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Intravenous allogeneic mesenchymal stromal cell therapy in 13 Pugs with presumptive early necrotizing meningoencephalitis

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Objective

To describe the tolerability and activity of IV allogeneic mesenchymal stromal cell (MSC) therapy in 13 Pugs with presumptive early necrotizing meningoencephalitis (NME).

Methods

255 Pugs were screened from 2021 to 2024 for neurological examination (NE) abnormalities suggestive of early NME. All dogs received a minimum of 2 NEs spaced 2 to 4 weeks apart. An NE score (NES) was assigned at each visit. Magnetic resonance imaging, CSF analysis, and infectious disease testing was obtained in all affected Pugs. Pugs with consistent or progressive NES and MRI or CSF findings supportive of early NME were eligible for MSC therapy.

Results

NE abnormalities prior to MSC therapy included spinal hyperesthesia (11 of 13 [85%]), paw placement deficit (11 of 13 [85%]), menace deficit (9 of 13 [69%]), obtundation (9 of 13 [69%]), seizures (7 of 13 [54%]), and ataxia (4 of 13 [31%]). The NES improved in all dogs within 24 hours of the first dose of MSC (mean improvement, 86%). Mild adverse events were noted after 3 of 30 MSC doses (10%). All 13 dogs are currently in remission (follow-up time, 5 to 43 months); 7 of 13 Pugs (54%) remained in remission after MSC therapy alone, and 6 of 13 (46%) required the addition of immunosuppressive therapy.

Conclusions

IV allogeneic MSC administration was well tolerated and resulted in immediate clinical benefit in this small cohort of Pugs with presumptive early NME. Strategies to maintain the long-term benefits of MSC therapy require further study.

Clinical Relevance

Immunomodulatory MSC therapy may be a potential treatment for neuroinflammatory disease in dogs. Further studies are needed to optimize long-term benefits.

Keywords: mesenchymal stromal cell, autoimmune, immune-mediated, meningitis, encephalitis

Necrotizing meningoencephalitis (NME) is a fatal neuroinflammatory disease affecting several small dog breeds, most notably the Pug (*Pug dog encephalitis*). Necrotizing meningoencephalitis shares several similarities to multiple sclerosis (MS) in humans, including genetic risk loci within the major histocompatibility

complex (MHC) class II region.¹⁻⁴ Necrotizing meningoencephalitis has historically been associated with rapid clinical onset and progression that closely resembles the rare fulminant forms of MS.⁵⁻¹¹ Most forms of MS exhibit a more insidious clinical course that progresses over 30 to 40 years, characterized by a preclinical phase in which brain pathology and MRI changes appear before overt clinical signs manifest.¹² On the basis of past published studies of NME,^{13,14} we identified a combination of neurological clinical signs in “apparently normal” young Pugs that were at genomic risk of developing NME that are not present in dogs at low

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genomic risk. These signs include multifocal spinal hyperesthesia, menace response deficits, paw placement deficits, and proprioceptive ataxia, which have been documented through screening over 250 Pugs over the past 3 years.^{13,14} These clinical signs mimic the earliest symptoms of MS in humans, suggesting that there may be an earlier stage of NME or a distinct novel, less aggressive immune-mediated neuroinflammatory disease in some Pugs.^{1,12,15} Although NME cannot be confirmed without brain histopathology, MRI and CSF changes in these Pugs with presumed early forms were consistent with NME.^{13,14} Identification of Pugs with an early manifestation of neuroinflammatory disease provides an important opportunity for therapeutic intervention before advanced signs occur.

The etiology of MS is complex and generally accepted to be associated with immune reactivity of T and B cells against myelin in the CNS resulting in inflammatory lesions, loss of oligodendroglia, and degeneration of axons.^{16,17} In the rare fulminant forms of MS such as the Marburg variant, necrosis is a prominent feature.^{6–8,18} Corticosteroids, immunosuppressants, interferon β , and monoclonal antibodies have been used to treat MS but often have negative side effects, and there is currently no cure for MS.^{17,19} Some MS drugs suppress inflammatory and neurodegenerative effects in early stages of the disease, but neuroregeneration or axon remyelination do not occur with standard disease-modifying therapies.¹⁶ Standard immunosuppressive therapy is also ultimately futile in treating NME, with median survival times of 6 to 7 months after diagnosis.^{5,20}

Mesenchymal stem cells, also called mesenchymal stromal cells (MSCs), have therapeutic potential in MS predicted by their anti-inflammatory and immunomodulatory effects.^{16,17,19} Several small phase I/II MSC studies have shown feasibility, tolerability, and improvement in disability in humans with MS.^{16,19,21,22} Larger and more rigorous studies are needed to understand the role of MSCs in MS.^{16,19,21,23} Given the similarities between MS and NME and promising results of MSC therapy in humans with early MS, MSC therapy may have similar anti-inflammatory and immunomodulatory effects in dogs in early stages of neuroinflammatory disease including NME.

The objective of this observational pilot study was to describe the tolerability and short- and long-term clinical response to IV allogeneic MSC therapy in Pugs with clinical and diagnostic abnormalities consistent with presumed early NME. In this small pilot study, MSC administration was well tolerated and resulted in clinical improvement in all dogs within 24 hours. Sustained improvement was more variable; however, nearly half of the dogs were in remission after MSC therapy alone. The results of this study may be used to inform the design of future larger prospective studies evaluating the use of MSCs in NME and other neuroinflammatory diseases.

Methods

Patient eligibility

Normal Pugs ranging from 4 months to 6 years of age and of any sex and weight were recruited

from Colorado and enrolled into the study through a single tertiary referral hospital from 2021 to 2024 (**Supplementary Figure S1**).

The study received Animal Care and Use Committee approval (Animal Clinical Investigation Biosciences), and informed consent was obtained from all owners prior to enrollment of their dog into the study. Use of MSC in client-owned animals was allowed under INAD No. 012882. Dogs with chronic (> 4 weeks) neurological abnormalities (gait deficits, seizures) that could be attributed to other neurological diseases including idiopathic epilepsy were excluded. No dogs had any prior treatments for a neurological condition. All Pugs received 2 physical and neurological examinations, spaced 2 to 4 weeks apart, by a board-certified veterinary neurologist blinded to NME genotype risk to confirm consistency in the clinical signs documented during the examinations.

All neurological examination abnormalities were recorded, and a structured ordinal scoring system was used to determine a total neurological examination score (NES), shown in **Supplementary Table S1**. The NES was compared at each visit to visualize improvement, progression (worsening), and regression (initial improvement followed by relapse) within an individual Pug over time.

