pubvet
MEDICINA VETERINÁRIA E ZOOTECNIA
ISSN 1982-1263
https://doi.org/10.31533/pubvet.v18n10e1672

# Mesenchymal stem cell treatment for feline medullary aplasia: Case report

**Rafaela Cassaniga[1]*, Roberta Cassaniga[1], Mariana Aimee Ramos Xavier da Silva[2], Edson Guimarães Lo Turco[3]**

*[1]Veterinarian specialized in General Surgery and Oncology, Electrochemotherapy, Immunology, Oncology, Geriatrics, Regenerative Medicine, Stem Cells, Integrative Medicine, and Wildlife Medicine. Graduate degree completed at Anclivepa, Brazil.*
*[2]Veterinarian with a graduate degree in Acupuncture from Qualittas, specializing in Public Health from UNESP Botucatu, Physiatry, and Ozone Therapy. Brazil.*
*[3]Scientific Director of the MedMep Cell Technology Center, São Paulo, São Paulo. Advisor to the Postgraduate Program of the Discipline of Urology EPM/UNIFESP. M.Sc. in Animal Reproduction from UNESP. PhD in Human Reproduction from the Federal University of São Paulo, Brazil.*
**Author for correspondence, email: rcassaniga@hotmail.com*

**Abstract.** Treatment with mesenchymal stem cells (MSC) is one of the emerging therapies in regenerative medicine. Administered intraosseously to access the bone marrow, it represents a promising treatment modality for both degenerative and non-degenerative diseases, such as medullary aplasia. This condition is characterized by pancytopenia and involves the replacement of hematopoietic tissue by pathological tissue. Medullary aplasia can be acute or chronic and may be caused by infections, neoplasms, drugs, toxins, radiation, or be idiopathic when other causes are excluded. Diagnosis necessitates a comprehensive hematopathological investigation. This paper reports the case of a female feline treated in July 2020, of no defined breed, neutered, aged two years and eight months, who presented with nonspecific clinical signs, including anorexia, apathy, and pallor of the mucous membranes. The presumptive diagnosis of medullary aplasia was made based on a thorough review of the patient's history and supportive diagnostic tests, which included hematological tests showing recurrent pancytopenia (anemia, thrombocytopenia, lymphopenia), ultrasounds, serum biochemistry, and positive serology for Feline Leukemia (FeLV); in addition to interventions like blood transfusions and prescribed treatments. Treatment with stem cells provided by the PET MEP Laboratory was administered intraosseously, resulting in symptomatic improvement and expedited control of hematological parameters. In conclusion, intraosseous stem cell therapy can serve as a supportive treatment for medullary aplasia, as demonstrated in this feline, who achieved excellent results. However, continued specialized veterinary monitoring is imperative, assessing the necessity for ongoing application maintenance.

**Keywords:** Medullary aplasia, mesenchymal stem cells, regenerative medicine

## Introduction

As a cornerstone of regenerative medicine, Jeung et al. (2024) elucidate that stem cell therapy has primarily been utilized to address cell damage and treatment-resistant diseases in dogs and cats (Kang & Park, 2020). The objective is to enhance treatments for various diseases that may benefit from such interventions. The application of stem cells contributes significantly to the quality of life, supports, or becomes the primary treatment choice in veterinary medicine.

In the field of hematology, the clinical routine initially employs blood transfusion to repair tissue lesions, enhance oxygen transport, and reconstitute hemostasis. The subsequent phase involves cell therapy using stem cells, which are categorized into two groups: hematopoietic stem cells (HSC), originating from the hematopoietic environment located within the bone marrow cavities of bones,

tissues, and organs (spleen, liver, lymph nodes, and thymus), and stromal or mesenchymal stem cells (MSC), which are found in various body tissues such as adipose tissue, liver, dental pulp, blood vessels, skeletal muscle, pancreas, skin, digestive epithelium, cornea, retina, and brain. These cells have the capacity to reconstitute a diseased or damaged system and restore tissue functionality (Brito et al., 2010; Heckler, 2014; Olsson, 2020).

MSCs are defined as undifferentiated cells characterized by their high potential for proliferation, self-renewal, and differentiation into various cell types, such as osteoblasts, chondrocytes, adipocytes, and cardiomyocytes. They are ubiquitous throughout the body, playing crucial roles in maintaining homeostasis and repairing tissues throughout an animal's life (Malard et al., 2020; Monteiro et al., 2010; Paim et al., 2021; Santos, 2018; Santos et al., 2019). When introduced into the body, they adopt both the morphology and functionality of the damaged cells to restore the injured tissue. This capability is attributed to the plethora of bioactive molecules they release, which modulate inflammatory responses, angiogenesis, and the mitosis of cells involved in tissue repair (Jeung et al., 2024). Additionally, MSCs can exhibit antitumor effects by inhibiting tumor growth and enhancing the efficacy of chemotherapeutic agents (Ghasempour et al., 2022; Jeung et al., 2024).

Studies have demonstrated that substances injected into the bone marrow are immediately dispersed into the systemic circulation. This is also applicable to the administration of various blood products, solutions, and pharmacological agents in emergency scenarios, as well as for the collection of materials for cyto- and histopathological examinations and transplants. Such materials can be obtained from the interiors of long bones, like the greater trochanter of the femur and the greater tuberosity of the humerus (Müller et al., 2009).

Moraes & Takahira (2010) describe medullary aplasia as characterized by pancytopenia in peripheral blood and hypoplasia of the three cell types (erythroid, myeloid, and megakaryocytic) in the bone marrow, which leads to the replacement of hematopoietic tissue by pathological tissue. The disease is classified as acute or chronic based on the presentation and evolution of cytopenias in peripheral blood and medullary hypoplasia. Despite conventional treatments and specific therapies related to the primary cause, the treatment for medullary aplasia remains limited and generally carries an unfavorable prognosis. Therefore, the justification for this report is to demonstrate alternative support within regenerative medicine for treating feline medullary aplasia, which has been showing excellent results (Biezus et al., 2019).

The clinical manifestations caused by the feline leukemia virus (FeLV) can be nonspecific and asymptomatic; however, research by Biezus et al. (2019) also demonstrated the oncogenic potential of the virus, capable of inducing the appearance of neoplasms. They highlighted that FeLV can cause dysplastic changes in the bone marrow. Furthermore, the immunosuppression induced by FeLV is notably complex, leading to profound lymphoid atrophy and suppression of the immune system. This, in turn, may result in severe chronic infections in the cutaneous region.

