

A Phase 2 Study of Allogeneic Mesenchymal Stromal Cells for Luminal Crohn's Disease Refractory to Biologic Therapy

Geoffrey M. Forbes,^{*,‡} Marian J. Sturm,^{§,||} Rupert W. Leong,[¶] Miles P. Sparrow,[#] Dev Segarajasingam,^{**} Adrian G. Cummins,^{‡‡} Michael Phillips,^{§§} and Richard P. Herrmann^{§,||}

^{*}Department of Gastroenterology and Hepatology, [§]Cell and Tissue Therapy Western Australia, Royal Perth Hospital, Perth; [‡]School of Medicine and Pharmacology, ^{||}School of Pathology and Laboratory Medicine, ^{§§}Western Australian Institute for Medical Research, University of Western Australia, Perth; [¶]Department of Gastroenterology, Concord Hospital, Sydney; [#]Department of Gastroenterology, The Alfred Hospital, Melbourne; ^{**}Department of Gastroenterology, Sir Charles Gairdner Hospital, Perth; and ^{‡‡}Department of Gastroenterology, The Queen Elizabeth Hospital, Adelaide, Australia

BACKGROUND & AIMS: Transplantation of peripheral blood stem cells has been successful therapy for small numbers of patients with Crohn's disease (CD), but requires prior myeloconditioning. Mesenchymal stromal cells (MSCs) escape immune recognition, so myeloconditioning is not required before their administration. We investigated the efficacy of allogeneic MSCs in patients with luminal CD.

METHODS: Our phase 2, open-label, multicenter study included 16 patients (21–55 y old; 6 men) with infliximab- or adalimumab-refractory, endoscopically confirmed, active luminal CD (CD activity index [CDAI], >250). Subjects were given intravenous infusions of allogeneic MSCs (2×10^6 cells/kg body weight) weekly for 4 weeks. The primary end point was clinical response (decrease in CDAI >100 points) 42 days after the first MSC administration; secondary end points were clinical remission (CDAI, <150), endoscopic improvement (a CD endoscopic index of severity [CDEIS] value, <3 or a decrease by >5), quality of life, level of C-reactive protein, and safety.

RESULTS: Among the 15 patients who completed the study, the mean CDAI score was reduced from 370 (median, 327; range, 256–603) to 203 (median, 129) at day 42 ($P < .0001$). The mean CDAI scores decreased after each MSC infusion (370 before administration, 269 on day 7, 240 on day 14, 209 on day 21, 182 on day 28, and 203 on day 42). Twelve patients had a clinical response (80%; 95% confidence interval, 72%–88%; mean reduction in CDAI, 211; range 102–367), 8 had clinical remission (53%; range, 43%–64%; mean CDAI at day 42, 94; range, 44–130). Seven patients had endoscopic improvement (47%), for whom the mean CDEIS scores decreased from 21.5 (range, 3.3–33) to 11.0 (range, 0.3–18.5). One patient had a serious adverse event (2 dysplasia-associated lesions), but this probably was not caused by MSCs.

CONCLUSIONS: In a phase 2 study, administration of allogeneic MSCs reduced CDAI and CDEIS scores in patients with luminal CD refractory to biologic therapy. ClinicalTrials.gov number, NCT01090817.

Keywords: Cellular Therapy; Intestine; Inflammation; Immune Response; Autoimmune.

Podcast interview: www.gastro.org/cghpodcast. Also available on iTunes.

Despite the advent of biologic therapy for Crohn's disease (CD), about one quarter of patients still need major abdominal surgery within 5 years after their diagnosis.¹ To avoid surgery, cellular therapy by bone marrow or peripheral blood stem cell transplantation, either allogeneic or autologous, has been used successfully in small numbers of patients, but requires prior myeloablative therapy or hematopoietic stem cell mobilization.²

By contrast, mesenchymal stromal cells (MSCs) are multipotent adult stem cells that are considered to lack

immunogenicity and hence escape immune recognition; they have low level HLA class I expression, lack HLA class II antigen, and do not express co-stimulatory molecules. Accordingly, in allogeneic administration, donor to recipient matching is not required, nor chemotherapeutic marrow conditioning.³

Abbreviations used in this paper: AQL, assessment of quality of life; CD, Crohn's disease; CDAI, Crohn's disease activity index; CDEIS, Crohn's disease endoscopic index of severity; CMV, cytomegalovirus; CRP, C-reactive protein; GVHD, graft-versus-host disease; IBDQ, inflammatory bowel disease questionnaire; MSC, mesenchymal stromal cell.