Necrotizing meningoencephalitis genetic risk assessment

Whole blood was collected from 77 dogs and transferred to 2-mL K₂ EDTA coated tubes (Becton, Dickinson and Co). Blood was stored in an on-site -80 °C freezer prior to overnight FedEx shipment on dry ice for genetic analysis at the Translational Genomics Research Institute. Saliva from the buccal mucosal fold was collected in 52 dogs and placed in an aqueous solution containing solvent for purification (PERFORMA-gene PG-100; DNA Genotek Inc). Oral samples were kept at room temperature and shipped within 2 days of collection. Genomic DNA was isolated from the whole blood and oral swabs following a previously published protocol.^{2,13,24} Post hoc genotyping confirmed Pug breed in all dogs in this prospective cohort. Dogs in the cohort were defined as having low genomic risk (no genomic risk alleles, designated A/A) or at genomic risk (presence of 1 [T/A] or 2 [T/T] at-risk alleles).

Magnetic resonance imaging

Magnetic resonance imaging was acquired in Pugs with reproducible or progressive neurological examination abnormalities with a 1.5-T MRI system (MAGNETOM Symphony; Siemens AG). Sequences of the brain included T1-weighted pre- and post-contrast sagittal, transverse, and dorsal planes; T2-weighted sagittal and transverse planes; and FLAIR and gradient echo transverse planes. Cervical and thoracolumbar spinal cord imaging was obtained with T2-weighted and STIR sagittal and transverse planes and postcontrast T1-weighted sagittal plane. Magnetic resonance imaging was reviewed and reported independently by 2 board-certified neurologists, one of whom was blinded to the abnormalities detected on neurological examination.

Cerebrospinal fluid analysis and infectious disease testing

Cerebrospinal fluid was collected aseptically from the cerebellomedullary cistern with a 22-gauge, 1.5-inch spinal needle. A sample of CSF was submitted for cytological analysis and clinical pathologist review to a commercial laboratory (Zoetis Reference Laboratory).

A neurological infectious disease PCR panel on the initial CSF was submitted to test for the presence of *Anaplasma* spp, *Blastomyces* spp, *Bartonella* spp, *Cryptococcus* spp, *Ehrlichia* spp, *Histoplasma capsulatum*, canine distemper virus, *Borrelia burgdorferi*, *Neospora caninum*, and *Rickettsia rickettsii*. Any dog positive for any infectious disease was removed from the study and treated at the discretion of the attending neurologist.

Intravenous MSC preparation and administration

Pugs of any genotype with persistent or progressive NES and MRI or CSF abnormalities were eligible to continue in the study. For these treatment-eligible patients, research-grade fresh allogeneic adipose-derived MSCs (AMSCs; University of California-Davis Regenerative Medicine Laboratory) were administered from October 2021 through September 2023. Frozen-thawed pharmaceutical-grade allogeneic uterine-derived MSCs (UMSCs; Gallant Therapeutics) were administered from November 2023 through July 2024.

Adipose-derived MSC preparation and administration

The AMSCs for the study were previously isolated from a single healthy canine donor's adipose tissue.²⁵ Canine adipose MSCs were thoroughly characterized and cryopreserved as previously described.^{25,26} Before each treatment, an aliquot of cryopreserved MSCs was thawed, expanded, and formulated for clinical use by the Regenerative Medicine Laboratory at the Veterinary Medical Teaching Hospital at the University of California-Davis. Briefly, cryopreserved MSCs were thawed in a 37 °C water bath and plated into tissue culture flasks with Dulbecco modified Eagle medium (Gibco low glucose; Thermo Fisher Scientific Inc), 10% fetal bovine serum (Atlanta Biologicals), and 1% penicillin/streptomycin (Gibco; Thermo Fisher Scientific Inc) and incubated at 37 °C in 5% CO₂ at 90% humidity. Prior to clinical preparation, MSCs were trypsinized, enumerated, and tested for bacterial sterility, viability (> 85% with trypan blue exclusion), confirmed negative for endotoxin (PYROGENT Plus LAL gel clot assay; Lonza Bioscience), and confirmed negative for Mycoplasma (Myco-Scope PCR kit; Genlantis Inc). Cultured MSCs were reconstituted in physiologic saline prior to shipment.

Adipose-derived MSCs were shipped overnight on dry ice and administered immediately upon arrival. A single vial containing approximately 20 X 10⁶ AMSCs in liquid suspension was visually examined to confirm appropriate volume. The vial was gently mixed prior to administration. Using an 18- to

22-gauge needle on a 3-mL polypropylene syringe, 0.5 mL of the MSC suspension was removed and mixed with 0.5 mL of sterile saline. Within 5 minutes, the MSCs were administered through a 22-gauge or greater peripheral IV catheter into the cephalic vein over 5 minutes. Once complete, the IV catheter was flushed with 0.25 mL of sterile saline. This process was repeated 3 more times at 10, 20, and 30 minutes until all cells were administered (a total 35-minute administration time). If an adverse reaction was noted, the treatment was slowed (given over 10 to 15 minutes) and 1 to 2 mg/kg of diphenhydramine was given IM. If a serious adverse reaction was noted, treatment was stopped.

Uterine-derived MSC preparation and administration

Uterine-derived MSCs were collected from healthy donor dogs tested free from relevant disease agents. Cells were expanded and cultured and frozen in liquid nitrogen. The formulation consisted of uterine stem cells (stromal and epithelial cells) formulated in a suspension containing 10 million cells/mL suspended in CryoStor CS5 (CryoStor base with 5% dimethylsulfoxide; BioLife Solutions Inc), packaged in a 5-mL vial.

Doses were shipped in a liquid nitrogen dry shipper (dewar) validated to hold a consistent temperature (< 130 °C [-130 to -200 °C]) for up to 8 days. Upon receipt, the shipping container was kept sealed and set aside in a secure location until the time of intended dosing (within 48 hours). At the time of administration, the dose was removed from the shipper and the cells were thawed by placing the cryovial in a tube rack at room temperature for approximately 15 minutes or until there was no visible ice left in the vial. Once the cells were thawed, the vial was kept upright and cleaned with an alcohol wipe. The vial containing a target dose of 20 X 10⁶ UMSCs was gently rolled for several seconds to resuspend the cells into solution. A 20-gauge needle attached to a 5-mL polypropylene syringe was inserted through the bottom of the cryovial to slowly draw 0.8 mL of the suspended cell solution into the syringe from the vial. The 0.8 mL of suspended cell solution was then diluted into 4.2 mL of sterile saline (total volume, 5 mL). This process was repeated 3 times to provide a total of 4 syringes for injection with a total injection volume of 20 mL. Each aliquot was slowly injected over 5 minutes (1 mL/min) into a primed T-port attached to a 22-gauge or greater cephalic vein IV catheter, followed by a flush with 0.5 to 1 mL of heparinized saline following each syringe of cells. Three additional aliquots were administered following the same timing protocol as the fresh allogeneic MSCs (10, 20, and 30 minutes).