## Case report

In July 2020, FULLVET in Bertioga, São Paulo, Brazil, treated SARA, a two-year and eight-month-old neutered female feline of no defined breed, weighing 2.5 kg, who presented with recurrent nonspecific clinical manifestations, including anorexia, apathy, and pallor of all mucous membranes (ocular, oral, genital, and nasal). The feline's body temperature was 37°C, and she had a history of chronic anemia that oscillated to pancytopenia, also exhibiting thrombocytopenia. This condition was confirmed by successive blood tests, total abdominal ultrasound, and serum hematological biochemistry (urea, creatinine, ALT, FA, glycemia, and others). On June 26, 2020, she tested positive for Feline Leukemia Virus (FeLV) through an ELISA serological test. She had received numerous blood transfusions and undergone various treatments, including corticosteroids (prednisolone, 1 mg/kg continuously), antibiotics (doxycycline, 10 mg/kg for 40 days), nutraceuticals (Erythros Cat®, 1 gram daily, continuous use), and others, including omeprazole (2 mg/kg), domperidone (0.45 mg/kg), and erythropoietin (4000 IU, 0.2 mL daily for 10 days). While she was responsive to treatment, the relapses remained significant. Ultrasound scans revealed no significant pathological findings, and the anatomical structures in the abdominal cavity were preserved.

The owner reported that, in mid-May 2019, Sara had presented with a similar condition and was treated under the diagnosis of Hemoparasitosis, as a Polymerase Chain Reaction (PCR) test for FeLV had returned negative at that time. Despite this, she exhibited anemia and thrombocytopenia and had been given blood transfusions on April 11, 2019, March 5, 2020, and May 12, 2020.

Given Sara's severe chronic condition—characterized by anemia, thrombocytopenia, and lymphopenia—evident in hematological tests, a presumptive diagnosis of Medullary Aplasia was made. This diagnosis, alongside positive FeLV serology, ultrasound and radiological images, clinical symptoms, and differential diagnoses, led to the recommendation of the Medullary Aplasia Protocol from the PET MEP Biotechnology Laboratory. This protocol involved four intravenous applications tailored for corticosteroid users, aimed at improving Sara's quality of life and serving as a support tool for managing her hematological and immunological condition caused by FeLV, with the possibility of future splenectomy surgery.

The first treatment, administered intravenously on July 17, 2020, was preceded by hematological parameters collected on June 6, 2020, which showed a hematocrit of 38%, erythrocytes of 7.55 x $10^6$/uL, hemoglobin at 12.18 g/dL, lymphocytes at 4488/uL, and platelets at 303 x $10^3$/$mm^3$. On July 23, 2020, the hematocrit remained at 38%, erythrocytes increased to 8.44 x $10^6$ /uL, hemoglobin measured at 11.5 g/dL, lymphocytes dropped to 1677/uL, platelets increased to 450 $10^3$/$mm^3$, and the absolute reticulocyte count was 8440 $10^3$/uL (Table 1). However, there was no expected positive hematological effect following this treatment.

The second treatment, administered on August 12, 2020, was also intravenous. At this point, corticosteroid therapy had been indefinitely suspended; however, following this treatment, the animal experienced a severe anemia crisis accompanied by dyspnea, necessitating emergency hematological support, including a blood transfusion and hospitalization. After evaluating the results from tests conducted following this treatment, the clinical presentation and laboratory findings did not reflect the anticipated improvements, leading to a renewed recommendation for splenectomy. In the interim, a protocol was instituted from September 2020 to July 2023, consisting of chlorambucil (2 mg/cat for an indefinite period) combined with prednisolone (2 mg/kg every 24 hours), which was then reduced to 1 mg/kg for 10 days and subsequently to 0.5 mg/kg for an indefinite period under the guidance of an oncologist.

On August 19, 2020, hematological parameters were as follows: hematocrit 25%, erythrocytes 5.27 x $10^6$ /uL, hemoglobin 7.18 g/dL, lymphocytes 2340/uL, and platelets 900 x $10^3$/$mm^3$. By August 26, 2020, these values had deteriorated, with the hematocrit at 13%, erythrocytes 4.2 x $10^6$ /uL, hemoglobin 7.1 g/dL, lymphocytes 1890/uL, and platelets 610 x $10^3$/$mm^3$. As a result, a packed red blood cell transfusion was performed on September 12, 2020, when the hematocrit had dropped further to 11%, erythrocytes to 1.84 x $10^6$ /uL, and hemoglobin to 3.1 g/dL (Table 1).

A subsequent blood transfusion was administered on September 30, 2020, following test results from September 28, 2020, which indicated: hematocrit 15%, erythrocytes 3.3 x $10^6$ /uL, hemoglobin 4.7 g/dL, lymphocytes 2780/uL, platelets 171 x $10^3$/$mm^3$, and an absolute reticulocyte count of 3.3 x $10^3$/uL. Consequently, a splenectomy and a third intraosseous treatment were indicated. The patient underwent general anesthesia to facilitate the intraosseous stem cell treatment, which was delivered via an intraosseous catheter into the bone marrow region. After these interventions, on October 5, 2020, the animal's hematocrit improved to 22.6%, erythrocytes to 4.7 x $10^6$/uL, hemoglobin to 6.6 g/dL, lymphocytes to 2140/uL, platelets to 119 x $10^3$/$mm^3$, and an absolute reticulocyte count of 12.1 x $10^3$/uL (Table 1).

The third treatment was administered on October 14, 2020. Due to the hematological risk, corticosteroid therapy was not suspended. The procedure was performed under general anesthesia, using the following protocol: ketamine (15 mg/kg), midazolam (0.2 mg/kg), tramadol (4 mg/kg), amoxicillin (22 mg/kg), dipyrone (25 mg/kg), and maropitant (Cerenia®, 1 mg/kg). The intraosseous access sites were shaved and antiseptically treated with 2% chlorhexidine and 5% alcohol. Sterile drapes were placed, followed by intraosseous access to the femur (Figure 1A), using a 20-gauge Nipro catheter (the soft part of the catheter was discarded, and only the needle was used). A total of 0.25 mL of the stem cell solution was administered at each access point. Additionally, 0.1 mL was administered epidurally between the vertebrae at the lumbar and iliac crests, and 0.1 mL was administered intravenously (Figure 1B).

