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ORIGINAL ARTICLE

A cell-based approach to the minimization of immunosuppression in renal transplantation

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Summary

Five renal transplant recipients were preoperatively treated with transplant acceptance-inducing cells (TAICs) in a Phase-I safety study of TAICs as an adjunct immune-conditioning therapy in living-donor kidney transplantation. Initially, patients received anti-thymocyte globulin induction therapy in combination with tacrolimus and steroid immunosuppression. Over the course of 12 weeks, steroids were withdrawn and tacrolimus therapy was minimized. Three of the five patients were able to tolerate low-dose tacrolimus monotherapy and one patient was withdrawn from all immunosuppression for over 8 months. No acute or delayed adverse events were associated with the infusion of TAICs. Monitoring of the recipient anti-donor reactivity of TAIC-treated patients in mixed lymphocyte cultures demonstrated that, during periods of clinically stable graft function, recipient T-cell proliferation and cytokine secretion in response to stimulation with donor alloantigen was relatively suppressed. Therefore, although the TAIC-II trial did not provide conclusive evidence of a beneficial effect of preoperative TAIC treatment, the results were encouraging because they suggest that TAICs promote a state of alloantigen-specific unresponsiveness, which might allow safe minimization of pharmacological immunosuppression.

Introduction

Administering tolerance-promoting cell types to transplant recipients prior to surgery in order to condition the immune response in favour of graft acceptance, in such a way that can be verified before transplantation, is a very appealing notion. Indeed, to appreciate the therapeutic potential of cell-based immunological preconditioning we need only to consider the beneficial effects previously attributed to donor-specific blood transfusion in solid organ transplantation [1–5]. The challenge is to achieve a similar benefit without the attendant complications of sensitizing the recipient to donor-antigens, introducing infection or malignancy, or risking emboli of cellular aggregates.

In animal models, tolerance of transplantation antigens is readily transferred with regulatory T cells, and both appropriately stimulated antigen-presenting cells and

certain T-cell subsets can establish a state of specific transplantation tolerance. Despite these being long-established experimental protocols, translation into the clinical setting has met with only limited success [6–9]. This is, perhaps, a reflection of the very substantial technical challenge of isolating and expanding rare, often ill-defined cells and administering those cells to the patient in such a way that they adequately engraft.

Work from this laboratory has concentrated on the potential of a subset of immunoregulatory macrophages, referred to as transplant acceptance-inducing cells (TAICs), to bring about operational transplantation tolerance [10–16]. The TAIC has a number of properties which make it particularly suitable for clinical use. First, the production of consistent, clinical-grade TAICs is relatively uncomplicated and the cells themselves are sufficiently robust to survive clinical handling procedures. Second, human TAICs appear to be directly equivalent to cells from mice and rats, which efficiently engraft in recipient tissues and prolong allogeneic, solid organ graft survival in nonimmunosuppressed animals (F. Fändrich, unpublished data). Third, the TAIC-I trial, a single-centre, open-label study of the administration of TAIC to renal transplant recipients concluded that TAIC treatment has no adverse effect on renal graft survival or function, and was without acute or medium-term complications; importantly, TAIC infusion did not sensitize recipients to graft antigens or otherwise accelerate rejection [10].

A second clinical trial, the TAIC-II study, using TAICs as an adjunct immune-conditioning therapy in living-related kidney transplantation concluded in December, 2007. The treatment of patients in TAIC-II differed from the TAIC-I study protocol in several important respects: TAICs were prepared according to a modified method, the TAIC infusion was given 5 days prior to transplantation, a greater number of TAICs were transferred, and the immunosuppressive induction therapy given to the TAIC recipients was different [10]. This initial report describes the clinical outcomes of the five patients enrolled in the TAIC-II study after a minimum follow-up period of 1 year.

Materials and methods

Patients

A protocol for a study titled, 'a multi-centre open-label study of the administration of allogeneic and autologous regulatory cells for the induction of donor-specific tolerance in renal allograft recipients (living donor)', was approved by the local ethic committees of the participating centres in Kiel, Düsseldorf and Essen. A total of five patients were recruited to the study according to the following inclusion criteria. Patients had to be 18–64 years of age with renal failure necessitating transplantation: only patients receiving the first renal transplant were eligible and a suitable living-donor had to be available. Both donor and recipient were required to have normal haematological parameters, particularly with regard to absolute monocyte and lymphocyte numbers. Patients had to give informed consent in writing.

Donor–recipient pairs were excluded from the trial according to the following criteria: Those with active infections, including HIV and hepatitis; donor–recipient pairs with cytomegalovirus and Epstein–Barr virus incompatibility; those with a history of alcohol, drug abuse or sepsis; those who were pregnant or nursing mothers; those with hypersensitivity or contraindication to immunosuppressives administered during the course of the study; those with a history or present symptoms of autoimmune vasculitis, including renal insufficiency on account of vasculitis; those with more than 5% human leukocyte antigen (HLA) antibodies (all current and historical values); those with a malignancy or history of malignancy; those whose condition required continuous systemic administration of immunosuppressives; those who were simultaneously participating (or planned to participate) in any other clinical study; patients with psychiatric or emotional problems, or lack of knowledge of the German language; those with corresponding donors presenting any one of the exclusion criteria documented in the Eurotransplant guidelines.

Patients enrolled in the TAIC-II study were monitored for indices of rejection and graft acceptance through the RISET network. For this purpose, the patients were coded as follows: patient 1. CRG, KI/T03/P01; patient 2. CFH, KI/T03/P02; patient 3. WG, KI/T03/P04; patient 4. AG, KI/T03/P05; patient 5. FI, KI/T03/P08. Patient CK, KI/T03/P06 was excluded from the trial owing to a respiratory infection, which manifested prior to TAIC treatment.

Preparation of the TAICs and TAIC-coculture cells

Figure 1 illustrates the preparation of donor-derived TAICs from leucapheresis products and their co-culture with recipient-derived lymphocytes. All *ex vivo* manipulations of cells were performed in accordance with GMP guidelines (Pharmaceutical Inspection Convention. Pic/s GMP guide for blood establishments. 1-7-2004) using laboratory facilities in the Department of Haematology at the University Hospital of Schleswig-Holstein (UKSH). A single, clinically qualified operator was responsible for the production, quality assessment and administration of the cellular product.

