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Impact of allogeneic feline uterine-derived mesenchymal stromal cell intravenous treatment on renal function of nephrectomized cats with chronic kidney disease

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ABSTRACT

Chronic kidney disease (CKD) is a common condition and leading cause of mortality in cats. Mesenchymal stromal cells (MSCs) may have a therapeutic effect on CKD. The aim of this pilot study was to determine efficacy of systemically-administered allogeneic uterine tissue-derived MSCs (UMSCs) in cats with CKD. Eighteen renal-compromised, unilaterally nephrectomized cats received two doses of 3×10^7 allogeneic UMSCs given intravenously (IV) with a 2-week dose interval. The primary endpoint was renal function, with treatment success defined by a 20% increase in glomerular filtration rate (GFR; iohexol clearance) and/or a 20% decrease in plasma creatinine in 50% of the cats. Secondary endpoints included diet and water consumption, body weight, urine characteristics, and adverse events. Treatment was well tolerated and associated with a statistically meaningful increase in GFR on Days 13, 28, 57, 99, 121 and 182, compared with baseline ($P < 0.0001$ for Days 13 to 99 inclusive; $P = 0.0029$ and $P = 0.0225$ for Days 121 and 182, respectively). Greater than 50% of the cats demonstrated a 20% increase in GFR on all days except Day 150, at which point GFR measurements were consistently above baseline. Statistically meaningful increases in diet and water consumption were observed. Substantial improvements in GFR were observed throughout the six-month evaluation period (excluding Day 150) in more than 50% of cats, thereby meeting the primary endpoint. Therefore, this IV-administered, allogeneic cellular therapy may support both renal function and clinical status of cats with CKD.

1. Introduction

Feline chronic kidney disease (CKD) is an inflammatory, degenerative condition with an overall prevalence of 4% (O'Neill et al., 2014a). This rate increases rapidly with age, reaching approximately 40% in cats greater than 10 years, and 80% after 15 years of age (Marino et al., 2014). Among cats five years and older, renal disease is the leading cause of mortality, at a rate of 13.6% (O'Neill et al., 2014b).

The etiology of CKD is unclear, and variable, with most cases remaining idiopathic (Sparkes et al., 2016). The kidney is responsible for an array of physiologic processes, ranging from endocrine production to waste excretion, and clinical manifestations of CKD are equally broad, but most often include weight loss and inappetence. Most CKD therapies do not impact long-term disease course; instead, they address clinical signs that impact Quality of Life (QOL), such as anti-nausea medication for gastrointestinal issues, or fluid administration for dehydration (Ross et al., 2006; Sparkes et al., 2016).

Mesenchymal stromal cells (MSCs) have been actively investigated as a cell-based therapeutic for CKD (Quimby et al., 2011, Quimby et al., 2016; Vidane et al., 2017; Thomson et al., 2019;). MSCs are plastic adherent (in culture), non-hematopoietic cells that have demonstrated therapeutic potential in both human and animal diseases (Black et al., 2007; Semedo et al., 2009; Quimby et al., 2011; Broeckx et al., 2014; Penha et al., 2014; Arnhold and Wenisch, 2015; Bittencourt et al., 2016; Kim et al., 2017; Tompkins et al., 2017). MSCs promote tissue regeneration via multiple mechanisms of action, including mediator secretion, cell-to-cell contact, transfer of trophic factors to injured cells and production of extracellular vesicles (Lai et al., 2010; Carrade and Borjesson,

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2013; Glenn and Whartenby, 2014; Fu et al., 2017).

Several rodent model studies of CKD have demonstrated the therapeutic potential of MSCs through improvement of blood pressure, proteinuria, albuminuria, creatinine, BUN, renal fibrosis and glomerulosclerosis (Cavaglieri et al., 2009; Semedo et al., 2009; Villanueva et al., 2013). In cats with CKD, some MSC studies have reported trends toward improvement in kidney function, as well as stabilization of body weight and owner-reported improvements in QOL (Quimby et al., 2011; Quimby et al., 2013; Quimby et al., 2016; Vidane et al., 2017; Thomson et al., 2019). In the only controlled feline trial to date ($n = 7$), three intravenous doses of allogeneic, adipose-derived MSCs did not significantly improve serum creatinine or glomerular filtration rate (GFR) (Thomson et al., 2019). However, due to differing manufacturing conditions, MSC sources, routes of administration, doses, intervals and small study population size, comparisons of efficacy among these studies remain challenging.

Across all studies in cats with CKD, MSC administration has been generally well tolerated. However, one study found that cryopreserved MSCs thawed and administered were associated with instant blood-mediated inflammatory reactions in cats; a reaction which was not observed in a separate cohort given MSCs obtained directly from culture, without cryopreservation of the cell dose (Quimby et al., 2013). This finding emphasizes the need to consider novel MSC therapies on an individual basis, as different preparations, cell lines and manufacturing methods may have an impact on treatment benefit and adverse event profile.

The purpose of this study was to evaluate the efficacy of uterine tissue-derived mesenchymal stromal cells (UMSCs) for improving renal function in cats with treatment-naïve International Renal Interest Society (IRIS) stage 2 or 3 CKD.