Monitoring after MSC administration

Heart rate, respiratory rate, mucous membrane color, capillary refill time, body temperature, and changes in mentation or behavior were recorded. The dogs were monitored continuously for 5 minutes after each injection of each aliquot. All dogs were

monitored for an additional 40 minutes after administration of the fourth aliquot of cells and were hospitalized overnight for further monitoring.

Follow-up

The same board-certified neurologist reevaluated each Pug at 24 hours, 2 to 3 weeks, 6 weeks, and every 3 months after MSC administration and was therefore unblinded to the initial neurological examination abnormalities. The NES for each Pug was totaled at each visit and used to create a visual representation of neurological status over time. A second dose of MSCs (MSC-2) was given 2 to 3 weeks after the first dose of MSCs (MSC-1) for the majority of dogs following similarly published protocols²⁷; however, AMSC availability restrictions limited the dosing interval for some dogs in the middle stage of the study (**Supplementary Table S2**). Three Pugs showing regression in NES at 5, 16, and 24 months after initially showing improvement received additional doses of MSCs (MSC-3 and MSC-4). Dogs that showed progression of their NES after additional MSC treatment were rescued with cytarabine arabinoside (CA) at 600 mg/m² divided into two 300-mg/m² SC doses given every 24 hours for 2 days and prednisone (1 mg/kg/d). Cytarabine arabinoside was initially given every 4 weeks, then tapered to every 5 to 6 weeks over 4 to 6 months. Prednisone was tapered over 6 to 8 weeks to a maintenance dose of 0.25 mg/kg every 2 to 3 days, with increases as needed based on clinical signs at rechecks every 4 to 6 weeks.

Results

Two hundred fifty-five Pugs were examined over 42 months from June 2021 through December 2024. Sixteen of 255 Pugs (6.3%) demonstrated a clinical phenotype consistent with early NME as previously described.¹³ The group included 8 spayed females and 8 males (6 castrated, 2 intact). Coat colors included 10 fawn, 3 black, and 1 each of silver, apricot, and white. Thirteen of these dogs were treated with MSCs; follow-up > 12 months is currently available for 11 dogs. Three dogs in the early stage of the screening study were not treated with MSCs, as the AMSCs being used were unavailable at the time of diagnosis (largely because identification of the abnormal clinical phenotype was not anticipated). These dogs were treated with CA and prednisone.

Genetic risk for NME was assessed in 129 dogs (including all 16 abnormal dogs); 13 (10%) had 2 copies of the at-risk haplotype, 48 (37%) had 1 copy of the at-risk haplotype, and 68 (53%) had no copies of the at-risk haplotype. Eleven of 16 (69%) of the neurologically abnormal cohort was at genetic risk for NME (1 at-risk allele in 8 Pugs; 2 at-risk alleles in 3 Pugs); 5 of 16 (31%) were at low genetic risk for NME. Three of the 5 abnormal dogs at low genetic risk were Pug-Chihuahua crosses. Ten genetically high-risk Pugs did not show signs of early NME and were rechecked every 3 months; none developed signs of early or advanced NME during the study follow-up time (12 to 43 months). One hundred

twenty-six Pugs screened at the Pug Dog Club of America national shows did not receive genetic testing with our group as they reported previous testing for NME risk (University of California-Davis Veterinary Genetics Laboratory). Those genetic risk results are not reported here, as breeders/owners did not provide written verification of test results at the time of neurological examination screening; however, the majority reported low genetic risk (N/N using the University of California-Davis reporting system) and none of those Pugs were neurologically abnormal.

Neurological examination findings prior to MSC therapy

At the time of publication, 13 Pugs had been treated with MSCs and 11 had been followed for a minimum of 365 days. Twelve of 13 dogs showed progression of the NES prior to receiving any MSC therapy, and 1 dog remained stable (**Supplementary Table S2**). Neurological abnormalities were consistent within the cohort and included spinal hyperesthesia (11 of 13 [85%]), paw placement deficit (11 of 13 [85%]), menace deficit (9 of 13 [69%]), obtundation (9 of 13 [69%]), seizures (7 of 13 [54%]), and ataxia (4 of 13 [31%]).

Mesenchymal stromal cell tolerability

Tolerability data is presented in **Table 1**. No serious adverse events (AEs) were noted in any of the 13 dogs treated with AMSCs or UMSCs. Mild AEs were noted in 3 dogs, totaling 3 of 30 MSC dose administrations (10%). One dog receiving AMSCs had a single episode of vomiting (resolved after Cerenia, 1 mg/kg, IV), 1 dog receiving AMSCs developed urticaria (resolved with diphenhydramine, 2 mg/kg, IM), and 1 dog receiving UMSCs showed evidence of mild nausea without vomiting (resolved after Cerenia, 1 mg/kg, IV). All observed AEs were reasonably attributed to MSC administration, and all dogs still received the full MSC dose.

Table 1—Intravenous mesenchymal stromal cell (MSC) administration was associated with few adverse events in Pugs with presumptive early necrotizing meningoencephalitis.

Adverse event ^a	AMSCs ^b	UMSCs ^c	Total doses
None	12 (85%)	15 (94%)	23 (88%)
Mild	2 (14%)	1 (6%)	3 (10%)
Moderate	0 (0%)	0 (0%)	0 (0%)
Severe	0 (0%)	0 (0%)	0 (0%)
Total doses	14	16	30

AMSC = Adipose-derived MSCs. UMSCs = Uterine-derived MSCs.
^aAdverse event scoring as described by the Veterinary Cooperative Oncology Group—Common Terminology Criteria for Adverse Events.²⁸ ^bAllogeneic research-grade AMSCs; University of California-Davis Regenerative Medicine Laboratory. ^cAllogeneic pharmaceutical-grade UMSCs; Gallant Therapeutics.

Short-term MSC activity

The short-term response to MSCs was pronounced, with all 13 dogs showing improvement in the NES within 24 hours after MSC-1 (**Figure 1**). The mean change in NES was 6 (range, 2 to 9) and mean percentage of improvement in NES was 86% (range,

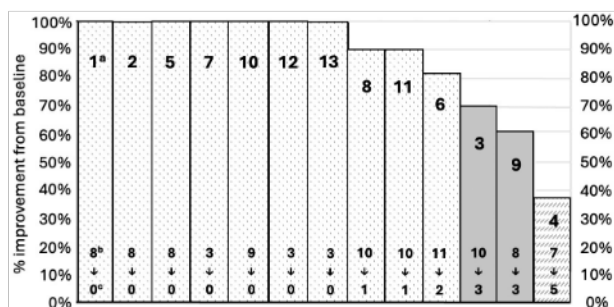


Figure 1—All dogs (numbered 1 through 13) showed an improved neurological examination score (NES) within 24 hours of mesenchymal stem cell (MSC) therapy. Ten of 13 (77%) showed marked improvement (> 75% improvement in NES; dotted bars), 2 of 13 (15%) showed moderate improvement (50% to 75% improvement in NES; filled bars), and 1 of 13 (7.6%) showed mild improvement (< 50% improvement in NES; dashed bars). Changes in the NES before and after the first dose of IV MSC (MSC-1) are listed at the bottom of the bar for each dog. ^aDog number. ^bNES before MSC-1. ^cNES after MSC-1

29% to 100%). Seven dogs (54%) showed 100% improvement in the NES. Twelve dogs (92%) remained improved at the 2-week recheck.

