual cases will provide insight into feasibility, safety aspects, and effects of each specific cell therapy product. These results will permit examination of issues such as cell production methods, CBMP characterisation, cell dosing, infusion scheduling, clinical outcomes, immunological features from kidney transplant biopsy specimens, and a comprehensive set of central immune monitoring results. Nonetheless, the current analysis of results from the combined CTG trials provides a uniquely broad evaluation of the safety and outlook perspective for cell therapy in organ transplantation, and shows that cell therapy was feasible in terms of logistics and cell manufacturing in the majority (38 [73%] of 52) of patients ready to receive the therapy.

One of the main motivations for seeking new therapies in organ transplantation is to reduce the need for general immunosuppressive drugs, which have substantial toxicities and incrementally expose recipients to dangers inherent from a suppressed immune system, most commonly infections. A set of guidelines and comprehensive review by Fishman[17] highlights the extent of the infection problem, and its direct relationship with immunosuppressive load. Results from the CTG trials indicate that lowering immunosuppression does appear to decrease the risk for viral infections. This finding was also supported by the immune monitoring results, as only patients in the RGT showed a tendency towards increased proportions of cytomegalovirus-specific memory T cells correlating with signs of chronic $CD8^+$ T-cell activation at the end of the observation period, as previously described.[18–20] What remains unknown is whether decreased infections were simply due to less immunosuppression in the CTG trials, or were related in some way to the cell therapy action itself; neither possibility can be ruled out yet. It should be noted that immunosuppressive burden was lower in the early stages after kidney transplant (no basiliximab induction), and in some patients, 9 months after kidney transplant (mycophenolate mofetil tapering), but that the infection rates were consistently lower across the spectrum of CTG trials during the entire observation period (figure 3C). Although reduction of mycophenolate mofetil treatment is within the prophylactic guidelines for patients at risk for developing viral infection,[17] the gap in reported infections did not show evidence of widening between the RGT and CTG trials after 9 months, leaving this issue unresolved. Nonetheless, our data encourage prospective randomised clinical trials to confirm an infectious disease benefit from regulatory cell therapy protocols.

Our immune monitoring results showed that patients with end-stage renal failure had major alterations in their peripheral immune cell composition compared with age-matched and sex-matched healthy controls, most likely reflecting their increased inflammatory state.[21–23] Standard immunosuppressive therapy in patients in the RGT did not reverse these alterations, but rather led to further immune cell imbalance as evidenced by a significant reduction in markers for stable Tregs.[24] Importantly, regulatory cell therapy mitigated this Treg reduction and was associated with a healthy control-like restoration of immune cell composition. In particular, marginal zone-like B-cell numbers, also discussed to have anti-inflammatory or regulatory function,[25,26] were increased in patients in the CTG trials at the end of the observation period. Thus, although both patients in the RGT and CTG trials had a reduction in donor-specific IFNγ-producing memory T cells, only the patients given cell therapy tended to have a re-establishment of immune cell homoeostasis, which is a major goal in organ transplantation. Importantly, these immune-related differences were independent of potential confounding factors such as donor relationships. Whether this effect is related to cell therapy itself, or is due to reduced immunosuppressive load in the CTG trials, will need to be investigated further in future trials.

To date, few reports have been published on the use of regulatory cell therapy in human organ transplantation,

some of which were pilot trials done previously by The ONE Study investigators. Hutchinson and colleagues have tested different preparations of regulatory macrophages in recipients of kidney transplants,[27–29] which provided essential lessons for designing the CTG trials. Additionally, polyclonal Tregs have been administered by the UCSF group to three recipients of kidney transplant with biopsy-proven subclinical inflammation 6 months after transplantation, showing that cell therapy is feasible in this circumstance.[30] Late administration of expanded polyclonal Tregs has also been reported by the Northwestern group in nine lymphodepleted recipients of kidney transplants.[31] In liver transplantation, Todo and colleagues have infused costimulatory blockade conditioned lymphocytes similar to those used by the Massachusetts General Hospital group in The ONE Study, and were able to achieve complete immunosuppression withdrawal in seven of the ten liver transplant recipients who were splenectomised and conditioned with cyclophosphamide.[32] Unfortunately, these pilot studies are highly variable in design, and did not incorporate a parallel trial with a similar group of patients not receiving cells to better appraise whether cell therapy is safe or shows indications of discernable effects. Importantly, The ONE Study trials were developed with the fundamental viewpoint that a reference trial, and also comparison to healthy control data, is absolutely necessary to make practical conclusions about regulatory cell therapy testing. Therefore, to advance the cell therapy field in organ transplantation, we aimed to evaluate cell therapy against a recognised standard-of-care (RGT) treatment by infusing different CBMPs near the time of kidney transplant as a replacement for conventional induction treatment (omitting basiliximab induction). Into this design we incorporated an option to wean mycophenolate mofetil starting at 9 months to further offer potential benefit to patients by reducing general immunosuppression, and to stress-test this cell therapy protocol under rigorous clinical monitoring. With this overall study strategy, and by doing the RGT as a multicentre study together with the CTG trials as parallel individual trials at the same sites, we uniquely delivered meaningful and reliable information about regulatory cell therapy to the organ transplantation community. Based on The ONE Study, the UK group has already initiated a randomised trial called the TWO Study with their polyclonal Treg cell product (ISRCTN11038572), and other ONE Study partners (Massachusetts General Hospital: NCT03577431 and UCSF Medical Center: NCT02188719) are doing trials in transplant recipients with cell products used in The ONE Study. Opening the way to these and other more advanced clinical trials was the unifying philosophy of The ONE Study.

For the **common controlled access principles** see https://www.ukri.org/funding/information-for-award-holders/data-policy/

Contributors

BS, EKG, PNH, PR, AM, JAH, DSG, CAB, JFM, TB, RC, WP, NA, HJS, PJF, RH, MB, BJ, JBvdN, MPH-F, UK, SJK, JG, PJM, LB, LAT, RIL, AB, JAB, GL, KJW, MCC, ASe, BB, GB, S-MK, and H-DV contributed to the study design. PNH, PR, JAH, DSG, CAB, JFM, TB, RC, WP, NA, HJS, PJF, RH, JBvdN, ASe, BB, GB, S-MK, NMO, and RÖ managed patient care. PR, AM, JAH, MB, AB, JAB, GL, KJW, MCC, QT, CS, ECG, LC-R, KC, ME, SK, and AS were involved in cell production. BS, EKG, PNH, PR, AM, JAH, MB, BJ, JBvdN, MPH-F, AB, MCC, H-DV, QT, CS, ECG, LC-R, KC, WJB, JLH, IM, FI, ISDR, MS, RJ, CB, ND, MK, and TM did biomarker development and data collection. BS, EKG, AM, JAH, BJ, MPH-F, AB, S-MK, QT, CS, WJB, JLH, IM, FI, ISDR, MS, RJ, CM, and SS did data analysis. BJ, CM, SS, and KJ were study statisticians. BS, EKG, PNH, PR, UK, SJK, JG, PJM, LB, LAT, RIL, AB, JAB, GL, KJW, MCC, ASc, BB, GB, S-MK, H-DV, ASe, ISDR, MS, RJ, CM, SS, and KJ interpreted data. EKG, BJ, and BS wrote the manuscript, which was reviewed by JAH, SS, and KJ, and all other authors. EKG was The ONE Study EU FP7 project coordinator.

