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Concise Review: Stem Cell Trials Using Companion Animal Disease Models

ANDREW M. HOFFMAN[a] STEVEN W. DOW[b]

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[a]Regenerative Medicine Laboratory, Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, Grafton, Massachusetts, USA; [b]Center for Immune and Regenerative Medicine, Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado, USA

Correspondence: Andrew Hoffman, D.V.M., D.V.Sc., Large Animal Internal Medicine, Regenerative Medicine Laboratory, Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts 01536, USA. Telephone: 508-887-4589; Fax: 508-887-4213; e-mail: andrew.hoffman@tufts.edu

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ABSTRACT

Studies to evaluate the therapeutic potential of stem cells in humans would benefit from more realistic animal models. In veterinary medicine, companion animals naturally develop many diseases that resemble human conditions, therefore, representing a novel source of preclinical models. To understand how companion animal disease models are being studied for this purpose, we reviewed the literature between 2008 and 2015 for reports on stem cell therapies in dogs and cats, excluding laboratory animals, induced disease models, cancer, and case reports. Disease models included osteoarthritis, intervertebral disc degeneration, dilated cardiomyopathy, inflammatory bowel diseases, Crohn's fistulas, meningoencephalomyelitis (multiple sclerosis-like), keratoconjunctivitis sicca (Sjogren's syndrome-like), atopic dermatitis, and chronic (end-stage) kidney disease. Stem cells evaluated in these studies included mesenchymal stem-stromal cells (MSC, 17/19 trials), olfactory ensheathing cells (OEC, 1 trial), or neural lineage cells derived from bone marrow MSC (1 trial), and 16/19 studies were performed in dogs. The MSC studies (13/17) used adipose tissue-derived MSC from either allogeneic (8/13) or autologous (5/13) sources. The majority of studies were open label, uncontrolled studies. Endpoints and protocols were feasible, and the stem cell therapies were reportedly safe and elicited beneficial patient responses in all but two of the trials. In conclusion, companion animals with naturally occurring diseases analogous to human conditions can be recruited into clinical trials and provide realistic insight into feasibility, safety, and biologic activity of novel stem cell therapies. However, improvements in the rigor of manufacturing, study design, and regulatory compliance will be needed to better utilize these models. STEM CELLS *2016; 00:000—000*

SIGNIFICANCE STATEMENT

Studies in veterinary medicine which have employed companion animals to evaluate safety and efficacy of stem cells have not been systematically reviewed for the human medical and biomedical research community. The goal of this review is to shed light on examples whereby companion animal spontaneous disease models (i.e., veterinary patients) were utilized to study novel stem cell therapies, and to stimulate further discussion on the potential value of these models in multidisciplinary studies for the dual benefit of human and veterinary medicine.

INTRODUCTION

The health care field is rapidly evolving with increasing importance placed on disease prevention, early detection, reduced invasiveness, and personalization of therapies. Fundamental knowledge about disease mechanisms is being unraveled by increasingly sophisticated technologies, such as cell reprogramming, gene editing, rapid whole genome sequencing, and multimodality imaging methods that were inaccessible only a few years ago. Evidence is now disseminated across the globe through vast information channels, increasing its potential influence on health care.

Many of these trends are impacting veterinary medicine, particular in the companion animal sector [1–5]. Companion animal diseases mirror many human conditions with respect to their symptoms, natural history, pathology, gene associations, molecular phenotype, environmental risk factors, and responses to medication [6]. Homology of gene sequences in healthy tissues and tumors is more extensive between humans and companion animals (e.g., dogs and cats), than between humans and rodents [7–12]. The epigenomic behavior (e.g., DNA methylation) of canine and human tumor cells strongly resembles each other [13–16]. Thus, data from companion animal research have significant potential to illuminate disease pathogenesis and mechanisms of treatment resistance, while also testing the potential benefits of human-ready, but untested therapies.

Yet, the inclusion of companion animal disease models in multidisciplinary studies lags behind steep gains in knowledge and technology. This may reflect the gradual pace by which veterinary medicine emerged from its agricultural roots in the 20th century, to become the multifaceted profession of today, providing health care to companion, laboratory, food and fiber, and zoo animals, wildlife, and leading efforts in food safety and public health. While medical research has remained the domain of human physicians and Ph.D.'s who have employed *experimental animal model systems* (including transgenic animals, injury models, induced infection and tumor models, and nonhuman primates), the veterinary profession has developed tremendous knowledge and expertise in the care and research of companion animals which naturally develop many of the diseases that researchers attempt to recreate artificially in the laboratory. Thus, while human and veterinary medical professions have seen tremendous advances in parallel, they interact more obliquely. This is exemplified by the fact that veterinary research is principally found in veterinary focused journals, limiting its dissemination. The calls for a more multidisciplinary approach to publication and access to literature are both timely and important (i.e., "One Health, One Literature") [17].

Companion animal disease models are compelling based on their resemblance to human diseases, but their natural complexity runs against the tide of reductionism in science, for example, "one molecule one target." Further, companion animal studies are logistically more byzantine compared to standard laboratory animal (i.e., rodent-based) research, involving veterinarians, owners and their wishes, owner-based observations and biases, and factors such as cost-containment, insurance, and euthanasia.

It is, therefore, worth asking the question: can companion animal disease models replace or strategically supplement more traditional laboratory studies employing purpose-bred animals? Likewise, can investments in companion animal spontaneous disease research return benefits to human patients? There is no question that observations and novel therapies developed from companion animal research have directly influenced human medicine [6, 18]. However, studies in veterinary medicine which have employed companion animals to evaluate safety and efficacy of stem cells have not been systematically reviewed for the human medical and biomedical research community. The goal of this review, therefore, is to shed light on examples whereby companion animal spontaneous disease models (i.e., veterinary patients) were utilized to study novel stem cell therapies, and to stimulate further discussion on the role of these models in multidisciplinary studies for the dual benefit of human and veterinary medicine.