**Table 1.** Hematological monitoring of the SARA case. IDEXX Laboratories, Brazil.

| Date | Hematocrit, % | Red blood cells, $10^6$/uL | Hemoglobin, g/dL | Absolute reticulocytes, $10^3$/uL | Lymphocytes, uL | Platelets, $10^3$/mm$^3$ |
|---|---|---|---|---|---|---|
| 10/28/2019 | 11 | 1.84 | 3.1 | - | 2592 | 288 |
| 11/1/2019 | 11 | 1.71 | 3.23 | - | 2622 | 328 |
| 11/11/2019 | 20 | 3.64 | 6.52 | - | 3519 | 335 |
| 11/22/2019 | 26 | 4.77 | 8 | - | 1716 | 301 |
| 12/6/2019 | 30 | 5.87 | 9.87 | - | 2112 | 405 |
| 4/30/2020 | 12 | 2.04 | 3.32 | - | 2770 | 332 |
| 5/2/2020 | 10 | 1.82 | 3.05 | - | 1312 | 232 |
| 5/9/2020 | 13 | 1.99 | 3.09 | 103,400 | 3660 | 255 |
| 5/12/2020 | 13 | 2.09 | 4.06 | - | 670 | 122 |
| 5/29/2020 | 37 | 7.1 | 12.15 | - | 2013 | 445 |
| 6/6/2020 | 38 | 7.55 | 12.18 | - | 4488 | 303 |
| 7/23/2020 | 38 | 8.44 | 11.5 | 8440 | 1677 | 450 |
| 8/5/2020 | 29.2 | 7.45 | 10.3 | 6705 | 2739 | 769 |
| 8/12/2020 | 26.2 | 6.51 | 8.5 | 2604 | 2139 | 332 |
| 8/19/2020 | 25.09 | 5.27 | 7.18 | - | 2340 | 900 |
| 8/26/2020 | 13 | 3.58 | 4.19 | - | 1911 | 610 |
| 9/1/2020 | 18 | 4.2 | 7.1 | - | 1890 | 400 |
| 9/5/2020 | 16 | 4.09 | 5.53 | - | 1359 | 692 |
| 9/12/2020 | 12 | 2.58 | 3.73 | - | 366 | 560 |
| 9/14/2020 | 24 | 5 | 8.2 | 19.1 | 1879 | 158 |
| 9/28/2020 | 15.2 | 3.3 | 4.7 | 3.3 | 2780 | 171 |
| 10/5/2020 | 22.6 | 4.7 | 6.6 | 12.1 | 2140 | 119 |
| 11/3/2020 | 19 | 4.3 | 6.3 | 62.5 | 960 | 261 |
| 12/1/2020 | 33.2 | 6.8 | 10 | 27.2 | 930 | 265 |
| 1/8/2021 | 36.8 | 8.2 | 11.3 | 18 | 820 | 278 |
| 2/17/2021 | 28.4 | 5.9 | 9.4 | 51.1 | 680 | 364 |
| 3/5/2021 | 32 | 6.8 | 10.2 | 28 | 620 | 310 |
| 4/28/2021 | 32 | 7.1 | 10.4 | 27.7 | 820 | 352 |
| 6/9/2021 | 36 | 7.2 | 11.5 | 42.9 | 650 | 373 |
| 7/6/2021 | 41.6 | 7.8 | 12.4 | 25.6 | 786 | 387 |
| 8/18/2021 | 36.7 | 6.8 | 10.8 | 47.4 | 390 | 331 |
| 9/15/2021 | 37 | 7.2 | 11.8 | 48.6 | 440 | 349 |
| 10/15/2021 | 39.6 | 7.8 | 12.2 | 27.2 | 497 | 326 |
| 11/11/2021 | 38.2 | 7.4 | 11.6 | 42.4 | 322 | 353 |
| 12/9/2021 | 39.2 | 7.2 | 11.3 | 33.7 | 440 | 293 |
| 1/13/2022 | 40.7 | 7.8 | 12.4 | 36.5 | 522 | 283 |
| 3/2/2022 | 40 | 8.3 | 13 | 19.9 | 580 | 214 |
| 4/13/2022 | 44.7 | 9 | 13.6 | 23.4 | 1640 | 296 |
| 5/18/2022 | 36 | 7.3 | 11.6 | 41.3 | 980 | 226 |
| 6/29/2022 | 45.7 | 9.3 | 14.1 | 22.2 | 736 | 27 |
| 7/27/2022 | 40.8 | 8.6 | 12.7 | 48.8 | 890 | 255 |
| 9/8/2022 | 45.9 | 10.7 | 14.5 | 32.1 | 530 | 75 |
| 10/15/2022 | 39.6 | 7.8 | 12.2 | 27.2 | 497 | 326 |
| 10/19/2022 | 50 | 11.1 | 15 | 44.2 | 790 | 134 |
| 11/11/2022 | 38.2 | 7.4 | 11.6 | 42.4 | 322 | 353 |
| 12/9/2022 | 39.2 | 7.2 | 11.3 | 33.7 | 440 | 293 |
| 7/17/2023 | 13 | 2.82 | 3.9 | - | 460 | 520 |
| 7/24/2023 | 19.4 | 4.2 | 6.2 | 9.3 | 601 | 274 |
| 7/31/2023 | 18.8 | 3.6 | 5.6 | 7.6 | 630 | 360 |
| 8/7/2023 | 21.7 | 3.8 | 6.4 | 13 | 385 | 276 |
| 8/17/2023 | 24.7 | 4.4 | 7.3 | 11.4 | 381 | 316 |
| 8/31/2023 | 22 | 4.4 | 7.7 | 4 | 366 | 251 |
| 9/13/2023 | 22.5 | 4 | 6.9 | 2.4 | 790 | 282 |
| 9/28/2023 | 27 | 5.1 | 8.7 | 6.1 | 740 | 234 |
| 10/11/2023 | 24.2 | 4.4 | 7.6 | 3.5 | 457 | 304 |
| 10/26/2023 | 23 | 4.4 | 8 | 12.4 | 720 | 274 |
| 11/9/2023 | 27 | 5.3 | 9.1 | 5.3 | 965 | 302 |
| 11/30/2023 | 29 | 5.6 | 9.4 | 4.4 | 601 | 213 |
| 12/18/2023 | 28 | 5.2 | 8.8 | 4.2 | 643 | 282 |
| 1/11/2024 | 27.9 | 5.7 | 9.3 | 5.6 | 379 | 208 |
| 2/15/2024 | 30.2 | 5.9 | 9.4 | 12.3 | 3110 | 262 |

**Reference values**: for hematocrit between 28.2 and 52.7%, red blood cells between 7.12 and 11.46 $10^6$ /uL, hemoglobin between 10.3 and 16.2 g/dL, absolute reticulocytes between 3.0 and 50 x $10^3$/uL, lymphocytes between 850 and 5850/uL, platelets between 155 and 641 x $10^3$/uL.