Spinal hyperesthesia, obtundation, and ataxia improved or resolved in all dogs within 24 hours; paw placement and menace deficit improved or resolved in 91% and 88% of dogs, respectively (**Figure 2**). Improvement was sustained for all clinical signs at the 2-week mark after MSC administration.

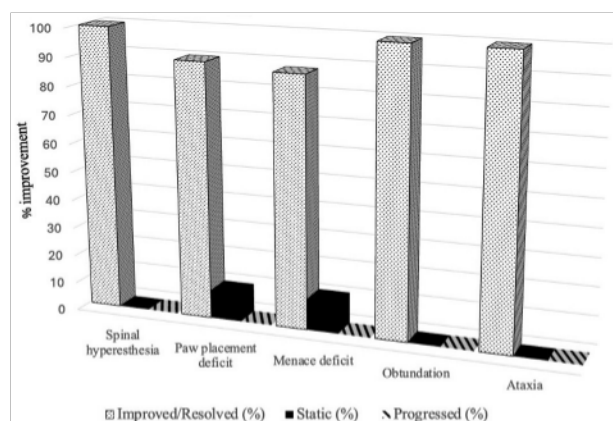


Figure 2—Individual neurological examination abnormalities improved markedly within 24 hours after administration of MSC-1, with improvement or resolution of spinal hyperesthesia, obtundation, and ataxia in 100% of dogs and improvement or resolution of paw placement and menace deficit in 90% of dogs.

Eleven Pugs received MSC-2. Dog 1 did not receive MSC-2 because clinical signs resolved and AMSCs were unavailable at the time of the second dose. Dog 7 did not receive MSC-2 because urticaria had developed during MSC-1 and clinical signs had progressed at the 2-week recheck (Supplementary Table S2). Given the improvement after MSC-1, all other 11 dogs had a lower NES before MSC-2. A similar rapid response was seen within 24 hours

after MSC-2. At the 2-week recheck, improvement or resolution of spinal hyperesthesia was noted in 5 of 8 dogs (63%), paw placement deficits in 5 of 7 dogs (72%), menace deficit in 4 of 4 dogs (100%), and obtundation in 2 of 2 dogs (100%). Dog 4 developed obtundation and a menace deficit and had a higher NES compared to the pretreatment score at the 2-week recheck following MSC-2.

Long-term MSC activity

Seven dogs (54%) showed sustained clinical improvement with no additional therapy needed (**Table 2**; Supplementary Table S2). At the time of publication, 6 of these dogs had shown no relapse of signs; 1 dog relapsed twice at 10 and 24 months but went into remission again after additional doses of MSCs. Four dogs (36%) showed initial marked response to MSCs before showing regression at variable time points (3, 5, 11, and 16 months) following MSC-1. **Supplementary Figure S2** outlines the utility of NESs to track progress in a single dog over time.

Cytarabine arabinoside and prednisone were added in 6 dogs after MSC therapy (range, 15 to 543 days following MSC-1). Clinical signs improved or normalized in all dogs (**Table 2**; Supplementary Table S2).

Seizure control following MSC therapy

Seven of 13 dogs (54%) had seizures in the 2 weeks prior to MSC therapy, with no prior history of seizures and no anticonvulsant therapy prior to study enrollment. Five of those dogs (71%) had no seizures after initiating MSC therapy. Two dogs had no seizures for 3 and 14 months before developing seizures every 1 to 2 weeks that correlated with NES progression, suggesting relapse. Those two dogs received MSC-3 and MSC-4 following the outlined protocol at that time and had no further seizures for 3 and 4 months before relapse of neurological signs and seizures began again every 2 to 4 weeks for both dogs. Both were started on CA, prednisone, and anticonvulsant therapy (zonisamide for one dog, phenobarbital for the other dog) at that time, and neurological signs and seizures resolved. At the time of publication, these dogs were being monitored and received CA every 4 to 6 weeks as described herein.

Magnetic resonance imaging before and after MSCs

Magnetic resonance imaging abnormalities were noted in all 13 Pugs before MSC administration and included patchy T2-weighted hyperintensities suggestive of inflammatory lesions or early necrosis in 10 dogs, multifocal meningeal enhancement in 7 dogs, and focal parenchymal enhancement in 5 dogs. Examples of these MRI abnormalities have been previously reported, with additional examples in **Figures 3 and 4**.^{13,14} A recheck MRI was acquired in 12 dogs at 3 months following MSC-2. Magnetic resonance imaging was not repeated in dog 7, as that dog received CA and prednisone within 3 weeks after MSC-1 due to progression of NES. The MRI lesions at the 3-month recheck were improved in 10 dogs and progressed in 2 dogs, those 2 dogs also showed

Table 2—Summary of long-term response to MSC therapy for each dog (numbered 1 to 13), including total follow-up time to date, time(s) to relapse of signs after first dose of MSC (MSC-1), time to addition of cytarabine arabinoside (CA) after MSC-1, overall percentage of improvement and numerical change in neurological examination score (NES) after MSCs alone, and percentage of improvement and numerical change in NES after addition of CA.

Dog No. ^a	Age/sex/genetic risk (No. of MSC doses)	Total follow-up time (mo)	Time to relapse after MSC-1	Time to add CA ^b	Long-term improvement in NES ^c after MSCs only	Long-term improvement in NES ^c after MSCs and CA
1	1.5 y FS T/A (1)	43	No relapse	NA ^d	75% (8–2) ^e	NA
2	2.5 y FS T/A (4)	43	10 and 24 mo	NA	100% (8–0)	NA
3	6 mo M T/A (2)	43	3 mo	3 mo	60% (10–4)	100% (4–0) ^f
4	1.5 y FS T/T (2)	43	6 wk	6 wk	–43% ^g (7–10)	100% (10–0)
5	1 y FS T/T (2)	38	11 mo	11 mo	12.50% (8–7)	100% (7–0)
6	4 mo F A/A (2)	35	No relapse	NA	100% (11–0)	NA
7	1 y FS T/T (1)	29	2 wk	2 wk	–33% ^g (3–4)	100% (4–0)
8	4 mo M A/A (4)	29	16 mo	18 mo	40% (10–6)	100% (6–0)
9	5 y MC T/A (2)	19	No relapse	NA	100% (8–0)	NA
10	4 y MC A/A (4)	17	5 mo	8 mo	33% (9–6)	100% (6–0)
11	4 mo M A/A (2)	12	No relapse	NA	100% (11–0)	NA
12	1 y MC T/A (2)	5	No relapse	NA	100% (3–0)	NA
13	3 y MC T/A (2)	5	No relapse	NA	100% (3–0)	NA

A/A = No genomic risk alleles. F = Female. FS = Female spayed. M = Male. MC = Male castrated. NA = Not applicable. T/A = Presence of 1 at-risk allele. T/T = Presence of 2 at-risk alleles.

