Original Article

jfms Journal of Feline Medicine and Surgery

Journal of Feline Medicine and Surgery
2015, Vol. 17(10) 901–908

DOI: 10.1177/1098612X14561105
jfms.com
SAGE

Stem cell therapy in cats with chronic enteropathy: a proof-of-concept study

Tracy L Webb and Craig B Webb

Abstract

Objectives The current treatment of cats with chronic enteropathy frequently includes use of a prescription diet and daily medication administration, with the potential for side effects or problems with owner compliance, and may still result in treatment failure in some cases. The objective of this study was to determine if stem cell therapy was a safe and viable treatment in cases of feline chronic enteropathy.

Methods Allogeneic adipose-derived feline mesenchymal stem cells (fMSC) were used to treat seven cats with diarrhea of no less than 3 months' duration, while four cats with a similar clinical condition received placebo, in a blinded manner. Three additional cats were treated with an identical fMSC protocol, but owners were not blinded to the treatment. Owners completed a questionnaire characterizing clinical signs both before entering the study and 2 weeks following the second of two fMSC or placebo treatments. Owners were also surveyed for similar input by email 1–2 months later before being unblinded to their cat's study group. Besides the fMSC or placebo treatment, no other changes were made in diet, supplement or medication administration during the study.

Results No adverse reactions or side effects were attributed to the fMSC therapy in any of the cats. Owners of 5/7 fMSC-treated cats reported significant improvement or complete resolution of clinical signs, while the owner of the remaining two cats reported modest but persistent improvement. Owners of placebo-treated cats reported no change or worsening of clinical signs. Of the owners not blinded to the treatment, one reported marked improvement, one reported no change and one was lost to follow-up.

Conclusions and relevance Although allogeneic adipose-derived fMSC therapy appears to be a safe and potentially effective treatment for cats suffering from chronic enteropathy, these preliminary results require significant follow-up study.

Accepted: 01 November 2014

Introduction

Other than food-responsive diarrhea, the treatment of chronic enteropathy in cats frequently involves lifelong medication with glucocorticoids, either alone or in combination with additional immunomodulatory drugs, antibiotics or chemotherapeutics.[1] The most commonly diagnosed chronic enteropathy in cats is idiopathic inflammatory bowel disease (IBD), a condition that is proposed to result from disruption of gastrointestinal (GI) mucosal immunity and loss of tolerance to intestinal antigens.[1,2] Treatment of chronic enteropathy in cats is challenging owing to complications associated with owner compliance when asked to administer daily medication(s) for prolonged periods of time, side effects of glucocorticoid or other anti-inflammatory or immunomodulatory medications, dietary manipulation, and the frequent need for adjustments in therapy in an effort to control adequately the clinical signs. In a number of patients these treatment options result in an unsatisfactory control of the clinical signs or unacceptable side effects – hence the need for a novel approach to therapy.

Mesenchymal stem cells (MSCs) have come to the forefront in recent years as a potential therapeutic option for chronic inflammatory diseases owing to their immunomodulatory properties. MSCs have been shown to alter immune responses and reduce inflammation through changes in cytokine production, direct interactions with

Clinical Sciences Department, Colorado State University, Fort Collins, CO, USA

Corresponding author:
Craig B Webb PhD, DVM, DACVIM, Colorado State University Veterinary Teaching Hospital, 300 West Drake Road, Fort Collins, CO 80523, USA
Email: cbwebb@colostate.edu

T cells, natural killer cells, neutrophils and dendritic cells, and by increasing regulatory T cells (Tregs).[3–5] MSCs can be generated from a multitude of adult tissues, including adipose tissue, and under many conditions do not induce a clinical immune response when delivered to allogeneic or zenogeneic recipients, in part owing to their lack of major histocompatibility complex (MHC) class II molecules. Experimental and clinical evidence shows that MSC therapy in humans is safe and induces long-lasting remission in many patients with active severe Crohn's disease that is otherwise refractory to standard treatments.[6,7] Adipose-derived MSCs are currently being used in phase III clinical trials for IBD in humans.[8]

Previous research in our laboratory has shown that feline adipose-derived MSCs (fMSCs) can be generated in large quantities for clinical use, and have been safely administered to cats with chronic kidney disease.[9–11] This study was designed to confirm that allogeneic adipose-derived freshly cultured fMSCs could be safely administered to cats with a chronic enteropathy, and to determine if such a therapy had any beneficial effect on the clinical manifestations of this condition in cats.