O material was collected for a myelogram, but the result indicated insufficient sample material. It was advised that SARA undergo weekly blood tests for four weeks, followed by monthly testing. A homeopathic formula containing *Avena sativa*, *Cardus marianus*, and *Nux vomica,* all at 15 CH potency, was prescribed to be administered every eight hours for 10 days, along with the Chinese herb Gui Pi Tang® every 12 hours for an indefinite period. Additionally, mirtazapine (2 mg/day) was prescribed as an appetite stimulant for 10 days. A splenectomy was not performed.

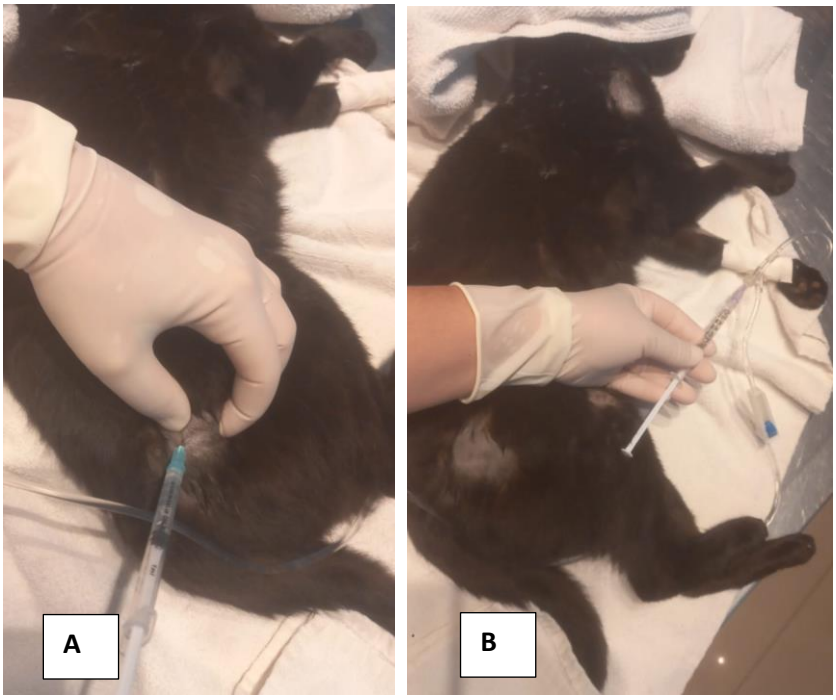


**Figure 1. A:** Photographic image from SARA's personal medical file, taken after the removal of sterile drapes, demonstrating intraosseous access in the right femoral region with palpation of the anatomical structures for needle insertion and stem cell administration. **B:** Photographic image from SARA's personal archive showing venous access via the cephalic vein in the left thoracic limb, demonstrating the intravenous application of stem cells.

Over the following three days, SARA's clinical-hematological condition stabilized, with an increase in hematological parameters (hematocrit, erythrocytes, and platelets), resulting in normoxia and a return to physical and behavioral vigor. The treatment achieved the expected results, successfully controlling the condition for three years until July 2023, without additional transfusions or hospitalizations.

However, after July 2023, her hematological parameters began to deteriorate once again, showing a hematocrit of 18.2% (reference: 28.2 - 52.7%), erythrocytes at 4.2 x $10^6$/uL (reference: 7.2 - 11.46 x $10^6$/uL), hemoglobin at 6.0 g/dL (reference: 10.3 - 16.2 g/dL), absolute reticulocytes at 1.3 x $10^3$/uL (reference: 3.0 - 50.0 x $10^3$/uL), and lymphocytes at 480/uL (reference: 850 - 5850/uL). In response, she was prescribed four new doses of treatment, administered approximately 30 days apart, while also undergoing corticosteroid therapy and having previously had a splenectomy. On July 18, 2023, SARA received a 120 mL blood transfusion of concentrated red blood cells for felines. Subsequently, on July 21, 2023, treatment with mesenchymal stem cells from the PET MEP Laboratory was initiated via intraosseous injection (Table 2).

The case progression was closely monitored by selecting key hematological parameters that exhibited the most significant alterations in complete blood count tests. These parameters included hematocrit (expressed as a percentage of total blood volume), red blood cell count (cells per field in $10^6$/uL), hemoglobin concentration (grams per dL), absolute reticulocyte count (cells per field in $10^3$/uL), lymphocyte count (cells per field in /uL), and platelet count (cells per field in $10^3$/uL). The reference ranges provided by the laboratory, determined by IDEXX Laboratories' automatic cell counters in Brazil, were used for comparison.

Currently, the feline receives weekly subcutaneous injections of erythropoietin (alpha-epoetin rHu Epo-Eritromax, 4,000 IU/mL), with a dosage of 0.1 mL. In addition, a supplementary support formula is being administered, which contains the following components per dosage: coenzyme Q10 (4 mg), resveratrol (3 mg), macrogard (50 mg), B12 methylcobalamin (300 mg), chelated zinc (10 mg), espinheira santa (20 mg), omega-3 (200 mg), and folic acid (1 mg). The patient is under continuous monitoring.

**Table 2**. Period, weight, and description of the procedure for administering the solution with mesenchymal stem cells in a feline.

| Date | Weight, Kg | Description of the procedure for administering the stem cell solution intraosseously. |
|---|---|---|
| 6/21/2023 | 4 | Fluid therapy with Ringer's lactate solution was administered in the lateral decubitus position via venous access in the cephalic vein of the right thoracic limb under general intramuscular anesthesia (ketamine 5 mg/kg, xylazine 1 mg/kg, maropitant/Cerenia®/1 mg/kg, dipyrone 25 mg/kg). The femoral region was shaved, followed by antisepsis of the skin using a 2% chlorhexidine solution and 5% alcohol. |
| 8/11/2023 | 4 | |
| 9/15/2023 | 4 | Intraosseous administration was performed in the cranio-lateral portion of the greater tuberosity of the left humerus using a 1 mL BD® disposable insulin syringe and a 25x7 cm BD® disposable needle. A total of 0.2 mL of the stem cell suspension was administered (containing for each 1 mL: 2.5 x $10^6$ mesenchymal stem cells, excipient q.s.p.: phosphate-buffered saline, feline plasma, penicillin, streptomycin, amphotericin B). Additionally, 0.2 mL of the stem cell solution was administered intravenously. |
| 10/27/2023 | 4.5 | |

## Discussion

This case report aims to present an additional supportive tool for current treatments in Regenerative Medicine, particularly in cases where chemotherapy, other oncological procedures, or drugs that induce medullary aplasia are employed. It also explores the application of this therapy for treating neoplasms, autoimmune diseases, infectious diseases, hematological support, and metabolic disorders. As noted by Moraes & Takahira (2010), many animals are euthanized soon after diagnosis due to the unfavorable prognosis associated with conditions such as medullary aplasia.