Companion Animal Origin and Definitions

"Companion animals" are domesticated animals that have a psychological bond with their owners. Dogs, for example, appear to have been domesticated >15,000 years ago in Central Asia about the time when dogs and wolves became genetically distinct [19]. In the process of domestication, humans and dogs developed mutual cooperation (social tolerance and attentiveness), for example, [20]. In the ensuing years, dogs and humans appear to have undergone convergent evolution, with positive selection of metabolic, neurologic, and cancer associated genes in both species concurrently [21]. For example, interactions between humans and animals have been shown to induce mutual physiologic benefits, mediated in part through oxytocin release by both partners in the interaction [22, 23].

Not surprisingly, companion animals are considered family members by people in the U.S. (66.7% for dogs, 56.1% for cats, and 34.5% of horses were considered family members in 2011) (https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-Pet-Ownership-Demographics-Sourcebook.aspx). This concept is further supported by the high numbers of pets (one for every two people, or one in three households in U.S.), the enjoyment people derive from traveling with pets, on the amount of money people spend on pets, and the physical risks that people take to rescue pets. Further underscoring their role as family members, abuse of companion animals is linked to violence toward children, spouses, and the elderly in families [24].

It is the nature of human-animal interactions which defines the term companion animal, not the animal species itself. To illustrate, animals raised for production of food and fiber including pigs, sheep, goats, chickens, and cattle, are not generally regarded as companion animals. Animals housed in laboratory settings or breeding colonies of animals (e.g., with genetic mutations) that do not have a specific owner or inhabit a household are excluded from this working definition. Animals that are raised for the purpose of commercial or noncommercial athletic competition (e.g., racehorses) while beloved by owners, are not necessarily considered companion animals or family members. While many competitive and noncompetitive horses are considered companion animals by their owners, equine studies are not considered in this article. Indeed, significant attention has been paid to stem cell-based therapies for musculoskeletal diseases in horses, a subject which has been reviewed elsewhere [25–31].

"Best in Show" Companion Animal Disease Models

To understand the potential for companion animals to contribute to the advancement of stem cell therapies, it is useful to estimate the prevalence of companion animal diseases that best represent candidates for clinical trials based on similarities to human diseases (Table 1). In the U.S. alone, there are ~70M dogs and ~74M cats, which are cared for by approximately 103,000 veterinarians, 11% of which are specialists (https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-veterinarians.aspx)

(versus 914,000 medical doctors, > 50% of which are specialists in 2014, http://kff.org/other/state-indicator/total-active-physicians/). Accordingly, millions of companion animals will develop diseases with close analogy to diseases of humans, including mitral valve disease (canine model of mitral valve prolapse, many progressing to congestive heart failure), canine cognitive dysfunction syndrome (model of Alzheimer's Disease), canine degenerative myelopathy (model of Amyotropic Lateral Sclerosis [ALS]), canine atopic dermatitis, keratoconjunctivitis sicca (KCS), feline chronic kidney disease (CKD), and osteoarthritis (OA; both dogs and cats) in their lifetime (Table 1). As many as 10,000 to 100,000 companion animals per year will develop epilepsy, intervertebral disc degeneration (IVDD; with or without disc herniation), or inflammatory bowel disease. In general, these data are skewed toward adult-onset diseases, which are prevalent in veterinary medicine. These statistics can serve as an important reminder of the significant opportunities for multidisciplinary research with companion animal disease models.

The prevalence of companion animals with specific diseases that will fulfill stringent clinical trial eligibility requirements is of course whittled down by common exclusion criteria that impose limits on age, body weight, body condition, comorbidities, stage of disease, or prior therapies, and the willingness of owners to have their pets participate in studies. In some disease models, biopsy confirmation is readily available, but in other conditions it may be challenging to get biopsy confirmation (e.g., in dogs with West Highland White pulmonary fibrosis syndrome [88]). Confirmation by specific imaging (magnetic resonance imaging - MRI, computed tomography - CT, positron emission tomography - PET) may or may not be accessible or affordable to owners. Conditions that are expressed at very low life-time prevalence, thwart their contribution to studies (e.g., idiopathic pulmonary hypertension in dogs [94]). To overcome these limitations, some investigators have established breeding colonies of companion animals with monogenic diseases (e.g., Duchenne Muscular Dystrophy [95]). Other companion animal spontaneous diseases are episodic, posing challenges to recruit animals during these exacerbations, leading to the development of laboratory colonies of animals of same species, for the purpose of reproducing the disease, for example, feline asthma induced by Bermuda Grass antigen [96, 97].

For many companion animal diseases, effective standard of care (SOC) treatment guidelines have been developed. In these disease models, there is significantly less interest in development of alternative therapies. However, a subpopulation of companion animal patients (based on their disease phenotype or chronicity) is partially or completely refractory to these SOC protocols, prompting owners to seek novel therapies such as stem cells for their animals. Accordingly, many veterinary clinical trials aim to evaluate stem cells as enhancers (adjuncts) rather than alternatives to SOC as reviewed below. It is noteworthy that SOC guidelines in veterinary medicine, not unlike human medicine, are not immutable; rather, the nature of SOC protocols is constantly evolving and in some instances reflects a variety of perspectives in veterinary medicine [98, 99]. Thus, when discussing clinical trial protocols it is important to address these variances.

