AP&T Alimentary Pharmacology and Therapeutics

The safety of autologous and metabolically fit bone marrow mesenchymal stromal cells in medically refractory Crohn's disease – a phase 1 trial with three doses

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Publication data
Submitted 16 February 2016
Resubmitted 29 April 2016
Resubmitted 13 June 2016
Accepted 15 June 2016
EV Pub Online 7 July 2016

The Handling Editor for this article was Professor Roy Pounder, and it was accepted for publication after full peer-review.

SUMMARY

Background
Mesenchymal stromal cells ability to reset immune functionalities may be useful in Crohn's disease.

Aim
To perform a first-in-human phase 1 safety clinical trial of metabolically fit autologous bone marrow-derived mesenchymal stromal cells in 12 subjects with Crohn's disease utilising three doses.

Methods
Autologous mesenchymal stromal cells were derived from marrow aspirate and propagated for 2–3 weeks with fibrinogen depleted human platelet lysate and subsequently administered to subjects without interval cryobanking. Twelve subjects received a single mesenchymal stromal cell intravenous infusion of 2, 5 or 10 million cells/kg BW(n = 4/group). Infused mesenchymal stromal cells were analysed for cell surface marker expression, IDO(indoleamine 2,3-dioxygenase) upregulation by IFNγ stimulation, and inhibition of third party peripheral blood mononuclear cell proliferation *in vitro*. The primary end point measured was safety and tolerability; clinical response was assessed as a secondary endpoint.

Results
All patients tolerated the mesenchymal stromal cell infusion well and no dose limiting toxicity was seen. Seven patients had serious adverse events of which five were hospitalisations for Crohn's disease flare. Two of these serious adverse events were possibly related to the mesenchymal stromal cells infusion. Five subjects showed clinical response 2 weeks after the infusion. Mesenchymal stromal cell phenotype, cytokine responsiveness, and peripheral blood mononuclear cell proliferation blockade were not different among the patients.

Conclusion
Single infusion of fresh autologous bone marrow mesenchymal stromal cells propagated *ex vivo* using human platelet lysate-supplemented media was safe and feasible at intravenous doses of up to 10 million cells/kg BW in patients with Crohn's disease.

doi:10.1111/apt.13717

INTRODUCTION

Crohn's disease and ulcerative colitis, collectively known as inflammatory bowel disease, are chronic, lifelong disorders with devastating consequences, including malnutrition, intestinal obstructions and perforations. Chronic inflammation and ulcerations of the intestine not only result in malnutrition and intestinal failure but also progression to fibrosis that results in complications such as obstruction and bowel perforations.[1, 2] Although current therapies may be effective in controlling inflammation, enthusiasm over these agents is tempered by their lack of treatment durability with time and risk of serious side effects including infection and malignancy. Standard anti-inflammatory regimes such as corticosteroids and mesalazine (mesalamine) are only effective in controlling the inflammation but do not halt the progression of the disease nor do they change the natural history of IBD. Although more aggressive, mucosal healing agents such as biological therapies have been developed, significant disadvantages exist to these such as immunogenicity[3] and lack of treatment durability with time.[3–5] Enthusiasm over the use of these agents is further tempered by concerns of serious side effects including life-threatening infections, severe allergic reactions and malignancies such as hepatosplenic T-cell lymphomas which are often lethal.[4, 5] An alternative approach to modulate chronic inflammation by nontoxic cell rather than protein-based therapy may serve as an attractive option. Such potential inflammatory bowel disease therapies include mesenchymal stromal cells, a cellular product that can be derived from bone marrow and propagated *ex vivo*. They have been shown to have a broad spectrum of immunomodulatory actions on both the innate and adaptive immune systems, which may aid in inflammatory bowel disease.[6–9] Mesenchymal stromal cells possess unique transcriptome and secretome, which collectively enforce immune modulation of innate and adaptive immune cells of the host.[7–9] Mesenchymal stromal cells are under intensive clinical investigation for treatment of auto- and allo-immune disorders including as IBD suppressor cell therapy.

