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# Stem cell therapy prior to full-mouth tooth extraction lacks substantial clinical efficacy in cats affected by chronic gingivostomatitis

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## Abstract

**Objectives** The aim of this pilot study was to determine the safety, efficacy and immunomodulatory function of systemically administered adipose-derived mesenchymal stem cells (ASCs) in cats affected by feline chronic gingivostomatitis (FCGS) prior to full-mouth tooth extractions.

**Methods** Five client-owned cats affected with FCGS that did not undergo full-mouth tooth extractions for FCGS treatment received two intravenous injections of 20 million fresh, allogeneic or autologous ASCs. An oral examination with photographs, a complete blood count, blood immune cell phenotyping and a biochemical profile were completed at 0 and 6 months after treatment.

**Results** Four cats completed the study and one cat exited the study 3 months after treatment. While the treatment was determined to be clinically safe, no positive clinical response was observed in three cats and a mild response was noted in two cats. Furthermore, none of the cats exhibited immune modulation, as evidenced by no alteration in circulating CD8<sup>+</sup> T cells, normalization of the CD4:CD8 ratio or neutrophil counts.

**Conclusions and relevance** Unlike the reported efficacy of ASCs in treating cats with non-responsive FCGS after full-mouth tooth extraction, the systemic administration of ASCs prior to full-mouth tooth extraction lacks substantial clinical efficacy and is not recommended at this time.

**Keywords:** Adipose-derived mesenchymal stem cells; gingivostomatitis; early intervention; efficacy; safety

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## Background

Feline chronic gingivostomatitis (FCGS) is a painful and debilitating chronic oral mucosal inflammatory condition in cats.<sup>1</sup> FCGS is diagnosed when the inflammation crosses the mucogingival junction and extends to the buccal and caudal oral mucosa, lateral to the palatoglossal folds.<sup>2,3</sup> There are two observed phenotypes: ulcerative/erosive and/or proliferative inflammatory mucosal lesions at the area lateral to the palatoglossal folds.<sup>2</sup> The etiology of FCGS is currently elusive. However, it is generally accepted that FCGS arises from an inappropriate immune response to oral antigenic stimulation. The etiology is potentially multifactorial in nature with a variety of proposed inciting causes.<sup>4–6</sup>

Mesenchymal stem cells (MSCs) have a profound regenerative capacity attributed, in part, to their ability

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to modulate both innate and adaptive immunity. Similar to other species, feline MSCs express immunomodulatory mediators, including hepatocyte growth factor and tumor necrosis factor-stimulated gene 6, secrete prostaglandin E<sub>2</sub>, interleukin-6 and tumor growth factor- $\beta$ , decrease T-cell activation and proliferation, and reduce tumor necrosis factor secretion.<sup>7,8</sup> Over the past 8 years, our group has demonstrated the efficacy of autologous and allogeneic adipose-derived MSCs (ASCs) for the treatment of refractory (ie, in edentulous cats) FCGS in a series of clinical trials.<sup>4-6</sup> In these studies, 32 cats with FCGS that did not respond to full-mouth tooth extraction were treated with two doses of  $20 \times 10^6$  ASCs, administered 1 month apart. In these clinical trials, a positive response rate (either substantial improvement or complete remission) was noted in approximately 70% of the cats within 3–12 months after ASC treatment.<sup>4-6</sup>

Currently, full-mouth tooth extraction is considered the standard of care to treat FCGS.<sup>2,3</sup> After extraction therapy, approximately 30% of cats experience complete remission, 40% improve and 30% will not respond.<sup>3</sup> Full-mouth tooth extraction is expensive and invasive, and cats that do not respond often need lifelong medical management such as corticosteroids, cyclosporine, antibiotics and analgesics. Some cats will not respond to medical management and/or will have a deteriorating quality of life and will be euthanized.<sup>2</sup> The objective of this pilot study was to determine if early intervention with ASC therapy (cells administered prior to full-mouth extraction) could be a successful therapy and result in substantial improvement or complete resolution of FCGS. We hypothesized that two intravenous (IV) injections of ASCs, administered 1 month apart, would reduce oral inflammation and improve the quality of life in cats with FCGS that have not yet undergone full-mouth tooth extraction.

## Materials and methods

### Study population

This study was approved by the Institutional Animal Care and Use Committee at the University of California, Davis (UCD), and the Clinical Trials Review Board at the School of Veterinary Medicine, UCD. All cat owners signed an informed consent prior to enrollment. Five cats affected with FCGS were enrolled. All cats had inflammation lateral to the palatoglossal folds and moderate-to-severe gingivitis. Cats had not undergone full- or near-full mouth tooth extraction and had pain and discomfort. All cats were otherwise healthy as determined by physical examination, blood work and urinalysis. Cats may have been treated with corticosteroids, antibiotics and/or pain medication; however, the cats were free of corticosteroids and/or antibiotic treatment for at least 14 days prior to ASC therapy. Pain treatment with opioids was continued as needed.