In conclusion, companion animal disease models with significant potential to contribute to multidisciplinary studies are those which (1) closely resemble a human disease (symptoms, pathology, gene associations, therapeutic responses, and biomarkers), (2) are sufficiently common to facilitate study recruitment, (3) have a well-established natural history (disease progression, survival data), (4) have an established range of SOC or there is no available treatment, and (5) may be refractory or intolerant to SOC, or SOC is prohibitively expensive. While this is not an exhaustive list, the disease models summarized in Table 1 satisfy most, if not all of these criteria and thus will serve as important models for stem cell clinical trial purposes in the future.

Owner Participation: Much More than a Human-Animal Bond

Implicit in the relationship between owners and companion animals is intense mutual attentiveness. The frequent and detailed observations made by owners of their companion animals are leveraged to record specific endpoints in clinical studies. Many owner-based observations are incorporated into clinical assessment and quality of life scales that have been validated against more objective endpoints [100–102]. Moreover, observations are made in the environment of the home, which can add context and insight into mentation (attitude, arousal, fear, aggression, affection), posture, appetite and eating behaviors, sleeping habits, ambulation (total mobility, stability, lameness, range of motion [ROM]), navigation, thermoregulation, elimination behavior, exercise tolerance, olfactory senses, coughing, vomiting and nausea, visual acuity (e.g., night, day, around familiar obstacles), and micturition behavior. The scope of observations by owners is unparalleled in the laboratory animal world.

Another unique feature of companion animal research is public visibility. Information about clinical trials involving client-owned animals is disseminated using publically accessible web sites and social media. Owners unlike investigators and institutions are in most cases not asked to sign nondisclosure agreements; therefore, participants are free to interact. Consent forms contain clauses that allow owners to voluntarily exit a clinical trial at any time, so patients may be lost due to circumstances beyond the control of the investigators.

Income disparities may also influence participation or compliance in companion animal clinical trials, especially where incentives are offered. SOC may be financially offset by pet insurance, but only a minority of pets (1.4 out of 179 million, or 0.78%, https://www.naphia.org/industry/) in the U.S. is insured. As most treatment costs are paid directly out of pocket, ethically applied incentives are important to promote participation in clinical trials.

Pet owners typically make end-of-life decisions for their companion animals, often in consultation with veterinarians. Owners elect euthanasia when they perceive their companion animals are unduly suffering or to contain costs, rather than at discreet experimental time points. Clinical trial incentives may absolve the immediacy with which the owner elects euthanasia for financial reasons, but incentives are not intended to induce owners to prolong life if they perceive their animal is suffering. At the same time, pets live naturally ~1/5 of the lifespan of humans, so studies involving survival endpoints are significantly compressed in time relative to humans. In sum, understanding the differences between companion and laboratory animals provides essential context to the design and critical review of stem cell trials using companion animal studies.

Table 1. Selected canine and feline companion animal disease models

System	Veterinary disease	Human disease	Lifetime prevalence	Pathology	Gene association	References
Cardiac	Myxomatous mitral valve disease (canine)	Mitral valve prolapse	21%-97% all breeds; increased in CKCS	Myxomatous valves, fibrosis, glycosaminoglycan accumulation	NA	[32–34]
	Arrythmogenic right ventricular cardiomyopathy (canine)	Arrythmogenic right ventricular dysplasia/ cardiomyopathy	NA (Increased in Boxers)	Fibrofatty infiltration and myocarditis right more than left ventricles	STRN, dilated cardiomyopathy form (Boxers)	[35–37]
	Dilated cardiomyopathy (canine)	Dilated cardiomyopathy	0.16% all breeds; $>$ 50% Doberman Pinschers	Attenuated wavy fibers, atrophy of myofibers, fibro or fibro-fatty infiltration, infarcts	DMD (German Short Haired), PDK4 (Doberman Pinschers)	[38–43]
	Hypertrophic cardiomyopathy (HCM) (feline)	Hypertrophic cardiomyopathy	NA all breeds; 41.5% Maine Coon cat	Asymmetric hypertrophy of the ventricular septum, marked disorganization of cardiac muscle cells, abnormal intramural coronary arteries, and ventricular septal fibrosis	MYBPC3-A31P—(Maine Coon cats)	[44–46]
Neurologic	Intervertebral disc degeneration/ herniation (canine)	Intervertebral disc herniation	3.5% all breeds	Annulus fibrosus or pulpy nucleus degeneration and cervical, thoracic, or lumbar region herniation, with spinal cord compression	NA	[47–49]
	Epilepsy (canine)	Epilepsy	0.6%-0.75% All breeds; 3.5% Labrador Retriever; 33% Belgian Shepherds	Neuronal cell loss, aberrant neurogenesis, microglial activation, blood brain barrier alterations	LGI2, ADAM23	[50–54]
	Canine cognitive dysfunction syndrome (canine)	Alzheimer's Disease	14%-60% (all breeds)	Amyloid-β_{42} fibrillar plaque cerebral amyloid angiopathy; neurodegeneration	NA	[55–58]
	Degenerative myelopathy (canine)	ALS	24% all mixed and purebred dogs; 37% German Shepherd; 94% Wire Fox Terrier	Insoluble SOD1 aggregates in neurons	SOD1:c.118A (mixed breeds, all breeds) and SOD1:c.52T (Bernese mountain dogs)	[59–62]
	GME (canine)	Neuroinflammatory features of MS	NA	Perivascular mononuclear cell whorling infiltrate in white matter of brain, spinal cord, and meninges; in acute cases, both white and gray matter affected	NA	[63, 64]
Gastrointestinal	Inflammatory bowel disease (canine, feline)	Inflammatory bowel disease	NA; higher in Weimeraner, Rottweiller, German Shepherd, Border Collie, and Boxer	Gastric, small intestinal, and colonic epithelial injury, intraepithelial and lamina propria infiltration with lymphocytes, plasma cells, eosinophils, neutrophils, and macropahges (colon), villus stunting (duodenum), crypt hyperplasia/dilation/ distortion	NOD2; TLR4; TLR5 (breed independent)	[65–70]