Clinical trials have demonstrated safety and likely utility of mesenchymal stromal cells for treating Crohn's disease.[10–13] However, cryobanked allogeneic mesenchymal stromal cells have been widely investigated in a number of industry-sponsored clinical trials as a cellular pharmaceutical for treatment of immune disorders, including inflammatory bowel disease (e.g. Multistem cells – NCT01240915 and Prochymal – NCT00294112). We have observed that mesenchymal stromal cells thawed from cryopreservation display attenuated immunosuppressive and *in vivo* distribution properties.[14, 15] In addition, we have also previously demonstrated that allogeneic mesenchymal stromal cells are susceptible to immune rejection by hosts with normal immune systems.[16] These findings provides a straightforward biological explanation for the negative outcome of a large prospective randomised Phase 3 and 2 clinical trials (NCT00366145, NCT01240915) of cryobanked industrial random donor allogeneic mesenchymal stromal cells for graft-versus-host disease and severe ulcerative colitis respectively.[17, 18] We have proposed that autologous noncryopreserved actively growing mesenchymal stromal cells would provide the best therapeutic effect unhindered by host rejection or cryo injury.[19] In congruence, we have demonstrated that mesenchymal stromal cells derived from Crohn's disease subjects can be expanded *in vitro* and deploy immunosuppressive mechanisms identical to healthy controls and thus can serve as an autologous cell pharmaceutical.[20] On the basis of this, we have informed the design and implementation of a phase 1 clinical trial examining the use of autologous and noncryopreserved mesenchymal stromal cells for treatment of Crohn's disease (NCT01659762). We aimed to determine the safety and tolerability of fresh autologous bone marrow derived, metabolically fit mesenchymal stromal cells in patients with Crohn's disease as a primary endpoint, and to study the efficacy of autologous bone marrow-derived mesenchymal stromal cells as measured by Crohn's Disease Activity Index and C-reactive protein as a secondary endpoint.

METHODS

Study design and participants

Our trial was a US Food and Drug Administration sanctioned [IND 14825, sponsor: Jacques Galipeau, MD] and Emory Investigational Review Board approved single centre, Phase 1 open label study utilising three doses designed to determine the safety, tolerability and efficacy of autologous bone marrow-derived mesenchymal stromal cells in patients with moderate to severe Crohn's disease. Patients were enrolled in the out-patient paediatric and adult gastrointestinal clinics at Emory University, Atlanta, Georgia between June 2012 and July 2015. Male and female patients were between the ages of 18–52 with firmly established Crohn's disease for at least 3 months as confirmed by endoscopic, radiological and/

or histological evidence with a Crohn's disease Activity Index score >220 and were refractory to conventional Crohn's disease treatment, defined as lack of response to immunomodulators (including mercaptopurine, azathioprine or methotrexate) and/or biologics (anti-TNF agents or anti-adhesion agents) for at least 3 months at some point in the course of their disease. Patients who were found to have intestinal strictures or need for near immediate surgery were excluded. Those with an ostomy, ongoing use of biological or immunomodulator therapy, concurrent infections requiring antibiotics, renal insufficiency, elevated transaminases 2× upper limit of normal, bilirubin >1.2 mg/dL, neutrophil count <1500/μL, haemoglobin <8 mg/dL, platelet count <100 000/μL, concurrent inflammatory eye disease, pyoderma gangrenosum, history of HIV, active/chronic hepatitis B or C, tuberculosis, active CMV viraemia, presence of enteric pathogens, current or previous history of opportunistic infection within 6 months prior to screening, serious infection in previous 3 months, and previous history of malignancy were excluded from the study. Per protocol, patients were permitted to continue corticosteroids (prednisone or budesonide), mesalazine, non-narcotic analgesics, nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors with stable doses for at least 2 weeks. Patients had at least a 4 week wash out of immunomodulators (azathioprine, mercaptopurine and methotrexate) and/or biologics prior to enroling in the study if they were on these agents. Subjects were screened and followed in the out-patient paediatric clinic in Emory Children's Center and/or the adult gastroenterology Emory clinic. Those patients meeting the inclusion and exclusion criteria who were willing to participate were enrolled. Informed consent was obtained after discussion with the patients. Upon signing the consent, participants underwent a rigorous screening process including physical examination and collection of lab data (complete blood count, comprehensive metabolic panel, C-reactive protein, erythrocyte sedimentation rate, faecal calprotectin, urinalysis, pregnancy test if applicable, CMV PCR serum assay, stool culture and *Clostridium difficile* toxin assay, HIV and hepatitis B and C testing). Tuberculosis screening was performed using a PPD. Patients underwent a baseline chest X-ray, pulse oximetry measurement, pulmonary function tests and a baseline electrocardiogram. Four cohorts of patients ($N = 4$ in each group) were included. The first group of four subjects was a control observational cohort who neither underwent a bone marrow aspirate nor the mesenchymal stromal cells infusions but went through the entire screening and follow-up procedures. This cohort was included based on the US Food and Drug Administration's recommendations. The remaining three cohorts received autologous mesenchymal stromal cells infusions at three different doses as described below. The study was posted on ClinicalTrials.gov under number NCT01659762.