^aDog number as seen in Figure 1. ^bReceived CA and prednisone at the time of regression. ^cNeurological examination score, as outlined in Supplementary Table S1. ^dCytarabine arabinoside not added. ^eNeurological examination score before MSC therapy and long-term follow-up score or score at time of conversion to CA. ^fNeurological examination score after MSC therapy before converting to CA and long-term follow-up score after starting CA. ^gA negative percentage indicates that the NES ultimately progressed after MSC therapy.

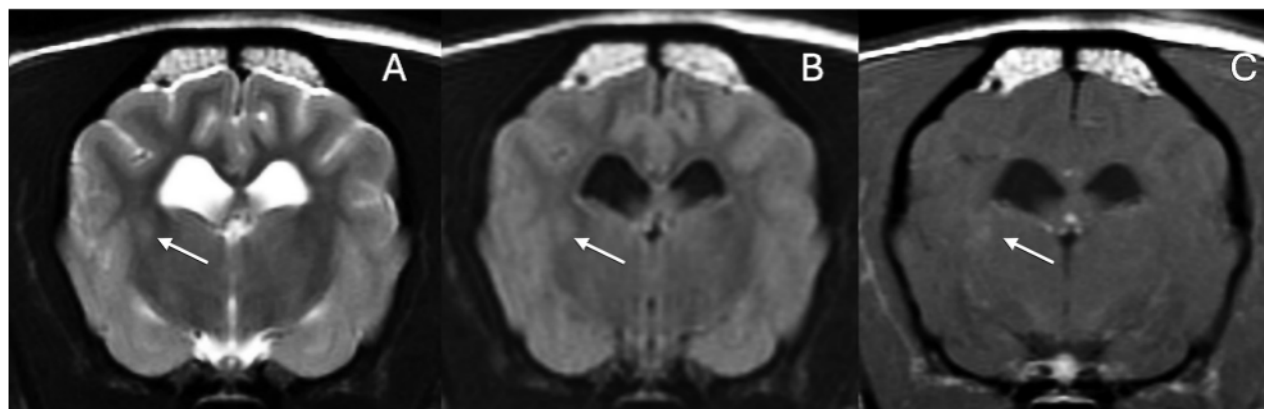


Figure 3—Example of an MRI lesion potentially consistent with early necrotizing meningoencephalitis. T2-weighted (A), FLAIR (B), and T1-weighted postcontrast (C) axial MRI images from dog 10, a 4-year-old castrated male Pug with seizures. The white arrows indicate a suspect inflammatory lesion.

progression of the NESs. Magnetic resonance imaging was rechecked again in 2 dogs: at the time of first relapse at 16 months in one dog, which showed a progressive lesion, and second relapse at 24 months in the other dog (MRI lesions still improved).

Cerebrospinal fluid before and after MSCs

Cerebrospinal fluid abnormalities were noted in 8 of 13 dogs (62%). Lymphocytic or mononuclear pleocytosis (defined as > 3 total nucleated cells/ μ L for a cisternal sample)²⁹ was noted in 7 of 13 dogs (54%; median, 5 cells/ μ L; range, 4 to 9 cells/ μ L), and elevated protein (defined as $\geq$ 25 g/dL) was noted

in 4 of 13 dogs (31%; median, 26.9 g/dL; range, 25.4 to 34.2 g/dL). Cerebrospinal fluid analysis was repeated in 12 dogs at 3 months following MSC-1. Cerebrospinal fluid recheck at 3 months was static or improved in 9 of 12 dogs (75%) but showed a progressive pleocytosis in 2 dogs and increase in protein in 1 dog (both dogs also showed regression or progression of NES). Cerebrospinal fluid was rechecked at the time of the first relapse at 16 months in dog 8 (WBCs, 2 cells/ μ L; protein, 22 g/dL) and at second relapse at 24 months in dog 2 (WBCs, 18 cells/ μ L); the CSF in dog 2 was normal at the 2-week recheck following MSC-3 (WBCs, 1 cell/ μ L).