Table 1. *Continued*

System	Veterinary disease	Human disease	Lifetime prevalence	Pathology	Gene association	References
Dermatologic	Atopic dermatitis (canine)	Atopic dermatitis	8.7% all breeds	Erythema, lichenification, and alopecia/excoriation	CFA27 and PKP2 (GS) PKP2 (GS); CFA17 and PTPN22 (WHWT).	[71–77]
	Peri-anal fistulas, "furunculosis" (canine)	Perianal fistulizing Crohn's, anal furunculosis	NA; increased in German Shepherds	Perianal fistulitis with dense sheets of plasma cells, perivascular lymphoid nodules; eosinophilic subcorneal pustules in duct epithelium	DLA-DRB1*001:01 haplotype; GWAS: ADAMTS16 and CTNND2	[78, 79]
	Pemphigus foliaceus (canine)	Pemphigus foliaceus	NA	IgG auto-antigen (Desmocollin-1) mediated subcorneal or intra-granular pustular dermatitis; pustules contain acantholytic cells which span over multiple hair follicles	NA	[80, 81]
Ophthalmologic	Keratoconjuncti-vitis sicca (canine)	Keratoconjunctivitis sicca, features of Sjogren's Disease	4%-20% all breeds	Goblet cell depletion, multifocal chronic adenitis, lymphoid infiltrations, focal acinous atrophy, increased fibrous tissue of lacrimal glands; Th, and B lymphocytic infiltration	NA	[82–84]
Musculoskeletal	Osteoarthritis (canine, e.g., elbow dysplasia, hip dysplasia)	Osteoarthritis	11% Labrador retrievers (hip dysplasia); 47% Chow-Chow (elbow dysplasia)	Loss of cartilage and chrondrocytes, decreased proteoglycans, loss of collagen integrity, tidemark crossed by vessels disappears, synovial proliferation/fimbriation/ thicking/ hypervascularity/ infiltration with lymphocytes, subchondral bone thickening, and meniscal tears	No major locus	[85–87]
Pulmonary	Idiopathic pulmonary fibrosis (canine)	Idiopathic pulmonary fibrosis (features of NSIP and UIP)	NA; increased in West Highland White Terriers	Diffuse mature fibrosis, resembling human NSIP > UIP; accentuated subpleural and peribronchiolar fibrosis with interspersed "honeycombing" and substantial alveolar epithelial changes, resembling UIP > NSIP; progressive intra-alveolar organizing fibrosis and inter-stitial mature collagen	NA	[88, 89]
	Asthma (feline)	Asthma	1%-5%; 4-5 years old		NA	[90–93]

Table 1. *Continued*

System	Veterinary disease	Human disease	Lifetime prevalence	Pathology	Gene association	References
				Eosinophilia, perivascular, peribronchial, peribronchiolar, and alveolar inflammation, smooth muscle hyperplasia, bronchial gland hyperplasia		
Renal	Chronic kidney disease (feline)	End-stage renal disease	80%-90% by age 15 years	Tubulointerstitial nephritis, with mononuclear cell infiltration (lymphocytes, macrophages) and progressive loss of proximal tubules	NA	

Abbreviations: ALS, amyotrophic lateral sclerosis; CKCS, Cavalier King Charles Spaniel; DMD, ; GME, granulomatous meninomyelo-encephalitis; GS, German Shepherds; MS, multiple sclerosis; NA, not available; NSIP, nonspecific interstitial pneumonia; STRN, Striatin; UIP, usual interstitial pneumonia; WHWT, West Highland White Terrier.

Review of Recent Clinical Trials of Stem Cell Therapies in Companion Animal Disease Models

Several studies have been performed to advance our understanding of the therapeutic potential of stem cells in companion animal disease models. For the purpose of this article, studies in the English language literature were identified using PubMed search terms "(canine or dog or feline or cat) and (stem cell)" between the years 2008-2015, yielding 118 publications. The search was further refined by omitting citations concerning laboratory animal studies or any study involving experimental induction of disease, or those concerning tumors or cancer (as these are not classically targets for "regenerative medicine"). Case reports employing mesenchymal stem cells (MSC) for novel treatment of large open wounds [103], fibrocartilaginous emboli and ischemic myelopathy [104], and pemphigus foliaceus [105] were identified but left out from further review because the findings have not been reproduced. The remaining studies ($n = 19$, Table 2) that were evaluated in this review were mostly (12/19) aimed at establishing feasibility (safety, route of administration, dosage, biologic responses) or preliminary efficacy of stem cell treatments, that is, the majority were done in an open label, baseline controlled fashion, and there was no blinding or placebo (or vehicle) control group. Exceptions include two studies that were performed as a double blind (owner and investigator blinded) randomized placebo controlled study [112, 128], one study as a double blind randomized comparative study [108], one study as an open label randomized controlled study [116], one study as a single blinded randomized controlled study [126], and two studies as double blinded baseline-controlled studies [106, 107]. In all studies reviewed, it was noted that study protocols were approved by internal review boards at the parent institution, and informed consent was obtained prior to initiation of study protocols. The exact nature of information contained within the informed consent, including stated risks of stem cell transplantation and incentives offered to clients were not disclosed in publications.