Dosing cohorts

Table 1 describes the baseline demographic and clinical characteristics of all subjects enrolled. The control cohort consisted of four Crohn's disease patients who were followed up for a 12-week period bud did not undergo a bone marrow harvest with mesenchymal stromal cell's *ex vivo* expansion. Cohort 1 consisted of four patients who received low dose of 2 million cells/kg BW, which

Table 1 | Patient characteristics and history of immunosuppressive regimen prior to MSC treatment

Subject	Dose (MSCs/kg body weight)	Age	Sex	Montreal classification	Prior biological/IM therapy
PRC005	2×10^6	18	F	L3, L4	IFX, ADA, MP, MTX
PRC006		20	F	L3	IFX, ADA, MP
PRC007		19	F	L2	IFX, ADA, MP
PRC008		52	M	L1	IFX, ADA, MP, MTX
PRC009	5×10^6	18	M	L3, L4	IFX, ADA
PRC010		22	F	L2	ADA, MP
PCR011		37	M	L1	IFX, ADA, MP, MTX
PCR012		22	M	L1	IFX, ADA, MP
PRC013	10×10^6	18	F	L2, L4	IFX, ADA, MP
PRC014		27	M	L3	IFX, ADA, MP
PRC015		32	M	L3	IFX, ADA, MP
PRC016		25	F	L3	IFX, ADA, MP, CZP, VEDO

IFX, infliximab; ADA, adalimumab; MTX, methotrexate; MP, mercaptopurine; VEDO, vedolizumab; CZP, certolizumab. None of the patients undergone prior surgery.

is the dose that has been used with a multitude of previous mesenchymal stromal cells trials and did not show significant toxicity.[21] Cohort 2 consisted of four patients who received a medium dose of 5 million cells/kg BW and Cohort 3 consisted of four patients who received a high dose of 10 million cells/kg BW. Following the successful enrolment of each noncontrol cohort, a request to the data safety monitoring board was made regarding a decision for dose escalation, maintenance or de-escalation. A minimum of 1 week between the final enrolment of one cohort and initiation of the new cohort was observed prior to the meeting. This was to assess for any dose limiting toxicity prior to enrolment with the next cohort and dose escalation. No significant dose limiting toxicity was noted, therefore, dose escalation was allowed for each noncontrol cohort.

Manufacturing of cellular product

Bone marrow aspirates (ranged from 10 to 75 cc) from the iliac crest were obtained under wakeful anaesthesia. The samples were transferred to a class 10 000 cGMP compliant clean room facility located at Emory University Hospital. Bone marrow mononuclear cells were isolated by Ficoll density gradient centrifugation, washed and then suspended in αMEM culture media containing 10% pooled human platelet lysate as a growth supplement. The mononuclear cells were then placed into a tissue culture flask and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 7 ± 2 days (Passage 0–P0). The adherent cells were detached enzymatically and re-plated at a density of 1000 cells/cm^2 in culture media containing 10% human platelet lysate for another 7 ± 2 days (Passage 1–P1). These cells were collected enzymatically, washed, counted and prepared for patient infusion. For the patient infusion, mesenchymal stromal cells were resuspended at a concentration of 4×10^6 cells/mL in a solution of Plasmalyte A containing 0.05% human serum albumin. These cells were injected into a standard blood transfusion bag and then transported from the manufacturing facility to the infusion centre following release criteria being met. The release criteria on the day of infusion were viability >70%, negative stat gram stain of infusion product, negative bacterial, fungal, endotoxin and mycoplasma test during first replating. Sterility testing of the final product was performed according to 21 CFR 610.12. Table 2 illustrates mesenchymal stromal cells manufacturing variables for each subject, the initial marrow volume and the final products.

Mesenchymal stromal cell infusion to human subjects

The infusion was initiated using a standard blood filter tubing set. The cells were infused via gravity over roughly 60 min, with the infusion rate controlled by the

Table 2 | Manufacturing summary of autologous MSC therapeutics derived from marrow of Crohn's patients

Subject	Dose (MSCs/kg)	Age	Sex	Marrow volume (mL)	Total WBC ($\times 10^6$)	Total MNC Post-Ficoll ($\times 10^6$)	MSC P0 ($\times 10^6$)*	MSCs reseeded ($\times 10^6$)	MSC passage 1 ($\times 10^6$)†	MSCs reseeded	MSC passage 2 ($\times 10^6$)	Doubling time‡ P0–P1 (h)
PRC005	2×10^6	18	F	54	1340	544	16	9	220	N/A	N/A	41.66
PRC006§		20	F	10	43	27	2	2	23	7.5	32	27.26
PRC007		19	F	50	1315	800	10	10	260	N/A	N/A	30.65
PRC008		52	M	63	737	395	10	7	74	10	250	35.29
PRC009	5×10^6	18	M	62.5	1956	1221	220	13	293	N/A	N/A	37.40
PRC010		22	F	55	1824	633	45	14	480	N/A	N/A	32.96
PCR011		37	M	62.5	1178	451	14	14	48	22	575	54.04
PCR012		22	M	75	408	267	54	22	700	N/A	N/A	33.67
PRC013	10×10^6	18	F	65	2135	1074	30	30	962	N/A	N/A	33.60
PRC014		27	M	65	NA	822	57	32	1040	N/A	N/A	38.25
PRC015		32	M	60	1196	715	247	32	1404	N/A	N/A	41.66
PRC016		25	F	60	1049	506	53	53	1550	N/A	N/A	27.26
												36.4 ± 7.36¶

NA, not available; N/A, not Applicable.

