DOI:
10.1016/S0140-6736(20)30167-7

Document Version
Peer reviewed version

Title:

The ONE Study: Evaluation of Regulatory Cell Therapy in Kidney Transplantation Using a Harmonized Trial Design

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Abstract

Background

Using cell-based medicinal products (CBMPs) represents a state-of-the-art approach to reducing general immunosuppression in organ transplantation. Accordingly, *The ONE Study* Consortium tested multiple regulatory CBMPs in kidney transplant (KTx) trials. Here, we report primary analysis results for safety of regulatory CBMPs when combined with reduced immunosuppressive treatment in this first *ONE Study* publication.

Methods

Seven investigator-led single-armed trials were conducted internationally in living-donor KTx recipients (60 week follow-up). One single-arm trial, the Reference Group Trial (RGT, n=66), represents a "standard-of-care" group given basiliximab, tapered steroids, mycophenolate mofetil (MMF) and tacrolimus. Data from six non-randomized phase I/IIa cell therapy group (CTG) trials were pooled and analyzed, where patients (n=38) 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 MMF tapering was allowed. The primary endpoint was biopsy-confirmed acute rejection (BCAR); adverse event (AE) coding was centralized.

Findings

Standard-of-care immunosuppression in the RGT recipients resulted in a 12·1% BCAR rate (expected range: 3·2-18·0%). The 6 CBMPs for the parallel CTG trials were administered to a combined total of 38 patients, with an overall BCAR rate of 15·8%. 15 CBMP-treated patients (39·5%) were successfully weaned from MMF and maintained on tacrolimus monotherapy. Combined AE data and BCAR episodes from all six CTG trials revealed no safety concerns versus 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 KTx recipients, and is associated with fewer infectious complications, but comparable rejection rates in the first year.

Funding

The ONE Study was funded by the 7th EU Framework Programme.

Introduction

Combinations of general immunosuppressive drugs have enabled the widespread application of life-saving organ transplantation today; however, transplant survival is limited and has plateaued over the last decade,[1] leaving the dilemma of needing to replace damaged transplanted organs in a world where not enough organs are available, while the morbidity and economic costs associated with life-long general immunosuppression accrue. To address this problem, the organ transplantation community is well-aware that new strategies are urgently needed to decrease our dependency on immunosuppressive drugs to prevent allograft rejection.[2] Indeed, international networks have been established with this explicit purpose in mind, notably including a series of European Union-funded programs and, in North America, the *Immune Tolerance Network*. Research from these expert networks, and from numerous research laboratories across the globe, 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 organ transplant recipients have been trialed 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.

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) that can then be infused into transplant recipients. Towards this aim, a European Union-funded consortium called *The ONE Study* was initiated with the aim of developing a range of CBMPs

and to test those cell products in early-phase clinical trials. The six CBMPs developed and tested in six parallel cell therapy group (CTG) trials (≤12 patients each) in *The ONE Study* included two polyclonal T regulatory (pTreg), two donor-antigen reactive Treg (darTreg), one tolerogenic DC (ATDC) and one regulatory macrophage (Mreg) cell products. Central to the concept of *The ONE Study* was that all CBMPs be tested using the equivalent patient population of living-donor kidney transplant (KTx) recipients that receive the 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 conducted on an equivalent patient population using standard-of-care immunosuppression. While the RGT is not strictly a true control group due to 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 recognized standard-of-care benchmark to assess whether currently expected outcomes are generally attainable with regulatory cell therapy with less immunosuppression. Second, with a standard-of-care RGT, performance of centralized immune monitoring allows for reliable detection of potential immunological changes caused by cell therapy. Here, we present the special design, clinical data, safety results and immune monitoring data of the *ONE Study* RGT and combined CTG group of trials, which is intended as a foundation for further regulatory cell therapy trials in organ transplantation.

Methods

Study design and participants

The *ONE Study* aimed to explore the safety and immunological effects of regulatory cell-based therapy as an adjunct immunosuppressive treatment in living-donor kidney transplant recipients through a series of clinical trials sharing the same general design. Therefore, we created a multi-trial design strategy to facilitate: 1) comparison of different cell therapy trials versus standard-of-care treatment, and 2) comparison of cell therapy trials to each other. In total, seven trials were performed, the first being the single-arm multi-center RGT conducted at all clinical sites that were planning to perform an individual cell therapy trial. The RGT formed the basis for the other six individual trials testing CBMPs (the CTG trials). Chronologically, enrollment 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.

CBMPs. 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 polyclonal natural T regulatory cells approved respectively in the United Kingdom ("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 (PBMCs) in Boston[9] (referred to as costimulatory blockade "darTreg-CSB") and the other product was developed in San Francisco where Tregs sorted from PBMCs 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, where monocytes were stimulated in Nantes with GM-CSF to produce autologous tolerogenic dendritic cells ("ATDC"),[11] or in Regensburg with M-CSF and IFN-γ 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. Table S1 provides an overview of the overall characteristics of the CBMPs, including a reference to cell production methods.

Patient selection for trials. Living-donor KTx recipients were selected for inclusion into all seven trials. Living donors were chosen for these trials to allow for maximal planning logistics with regard to 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 Table S2. The main exclusion criteria were patients transplanted previously, high risk recipients (PRA >40%) and HLA identical donor-recipient mismatches (0-0-0 mismatches); all patients needed to be $\geq$18 years old.

RGT treatment protocol. The *ONE Study* group of clinicians developed the RGT immunosuppression design based on their own local standard-of-care protocols, which included some features of the ELITE-Symphony study[13], for the selected non-high risk KTx patient population. The study protocol (clinicaltrials.gov: NCT01656135) consisted of: basiliximab administration $\leq$2 hours before transplant surgery and on day 4 after surgery (20mg i.v.); prednisolone starting on day 0 (day of KTx) and gradually tapered away by week 15; mycophenolate mofetil (MMF) at 2 g/day from day -1 to day +14 and 1.5 g/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 can be found in Fig. S1. Patient follow-up was continued for 60 weeks. The target recruitment figure for the RGT was 60 patients.