Materials and methods

Study design and entry criteria

Clients signed a consent form as a requirement for study participation, and all aspects of this study were done with the review and approval of the Colorado State University (CSU) Animal Care and Use Committee requirements and guidelines.

For 11 cats this was a prospective, single-blinded, placebo-controlled study. Cats presenting with clinical signs consistent with a chronic enteropathy (diarrhea, vomiting or both for a duration of >3 months) were recruited for the study. All cats were screened at CSU for the presence of other chronic diseases with history, physical examination, body weight, complete blood count (CBC), biochemical profile, urinalysis, total thyroid hormone level (TT4), feline pancreatic lipase immunoreactivity (fPLI), feline trypsin-like immunoreactivity (fTLI), folate and cobalamin. All participants were indoor-only adult cats that had undergone fecal screening, feline leukemia virus/feline immunodeficiency virus testing and prophylactic deworming by their referring veterinarian during a previous work-up. The presence of a concurrent disease did not exclude a cat from participation if the condition was chronic, stable and manifested with minimal or no clinical signs. Any changes in ongoing therapy or diet during the full course of the study, including the final survey of clinical signs 1–2 months following treatment, would disqualify the cat from the study. Eleven cats met the criteria for study inclusion and all 11 complied with the requirements through the duration of the study. Three additional cats were treated with fMSCs in an unblinded but otherwise identical protocol following completion of the blinded portion of the study.

Derivation and preparation of the fMSCs

All fMSCs used in all the study cats were derived from a single collection of peripheral fat harvested from a single specific pathogen-free cat during a routine ovariectomy procedure. Briefly, subcutaneous adipose tissue from the area of the ventral abdomen was collected and stored in liquid nitrogen. Frozen adipose tissue was then thawed, washed, minced and digested with collagenase. The samples were centrifuged, and the resultant stromal vascular fraction was plated in sterile plastic tissue flasks in MSC media consisting of low-glucose Dulbecco's modified eagle medium supplemented with penicillin, streptomycin, L-glutamine, essential and non-essential amino acids, bicarbonate and heat-inactivated fetal bovine serum as previously described.[9] The cells were incubated for 72 h at 37°C and 5.0% CO_2, after which time the medium was removed and fresh medium added. The remaining plastic-adherent cells were incubated until approximately 80% confluency with media changes every 2–3 days. The cells were then harvested with trypsin-EDTA for passage to larger flasks to allow for expansion. Culture-expanded fMSCs at passage 2–4 were then harvested, washed three times in Dulbecco's phosphate-buffered saline (DPBS), and viable cell numbers determined prior to administration in DPBS and heparin sulfate, which is used to decrease cell clumping.

Appointments and injection protocol

Cats were randomly assigned to either the fMSC or placebo control group, and the owners of 11 cats were blinded to group assignment. At the first study appointment owners signed a consent form and filled out an initial questionnaire covering medical history and medications, dietary history, appetite, supplement use and clinical signs, including quantification of the frequency and consistency of diarrhea, and the presence and frequency of vomiting. Following the history and physical examination, a fasted blood and urine sample was collected for a CBC, biochemical profile, TT4 level, Texas A&M GI panel (fPLI, fTLI, folate and cobalamin) and urinalysis. A peripheral catheter was placed and the calculated dose of 2×10^6 cells/kg allogeneic fMSCs or the same volume of sterile 0.9% saline was injected intravenously over 20 mins followed by a heparinized saline flush. Syringes with fMSCs were gently and continuously agitated during the 20 mins injection period; the injections were administered by a veterinarian (CBW) who remained with the cat during the entire duration of the injection, and the cat was monitored for 60 mins following completion of the injection, prior to discharge. The injection protocol was repeated with the same dose of fMSCs or placebo 2 weeks later. Two weeks after the second injection, a fasted blood sample was collected for a repeat Texas A&M GI panel, and the owner completed

the final study questionnaire (same questions as the initial questionnaire). Owners were asked to quantify fecal consistency using the following scale: 1 (very hard), 2 (firm), 3 (normal), 4 (moist), 5 (soggy), 6 (no shape), 7 (watery).

Follow-up

One to 2 months following completion of the study a follow-up email was sent to all owners asking them to comment on their cat's general health and, specifically, those clinical signs associated with the chronic enteropathy.