Stem cells employed were either MSC (17/19 trials), olfactory ensheathing cells (OEC, 1 trial), or neural lineage cells derived from MSC (1 trial). Sixteen out of 19 studies were performed in dogs and 3 in cats. Thirteen out of 17 of the MSC studies used adipose tissue-derived MSC (AD-MSC), either allogeneic (8/13 trials) or autologous (5/13 trials) AD-MSC. Characterization of stem cells varied in scope, but those studies employing MSC followed International Society for Cellular Therapy (ISCT) consensus guidelines [130] including trilineage differentiation (chondrocyte, adipocyte, and osteocyte) and immunophenotype. Processing and manufacturing to obtain stem cells was conducted mainly in academic laboratories (non-GMP or GMP-like) with exceptions noted under subheading "Manufacturing" in Table 2.

Study Disease Targets

Osteoarthritis

OA which is one of the most prevalent disease in companion animals was addressed in several veterinary stem cell-based clinical trials (Table 1). Four of five stem cell trials focused on canine hip [106–109] and one on canine elbow [110] OA (loci

Table 2. Summary of stem cell clinical trials in canine and feline species from 2008 to 2015

Disease	Species	Study type	Arms	Manufacturing	Interventions	Endpoints	Results	Follow-up period	References
Osteoarthritis (hip joint)	Canine	Blinded, baseline controlled study with two single groups; after washout of medical therapies	Lameness group ($n = 9$), not lame ($n = 5$)	AD-MSC from inguinal fat using DogStem® kit and GMP lab (Fat-Stem, Buggenhout, Belgium)	Autologous AD-MSC, 30×10^6 single unilateral hip joint injection	Blinded force plate (lameness) evaluation	Significant Improvement in force plate variables compared to pretreatment for 3 months	6 months	[106]
Osteoarthritis (hip joint)	Canine	Blinded, baseline controlled with two single groups; after washout of medical therapies	Lameness group ($n = 8$), not lame ($n = 5$)	AD-MSC from inguinal fat, using GMP lab (Fat-Stem,					
(Buggenhout,Belgium)	Autologous AD-MSC 30×10^6, plus platelet rich growth factors; single unilateral hip joint injection	Blinded force plate (lameness) evaluation	Significant improvement in force plate (objective) variables at 6 months compared to pretreatment	6 months	[107]				
Osteoarthritis (hip joint)	Canine	Double blind randomized comparative study; after washout of medical therapies	Autologous AD-MSC or plasma rich in growth factors	AD-MSC from inguinal fat using DogStem® kit and GMP lab (Fat-Stem, Aalst, Belgium)	Autologous AD-MSC 30×10^6 ($n = 18$) or plasma rich in growth factors ($n = 17$)	Functional limitation, ROM, owner's and veterinary investigator VAS, and patient's QOL	Functional limitation, ROM, owner's and investigator's VAS, and QOL improved 1-6 months after either treatment. AD-MSC produced better pain relief at the 6-month time point	6 months	[108]
Hip dysplasia (partially refractory to SOC)	Canine	Open label baseline-controlled study with two single groups; after washout of medical therapies	Autologous SVF ($n = 4$) or allogeneic AD-MSC ($n = 5$) treatment groups	SVF and AD-MSC from inguinal fat by collagenase digestion, expansion, non-GMP	Injections at three acupuncture sites with autologous SVF ($2\text{-}5 \times 10^6$ total) or allogeneic AD-MSC ($2\text{-}8 \times 10^5$ total cells)	Results were scored as: worse, no modification, or improvement	Improvement in clinical scores in all patients; results more favorable for SVF than allogeneic AD-MSC	3 months	[109]
Osteoarthritis humero-radial joint (refractory)	Canine	Open label baseline-controlled study with two single groups; after washout of medical therapies	Autologous AD-MSC with PRP or Hyaluronic acid (HA)	AD-MSC from subcutaneous inquinal, and visceral fat by collagenase digestion, expansion, non-GMP	Autologous AD-MSC ($3\text{-}5 \times 10^6$) plus either PRP ($n = 2$ dogs) or HA 10 mg/ml ($n = 2$ dogs)	Clinical observations (lameness, ROM)	Clinical improvement in lameness and pain on manipulation (not quantified)	1 month	[110]
Intervertebral disc degeneration (IVDD), chronic, (>6 months), no deep pain	Canine	Open label baseline-controlled study: adjunct to decompressive surgery	Stem cell treatment group ($n = 4$)	BM-MSC from iliac crest, non-GMP	Dimethyl sulfoxide (DMSO) plus autologous BM-MSC (1×10^6 per 1 mm 3 lesion site) into spinal cord lesions (over 5 mm) sealed with gelfoam	Neurologic exam, MRI	Improvements in pain sensation, reflexes, and ataxia; no change in features of MRI	12 months (18 months clinical)	[111]