On day 14 preoperatively, the donor underwent leucapheresis. Normosol-sodium citrate solution was freshly

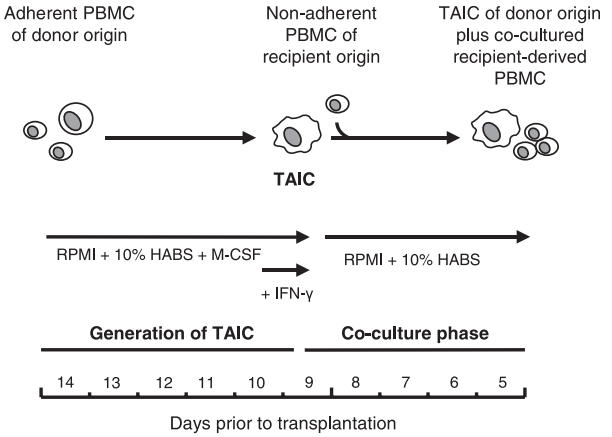

Figure 1 The production of human TAICs for clinical purposes under GMP conditions.

prepared from 1000 ml Normosol solution and 140 ml of 4% sodium citrate solution (Baxter, Unterschleißheim, Germany). Leucapheresis products were diluted 1:6 in Normosol-sodium citrate solution. 15 ml of Ficoll (Biochrom AG, Berlin, Germany) was dispensed into 50-ml centrifugation tubes (Becton Dickinson, Heidelberg, Germany) and overlaid with 20 ml of the diluted leucapheresis product. The mononuclear cell fraction was isolated from the leucapheresis product by centrifugation at 400 *g* for 20 min without break before carefully taking the cells at the serum-Ficoll interface by pipetting. The isolated mononuclear cells were pooled into 50-ml centrifugation tubes and pelleted by centrifugation at 300 *g* for 6 min. The pelleted cells were subsequently washed twice in Normolsol-sodium citrate solution, centrifuging at 200 *g* for 6 min each time. The resultant pellet was resuspended in 50 ml of TAIC medium and a differential viable cell count was performed. Samples of the isolated peripheral blood mononuclear cells (PBMCs) were sent for independent microbiological screening.

Transplant acceptance-inducing cell medium was prepared from RPMI-1640 without phenol red (Cambrex Bioscience Verviers, Verviers, Belgium) supplemented with 2 mM L-glutamine (Cambrex Bioscience), 100 U/ml Penicillin (Cambrex Bioscience), 100 μg/ml Streptomycin (Cambrex Bioscience) and 10% heat-inactivated human AB serum (Cambrex Bioscience). To this basic medium, an end concentration of 5 ng/ml recombinant human M-CSF (rhM-CSF) was added. Clinical-grade, carrier-free, lyophilized rhM-CSF was obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany), from which 5μg/ml (1000-fold) stock solutions were prepared in additive-free RPMI-1640 without phenol red (Cambrex Bioscience) containing 0.1% human serum albumin (Aventis, Frankfurt, Germany).

The concentration of isolated donor PBMC was adjusted to 5×10^7/ml viable cells (including all cell types present in the mixture except contaminating erythrocytes and thrombocytes) in TAIC medium before plating in T175 'Cell+'-coated tissue culture flasks (Sarstedt, Nürnbrecht, Germany) in a final volume of 30 ml, which is the equivalent of 1.5×10^9 viable PBMC per flask. The plated cells were incubated overnight (a period of 10–15 h) at 37 °C with 5% CO_2. The following day, 20 ml of the nonadherent cell fraction was removed by gentle agitation of the flask and pipetting; this supernatant was transferred into fresh T175 flasks and supplemented with a further 10 ml of fresh TAIC medium; the 20 ml of medium removed from the initial cultures was replaced with fresh TAIC medium. The cultures were then returned to the incubators (37 °C, 5% CO_2) for a further 24 h. On day 12 preoperatively, the entire supernatant was removed from each culture flask and the medium volume was replaced by freshly prepared TAIC medium. Any cells present in the nonadherent fraction were discarded.

On the 10th preoperative day, the TAIC cultures were pulsed for 24 h with recombinant human interferon-γ (IFN-γ). IFN-γ was obtained from Boehringer Ingelheim (Imukin®; Boehringer Ingelheim, Ingelheim am Rhein, Germany) and used as a solution at a concentration of 200 μg/ml (4×10^6 IU/ml). Fresh TAIC medium was prepared as described above and supplemented with 25 ng/ml IFN-γ. A complete exchange of medium was made in each of the tissue-culture flasks, replacing the 30 ml of supernatant with 30 ml of fresh TAIC medium supplemented with IFN-γ; any cells in the supernatant fraction were discarded. The TAIC cultures were returned to the incubators for a further 24 h.

Recipients underwent leucapheresis on day 9 preoperatively using the same leucapheresis apparatus and settings as described for the donors. PBMC were isolated from these leucapheresis products as described above, before being resuspended in basal medium (RPMI-1640 without phenol red/10% heat-inactivated human AB serum/2 mM L-glutamine/100 U/ml penicillin/100 μg/ml streptomycin) at a density of 2×10^7 cells/ml. PBMC were then plated in T175 Cell+ culture flasks at 6×10^8 viable PBMC per flask in 30 ml of medium, before being incubated at 37 °C in 5% CO_2 for precisely 1 h. After this 1-h incubation step, the nonadherent cell fraction was collected and washed once in basal medium, centrifuging at 200 *g* for 10 min. The resulting pellet of recipient cells, which was largely depleted of monocytes, was resuspended at a concentration 2×10^7 cells/ml in basal medium. The recipient-derived adherent cell fraction was discarded.

Supernatant from the IFN-γ-treated donor-derived TAIC cultures was discarded, along with any nonadherent

cells which it contained, and was replaced by 30 ml of recipient-derived nonadherent cell suspension. These co-cultures were allowed to proceed uninterrupted for a further 4 days, except for the addition of 20 ml fresh basal medium to each flask on day 7 preoperatively.

Cells for infusion were harvested on day 5 preoperatively. Each flask was taken from the incubator and gently rocked to resuspend nonadherent cells that had precipitated onto the adherent layer. The culture supernatant was collected into a 50 ml centrifugation tube and was replaced in the flask with 10 ml of Dulbecco's PBS (DPBS) at 4 °C (Cambrex Bioscience). The adherent cells were then removed from the flask by careful scraping. These cells were pooled with the cells from the nonadherent fraction before centrifugation at 200 *g* for 6 min. The resulting pellet was resuspended in a final volume of 40 ml of 5% human albumin solution at 4 °C. Samples were sent for independent microbiological investigation and other quality-control assays.