The immunomodulatory properties of MSC have been applied successfully to steroid-refractory graft-versus-host disease (GVHD)^{4,5} and are under evaluation in other diseases.⁶ Locally administered allogeneic or autologous MSCs were effective in small numbers of patients with perianal fistulous CD^{7,8}; intravenous autologous MSCs were safe when used in biologic-refractory luminal CD.⁹ Intravenous allogeneic MSCs led to clinical response at 14 days in 3 of 9 patients with biologic-refractory CD.¹⁰ In determining whether allogeneic or autologous MSCs should undergo evaluation, we took into account international and local evidence of the efficacy of allogeneic MSCs in GVHD, the 6-week time-frame required to manufacture autologous cells, and an uncertain effect of immunosuppressant therapy on in vivo autologous MSCs.

Accordingly, our hypothesis was that allogeneic MSCs would lead to clinical improvement of medically refractory luminal CD. The aims of this study were to establish the efficacy and safety of allogeneic MSCs in luminal CD.

Methods

Study Overview

This phase II study was an open-label, multicenter, Australian, nonrandomized evaluation of subjects with active luminal CD (CD activity index [CAI], >250) who had failed anti-tumor necrosis factor therapy. Subjects received 4 MSC infusions (2×10^6 cell/kg body weight) each 1 week apart (Figure 1). The primary outcome measure was clinical response (CAI reduction, >100 points) at day 42; secondary outcome measures at day 42 were clinical remission (CAI, <150), endoscopic improvement (CD endoscopic index of severity [CDEIS]¹¹ decrease of >5, or CDEIS <3), improved quality of life (inflammatory bowel disease questionnaire [IBDQ]¹² and assessment of quality of life [AQoL]¹³ scores), normalization of C-reactive protein (CRP) level, and safety.

Eligibility Criteria

The following inclusion criteria were applied: colonic or small-bowel CD (based on endoscopy and histology); CDAI of more than 250; endoscopically active disease;

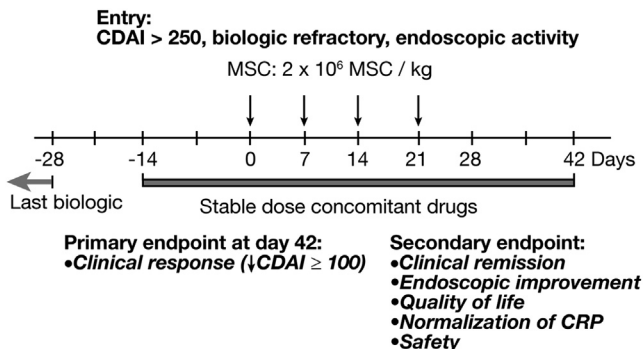


Figure 1. Study overview.

disease refractory to induction with infliximab or adalimumab, or loss of response to both, or side effects to either or both of these drugs precluding further use. Failure to induce remission was defined as failure of clinical remission 4 weeks after a minimum of 3 doses of infliximab (5 mg/kg) at 0, 2, and 6 weeks; or 3 doses of adalimumab (160 mg at week 0, 80 mg at week 2, 40 mg at week 4).

The following exclusion criteria were applied: chronic stricturing disease without inflammation; co-existent bacterial enterocolitis or cytomegalovirus (CMV) infection; prior malignancy; pregnancy or unwilling to use birth control during therapy; breastfeeding; stoma (CDAI not measurable); or active sepsis, including perianal sepsis or perforating disease.

Regarding concomitant drug therapy during the study, we established the following rules: last biologic (infliximab or adalimumab) 4 or more weeks previously; and between time = -14 days and day 42, stable doses of corticosteroid (within 10 mg prednisolone variation), immunomodulator (azathioprine, 6-mercaptopurine, methotrexate), or antidiarrheal.

Investigational Treatment and Study Conduct

Subjects received MSC weekly for 4 weeks (t = 0, 7, 14, and 21 d); study visits were at these time points, and at days 28 and 42. Thawed MSCs (volume, 40–80 mL) were administered over 15 to 20 minutes through a peripheral intravenous cannula without a leukocyte filter.

In respect to determining the relationship of adverse events with study therapy, possible adverse events were discussed between chief investigators. Further, interim safety reports generated after every 5 patients were reviewed by an independent committee, comprising 2 independent clinicians, and an ethics committee chairperson. Institutional ethics committee approval was obtained from all participating centers, and signed informed consent was obtained from participating subjects.

Mesenchymal Stromal Cell Preparation

Bone marrow-derived MSCs were manufactured in a government-funded facility within Royal Perth Hospital; the facility is licensed by the Australian regulator, the Therapeutic Goods Administration. Bone marrow was obtained from donors meeting strict selection criteria, including formal medical assessment, age younger than 30 years, and negative testing for mandatory infectious disease markers. There is a local policy for CMV-negative recipients to receive MSCs from CMV-negative donors.