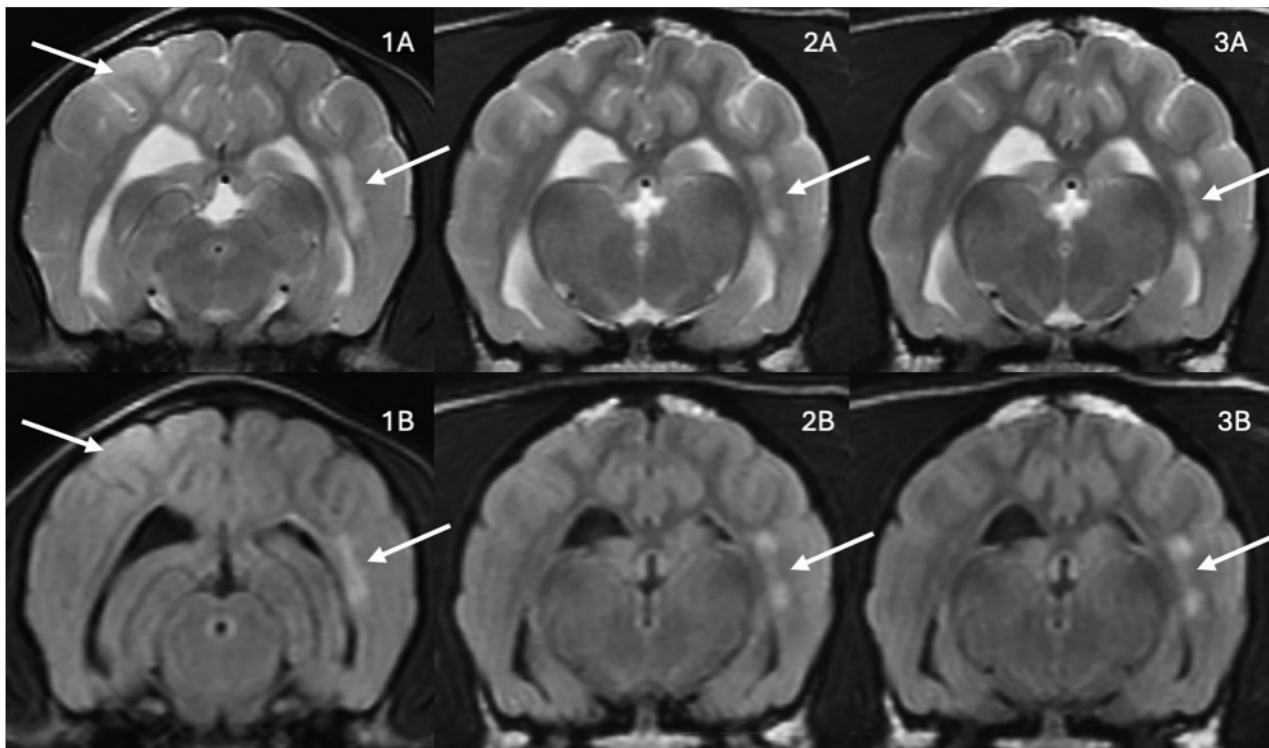


Figure 4—Serial MRI showing improvement in the lesions after MSC therapy. T2-weighted (A images) and FLAIR (B images) axial MRI images of dog 8, a 4-month-old male Pug, taken at the time of diagnosis (1A and 1B), 5 months after MSC-1 (2A and 2B), and at the time of the first relapse 16 months after MSC-1 (3A and 3B). The white arrows indicate a suspect inflammatory lesion.

Discussion

We demonstrated that IV allogeneic MSCs were well tolerated and associated with clinical benefits within 24 hours of administration. However, long-term duration of MSC activity was more varied between dogs. No dog experienced a serious AE, and the mild AEs observed were brief and resolved with treatment when needed. The results of this pilot study suggest that IV MSC therapy warrants further exploration in dogs with NME and other forms of neuroinflammatory disease.

It remains unclear whether the identified cohort of Pugs had an early form of NME or a previously unrecognized, more subtle immune-mediated neuroinflammatory disease with unknown natural history. The progression of neurological abnormalities over weeks prior to treatment and immediate MSC activity in all dogs suggests that spontaneous remission was unlikely. Nonetheless, further studies of this presumed early presentation of NME are needed. Several inflammatory conditions including NME in dogs resemble parts of human disease, suggesting an opportunity to study MSCs in dogs for the purpose of human translation.^{30,31} Mesenchymal stem cell administration has demonstrated promising results in murine models of experimental autoimmune encephalitis, the most common animal model of MS.^{17,32,33} The strong breed predisposition and narrow age of onset of NME in the Pug allows for early screening and therapeutic intervention and prevention studies in a naturally occurring

rather than experimentally induced disease (such as experimental autoimmune encephalitis).³¹ Diagnostic monitoring with repeat MRI, CSF, and biomarker analysis is more feasible in the dog than the rodent but also more costly.

Intravenous administration was immediately effective in all dogs. The mechanisms for this observed therapeutic benefit are unclear and deserve further study. It is reasonable that MSCs may exert a local immunomodulatory benefit after homing to diseased brain tissue or may reduce systemic inflammatory mediators without homing to the brain. More knowledge on these mechanisms may allow rational iteration in the optimal route of administration (ie, intrathecal [IT] versus IV) and the optimal schedule/regimen to achieve sustained or curative modulation of neuroinflammation.^{16,17} The immunosuppressive effects of MSCs may have stalled disease progression in some dogs, but the time to and severity of regression was variable. Further research is necessary to understand long-term responder and nonresponder differences.

This small pilot study did not allow us to compare tolerability or therapeutic benefit between allogeneic AMSCs and UMSCs. Mesenchymal stromal cells derived from various sources have identical morphologies and surface markers; however, differences in differentiation capacity and immunomodulatory processes are still being explored.³⁴ Unlike adipose-derived stromal cells, uterine-derived stem cells contain 2 populations of stromal cells—stromal and epithelial—and may have a varied potency and efficacy

for certain indications. Adverse events with these allogeneic MSCs were uncommon (mild AEs with 10% of MSC doses). Allogeneic MSCs exhibit low levels of MHC class I molecules and rarely express cell surface MHC class II, allowing them to avoid T cell recognition. Allogeneic MSC administration has benefits over autologous MSC administration, which requires harvesting and culturing fresh MSCs from a sick individual (with potentially less healthy or viable MSCs) that may need MSC therapy more urgently than the 2 to 3 weeks necessary to prepare autologous MSCs.³⁵

A previous study³⁶ used IT and intra-arterial or IT and IV autologous MSCs in a small group of dogs with refractory meningoencephalitis of unknown origin with promising results. Although IT administration of MSCs appears safe and can impose more direct effects on the CNS, several complications including meningeal injury, aseptic meningitis, acute encephalomyelitis, and seizures have been reported in humans.¹⁶ Intravenous MSC doses of 1×10^6 to 5×10^6 cells/kg of body weight are typically recommended in humans to allow adequate brain penetration.^{16,21,37} Our dose averaged to be approximately 2×10^6 cells/kg in a Pug-sized dog. Further studies are needed to determine the optimal MSC dose.

From our limited clinical experience thus far, we recommend repeat MSC dosing at the time of clinical regression rather than automatically redosing at 2 to 3 weeks. Repeated dosing of MSCs is well tolerated, with no apparent immunologic factors affecting continued efficacy. Repeated MSC administration is often necessary to achieve optimal results in people, but the exact timing of these repeated doses is unknown and likely dependent on the individual.¹⁶ Pilot MSC therapy trials in animals with other inflammatory conditions have shown efficacy with a single MSC dose.³⁸ We may have achieved longer remission in dogs 3 and 5 using MSC therapy alone with repeated dosing; however, limited availability of AMSCs in the early phase of the study restricted redosing options. The frozen allogeneic UMSC product used in the latter part of the study was readily available, allowing us to repeat MSC dosing quickly as needed. Future larger-scale trials are necessary to determine optimum dosing and timing of MSC administration and how MSCs can be used with other therapies to control disease.

Disease-specific biomarker analysis would be ideal to guide MSC administration in future studies. A reduction in peripheral blood proinflammatory cytokines and an increase in anti-inflammatory cytokines have been described in humans with MS following MSC therapy, and a shift toward a proinflammatory cytokine signature has been documented at the time of clinical relapse.³⁹ It is possible that similar fluctuations occur in Pugs with presumptive early NME, and MSC therapy may shift toward an anti-inflammatory state. In a previous study,²⁴ we identified lower CD4⁺ T cell and higher IL-10 plasma levels in genetically at-risk Pugs that did not develop NME, suggesting that this anti-inflammatory signature may have been protective. In a subsequent study (R Windsor DVM, DACVIM, unpublished data, February 2023), we did not find a consistent proinflammatory cytokine signature in Pugs with presumptive early NME;

however, other cytokines including IL-17 and other different systemic biomarkers should be explored.⁴⁰

The expanded disability status scale was established in humans with MS to quantify degree of disability and is used to monitor response to MSCs and other therapies. We started using the NES as our nonvalidated visualization system to monitor patient progression in 2021. In 2023, a similar scoring system called the neurodisability scale was published that has subsequently been used to compare neurological examination abnormalities in dogs with meningoencephalitis of unknown origin.^{41,42} Creation of the NES and neurodisability scale highlights the need for a validated neurological examination scoring system to allow for comparison of neurological examination abnormalities associated with various inflammatory conditions across clinicians. While repeated neurological examinations by a single observer enhances consistency, unblinded evaluations may contain inherent bias. Although examinations were performed multiple times during each visit, dogs with stable disease may still exhibit fluctuations in their neurological status reflected in the NES.