The cell suspension was taken for immediate infusion into the recipient via a central venous catheter. Patients receiving TAIC infusions were treated with prophylactic low molecular weight heparin to reduce the risk of pulmonary embolus. The total viable cell doses administered to individual patients are listed in Table 1. Before administration to the patients, an aliquot of each TAIC preparation was taken for analysis by flow cytometry (Fig. 2).

Immunosuppressive protocol

In addition to treatment with TAICs, patients were immunosuppressed with anti-thymocyte globulin, (ATG; ATG-FreseniusS®; Fresenius AG, Bad Homburg, Germany), tacrolimus (Prograf® Capsules; Astellas Pharma AG, Munich, Germany) and prednisolone (Solu-Decortin® H, Decortin H®; Merck Pharma GmbH, Darmstadt, Germany). ATG was administered on day 0, day 1 and day 2 after transplantation. Initially, all patients were treated with the combination of steroids and tacrolimus aiming for trough levels of 8–12 ng/ml. Eight weeks after transplantation, steroids therapy was tapered off over a period of 14 days, provided that serum creatinine levels stayed below 2.0 mg/dl, histological signs of rejection were absent and the transplanted kidney was clinically and sonographically normal. Following cessation of corticosteroid treatment, creatinine clearance (CL_{Cr}) was monitored tightly; unless a reduction in CL_{Cr} of more than 25% occurred, and provided there was no other evidence of graft compromise, tacrolimus trough levels were reduced over a period of 1 week to levels of 5–8 ng/ml. Further reduction of tacrolimus doses were made at the discretion of the responsible physician, depending on CL_{Cr}, histological and clinical findings. The reductions were made in a stepwise fashion, such that administration of tacrolimus was first reduced to once daily, then to three times a week, twice a week and finally complete cessation. As detailed below, patients were tightly monitored for signs of rejection and a low threshold for reinstating conventional immunosuppressive therapy was adopted. In the case of WG, rescue therapy with Rituximab (Mabthera®; Hoffmann LaRoche AG, Basel, Switzerland) and intravenous immunoglobulins (Intratec®; Biotest AG, Dreieich, Germany) was necessary.

Renal biopsy

A renal core biopsy of the transplanted organ was performed intra operatively (before reperfusion) and at weeks 8, 24 and 52. Additional biopsies were taken whenever a rejection reaction was suspected. The biopsies were evaluated blindly by Prof. Dr M. Mihatsch, Director of the Institute of Pathology of the University Basel, Switzerland, as an independent expert.

Flow cytometry

Harvested TAICs were washed twice in ice-cold staining buffer (DPBS with 10% BSA and 0.02% NaN_3) before blocking with 10% FcR Block (Miltenyi, Bergisch Gladbach, Germany) for 30 min on ice at a density of 10^7 cells/ml. Directly conjugated primary antibodies were applied at a final concentration of 1 μg/10^6 cells. Anti-

Table 1. Patient and donor characteristics.

Patient	Age	Body weight (kg)	Sex	Disease	Donor age	Donor BW (kg)	Donor	Cell-dose (vital cells/kg BW ×10^7)
CRG	42	80	Male	IgA nephritis	40	100	Brother	1.74
CFH	39	57	Female	Glomerulonephritis	38	84	Husband	10.39
WG	48	83	Male	Polycystic kidney disease	38	98	Brother	3.95
AG	59	77	Male	Glomerulonephritis	24	60	Daughter	1.82
CK	37	95	Female	Polycystic kidney disease	36	118	Husband	2.08
FI	48	94	Male	Polycystic kidney disease	40	56	Wife	2.54

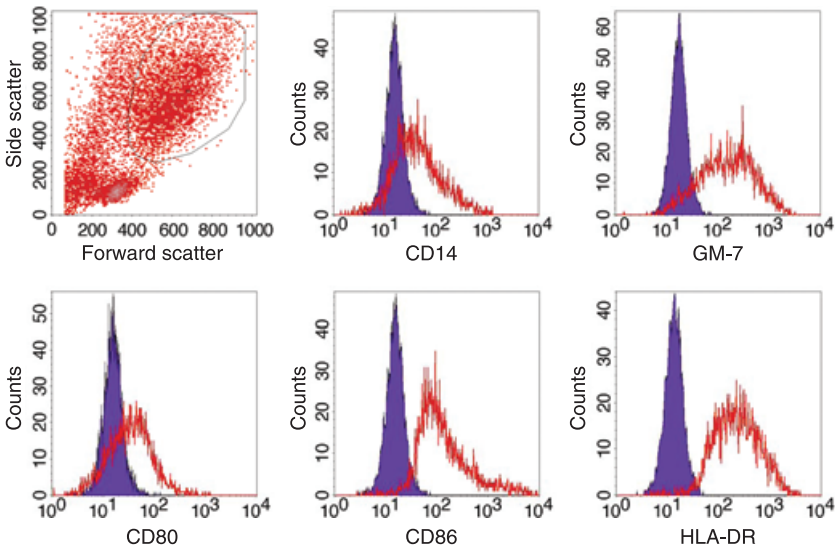

Figure 2 A representative phenotypic analysis of the TAICs given to patient CFH. Prior to administration to the patients, a sample of TAICs was analyzed by flow cytometry. As described previously, the TAICs expressed low levels of CD14 and stained positively for the GM-7 antigen. The cells exhibited a partially matured phenotype with relatively weak expression of CD80, CD86 and HLA-DR.

bodies with the following specificities were used: CD14 [Becton Dickinson (BD), Heidelberg, Germany; catalogue #555399], CD13 (BD, #555394), HLA-DR (BD, #555811), CD80 (BD, #557227), CD86 (BD, #555658). GM-7 is an incompletely characterized TAIC-specific monoclonal antibody produced in-house [14]. 7-AAD (BD, #559925) was used for dead cell exclusion. FACS analyses were performed with a BD FACS Calibur machine and data was recorded and analyzed with Cell Quest software.

Antibody screening

Patients were screened pre- and postoperatively for the presence of HLA-specific antibodies by ELISA and complement-dependent cytotoxicity (CDC) assays. Dithiothreitol (DTT) sensitivity and immunoglobulin G (IgG)-specific ELISA were used to discriminate between IgG and IgM antibodies.