As previously described,⁵ cells were isolated from mononuclear cell preparations by adherence to plastic, then culture expanded in Dulbecco's modified Eagle medium (Life Technologies, Mulgrave, Australia), supplemented with 10% fetal bovine serum (SAFC Bio-Sciences, Melbourne, Australia), which was removed in the final stages of preparation. Cells were harvested at

80% confluence and passaged to passage 5. Cell doses were cryopreserved in 10% dimethylsulfoxide (WAK-Chemie, Steinbach, Germany), 50% PlasmaLyte (Baxter Health Care, Sydney, Australia), 20% sodium chloride, and 20% human serum albumin (Commonwealth Serum Laboratories, Melbourne, Australia), and stored at less than -150°C . Three unrelated donors provided marrow for manufacturing MSCs; no individual patient received MSCs from more than 1 donor.

Before release for therapy, MSC products were required to have cell viability greater than 70%, negative microbial contamination testing, and show MSC characteristics according to the International Society for Cellular Therapy.¹⁴ Cytogenetic abnormalities were excluded by studies at passage 5.

Dose Determination

Local experience in treating GVHD had been with a dose of 1 to 2 (median, 1.7×10^6 cell/kg body weight, administered as 2 doses weekly for 2 weeks, and retreatment if there was no response.⁵ Internationally, 1.4×10^6 cell/kg had been used for GVHD.⁴ For CD, in a study of autologous MSCs, 2 infusions of 1 to 2×10^6 cell/kg a week apart were used.⁹ In an allogeneic MSC study, patients received 2 infusions of either 2×10^6 cell/kg ($n = 4$) or 8×10^6 cell/kg ($n = 5$).¹⁰

Accordingly, we determined that our study would use 4 doses, each a week apart, of a minimum of 2×10^6 cell/kg. Our MSCs were prepared and stored in aliquots of 50×10^6 cells; accordingly, patients were to receive 2 to 2.7×10^6 cell/kg per infusion.

Statistical Methods

Data are described using proportions and mean or median scores together with the 95% confidence intervals as appropriate to the variable and its distribution. The primary outcome measure was the change over time in CDAI. Because there was no control group for comparison, a longitudinal random-effects regression was an efficient and appropriate method of analysis. The regression analysis used maximum likelihood estimation with time as the single predictor. The assumption of linearity was assessed using restricted cubic splines. For the outcome measures of IBDQ and AQoL, the related samples nonparametric Wilcoxon signed-rank test was used.

A power analysis based on a longitudinal random-effects model indicated that a sample size of 20 subjects was sufficient to show a statistically significant reduction in the CDAI of 100 points in 20% of patients (critical $\alpha = .05$) with power in excess of 80%. The power analysis incorporated a penalty for 2 interim analyses. We determined that if at least 1 of the first 10 patients had a clinical response at the primary study end point, then recruitment would continue until 30 patients

had been recruited. However, the second interim analysis led to the decision to report outcome data after the accrual of 16 patients.

Results

Demographics

This study included 16 patients, aged 21 to 55 years (7 men), with a mean CDAI of 371 (range, 256–603). Thirteen patients had Crohn's colitis, 2 patients had ileocolitis, and 1 patient had ileal disease alone (Table 1). Three patients had primary failure of a single biologic; the remainder failed both infliximab and adalimumab. The most recently administered biologic was between 4 and 8 weeks before MSC administration in 11 patients (adalimumab, $n = 10$; infliximab, $n = 1$), and between 8 weeks and 12 months in 5 patients (adalimumab, $n = 3$; infliximab, $n = 2$). Fourteen of 16 patients took other immunosuppressive therapy (corticosteroid or immunomodulator) during the study, which was within protocol.

Protocol Deviations or Variations

Protocol deviations or variations occurred in 4 instances, and adversely impacted outcome measures with certainty in 2 patients (patients 11 and 14), and possibly in 1 patient (patient 6).

In patient 11, with a past history of a known proximal rectal stricture with mucosal atypia and recommendation for surgery, low-grade dysplasia was found on a mucosal biopsy specimen before study entry. However, this was not brought to the attention of the chief investigators until after completion of the study, at which time a stage I colorectal cancer was diagnosed. This is detailed in the Safety section later.

Patient 14 attended a nonstudy hospital after receiving 4 MSC infusions, and received infliximab and further follow-up evaluation at the nonstudy hospital. Although the CDAI decreased from 397 to 202 after 3 of 4 MSC infusions, no outcome data are available after this, and the patient was withdrawn from analysis for all outcome variables except safety.

Patient 6 used herbal preparations containing slippery elm and olive leaf extract on day 21, without investigator endorsement, after receiving 4 MSC infusions. Although data are lacking on this preparation in CD, an effect on outcome data cannot be discounted.

