Gallant

Summary: Canine Uterine-Derived Allogeneic Mesenchymal Cells Intravenous Dose Escalation Safety Study

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Background

Mesenchymal stem cells (MSCs) have been used in veterinary medicine since 2002. Most of the safety and efficacy work has been reported on adipose-derived MSCs. Although other types of MSCs, including cord blood-derived, bone marrow-derived, and amniotic tissue-derived have also been tested, there is far less information for practitioners on the safety and efficacy of reproductive tissue-derived stem cells.

The purpose of this study was to evaluate the tolerability of a single, high dose, intravenous infusion of canine allogeneic mesenchymal like stem cells. This study was conducted under Good Laboratory Practices (GLP) and conducted at a contract research organization (CRO).

Materials and Methods

The test article was a suspension of canine uterine-derived allogeneic MSCs. The cells were extracted from the uterus of a healthy, young canine who was infectious disease tested and was undergoing her routine spay procedure. The uterus was collected in a sterile container and transported overnight, on ice, to the lab. The tissue was processed by enzymatic digestion, washing and centrifugation, and the primary cells plated for culture.

The cells were cultured prior to cryopreservation in a specific cell therapy vial (CellSeal) at a concentration of 10 million (M) cells per ml then transported via validated dry shipper to the test site. The cells were thawed in a 37 degree water bath and suspended at a concentration of 1 million cells per ml in a solution of saline and trehalose immediately prior to transfusion.

Twelve (12) dogs were divided into four groups of three animals per group. One group (Group 4) had an accelerated infusion rate of 2ml/min verses the standard of 1ml/min. Each dog was prepared for the infusion of cells by placing a 20g peripheral cephalic catheter. No medications were used in the dogs prior to the transfusion. Cells were infused at a rate of 1M cells/min.

The four groups were as follows:

Group 1 (3 dogs)	20M cells	20 min infusion (1ml/min)
Group 2 (3 dogs)	50M cells	50 min infusion (1ml/min)
Group 3 (3 dogs)	80M cells	80 min infusion (1ml/min)
Group 4 (3 dogs)	50M cells	25 min infusion (1ml/min)

The dogs were monitored for any physiologic abnormalities, blood pressure, heart rate, temperature and ECG were recorded at least every 30 minutes during the infusion and for two hours after infusion. Body weight checks performed as well as blood and urine samples for clinical pathology were gathered two hours after the infusion then once weekly for eight (8) weeks.

Results

Clinical pathology results showed no significant excursions in any of the dogs. Mild, clinically insignificant decreases were noted within the red blood cell indices, total bilirubin, creatinine, GGT and AST. ALP was above normal at baseline measurements in 10 of 12 young, large breed dogs who averaged eight months in age. After the infusion, ALP was mildly elevated at a clinically insignificant level in 11 of the 12 dogs, this also may be correlated with the young age of the colony or with the cell infusion. ALT, or any other liver indicator, was never increased in any of the dogs therefore stem cell induced hepatotoxicity is unlikely.
It is noteworthy that the CRO colony of dogs, including this colony, were found to be infected with giardia while this study was ongoing. Some of the dogs throughout the CRO site had clinical signs of giardia and were being treated. The study was not halted or altered due to this finding.

Clinical abnormalities were noted in three dogs at the higher doses of 50 and 80 million cells (Table 1).

Table 1: Clinical abnormalities in three of twelve dogs.

Dog 003	Group 2: 50M/50 min	Ataxia, tachypnea pale mucus membranes, hypersalivation	Stop infusion. Signs resolved. Treated with diphenhydramine and dexamethasone. Dog recovered within minutes. Continued infusion.
Dog 006	Group 3: 80M/80 min	One episode of vomiting/diarrhea two hours after infusion	Potentially attributable to giardiasis (+) status of the colony or to the cell administration.
Dog 007	Group 3: 80M/80min	Mild lethargy, injected mucus membranes	May be attributable to excitement/activity or to cell infusion. The dog was not treated, completed infusion without consequence

Histopathological evaluation showed no abnormalities of any tissues, including those of dogs that demonstrated clinical abnormalities in Table 1.

Conclusion

There were no significant excursions in any clinical pathology data and no histological findings in any tissue examined. Three dogs had brief clinical abnormalities that resolved with minor treatment to one dog. Two of the episodes could not readily be tied to the infusion due to concomitant giardiasis and anxiety/excitation. No other abnormalities were noted in any animals during the eight-week period. These findings along with the lack of histopathological and clinical pathology findings suggest an acceptable safety profile for all four doses and accelerated infusion dose.