Four dogs in this cohort were at low genetic risk for NME, 3 of which were Pug-Chihuahua crosses. Necrotizing meningoencephalitis has been reported in Chihuahuas,¹⁰ and the genetic risk loci in crossbreeds likely differ from purebred Pugs or Chihuahuas.⁴³ Our assessment of genetic risk for NME using the currently identified loci in Pugs suggests there may be additional genetic risk loci, and genetic risk may vary geographically. Ten of 13 Pugs at high genetic risk in our studies did not develop signs of NME over a 3-year follow-up period. Genetic risk is only 1 factor in determining overall NME risk and may not factor into therapeutic response. A more extensive description of the genetic risk analysis from our larger cohort of dogs will follow.

The ideal optimal MSC dose, schedule, and regimen remain elusive and warrant a larger blinded clinical trial. Given that NME is a relatively uncommon disease, case enrollment for this study was slow. Large multicenter prospective studies are necessary to evaluate the use of MSCs for NME and other immune-mediated neuroinflammatory diseases to achieve any reliable assessment of long-term efficacy in a large group of dogs. We evaluated MSC therapy early in the course of the disease; the efficacy of MSCs for more advanced NME is unknown.

Our study demonstrated that IV allogeneic MSC administration was well tolerated and immediately effective in all Pugs with presumptive early NME. Long-term MSC activity was variable; however, nearly half of the dogs were markedly improved, with a durable response of 12 to 43 months after MSC therapy alone. The results of this pilot study support future studies of MSCs for neuroinflammatory diseases in dogs and suggest that the dog may be a useful animal model for humans with MS.

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References

1. Sawcer S, Hellenthal G, Pirinen M, et al; International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011;476(7359):214–219. doi:10.1038/nature10251
2. Barber RM, Schatzberg SJ, Corneveaux JJ, et al. Identification of risk loci for necrotizing meningoencephalitis in Pug dogs. *J Hered*. 2011;102(suppl 1):S40–S46. doi:10.1093/jhered/esr048
3. Pedersen N, Liu H, Millon L, Greer K. Dog leukocyte antigen class II-associated genetic risk testing for immune disorders of dogs: simplified approaches using Pug dog necrotizing meningoencephalitis as a model. *J Vet Diagn Invest*. 2011;23(1):68–76. doi:10.1177/104063871102300110
4. Greer KA, Wong AK, Liu H, et al. Necrotizing meningoencephalitis of Pug dogs associates with dog leukocyte antigen class II and resembles acute variant forms of multiple sclerosis. *Tissue Antigens*. 2010;76(2):110–118. doi:10.1111/j.1399-0039.2010.01484.x
5. Levine JM, Fosgate GT, Porter B, Schatzberg SJ, Greer K. Epidemiology of necrotizing meningoencephalitis in Pug dogs. *J Vet Intern Med*. 2008;22(4):961–968. doi:10.1111/j.1939-1676.2008.0137.x
6. Siddiqui N, Sharma R, Bell D, et al. Marburg variant of multiple sclerosis. Reference article. Radiopaedia.org. doi:10.5334/rID-12276
7. Anson D, Bartnik N, Shamim D, Wang Y. Rapidly progressive demyelinating disease secondary to the Marburg variant of multiple sclerosis: a case report (P9-4.004). *Neurology*. 2022;98(18 suppl):1272. doi:10.1212/WNL.98.18_supplement.1272
8. Capet N, Levraut M, Delourme A, et al. Marburg multiple sclerosis variant: complete remission with very early administration of mitoxantrone: a case report. *Neurol Ther*. 2022;11(1):507–513. doi:10.1007/s40120-021-00308-6
9. Flegel T. Breed-specific magnetic resonance imaging characteristics of necrotizing encephalitis in dogs. *Front Vet Sci*. 2017;4:203. doi:10.3389/fvets.2017.00203
10. Higgins RJ, Dickinson PJ, Kube SA, et al. Necrotizing meningoencephalitis in five Chihuahua dogs. *Vet Pathol*. 2008;45(3):336–346. doi:10.1354/vp.45-3-336
11. Flegel T, Henke D, Boettcher IC, Aupperle H, Oechtering G, Matiassek K. Magnetic resonance imaging findings in histologically confirmed Pug dog encephalitis. *Vet Radiol Ultrasound*. 2008;49(5):419–424. doi:10.1111/j.1740-8261.2008.00400.x
12. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple sclerosis: mechanisms and immunotherapy. *Neuron*. 2018;97(4):742–768. doi:10.1016/j.neuron.2018.01.021
13. Windsor R, Stewart S, Schmidt J, et al. A potential early clinical phenotype of necrotizing meningoencephalitis in genetically at-risk pug dogs. *J Vet Intern Med*. 2022;36(4):1382–1389. doi:10.1111/jvim.16444
14. Windsor R, Stewart S, Huentelman M, Keller S, Khanna C. An early clinical phenotype of necrotizing meningoencephalitis in the Pug reveals similarities to multiple sclerosis in humans. *Am J Vet Res*. 2023;85(1):ajvr.23.07.0164. doi:10.2460/ajvr.23.07.0164
15. Ransohoff RM. Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat Neurosci*. 2012;15(8):1074–1077. doi:10.1038/nn.3168
16. Barati S, Tahmasebi F, Faghihi F. Effects of mesenchymal stem cells transplantation on multiple sclerosis patients. *Neuropeptides*. 2020;84:102095. doi:10.1016/j.npep.2020.102095
17. Sherman LS, Romagano MP, Williams SF, Rameshwar P. Mesenchymal stem cell therapies in brain disease. *Semin Cell Dev Biol*. 2019;95:111–119. doi:10.1016/j.semdb.2019.03.003
18. Ludwig A. Marburg's variant of multiple sclerosis with extensive brain lesions: an autopsy case report. *Int J Neurol Neurother*. Published online April 10, 2015. doi:10.23937/2378-3001/2/1/1027
19. Smith JA, Nicaise AM, Ionescu RB, Hamel R, Peruzzotti-Jametti L, Pluchino S. Stem cell therapies for progressive multiple sclerosis. *Front Cell Dev Biol*. 2021;9:696434. doi:10.3389/fcell.2021.696434
20. Park ES, Uchida K, Nakayama H. Comprehensive immunohistochemical studies on canine necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE), and granulomatous meningoencephalomyelitis (GME). *Vet Pathol*. 2012;49(4):682–692. doi:10.1177/0300985811429311
21. Petrou P, Kassis I, Levin N, et al. Beneficial effects of autologous mesenchymal stem cell transplantation in active progressive multiple sclerosis. *Brain*. 2020;143(12):3574–3588. doi:10.1093/brain/awaa333
22. Zhou Y, Zhang X, Xue H, Liu L, Zhu J, Jin T. Autologous mesenchymal stem cell transplantation in multiple sclerosis: a meta-analysis. *Stem Cells Int*. 2019;2019:8536785. doi:10.1155/2019/8536785
23. Tremblay F, Ansari Y, Remaud A, Freedman MS. Neurophysiological outcomes following mesenchymal stem cell therapy in multiple sclerosis. *Clin Neurophysiol*. 2022;136:69–81. doi:10.1016/j.clinph.2022.01.125
24. Windsor R, Stewart SD, Talboom J, et al. Leukocyte and cytokine variables in asymptomatic Pugs at genetic risk of necrotizing meningoencephalitis. *J Vet Intern Med*. 2021;35(6):2846–2852. doi:10.1111/jvim.16293
25. Kol A, Foutouhi S, Walker NJ, Kong NT, Weimer BC, Borjesson DL. Gastrointestinal microbes interact with canine adipose-derived mesenchymal stem cells in vitro and enhance immunomodulatory functions. *Stem Cells Dev*. 2014;23(16):1831–1843. doi:10.1089/scd.2014.0128
26. Chung DJ, Hayashi K, Toupadakis CA, Wong A, Yellowley CE. Osteogenic proliferation and differentiation of canine bone marrow and adipose tissue derived mesenchymal stromal cells and the influence of hypoxia. *Res Vet Sci*. 2012;92(1):66–75. doi:10.1016/j.rvsc.2010.10.012
27. Black L, Zacharias S, Hughes M, Bautista R, Taechangam N, Sand T. The effect of uterine-derived mesenchymal stromal cells for the treatment of canine atopic dermatitis: a pilot study. *Front Vet Sci*. 2022;9:1011174. doi:10.3389/fvets.2022.1011174
28. LeBlanc AK, Atherton M, Bentley RT, et al. Veterinary Cooperative Oncology Group—Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) following investigational therapy in dogs and cats. *Vet Comp Oncol*. 2021;19(2):311–352. doi:10.1111/vco.12677
29. Bailey CS, Vernau W. Cerebrospinal fluid. In: Kaneko JJ, Harvey JW, Bruss ML, eds. *Clinical Biochemistry of Domestic Animals*. Elsevier; 1997:785–827. doi:10.1016/B978-012396305-5/50028-2
30. Gugjoo MB, Amarpal A, Sharma GT. Mesenchymal stem cell basic research and applications in dog medicine. *J Cell Physiol*. 2019;234(10):16779–16811. doi:10.1002/jcp.28348
31. Kol A, Walker NJ, Nordstrom M, Borjesson DL. Th17 pathway as a target for multipotent stromal cell ther-