In patient 5 we could not discount the possibility that MSCs led to transient worsening of a pre-existing increased serum alanine aminotransferase level, leading to withholding 1 of 4 MSC infusions. This is detailed further in the Safety section later.

With the exception of patient 14, all patients with protocol deviations had outcome data analyzed on an intention-to-treat basis.

Table 1. Patient Demographics and Details

Patient number	Age, y	Sex	Distribution of disease	Entry CDAI	Prior biologic/immunomodulator therapy	Prior surgery	Anti-TNF before MSC (weeks before last dose) primary failure ^a	Concomitant drug therapy during study ^b	Total MSC dose
1	25	F	Colitis	414	Infliximab, adalimumab, azathioprine	Perianal abscess drainage/insertion of seton	Adalimumab 4 wk		9.4×10^6 /kg
2	55	M	Colitis	524	Infliximab, adalimumab, azathioprine	Nil	Adalimumab 4 wk	Azathioprine, prednisolone 10 mg	8.2×10^6 /kg
3	31	F	Colitis	269	Infliximab, adalimumab, azathioprine	Subtotal colectomy	Adalimumab 8 wk	Prednisolone 12.5–15 mg, ^b prednisolone enema	9.8×10^6 /kg
4	32	F	Colitis	319	Infliximab, adalimumab, azathioprine	Nil	Adalimumab 4 wk ^a	Azathioprine	9.8×10^6 /kg
5	23	F	Colitis	325	Infliximab, adalimumab, azathioprine	Nil	Adalimumab 4 wk	Prednisolone 20 mg	7.1×10^6 /kg
6	39	F	Colitis	603	Infliximab, adalimumab, azathioprine	Perianal abscess drainage/insertion of seton	Adalimumab 6 mo	Mycophenolate mofetil, ciprofloxacin	8.0×10^6 /kg
7	21	M	Colitis	366	Infliximab, 6-MP	Nil	Infliximab 4 wk ^a	6-MP, prednisolone 25–15 mg ^b	8.6×10^6 /kg
8	27	F	Colitis	344	Infliximab, adalimumab, azathioprine	Perianal abscess drainage/insertion of seton	Adalimumab 3 mo	Azathioprine, prednisolone 15 mg	8.3×10^6 /kg
9	52	M	Colitis	256	Infliximab, adalimumab, azathioprine	Perianal abscess drainage	Adalimumab 4 wk	6-MP	8.0×10^6 /kg
10	39	F	Colitis + perianal	498	Infliximab, adalimumab, azathioprine, methotrexate	Nil	Infliximab 12 mo	Azathioprine, prednisolone 35 mg	9.3×10^6 /kg
11	31	M	Colitis + perianal	295	Infliximab, adalimumab, 6-MP	Nil	Infliximab 4 mo	6-MP, prednisolone 35 mg	10.1×10^6 /kg
12	41	M	Ileocolitis	285	Infliximab, adalimumab, azathioprine	Ileocolonic resection	Adalimumab 4 wk	Azathioprine	8.6×10^6 /kg
13	46	F	Ileocolitis	327	Infliximab, adalimumab, azathioprine	Nil	Adalimumab 10 wk	Prednisolone 20–15 mg ^b	9.0×10^6 /kg
14	35	M	Colitis + perianal	397	Infliximab, adalimumab, azathioprine, methotrexate	Perianal abscess drainage/insertion of seton and temporary ileostomy	Adalimumab 6 wk	Methotrexate, prednisolone 10 mg	9.4×10^6 /kg
15	23	M	Colitis	259	Infliximab, adalimumab, azathioprine	Nil	Adalimumab 4 wk	Prednisolone 10 mg	9.7×10^6 /kg
16	52	F	Ileal	460	Adalimumab, azathioprine, 6-MP	Ileocolonic/ileal resections $\times 3$	Adalimumab 4 wk ^a	Loperamide	8.8×10^6 /kg

F, female; M, male; 6-MP, 6-mercaptopurine; TNF, tumor necrosis factor.

^aPrimary failure of a single biologic; all other patients failed both biologics.

^bWhen variation of the prednisolone dose is given, the initial dose figure represents the study entry dose, and the last figure represents the study end point dose.