* 7 ± 2 (average ± s.d.) days in culture for MNC to P0.

† 6 ± 1 (average ± s.d.) days in culture for P0 to P1.

‡ Expanded MSCs display normal karyotypes in cytogenetic analysis. Sterility of the expanded MSCs was confirmed before infusion.

§ PRC006 marrow volume was low and hence third passage was required to get the yield of 772×10^6.

¶ Average ± s.d. doubling time of 12 MSC populations is given.

investigator. Patients were monitored closely for any changes in respiratory or cardiovascular parameters during the 1 h infusion and for 4 h after the start of the infusion. Vitals including temperature, heart rate, mean arterial pressure and respiratory rate were assessed at 15 min, 30 min, 1 h, 2 h, 3 h and 4 h.

Post-mesenchymal stromal cell Infusion clinical endpoint studies

Follow-up clinic visits after the infusion took place at 1, 5 and 9 weeks after the infusion. Repeat laboratory data were collected including complete blood count, comprehensive metabolic panel, erythrocyte sedimentation rate, C-reactive protein and urinalysis. A complete history and physical exam were performed by one of the physician investigators and adverse events were noted.

Patients were assessed for clinical response by measuring Crohn's disease Activity Index and C-reactive protein in blood prior to the infusion and 2 weeks post-infusion. Clinical safety data were collected up to 9 weeks after the infusion.

Phenotyping of mesenchymal stromal cells by flow cytometry

Mesenchymal stromal cells were subjected to flow cytometry analysis for the expression of CD105PE, CD73PE, CD90APC or CD45PE and appropriate isotype controls (BD Biosciences, St Jose, CA, USA). Histogram analysis for the marker expression was performed with Flow Jo software.

Real-time quantitative IDO1 PCR

Mesenchymal stromal cells were cultured for a minimum of 7 days and activated for 4 h with 10 ng/mL recombinant human interferon-gamma (rhIFN-γ). DNA-free total RNA was extracted and reverse transcribed as described.[22] Real-time qPCR assays were performed in duplicate on an ABI 7500 Fast Real-Time PCR system thermal cycler and SYBR Green Mastermix (Applied Biosystems, Foster City, CA, USA) with human primer sequences for IDO, and actin. Primers were designed using the NCIB/Primer Blast designing tool. Primary CT values were represented as heat map in JMP software.

Mesenchymal stromal cells and T-cell proliferation assay

Blood was obtained from healthy volunteers after informed consent on an investigational review board-approved protocol. Human peripheral blood mononuclear cells (PBMCs) were isolated using a Ficoll density gradient. PBMCs were cultured at 500 000 cells/well in a 24 well-plate with cultured 10% FBS RPMI and were stimulated using anti-CD3/anti-CD28 Dyna beads (Invitrogen Carlsbad, CA, USA) in the presence and absence of mesenchymal stromal cells at the indicated ratios, Co-culture of mesenchymal stromal cells and T cells for proliferation was well described previously and T-cell proliferation was determined 4 days later by flow cytometry analysis of Ki67 expression.[23]

Statistical analysis

In this early phase 1 study, a sample size calculation was not performed. The study was not powered to evaluate efficacy so no statistical analysis was performed.

RESULTS

Study population

A total of 12 patients aged 18–52 (six men) were enrolled into treatment cohorts; four received the low dose (2 million cells/kg BW), four received the intermediate dose (5 million cells/kg BW), and four received the high dose (10 million cells/kg BW). Prior to the dosing of the 12 subjects, there were four additional subjects were enrolled as control and the data on them are not shown. Six of the noncontrol patients were female (50%) and the mean age for the noncontrol patients was 26 years (range, 18–52). Aggregate demographic and clinical characteristics of the each cohort are detailed in Table 1. None of the patients were on concomitant immunosuppressive therapy including steroids at the time of enrolment.

Mesenchymal stromal cells manufacturing

Marrow volume collected ranged from 10 to 75 mL. Individual clinical doses of up to 10 million cells/kg BW were achieved in all subjects as outlined in Table 2. Average mesenchymal stromal cell doubling time was 36 ± 7 h. Cytogenetic analysis on all samples was normal.