A minimum database (complete blood count [CBC] and biochemical profile) was obtained prior to treatment and at 6 months post-ASC administration (exit

examination). All cats received full-mouth radiographs, periodontal charting and periodontal treatment. Selected extractions were performed in all five cats as needed (ie, teeth that had periodontal disease or resorptive lesions). Cats that had periodontal disease, resorptive lesions or tooth fractures severe enough to necessitate extraction of >50% of the teeth were excluded from the study. The Stomatitis Disease Activity Index (SDAI) was completed prior to treatment and at 6 months, as previously described.<sup>5,9</sup>

### ASC isolation, expansion and phenotyping

With the exception of one cat, adipose tissue was obtained from specific pathogen-free cats that were euthanized for reasons unrelated to this study. In one cat receiving autologous ASCs, the adipose tissue was obtained from the subcutaneous abdominal fat, as previously described.<sup>5</sup> The isolation and expansion was performed at the Regenerative Medicine Laboratory at the William T Pritchard Veterinary Medical Teaching Hospital, UCD, exactly as previously described.<sup>4,5,10</sup> Fresh expanded early passage (P2 or P3) cells that passed quality assurance and control criteria were used for treatment.

### ASC treatment

All cats received two IV transfusions of  $20 \times 10^6$  (~5 million ASCs/kg) fresh (ie, cryopreserved ASCs that were revived in culture for ~72 h prior to administration) allogeneic (n = 4) or autologous (n = 1) ASCs, 1 month apart, as previously described.<sup>4-6</sup> All ASCs were administered by direct injection over a period of 20–40 mins by dividing the total dose into four separate aliquots (~5 million cells at a time) to prevent ASC adherence to syringe plastic and to prevent reactions associated with rapid cell infusion. All cats were hospitalized for 24 h post-treatment to monitor for adverse reactions.

### Blood work and lymphocyte phenotyping

A CBC and biochemical profile were run on automated analyzers (Bayer ADVIA 120 [Bayer Diagnostics] and Cobas c501 [Roche Diagnostics International], respectively). Lymphocytes were phenotyped (CD4, CD8, CD21) as previously described.<sup>4,5</sup>

## Results

### Early-intervention ASC therapy did not induce clinical improvement in cats with FCGS

A total of five cats that met the inclusion criteria were enrolled and four cats completed the study (Table 1). Three cats did not exhibit clinical improvement, as determined by oral examination and photographs, and two cats exhibited mild clinical improvement. Clinical assessment of disease severity, the SDAI, was in agreement with our clinical observations. The lack of improvement of clinical signs corresponded with a lack of improvement of the oral mucosal lesions. During the study, the cats

**Table 1** Signalment of enrolled cats with feline chronic gingivostomatitis

Patient number	Age (years)	Breed	Sex	Weight (kg)	SDAI		Cell source	Follow-up (months)
					Pre-tx	Post-tx		
1	2	DSH	MC	5.7	20	20	Autologous	12
2	13	DSH	MC	4.7	15	16.5	Allogeneic	7
3	1	DSH	FS	3.5	20	–	Allogeneic	10
4	1.5	DSH	MC	4.3	10	6	Allogeneic	6
5	6.4	Maine Coon	MC	7.3	–	–	Allogeneic	14

SDAI = Stomatitis Disease Activity Index; DSH = domestic shorthair; MC = male castrated; FS = female spayed; tx = treatment

**Table 2** Blood cell and biochemical data in cats with feline chronic gingivostomatitis (FCGS) prior to and after allogenic adipose-derived mesenchymal stem cell treatment, comparison between cats in the current study (no full-mouth tooth extraction) and historical data from cats enrolled in our prior study (with full-mouth tooth extraction)<sup>4</sup>

Parameters	Time point	FCGS cats prior to tooth extractions (n = 5)			FCGS cats with full-mouth tooth extractions (n = 7)		
		Mean	SD	Range	Mean	SD	Range
White blood cell count (/μl)	Pre-tx	10,006	347	4650–13,780	14,026	4621	7700–23,300
	6 month	8234	295	5830–10,310	11,036	3742	7830–18,350
CD4:CD8 T cell ratio	Pre-tx	1.24	0.39	0.62–1.70	0.73	0.45	0.3–1.79
	6 month	1.28	0.31	0.90–1.65	0.85	0.64	0.4–2.36
CD8 <sup>lo</sup> T cells (%)	Pre-tx	4.40	1.53	2.00–6.20	5.99	2.59	1.15–8.63
	6 month	3.82	1.54	2.23–5.91	7.73	3.18	3.15–13.09
PMN count (/μl)	Pre-tx	7375	4598	3320–13,989	9855	2861	6255–14,550
	6 month	5487	2269	3197–7836	7392	2696	3860–13,212
Albumin (g/dl)	Pre-tx	3.62	0.17	3.6–3.8	3.15	0.63	2.1–4.4
	6 month	3.25	0.46	2.7–3.8	2.85	0.60	2.0–3.5
Globulin (g/dl)	Pre-tx	5.0	1.1	3.9–7.0	4.8	0.92	3.0–6.0
	6 month	6.0	1.4	4.2–7.6	5.4	0.86	3.9–6.6

tx = treatment; PMN = polymorphonuclear leukocyte

did not gain weight and did not return to normal eating behavior, grooming and sociability. Finally, none of the cats exhibited adverse events during the ASC administration or for the duration of the study.