Table 2. CDAI and Endoscopic Outcome Data

Patient number	CDAI entry	CDAI day 7	CDAI day 14	CDAI day 21	CDAI day 28	CDAI day 42	Clinical response	Clinical remission	CDEIS entry	CDEIS day 42	Endoscopic improvement
1	414	315	330	329	324	466	N	N	9.75	17.5	N
2	524	504	392	320	338	422	Y	N	19	18.5	N
3	269	160	143	-	147	122	Y	Y	19.5	19.5	N
4	319	149	186	201	193	130	Y	Y	23	18.5	Y
5	325	76	180	108	88	44	Y	Y	17	9.5	Y
6	603	362	437	169	160	236	Y	N	26	25.3	N
7	366	189	113	68	107	114	Y	Y	27.5	17.5	Y
8	344	382	223	256	177	85	Y	Y	3.3	0.3	Y
9	256	182	159	80	46	81	Y	Y	19	9.75	Y
10	498	338	256	281	135	275	Y	N	32.5	31	N
11	295	245	234	222	175	180	Y	N	28	8.6	Y
12	285	210	227	169	169	128	Y	Y	33	13	Y
13	327	97	8	20	0	50	Y	Y	6.1	10.2	N
14	397	389	291	202	-	-	-	-	18	-	-
15	259	277	276	244	236	282	N	N	31.5	32	N
16	460	454	390	472	436	436	N	N	9.1	6.25	N

Outcome Evaluations

Clinical response: decrease in Crohn's disease activity index by more than 100 points. The mean CDAI ($n = 15$) decreased from 370 (median, 327; range, 256–603) at entry to 203 (median, 129; range, 44–466) at day 42 ($P < .0001$) (Table 2). Clinical response occurred in 12 (80%, 95% confidence interval, 72%–88%) of 15 patients (mean CDAI reduction, 211; range, 102–367) (Figure 2). The mean CDAI decreased after each MSC infusion (day 0, 371; day 7, 269; day 14, 240; day 21, 209; day 28, 182; and day 42, 203) (Figure 3).

Clinical remission: decrease in Crohn's disease activity index to less than 150. Clinical remission occurred in 8 (53%, 95% confidence interval, 43%–64%) (mean CDAI at day 42, 94; range, 44–130) of 15 patients at day 42.

Endoscopic improvement: a Crohn's disease endoscopic index of severity decrease of more than 5, or a Crohn's

disease endoscopic index of severity of less than 3. Endoscopic improvement occurred in 7 (47%, 95% confidence interval, 36%–57%) (mean CDEIS, 21.5 [range, 3.3–33] to 11.0 [range, 0.3–18.5]) of 15 patients at day 42.

Quality of life. Improved quality of life is reflected by an increase in the 32-item IBDQ score, or a decrease in the 35-item AqoL-8D score. In 14 evaluable patients, mean IBDQ scores improved from 119 to 150 ($P = .014$), and mean AqoL scores improved from 81 to 70 ($P = .013$) (Table 3). For patients with a clinical response ($n = 11$), IBDQ scores improved from 118 to 158 and the AqoL improved from 80 to 63; in patients with clinical remission and endoscopic improvement ($n = 7$) the IBDQ scores improved from 125 to 163 and the AqoL improved from 82 to 60.

C-reactive protein. CRP data were complete in 13 of 16 patients. Seven patients had a study entry CRP level greater than 10, which became normal in 2 patients.

Safety. One serious adverse event occurred (Table 4). Patient 11 had 2 dysplasia-associated lesions or masses diagnosed in the rectosigmoid at the day 42 endoscopy, and subsequent surgery found a stage I well-differentiated sigmoid adenocarcinoma. This occurred in the aftermath of 2 events that the chief investigators had not been made aware of until study completion: (1) low-grade dysplasia on sigmoid mucosal biopsy specimen at entry endoscopy, and (2) previous recommendation for colectomy in light of mucosal atypia within proximal rectal stricturing 12 months previously. It is the view of the chief investigators that it is likely that cancer was present at the index endoscopy 8 weeks before the day 42 examination but was not appreciated endoscopically in the presence of active colitis and that this patient should not have been permitted to enter the study, but the possibility of MSC contributing to progression of dysplasia to cancer cannot be entirely discounted.

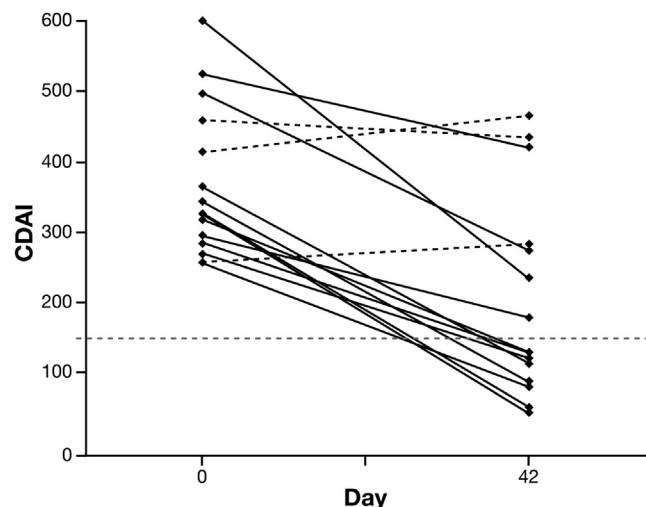


Figure 2. CDAI score of 15 subjects at study entry and at day 42. Broken lines indicate patients without clinical response.