CTG treatment protocol. The clinical protocol for the six CTG trials closely mirrors the regimen for the RGT (Fig. S1). All cell products were delivered once intravenously between day -7 and day +10

relative to the day of KTx; within this timeframe, monocyte-derived cell products were administered before KTx and T cell-derived products were given after KTx. 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 x 10^6 cells/Kg BW for all cell products except darTreg-CSB, where a range between 2 x 10^3 - 2 x 10^6 cells/Kg BW 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-KTx an option was included to completely taper away MMF by one year post-KTx; with MMF cessation, tacrolimus continued as a monotherapy. Tapering of MMF was not allowed if an immediately prior KTx biopsy 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 cell therapy-treated patients did not exceed 12 in any individual CTG trial. All CTG trials are registered on ClinicalTrials.gov (NCT02252055, NCT02085629, NCT02244801, NCT02371434, NCT02129881 and NCT02091232).

Sites performing trials. The multicenter RGT was performed at eight international locations, 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) and UCSF Medical Center (San Francisco, CA) (Fig. 1). After completing enrollment for the RGT, seven centers conducted a separate CTG trial with one of six regulatory cell products (see above). Unlike the five centers that recruited patients into their respective single-center 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, since their cell product was not approved for clinical trial testing during *The ONE Study*.

Endpoints. Biopsy-confirmed acute rejection (BCAR) was the primary endpoint. Histopathological grading of KTx biopsies was performed by a central pathologist (Prof. Ian Roberts, Oxford University) for all trials within *The ONE Study*, with the standard assessment performed according to the Banff criteria.[14] Notably, a case of borderline histological change in a for-cause biopsy with clinical evidence of acute rejection was considered a BCAR. However, histological changes consistent with acute rejection that were not accompanied by clinical evidence of rejection were not recorded as a BCAR, but were logged as a secondary endpoint. Estimated glomerular filtration rate (eGFR: MDRD method) was recorded as a secondary endpoint.

For the RGT, we estimated a BCAR rate of approximately 10% after 60 weeks under standard immunosuppressive therapy in the select KTx patient population. With this assumption, a two-sided 95% confidence interval 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 collection and monitoring. Clinical data from all trials were entered into a web-based data capture platform consisting of electronic case report forms (eCRF) 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 performed by qualified CRAs. 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 (AEs) and serious adverse events (SAEs) were coded centrally using version 20.1 of the Medical Dictionary for Regulatory Activities (MedDRA) and quality-controlled to ensure consistency of coding across all trials and study sites. To compare safety events reported from cohorts of different sizes, (S)AE frequencies were normalized using a cohort-specific “Patient Study

Years" (PSY) denominator. PSY is the cumulative amount of time spent by trial participants in study follow-up and was calculated and applied for RGT and CGT separately. A safety advisory board (SAB) received SAE reports for all CTG trials as they occurred and reviewed all safety data twice per year. To be sure of open communication within *The ONE Study* trial series, safety alerts or conclusions from the SAB were shared with all centers performing CTG trials.

Immune monitoring. We used a mixed model of locally and centrally performed assays to compare pre- and post-transplant immune status of RGT and CTG trial patients.[15] The following analyses were performed as provided in supplementary materials: immune cell composition by whole blood flow cytometry, TSDR demethylation gene expression (see Supplementary Methods) and anti-donor as well as anti-CMV IFNg 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 performed comparative analyses with age-and gender-matched healthy controls from our recently generated cohort data set.[16]

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

For clinical data, results for baseline characteristics, safety and transplant function or rejection endpoints were summarized descriptively. No formal testing was performed. In addition to crude rejection rates, time to first BCAR was analyzed using Kaplan-Meier methods. The primary BCAR endpoint is reported descriptively for the intention-to-treat population (RGT, n=66; CTG, n=38); the time-to-event Kaplan-Meier BCAR analysis is presented for both the intention-to-treat (66/38, respectively) and per-protocol (47/32, respectively) populations. All other variables (DSA, eGFR,

tacrolimus levels) are summarized for the number of patients who were tested at the relevant study time points. Incidence rates of adverse events normalized per 100 patient study years were calculated and based on the intention-to-treat population.

Differences in immune monitoring results between RGT patients prior to transplantation and healthy controls were analyzed applying Kruskal Wallis tests followed by Dunn-Bonferroni tests. Changes between pre-transplant and post-transplant time points of the same patient were analyzed applying Wilcoxon matched-pairs signed rank test. To reveal differences in immune cell composition or TSDR changes after transplantation between RGT and CTG patients, we employed a Kruskal Wallis and a post-hoc Dunn's multiple comparison test. P values <0·05 were considered as significant.

Role of the funding source. The funders had no role in data collection, analysis, interpretation or writing of the manuscript. EKG, as the ONE Study Consortium FP7 project coordinator, had access to all the data in the study; BS also had access to the full data set. 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 the final responsibility in this decision.

Results

Results from clinical trials

RGT and CTG trials conduct. Recruitment to the RGT began in December 2012, with the last patient-last visit in December 2015. Fig. 1 shows that 70 patients were enrolled in the RGT in total (red arrow bars), with 66 receiving a KTx. Of the four pre-KTx withdrawals, two 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 RGT patients 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 ATG 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 weeks). Fig. 1 also summarizes patient recruitment into the six individual CTG trials (non-red arrow bars), where a total of 60 patients were recruited into the various trials, with the first patient-first visit conducted in May 2014 and the last patient-last visit done in November 2018. Of the 60 enrolled patients, 38 received a KTx and the designated cell therapy. All of these patients completed the 60 week follow-up planned in the *ONE Study*. The 22 patients withdrawn were due to one of the following: cell manufacturing failures (14), early development of acute rejection before the planned cell infusion (5), discovery of ineligibility criteria after enrollment (2) or requirement for a second abdominal surgery shortly after KTx (1). Cell manufacturing failures were because of failure to meet release criteria (9), cancellation (2), microbiology testing positive (2) and leucapheresis side effects (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 weeks). A summary of the recipient and donor demographic data for the RGT and CTG trials is provided (Tables S3 and S4). Data on recipient and donor age, gender, ethnicity, renal replacement therapy, relationship of donor and recipient, and underlying diagnosis show that the RGT and combined CTG trials were well-balanced. Notably, both the RGT and combined CTG trials have a nearly identical

over-representation of male recipients; since gender-related effects are known in transplantation, this should be taken into consideration when interpreting the results.