Table 2. *Continued*

Disease	Species	Study type	Arms	Manufacturing	Interventions	Endpoints	Results	Follow-up period	References
IVDD, chronic (>3 months), no deep pain	Canine	Double blind randomized controlled clinical trial; dogs with no prior surgery	OEC, ($n = 23$) vs. vehicle ($n = 11$)	Olfactory mucosa, collagenase digestion, expansion, non-GMP	Autologous transcutaneous olfactory ensheathing (p75+) cells (5×10^6) vs. cell transport solution	Fore limb–hind limb temporal coordination using computerized analysis of digitized kinematic data, somatosensory evoked potential, bladder compliance	Significant improvement of fore-hind coordination in treatment group	6 months	[112]
IVDD, chronic (>60 days), no deep pain	Canine	Open label baseline controlled study; adjunct to prior decompressive surgery	Spinal cord injury group: ($n = 6$)	50-60 days gestation fetal canine BM-MSC, non-GMP citing [113]	Transcutaneous intraspinal allogeneic fetal bone marrow stem cells (1×10^6)	MRI; independent assessment of locomotory function by three blinded physiotherapists	Clinically improved neurologic-locomotory function in 6/6 dogs	90 days	[114]
IVDD, acute, no deep pain	Canine	Open label randomized controlled clinical trial; adjunct to decompressive surgery	AD-MSC treatment ($n = 9$) or control ($n = 25$)	AD-MSC from hip fat citing [115], non-GMP	Intraoperative intraspinal allogeneic AD-MSC (2×10^7 cells) vs. no treatment	Neurologic exam, MRI, or CT	Significantly higher proportion of neurologic recovery in AD-MSC treatment group	6 months	[116]
IVDD, no deep pain >42 days after hemilaminectomy-discectomy	Canine	Open label baseline controlled study; adjunct to prior decompressive surgery	BM-MSC differentiated to neural lineage (NIBM-MSC) ($n = 7$)	BM-MSC from iliac crest; differentiation to neural lineage [117], non-GMP	At 42 and 63 days after surgery, 5×10^6 autologous percutaneous intraspinal NIBM-MSC at surgery sites	Texas spinal cord injury score'; MRI; somatosensory and motor evoked potentials	Improvements at 4-8 months after intraspinal treatments in gait, proprioception, and evoked potential in five dogs	4-8 months	[118]
MUO	Canine	Open label baseline controlled study with two single groups; adjunct to immunosuppressive agents	Autologous BM-MSC IT plus IA (carotid) or IV in steroid refractory disease	BM-MSC from proximal humerus; non-GMP	BM-MSC IT plus IA $n = 3$, or IT plus IV $n = 4$	Neurologic signs	IT plus IA stem cells shorter time to response than IT plus IV stem cells	6-24 months	[119]
Dilated cardiomyopathy	Canine	Open label, historical controls	Single treatment group ($n = 15$); adjunct to SOC	Commercial source of non-Dobermann AD-MSC (ad-MSC; Sciencell™ Research Laboratories)	Tyrosine mutant adeno-associated virus 2-stromal derived factor-1 allogeneic adipose-derived mesenchymal stem cells	Echocardiography, Holter monitoring of ECG, AAV titers	Median survival 620 days (all dogs) and 652 days (excluding dogs in CHF), no different from historical controls	2 years	[120]
Atopic dermatitis (nonseasonal)	Canine	Open label, baseline controlled study	Single treatment group ($n = 5$); adjunct to SOC	AD-MSC from interscapular fat, collagenase digestion, non-GMP	Autologous AD-MSC (1×10^6) intravenous	CADESI-03 and Visual Pruritus Scores	No significant change	12 weeks	[121]

Table 2. *Continued*

Disease	Species	Study type	Arms	Manufacturing	Interventions	Endpoints	Results	Follow-up period	References
Perianal fistulas (furunculosis) refractory to cyclosporine	Canine	Open label, baseline controlled study	Single treatment group ($n = 6$); adjunct to SOC	MSC derived from FDA approved human embryonic stem cell line MA09 by single blastomere technology [122]	Xenogeneic human embryonic stem cell derived MSC, single treatment, fibrin sealant	Peri-anal fistula closure, cyclosporine requirements	Complete closure of fistulas in all dogs (3 months); relapse in 2 dogs (6 months); $>50\%$ reduction in cyclosporine usage in 5/6 dogs at 3 months and 4/6 dogs at 6 months	1 year	[123]
Inflammatory bowel disease (5 months-1 year duration), partially refractory	Canine	Open label, baseline controlled study	Single treatment group ($n = 12$); after washout of medical therapies	AD-MSC from abdominal fat of single donor 2.5-year-old donor; non-GMP	Allogeneic AD-MSC, single injection 2×10^6/kg bwt IV	Clinical Inflammatory Bowel Disease Activity Index and Canine Chronic Enteropathy Clinical Activity Index, C-reactive protein, albumin, folate, and cobalamin on day 42 post-treatment	Improvements in clinical scores, albumin, folate, and cobalamin but not C-reactive protein	42 days	[124, 125]
Chronic enteropathy (>3 months)	Feline	Single (owner) blind randomized controlled clinical trial	Allogeneic AD-MSC ($n = 7$) vs. vehicle ($n = 4$); additional AD-MSC unblinded for additional 3 months	AD-MSC from abdominal fat of single <1 year old SPF feline donor, collagenase digestion, expansion; non-GMP	Allogeneic feline AD-MSC (2×10^6/kg bwt) or saline vehicle twice 2 weeks apart	Owner reported fecal consistency using the following scale: 1 (very hard), 2 (firm), 3 (normal), 4 (moist), 5 (soggy), 6 (no shape), 7 (watery)	Improvements in clinical scores but not laboratory data (albumin, cobalamin, folate) in AD-MSC-treated cats	2 months	[126]
Chronic kidney disease (serum creatinine 1.6-5 g/dl)	Feline	Open label, baseline-controlled study with two single groups; adjunct to SOC	Allogeneic AD-MSC (low dose, high dose cryopreserved AD-MSC, or high dose AD-MSC from cryopreserved adipose tissue)	AD-MSC from abdominal fat of single <1 year old SPF feline donor, collagenase digestion, expansion; non-GMP	Allogeneic AD-MSC, 3 biweekly treatments. Group 1: 4.1×10^5/kg bwt cryopreserved AD-MSC ($n = 6$); Group 2: 8.3×10^5/kg bwt cryopreserved AD-MSC ($n = 5$); Group 3: 8.4×10^5/kg bwt AD-MSC expanded from cryopreserved adipose tissue ($n = 5$)	Serum biochemistry, complete blood count, urinalysis, urine protein, glomerular filtration rate, and urinary cytokines	Adverse events: Group 2: 4/5 cats (vomiting, tachypnea); serum Cr: Group 1, significant decrease in Serum Cr (-0.5 g/dl); GFR: trend toward increase GFR in Group 2; urinary MCP1 and IL-8 decreased significantly	8 weeks	[127]