- apy in dogs: implications for translational research. *PLoS One*. 2016;11(2):e0148568. doi:10.1371/journal.pone.0148568
32. Hu H, Li H, Li R, Liu P, Liu H. Re-establishing immune tolerance in multiple sclerosis: focusing on novel mechanisms of mesenchymal stem cell regulation of Th17/Treg balance. *J Transl Med*. 2024;22(1):663. doi:10.1186/s12967-024-05450-x
 33. Brown C, McKee C, Halassy S, Kojan S, Feinstein DL, Chaudhry GR. Neural stem cells derived from primitive mesenchymal stem cells reversed disease symptoms and promoted neurogenesis in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis. *Stem Cell Res Ther*. 2021;12(1):499. doi:10.1186/s13287-021-02563-8
 34. Costela-Ruiz VJ, Melguizo-Rodríguez L, Bellotti C, et al. Different sources of mesenchymal stem cells for tissue regeneration: a guide to identifying the most favorable one in orthopedics and dentistry applications. *Int J Mol Sci*. 2022;23(11):6356. doi:10.3390/ijms23116356
 35. Figueroa FE, Carrión F, Villanueva S, Khoury M. Mesenchymal stem cell treatment for autoimmune diseases: a critical review. *Biol Res*. 2012;45(3):269–277. doi:10.4067/S0716-97602012000300008
 36. Zeira O, Asiag N, Aralla M, et al. Adult autologous mesenchymal stem cells for the treatment of suspected non-infectious inflammatory diseases of the canine central nervous system: safety, feasibility and preliminary clinical findings. *J Neuroinflammation*. 2015;12(1):181. doi:10.1186/s12974-015-0402-9
 37. Galipeau J, Sensébé L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell Stem Cell*. 2018;22(6):824–833. doi:10.1016/j.stem.2018.05.004
 38. Febre M, Maddens S, Robert C, Rakic R, Plantier N, Saulnier N. Single infusion of allogeneic neonatal mesenchymal stromal cells to manage refractory feline gingivostomatitis: a clinical pilot study. *Cytotherapy*. 2020;22(5 suppl):S102. doi:10.1016/j.jcyt.2020.03.182
 39. Hendrawan K, Khoo MLM, Visweswaran M, et al. Long-term suppression of circulating proinflammatory cytokines in multiple sclerosis patients following autologous haematopoietic stem cell transplantation. *Front Immunol*. 2022;12:782935. doi:10.3389/fimmu.2021.782935
 40. Barber R, Barber J. Differential T-cell responses in dogs with meningoencephalomyelitis of unknown origin compared to healthy controls. *Front Vet Sci*. 2022;9:925770. doi:10.3389/fvets.2022.925770
 41. Gonçalves R, De Decker S, Walmsley G, Maddox TW. Prognosis in meningoencephalitis of unknown origin in dogs: risk factors associated with survival, clinical relapse, and long-term disability. *J Vet Intern Med*. 2024;38(3):1583–1590. doi:10.1111/jvim.17037
 42. Gonçalves R, Maddox TW, Philipps S, et al. Development of a reliable clinical assessment tool for meningoencephalitis in dogs: the neurodisability scale. *J Vet Intern Med*. 2023;37(3):1111–1118. doi:10.1111/jvim.16717
 43. Oshima A, Ito D, Katakura F, et al. Dog leukocyte antigen class II alleles and haplotypes associated with meningoencephalomyelitis of unknown origin in Chihuahuas. *J Vet Med Sci*. 2023;85(1):62–70. doi:10.1292/jvms.22-0116

Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.