Declaration of interests

BS, PR, AM, JAH, DSG, QT, ECG, MB, WJB, ISDR, MS, RJ, JFM, CB, BJ, LC-R, RC, IM, NMO, MPH-F, CM, SK, LAT, JAB, RJL, HJS, MCC, SS, S-MK, BB, GB, H-DV, GL, KJW, and EKG report grants from the EU (FP7 ONE Study) during the conduct of the study. PR and H-DV report grants from the BMBF, outside the submitted work. JAH reports other support from Trizell, personal fees from Finvector Oy during the conduct of the study. DSG reports non-financial support and other from Sandoz, non-financial support and other from Chiesi, non-financial support and other from Astellas, outside the submitted work. QT has a patent US14/382,537 issued and she is a co-founder of Sonoma. MB is one inventor of a patent for in-vitro generation and expansion of $CD4^+CD25^+$ T regulatory cells by rapamycin (# WO 2006/090291A2). The patent was licensed for non-exclusive usage to Miltenyi Biotech to develop a commercial kit for the ex-vivo expansion of Treg cells with rapamycin. ND reports other from Beckman Coulter Life Sciences, during the conduct of the study; other from Beckman Coulter Life Sciences, outside the submitted work. MK reports other from Beckman Coulter Life Sciences, during the conduct of the study; other from Beckman Coulter Life Sciences, outside the submitted work. MPH-F reports other from UCB Pharma, outside the submitted work. LAT reports personal fees from Third Rock Ventures, personal fees from Rheos Medicine, outside the submitted work; LAT is employed by Rubius Therapeutics. JAB has a patent US 7722862 B2 issued, a patent US 20080131445 A1, 9,012,1 issued, and a patent US 20150110761 A1 issued and is a founder and current CEO of Sonoma Biotherapeutics, which works on Tregs as therapeutics. HJS reports grants and personal fees from Novartis Pharma, grants and personal fees from Chiesi, outside the submitted work. TM reports other from Beckman Coulter Life Sciences, during the conduct of the study and outside the submitted work. RH reports personal fees and non-financial support from Chiesi, outside the submitted work. EKG reports grant support from Trizell and speaking fees from Novartis Pharma and Chiesi, outside the submitted work. All other authors declare no competing interests.

Data sharing

We will follow the common controlled access principles outlined by the Medical Research Council Clinical Trials Unit. According to those principles, we will acknowledge that data with long-term value be preserved, and usable for future research. We do, however, want to ensure that there are legal, ethical, and commercial constraints maintained on the release of research data according to the following code. Research teams are entitled to receive appropriate recognition for their efforts in collecting and analysing data and should be given at least a limited period of sole access to use and publish the data, before key trial data are open for use by other researchers. If such requests are made to access the data, resources need to be available to process the request and prepare the data in a timely manner, if possible. Because of these demands, there must be an important scientific objective behind each request. Especially in the case of our international project, The ONE Study, any request must comply with regulations set by the competent authorities in the relevant countries that govern data security policies.

Acknowledgments

This work was also supported by funds from IHU-CESTI (Investissement d'Avenir ANR-10-IBHU-005, Région Pays de la Loire and Nantes Métropole), the Labex IGO project (ANR-11-LABX-0016-01), and a donation from John Lang and Nancy Merrell. We also thank the Cell and Gene Therapy Manufacturing facility from Centre Hospitalier

Universitaire de Nantes for the ATDC production. In addition, we thank apceth Biopharma (Munich, Germany) for Mreg production in the study. We thank the nurses, physicians, and patients who contributed to the study. The research leading to these results has received funding from the EU Seventh Framework Programme (FP7) under grant agreement number 260687.

References

1 Wekerle T, Segev D, Lechler R, Oberbauer R. Strategies for long-term preservation of kidney graft function. *Lancet* 2017; **389:** 2152–62.

2 Bamoulid J, Staeck O, Halleck F, et al. The need for minimization strategies: current problems of immunosuppression. *Transpl Int* 2015; **28:** 891–900.

3 Rickert CG, Markmann JF. Current state of organ transplant tolerance. *Curr Opin Organ Transplant* 2019; **24:** 441–50.

4 Safinia N, Grageda N, Scottà C, et al. Cell therapy in organ transplantation: our experience on the clinical translation of regulatory T cells. *Front Immunol* 2018; **9:** 354.

5 Marín E, Cuturi MC, Moreau A. Tolerogenic dendritic cells in solid organ transplantation: where do we stand? *Front Immunol* 2018; **9:** 274.

6 Hutchinson JA, Geissler EK. Now or never? The case for cell-based immunosuppression in kidney transplantation. *Kidney Int* 2015; **87:** 1116–24.

7 Fraser H, Safinia N, Grageda N, et al. A rapamycin-based gmp-compatible process for the isolation and expansion of regulatory T cells for clinical trials. *Mol Ther Methods Clin Dev* 2018; **8:** 198–209.

8 Landwehr-Kenzel S, Zobel A, Hoffmann H, et al. Ex vivo expanded natural regulatory T cells from patients with end-stage renal disease or kidney transplantation are useful for autologous cell therapy. *Kidney Int* 2018; **93:** 1452–64.

9 Guinan EC, Cole GA, Wylie WH, et al. Ex vivo costimulatory blockade to generate regulatory t cells from patients awaiting kidney transplantation. *Am J Transplant* 2016; **16:** 2187–95.

10 Putnam AL, Safinia N, Medvec A, et al. Clinical grade manufacturing of human alloantigen-reactive regulatory T cells for use in transplantation. *Am J Transplant* 2013; **13:** 3010–20.

11 Marin E, Bouchet-Delbos L, Renoult O, et al. Human tolerogenic dendritic cells regulate immune responses through lactate synthesis. *Cell Metab* 2019; **30:** 1075–90.e8.

12 Hutchinson JA, Ahrens N, Geissler EK. MITAP-compliant characterization of human regulatory macrophages. *Transpl Int* 2017; **30:** 765–75.

13 Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357:** 2562–75.

14 Streitz M, Miloud T, Kapinsky M, et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. *Transplant Res* 2013; **2:** 17.

15 Kverneland AH, Streitz M, Geissler E, et al. Age and gender leucocytes variances and references values generated using the standardized ONE-Study protocol. *Cytometry A* 2016; **89:** 543–64.

16 Roufosse C, Simmonds N, Clahsen-van Groningen M, et al. A 2018 reference guide to the Banff classification of renal allograft pathology. *Transplantation* 2018; **102:** 1795–814.

17 Fishman JA. Infection in Organ Transplantation. *Am J Transplant* 2017; **17:** 856–79.

18 Meijers RW, Litjens NH, de Wit EA, et al. Cytomegalovirus contributes partly to uraemia-associated premature immunological ageing of the T cell compartment. *Clin Exp Immunol* 2013; **174:** 424–32.