Table 2. *Continued*

Disease	Species	Study type	Arms	Manufacturing	Interventions	Endpoints	Results	Follow-up period	References
Chronic kidney disease	Feline	Placebo-controlled randomized trial; adjunct to SOC	Allogeneic, culture expanded fresh MSC	AD-MSC from abdominal fat of single <1 year old SPF feline donor, collagenase digestion, expansion; non-GMP	Allogeneic AD-MSC 4×10^6/kg bwt, 3 IV infusions at 2 week intervals; expanded from cryopreserved	Serum creatinine; GFR determined by nuclear scintigraphy; serum biochemistry, and CBC	Adverse events not noted; no significant difference in renal functional parameters or serum Cr between MSC treated and placebo-treated cats	8 weeks	[128]
KCS refractory to SOC	Canine	Open label, baseline controlled study; after washout of medical therapies.	Allogeneic AD-MSC	AD-MSC from gluteal fat of 3 2-year-old canines, collagenase digestion, expansion; non-GMP	Allogeneic AD-MSC ($n = 12$, or 24 eyes), 5×10^6 cells around lacrimal gland and 3×10^6 surrounding gland of third eyelid	Schirmer tear test, ocular discharge, hyperemia, corneal opacity	Significant improvements in all endpoints compared to baseline; absence of disease progression	9 months	[129]

Abbreviations: AD-MSC, adipose tissue derived mesenchymal stem cells; BM-MSC, bone marrow mesenchymal stem cell; CHF, congestive heart failure; Cr, creatinine; CT, computed tomography; DMSO, dimethyl sulfoxide; GFR, glomerular filtration rate; GMP, good manufacturing practices; IA, intra-arterial; IV, intravenous; IVDD, intervertebral disc degeneration; IT, intrathecal; KCS, keratoconjunctivitis sicca; MUO, meningoencephalitis of unknown origin; MRI, magnetic resonance frequency; NIBM-MSC, neural lineage induced bone marrow derived mesenchymal stem cells; OEC, olfactory ensheathing cells; PRP, platelet rich plasma; QOL, quality of life; ROM, range of motion; SOC, Standard of Care; SPF, specific pathogen free; SVF stromal vascular fraction; VAS, visual analogue scale.

of highest prevalence), and all trials employed single injections of intra-articular AD-MSC. AD-MSC was used alone [106], or in conjunction with either intra-articular autologous platelets rich in growth factors (PrP) [107] or hyaluronic acid (HA) [110] as a chondroprotective agent [131] (Table 2) Comparisons were made between cultured AD-MSC plus PrP versus AD-MSC plus HA [110], or AD-MSC versus fresh stromal vascular fraction (SVF) [109]. Interestingly, one trial employed injections at acupuncture points rather than intra-articular injections [109]. In all of these OA trials, treatments were evaluated as alternatives to SOC (i.e., analgesics, anti-inflammatories were "washed out" prior to onset of trial). The use of AD-MSC (between 2 and 30 million cells per administration) admixed with PrP resembled a clinical trial underway to evaluate intra-articular AD-MSC in humans with OA (e.g., NCT01739504, http://www.clinicaltrials.gov). Clinical endpoints included canine-modified visual analog scales (VAS) which assess musculoskeletal pain, ROM, and quality of life scores (including pain assessment) similar to studies in humans, or alternatively a subjective clinical assessment [109]. Blind force plate analysis, considered the gold standard in objective gait analysis, was performed in two of five of the canine OA studies [106, 107]. Only one study employed a double blind randomized controlled study design [108], and none of the studies employed placebo controls.

The studies consistently demonstrated improved endpoints (pain, ROM, VAS) in dogs treated with AD-MSC, AD-MSC plus PrP, AD-MSC plus HA, or SVF. One study [108] demonstrated superiority of AD-MSC over PrP alone at the 6-month time point. Duration of improvement was observed to be 3-6 months, although animals were not observed beyond these time points. No adverse events were recorded for any of the study animals; complete blood counts, serum chemistries, and lameness evaluations were performed to evaluate safety informally. These studies demonstrate feasibility, safety, and preliminary evidence of biological activity of intra-articular MSC at the dosages employed in severe OA in dogs. Additional studies to evaluate the feasibility of multiple intra-articular injections or the additional of systemic injections for this disease. Clearly, placebo controlled studies will be important to further establish efficacy of therapies based on MSC, PrP, and chondroprotective agents either alone or in combination.

Intervertebral Disc Degeneration

IVDD with or without disc herniation is a common problem in smaller chondrodystrophic breeds (e.g., Dachshunds). Clinical features including back pain, paresis or paralysis, or a subclinical course; moreover, histological and biochemical features resemble human IVDD [47] (Table 1). Canines with IVDD are the only species that are diagnosed and managed, using both medical and surgical approaches, in similar ways to humans. Spinal cord contusion varies in depth, extent, and chronicity in canine IVDD (akin to humans), unlike the type of contusion that is experimentally generated to create spinal cord injury (SCI) in laboratory animals, the latter which also invokes ethical concerns. For these reasons, canine IVDD is considered a valuable disease model for human IVDD in the quest for novel and effective therapies.