Mixed lymphocyte culture with multiple cytokine analysis

Cultures of responder PBMC (10^5 cells/well) and stimulator cell (PBMC, 10^5 cells/well) were set up in triplicates in U-bottom trays. After 5 days of incubation at 37 °C, 5% CO_2, 100 µl of the supernatant was removed from the cultures, transferred to siliconized eppendorf tubes and stored at −70 °C until required further use. For proliferation assays, ^{3}H-Thymidine was added to the cultures and the cultures were harvested 16 h later. The filters were counted with a beta-counter (PE-Wallac, Turku Finland). For the Luminex bead array-based multiple cytokine assays, the Bio-Plex Human Th1/Th2 panel (9-plex) (Cat. #171-A11081; Bio-Rad, Munich, Germany) was used according to the Bio-Rad protocol. Results of anti-donor reactivity in MLR (recipient responder lymphocytes against irradiated donor leucocytes) was compared with the background response (either medium-only or autologous stimulator cells) and a nonspecific third-party response (fully mismatched PBMC and/or PBMC with a similar number of HLA class I and class II mismatches as the donor). Proliferative responses have been expressed in terms of the *stimulation index* (the ratio of the stimulated response to the unstimulated (medium-only) response). Quoted values are mean ± SD.

Results

Recruitment and treatment of patients in the TAIC-I trial

Eight living-donor and recipient pairs were considered for enrolment in the TAIC-II study, of which six were admitted to the study and received treatment with TAICs (Tables 1 and 2). TAIC infusion was tolerated well by all the patients: no acute complications of the cell infusion arose and no delayed complications have yet occurred. One patient, CK, developed a respiratory tract infection prior to TAIC transfusion, which delayed her subsequent surgery and altered her planned management; accordingly, this patient has been excluded from the trial analysis, leaving five for evaluation.

Case 1 – Patient CRG

CRG, a 42-year-old man with renal failure on account of IgA nephropathy, received a kidney transplant from his HLA-identical 40-year-old brother (Fig. 3). As per the protocol, donor-derived TAICs were infused via a central venous catheter 5 days before transplantation. Surgery

Table 2. HLA-matching, blood group and crossmatch.

Patient	HLA type	Blood group	Crossmatch
CRG	A2, 68; B27, 53; DR13, 15	0 RhD^+	Negative
Donor	A2, 68; B27, 53; DR13, 15	0 RhD^+	
CFH	A2, –; B7, 44; DR12, 15	A RhD^-	Negative
Donor	A11, 25; B18, 44; DR7, 11	0 RhD^-	
WG	A2, 68; B8, 35; DR3, 7	B RhD^+	Negative
Donor	A1, 68; B8, 35; DR3, 7	B RhD^+	
AG	A3, 26; B7, 57; DR7, 11	A RhD^+	Negative
Donor	A2, 26; B44, 57; DR4, 7	A RhD^+	
CK	A2, 3; B51, –; DR11, 8	A RhD^+	Negative
Donor	A2, –; B39, 58; DR1, 3	A RhD^+	
FI	A31, 32; B60, 44; DR3, 15	A RhD^+	Negative
Donor	A1, 3; B7, 8; DR3, 15	A RhD^+	

was successful and the patient registered excellent early graft function. Corticosteroids were weaned after 8 weeks, so that by week 10, the patient was receiving tacrolimus monotherapy with trough serum levels in the range 8–10 ng/ml. At week 10, patient CRG showed no clinical signs of rejection, so between weeks 10 and 24, tacrolimus therapy was gradually tapered until the patient was stably established on tacrolimus monotherapy with trough serum levels of 4–8 ng/ml.

Graft function at week 24 was stable and a second protocol biopsy also showed no sign of rejection (Fig. 3c). Tacrolimus doses were further reduced, such that trough serum levels were <4 ng/ml from week 25 onwards. Patient CRG remained on this immunosuppressive regimen until week 43, when it was decided to withdraw tacrolimus treatment completely. This withdrawal was well-tolerated and renal function remained stable with no indication of rejection for the following 8 months (weeks 44–76).

A routine graft biopsy at week 50 revealed a marked, focal, interstitial cellular infiltrate (Fig. 3d and e) with patchy positive HLA-DR staining of the tubular epithelium. These histological findings were not clearly indicative of a rejection episode and, at that time, there was no clinical suspicion of rejection. It was noted that similar histological findings had been reported in renal transplant recipients treated with long-term, low-dose tacrolimus monotherapy and that these infiltrates were not necessarily associated with an adverse outcome [10,17]. Two subsequent graft biopsies, taken 25 and 65 days later, showed similar results. In light of the absolutely stable clinical condition of the patient, it was decided not to recommence immunosuppressive treatment, but to monitor his clinical condition closely.

During week 61, a mixed lymphocyte culture with multiple cytokine analysis (MLC-MCA) was performed to compare recipient reactivity to donor or third-party, fully mismatched stimulation (Fig. 3f–h). There was no measurable anti-donor response, both in terms of proliferation and secretion of interleukin-2 (IL-2) and IFN-γ, but the recipient did react to third-party cells. In week 77, patient CRG suffered an acute rejection episode, which was managed with corticosteroids and the reintroduction of tacrolimus. The patient's renal function recovered well and he is now maintained with tacrolimus monotherapy (trough serum levels of 6–8 ng/ml) with stable graft function. No HLA-specific antibodies were detected at any point during the study.

Case 2 – Patient CFH

Patient CFH, a 39-year-old female, presented with renal insufficiency owing to glomerulonephritis (Fig. 4). She received an organ from her 38-year-old husband, with whom she had five of six HLA mismatches. Surgery was without complication and the initial graft function was good, with normalized creatinine values within the first week postoperatively. Corticosteroids were withdrawn between weeks 8 and 10, and trough serum tacrolimus levels were adjusted into the range 6–12 ng/ml by week 24.

Trough tacrolimus levels were lowered to ≤4 ng/ml by week 26 and, subsequently, into the range of 2–4 ng/ml by week 29. The patient's clinical status remained unchanged for the subsequent six-and-a-half weeks, when the patient abruptly discontinued all immunosuppression of her own volition. Ten days later, the patient underwent an episode of histologically confirmed acute cellular rejection, which was managed with corticosteroids. The patient now is maintained on tacrolimus monotherapy (2.5 mg OM, 2 mg ON) and her creatinine is stable at approximately 0.9 mg/dl. No HLA-specific antibodies were detected at any point during the study. Given that the patient CFH received an organ from a donor with whom she shared only a single HLA match, her current, stable graft function on tacrolimus monotherapy is very encouraging.