2. Materials and methods

2.1. Tissue processing and UMSC dose production

2.1.1. Tissue acquisition

The uterus, in toto, was harvested from a feline donor during a routine ovariohysterectomy at a humane society facility. The donor was younger than 24 weeks, had an unremarkable physical exam and tested negative for a panel of infectious diseases prior to manufacturing of the uterine tissue-derived cells. The donor tissue was transported to the processing laboratory in a sterile receptacle containing sterile 0.9% sodium chloride solution.

2.1.2. Tissue processing and culture expansion to uterine tissue-derived clinical dose (UMSC)

Within 24 h of receipt, the ovaries were resected and discarded, and the uterine tissue was transferred into a biological safety cabinet (SterilGard, Baker, Sanford, ME, USA), manually dissected, minced, and digested with collagenase and thermolysin (VitaCyte, Indianapolis, IN, USA; at 13 mg collagenase and 0.2 mg thermolysin per gram of uterine tissue). Digestion was performed in 4.5 mL Dulbecco's Phosphate Buffered Saline (DPBS; Gibco, Grand Island, NY, USA) at 37 °C for 1 h with periodic manual shaking. Dulbecco's Modified Eagle Medium (DMEM; Lonza, Walkersville, MD, USA) culture medium containing 10% fetal bovine serum (FBS; Atlas Biologicals, Fort Collins, CO, USA) was added to limit further enzymatic digestion. The digest, including single cells and tissue explants, was plated in T225 flasks (0.1 g tissue/flask; Corning, Durham, NC USA) and incubated at 37 °C, 5% CO_2 and 100% humidity (ThermoFisher Scientific, Waltham, MA, USA). The culture medium contained 0.001% amphotericin B (Fisher Scientific, San Diego, CA, USA) and 0.05 mg/mL gentamicin (Fisher Scientific) and was supplemented with 8 ng/mL GMP recombinant human fibroblast growth factor-basic active protein (rhFGF-2; CellGenix, Portsmouth, NH, USA), 6 mM L-glutamine (Fisher Scientific) and 10% FBS. After approximately 72 h, the cells were fed by replacing the tissue explants and spent medium with fresh culture medium. Once the flasks reached 70–80% confluence, the cells were detached via addition of TrypLE™ (Gibco, USA), washed, filtered (Cell Strainer; Corning) and counted (NucleoCounter NC-200, ChemoMetec, La Jolla, CA, USA). Detached cells were cryopreserved. Each cryovial contained 5×10^6 cells cryopreserved in 1-mL of cryopreservation medium (1:1 *v/v* 20% DMSO [Fisher Scientific, USA; in DMEM with 10% FBS] and DMEM with 10% FBS). These cryovials (CellSeal vials, Sexton Biotechnologies, Indianapolis, IN, USA) were placed in a controlled rate freezer (CryoMed 7452, Thermo Fisher Scientific, USA), and the temperature was reduced to approximately −60 °C prior to transfer to the vapor phase of monitored liquid nitrogen (LN_2) storage. These cryopreserved cells were designated as Passage 0 (P0). Expansion of cells occurred in T225 flasks through Passage 2. Subsequently, culturing took place in a 250-mL spinner flask (Bellco Glass, Vineland, NJ, USA) with FACT III microcarrier particles (Sartorius, Bohemia, NY, USA), which was placed in a 37 °C incubator (7% CO_2, 100% humidity) on a stirring platform (Bellco Glass) with a rotational velocity of 35 rpm. Cells were harvested from the spinner flask after 4–5 days in culture and cryopreserved (P2) as previously indicated. Additional cell expansion was performed by seeding multiple 250-mL spinner flasks. Sufficient Passage 3 cells obtained from multiple 250-mL spinner flasks were harvested to seed fresh, multiple 250-mL spinner flasks without cryopreservation, until Passage 4 cells were detached from the FACT III microcarrier particles via TrypLE™, collected, washed twice with DPBS, counted and then resuspended in a cryopreservation medium composed of 6% hydroxyethyl starch (HESPAN® in 0.9% NaCl; B. Braun Medical, Bethlehem, PA, USA) and 2% DMSO. Clinical doses (Passage 4) were generated by placing 3×10^7 cryopreserved cells in each 5-mL CellSeal cryovial (Sexton Biotechnologies) and handled as described above prior to transfer to vapor phase liquid nitrogen (LN_2) storage. Representative vials, including clinical doses, were sterile and negative for endotoxin and mycoplasma. Clinical doses were thawed and the recovered cells routinely had a viability greater than 90% (data not shown; counts and viability determined with the NucleoCounter NC-200 method).

Table 1
Pretreatment IRIS[a] stage 2 and 3 values.

IRIS Stage[b]	IRIS Substage	Number of Animals
2: Creatinine 140 — 250 μmol/L	1: UPC[c] < 0.2 (non-proteinuric)	13
	2: UPC 0.2 — 0.4 (borderline proteinuric)	1
3: Creatinine 250 — 440 μmol/L	1: UPC <0.2 (non-proteinuric)	1
	2: UPC 0.2 — 0.4 (borderline proteinuric)	2
	3: UPC >0.4 (proteinuric)	1

[a] IRIS = International Renal Interest Society.
[b] Symmetric dimethylarginine (SMDA) was not part of the staging criteria at the time of the study.
[c] UPC = Urine protein:creatinine ratio.