19 Meijers RW, Litjens NH, Hesselink DA, Langerak AW, Baan CC, Betjes MG. Primary Cytomegalovirus Infection Significantly Impacts Circulating T Cells in Kidney Transplant Recipients. *Am J Transplant* 2015; **15:** 3143–56.

20 Makwana N, Foley B, Fernandez S, et al. CMV drives the expansion of highly functional memory T cells expressing NK-cell receptors in renal transplant recipients. *Eur J Immunol* 2017; **47:** 1324–34.

21 Ulrich C, Heine GH, Gerhart MK, Köhler H, Girndt M. Proinflammatory CD14+CD16+ monocytes are associated with subclinical atherosclerosis in renal transplant patients. *Am J Transplant* 2008; **8:** 103–10.

22 Vereyken EJ, Kraaij MD, Baan CC, et al. A shift towards pro-inflammatory CD16+ monocyte subsets with preserved cytokine production potential after kidney transplantation. *PLoS One* 2013; **8:** e70152.

23 van den Bosch TPP, Hilbrands LB, Kraaijeveld R, et al. Pretransplant numbers of $CD16^+$ monocytes as a novel biomarker to predict acute rejection after kidney transplantation: a pilot study. *Am J Transplant* 2017; **17:** 2659–67.

24 Braza F, Dugast E, Panov I, et al. Central role of CD45RA- Foxp3hi memory regulatory T cells in clinical kidney transplantation tolerance. *J Am Soc Nephrol* 2015; **26:** 1795–805.

25 Gray M, Gray D. Regulatory B cells mediate tolerance to apoptotic self in health: implications for disease. *Int Immunol* 2015; **27:** 505–11.

26 Appelgren D, Eriksson P, Ernerudh J, Segelmark M. Marginal-zone B-cells are main producers of IgM in humans, and are reduced in patients with autoimmune vasculitis. *Front Immunol* 2018; **9:** 2242.

27 Hutchinson JA, Brem-Exner BG, Riquelme P, et al. A cell-based approach to the minimization of immunosuppression in renal transplantation. *Transpl Int* 2008; **21:** 742–54.

28 Hutchinson JA, Riquelme P, Brem-Exner BG, et al. Transplant acceptance-inducing cells as an immune-conditioning therapy in renal transplantation. *Transpl Int* 2008; **21:** 728–41.

29 Riquelme P, Haarer J, Kammler A, et al. $TIGIT^+$ iTregs elicited by human regulatory macrophages control T cell immunity. *Nat Comms* 2018; **9:** 2858.

30 Chandran S, Tang Q, Sarwal M, et al. Polyclonal regulatory T cell therapy for control of inflammation in kidney transplants. *Am J Transplant* 2017; **17:** 2945–54.

31 Mathew JM, H-Voss J, LeFever A, et al. A phase I clinical trial with ex vivo expanded recipient regulatory T cells in living donor kidney transplants. *Sci Rep* 2018; **8:** 7428.

32 Todo S, Yamashita K, Goto R, et al. A pilot study of operational tolerance with a regulatory T-cell-based cell therapy in living donor liver transplantation. *Hepatology* 2016; **64:** 632–43.

APPENDIX

ORGAN RECIPIENT	ORGAN DONOR
MAIN INCLUSION CRITERIA	
• Chronic renal insufficiency necessitating kidney transplantation and approved to receive a primary kidney allograft from a living donor • Age ≥ 18 years • Signed and dated written informed consent	• Eligible for live kidney donation • Age ≥ 18 years • Signed and dated written informed consent
MAIN EXCLUSION CRITERIA	
• Any previous tissue or organ transplant • Genetically identical to the prospective organ donor at the HLA loci (0-0-0 mismatch) • PRA grade > 40% within six months prior to enrolment • Previous treatment with any desensitisation procedure (with or without IVIg) • HIV-positive, EBV-negative or chronic viral hepatitis • Significant liver disease, defined as persistently elevated AST and/or ALT levels > 2 x upper limit of normal range • Malignant or pre-malignant haematological conditions • Concomitant malignancy or history of malignancy within five years prior to planned study entry (excluding successfully-treated non-metastatic basal/squamous cell carcinoma of the skin) • Evidence of significant local or systemic infection • Ongoing treatment with systemic immunosuppressive drugs at study entry • Known contraindication to the study medications • Female patients with a positive pregnancy test at enrolment • Female patients who are breast-feeding • Female patients of child-bearing potential, unless the patient maintains a highly effective method of birth control or the career, lifestyle, or sexual orientation of the patient ensures that there is no risk of pregnancy • Patients unable to freely give informed consent (e.g. individuals under legal guardianship)	• Genetically identical to the prospective organ recipient at the HLA loci (0-0-0 mismatch) • Exposure to any investigational product at the time of kidney donation, or within 28 days prior to kidney donation • Subjects unable to freely give informed consent (e.g. individuals under legal guardianship)

Core eligibility criteria applied to the selection of donor-recipient pairings enrolled in The ONE Study clinical trials. Each CTG trial additionally applied a set of customised exclusion criteria specific to the infused cell product. HLA = human leukocyte antigen; PRA = panel reactive antibody; IVIg = intravenous immunoglobulin; HIV = human immunodeficiency viruses; EBV = Epstein-Barr virus; AST = aspartate transaminase; ALT = alanine transaminase.

CBMP	TRIAL LOCATION	CELL ORIGIN	DESCRIPTION	REFERENCE TO CBMP CHARACTERISTICS
Regulatory T cell-derived products				
pTreg-1	London/Oxford	Autologous	Polyclonal regulatory T cells	[7] Fraser H et al.
pTreg-2	Berlin	Autologous	Polyclonal regulatory T cells	[8] Landwehr-Kenzel S et al.
darTreg-sBC	San Francisco	Autologous	Donor antigen-reactive Treg stimulated with donor B cells	[10] Putnam AL et al.
darTreg-CSB	Boston	Autologous	Donor antigen-reactive Treg generated under costimulatory blockade	[9] Guinan EC et al.
Monocyte-derived cell products				
ATDC	Nantes	Autologous	Autologous tolerogenic dendritic cells	[11] Marin E et al.
Mreg	Regensburg	Allogeneic (organ donor)	Regulatory macrophages	[12] Hutchinson JA et al.

Overview of CBMPs used in the ONE Study CTG trials. CBMP = cell-based medicinal product.