Statistical analysis

The non-parametric Mann–Whitney test was used to compare the medians of variables between groups, both before and after treatment, because of the small sample size. Results are reported as median and range. The owner's quantification of frequency and consistency of the stool character was examined using a non-parametric test (Wilcoxon matched pairs test) looking for differences within groups pre- and postintervention, while the mean Δ for change in fecal consistency between groups was analyzed using an unpaired *t*-test.

Results

Eleven cats were entered into the blinded portion of the study; seven cats completed the fMSC treatment protocol and four cats completed the same protocol but received the sterile saline placebo, as randomly assigned. Table 1 lists the initial clinical variables for all cats, grouped by protocol. There was no significant difference between the fMSC-treated and placebo groups for age, body weight or body condition scores. Nine of the eleven cats had diarrhea as the main presenting complaint, with one cat in each group having normal stool. The initial median quantified fecal consistency score for the fMSC-treated group (4.5, range 4.0–6.5) and the placebo group (4.0, range 3.0–6.0) was not significantly different ($P = 0.52$). Five of the fMSC-treated cats and three of the placebo-treated cats were reported to have vomiting as a clinical sign. The majority of owners were unsure if their cat had lost weight and previous records were not available to make that determination, while in three fMSC-treated cats and two placebo-treated cats there was recorded evidence of weight loss. Appetite and diet were variable (see Table 1). Three of the fMSC-treated cats and two of the placebo cats were receiving medication, and five cats were being supplemented with either FortiFlora (Purina probiotic) or vitamin B12 injections. All medications and supplements had been in place for at least 1 month prior to entry into the study, and no changes in diet, medication or supplementation were made for any of the cats during the study. One cat in the fMSC-treated group had been previously diagnosed with hyperthyroidism but was well controlled on methimazole, as judged by a TT4 of 1.0 µg/dl both before and after the study period. No other study cat was found to be hyperthyroid, and none of the study cats had changes on CBC, biochemical profile or urinalysis that suggested another concurrent disease was present.

Table 2 shows the initial values for selected biochemical parameters most relevant to characterizing a chronic enteropathy. Only 3/11 cats had upper GI endoscopy performed and a histopathologic diagnosis of their condition: moderate lymphocytic–plasmacytic enteritis in all three cats. Three cats in the fMSC-treated group had serum albumin levels just below the reference range, but the median serum albumin concentration for the fMSC-treated group (3.2 gm/dl, range 2.9–3.7) was not significantly different from the median for the placebo-treated group (3.6 gm/dl, range 3.3–4.6) ($P = 0.16$). Three cats in the fMSC group had fasted serum cobalamin concentrations below the reference range, but the median serum cobalamin concentration for the fMSC-treated group (425 ng/l, range 150–1000) was not significantly less than the median for the placebo group (999 ng/l, range 890–1000) ($P = 0.41$). Two of the cats in the fMSC-treated group and one cat in the placebo group had fPLI values above the reference range, although none of these cats were reported as being either lethargic or anorectic; there was no significant difference in folate, fPLI or fTLI concentrations between the fMSC-treated group and the placebo group.

Table 3 shows the relevant parameters collected at the final appointment, 2 weeks after the second injection of either fMSC or placebo. Initially, there was no significant difference in body weight between the two groups, although three cats in the fMSC-treated group had gained a small amount of weight, while three cats in the placebo group had lost weight. One cat in the fMSC group showed a marked increase in serum cobalamin concentration, while one cat in the placebo-treated group showed a marked decrease in cobalamin concentration. Two cats in the fMSC-treated group showed a notable change in serum folate concentration, with one cat showing an increase and one cat showing a decrease in that parameter. There were no significant changes within the two groups for cobalamin level, or frequency of diarrhea from pretreatment to this point in the protocol, 2 weeks following the second injection of fMSC or placebo. Comparing the change (Δ) in fecal consistency scores pre- and post-treatment between the two groups showed an improvement in mean fecal consistency in the fMSC group (0.9 ± 0.5 SEM) compared with a decrease in mean fecal consistency in the placebo group (–1.0 ± 0.6 SEM) ($P = 0.03$).