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 (criteria listed in Table S5). In the RGT, 47 of 66 KTx patients (71·2%) received treatment that closely followed the clinical protocol, whereas 32 of the 38 patients (84·2%) 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 performing the CTG trials tapered immunosuppression to tacrolimus monotherapy (optional) in 17 of 38 (44·7%) patients. The immunosuppression was successfully tapered in all but two cases, where triple therapy was later reinstated due to a BCAR and detection of recurrent IgA nephropathy, respectively.

Outcomes (BCAR rate, GFR, DSA, tacrolimus levels)

BCAR rate in the RGT was 12·1% (8/66), which is within the expected range of 3·2-18·0%. BCAR occurred in 15·8% (6/38) 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 Fig. 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 (Fig. 2B); one patient in the RGT experienced 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 after two weeks post-KTx; similarly, 4 of 6 episodes of BCAR in the CTG group trials occurred before three weeks post-KTx. Specific BCAR data from individual sites will be published separately for each CTG trial. In addition, we also performed a Kaplan-Meier analysis for the “per protocol” patients in the RGT and group of CTG trials (Fig. 2C); the rate and timing of the BCAR episodes were essentially the same.

A set of tests was performed at study end (60 weeks) to further assess outcomes in the trials, including DSA detection, eGFR and tacrolimus blood levels. At study end, DSA testing revealed that 13·7% (7/51 tested) of RGT recipients had a DSA, with 15·2% (5/33 tested) showing DSA in the combined CTG trials; of the CTG patients tapered to monotherapy, 13·3% (2/15 tested) had a new DSA. Regarding kidney function (Fig. S2), eGFR measurements in the RGT and CTG trials showed an almost identical increase over the study period (20·4% and 20·8%, respectively) when comparing median eGFR at 60 weeks post-KTx to median eGFR at one week post-KTx. As a reflection of immunosuppressive load at study end, tacrolimus trough levels were found to be similar in the RGT and combined CTG trials, at 6·1 ± 2·1 (mean±SD; n=44 tested) and 6·6 ± 1·6 ng/ml (mean±SD; n=32 tested), respectively. Furthermore, immunosuppressive burden with tacrolimus (trough level: Fig. S3A, B) and MMF (dose: Fig. S3C, D) was similar or even tended to be lower throughout the study period in the CTG versus RGT patients. Together, these data should be considered with the understanding that 15 patients (39·5%) in the CTG trials were on tacrolimus monotherapy at study end, whereas 98·4% (60/61) of patients in the RGT continued on at least dual immunosuppression.

Safety Data

The normalized incidence rates of treatment-emergent SAEs/AEs in the RGT (n=66) and CTG trials (n=38) were 91·2/1614.6 and 70·7/1452.0 events per 100 PSY, respectively, indicating no increase in adverse events with cell therapy (Table S6). In the CTG trials, there was special attention given to identifying SAEs/AEs related to cell therapy infusion. Overall, there were 12 AEs reported with a possible relationship to the cell infusion, only one of which was a serious incident (an SAE; increased creatinine) (Table S7). All potentially related adverse events only occurred once, so no specific pattern was exposed in the 38 patients treated with CBMPs. No deaths were reported in any of the trials.

A descriptive analysis of normalized data comparing MedDRA-coded SAEs in the RGT versus the combined data from the CTG trials revealed that most serious medical problems were similar in frequency (Fig. 3A). However, there was one substantial difference that emerged which is worth considering in detail. The incidence rate of SAEs in the RGT related to infections and infestations was nearly six-fold higher compared to the combined CTG trials. After examining all infection-related adverse events (AEs) recorded in the trials, this pattern of decreased infections in the CTG trials was consistently observed across the CTG trials (Fig. 3B) and was evident during the entire post-KTx observation period (Fig. 3C). Also interestingly, we found that the main difference was with regard to a reduced number of viral infections in the CTG trials (Fig. 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 AEs, the main decreases in viral infections in the CTG trials were with regard to CMV, herpes (including herpes simplex, herpes-zoster, oral herpes, nasal herpes and Varicella-zoster) and polyoma virus (Fig. 3E). The decreased rate of viral infection in the CTG was not due to more preventive measures, since 65·2% (43/66) RGT and 52·6% (20/38) CTG patients received anti-viral prophylaxis in the first three months after KTx; also, notably, the percentage of CMV^+ to CMV^- donor to recipient transplants was 18·2% and 21·1% in the RGT and CTG trials, respectively. Therefore, patients receiving cell therapy in general developed fewer viral infections compared to patients receiving standard-of-care treatment.

Immune monitoring results

Identical standardized immune monitoring testing of peripheral blood cells was performed in all patients of the seven trials. In general, principal component analyses show that RGT patients prior to KTx have major alterations in absolute and relative blood immune cell population composition compared to age- and gender-matched healthy controls (Fig. 4A). Populations contributing most to those alterations were granulocytes, $CD16^+$ mDCs and $CD14^{high}CD16^+$ intermediate monocytes, which