	RGT (N = 66)	CTG (N = 38)
Recipient age (years)		
20 – 29	6 (9·1 %)	2 (5·3 %)
30 – 39	15 (22·7 %)	10 (26·3 %)
40 – 49	17 (25·8 %)	11 (28·9 %)
50 – 59	15 (22·7 %)	8 (21·1 %)
60 – 69	10 (15·2 %)	6 (15·8 %)
70 – 79	3 (4·5 %)	1 (2·6 %)
Mean ± SD	47·5 ± 13·1	47·6 ± 12·7
Median	47·3	45·3
Min - Max	23·3 – 72·6	24·4 – 71·3
Recipient sex		
Female	18 (27·3 %)	11 (28·9 %)
Male	48 (72·7 %)	27 (71·1 %)
Recipient race		
White	59 (89·4 %)	36 (94·7 %)
Asian	6 (9·1 %)	1 (2·6 %)
Other	1 (1·5 %)	1 (2·6 %)
Renal replacement therapy (RRT)		
No RRT (pre-emptive transplantation)	27 (40·9 %)	18 (47·4 %)
Haemodialysis	30 (45·5 %)	16 (42·1 %)
Peritoneal dialysis	9 (13·6 %)	4 (10·5 %)
RRT time on dialysis (months)		
< 6	12 (18·2 %)	5 (13·2 %)
6 – 12	9 (13·6 %)	4 (10·5 %)
12 – 24	6 (9·1 %)	4 (10·5 %)
24 – 36	4 (6·1 %)	3 (7·9 %)
36 – 48	1 (1·5 %)	0 (0·0 %)
> 48	6 (9·1 %)	4 (10·5 %)
Unknown	1 (1·5 %)	0 (0·0 %)
Underlying CKD diagnosis		
Polycystic kidney disease	16 (24·2 %)	8 (21·1 %)
IgA nephropathy	13 (19·7 %)	8 (21·1 %)
Diabetic nephropathy	8 (12·1 %)	2 (5·3 %)
Hypertensive nephrosclerosis	5 (7·6 %)	3 (7·9 %)
Idiopathic focal segmental glomerulosclerosis	3 (4·5 %)	2 (5·3 %)
Congenital obstructive uropathy	2 (3·0 %)	2 (5·3 %)
Chronic pyelonephritis (incl. reflux nephropathy)	2 (3·0 %)	1 (2·6 %)
Alport syndrome	2 (3·0 %)	1 (2·6 %)
Membranoproliferative glomerulonephritis	2 (3·0 %)	0 (0·0 %)
Membranous glomerulonephritis	2 (3·0 %)	0 (0·0 %)
Henoch-Schonlein purpura	1 (1·5 %)	0 (0·0 %)
Toxic or drug-related tubulointerstitial disease	1 (1·5 %)	0 (0·0 %)
Idiopathic tubulointerstitial disease	1 (1·5 %)	0 (0·0 %)
Thrombotic microangiopathy	0 (0·0 %)	1 (2·6 %)
Other disease	8 (12·1 %)	10 (26·3 %)

Baseline characteristics of organ recipients in The ONE Study. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; CKD = chronic kidney disease; SD = standard deviation; RRT = renal replacement therapy. Age calculated at time of transplantation, dates of birth with incomplete day information set to the first of the respective month. All categorical variables shown as number (%) by group.

	RGT (N = 66)	CTG (N = 38)
Donor age (years)		
20 – 29	3 (4·5 %)	5 (13·2 %)
30 – 39	8 (12·1 %)	4 (10·5 %)
40 – 49	14 (21·2 %)	8 (21·1 %)
50 – 59	25 (37·9 %)	13 (34·2 %)
60 – 69	16 (24·2 %)	7 (18·4 %)
70 – 79	0 (0·0 %)	1 (2·6 %)
Mean ± SD	51·8 ± 11·3	49·2 ± 12·8
Median	53·3	51·1
Min - Max	23·8 – 69·0	23·8 – 70·7
Donor sex		
Female	41 (62·1 %)	22 (57·9 %)
Male	25 (37·9 %)	16 (42·1 %)
Donor race		
White	58 (87·9 %)	36 (94·7 %)
Asian	6 (9·1 %)	1 (2·6 %)
Other	2 (3·0 %)	1 (2·6 %)
Donor's relationship to recipient		
Mother	10 (15·2 %)	7 (18·4 %)
Father	10 (15·2 %)	1 (2·6 %)
Daughter	3 (4·5 %)	0 (0·0 %)
Son	1 (1·5 %)	3 (7·9 %)
Sibling	16 (24·2 %)	6 (15·8 %)
Niece / Nephew / First cousin	2 (3·0 %)	0 (0·0 %)
Spouse / Unrelated	24 (36·4 %)	21 (55·3 %)

Baseline characteristics of organ donors in The ONE Study. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; SD = standard deviation. Age calculated at time of transplantation, dates of birth with incomplete day information set to the first of the respective month. All categorical variables shown as number (%) by group.

The ONE Study RGT and CTG

PER-PROTOCOL CRITERIA

- No major violation of trial eligibility criteria (e.g. HLA 0-0-0 mismatch)
- Treatment with cell infusion (CTG only)
- Treatment with at least one dose of basiliximab (RGT only)
- Duration of treatment with oral prednisolone not exceeding 20 weeks post-transplantation
  - Concomitant therapy with steroids taken for other indications (prior to or after 20 weeks) is permitted
  - Concomitant therapy with topical / inhaled steroids is permitted
  - Any steroid treatment given for anti-rejection prophylaxis after 20 weeks is not permitted
- MMF/MPA and tacrolimus (or acceptable substitutes) dosed continuously until study end / BCAR / drop out (interruptions of <2 months duration permitted)
- CTG only: MMF/MPA (or acceptable substitute) dosed continuously until study end / BCAR / drop out or until deliberate dose tapering starting from 30 weeks (interruptions of <2 months duration permitted)
- No high-dose immunosuppressive agents or potent anti-inflammatory treatments given as supplementary therapy in the absence of a confirmed primary endpoint

Criteria applied to the per-protocol analysis set. Patients who registered a primary endpoint of BCAR or dropped out prematurely were included in the per-protocol set if compliant with these criteria up to the date of first BCAR diagnosis / date of withdrawal. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; HLA = human leukocyte antigen; MMF = mycophenolate mofetil; MPA = mycophenolic acid; BCAR = biopsy-confirmed acute rejection.

Severity of 1st BCAR episode	RGT (N = 8)	CTG (N = 6)
Central pathological diagnosis		
Acute TCMR IA	1 (12·5 %)	1 (16·7 %)
Acute TCMR IIA	3 (37·5 %)	2 (33·3 %)
Acute TCMR IB	1 (12·5 %)	1 (16·7 %)
Acute TCMR IIB	0 (0·0 %)	2 (33·3 %)
Borderline changes	3 (37·5 %)	0 (0·0 %)
ABMR diagnosed locally?		
Yes	1 (12·5 %)	2 (33·3 %)
No	7 (87·5 %)	4 (66·7 %)
Response to treatment		
Glucocorticoid-responsive	4 (50·0 %)	3 (50·0 %)
Responsive to depleting antibody treatment	3 (37·5 %)	3 (50·0 %)
Not applicable*	1 (12·5 %)	0 (0·0 %)

Primary endpoint (BCAR) data. Severity of first BCAR episode by central pathological diagnosis and response to treatment. * One patient treated with low-dose oral steroids and by not tapering immunosuppression. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; BCAR = biopsy-confirmed acute rejection; TCMR = T cell-mediated rejection; ABMR = antibody-mediated rejection.