2.2. Cell characterization by immunophenotyping

Cells were analyzed with commercially available, directly-labelled antibodies reactive with CD34, CD45, CD44, CD105 (ThermoFisher Scientific) and 7-aminoactinomycin D red fluorescent live/dead stain (7-AAD, Invitrogen, Carlsbad, CA, USA). The cells were incubated in the previously optimized reagents for 30 min in darkness and on ice, prior to being assessed for reactivity on a BD Accuri C6 Flow Cytometer (with C6 Software; Becton Dickinson, Ann Arbor, MI, USA).

2.3. Nephrectomized CKD model and administration

At approximately 6-months of age, each enrolled cat underwent unilateral nephrectomy with contralateral induced ischemic renal

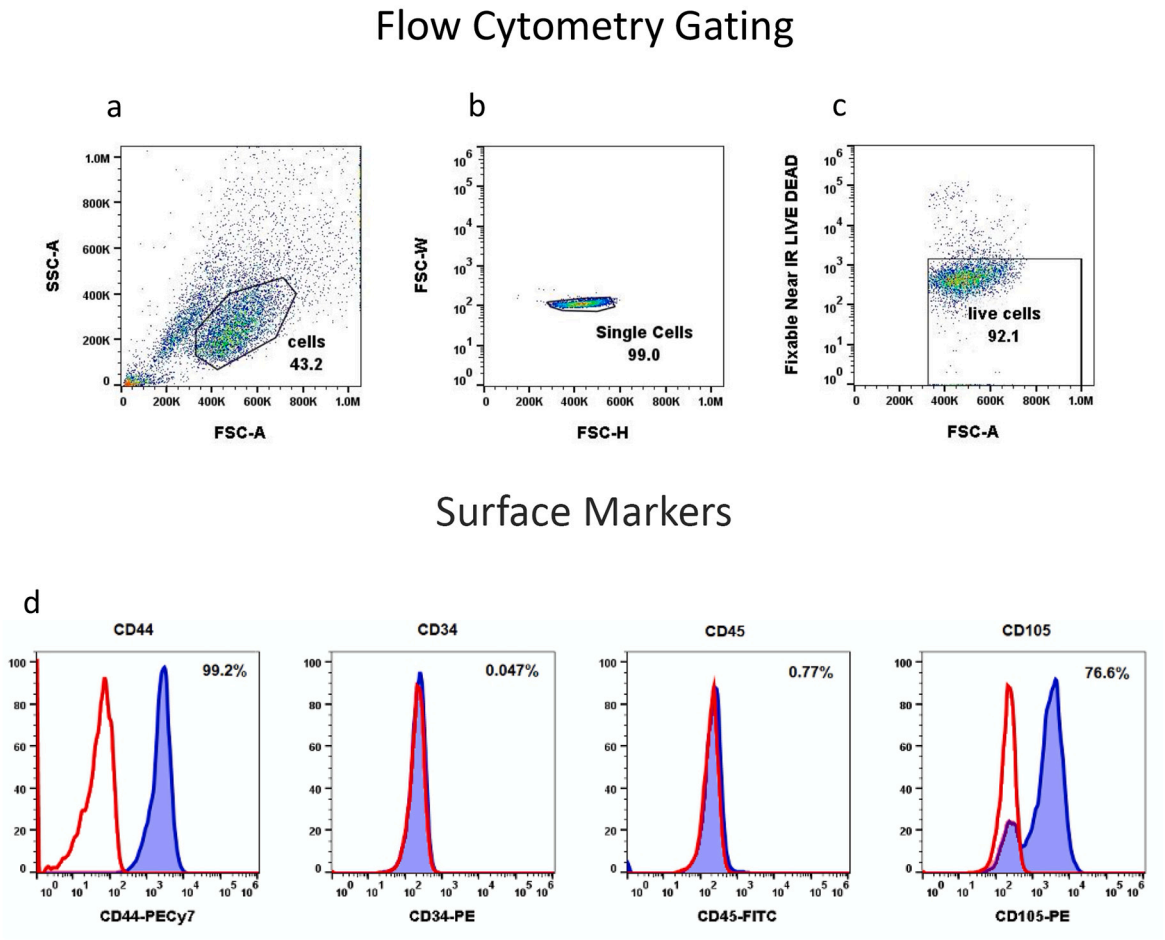

Fig. 1. (A) Scatter plot of all cells from a representative UMSC preparation. (B) Gating strategy for excluding dead, apoptotic or aggregated cells. Cells were stained with 7-aminoactinomycin D red fluorescent live/dead stain (7-AAD). (C) Scatter plot of single, viable cells used for determining single color reaction frequencies. (D) Single-color histograms of UMSCs demonstrating reactivity for CD44, CD35, CD45 and CD105, with unstained (red/open) and stained (blue/shaded) histograms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

damage. Consequently, the period from nephrectomy to enrollment varied from 6 to 7.5 years, depending on the age of the cat. At the start of this study, all subjects were spayed or neutered and had progressive renal disease classified as IRIS stage 2 or 3 CKD (Table 1), which was previously untreated. The protocol for this study was reviewed and approved by the Ethical Review Committee at Charles River Laboratories prior to its initiation. Study enrollment required animals to meet all inclusion criteria: conformation to required animal description (6.8–8.5 years of age, male or female, reproductively intact or sterilized, European Mixed Breed, male bodyweight of 4.1–4.9 kg on day −7, female bodyweight of 2.6–5.6 kg on day −7, nonpregnant and non-lactating), serum creatinine of at least 140 μmol/l on day −7, GFR less than 1.94 ml/min/kg on day −7, appropriate temperament for compliance with study procedures, and sufficient clinical stability to survive the study. Animals were excluded if the above criteria were not met, or if the animal had an active urinary tract infection, a previous or current diagnosis of cardiomyopathy, or a previous or current diagnosis of hyperthyroidism (based on tetraiodothyronine [T4] concentration greater than 5.4 μg/dl, elevated liver enzymes, and/or other indications of the disease).