Table 4 shows the clients' commentary on their cats' conditions 1–2 months following the last study

Table 1 Initial parameters

	fMSC-treated cats							Placebo cats			
	1	2	3	4	5	6	7	1	2	3	4
Age (years)	7	13	13	15	14	13	7	15	11	12	10
Sex	MC	FS	FS	FS	MC	FS	MC	FS	FS	FS	FS
Breed	DSH	DSH	DSH	DSH	DSH	DSH	DSH	DLH	DSH	DSH	DSH
Weight (kg)	3.3	4.0	3.4	3.6	3.1	2.5	4.0	3.0	6.0	3.7	4.6
BCS	3/9	5/9	4/9	4/9	3/9	3/9	4/9	3/9	7/9	3/9	4/9
Duration (months)	>12	>12	>12	>12	>12	>12	>12	>6	>12	>12	>6
Diarrhea	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
Frequency	Once daily	Five times per week	Five times per week	Twice daily	Twice daily	Once daily	Three times daily	Three times daily	Once daily	Once daily	Once daily
Consistency	5.0	4.5	4.5	4.5	6.5	4.0	6.0	5.0	3.0	6.0	3.0
Vomiting	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes
Frequency	Three times daily	Twice weekly	Once weekly	Twice monthly	Five times weekly	NA	NA	Twice weekly	NA	Once daily	Twice weekly
Weight loss (kg)	1.5	NR	NR	NR	1.0	1.5	NR	0.5	None	1.0	NR
Appetite	Decr	Decr	Decr	Norm	Norm	Norm	Norm	Norm	Norm	Incr	Norm
Diet	Nat Bal	Hill's z/d	Hill's z/d	RC HF	Nat Bal	Nat Bal	Friskies	Hill's k/d	Sci Diet	Pur HA	Hill's i/d
Medications	None	Pred,* Met†	Pred*	None	Pred,† Leuk§	Leuk§, Depo¶	None	None	Bude∞	Pred#	None
Supplements	None	FF	FF	FF	V B12	None	V B12	None	None	None	None

*5 mg q48h
†2.5 mg q12h
‡5 mg q12h
§2 mg twice weekly
¶20 mg every 6–8 weeks
∞1 mg q24h
#5 mg q24h
BCS = body condition score; Bude = budesonide; Decr = decreased; Depo = Depo-Medrol injection (Pfizer); DLH = domestic longhair; DSH = domestic shorthair; fMSC = feline adipose-derived mesenchymal stem cells; FF = FortiFlora (Purina probiotic) once daily; Friskies = Friskies (Purina)/9Lives (Big Heart Pet Brands); FS = female spayed; Incr = increased; Leuk = Leukeran (GlaxoSmithKline); MC = male castrated; Met = methimazole; NA = not applicable; Nat Bal = Natural Balance (Big Heart Pet Brands); Norm = normal; NR = not recorded; Pred = prednisolone; Pur HA = Purina Hypoallergenic; RC HF = Royal Canin High Fiber; Sci Diet = Science Diet Adult Maintenance (Hill's); V B12 = vitamin B12 injections 250 µg twice monthly

Table 2 Initial diagnostics

	fMSC-treated cats							Placebo cats			
	1	2	3	4	5	6	7	1	2	3	4
Histopathology	Mod	NA	NA	NA	NA	NA	NA	NA	Mod	Mod	NA
Albumin*	2.9	3.5	3.7	3.2	2.9	3.5	3.0	3.4	4.6	3.3	3.8
Cobalamin†	166	425	>1000	>1000	>1000	151	<150	>1000	890	997	>1000
Folate	22.3	37.8	30.4	58.2	24.2	15.9	23.4	16.1	25	14.3	11.7
fPLI‡	0.6	1.0	3.0	1.8	4.0	2.4	9.2	1.6	2.4	5.2	2.3
fTLI§	10.7	126.1	47.5	41.6	39.6	47.8	55.0	73.0	38.9	34.0	31.5

*3.1–4.4 g/dl
†290–1400 ng/l
‡0.1–3.5 µg/l
§12–82 µg/l
fMSC = feline adipose-derived mesenchymal stem cells; fPLI = feline pancreatic lipase immunoreactivity; fTLI = feline trypsin-like immunoreactivity; Mod = moderate lymphocytic–plasmacytic inflammation; NA = not available