were increased in RGT patient samples, but also plasmacytoid DCs (pDCs), marginal zone-like B cells (MZB) and $CD8^+CD28^+$ T cells which were higher in samples of healthy controls (Fig. 4B). Post-KTx longitudinal analysis revealed only moderate or absent normalization of CD16-expressing monocytes and MZB, respectively (Fig. 4C). Furthermore, whereas composition of conventional $CD4^+$ T cells subsets remained normal and comparable to healthy controls, $CD8^+$ T cells subset composition showed major alterations over the post-KTx course. Although naïve T cells increased early after transplantation, we observed a skewing towards terminal differentiation of $CD8^+$ T cells in the long-term (Fig. 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 15 months post-KTx (Fig. 5A). A significant reduction in TSDR demethylation occurred in RGT patients, but not in CTG trial patients. Furthermore, only RTG patients showed a significant increase in $CD8^+$ T_{EMRA} cells and $CD8^+CD57^+$ chronically-activated T cells (Fig. 5B), whereas in samples from CTG patients we observed more $CD8^+CD28^+$ T cells. Both patient groups showed a reduction of donor-specific IFNγ producing memory T cells after KTx (Fig. S4A). However, RGT patients in contrast to CTG patients showed higher anti-CMV T cell responses (Fig. S4B), which correlated with absolute $CD8^+$ T_{EMRA} numbers (Fig. S4C). This increase is well known in KTx patients and is likely related to inflammation triggered subclinical reactivation of CMV, which we also only observed in RGT but not CTG patients (Fig. 3E). Although both patient groups had more pDCs 15 months post-KTx, we only observed a normalization of MZB numbers and a significant reduction of $CD14^{high}CD16^+$ monocytes in CTG patients (Fig. 5C). In addition, CTG patients showed increased mRNA expression of genes described to be high in immunosuppression-free operationally tolerant kidney transplant patients (e.g. Ms4A1) and co-inhibitory molecules (CD200), but reduced expression of rejection-associated genes (HMMR, Fig. S4D). Together, these data suggest that regulatory cell therapy within our trials CTG patients show a more healthy control-like restoration of immune cell composition.

Discussion

The *ONE Study* consortium has taken the unique approach of performing side-by-side trialing of different T cell, DC and macrophage regulatory cell products in low to medium risk KTx recipients. In this coordinated group of six international early phase clinical trials (the CTG trials), we show that CBMP application in this patient population is feasible for multiple regulatory cell types, and their categorical application near the time of KTx reveals no apparent safety concerns, including allograft rejection rate. Furthermore, 15 of the 38 patients treated with CBMPs were successfully weaned to tacrolimus monotherapy during the 60 week observation period. The conduct of a parallel 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 critical safety and immunological parameters, and also to evaluate whether reduction of immunosuppression through CBMP application could have potential benefits to patients. Remarkably, in this regard, the rate of viral infections was considerably lower in patients treated with regulatory cell products compared to standard-of-care treatment, particularly with regard to viral infections. Furthermore, centralized immune monitoring of peripheral blood leucocyte populations suggests a return of CBMP-treated (CTG), but not conventionally-treated (RGT), recipients towards a state of immune homeostasis. Therefore, results from the *ONE Study* establish a fundamental basis for further testing of regulatory cell CBMP therapy in organ transplantation, and provide initial evidence that reducing general immunosuppressive burden through cell therapy could potentially decrease serious side effects in KTx recipients.

This initial *ONE Study* report focusses 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 with regard to 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.

Indeed, forthcoming details from the individual cases will provide insight into interesting feasibility, safety aspects and effects of each specific cell therapy product, permitting examination of issues such as cell production methods, CBMP characterization, cell dosing, infusion scheduling, clinical outcomes and immunological features from KTx biopsy specimens, as well as 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 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 (38/52: 73%) 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 recent set of guidelines and comprehensive review by Fishman[17] highlights the extent of the infection problem, and its direct relationship to immunosuppressive load. Results from the *ONE Study* CTG trials indicate that lowering immunosuppression does appear to decrease the risk for viral infections. This was also supported by the immune monitoring results, as only RGT patients showed a tendency towards increased proportions of CMV-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 at this point 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. It should be noted that immunosuppressive burden was lower early-on post-KTx (no basiliximab induction) and in some patients after nine months post-KTx (MMF tapering), but that the infection rates were consistently less across the spectrum of CTG trials during the entire observation period (Fig. 3C). While reduction of MMF 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 nine months, leaving this issue an

492 open question. Nonetheless, our data encourage the performance of prospective randomized clinical
493 trials to confirm an infectious disease benefit from regulatory cell therapy protocols.
494
495 Our immune monitoring results showed that patients with end-stage renal failure exhibit major
496 alterations in their peripheral immune cell composition compared to age- and gender-matched
497 healthy controls, most likely reflecting their increased inflammatory state.[21-23] Standard
498 immunosuppressive therapy in RGT patients did not reverse these alterations, but rather led to
499 further immune cell imbalance as evidenced by a significant reduction in markers for stable Tregs.[24]
500 Importantly, regulatory cell therapy mitigated this Treg reduction and correlated with a healthy
501 control-like restoration of immune cell composition. In particular, MZB numbers, also discussed to
502 have anti-inflammatory or regulatory function,[25,26] were increased in CTG patients at the end of the
503 observation period. Thus, although both RGT and CTG trial patients had a reduction in donor-specific
504 IFNγ-producing memory T cells, only the cell therapy-treated patients tended to experience a re-
505 establishment of immune cell homeostasis, which is a major goal in organ transplantation.
506 Importantly, these immune-related differences were independent of potential confounding factors
507 such as donor relationships. Whether this effect is related to cell therapy itself, or is due to reduced
508 immunosuppressive load in the CTG trials, will need to be investigated further in future trials.
509
510 To date, there are few published reports on the use of regulatory cell therapy in human organ
511 transplantation, some of which were pilot trials conducted previously by *ONE Study* investigators
512 [recently reviewed by Romano 2019]. Hutchinson and colleagues have tested different preparations
513 of regulatory macrophages in KTx recipients,[27-29] which provided critical lessons for designing the
514 *ONE Study* CTG trials. Additionally, polyclonal Tregs have been administered by the UCSF group to
515 three KTx recipients with biopsy-proven subclinical inflammation six months after transplantation,
516 showing that cell therapy is feasible in this circumstance;[30] late administration of expanded
517 polyclonal Tregs has also been reported by the Northwestern group in nine lymphodepleted KTx

recipients.[31] In liver transplantation, Todo et al. have infused costimulatory blockade conditioned lymphocytes similar to those used by the MGH group in the *ONE Study*, and were able to achieve complete immunosuppression withdrawal in seven of the ten splenectomized and cyclophosphamide-conditioned recipients.[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 recognized standard-of-care (RGT) treatment by infusing different CBMPs near the time of KTx as a replacement for conventional induction treatment (omitting basiliximab induction). Into this design we incorporated an option to wean MMF starting at nine months to further offer potential benefit to patients from general immunosuppression, and to stress-test this cell therapy protocol under rigorous clinical monitoring. With this overall study strategy, and by performing the RGT as a multicenter study together with the CTG trials as parallel individual trials at the same sites, the *ONE Study* consortium uniquely delivers 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 randomized 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 conducting 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*.