Cats were given two intravenous (IV) infusions (3×10^7 cells each) of allogeneic, feline UMSCs, with a dose interval of 2 weeks. Cryopreserved doses were thawed in a water bath at 37 °C until there was a thin piece of ice visible. A 20 g needle on a 5 mL syringe was used to obtain the thawed cell preparation from the CellSeal vial via a septated port (swabbed with an alcohol wipe). The thawed cells were transferred to a delivery syringe containing sufficient diluent (3.3% *w/v* Trehalose in 0.9% NaCl and 0.9% NaCl) to give a final volume of 30 mL. Each dose was infused into the cephalic vein at a rate of 1×10^6 cells per minute (1 mL/min) with the initial 5 min of infusion at a rate of 0.5 mL/min. The cats did not resist the approximately 33-min infusion, and were held/petted on a treatment table, outside of the cage, throughout. The cats enrolled in the study were returned to the facility colony at the conclusion of the study.

2.4. Monitoring of diet consumption, water consumption and body weight

Diet and water consumption were monitored on a weekly basis. Body weight was recorded prior to study initiation and approximately every four weeks starting after D56 post-treatment.

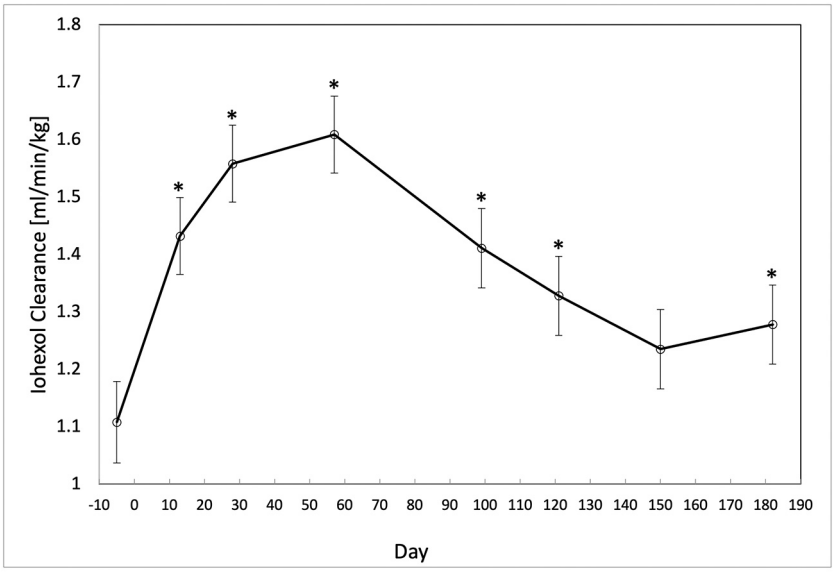

Fig. 2. Mean (SD) iohexol clearance rates over the course of the study. Asterisks denote significant ($P < 0.05$) increase for the cohort compared with baseline. An increase in iohexol clearance rate of at least 20% was observed in more than 50% of the cats on Days 13, 28, 57 and 99.

2.5. Blood and urine collection

Blood was collected on Day −7, 7, 13, 29, 56, 97, 120, 149 and 181 for clinical parameter (e.g., plasma creatinine) analysis. Urine was collected via cystocentesis on Day −7, 7, 14, 28, 56, 87, 120, 149 and 181. GFR was assessed by blood collection prior to iohexol administration and at 2, 3 and 4 h post-iohexol administration (Omnipaque, Nycomed, Oslo, Norway) on Days −5, 13 and 28; GFR was assessed at 2, 3 and 4 h post-iohexol administration on Days 57, 99, 121, 150 and 182.

2.6. Statistics

A comparison of values for each parameter at each timepoint with baseline values was performed. These values were analyzed with a Repeated Measures Analysis of Covariance (RMANCOVA). A 2-sided significance of 0.05 was used. Statistical analysis was performed with SAS, Version 9.2.2 (SAS Institute, San Diego, CA, USA).

3. Results

3.1. Study cats

Eighteen (15 female and 3 male) renal-compromised, unilaterally nephrectomized cats with CKD were enrolled in the study, all drawn from the CKD colony of Charles River Laboratories Ireland Ltd. All cats received two doses of allogeneic UMSCs on Day 0/1 and Day 14/15 per