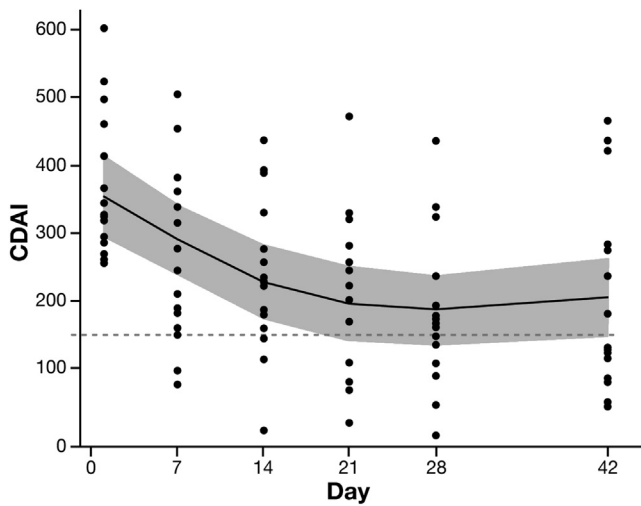


Figure 3. Mean CDAI by study visit (n = 15). Restricted cubic spline mean regression line with 95% confidence intervals (shaded area). Data points for all patients are shown.

All patients experienced dysgeusia, or distortion of taste, which is known to occur from the cryopreserving agent dimethylsulfoxide, which resolved within 36 hours of infusion in all patients.

Other adverse events, possibly related to study treatment, were as follows. Three patients noticed a low-grade, self limiting headache for up to 2 hours, after receiving MSCs on 2 occasions (n = 1) or 1 occasion (n = 2). Two patients noticed mild nausea at the time of headache on one occasion after receiving MSCs. One patient developed a 3-day, self-limiting coryzal illness between the first 2 MSC infusions, which did not lead to a protocol change. Vaginal candidiasis was noted by patient 13 at day 8 of the study. Patient 15 developed a self-limiting, 5-day episode of culture-negative, presumed viral gastroenteritis, within 2 days of a similar illness in 2 close relatives, occurring between days 9 and 13. There were no other infections. One patient with previous viral

hepatitis B and 1 patient with previous genital herpes had no recrudescence of disease after MSC therapy.

One patient with pre-existing lymphopenia had a further decrease in lymphocyte count, with a return to prestudy lymphocyte count after the study. Two patients developed mild lymphopenia; in 1 patient it spontaneously resolved within the 6-week study period. Three patients had normalization of pre-existing mild lymphopenia during the study. These changes were not considered directly attributable to MSC therapy. No instances of neutropenia or thrombocytopenia occurred.

One patient with pre-existing increased alanine aminotransferase levels had a further enzyme increase to 10 times normal during the study. Although MSC administration did not cause abnormal liver enzyme levels, at the time we were unable to discount the possibility that MSCs led to transient worsening of the previously increased enzyme level. Subsequent investigations have led to a diagnosis of primary sclerosing cholangitis, and it is considered that variation in liver enzyme levels was not related directly to MSC. A further 3 patients had transient increased alanine aminotransferase levels to less than twice normal, and subsequent normalization. These changes were not considered directly attributable to MSC.

No infusion reactions occurred.

Discussion

This phase II study suggests efficacy of intravenous allogeneic MSCs in luminal CD. In 15 patients with moderate to severe active disease, refractory to anti-tumor necrosis factor therapy, 4 infusions of 2×10^6 cell/kg at weekly intervals led to clinical response in 12 patients (80%), clinical remission in 8 patients (53%), and endoscopic improvement in 7 patients (47%). Quality of life improved, in parallel with improvement in CDAI.¹²

Table 3. Quality of Life and CRP Outcome Data

Patient number	IBDQ entry	IBDQ day 42	AQoL entry	AQoL day 42	CRP entry	CRP day 42
1	136	115	87	95	23	45
2	111	148	73	67	4.1	3.1
3	151	138	73	67	48	Not measured
4	104	160	85	64	9.5	5.5
5	107	184	98	58	6.6	4.4
6	85	143	79	79	21	16
7	136	207	67	41	13	2.9
8	99	161	90	72	2.9	5
9	150	115	58	43	20	1.9
10	103	148	73	61	36.9	36
11	129	162	85	67	3.1	1.4
12	125	176	100	77	13	18
13	91	Not available	80	Not available	6	Not available
14	88	Not measured	117	Not measured	38	Not measured
15	142	152	76	81	30	13
16	93	94	96	104	2.3	6.1