Schimanski et al. (2023) show that immunosuppression is a factor that predisposes dogs and cats to tumors, and that in cats, retrovirus infections such as feline viral immunodeficiency (FIV) and feline viral leukemia (FeLV) are predisposing factors to lymphoma, although the practice of vaccination has reduced the incidence of these viruses. In cases of progressive infection, bone marrow suppression can occur as a result of active viral replication or due to viral disruption and inactivation of genes in bone marrow stromal cells (Biezus et al., 2019).

Reinacher (1989) described that the clinical manifestations in cats with persistent infection vary significantly, with anemia, immunosuppression, lymphoma, and leukemia being the primary disease presentation. Additionally, infected animals become susceptible to secondary infections and may exhibit a range of symptoms, including stomatitis, gingivitis, skin lesions, abscesses, enteritis, respiratory alterations, nasal discharge, pneumonia, weight loss progressing to cachexia, persistent diarrhea, leukopenia, and varying degrees of anemia.

Oliveira et al. (2020) provide a comprehensive description of the etiopathogenesis of FeLV, which significantly alters the bone marrow microenvironment and is caused by four gammaretrovirus subtypes: A, B, C, and T. Only subtype A is transmitted between cats, meaning that all cats infected with FeLV-B, FeLV-C, or FeLV-T are also co-infected with FeLV-A, which, through mutations in its genetic material, gives rise to these other subtypes. FeLV-A is found in various tissues and cells and has the potential to induce lymphoma. FeLV-B is primarily associated with lymphoma, leukemia, and myelodysplasia, while FeLV-C is considered the most pathogenic and commonly linked to aplastic anemia. FeLV-T, isolated from a cat with FeLV-induced immunodeficiency, demonstrates T-lymphocyte lymphotropism. Viral replication, being immunosuppressive in nature, promotes the emergence of mutant cell lines, and it is believed that the compromised immune system allows for the proliferation of neoplastic clones. The infection of stromal cells that support the bone marrow microenvironment and the cytopathic effects of the virus further contribute to these alterations. This results in defective proliferation and replacement of active hematopoietic tissue with neoplastic tissue, manifesting systemically as cytopenias and dysplastic changes.

Erythroid lineage disorders may arise in response to immune-mediated hemolytic anemia, systemic inflammatory conditions, hematopoietic disorders, or neoplasms. Abnormal proliferation of bone marrow mesenchymal cells can lead to myelofibrosis, where viral action on fibroblast precursors within the bone marrow affects cell growth. A reactive inflammatory process triggered by cytokine activity within the bone marrow microenvironment can result in lesions and subsequent scarring. Additionally, medullary osteosclerosis is characterized by the excessive growth of bone trabeculae within the medullary cavity of long bones. The virus's direct impact on the bone marrow microenvironment leads to a deregulation of cell production mechanisms, resulting in significant alterations such as aplastic anemia, myelodysplastic syndrome, leukemia, and lymphoma.

According to Biezus et al. (2019), the FeLV virus is transmitted through oronasal exposure to infectious viral particles present in secretions. Once in the body, the virus replicates in the lymphocytes and macrophages of regional lymphoid tissues before spreading by infecting the bone marrow. This can lead to pancytopenia, and the progression of the infection can follow one of four courses: progressive, regressive, abortive, or focal. The clinical pathological manifestations that develop depend on the specific characteristics of the virus involved and the animal's individual immune response.

Moraes & Takahira (2010) demonstrated that the mechanisms underlying medullary aplasia can be divided into several categories: destruction of stem or progenitor cells, genetic mutations that result in a reduced proliferative capacity of stem cells, deregulation of hematopoietic cytokines, and alterations in the bone marrow stroma. Regardless of the primary cause, medullary aplasia can present in either an acute or chronic form. The acute form begins with leukopenia, thrombocytopenia, and neutropenia within two weeks of medullary injury, often accompanied by mild or absent anemia. A myelogram typically reveals multifocal areas of necrosis, degenerated hematopoietic cells, and an increased number of macrophages. The chronic form is characterized by neutropenia, thrombocytopenia, and moderate to severe anemia. Medullary repopulation is uncertain, and when it does occur, the process can take weeks to months.

Various studies have been published on the causes of medullary aplasia, including infectious agents, immunological mechanisms, drugs, idiopathic causes (by exclusion), and those associated with toxins and radiation. A contagious origin causes FeLV, as noted by Biezus et al. (2019). Immunological mechanisms can involve hematopoietic neoplasms or conditions such as estrogen excess, either of endogenous origin—resulting from Sertoli cell tumors in males or cystic ovaries in females—or of exogenous origin, where estrogen is administered to treat urinary incontinence or prevent pregnancy. Other immunological factors include thymic secretions that inhibit stem cell activity, suppression of erythrocyte-stimulating factor production, and iron metabolism disorders affecting erythroid precursors, leading to a reduction in hematopoietic cells, inhibition of differentiation, and decreased response to erythropoietin. Immune-mediated or idiosyncratic reactions also contribute to medullary aplasia but can be challenging to diagnose.

In terms of drug-induced causes, chemotherapeutic agents are significant contributors, with doxorubicin recognized as having the highest myelosuppressive potential alongside lomustine (Schimanski et al., 2023). Other drugs associated with medullary aplasia include the immunosuppressant azathioprine and various classes of compounds, such as phenylbutazone, trimethoprim/sulfadiazine, griseofulvin, cephalosporins, phenothiazine, captopril, albendazole, fenbendazole, chloramphenicol, quinidine, griseofulvin, and amphotericin B, which may cause idiosyncratic reactions (Moraes & Takahira, 2010). Although idiopathic causes are relatively rare in veterinary cases, these authors also report that they represent the leading cause of medullary aplasia in humans. In these instances, an immune reaction—mediated by T lymphocytes—targets hematopoietic stem cells, with interferon in particular exerting an antiproliferative effect on progenitor cells.