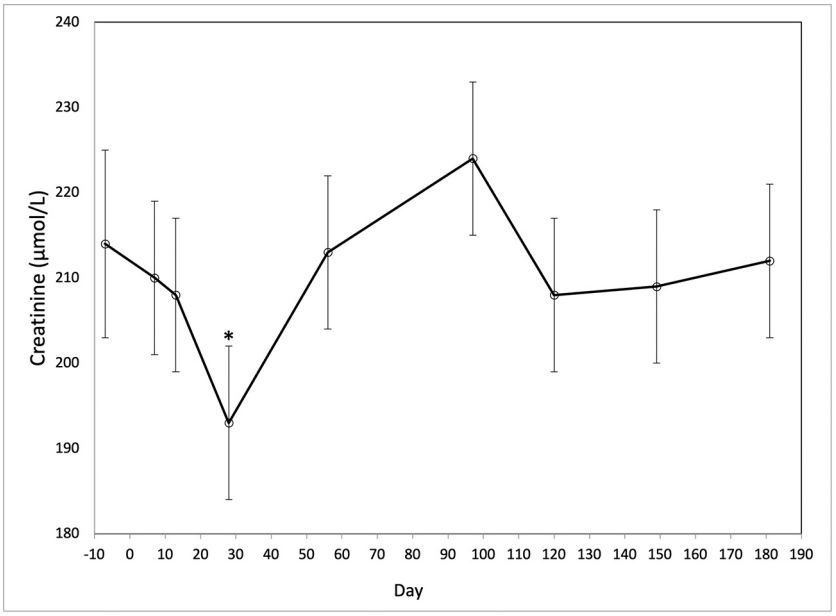

Fig. 3. Mean (SD) plasma creatinine levels compared with baseline values over the course of the study. The significant value on Day 28 ($P < 0.05$) represents four cats with at least 20% decrease in their plasma creatinine level compared with baseline.

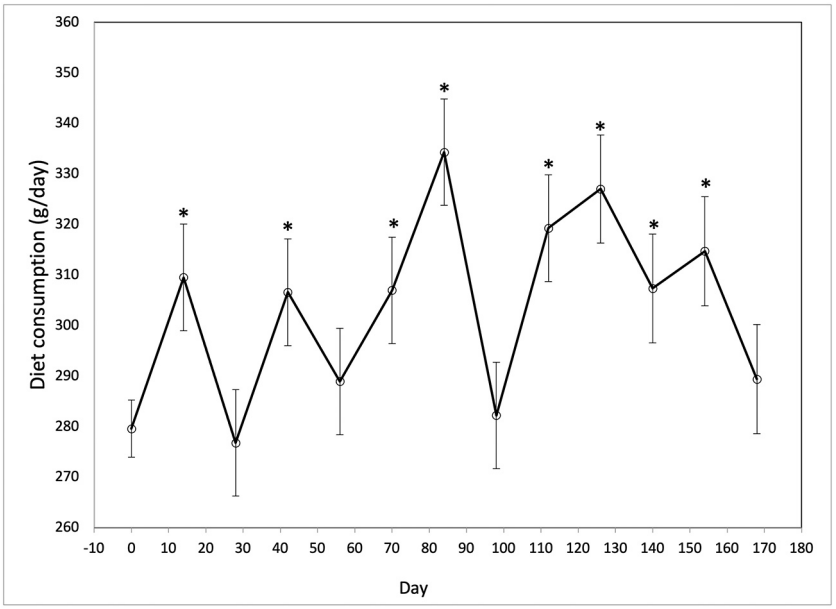

Fig. 4. Mean (SD) diet consumption over the course of the study. Asterisks denote significantly ($P < 0.05$) increased consumption compared with baseline.

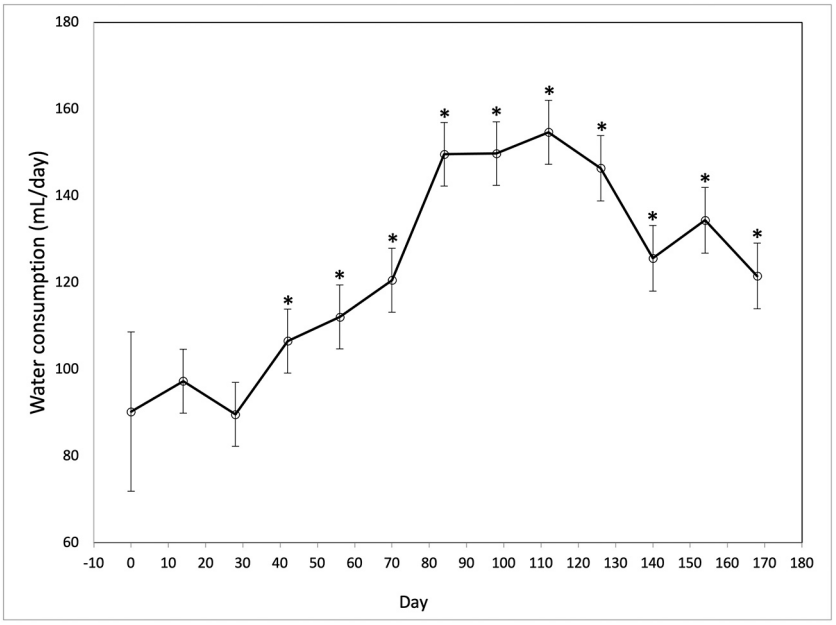

Fig. 5. Mean (SD) water consumption over the course of the study. Water consumption showed a significant increase ($P < 0.05$) over baseline starting on Day 42 and lasting through Day 168.

protocol. The study cats were classified according to stages 2 or 3 and, within each stage, substages 1, 2 or 3 (Table 1).

3.2. Cell characterization

The UMSCs were negative (<2% reactivity) for CD34 and CD45, and positive for CD105 (>70%), and CD44 (99%). A set of dot plots showing the gating strategy used and the single-color histograms for the CD markers for a representative preparation of the UMSCs are shown in Fig. 1. Percentage level of staining is shown for each CD marker. The cells also demonstrated tri-lineage differentiation for adipogenic, osteogenic and chondrogenic lineages (data not shown).