Table 4. Adverse Events

	n
Serious adverse events	
Adenocarcinoma arising in a dysplasia-associated lesion or mass	1
Adverse event definitely related to study treatment	
Dysgeusia	16
Adverse events possibly related to study treatment	
Headache	3
Infection: self-limiting presumed viral/vaginal candidiasis	2/1
Nausea	1
Lymphopenia	3
Normalization of pre-existing lymphopenia	3
Increased alanine aminotransferase level	3

Locally injected allogeneic MSCs are effective in perianal fistulous CD,⁷ and our data show effectiveness for luminal disease in a convincing way.

The transplantation of marrow-derived, or peripheral blood-derived, stem cells requires cytotoxic myeloconditioning, and appears only in a minority of patients to confer long-term remission of CD, and with significant treatment toxicity.^{15,16} MSCs are progenitor cells with immunologic properties that allow allogeneic administration without tissue-matching or myeloconditioning.³ An anti-inflammatory effect is believed to occur in association with an ability to home to sites of inflammation.¹⁷

Rigorous safety evaluations of new therapies are essential. For MSCs, clinical risk data largely stem from studies in steroid-refractory GVHD. Other than the possibility of an increased short-term risk of pneumonia in these highly immunocompromised patients, MSCs have been considered safe.¹⁸ There are no reports of increased risk from other infections or tumors, despite concerns based on theoretical mechanistic grounds.¹⁹ No significant adverse effects have been reported from CD studies of locally administered allogeneic MSCs,⁷ intravenous autologous MSCs,⁹ or intravenous allogeneic MSCs.²⁰

We describe the occurrence of a stage I adenocarcinoma within a dysplasia-associated lesion or mass diagnosed 3 weeks after receiving 4 infusions of MSCs. Although we cannot discount the possibility that MSCs contributed to this event, we believe that the cancer likely was present at index endoscopy; furthermore, that the patient would not have been permitted to enter the study had investigators been aware of the patient's background of colonic mucosal dysplasia and stricturing CD. No other serious adverse events occurred. Cryopreserved cells are stored in dimethylsulfoxide, which is required for cellular stability on thawing. It leads to an altered taste sensation for 24 hours until excretion is complete; patients were warned that this was likely to occur.

The immunopathogenetic mechanism of MSCs in treating CD is speculative, and is based on an

understanding of MSC function in animal models, in vitro, and in patients with GVHD.^{3,6,17} In CD there is an imbalance in T-cell subsets, including relative inactivity of anti-inflammatory regulatory T-cell function, compared with proinflammatory Th1 and Th17 cells.²¹ MSCs are known to have inhibitory action on various cell lines including effector and cytotoxic T cells, B cells, natural killer cells, dendritic cells, and macrophages. By contrast, a facilitatory effect on regulatory T cells by MSCs might help to re-establish the cellular milieu for disease quiescence.¹⁷

However, there is much to learn and understand about MSC function. In part, this relates to how an MSC is defined and differences between MSC preparations used in clinical trials. Relatively tight international guidelines require MSCs to be plastic-adherent in culture, express specific cell surface markers and lack others, and retain the capability for trilineage differentiation in vitro.¹⁴ Beyond these requirements, a variety of differences in MSC function may exist owing to, for example, variations in healthy donor immune function, and the number of cell doses obtained from a single donor.²² To minimize these risks, our donors typically are younger than age 30 years and have no concurrent medical illness; cells are cultured to no more than 5 cycles. Further, functional differences in MSCs used in our study compared with MSCs from other facilities may relate to our policy to use CMV-negative donors, or our laboratory practice to remove fetal bovine serum in the final stages of MSC preparation. A high prevalence of antibovine antibodies otherwise might lead to deleterious in vivo effects on fetal bovine serum-cultured MSC function.²²

Beyond possible functional differences in MSCs prepared in different laboratories and from different donors, it is reasonable to speculate that in vivo differences exist between autologous and allogeneic MSCs. For CD patients in whom their disease is predicated by immune dysregulation, autologous MSCs might be less effective than allogeneic MSCs. Conversely, although MSCs are considered to lack immunogenicity because of unique HLA expression, it is conceivable that donor-specific anti-HLA antibodies might develop with repeated infusions, particularly in CD patients, in contrast to the highly immunocompromised patient with GVHD.

We recognize the limitations of an unblinded non-comparator study of a novel therapy. In establishing this study, we considered it appropriate that preliminary efficacy and safety data should arise from such a phase II evaluation; furthermore, that it be undertaken initially in medically refractory disease. Despite the limitations of this study design, we believe the validity of our conclusions are supported by the positive results from the primary outcome, and various subjective and objective secondary outcome measures. In addition, although placebo response rates may be significant in CD studies, it can be argued that the placebo response in CD patients with biologic refractory moderate-to-severe disease is very low.