Case 3 – Patient WG

Patient WG, a 48-year-old man, developed progressive renal failure as a consequence of polycystic kidney disease. He received a renal transplant from his 38-year-old brother, with whom he had only a single HLA-mismatch. TAIC infusion and surgery passed without complications, and early graft function was good (Fig. 5). The first protocol biopsy at week 8 showed no pathological changes; accordingly, steroids were weaned. Trough serum tacrolimus levels were gradually lowered to <4 ng/ml over a period of 3 months, during which time there was no evidence of compromise to the graft. However, after 9 weeks of maintenance on tacrolimus

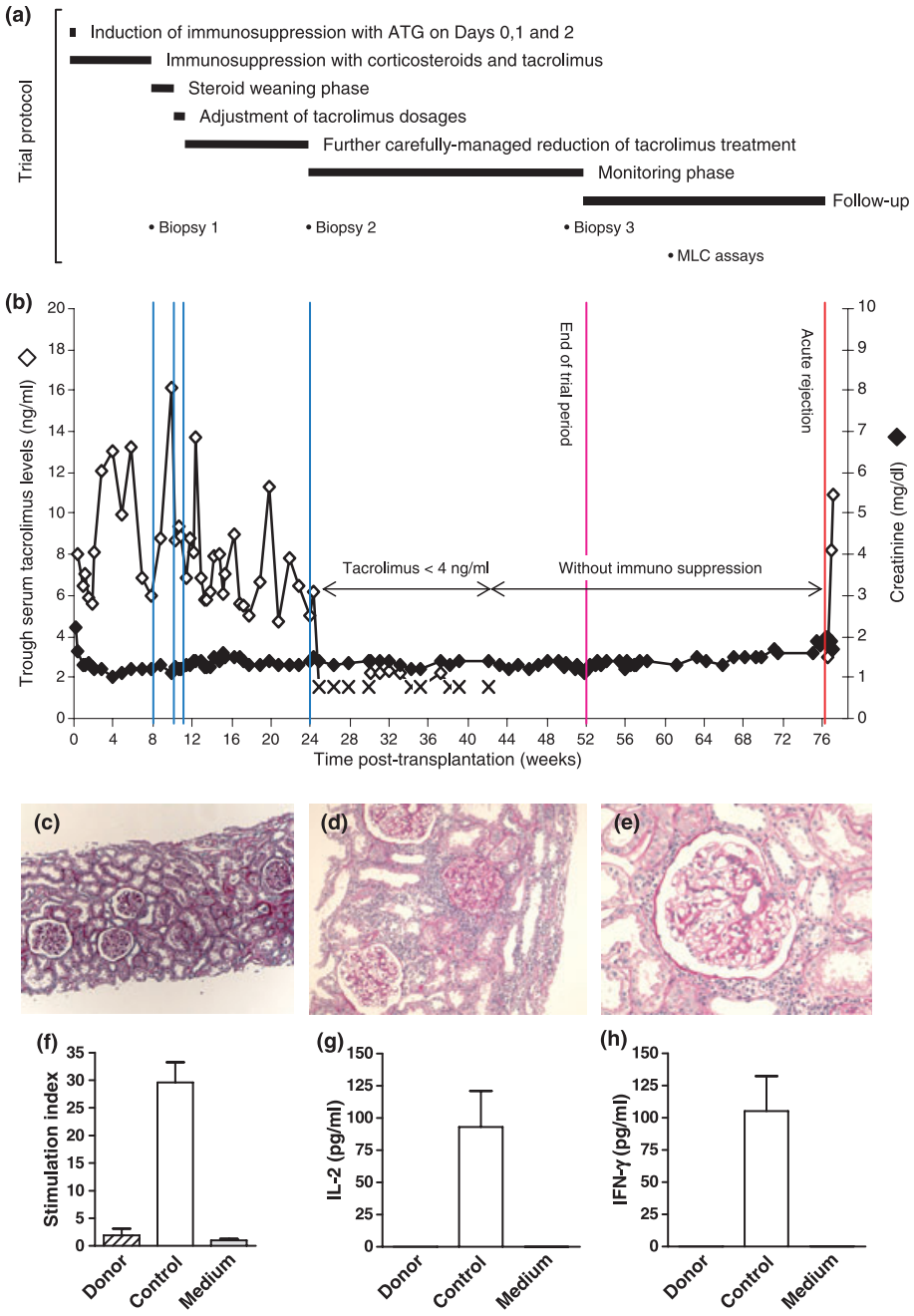

Figure 3 Summary of the clinical outcome of patient CRG. Patient CRG, a 42-year-old man, received a renal transplant from his HLA-identical younger brother. (a) Postoperative immunosuppressive therapy was administered according to the protocol shown. (b) Tacrolimus monotherapy was well-tolerated and, by the 26th week, the patient's trough serum tacrolimus levels were in the range 2–4 ng/ml. In week 43, all pharmacological immunosuppressive therapy was withdrawn. The patient's graft function remained stable for the subsequent 34 weeks, after which he experienced an acute rejection episode. (c) PAS-stained renal core biopsy from week 24 with no sign of rejection; original magnification: 100×. (d,e) By week 50, a marked lymphocytic infiltrate had accumulated, although no corresponding decline in graft function was noted; original magnifications: 100× and 200×, respectively. Biopsies taken 25 and 65 days later showed similar findings. During week 60, recipient anti-donor responses were assessed in MLC by measuring proliferation responses (f), IL-2 secretion (g) and IFN-γ production (h), and comparing these to recipient responses against a fully mismatched third-party stimulator (control) and in the absence of allogeneic stimulation (medium).

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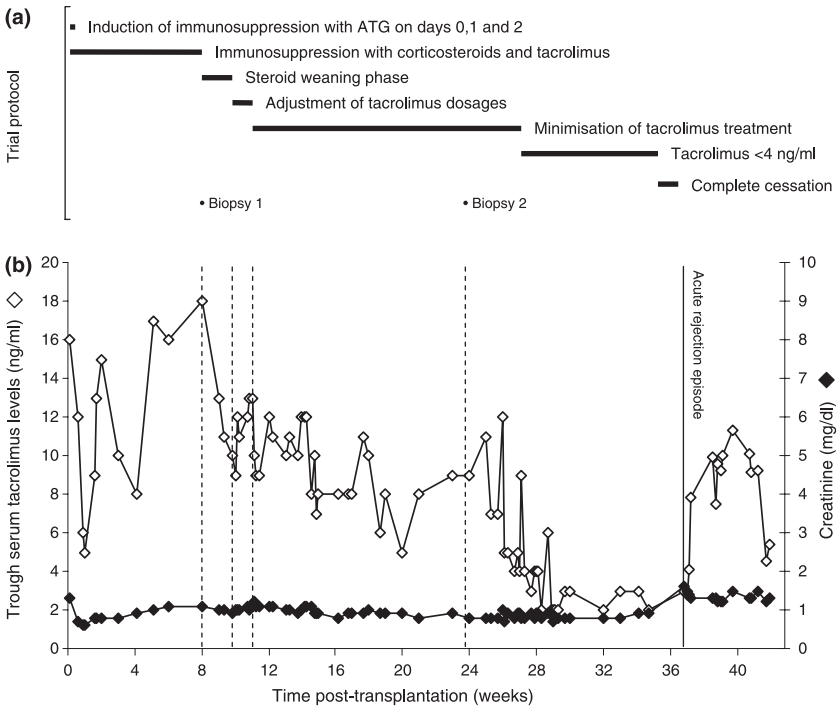