#### *ASC therapy did not result in decreased globulin concentration, a lower percentage of circulating CD8<sup>lo</sup> T cells or normalization of the CD4:CD8 ratio*

Differences were not noted in hematologic or biochemical parameters as a result of ASC therapy between the initial blood analysis (time 0) and at the 6-month exit examination (Table 2). Cats with FCGS had a variable leukocytosis owing to a neutrophilia, as well as variable levels of globulins with a tendency toward hyperglobulinemia. In the main, the cats did not differ in basic white blood cell parameters, total protein concentration or albumin concentration over time (Table 2). The majority of the cats (3/5) had sustained elevation of CD8<sup>+</sup> T cells and decreased CD4:CD8 ratio in blood prior to treatment and did not improve at exit examination. The percentage of

circulating CD8<sup>+</sup> T cells was stable over time in all of the cats, suggesting sustained cytotoxic immune activation. In addition, similar to other parameters, the percentage of CD8<sup>lo</sup> cells did not change during the course of the ASC therapy. Data from these cats were largely similar to our previously published data on FCGS cats with full-mouth tooth extraction prior to ASC therapy (Table 2).<sup>4</sup> Cats in the current study generally had a slightly lower white blood cell count and neutrophil count on study entry.

## Discussion

This pilot study is the first to test whether ASC therapy could be broadly expanded to treat cats with FCGS prior to the relatively invasive practice of full-mouth tooth extraction. Our data suggest that ASC therapy does not provide substantial clinical efficacy or meaningful immunomodulatory effects in cats with FCGS prior to full-mouth tooth extractions. This is in sharp contrast to the therapeutic effects we have demonstrated in non-responsive (ie, edentulous) FCGS-affected cats.<sup>4–6</sup> Furthermore,

although in two cats a small clinical response was noted, the cats continue to exhibit clinical signs of FCGS and are being maintained on other medications to control pain and inflammation.

While the reason for the lack of substantial clinical response noted in this pilot study remains elusive, there are several confounding factors that may influence these outcomes. Primarily, our previous work was focused on FCGS-affected cats that were edentulous and did not respond to extraction therapy, and the present work was performed on cats with full or near-full dentition.<sup>4–6</sup> It is plausible that the presence of teeth and the ongoing development of concurrent periodontitis (ie, even when periodontally affected teeth were extracted prior to treatment) results in chronic antigenic stimulation.<sup>1,11,12</sup> This may result in the ongoing generation of activated effector T lymphocytes and reduce the efficacy of short-lived ASCs to effectively reprogram the immune response. Given that all cats with FCGS typically have concurrent moderate-to-severe periodontitis, the presence of diseased teeth may counteract or diminish the response to ASC therapy.<sup>1</sup> In addition, cats with FCGS and periodontitis typically also have a complex subgingival microbiota that may play a role in FCGS and possible response to ASC therapy.<sup>13</sup>

Finally, although, to date, a causal relationship between viral infection and FCGS has not been proven,<sup>14,15</sup> it is plausible that an underlying chronic viral infection, specifically calicivirus, may play a role in determining ASC efficacy for this disease. Although we did not specifically determine the viral status of these cats, other than for feline leukemia virus and feline immunodeficiency virus, it is possible that a persistent viral load in these cats may have influenced the lack of clinical response.

## Conclusions

Taken together, and given the limitation of sample size of this pilot study, the results indicate that our hypothesis was not valid. It is currently recommended that cats affected by FCGS will first have full-mouth dental radiographs followed by full-mouth or premolar–molar tooth extractions prior to ASC therapy.<sup>2,3</sup> When these cats fail to respond to full-mouth tooth extraction (typically about 30% of cats), ASC therapy may be advised.

**Acknowledgements** The Stomatitis Disease Activity Index used in this study is a modified version of the Stomatitis Disease Activity Index originally developed by Dr Jamie Anderson.

**Author note** The work was performed at the Department of Surgical and Radiological Sciences, the Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA. In addition, the work was undertaken at Aggie Animal Dental Center, Mill Valley, CA, USA.

**Conflict of interest** The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Ethical approval** This work involved the use of experimental animals and the study therefore had ethical approval from an established committee as stated in the manuscript.

**Informed consent** Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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