Primary clinical endpoint safety

All patients tolerated the mesenchymal stromal cell infusion based on the observed averages of temperature, pulse, respiratory rate and mean arterial pressure during the 1 h infusion process (Figure 1). No patient developed a significant infusion reaction. Of the 12 patients enrolled in the treatment groups, long-term follow-up safety data after mesenchymal stromal cell infusion were

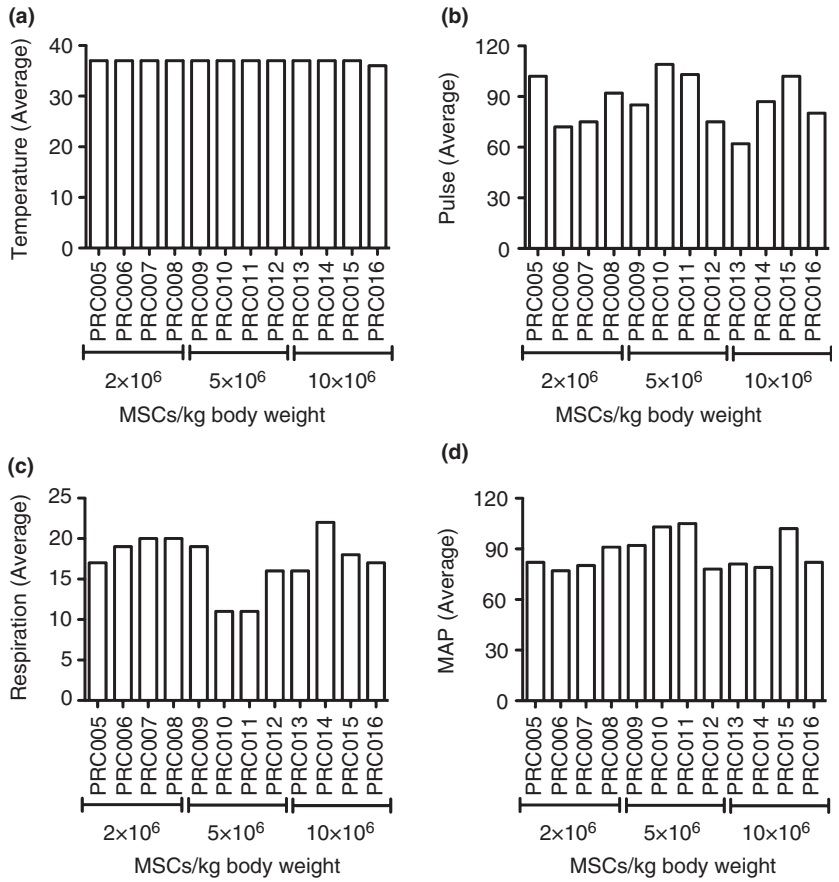

Figure 1 | Haemodynamic parameters of Crohn's disease patients receiving autologous mesenchymal stromal cell product at the time of infusion. Temperature, pulse, respiratory rate and mean arterial presume were measured at time of autologous mesenchymal stromal cell infusion. (a) Average temperature in degrees Celsius, (b) average pulse in beats/min, (c) average respiratory rate in breaths/min, (d) average MAP (mean arterial presume) in mmHg of each patient was plotted in prism software.

available on 11 patients; one patient was lost to follow-up. Safety laboratory values (mean serum creatinine, total bilirubin and aspartate aminotransferase and alanine aminotransferase) were not significantly changed for any of the three dosing groups (data not shown) during follow-up clinic visits. Seven patients developed serious adverse events (Table 3). One patient (2 million cells/kg BW group) developed acute appendicitis and underwent an appendectomy 9 days after the mesenchymal stromal cell infusion and underwent complete colectomy for medically refractory Crohn's disease 120 days after the infusion. One patient (5 million cells/kg BW group) developed *C. difficile* colitis 30 days after the infusion. Remaining five patient's (two in 2 million cells/kg BW group, two in 5 million cells/kg BW group and one in 10 million cells/kg BW group) SAEs were related to hospitalisation due to worsening of CD felt to be unrelated to MSC infusion.

Secondary endpoint of efficacy

Crohn's disease Activity Index values and C-reactive protein levels were measured at screening, day of infusion, and again during the clinic follow-up visits post-infusion. Figure 2a depicts the Crohn's disease Activity Index and C-reactive protein scores available at baseline and at 2 weeks of post-infusion follow-up. As defined by Crohn's disease Activity Index change in 100 as a clinical response, we had 5/11 subjects who showed a clinical response while 6/11 did not. Figure 2b display C-reactive protein levels plotted for each subject over time.

Mesenchymal stromal cell functional characterisation

As outlined in Figure 3a, mesenchymal stromal cells from all subjects deployed a characteristic mesenchymal stromal cell phenotype of $CD73^{+}CD90^{+}CD105^{+}CD45^{-}$. All cultured mesenchymal stromal cells were able to upregulate expression of IDO1 RNA upon interferon-γ