3.3. GFR and creatinine

There was a significant increase in GFR on Days 13, 28, 57, 99, 121 and 182 compared with baseline ($P < 0.0001$ for Days 13 to 99 inclusive; $P = 0.0029$ and $P = 0.0225$ for Days 121 and 182, respectively), but there was no significant difference in iohexol clearance on Day 150 compared with baseline ($P = 0.0925$) (Fig. 2). Greater than 50% of the cats demonstrated a 20% increase in GFR on Days 13, 28, 57 and 99.

Compared with baseline, there was a significant decrease in plasma

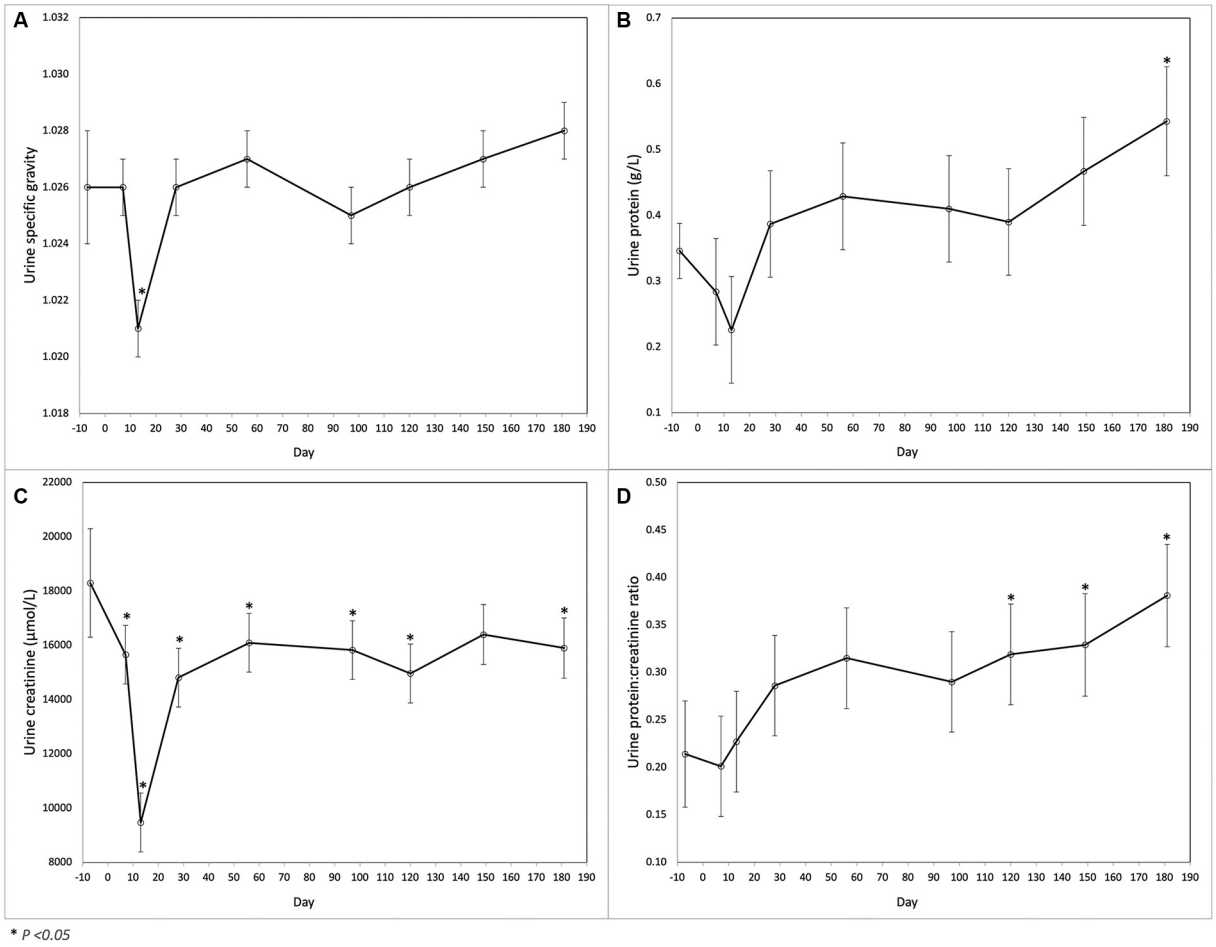

Fig. 6. (A) Mean (SD) urine specific gravity levels over the course of the study. The sole asterisk on Day 13 denotes a significant decrease ($P < 0.05$) in urine specific gravity for the cohort compared with baseline. (B) Mean (SD) of urine protein levels over the course of the study, with the sole asterisk on Day 181 indicating a meaningful decrease ($P < 0.05$) in urine protein on that day only. (C) Mean (SD) of urine creatinine levels over the course of the study. Asterisks denote significant decreases ($P < 0.05$) in urine creatinine compared with baseline that are consistently lower through the duration of the study (Day 181). (D) Mean (SD) of urine protein:creatinine ratio levels for the cohort over the course of the study. Asterisks associated with the values on Days 120, 149 and 181 represent significant increases ($P < 0.05$) in the ratios compared with baseline.

creatinine level on Day 28 only ($P = 0.0283$); however, on this day, just four animals (22%) had a 20% decrease of this parameter. On all other days, there was no significant decrease in plasma creatinine level compared with baseline (Fig. 3).