Table 3 Final variables and diagnostics

	fMSC-treated cats							Placebo cats			
	1	2	3	4	5	6	7	1	2	3	4
Weight (kg)	3.5	4.1	3.4	3.6	2.8	2.4	4.1	2.7	5.4	3.6	4.6
Δ Weight*	0.2	0.1	NC	NC	−0.3	−0.1	0.1	−0.3	−0.6	−0.1	NC
Diarrhea	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Frequency	Once daily	Four times weekly	Five times weekly	Once daily	Twice daily	Once daily	Once a fortnight	Three times daily	Twice daily	Once daily	Once daily
Consistency	3.5	4.5	5.0	3.0	6.5	3.0	3.0	5.0	5.0	6.0	5.0
Δ Consistency*	1.5	0	−0.5	1.5	0	1.0	3.0	0	−2.0	0	−2.0
Vomiting	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes
Frequency	Once daily	Twice weekly	Once weekly	Once monthly	Three times weekly	NA	Twice weekly	Twice weekly	NA	Once daily	Twice weekly
Appetite	Incr	Norm	Norm	Norm	Norm	Norm	Norm	Norm	Norm	Decr	Norm
Diagnostic											
Cobalamin	528	346	>1000	>1000	>1000	173	<150	>1000	980	669	>1000
Δ Cobalamin*	362	−79	NC	NC	NC	22	NC	NC	−20	−238	NC
Folate	72.1	27.1	31.5	28.9	14.5	12.3	31.3	20.9	24.0	11.9	14.7
Δ Folate*	49.8	−10.7	+1.1	−29.3	−9.7	−3.6	7.9	4.8	−1.0	−2.4	3.7

*Change in variable from initial value
fMSC = feline adipose-derived mesenchymal stem cells; NC = no change; NA = not applicable; Incr = increased; Norm = normal; Decr = decreased

appointment, or 6–8 weeks following the second injection of fMSC or placebo. Several owners offered the comments without any prompting, while others were responding to an email sent by one author (CBW), inquiring as to their cat's condition in general (ie, 'Checking in to see how [cat] is doing'), and whether they had noted any adverse side effects since completion of the study. At this time all the owners were still blinded as to their cat's treatment group. Only those portions of the comments relevant to vomiting, diarrhea, appetite or activity level are included.

No acute reactions to fMSC or placebo injection were witnessed in any of the cats at any appointment, nor were any chronic adverse reactions or side effects reported by any of the owners during the study.

Owners of 5/7 fMSC-treated cats reported significant improvement or complete resolution of clinical signs at the 1–2 month follow-up, while the owner of the remaining two cats reported modest but persistent

Table 4 Follow-up (1–3 months) owner commentary (still blinded as to protocol)

Cat	Relevant owner quotes
fMSC-treated	
1	I wanted to give you an update on (cat 1). The bloating in his mid-section is pretty much gone. He is still eating somewhat normally and for the last few days I have not found any stool on the floor.
2*	I'll just tell you up front that they both seem to be doing significantly better! The improvement seems to be holding :) …definitely improved consistency of (both cats') stools (ie, firmer).
3*	
4	(Cat 4) is doing great. One episode of soft stool was stress-related. That has cleared up and her stool is firm and formed…completely normal stool with no cling-ons.
5	Overall, I think (cat 5) seems a bit better. Still vomits…but less fecal incontinence and seems very interested in eating.
6	(Cat 6) seems to be about the same maintaining.
7	(Cat 7) rarely has diarrhea.
Placebo	
1	(Cat 1) is the same – still having diarrhea, no noticeable vomiting and good appetite.
2	Lost a pound, nauseated and gassy…has a bit of diarrhea.
3	I just gotta say I hope she is in your control group! Overall, I would say little to no change.
4	(Cat 4) vomiting increases with stress or change in environment.

fMSC = feline adipose-derived mesenchymal stem cells
*Cats were from the same house

improvement. Owners of three of the placebo-treated cats reported no change in clinical signs, while the owner of the fourth placebo-treated cat reported a worsening of clinical signs.

Three additional cats were treated with fMSCs, but owners were not blinded to that treatment: one cat was from the study placebo group and the owner requested fMSC treatment following study completion; one cat was traveling from a considerable distance to receive treatments; and one cat was from an area shelter and its adoption had been delayed considerably owing to chronic vomiting. One of the three cats (the shelter cat) was lost to follow-up (successfully adopted out). The cat that was crossed over from the placebo group showed no change in clinical signs, while the owner of the cat that traveled to receive therapy (with histopathology-confirmed moderate lymphoplasmacytic IBD) wrote '(My cat) is doing great! No vomiting or diarrhea at all. He has actually gained a pound since his treatments, and has a voracious appetite.' None of the cats showed any adverse reactions or side effects to the stem cell therapy at any time point.