The staging and diagnosis of medullary aplasia, as described by Moraes & Takahira (2010), require a thorough anamnesis and a detailed evaluation of the animal's medical history. This process involves investigating any drugs administered at least four weeks prior, potential exposure to radiation, the presence of ectoparasites, serology for diseases linked to medullary aplasia, and the persistence of pancytopenia for the last two weeks or following treatment for septicemia or endotoxemia. Evaluating the complete blood count and confirming medullary involvement through aspiration puncture or bone marrow biopsy is essential for diagnosis. These methods allow for the assessment of normal blood

production, identification of changes in cellular morphology, and detection of neoplastic cells (Müller et al., 2009; Schimanski et al., 2023). According to Moraes & Takahira (2010), when more than 75% of an adult dog's bone marrow consists of fat, combined with the reduction or absence of the three main cell lines (erythroid, myeloid, and megakaryocytic), the condition can be considered aplastic. The blood count in such cases typically reveals normocytic, normochromic regenerative anemia, leukopenia with neutropenia, and thrombocytopenia. During slide preparation, the myelogram may show particular changes caused by chemotherapy drugs, including degenerative changes, such as nuclear fragmentation and dysplastic alterations. Further diagnostic tools, such as immunohistochemistry, flow cytometry, and conventional cytogenetics, can provide additional insights to confirm the diagnosis of medullary aplasia (Müller et al., 2009).

Important differential diagnoses for medullary aplasia include myelodysplastic syndrome (MDS), myelofibrosis, conditions such as leukemia, myelonecrosis, pure red cell aplasia, hemophagocytic syndrome, chronic kidney disease, ectopic testicular or scrotal sac tumors, and neoplastic stages such as lymphoma and mastocytoma. Additionally, medullary iron stores must be evaluated in the diagnosis of leishmaniasis, systemic fungal diseases, and in animals presenting with fever of unknown origin, unexplained weight loss, and general malaise (Moraes & Takahira, 2010; Müller et al., 2009).

In dogs and cats, Müller et al. (2009) suggest that bone marrow can be collected from the epiphysis of long bones and regions of the ileum, such as the iliac crest or acetabular rim. They also recommend Jamshidi needles or myelogram needles as the preferred equipment for intraosseous infusions.

Silva et al. (2020) reviewed FeLV as one of the most significant infectious diseases in feline medicine, causing clinical manifestations related to its oncogenic, cytopathic, and immunosuppressive effects. (Schimanski et al., 2023) note that chemotherapy is the treatment of choice for most feline lymphomas, with antineoplastic agents such as doxorubicin, vincristine, cyclophosphamide, methotrexate, L-asparaginase, lomustine, and prednisolone. According to (Silva et al., 2020), chlorambucil is also used. These agents are often used in combination, leading to different treatment protocols of varying duration and outcomes. However, long-term chemotherapy is associated with side effects, including chronic hematological toxicity, decreased appetite, weight loss, and other gastrointestinal symptoms (Schimanski et al., 2023).

There is no standard treatment protocol for feline lymphomas, and various protocols are applied depending on the stage of the disease and the clinical manifestations. Some studies suggest that there is still no consensus on a definitive protocol, presenting this as a therapeutic challenge with a guarded prognosis. Similarly, other studies also emphasize the lack of consensus on a treatment protocol, reinforcing that this represents a therapeutic challenge with a guarded prognosis (Schimanski et al., 2023; Silva et al., 2020) (Almeida et al., 2019; Almeida et al., 2024). Regardless of the treatment approach chosen, rigorous case monitoring is essential. Protocols may need to be replaced as necessary, considering the drugs' effects and the desired outcomes at each stage. An initial, more aggressive, and effective protocol may be employed to reduce clinical manifestations, followed by a less toxic regimen that maintains the animal's quality of life (Silva et al., 2020).

Schimanski et al. (2023) and Silva et al. (2020) note that one of the most commonly used protocols is CHOP, which includes vincristine, cyclophosphamide, doxorubicin, and prednisone or prednisolone. A regimen of high-dose cyclophosphamide, vincristine, and prednisone or prednisolone is known as COP. Almeida et al. (2019) and Almeida et al. (2024) reported the use of prednisolone, thymomodulin, and chlorambucil in their treatment approach. Schimanski et al. (2023) and Silva et al. (2020) also indicate the combination of chlorambucil and prednisone, emphasizing its advantage for at-home administration by the guardian, as it is available in tablet form. However, there is no consensus on the duration of this protocol, with recommendations ranging from six months to two years or via pulse therapy over three consecutive days with 21-day intervals, allowing for the destruction of tumor cells and the recovery of normal cells.

Many authors have demonstrated the therapeutic efficacy of MSCs in animals affected by various conditions, such as medullary aplasia (Schleuning, 2000), tendinitis, osteoarthritis, medullary injury, corneal lesions, skin lesions, and ulcers (Santos, 2018). Webb (2020) also described their use in treating allergic asthma, feline eosinophilic keratitis, feline gingivostomatitis complex, and chronic

inflammatory bowel disease. Gugjoo et al. (2019) present promising results in treating neoplasms, nephropathies, hepatopathies, diabetes, discopathies, neuropathies, anal fistula, vocal fold injury, myocarditis, tendon and ligament injuries, muscle ruptures, periodontal disease, and osteoarthritis.

(2020) notes that MSCs have been successfully isolated from bone marrow and peripheral blood in cats, and the tissue source may directly influence the success of cell therapy. Among the most commonly used sources of stem cells in veterinary medicine are bone marrow, adipose tissue, and the umbilical cord. Cells derived from adipose tissue are particularly notable due to their ease of extraction, abundance, and minimal discomfort for the animal (Santos, 2018).