Figure 4 Summary of the clinical outcome of patient CFH. Patient CFH, a 39-year-old woman, received a 5/6 HLA-mismatched renal transplant. (a) Postoperative immunosuppression was administered as illustrated. (b) Trough serum tacrolimus levels were reduced to 4 ng/ml within the first 26 weeks and further lowered into the range 2–4 ng/ml by the 28th week. The patient's graft function remained stable throughout this period and for the subsequent six-and-a-half weeks when, of her own volition, the patient abruptly halted her immunosuppressive therapy. Ten days later, the patient experienced an acute rejection episode.

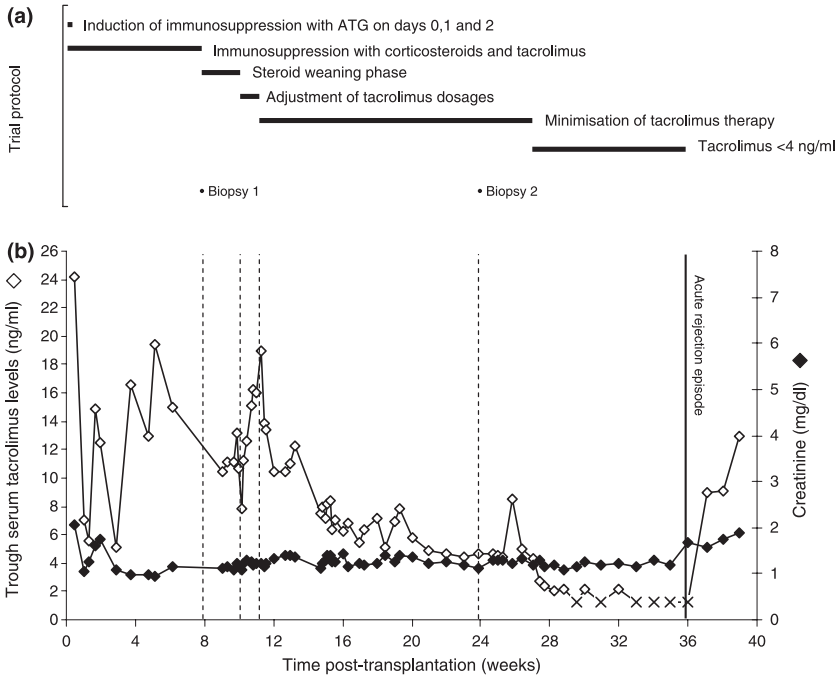

Figure 5 Summary of the clinical outcome of patient WG. Patient WG, a 46-year-man with polycystic kidney disease, received a transplant from his brother, with whom he shared a single HLA mismatch. (a) The patient received immunosuppressive treatment as shown. (b) By the 20th week postoperatively, the patient had been weaned to tacrolimus monotherapy with trough serum levels in the range of 4–6 ng/ml and his graft function remained stable under this regimen for 7 weeks. In the 27th week, tacrolimus doses were further reduced. Patient WG remained well with tacrolimus levels of <2 ng/ml for 9 weeks, when he underwent a severe, acute rejection episode.

monotherapy with trough serum levels of 2–4 ng/ml, the patient suffered a severe, acute rejection episode. The rejection was resistant to treatment with corticosteroids, so the patient was treated with ATG, Rituximab, plasma separation and intravenous immunoglobulins. Graft function has now been restored satisfactorily (creatinine approximately 2.0 mg/dl) and the patient is stably immunosuppressed with tacrolimus (trough levels: 8–10 ng/ml), prednisolone (10 mg OM) and mycophenolic acid (180 mg TDS). No HLA-specific IgG antibodies were detected at any point during the study.

Case 4 – Patient AG

Patient AG, a 59-year-old man, presented with renal insufficiency resulting from chronic glomerulonephritis (Fig. 6). He received a living-related kidney graft from his 24-year-old daughter with whom he had three HLA-mismatches. Unfortunately, after successful transplantation and treatment with TAICs, it was not clinically feasible to fully minimize the patient's immunosuppression because his rheumatoid arthritis necessitated continuing low-dose corticosteroid therapy. Anti-donor reactivity was assayed in MLC at weeks 42 and 54, and was found to be substantially suppressed when compared to a fully mismatched third-party control stimulator (data not shown). No HLA-specific antibodies were detected at any point during the study. At present, the patient's creatinine level is approximately 1.6 mg/dl and his immunosuppression consists of tacrolimus (trough levels: 3–4 mg/dl) and corticosteroids (prednisolone, alternating 5 and 2.5 mg OD).

Case 5 – Patient FI

Patient FI, a 48-year-old man, presented with renal insufficiency owing to adult polycystic kidney disease. He received a renal transplant with four HLA-mismatches from his 40-year-old long-term, female partner. The transplantation and postoperative course were uneventful, although the patient's serum creatinine levels remained persistently above 2 mg/dl (Fig. 7). Graft biopsies taken shortly after transplantation showed no pathology, so we attribute this patient's moderately elevated post-transplantation creatinine levels to the possibility that the transplanted organ, from a 56 kg donor, did not have adequate capacity for its muscular 94 kg recipient. In accordance with the trial protocol, steroids were successfully withdrawn, since which time the patient has been maintained on tacrolimus monotherapy, with trough levels in the range of 6–12 ng/ml. Routine biopsies taken at weeks 8 and 24 revealed no signs of rejection (Fig. 7c). A further biopsy taken at week 54 also showed no signs of rejection (Fig. 7d and e). As this patient, who is now in his 55th week postoperatively, has been very successfully established on low-dose tacrolimus monotherapy with stable graft function, there is no intention to attempt further reduction in his immunosuppressive therapy.