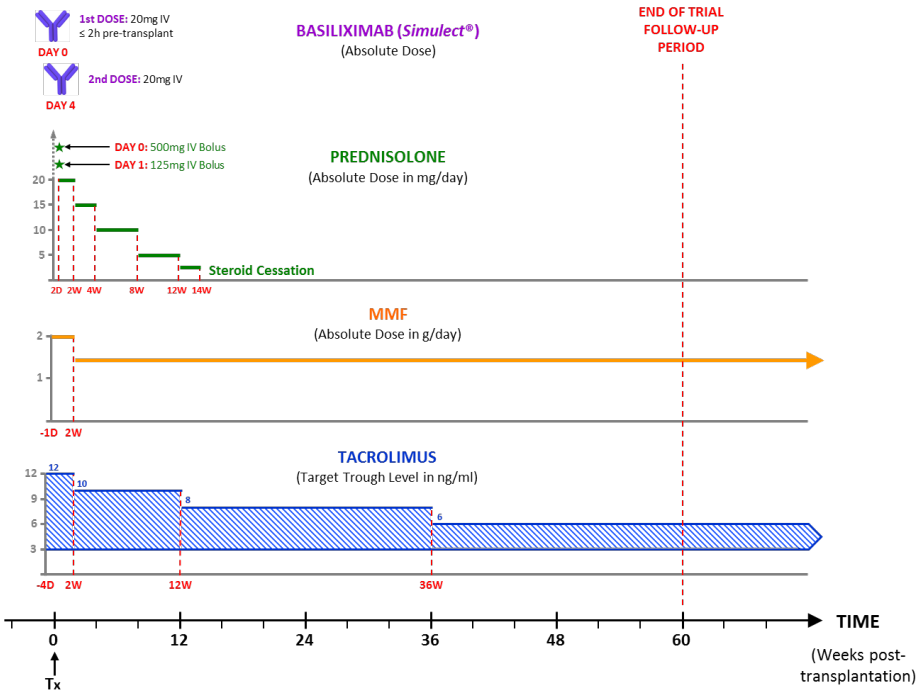
	RGT (N = 66; PSY = 72.34)			CTG (N = 38; PSY = 43.87)		
	Total Events	Events per 100-PSY	Patients (%) with events	Total Events	Events per 100-PSY	Patients (%) with events
All Body Systems						
Any serious adverse event (SAE)	66	91·2	36 (54·5 %)	31	70·7	16 (42·1 %)
Any adverse event (AE)	1168	1614·6	65 (98·5 %)	637	1452·0	38 (100·0 %)
Any SAE possibly related* to immunosuppression	21	29·0	15 (22·7 %)	7	16·0	7 (18·4 %)
Any AE possibly related* to immunosuppression	210	290·3	53 (80·3 %)	119	271·3	33 (86·8 %)

***Summary of all treatment-emergent (S)AEs in* The ONE Study.** Treatment-emergent (S)AEs are events with onset date equal to or after first dose of any study drug (i.e. basiliximab, prednisolone, MMF or tacrolimus in RGT; cell therapy, prednisolone, MMF or tacrolimus in CTG). All events coded to the MedDRA PT: "transplant rejections" are excluded, since rejection was measured as the primary efficacy endpoint. Incidence rates are expressed as "Events per 100-PSY" and use the cohort-specific cumulative patient-time observed in the RGT (72.34) and CTG (43.87) as denominator. * Relationship to immunosuppression was assessed by the reporting Investigator and defined as a reasonable possibility of a causal relationship to any of the study drugs. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; PSY = patient study years.

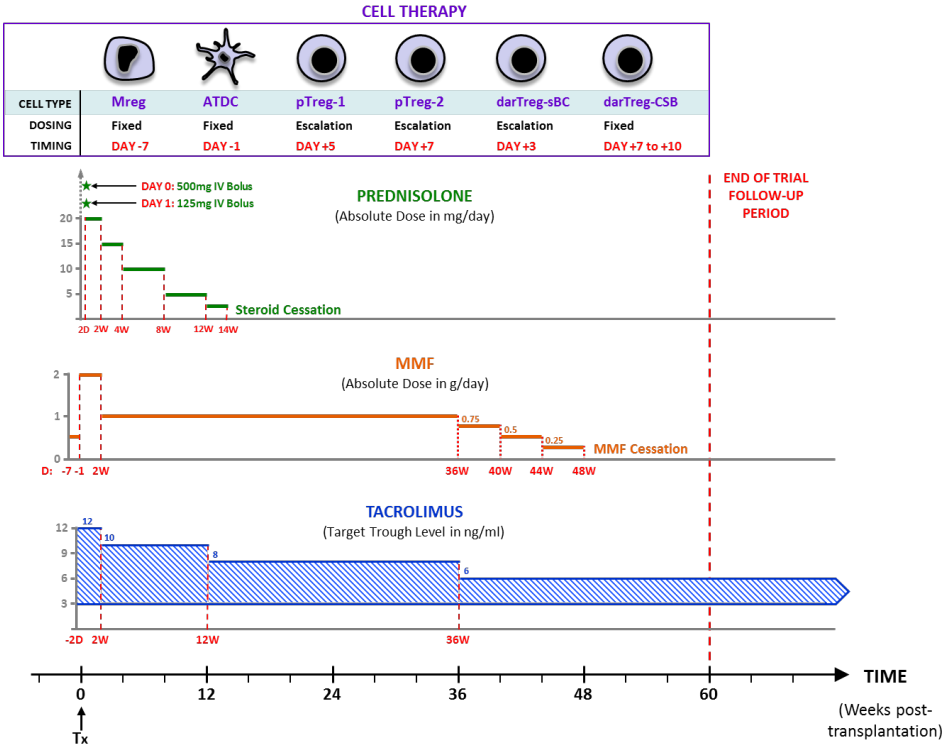
MedDRA System Organ Class (SOC) MedDRA Preferred Term (PT)	**Number of events**
Blood and lymphatic system disorders	
Lymphopenia	1
SOC SUB-TOTAL	**1**
General disorders and administration site conditions	
Feeling hot	1
SOC SUB-TOTAL	**1**
Investigations	
Alanine aminotransferase increased	1
Blood creatinine increased (SAE)	1
C-reactive protein increased	1
Donor-specific antibody present	1
Gamma-glutamyltransferase increased	1
SOC SUB-TOTAL	**5**
Musculoskeletal and connective tissue disorders	
Muscle spasms	1
SOC SUB-TOTAL	**1**
Nervous system disorders	
Dysgeusia	1
Headache	1
Paraesthesia	1
SOC SUB-TOTAL	**3**
Skin and subcutaneous tissue disorders	
Pruritus	1
SOC SUB-TOTAL	**1**
TOTAL:	**12**

All (S)AEs assessed as possibly related to a cell product in The ONE Study CTG trials. 12 events were assessed by the reporting investigator as having a reasonable possibility of a causal relationship with the cell product. Events are categorised by primary SOC and coded with MedDRA version 20·1. SOC = System Organ Class; PT = preferred term.