Contributors

BS, EKG, PNH, PR, AM, JAH, DSG, CAB, JFM, TB, RC, WP, NA, HJS, PJF, RH, MB, BJ, JBN, MPH-F, UK, SJK, JG, PJM, LB, LAT, RJL, AB, JAB, GL, KJW, MCC, AS, BB, GB, SMK, and HDV contributed to the study design. PNH, PR, JAH, DSG, CAB, JFM, TB, RC, WP, NA, HJS, PJF, RH, JBN, AS, BB, GB, SMK, 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, JBN, MPH-F, AB, MCC, HDV, QT, CS, ECG, LC-R, KC, WJB, JLH, IM, FI, ISDR, MS, RJ, CB, ND, MK, and TM did biomarker development / data collection. BS, EKG, AM, JAH, BJ, MPH-F, AB, SMK, QT, CS, WJB, JLH, IM, FI, ISDR, MS, RJ, CM, and SS performed data analysis. BJ, CM, SS, and KJ were study statisticians. BS, EKG, PNH, PR, UK, SJK, JG, PJM, LB, LAT, RJL, AB, JAB, GL, KJW, MCC, AS, BB, GB, SMK, HDV, AS, ISDR, MS, RJ, CM, SS, and KJ interpreted data. EKG and BS wrote the manuscript, which was reviewed by JAH, BJ, SS, and KJ, as well as the other authors. EKG was the *ONE Study* EU FP7 project coordinator.

Declaration of interest

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, SMK, BB, GB, HDV, GL, KJW and EKG report grants from the EU (FP7 ONE Study) during the conduct of the study. PR and HDV report grants from the BMBF, outside the submitted work. JAH reports other support from Trizell GmbH, 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. Dr. Tang has a patent US14/382,537 issued and she is a co-founder of Sonoma. MB has a patent In vitro generation/expansion of CD4+CD25+ T regulatory cells by rapamycin. WO 2006/090291A2 licensed to non-exclusive license to Miltenyi Biotech for the development of 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. 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; other from Beckman Coulter Life Sciences, outside the submitted work. RH reports personal fees and non-financial support from Chiesi Ltd, outside the submitted work. EKG reports grant support from Trizell GmbH 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 (https://www.ukri.org/funding/information-for-award-holders/data-policy/).

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 analyzing data and should be given at least a limited period of privileged 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 in order 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 our internationally conducted *ONE Study*, any request must comply with regulations set by the competent authorities in the relevant countries that govern data security policies.

Acknowledgements

ONE Study work was also supported by funds from IHU-CESTI (Investissement d'Avenir ANR-10-IBHU-005, Région Pays de la Loire and Nantes Métropole) and the Labex IGO project (ANR-11-LABX-0016-01). 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 GmbH (Munich, Germany) for Mreg production in the study.
We would like to thank the nurses, physicians and patients who contributed to the study.

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Fig. 1: *ONE Study design and patient disposition for the multicenter RGT and six monocenter CTG trials.* RGT = Reference Group Trial; CTG = Cell Therapy Group trials; Mreg: regulatory macrophages; ATDC: autologous tolerogenic dendritic cells; pTreg-1 / pTreg-2: polyclonal regulatory T cells; darTreg-sBC: donor-alloantigen reactive Treg; darTreg-CSB: costimulatory blockade generated Treg.

Fig. 2: *Primary endpoint (BCAR) data.* 2A). Kaplan-Meier estimates of the cumulative BCAR-free survival probability in the RGT (N=66) and CTG (N=38) intention-to-treat analysis sets (87.7 % vs. 84.2 % at 60 weeks). Censored patients marked with ticks. 2B). 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. 2C). Kaplan-Meier estimates of the cumulative BCAR-free survival probability in the RGT (N=47) and CTG (N=32) per-protocol analysis sets (82.8 % vs. 81.3 % at 60 weeks). Censored patients marked with ticks. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; BCAR = biopsy-confirmed acute rejection; TCMR = T cell-mediated rejection; ABMR = antibody-mediated rejection.

Fig. 3: *ONE Study safety data (normalized).* 3A) Incidence rate of treatment-emergent SAEs by MedDRA primary SOC. 3B) Incidence rate of treatment-emergent infections (all AEs) by study site. 3C) Incidence proportion of treatment-emergent infections (all AEs) over time. 3D) Incidence rate of treatment-emergent infections (all AEs) by MedDRA HLGT. 3E) Incidence rate of treatment-emergent viral infections (all AEs) by MedDRA HLT. All adverse events coded using MedDRA version 20.1. Treatment-emergent (S)AEs are events with onset date equal to or after first dose of any study drug. All events coded to the MedDRA PT: "Transplant rejection" are excluded, since rejection was measured as the primary efficacy endpoint. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; SOC = System Organ Class; HLGT = High Level Group Term; HLT = High Level Term; PSY = Patient study years; NEC = Not elsewhere classified.