3.4. Diet and water consumption

There was a significant increase in diet consumption on Days 14, 42, 70, 84, 112, 126, 140 and 154 compared with baseline ($P < 0.05$) (Fig. 4). Water consumption increased significantly from Day 42 to Day 168, inclusive, compared with baseline (Fig. 5). Cats were housed in a temperature-controlled environment, so changes in diet and water consumption were not a response by the cats to fluctuations in environmental conditions.

3.5. Urinalysis

Urine specific gravity (USG) was significantly decreased on Day 13 only ($P < 0.0001$) (Fig. 6A). Urine protein was significantly increased on Day 131 only ($P = 0.0221$) (Fig. 6B). Urine creatinine was significantly decreased on Days 7, 13, 28, 56, 97, 120 and 181 ($P < 0.05$) (Fig. 6C). Urine protein:creatinine ratio was significantly increased on Days 120, 149 and 181 ($P < 0.05$) (Fig. 6D).

3.6. Body weight

Over the course of the study, 9 cats gained weight, 3 maintained weight, and 5 lost weight. However, average body weight of the cohort showed no significant changes over the course of the study compared with baseline (Fig. 7).

3.7. Adverse events

Sixteen adverse events (AEs) occurred during the study. The AEs were classified as mild, not considered to be related to treatment with UMSCs and resolved before the end of the study: otitis externa (2), otitis exudate (1), diarrhea (2), loose feces (3), alopecia (1), mild topical wound (4), trace frank blood in litter (2) and anisocoria (1). One serious AE occurred on Day 120 for a cat that died during urine collection. A post mortem examination revealed that the cause of death was propofol overdose. Following this occurrence, all remaining cats received doses of propofol to achieve a lighter degree of anesthesia. Subsequent doses of propofol were given at a dose less than the recommended 8 mg/kg. The additive effect of propofol in repeated administration in cats was not taken into account in the original dose calculation.

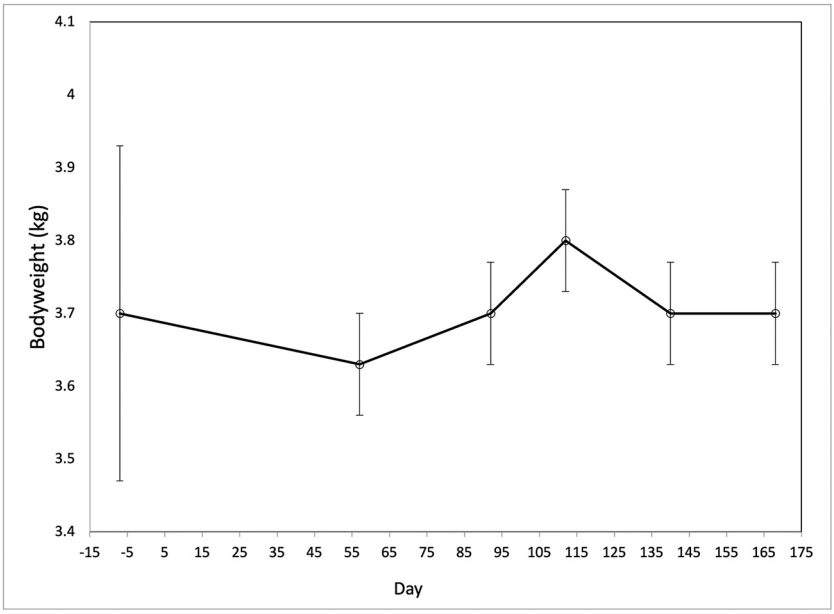

Fig. 7. Mean (SD) body weights over the course of the study. While none of the results were significantly different from baseline, nine cats gained weight, three cats maintained weight and five cats lost weight.

4. Discussion

This pilot study is the first to test the effects of UMSCs on feline CKD. Our data suggest that repeated, IV doses of allogeneic UMSCs are well tolerated in cats with IRIS stage II or III CKD, and provide significant improvement in renal function, as measured by GFR. Although plasma creatinine did not significantly improve with treatment, this could be due to variations in hydration status between timepoints and individuals. As GFR remains the standard measure of renal function, changes in iohexol clearance can be interpreted with greater confidence than those of plasma creatinine. Greater than 50% of the cats had at least 20% increased iohexol clearance at four timepoints through Day 99, thereby meeting the primary endpoint. UMSC therapy also was associated with significantly increased diet and water consumption, and decreased urine creatinine. A trend toward bodyweight support was also observed, with 12 out of 17 cats gaining or maintaining weight, versus 5 cats losing weight at study close. Some variability in the response of individual study cats to the UMSC therapy is to be expected, but overall, these findings suggest a significant clinical benefit, as improvements in appetite, along with weight gain or maintenance of body weight, are well-supported prognostic indicators for cats with CKD. As this was a pilot study, an untreated group was not included.

The greatest improvements in GFR occurred from approximately Week 2 to Week 8, followed by a plateau significantly above baseline that lasted until the end of the study, at Week 26 (with the exception of Day 150, when mean iohexol clearance was not significantly greater than baseline) (Fig. 2). Simultaneously, significant improvements in clinical signs, such as diet and water consumption, began as early as Week 2 and persisted until Week 24 (Figs. 4 and 5). These trends suggest that the therapeutic effects of UMSCs have a rapid onset that is clinically meaningful, though additional doses may be needed for longer-lasting benefits.