Gugjoo et al. (2019) suggest that stem cells primarily exert therapeutic effects through their immunomodulatory, anti-inflammatory, antiapoptotic, and chemotactic actions. Additionally, these effects—such as the angiogenesis of MSCs—can occur through both cell-cell contact and paracrine signaling (Hayretdağ & Coşkunpinar, 2019; Ilic & Polak, 2011; Rajabzadeh et al., 2019). These mechanisms involve the secretion of soluble immunosuppressive factors such as transforming growth factor-β1 (TGF-β1), prostaglandin E2 (PGE2), hepatocyte growth factor, indoleamine 2,3-dioxygenase (IDO), vascular endothelial growth factor (VEGF), adenosine, cyclooxygenase-2 (COX-2), and interleukin-68 (IL-68). Schleuning (2000) and Webb (2020) highlight that, unlike conventional treatments, MSCs can treat diseases that have no existing therapies or fail to respond to current treatments. Raghavachar et al. (1983) previously noted that MSC therapy is capable of reconstituting hematopoietic tissue.

MSCs can be administered through various access routes (Santos, 2018), including topical, ophthalmic, intraocular, intravenous, subcutaneous, intrathecal, epidural, intralesional, perilesional, and intraarticular methods. Some authors also indicate the intraosseous route (via the distal ulna or proximal tibia) as an efficient method for rapidly absorbing fluids or medications into the bloodstream (Dodson et al., 2010; Hayretdağ & Coşkunpinar, 2019; Ilic & Polak, 2011; Monteiro et al., 2010; Santos et al., 2019; Souza et al., 2022; Tanna & Sachan, 2014). Müller et al. (2009) suggest that the proximal end of the femur, iliac crest, proximal end of the humerus, sternum, and proximal epiphysis of the tibia are viable sites for accessing and collecting bone marrow.

There are potential complications associated with the procedure, as noted by Müller et al. (2009). These include bone fractures, perforation of blood vessels or organs due to needle misplacement, and contamination of the spinal canal, which could lead to osteomyelitis. Additionally, there may be instances where no material is collected, underscoring the importance of thorough pre- and post-operative antisepsis and proper training to ensure the safe execution of the procedure.

## Conclusion

Treatment with mesenchymal stem cells (MSCs) has emerged as an effective and innovative tool in veterinary medicine, yielding excellent results as a primary or supportive therapy for various conditions. These include degenerative diseases such as medullary aplasia, neoplasms, chemotherapy support, infectious diseases, osteoarticular diseases, trauma, poisoning, nephropathies, heart disease, and autoimmune disorders. MSCs can be administered through different routes, with the intraosseous route proving essential in this case report to stabilize and control the feline's immunological, hematological, and overall quality of life. Intravenous applications did not produce the desired effects for this specific treatment. However, while effective, it is essential to acknowledge the potential complications associated with this access route. Even with the continuous use of corticosteroids, the treatment proved effective.

The treatment protocol should be individualized and consider the animal's clinical, hematological, immunological, and physiological manifestations. For medullary aplasia, the standard protocol begins with three consecutive intraosseous applications spaced 30 days apart, except in cases where animals are on immunosuppressants or have hypoadrenocorticism, which may require a minimum of four applications.

It is crucial for veterinarians to inform owners considering intraosseous mesenchymal stem cell treatment for medullary aplasia that lifelong monitoring of the animal's clinical and hematological status is necessary, regardless of the underlying cause. Owners should also be made aware of the potential need for additional treatment sessions, which can be costly. Once stem cells are requested from the

laboratory, the delivery time is typically one week, as the cells must undergo laboratory processing and preparation before use by the veterinarian.

**Acknowledgments**

We extend our gratitude to the PET MEP Biotechnology Laboratory for its remarkable contributions, providing outstanding results in numerous cases through the use of stem cells. Their innovative approach has significantly improved the quality of life of animals and aided in controlling diseases with previously unfavorable prognoses and limited treatment options. The availability of their products to veterinarians has yielded exceptional outcomes in cases once deemed challenging.

**Translation:** Todd Anthony Harkin, Harkin Translations

**References**

Almeida, G. B., Zamian, T. R. O., Reis, F. M., Borges, Y. N. C., Godoy, J. V. F. T., & Barros, M. A. (2024). Linfoma alimentar de pequenas células em felinos. *PUBVET*, *18*(3). https://doi.org/10.31533/pubvet.v18n03e1568.

Almeida, T. M., Sousa Filho, R. P., Rodrigues, I. L., Cruz, R. O., Rodrigues, A. P. R., & Silva, I. N. G. (2019). Linfoma leucemizado em felino coinfectado com os vírus da imunodeficiência felina e da leucemia felina: Relato de caso. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *71*(1). https://doi.org/10.1590/1678-4162-10382.

Biezus, G., Ferian, P. E., Pereira, L. H. H. S., Withoeft, J. A., Antunes, M. M., Nunes Xavier, M. G., Volpato, J., Cristo, T. G., Fonteque, J. H., & Casagrande, R. A. (2019). Clinical and haematological disorders in cats with natural and progressive infection by Feline Leukemia Virus (FELV). *Acta Scientiae Veterinariae*, *47*(1). https://doi.org/10.22456/1679-9216.90027.

Brito, H. F. V, Corat, M. A. F., Santos, M. R., Gilioli, R., Passos, L. A. C., Lancellotti, M., Ferreira, F., & Min, L. L. (2010). Tratamento de sequelas neurológicas em cães, causadas por infecção pelo vírus da cinomose, através do transplante alogênico de células mononucleares de medula óssea. *Revista Científica de Medicina Veterinária-Pequenos Animais e Animais de Estimação*, *8*(24), 27–29.

Dodson, M. V, Hausman, G. J., Guan, L., Du, M., Rasmussen, T. P., Poulos, S. P., Mir, P., Bergen, W. G., Fernyhough, M. E., McFarland, D. C., Rhoads, R. P., Soret, B., Reecy, J. M., Velleman, S. G., & Jiang, Z. (2010). Skeletal muscle stem cells from animals I. basic cell biology. *International Journal of Biological Sciences*, *6*(5), 465–474. http://dx.doi.org/10.7150/ijbs.6.465

Ghasempour, E., Hesami, S., Movahed, E., Keshel, S. H., & Doroudian, M. (2022). Mesenchymal stem cell-derived exosomes as a new therapeutic strategy in the brain tumors. In *Stem Cell Research and Therapy* (Vol. 13, Issue 1). https://doi.org/10.1186/s13287-022-03212-4.

Gugjoo, M. B., Amarpal, A., & Sharma, G. T. (2019). Mesenchymal stem cell basic research and applications in dog medicine. *Journal of Cellular Physiology*, *234*(10), 16779–16811. https://doi.org/10.1002/jcp.28348.