Our data do not address long-term efficacy in this clinical setting, or allow for a determination of efficacy in earlier-stage disease for inducing or maintaining disease remission. Should subsequent evaluations in these settings show effectiveness and relative safety compared with current therapies, dose ranging or dose interval evaluations also are required. Further, our safety data should be considered preliminary because we cannot yet provide long-term information on this small number of patients. Mindful of these limitations, allogeneic MSCs may represent a significant therapeutic alternative or advance in treating luminal CD.

References

- Bouguen G, Peyrin-Biroulet L. Surgery for adult Crohn's disease: what is the actual risk? *Gut* 2011;60:1178–1181.
- Hommes DW, Duijvestein M, Zelinkova Z, et al. Long-term follow-up of autologous hematopoietic stem cell transplantation for severe refractory Crohn's disease. *J Crohns Colitis* 2011;5:543–549.
- Le Blanc K, Tammik C, Rosendahl K, et al. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003;31:890–896.
- Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008;371:1579–1586.
- Herrmann R, Sturm M, Shaw K, et al. Mesenchymal stromal cell therapy for steroid-refractory acute and chronic graft versus host disease: a phase 1 study. *Int J Hematol* 2012;95:182–188.
- Figueroa FE, Carrion F, Villanueva S, et al. Mesenchymal stem cell treatment for autoimmune diseases: a critical review. *Biol Res* 2012;45:269–277.
- Garcia-Olmo D, Herreros D, Pascual I, et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum* 2009;52:79–86.
- Ciccocioppo R, Bernardo ME, Sgarella A, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut* 2011;60:788–798.
- Duijvestein M, Vos AC, Roelofs H, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut* 2010;59:1662–1669.
- Onken J, Gallup D, Hanson J, et al. Successful outpatient treatment of refractory Crohn's disease using adult mesenchymal stem cells. Available at: http://www.osiris.com/pdf/Crohn's_Ph_II_Handout.pdf. Accessed February 10, 2013.
- Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease. Group d'Etudes Therapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). *Gut* 1989;30:983–989.
- Irvine EJ, Feagan B, Rockon J, et al. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. *Gastroenterology* 1994;106:287–296.
- AQoL. Assessment of Quality of Life. Available at: www.aqol.com.au. Accessed February 10, 2013.
- Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315–317.
- Burt RK, Craig RM, Milanetti F, et al. Autologous non-myeloablative hematopoietic stem cell transplantation in patients with severe anti-TNF refractory Crohn's disease: long-term follow-up. *Blood* 2010;116:6123–6132.
- Hawkey C, Allez M, Ardizzone S, et al. Clinical and endoscopic improvement following hemopoietic stem cell transplantation in the ASTIC trial. *J Crohn's Colitis* 2013;7(Suppl 1):S4.
- Newman RE, Yoo D, Le Roux MA, et al. Treatment of inflammatory diseases with mesenchymal stem cells. *Inflamm Allergy Drug Targets* 2009;8:110–123.
- Forslow U, Blennow O, Le Blanc K, et al. Treatment with mesenchymal stromal cells is a risk factor for pneumonia-related death after allogeneic haematopoietic stem cell transplantation. *Eur J Haematol* 2012;89:220–227.
- Mishra PJ, Mishra PJ, Glod JW, et al. Mesenchymal stem cells: flip side of the coin. *Cancer Res* 2009;69:1255–1256.
- Onken J, Jaffe T, Custer L. Long-term safety of prochymal adult mesenchymal stem cells in Crohn's disease. *Gastroenterology* 2008;134:A661.
- Brand S. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut* 2009;58:1152–1167.
- Galipeau J. The mesenchymal stromal cell dilemma—does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? *Cytotherapy* 2013;15:2–8.

Reprint requests

Address requests for reprints to: Geoffrey M. Forbes, MD, Department of Gastroenterology and Hepatology, Royal Perth Hospital, Box X2213 GPO Perth, Western Australia 6847, Australia. e-mail: geoff.forbes@health.wa.gov.au; fax: (61) 8-92241329.

Acknowledgments

The authors acknowledge and thank the following: Janina Pawlik, Janice Fogarty, Kath Shaw, Lisa Kaminskis, Adrian Pannekoek, and the staff of the Department of Haematology at The Alfred Hospital.

Conflicts of interest

The authors disclose no conflicts.

Funding

Supported by the Broad Medical Research Program of The Broad Foundation, and Therapeutic Innovation Australia (formerly Research Infrastructure Support Services, Inc).