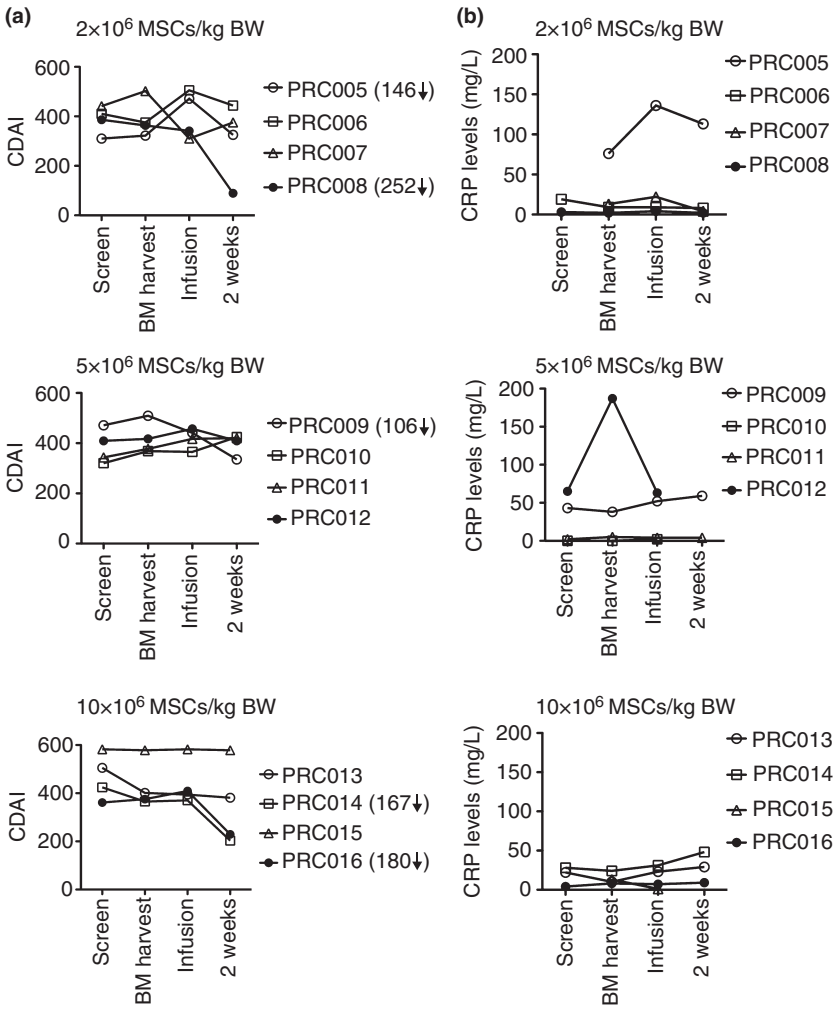

Figure 2 | Crohn's Disease Activity Index and C-reactive protein levels in Crohn's disease patients pre- and post-mesenchymal stromal cell infusion. Single dose of autologous mesenchymal stromal cell was infused into Crohn's disease patients (n = 4/cohort) through peripheral vein. (a) Crohn's Disease Activity Index scores and (b) C-reactive protein (mg/L) levels were measured at the indicated time points pre- and post-mesenchymal stromal cell infusion. Results were plotted in Prism software.

stimulation (Figure 3b) and of samples amenable to analysis we found that mesenchymal stromal cells could suppress proliferation of allogeneic PBMCs *in vitro* (Figure 3c).

DISCUSSION

Enthusiasm over the use of marrow-derived or peripheral blood derived stem cells has been tempered by risk of significant treatment toxicity.[24] The use of mesenchymal stromal cells which also possess immunomodulatory properties but do not require myeloconditioning provides an attractive option over stem cell based therapies. Here, we demonstrated that intravenous administration of a single dose of fresh autologous bone marrow-derived human mesenchymal stromal cells was well tolerated in this phase 1 trial in 12 patients with moderate to severe Crohn's disease.

Table 3 | Serious adverse events possibly related and/or nonrelated to autologous MSC infusion

	N	Possibly related	Not related
Adverse events post-MSC infusion			
1. Appendicitis	1	Yes	
2. *Clostridium difficile* colitis	1	Yes	
3. Hospitalised for disease flare	5	No	Yes