Hayretdağ, C., & Coşkunpinar, E. M. (2019). Stem cells and regenerative medicine. In *Turkish Journal of Immunology* (Vol. 7, Issue 1). https://doi.org/10.25002/tji.2019.856.

Heckler, M. C. T. (2014). *Efeitos biológicos do laser de baixa potência sobre o cultivo de células-tronco mesenquimais provenientes do tecido adiposo e da medula óssea de cães*. Universidade Estadual Paulista (Unesp).

Ilic, D., & Polak, J. M. (2011). Stem cells in regenerative medicine: Introduction: In *British Medical Bulletin* (Vol. 98, Issue 1). https://doi.org/10.1093/bmb/ldr012.

Jeung, S., Kim, S., Ah, J., Seo, S., Jan, U., Lee, H., & Lee, J. I. (2024). Exploring the Tumor-Associated risk of mesenchymal stem cell therapy in veterinary medicine. *Animals*, *994*(14). https://doi.org/10.3390/anil4070994.

Kang, M. H., & Park, H. M. (2020). Challenges of stem cell therapies in companion animal practice. In *Journal of Veterinary Science* (Vol. 21, Issue 3). https://doi.org/10.4142/JVS.2020.21.E42.

Malard, P. F., Peixer, M. A. S., Santana, L. R., Dallago, B. S. L., Milistetd, M., Queiroz, L. M., & Brunel, H. dos S. S. (2020). Avaliação da terapia com células-tronco mesenquimais halógenas em

doença renal crônica de cães e gatos. *PUBVET*, *14*(11), 1–8. https://doi.org/10.31533/pubvet.v14n11a700.1-8.

Monteiro, B. S., Argolo Neto, N. M., & Del Carlo, R. J. (2010). Células-tronco mesenquimais. *Ciência Rural*, *40*, 238–245. https://doi.org/10.1590/S0103-84782010000100040.

Moraes, L. F., & Takahira, R. K. (2010). Aplasia medular em cães. *Revista de Ciências Agroveterinárias*, *9*(1), 99–108.

Müller, D. C. M., Pippi, N. L., Basso, P. C., Olsson, D. C., Santos Junior, E. B., & Guerra, A. C. O. (2009). Técnicas e sítios de coleta de medula óssea em cães e gatos. *Ciência Rural*, *39*(7), 2243–2251.

Oliveira, A. F. X., Stocco, N. V, Guimarães, A., Marcelo, G. C. G., & Baldani, C. D. (2020). Fiv e FeLV e as consequências na medula óssea. *Vet Science Magazine*, *26*, 10–12.

Olsson, D. C. (2020). Medula Óssea e as Células Tronco – O que há de novo na Medicina Veterinária. VetScience Magazine. Pg 6-9. V26.

Paim, L. L., Costa, J. M. P., & Consul, P. M. (2021). Atualidades no uso de células-tronco para o tratamento de sequelas neurológicas decorrentes da cinomose canina. *PUBVET*, *16*(5), 1–4. https://doi.org/10.31533/pubvet.v16n05a1122.1-4.

Raghavachar, A., Prümmer, O., Fliedner, T. M., Calvo, W., & Steinbach, I. B. E. (1983). Stem cells from peripheral blood and bone marrow: A comparative evaluation of the hemopoietic potential in the dog. *The International Journal of Cell Cloning*, *1*(4), 191–205. https://doi.org/10.1002/stem.5530010401.

Rajabzadeh, N., Fathi, E., & Farahzadi, R. (2019). Stem cell-based regenerative medicine. *Stem Cell Investigation*, *6*(July). https://doi.org/10.21037/sci.2019.06.04.

Reinacher, M. (1989). Diseases associated with spontaneous feline leukemia virus (FeLV) infection in cats. *Veterinary Immunology and Immunopathology*, *21*(1), 85–95. https://doi.org/10.1016/0165-2427(89)90132-3.

Santos, E. (2018). Biologia das células-tronco mesenquimais de felinos obtidas a partir de nichos presentes no tecido adiposo objetivando sua aplicação terapêutica na medicina veterinária. *Revista Eletrônica Científica Da UERGS*, *4*(3), 379. https://doi.org/10.21674/2448-0479.43.368-379.

Santos, E. J. C., Winck, C. P., Alves, C. A. M., & Fernandes, R. A. (2019). Células-tronco mesenquimais alogênicas no tratamento de sequelas neurológicas de cinomose canina. *Revista Científica de Medicina Veterinária - Pequenos Animais e Animais de Estimação*, *49*(3), 32–40. http://dx.doi.org/10.31533/pubvet.v16n05a1122.1-4

Schimanski, L., Moraes, F., & Moya, C. (2023). Linfoma mediastinal em felino FELV - Relato de caso. *Enciclopédia Biosfera*, *20*(45). https://doi.org/10.18677/encibio_2023c15.

Schleuning, M. (2000). Adoptive allogeneic immunotherapy - History and future perspectives. *Transfusion Science*, *23*(2), 133–150. https://doi.org/10.1016/S0955-3886(00)00078-3.

Silva, T. F., Amaral, A. V. C., Ferraz, H. T., Lopes, D. T., Braga, Í. A., Saturnino, K. C., Romani, A. F., & Ramos, D. G. S. (2020). Comparação de tratamentos quimioterápicos em felino com vírus da leucemia felina (FELV). *Brazilian Journal of Health Review*, *3*(3), 4135–4148. https://doi.org/10.34119/bjhrv3n3-020.

Souza, J. A., Eduardo, L. S., Gomes, G. V. A., Silva, R. B., Nascimento, D. R., & Nascimento, J. W. A. (2022). Principais evidências clínicas da terapia com células-tronco na cicatrização de queimaduras: Uma revisão sistemática. *Research, Society and Development*, *11*(10), e184111032781. https://doi.org/10.33448/rsd-v11i10.32781.

Tanna, T., & Sachan, V. (2014). Mesenchymal stem cells: potential in treatment of neurodegenerative diseases. *Current Stem Cell Research & Therapy*, *9*(6), 513–521. https://doi.org/10.2174/1574888x09666140923101110.

Webb, T. L. (2020). Stem cell therapy and cats: What do we know at this time. In *Veterinary Clinics of North America - Small Animal Practice* (Vol. 50, Issue 5, pp. 955–971). https://doi.org/10.1016/j.cvsm.2020.06.002.

**Article history:**
**Received:** 10 August 2024
**Approved:** 4 September 2024