Recipient anti-donor reactivity was assayed in MLC at four points during patient FI's treatment: pretransplantation and at 6, 7 and 9 months postoperatively (Fig. 7f–h). Recipient anti-donor responses were compared to the response of the recipient to a Class I-mismatched, HLA-DR-identical third-party control and to a fully mismatched third-party control. Patient FI consistently reacted more strongly to both Class I-disparate and fully mismatched control stimulators than to donor stimulation, in terms of cell proliferation in MLC and secretion of IFN-γ and IL-2. No HLA-specific antibodies were detected at any point during the study.

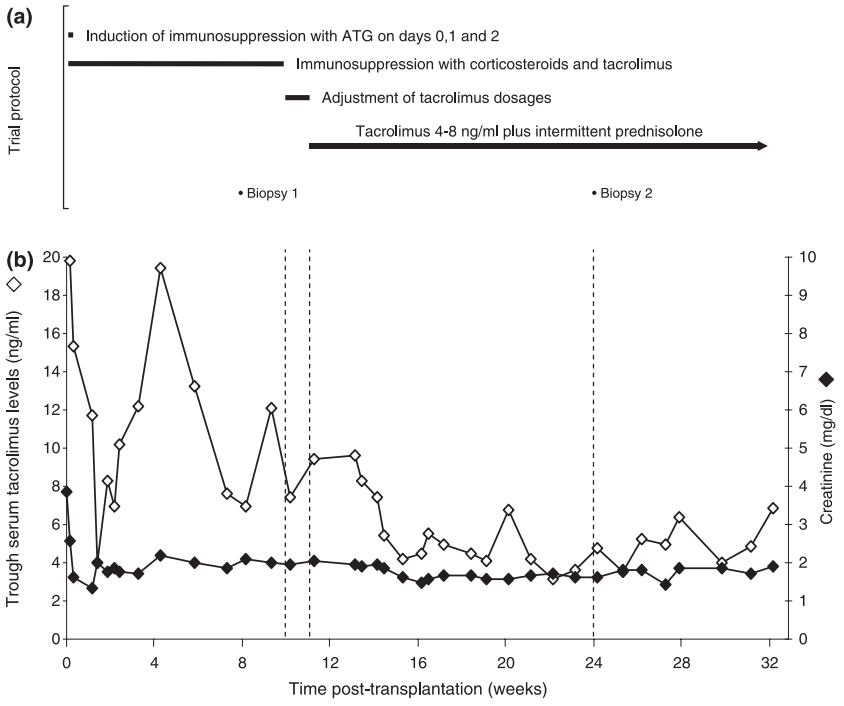

Figure 6 Summary of the clinical outcome of patient AG. Patient AG, a 59-year-old man with chronic glomerulonephritis received a transplant from his daughter. (a) Patient AG was treated according to the illustrated protocol and (b) remains very well with good graft function.