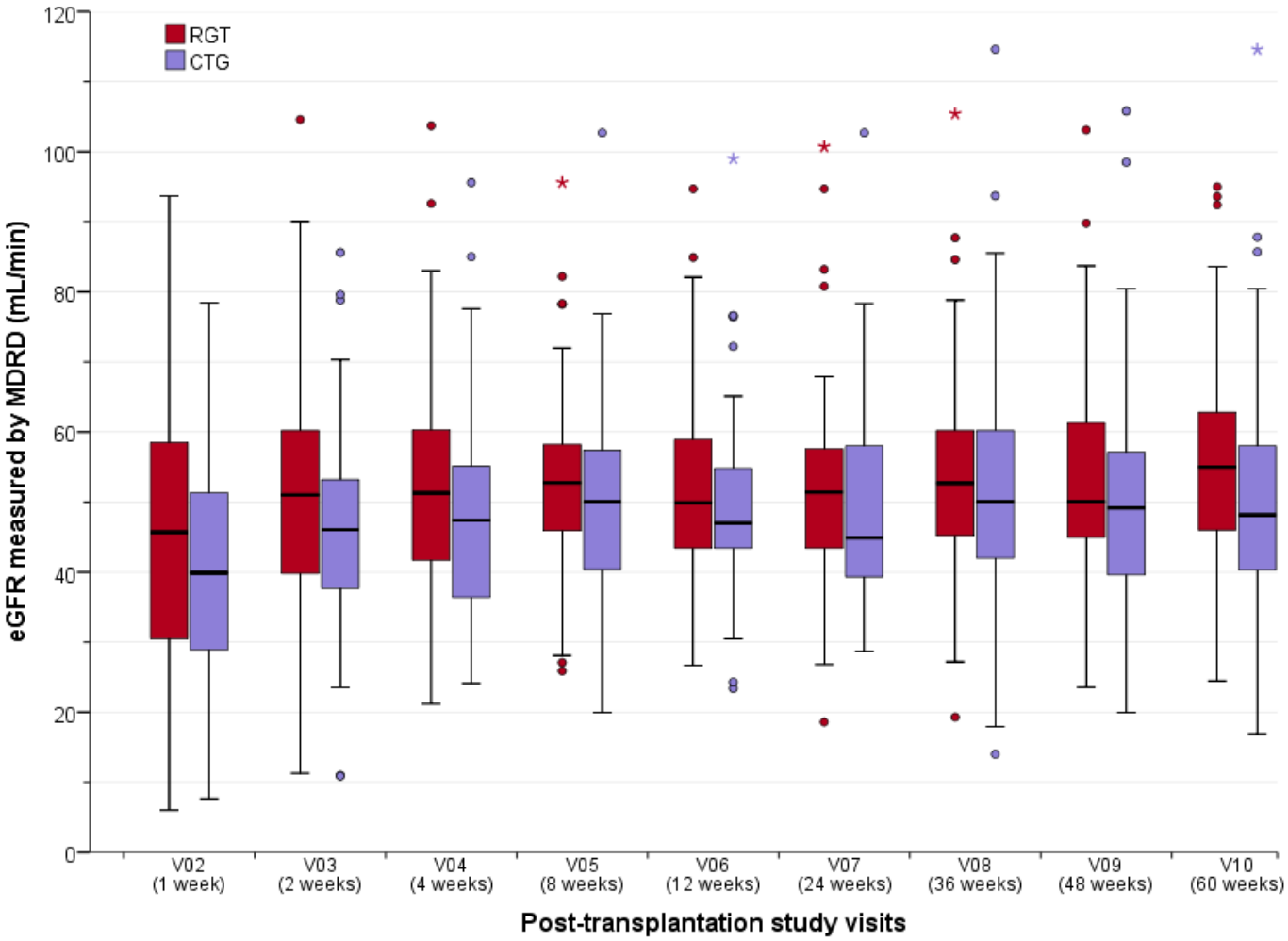
RGT



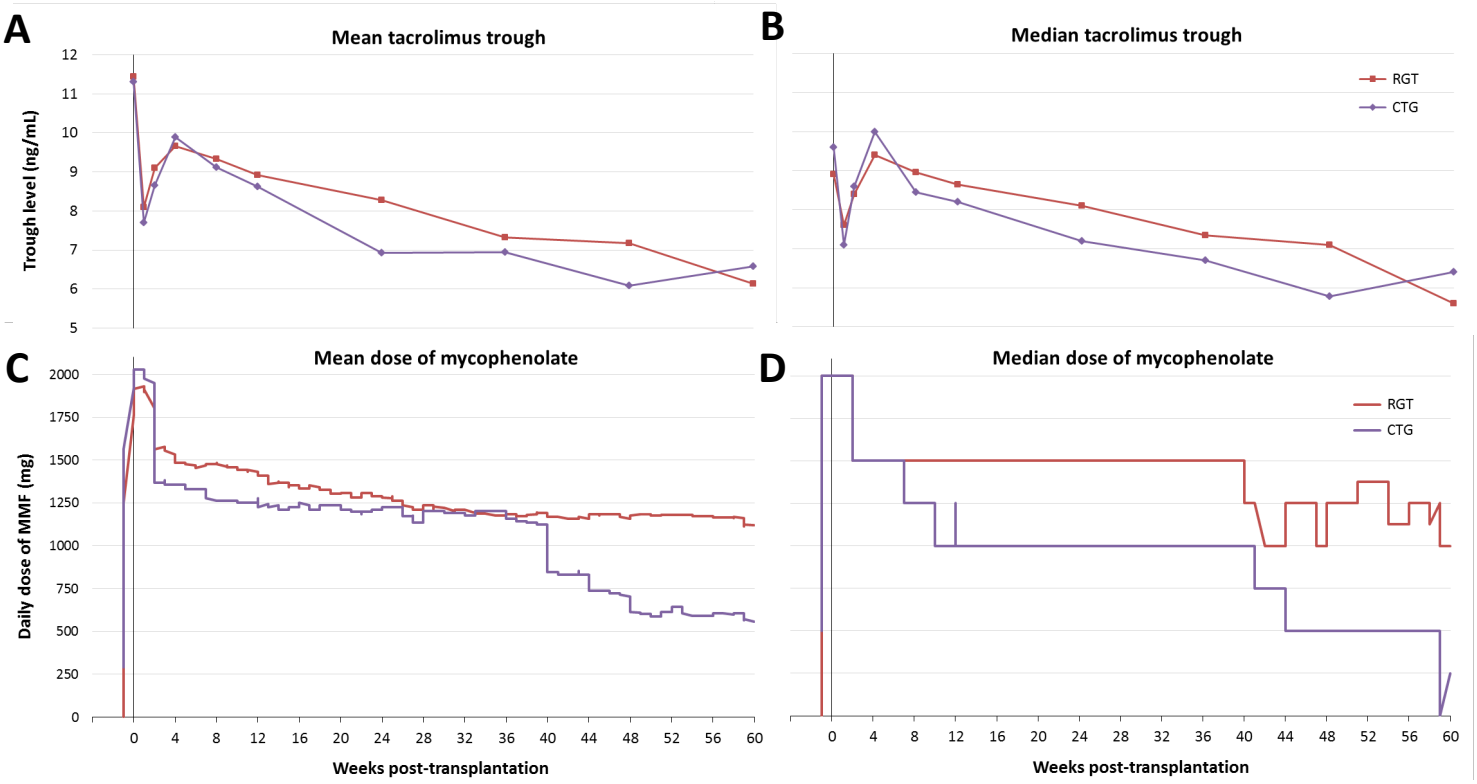
CTG trials



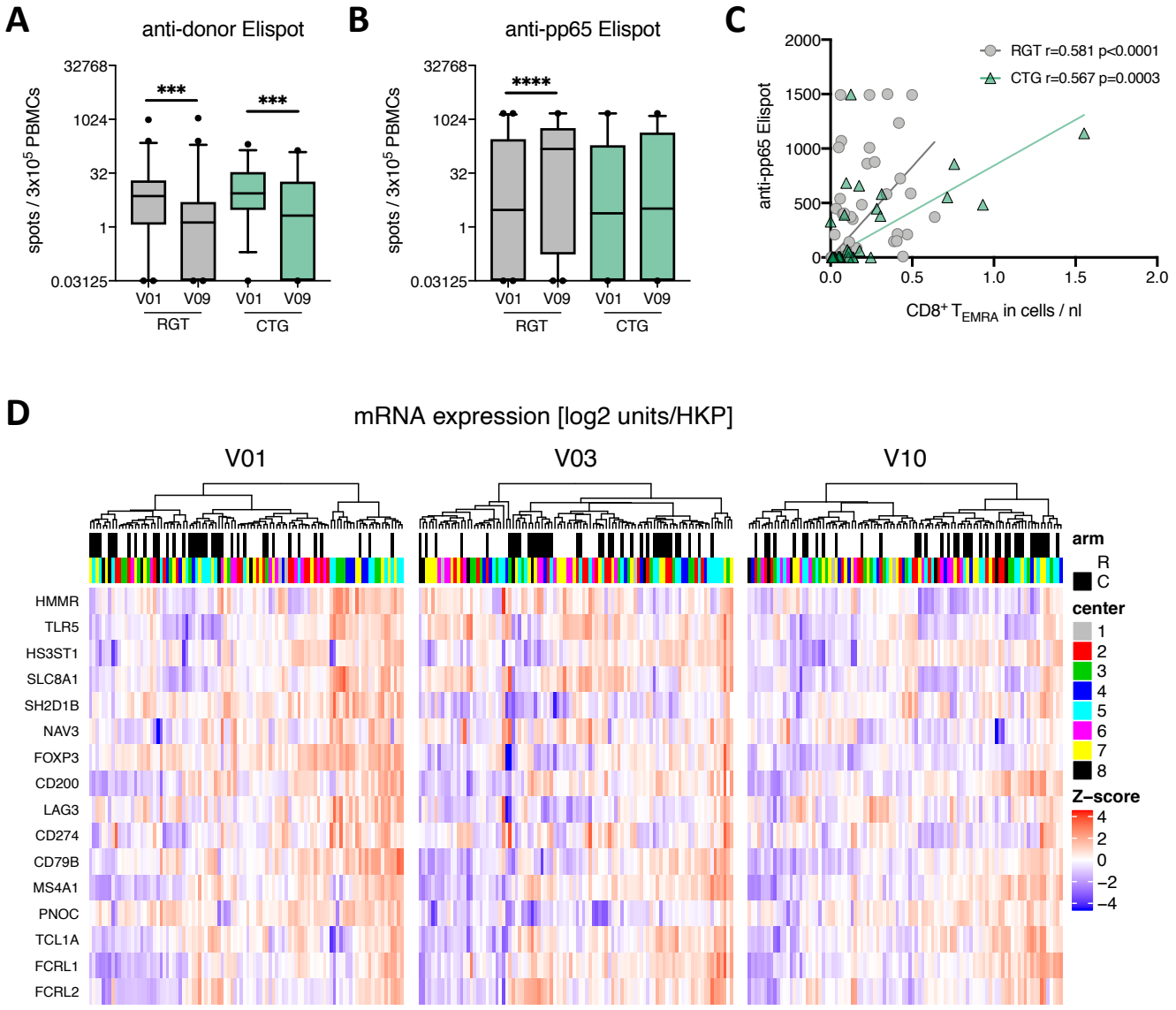
Treatment protocol for the RGT and CTG trials. IV = intravenous, D = day; W = week; MMF = mycophenolate mofetil; Tx = transplantation.



ONE Study kidney function post-transplantation. MDRD eGFR measured in the RGT and CTG trials at each study visit post-transplantation. Points mark outliers beyond inner fences set at 1.5 x IQR (interquartile range); asterisks mark extreme outliers beyond outer fences set at 3 x IQR. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; eGFR = estimated glomerular filtration rate; MDRD = Modification of Diet in Renal Disease formula; V = Visit.



Immunosuppressive burden of tacrolimus and mycophenolate over time. Mean (S3A) and median (S3B) blood trough levels of tacrolimus calculated at 10 time points using all available readings within the following time windows: day 0 (± 1 day), 7 (± 1), 14 (± 1), week 4 (± 1 week), 8 (± 1), 12 (± 1), 24 (± 1), 36 (± 1), 48 (± 1) and 60 (± 1); total number of data points = 771 (RGT) and 496 (CTG). Mean (S3C) and median (S3D) daily doses of mycophenolate calculated continuously from one week pre-transplantation to 60 weeks post-transplantation. Doses of mycophenolate sodium converted to biologically equivalent doses of mycophenolate mofetil. Patients switched to azathioprine were censored at the time of last dose of mycophenolate; if mycophenolate was discontinued permanently or temporarily without switching to an alternative anti-proliferative agent, the dose was set to zero for the period during which mycophenolate was withheld. Incomplete or unknown start / stop dates were imputed to the mid-point of two sequential doses (if in the middle of a dosing regimen) or to the protocol-specified start date (for the very first dose). RGT = Reference Group Trial (N=66); CTG = Cell Therapy Group trials (N=38); MMF = mycophenolate mofetil.



Anti-donor and anti-CMV IFNg EliSpot analyses as well as gene expression analyses.