Fig. 4: *Leukocyte subset alterations in ESRD patients and time-dependent changes after kidney transplantation.* A) Principal component analysis revealing the differences in leukocyte subset between whole blood samples from end stage renal renal disease (ESRD, n= 70) and healthy controls (HC, n= 98). B) Box-and-whiskers plots of absolute numbers from leukocyte subpopulations with highest influence at the PCA shown in A. C) Time-dependent changes from visit 1 prior to 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 RGT patients (n=59). Statistical analysis by Kruskal-Wallis-Test. * $p<0.05$, ** $p<0.01$

Fig. 5: *Differences in post-transplant changes between RGT and CTG patients.* 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 % $CD4^+$ T cells with demethylated TSDR in whole blood samples collected pre-transplant (V01) and at the end of the observation period (15 months post-transplant, V10) from RGT (n=59) and CTG patients (n=38) measured as described in material and methods. 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 (15 months post-transplant, V10) from RGT (n=59) and CTG patients (n=38). C) Differences in post-transplant changes in marginal zone-like B cells and dendritic cell subpopulation. Box and whisker plots of absolute numbers and proportions of marginal zone-like B cells, $CD16^+$ mDCs and pDCs in whole blood samples collected pre-transplant (V01) and at the end of the observation period (15 months post-transplant, V10) from RGT (n=59) and CTG patients (n=38). Statistical analysis by by Wilcoxon matched-pairs signed rank and Dunn's multiple comparison test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

Supplementary material

Supplementary methods

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.

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.

Table S1: ***Overview of CBMPs used in the ONE Study CTG trials.*** CBMP = cell-based medicinal product.

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)

Table S2: ***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.

	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 %)

Table S3: ***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 %)

Table S4: ***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

Table S5: ***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.

	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 %)

Table S6: ***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**

Table S7: ***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.

Supplementary figures

Figure S1

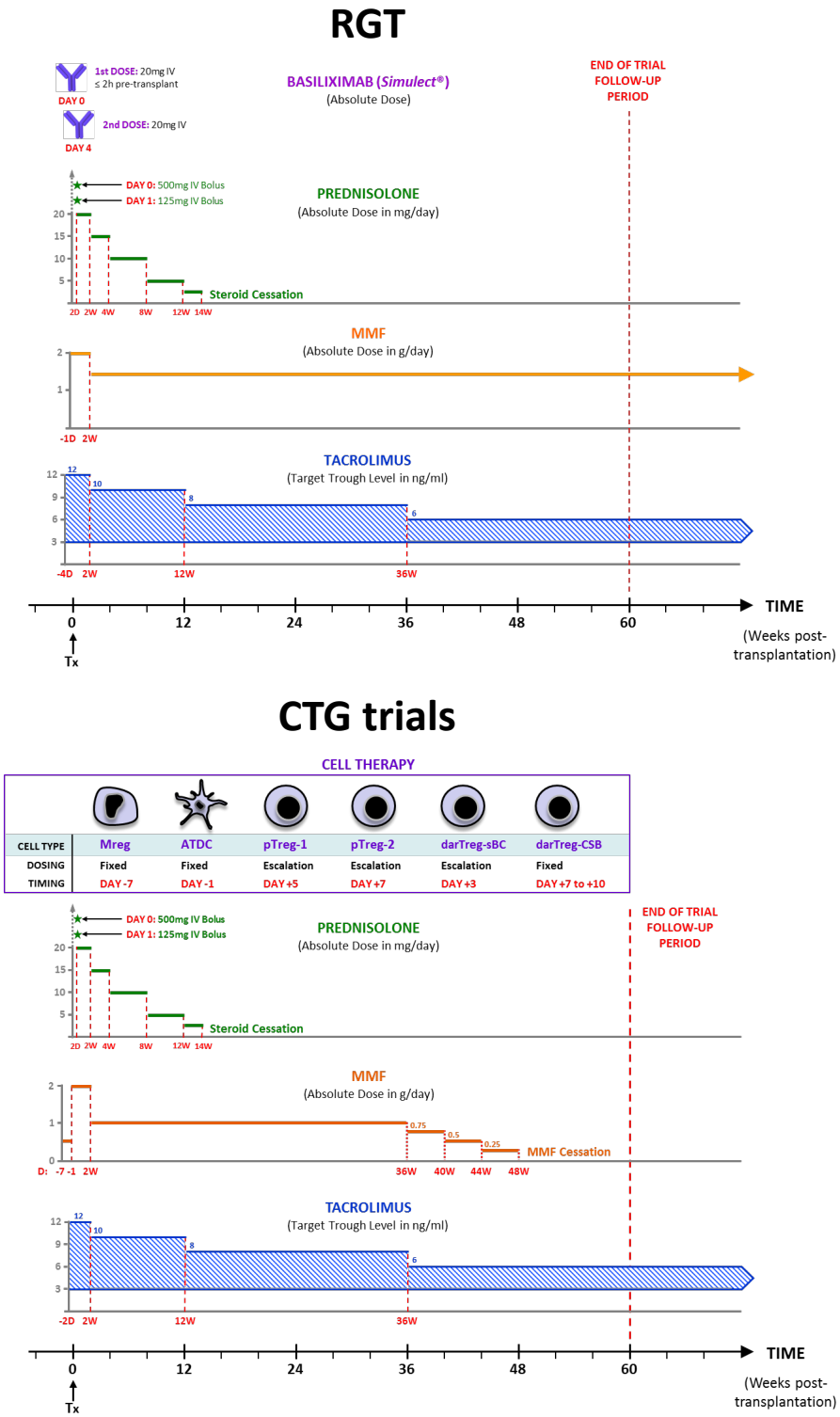

Fig. S1: ***Treatment protocol for the RGT and CTG trials.*** IV = intravenous, D = day; W = week; MMF = mycophenolate mofetil; Tx = transplantation.