Discussion

MSCs are considered a potential therapeutic tool for diseases involving chronic inflammation or immune dysregulation because of their anti-inflammatory and immunomodulatory properties Although their mechanisms of action are not completely understood, MSCs have been shown to stimulate significant changes in immune responses and a reduction in inflammation through direct interactions with inflammatory cells, as well as through the release of cytokines.[3,4] In mouse models of acute colitis, administration of a single injection of adipose-derived MSC has been shown to ameliorate clinical and microscopic signs of colitis, reduce systemic and mucosal pro-inflammatory cytokine production, increase interleukin-10 secretion and induce Tregs in mesenteric lymph nodes.[12,13] A portion of our study was a prospective, single-blinded, placebo-controlled study to confirm the safety and assess the potential effectiveness of using allogeneic freshly cultured adipose-derived fMSC therapy in cats with clinical signs consistent with a chronic enteropathy (diarrhea, vomiting or both) for a duration of >3 months.

An expected but crucial observation in this study was that no acute reactions to two allogeneic, freshly-cultured fMSC injections at 2×10^6 cells/kg body weight, a similar dose to human studies, or placebo intravenous (IV) injection were witnessed in any of the cats at any appointment, nor were any chronic adverse reactions or side effects reported by any of the owners during the study.[6] The cats' attitude, demeanor, heart and respiratory rate, and body temperature were not affected during or for 60 mins following the injections. None of the owners reported any changes or side effects following IV administration of allogeneic fMSC for either the first or second injection. This is consistent with the absence of antigenicity seen with MSCs under these conditions; these are immunoprivileged cells that lack class II MHC and co-stimulatory molecules on their cell surface.[14,15]

At the 2 week postinjection appointment there appeared to be minimal beneficial effect of the fMSC treatment, although the consistency of the stool for the fMSC-treated group improved, while the consistency of the stool for the placebo group got worse. The frequency

of diarrhea for cat 7 had decreased from three times daily to twice a week, and the owners of cats 1, 2 and 3 all reported an increase in their cat's appetite. The contrast between the paucity of reported improvement or change in other quantified variables at this time point, and the commentary offered by owners 1–2 months later, would suggest that our original study design 'missed' the optimal time frame for follow-up with fMSC treatment. Ideally, we would have repeated the questionnaire, the quantification of fecal consistency, and the measurement of important clinical and biochemical variables a full 1–2 months after the final fMSC injection instead of doing so just 2 weeks after that treatment.

The most important clinical aspect of this study was the clients' commentary on their cat's condition 1–2 months following the last study appointment, shown in Table 4; 5/7 owners claimed a marked reduction in clinical signs following allogeneic fMSC treatment. At this time all the owners were still blinded as to their cat's treatment group. Of special note, shortly after the communication from the owner of fMSC cat 1 shown in Table 4, the cat presented to the CSU emergency service acutely dyspneic and minimally responsive to supportive efforts. The cat's owner elected euthanasia and agreed to a necropsy. On necropsy it was found that cat 1 died of 'classic features of cardiomyopathy', 'features of acute-on-chronic congestive heart failure' and 'diffuse pulmonary edema' (CSU Diagnostic Lab ID #F1253804). Notably, the report also states that 'Histologic evidence of significant IBD was not identified in routine sections of intestine, perhaps due to treatment or resolution of this condition'. Although the CSU pathologist was aware of the prior histopathologic diagnosis of moderate lymphocytic–plasmacytic IBD, the pathologist was unaware of the cat's treatment group in this study.