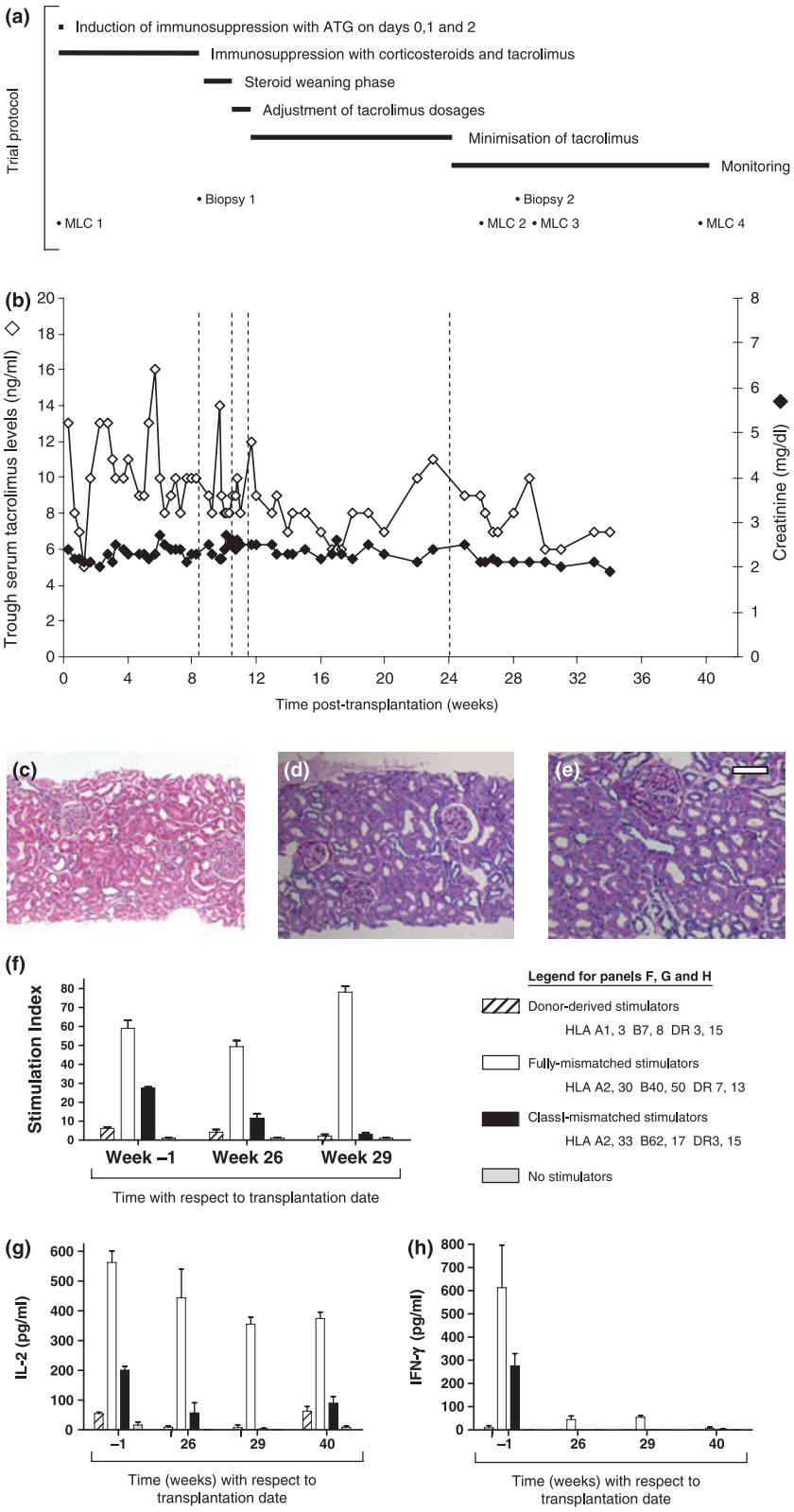

Figure 7 Summary of the clinical outcome of patient FI. Patient FI, a 48-year-old man with polycystic kidney disease, received a 4/6-mismatched renal transplant from his female partner. (a) Patient FI was treated according to the protocol shown. (b) Throughout the postoperative course, patient FI had stable graft function. (c) A PAS-stained section of a graft biopsy from week 24; original magnification 100×. (d) A PAS-stained section from graft biopsy taken at week 54 showed no signs of rejection; original magnification, 100×. (e) Detail of the graft biopsy taken at week 54; bar = 300 μm. (f) At weeks 1, 26 and 29 with respect to the date of transplantation, patient FI (HLA A31, A32; HLA B60, B44; HLA DR3, DR15) made less proliferative responses against donor-derived stimulator cells than to either Class I-mismatched or fully mismatched control stimulators. Similarly, IL-2 (g) and IFN-γ (h) production in response to donor-stimulation was relatively suppressed.

Discussion

The TAIC-II study was primarily a safety trial designed to test TAIC cells produced and administered under a protocol that differed from the TAIC-I safety study. In TAIC-II, no acute or delayed complications were observed and there was no evidence that the infusion of TAICs prior to transplantation could sensitize recipients against donor antigens or otherwise accelerate graft rejection. Therefore, we conclude that the preoperative treatment of living-donor kidney transplant recipients with TAICs is clinically practicable and safe in the acute and intermediate term.

The TAIC-II trial was not designed to test the efficacy of TAICs in establishing transplant recipients on low-dose tacrolimus monotherapy: the patient cohort was small and there was no untreated control group. Nevertheless, it is clear that patients enrolled in the TAIC-II trial experienced a higher rate of early, acute rejection episodes than would have been expected under conventional management. TAIC treatment, as it was administered in the TAIC-II study, is clearly not an adequate protocol for the induction of operational tolerance to renal allografts. Nevertheless, the very fact that the patients treated with TAICs were able to tolerate substantial reductions in their immunosuppressive therapy, albeit temporarily, runs contrary to our usual clinical expectation and warrants further consideration.

Of the five patients included in the TAIC-II trial, four were successful weaned from a conventional immunosuppressive regime to tacrolimus monotherapy; three of these patients registered trough serum tacrolimus levels in the range 6–8 ng/dl within 24 weeks of transplantation. Compared to previous studies, which sought to establish renal transplant recipients on tacrolimus monotherapy [17–24], low-dose tacrolimus treatment was achieved earlier in the TAIC-II study. Moreover, this weaning was well-tolerated: it was only after the complete withdrawal of immunosuppression that CFH underwent rejection, and patient WG's rejection crisis was precipitated by reduction of tacrolimus trough serum levels to <4 ng/ml. Patient CRG was successfully maintained on low-dose tacrolimus monotherapy for 18 weeks and subsequently received no immunosuppression for over 8 months.

Clinical experience teaches us that transplant recipients need not either be undergoing rejection or be operationally tolerant of their graft, but may occupy a metastable state of donor-specific hypo-responsiveness, in which they have a low requirement for maintenance immunosuppression, without being able to tolerate complete withdrawal of immunosuppressive therapy [25]. Necessarily, the immunological basis of such transient states of nonreactivity has not been well characterized, but it is recognized that pretransplantation exposure to donor alloantigen and early withdrawal of conventional immunosuppression may contribute to their establishment [26–29]. We contend that the clinical outcomes of the TAIC-II participants, in particular patients CRG and CFH, are consistent with TAIC treatment having induced a clinically relevant degree of anti-donor unresponsiveness. In support of this contention, T-cell proliferation and cytokine secretion in MLC confirmed that, during periods of clinically stable graft function, recipient-anti-donor reactivity appeared to be relatively suppressed.

At present, the cellular mechanisms by which TAICs might influence recipient anti-donor reactivity are not well characterized, but there may be informative parallels to be drawn between treatment with TAICs and longer-established techniques, such as donor-specific blood transfusion or donor-specific bone marrow transplantation [1,30,31]. In this context, it is interesting to note that the TAIC was originally identified as the principal derivative of a rat embryonic stem cell line, which induced tolerance to allogeneic heart grafts [10,12,13]. Recently, there have been impressive reports of the potential of both donor blood transfusion and bone marrow transplantation to induce tolerance in the setting of renal transplantation [30] and intestinal transplantation [32], but no such demonstration of the efficacy of TAIC treatment has yet been made. However, there are a number of theoretical advantages to the use of TAICs rather than crude blood products or conventional bone marrow transplants as immune conditioning therapies, perhaps mostly importantly that the composition, quality and dose of TAIC preparations can be tightly controlled. In addition, unlike bone marrow transplantation, TAIC treatment has the very substantial clinical advantage that it does not necessitate that patients be rendered temporarily lymphocytopenic in order that the tolerance-inducing cells adequately engraft [16,30]. Furthermore, TAIC treatment may be safer than either blood transfusion or bone marrow transplantation: None of the patients treated in either the TAIC-I or TAIC-II trial (a total of 16 recipients) were sensitized against donor antigens by the administration of TAICs [10,11]. By contrast, some 7% of patients are sensitized by donor-specific blood transfusion under cover of azathioprine [2,4] and one of five patients suffered irreversible humoural rejection in the combined bone marrow and renal transplant series reported by Kawai *et al.* [30].

Better understanding the cellular and molecular mechanisms underlying human TAIC development, and further characterization of TAICs in terms of marker expression and functional capabilities, should allow improvements in the clinical manufacture of TAICs, resulting in a more uniform, better defined *cellular medicine*. With this prospect, and a view to the more encouraging aspects of the

TAIC-I and TAIC-II clinical trials, we consider that the administration of TAICs as an adjunct immunosuppressive therapy in human solid organ transplantation deserves further investigation.

Authorship

JAH: data analysis, wrote paper. BGB-E: trial coordinator. PR: data analysis, edited manuscript. DR: performed MLC analyses. MS, KI, BG, OW, TP, LR, AH and AS: participating clinician. MM: trial coordinator nurse. AH, TS and FG: doctoral students. DK and FC: senior scientific participant. EKG: senior scientific participant, editing manuscript. UK: principal investigator. FF: senior scientific and clinical participant.

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