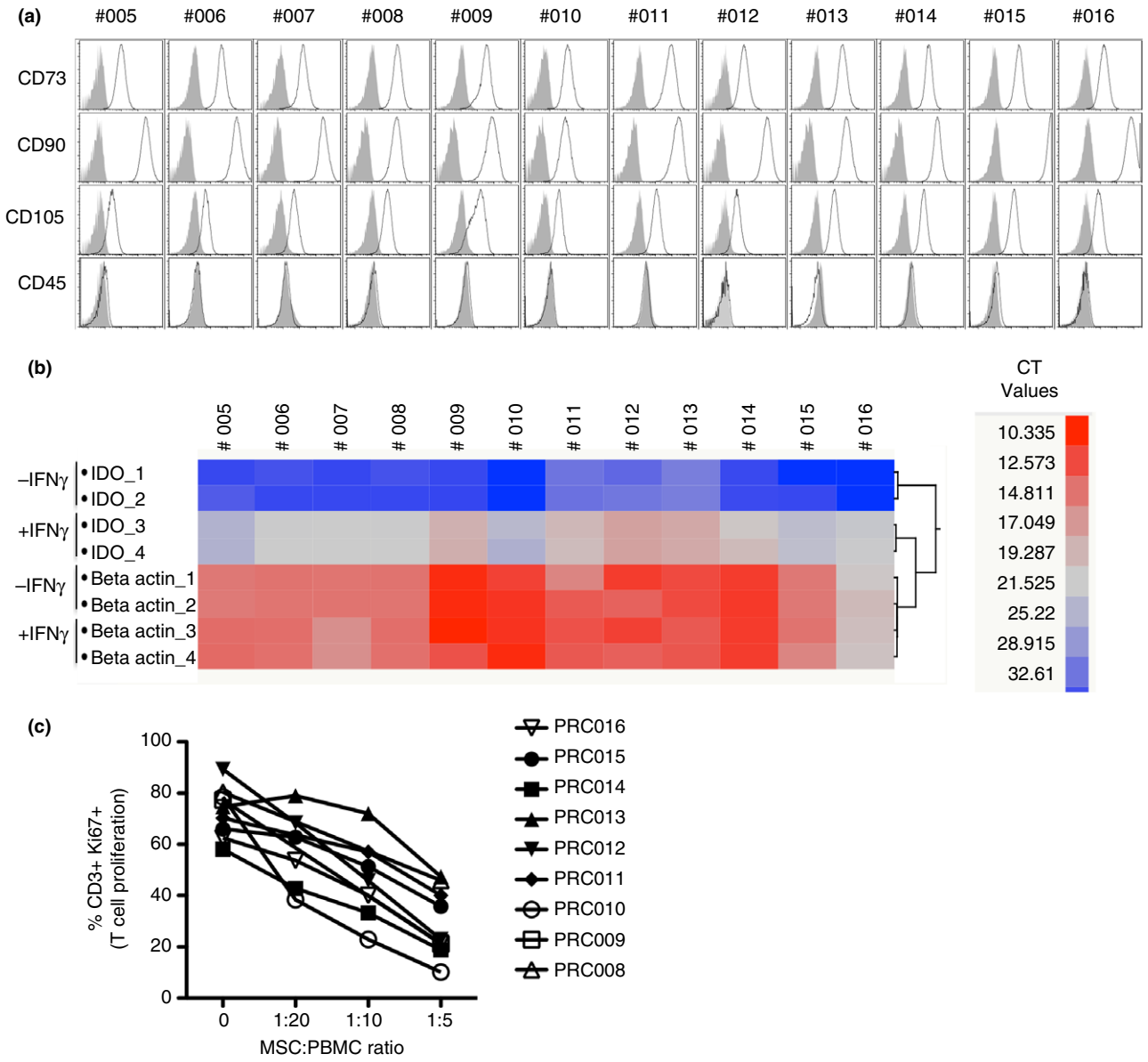

Figure 3 | Phenotype and function of autologous mesenchymal stromal cells derived from Crohn's disease patients at the time of infusion. (a) Autologous mesenchymal stromal cells derived from the bone marrow of Crohn's disease patients were subjected to staining for cell surface markers. Open and grey histograms represent marker and isotype controls respectively. (b) IFNγ responsiveness of autologous mesenchymal stromal cells derived from Crohn's disease patients at the time of infusion. Each mesenchymal stromal cell population was stimulated with IFNγ for 2 h. Expression level of IDO(indoleamine 2,3-dioxygenase) mRNA relative to beta actin was evaluated by quantitative SYBR green real-time PCR. Cumulative heat map was generated in JMP software with the primary CT values obtained from each experiment. (c) Immunosuppressive potential of mesenchymal stromal cells derived from Crohn's disease patients at the time of infusion. Peripheral Blood Mononuclear cells (PBMCs) derived from healthy donors were co-cultured in the presence and absence of each mesenchymal stromal cell population at the indicated ratios and were stimulated with αCD3αCD28 coated Dynabeads 4 days post culture. T-cell proliferation (CD3+ Ki67+) was measured in flow cytometry by Ki67 intracellular staining and results were analysed in flow Jo software.

The majority of studies of mesenchymal stromal cells have supported an overall safety and a few adverse events have been reported. A single occurrence of adenocarcinoma has been reported, however, this may have not been directly related to mesenchymal stromal cell infusion.[13] Studies of mesenchymal stromal cells in

graft-versus-host disease patients have raised the possibility of increased risk of pneumonia, however, an increased risk of infection has not been seen in Crohn's disease patients.[25] Reports of temporary dysgeusia and allergic reactions with the use of dimethylsulphoxide cryopreservant have been reported in mesenchymal stromal cell clinical trials in Crohn's disease.[13] However, our study used noncryopreserved mesenchymal stromal cells and hence no cryoprotectant associated toxicity has been observed.