A) Anti-donor IFNg EliSpot analysis prior to transplantation (V01) and 12 months post-transplantation (V09) of PBMCs from RGT (n=45) and CTG (n=33) patients. B) Anti-CMV (pp65) IFNg EliSpot analysis prior to transplantation (V01) and 12 months post-transplantation (V09) of PBMCs from RGT (n=45) and CTG (n=33) patients. C) Correlation between anti-CMV (pp65) Elispot spot counts and absolute numbers of $CD8^+$ T_{EMRA} ($CD8^+CD45RA^+CCR7^-$ T cells) at 12 months post-transplantation (V09). Statistical analysis by Wilcoxon matched-pairs signed rank test and Spearman's correlation. *** p<0.001, **** p<0.0001

D) Heat maps upon unsupervised clustering summarizing gene expression results of previously defined tolerance- (HS3ST1, SH2D1B, CD79B, MS4A1, PNOC, TCL1A, FCRL1, FCRL2) and rejection-associated (HMMR, TLR5, SLC8A1, VAV3) genes as well as FOXP3 and co-inhibitory molecules (CD200, LAG3, CD274) measured by qRT-PCR of whole blood samples from RGT patients (n=60, arm R, white bars) and CTG patients (n=38, arm C, black bars) collected pre-transplant (V01), two weeks post-transplant (V03) or 60 weeks post-transplant (V10).

Flow cytometry
Measurements were done locally at each study site upon training by central immune monitoring lab personnel and interlab comparisons. Blood samples collected into EDTA tubes were stained within 4 hours and analysed by flow cytometry using the previously published antibody panels and protocols (Kverneland Cytometry A 2016). Briefly, 100 µl EDTA blood were directly stained with prepared panel antibody mixes and incubated before lysing erythrocytes with lyse-fix solution composed of Versa Lyse™ and IOTest® Fixative Solution (Beckman Coulter GmbH). For the B cell panel (panel 4) 300 µl EDTA blood was first lysed with Red Blood Cell Lysis Solution (Miltenyi Biotec GmbH) prior to antibody staining. The dendritic cell panel was prepared twice and combined after staining. Samples were measured on a 10 colour Navios flow cytometer (Beckman Coulter). Calibration with "Flow-Set Pro Beads" and "Flow Check Pro Beads" (both Beckman Coulter) was performed daily. Acquired LMD files were centrally analysed by central immune monitoring lab personnel. Analysis of LMD files was done with Kaluza version 1.2 (Beckman Coulter). To calculate absolute cell numbers of all reported immune cell subsets, leucocyte cell count was obtained from the local clinical chemistry and related to the $CD45^+$ count within each panel. The corresponding proportions of all reported immune cell subsets were calculated in Excel.

Real-time quantitative reverse transcription PCR and TSDR-demethylation analysis
Patient blood samples were collected in Tempus Blood RNA Tubes (Thermo Fisher Scientific, Schwerte, Germany) and stored at -20°C until shipment as batches into central immune monitoring lab. RNA was isolated using the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Thermo Fisher Scientific). Up to 1000 ng RNA was transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). Hypothesis-driven expression of genes whose expression have been previously shown to be increased in samples from immunosuppression-free operationally-tolerant kidney transplant patients, such as HS3ST1, SH2D1B, CD79B, MS4A1, PNOC, TCL1A, FCRL1 and FCRL2, or in patients with rejection, such as HMMR, TLR5, SLC8A1 and VAV3 (Sagoo et al., *J Clin Invest* 2010, PMID: 20501943; Sawitzki et al., *Am J Transpl* 2007, PMID: 17456197; Viklicky et al., *Transplantation* 2013, PMID: 23222918; and Krepsova et al., *BMC Nephrol* 2015, PMID: 26286066) was measured using TaqMan Gene Expression Assays (Thermo Fisher Scientific, *Hypoxanthine-guanine phosphoribosyltransferase (HPRT)* = Hs02800695_m1, *beta-2-microglobulin (B2M)* = Hs00984230_m1, *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* = Hs99999905_m1, *(HMMR)* = , *toll-like receptor 5 (TLR5*) = Hs01019558_m1, *heparan sulfate-glucosamine 3-sulfotransferase 1 (HS3ST1)* = Hs01099196_m1, *solute carrier family 8 member A1 (SLC8A1)* = Hs01062258_m1, *SH2 domain containing 1B (SH2D1B)* = Hs01592483_m1, *neuron navigator 3 (NAV3)* = Hs00372108_m1, *forkhead box P3 (FOXP3)* = Hs00203958_m1, *CD200* = Hs01033303_m1, *lymphocyte activating 3 (LAG3)* = Hs00158563_m1, *CD274* = Hs01125301_m1, *CD79B* = Hs00236881_m1, *membrane spanning 4-domains A1 (MS4A1)* = Hs00544818_m1, *prepronociceptin (PNOC)* = Hs00918595_m1, *T cell leukemia/lymphoma 1A (TCL1A)* = Hs00172040_m1, *Fc receptor like 1 (FCRL1)* = Hs00957541_m1, *Fc receptor like 2 (FCRL2)* = Hs00229156_m1), microfluidic cards and TaqMan Universal Master Mix (Thermo Fisher Scientific) on the ViiA7 Real Time PCR System (Thermo Fisher Scientific). Reactions were run in duplicates using 384-well microfluidic Custom TaqMan® Array Cards and obtained data were analyzed applying the respective ViiA7 Software v 1.2.2. Gene expression was calculated relative to median expression of three reference genes (*HPRT, B2M, GAPDH)* using the $2^{-\Delta\Delta Ct}$ method.
TSDR Analysis was done centrally at the central immune monitoring lab in batches upon shipment of frozen EDTA blood samples. First, genomic DNA was isolated from EDTA blood using the QIAamp DNA Mini Kit (Qiagen). Up to 2 µg DNA were used for bisulfite treatment (EpiTect, Qiagen). Real-time PCR was done in a final reaction volume of 20 µl with 10 µl FastStart Universal Probe Master (ROX, Roche Diagnostics, Mannheim, Germany), 100 ng Lamda DNA (NEB, Frankfurt a.M., Germany), 5 pmol methylation or non-methylation specific probe, 30 pmol methylation or non-methylation specific primers and at least 15 ng bisulfite-treated DNA or plasmid standard (all Epiontis GmbH, Berlin, Germany). Samples were analyzed in triplicates on an ABI 7500 Cycler (Thermo Fisher Scientific). The percentage of $CD4^+$ T cells with demethylated TSDR was calculated by division of non-methylated by total genomic FoxP3 copy-number and normalization to the proportion of total $CD3^+CD4^+$ T cells as determined by flow cytometry.

IFNg EliSpot
Local immune monitoring labs performed isolation and cryopreservation of donor and recipient peripheral blood mononuclear cells (PBMCs). For donor PBMCs isolation RosetteSep Human CD3 Depletion Cocktail (Stemcell) was added to collected citrate blood prior to Ficoll (Ficoll-Paque Plus, GE Healthcare Life Sciences) gradient centrifugation to remove T cells.
EliSpot analyses were done centrally by central immune monitoring lab. Stimulation (24h) was done using the EliSpot Interferon-gamma Assay Kit (AID, Strassberg, Germany). For anti-donor responses $3x10^5$ recipient PBMCs were stimulated with $3x10^5$ T cell-depleted PBMCs in triplicates. Anti-CMV were quantified upon stimulation of $3x10^5$ recipient PBMCs with a CMV pp65 peptide pool (1.25µg/ml; Jerini peptide Technologies, Berlin, Germany) in duplicates. Unstimulated PBMCs served as controls.