To date, four studies have investigated the effects of MSCs on feline CKD (Quimby et al., 2011; Quimby et al., 2016; Vidane et al., 2017; Thomson et al., 2019). In another report, the results of three individual pilot studies were summarized (Quimby et al., 2013). Yet none of these studies has met a primary endpoint through improved GFR, or achieved clinically meaningful secondary endpoints, such as diet consumption, as this study has demonstrated. A number of factors may have contributed to these differences in efficacy, though the most significant were likely MSC source and dose, as these variables were most distinct from the protocols used in previous trials.

The present investigation was the first and only feline CKD study to administer uterine tissue-derived MSCs, instead of adipose-, bone marrow-, or amniotic membrane-derived MSCs, suggesting that uterine tissue-derived MSCs may possess unique properties beneficial to renal pathology. The UMSCs used in the current study were positive for CD markers associated with cultured mesenchymal stromal cells (CD105 and CD44) and were negative for CD34 and CD45. The UMSCs aligned with established standards for mesenchymal stromal cells as follows: 1) the cells grew as attached cells in culture; and 2) the cells underwent trilineage differentiation into adipo-, osteo- and chondrogenic cell lineages (data not shown) (Dominici et al., 2006). While the UMSCs were negative (i.e., <2% positive frequency) for CD34 and CD45, thereby meeting the standards set forth by Dominici and colleagues, the cells were not uniformly highly positive for CD105 (Fig. 1), which contrasts with the same set of standards (i.e., CD105 > 95%). It is worth noting, however, that these standards were based on characterization studies involving human adipose-derived and bone marrow-derived MSCs, leading Dominici and colleagues to acknowledge that cells from other species and other tissues might deviate from these values (as appears to be the case here).

The present data suggest that feline UMSCs, while similar to the "standard" MSC, aren't identical to human-associated MSCs, or even feline MSCs from other tissue sources, and their unique character may be associated with the observed therapeutic response. This kind of relationship—between MSC source and therapeutic potential—has been observed previously. For example, in 2018, Mohamed-Ahmed and colleagues performed a human donor-matched comparison of MSCs derived from adipose versus bone marrow, and found that the former were more proliferative and adipogenic while the latter were more osteogenic and chondrogenic (Mohamed-Ahmed et al., 2018). Similar findings have been reported in animals (Webb et al., 2012; Bortolotti et al., 2015; Sasaki et al., 2019). Other studies suggest that MSC source-dependent properties observed in vitro (e.g., greater osteogenicity) predict in vivo effects (e.g., greater osteogenesis) (Kuznetsov et al.,

1997; Yang et al., 2010; Jiang et al., 2014). While relevant studies are lacking in cats, equine research suggests that uterine tissue-derived MSCs may display a potential for migration, immunomodulation, and smooth muscle differentiation, all of which may contribute to relatively greater therapeutic effect in CKD, compared with MSCs from other sources (Rink et al., 2017; Cabezas et al., 2018; Cortés-Araya et al., 2018).

In addition to using a unique tissue source, the present study also delivered notably higher intravenous doses of UMSCs than previous studies, at approximately 5–12 $\times$ 10^6 cells per kilogram, compared with a previous study in which a maximum of approximately 2 $\times$ 10^6 cells per kilogram (Quimby et al., 2016) was delivered. This may be a crucial variable, as previous in vitro and animal studies have suggested that the effects of MSCs may be partly dose-dependent, and a recent meta-analysis of dose-response data from human clinical trials reported that IV doses were most effective in a narrow range, with lower and higher doses demonstrating less efficacy (Wolbank et al., 2007; Duffy et al., 2009; Joo et al., 2010; Krupa et al., 2018; Kabat et al., 2019). Thus, the present trial—by delivering a dose (per body weight) of UMSCs up to almost 3-fold higher than the highest dose delivered in previous studies—supports a higher minimum effective dose for feline CKD. There are several limitations to the study. The study didn't include a control group. However, it was performed as a pilot study, and in view of the positive outcomes, will contribute to setting the conditions and power of a randomized study. Also, 18 cats were enrolled in the study, which might have an impact on the interpretation of some of the clinical parameters due to a relatively small number of cats.

The present findings suggest that UMSCs have a positive effect on both renal function and clinical status, yet more trials are needed to answer a number of questions essential to achieving sustained therapeutic benefit, such as optimal dose, frequency and initiation timing (in relation to IRIS stage of CKD). Further research also is needed to determine the relationship between UMSC therapy and quality of life, as this is the central goal of veterinary medicine. Our next study, currently underway, will investigate this endpoint among client-owned cats with CKD.

5. Conclusions

This is the first study out of at least seven previous cell-based therapy studies in feline CKD to satisfy a primary endpoint by demonstrating a marked improvement in the measurement of renal function (GFR). The current study also demonstrated net weight gain and improved diet consumption, in contrast to previous reports (Quimby et al., 2011; Quimby et al., 2013; Thomson et al., 2019; Vidane et al., 2017). More studies are needed to confirm the demonstrated positive outcomes, as well as the promise of using uterine tissue-derived, allogeneic mesenchymal stem cells to treat cats with CKD.

Declaration of Competing Interest

Dr. Zacharias, Dr. Sand, and Dr. Black are employed by Gallant, which is developing the UMSCs as an investigational product for clinical studies and commercial use.

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