In our study, two patients developed serious adverse events, which were felt to be possibly related to the mesenchymal stromal cell infusions. Five patients required hospitalisations that were likely due to the moderate to severe nature of their underlying Crohn's disease and not the mesenchymal stromal cell infusions. Of the two patients who had serious adverse events that were possibly related to the mesenchymal stromal cells, one patient had severe Crohn's colitis based on her Crohn's disease Activity Index score and developed appendicitis 9 days after the infusion and underwent an uncomplicated appendectomy. She subsequently required a colectomy 120 days after the infusion due to the medically refractory nature of her disease. The second patient developed *C. difficile* colitis on a background of severe Crohn's colitis 30 days after the mesenchymal stromal cell infusion and required hospitalisation. Both patients had failed immunomodulatory and anti-tumour necrosis factor alpha therapy. While it is plausible, the mesenchymal stromal cells may have caused these events, we feel it is more likely they may have been a manifestation of the patients' underlying severe Crohn's disease given that complications including need for surgery and risk of infections such as *C. difficile* are known to be elevated in moderate to severe Crohn's disease patients, especially in those not responding to medical therapy.

Although this phase 1 study was not powered to test for efficacy, five patients did show a clinical response as assessed by Crohn's disease Activity Index score. However, when correlating the Crohn's disease Activity Index scores with C-reactive protein levels, discordant results were seen with improvements in Crohn's disease Activity Index scores but worsening of C-reactive protein levels in some patients. Such variable results may be a manifestation of the limitations of the Crohn's disease Activity Index score which utilises more subjective assessments rather than objective measurements such as endoscopic scores and faecal calprotectin levels. However, two patients receiving higher doses of mesenchymal stromal cells (one in the 5 million cells/kg BW cohort and one in the 10 million cells/kg BW cohort) showed clinical response based on Crohn's disease Activity Index scores as well as decreases in C-reactive protein levels, which suggests a potential benefit of mesenchymal stromal cells even with a single infusion. We were also unable to study long-term side effects as we followed patients only for 9 weeks post-infusion. However, the relevance of this is unclear when administering only a single infusion. Despite these limitations, we believe that our primary outcome of safety at least in the short term was supported through assessment of objective measures. Further larger scale studies utilising more accurate primary efficacy endpoints are needed to determine effectiveness of this therapy.

Our novel method of mesenchymal stromal cell preparation overcomes many of the pitfalls seen in other mesenchymal stromal cell clinical trials. The majority of studies have utilised allogenic mesenchymal stromal cells, which are felt to be immune privileged. However, it is now well established that allogeneic mesenchymal stromal cells are immune rejected.[16, 26] Our study demonstrates that manufacturing of autologous mesenchymal stromal cells is not only feasible but also safe to administering to Crohn's patients. Second, we utilised actively growing rather than cryopreserved mesenchymal stromal cells to overcome the limitation of thawing induced defect in mesenchymal stromal cell's immunomodulatory and biodistributive properties.[14, 15] Another novel approach of this trial is the utilisation of mesenchymal stromal cells derived from non xenogeneic culture expansion conditions. To date, the majority of cellular therapies involving *ex vivo* manipulation has relied on foetal bovine serum to propagate cells. Spees *et al.*, demonstrated that mesenchymal stromal cells in culture will internalise foetal bovine serum in sufficient quantities that a clinical dosage of cells could potentially carry up to 30 mg of xenoantigens.[27] Consequently, transplanted mesenchymal stromal cells could release these xenoantigens *in vivo* to produce a humoral response and potentially present xenoantigens in the context of MHC class I or class II evoking a cellular response resulting in rejection of transplanted mesenchymal stromal cells. Our preparation of autologous mesenchymal stromal cells grown in a pooled human platelet lysate growth media was designed to address this issue.

In conclusion, a single infusion of fresh autologous bone marrow-derived mesenchymal stromal cells propagated *ex vivo* using a non xenogeneic human platelet lysate growth supplement at doses ranging 2–10 million cell/kg BW was well tolerated in patients with medically

refractory moderate to severe Crohn's disease in this preliminary study. Our data neither addressed long-term safety nor sustained efficacy. However, this study informs that a future phase 2 study with multiple dosing and metabolically fit autologous mesenchymal stromal cell infusions at cell doses up to 10 million cell/kg BW per infusion is likely to be safe and well tolerated and thus may be tested in early stages of the disease process where such cell based therapies are likely to have the greatest benefit to determine if mucosal healing and sustainability of clinical response/remission can be improved.

AUTHORSHIP

Guarantor of the article: Subra Kugathasan.

Author contributions: Subra Kugathasan takes responsibility for the integrity of the work as a whole, from inception to published article. TD and SK recruited the subjects. TD, IBC, MG, KYC, RC, MP and JG have participated in the inception of the idea, conducted the experiments, analysis and writing. JG holds the IND for this trial. All the authors have approved the final version of this manuscript.

ACKNOWLEDGMENTS

Declaration of personal interests: None.

Declaration of funding interests: This trial was supported through a Crohn's and Colitis Foundation Award to SK.

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