Figure S2

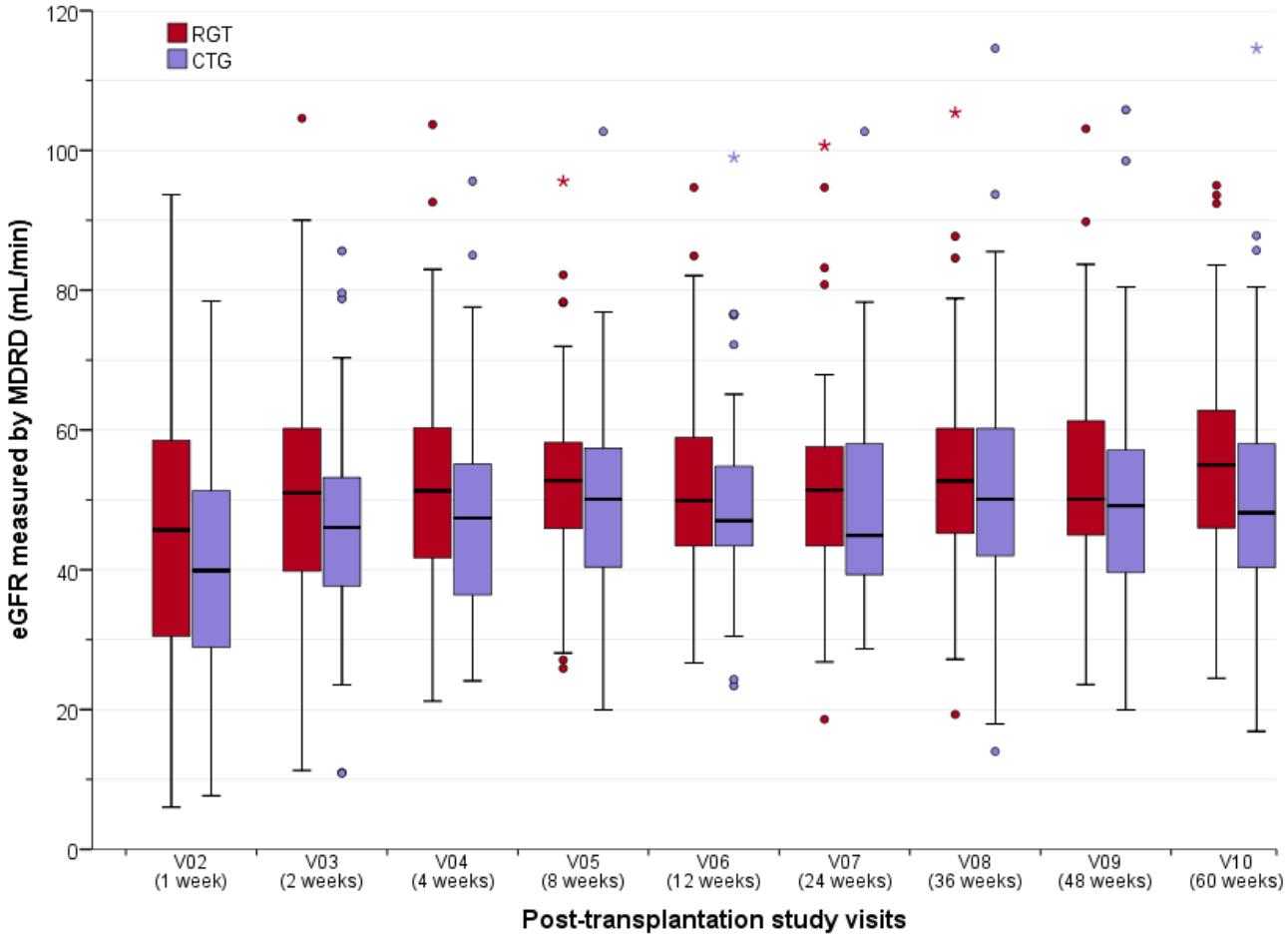

Fig. S2: ***ONE Study kidney function post-transplantation.*** MDRD eGFR measured in the RGT and CTG trials at each study visit post-transplantation. Points mark outliers beyond inner fences set at 1.5 x IQR (interquartile range); asterisks mark extreme outliers beyond outer fences set at 3 x IQR. RGT = Reference Group Trial; CTG = Cell Therapy Group trials; eGFR = estimated glomerular filtration rate; MDRD = Modification of Diet in Renal Disease formula; V = Visit.