Conclusions

Feline MSC therapy, as developed and administered in this study, appears to be a safe and potentially effective treatment for a number of cases of feline chronic enteropathy. A large number of important questions regarding fMSC therapy in these cats remain, including steps to optimize the desirable properties of fMSCs prior to administration, identification of variables that would predict the likelihood of a positive response in individual cats, and further refinement of the most effective treatment protocol, just to highlight a few. As a proof-of-concept study the entry criteria were not rigid and there was not a requirement for a histopathologic diagnosis prior to study entry. These study conditions limit our ability to categorize accurately an individual cat's specific disease, which is a weakness of this study. The cats in the study population were very likely not uniform in their underlying disease process. Without imaging and histopathology it is also possible that some of these cats had non-GI disease(s) contributing to their clinical signs. Pancreatitis, IBD and cholangitis, or 'triaditis' is thought to be more common than currently recognized. But when taken in total, results of the diagnostic work-up were not consistent with the most prevalent diseases in this age group other than a primary GI condition, and it mimics many of the clinical case work-ups we experience that result in a working diagnosis of chronic enteropathy. We also required that no changes in diet, medication or supplementation were made during the entire course of the study, including in the 2 months following the last appointment. These conditions, the blinded nature of the study protocol for 11 cats, the fact that two fMSC-treated cats did not improve, and the stark contrast between reports from owners of the other five fMSC-treated and owners of placebo-treated cats lend strength and validity to the likely impact of fMSC therapy on the clinical signs. This is only the third published study of the use of MSC therapy in a spontaneous feline disease, and the first to demonstrate a positive clinical response.[10,11]

We wish to highlight that this study is just an initial step in what must be an ongoing effort to assure the safety of what might be a positive therapeutic intervention. We are by no means prepared to endorse the widespread use of fMSCs in client-owned cats with signs of chronic enteropathy at this time as far too many important questions remain to be addressed in a thoughtful and carefully controlled manner.

Acknowledgements We would like to acknowledge the generous and ongoing support provided by the Naniboujou Research Legacy, and the important input from Dr Jessica Quimby, Colorado State University, at the initiation of this study.

Conflict of interest The authors do not have any potential conflicts of interest to declare.

Funding This work was supported by the Winn Feline Foundation (grant number 118630).

References

1 Trepanier L. **Idiopathic inflammatory bowel disease in cats. Rational treatment selection**. *J Feline Med Surg* 2009; 11: 32–38.
2 Janeczko S, Atwater D, Bogel E, et al. **The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease**. *Vet Microbiol* 2008; 128: 178–193.
3 Panés J and Salas A. **Mechanisms underlying the beneficial effects of stem cell therapies for inflammatory bowel diseases**. *Gut* 2009; 58: 898–900.
4 Uccelli A, Moretta L and Pistoia V. **Mesenchymal stem cells in health and disease**. *Nat Rev Immunol* 2008; 8: 726–736.
5 Kono S, Kazama T, Kano KI, et al. **Phenotypic and functional properties of feline dedifferentiated fat cells and adipose-derived stem cells**. *Vet J* 2014; 199: 88–96.

6 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.
7 Panés J, Ordás I and Ricart E. **Stem cell treatment for Crohn's disease**. *Expert Rev Clin Immunol* 2010; 6: 597–605.
8 Lanzoni G, Alviano F, Marchionni C, et al. **Isolation of stem cell populations with trophic and immunoregulatory functions from human intestinal tissues: potential for cell therapy in inflammatory bowel disease**. *Cytotherapy* 2009; 11: 1020–1031.
9 Webb TL, Quimby JM and Dow SW. **In vitro comparison of feline bone marrow-derived and adipose tissue-derived mesenchymal stem cells**. *J Feline Med Surg* 2012; 14: 165–168.
10 Quimby JM, Webb TL, Gibbons DS, et al. **Evaluation of intrarenal mesenchymal stem cell injection for treatment of chronic kidney disease in cats: a pilot study**. *J Feline Med Surg* 2011; 13: 418–426.
11 Quimby JM, Webb TL, Habenicht LM, et al. **Safety and efficacy of intravenous infusion of allogeneic cryopreserved mesenchymal stem cells for treatment of chronic kidney disease in cats: results of three sequential pilot studies**. *Stem Cell Res Ther* 2013; 4: 48.
12 González MA, Gonzalez-Rey E, Rico L, et al. **Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses**. *Gastroenterology* 2009; 136: 978–989.
13 Gonzalez-Rey E, Anderson P, González MA, et al. **Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis**. *Gut* 2009; 58: 929–939.
14 Ong WK and Sugii S. **Adipose-derived stem cells: Fatty potentials for therapy**. *Int J Biochem Cell Biol* 2013; 45: 1083–1086.
15 Lindroos B, Suuronen R and Miettinen S. **The potential of adipose stem cells in regenerative medicine**. *Stem Cell Rev* 2011; 7: 269–291.