Figure S3

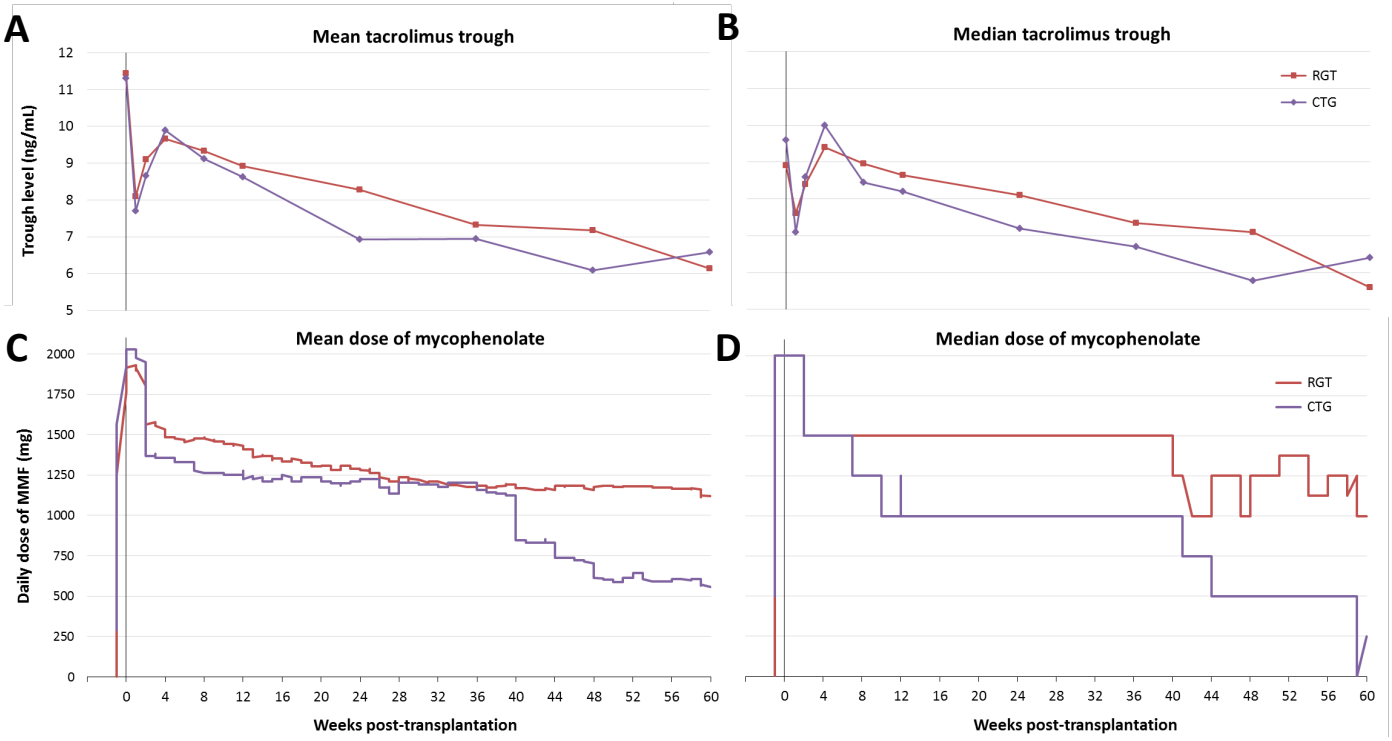

Fig. S3: ***Immunosuppressive burden of tacrolimus and mycophenolate over time.*** Mean (S3A) and median (S3B) blood trough levels of tacrolimus calculated at 10 time points using all available readings within the following time windows: day 0 (± 1 day), 7 (± 1), 14 (± 1), week 4 (± 1 week), 8 (± 1), 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.

Figure S4

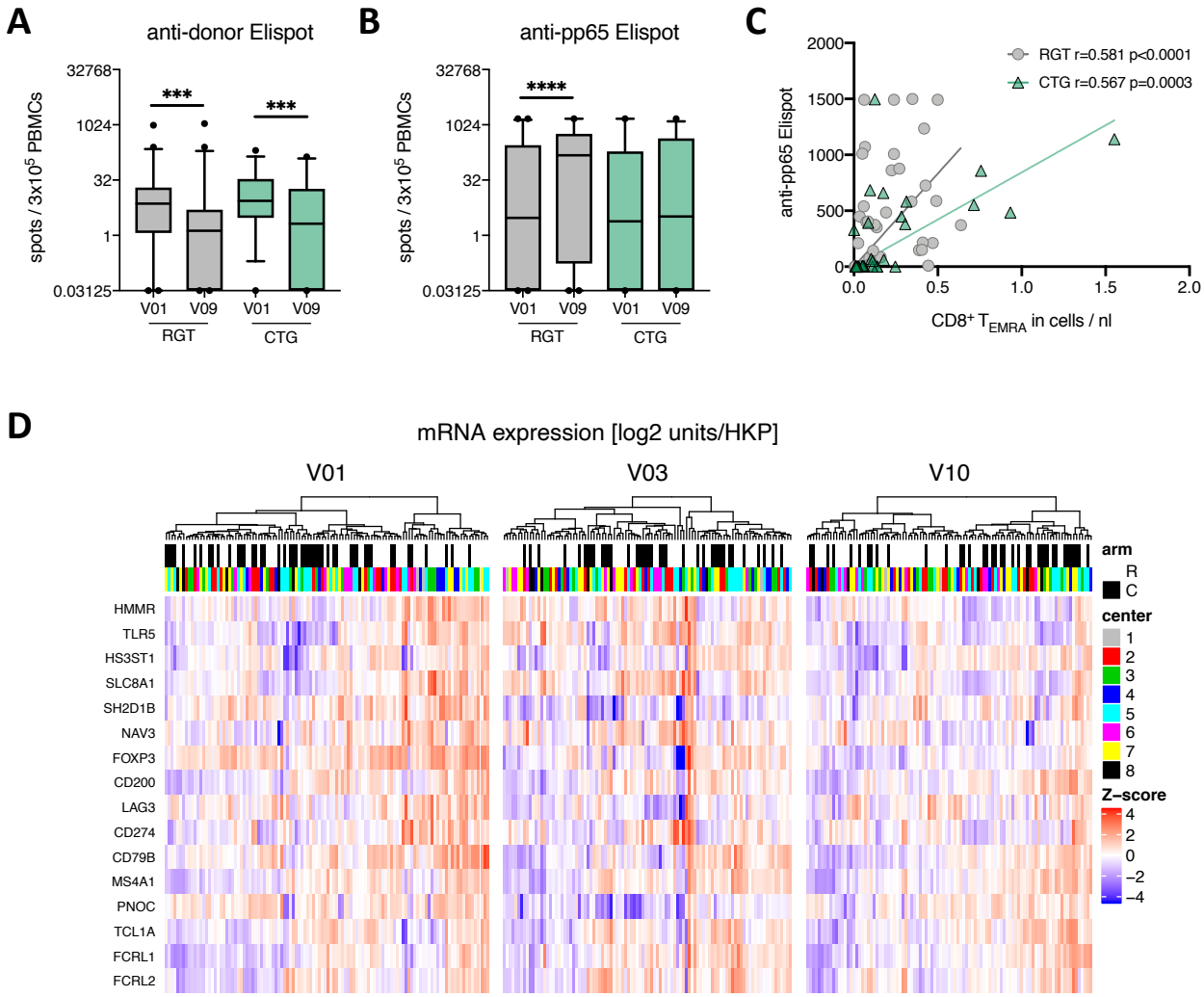

Fig. S4: ***Anti-donor and anti-CMV IFNg EliSpot analyses as well as gene expression analyses.***
A) Anti-donor IFNg EliSpot analysis prior to transplantation (V01) and 12 months post-transplantation (V09) of PBMCs from RGT (n=45) and CTG (n=33) patients. B) Anti-CMV (pp65) IFNg EliSpot analysis prior 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=66, 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). Statistical analysis was done by Kruskal-Wallis-Test.