Unlike the OA trials described above, patient characteristics, cell sources, routes of administration, and endpoints were diverse for clinical trials of stem cell therapeutics for IVDD in dogs (Table 2). In four studies, dogs experienced severe compressive herniation of the spinal cord and lacked any deep pain sensation for as long as 42 days, 2, 3, or 6 months prior to the institution of treatments, consistent with chronic SCI. In one such study of surgically refractory, chronic IVDD (>60 days) in four dogs [111], investigators delivered autologous bone marrow MSC (BM-MSC, 5×10^6 total cells) intralesionally at five sites during a second laminectomy and monitored progress using neurologic examination (18 months) and MRI (12 months) as endpoints. DMSO was administered to the cord immediately prior to BM-MSC treatments. No control arm was employed in this study. Treated canines showed improved pain, ataxia, and reflexes, although MRI appearance was unchanged. In another study of chronic (>30 days after decompressive laminectomy) IVDD in dogs [114], a single intralesional injection of allogeneic fetal canine BM-MSC (1×10^6 cells) [113] was delivered transcutaneously under fluoroscopic guidance to all dogs ($n = 6$). Blind evaluations led to the conclusion that all patients experienced degrees of neurologic-locomotory recovery (support of body weight, small uncoordinated steps, return of tail tone, deep pain reflexes, defecation, muscle tone) at 90 days after implantation of cells; however, no changes in MRI were noted in this study. It is not clear from these data whether there was any advantage to the use of fetal (vs. adult) BM-MSC. Besalti et al. [118] evaluated the therapeutic potential of autologous BM-MSC that were differentiated to neurospheres ($nestin^{pos}$), then dispersed and differentiated to neural lineage cells (NLBM-MSC, expressing CNPase, MAP-2, GFAP, and beta III tubulin) in dogs ($n = 7$) with chronic (>42 days) SCI secondary to IVDD. Dogs received two percutaneous intraspinal injections 2 weeks apart starting 42 days after hemilaminectomy. Proprioception and nociception did not improve but gait improved in one dog at 4 months after final injection. At 8 months, there was 1-2 point improvement in gait, proprioception, and nociception in three of the four dogs which remained in the study. Change in somatosensory and motor evoked potentials were minor. The overall conclusion is that the approach was safe and feasible, but the benefits compared to no stem cell therapy (based on historical controls) are inconclusive at this stage. In a double blind, randomized vehicle controlled clinical trial by Granger et al. [112], dogs with chronic (>3 months) IVDD with no deep pain equivalent to human ASIA grade A injury, that had not received decompressive surgery were randomized to receive either percutaneous autologous enriched OEC or vehicle transplantations. OEC were isolated by surgical biopsy of olfactory mucosa within the frontal sinus, enzymatic digestion, and expansion. Ultimately, they included ~50% $p75^{pos}$ cells plus ~50% fibronectin (Fn) expressing fibroblastic cells. Dogs received either 5×10^6 OEC ($n = 25$ dogs) or vehicle injections ($n = 11$ dogs). Objective endpoints included kinematic digitized analysis of gait (fore limb-hind limb coordination, ataxia), as well as somatosensory evoked potentials and measures of bladder compliance. At the 6-month time point (end of study), the OEC-treated group showed significantly better fore-hind limb temporal coordination than the vehicle treated group, although there was no effect on long track function (spasticity, bladder function). This study is the first to provide objective evidence of the feasibility and therapeutic potential of

intraspinal (nonsurgical) OEC transplantation in chronic spinal cord injury (SCI). Along with the study of allogeneic fetal canine BM-MSCs in IVDD [114] and NLBM-MSC [118], this study demonstrates that use of percutaneous delivery represents a viable nonsurgical route of administration that has not been fully appreciated in past studies. Questions remain concerning the method of isolation and characterization of OEC, given the challenge to access lamina propria tissue from olfactory mucosa; biopsy of olfactory bulb has been proposed as an alternative source of OEC (in humans) [132]. In the novel study employing NIBM-MSC, no comparison was made with undifferentiated BM-MSC [118], so the findings are inconclusive with regard to the specific role and mechanisms of neural lineage cells in mitigation of SCI due to IVDD and herniation. Further, one might ask whether the benefits observed of OEC require a heterogeneous population of $p75^{pos}$ and Fn expressing cells. If so, what is the role for each cell type in controlling injury or stimulating repair or regeneration? Multiarm studies in companion animals may be effective in elucidating this question. Another question is whether OEC were retained or engrafted into the neuronal population, or acted primarily as reservoirs of paracrine signals? Cell tracking studies may be effective in evaluating the fate of OEC and other cell types transplanted into the spinal cord in companion animals [133], notwithstanding the challenges to interpret whether signals arise from viable donor cells or residual labels. Furthermore, given that autopsy material in companion animals is often not available, in vivo methods to track injected cells will be an important area of development.

Recently Kim et al. [116] reported the results from a randomized controlled clinical trial employing a single injection of intraoperative, intraspinal allogeneic AD-MSC (2×10^7 cells, $n = 9$ patients) versus decompressive surgery alone ($n = 25$ patients) in canine IVDD patients with *acute* hind limb paraplegia and absence of deep pain responses. The investigators found that AD-MSC treated patients had a significantly higher rate of recovery (full recovery 55.6% vs. surgery alone 16%, $p < .05$) at 6 months after treatment. The strength of this study is the randomized control design with sufficient study power to derive therapeutic endpoints. Potential confounding factors in this study included the use of multiple and varying adjunctive treatment modalities (i.e., electroacupuncture, therapeutic laser therapy, physical therapy), and questions remain concerning the influence of baseline neurologic grade. The study was conducted without blinded evaluations, and the number and expertise of the evaluators was unclear. However, this is a landmark study demonstrating both the feasibility, early safety, and therapeutic potential of AD-MSC in acute IVDD with herniation, paving the way for further development of allogeneic donor sources, optimization of intraspinal delivery methods, selection of patients, and studies which define the mechanisms of action.

Based on these studies in dogs with IVDD, further evaluation of stem-progenitor cells (MSC, OEC, others) in prospective double blind randomized controlled studies for IVDD is warranted. Canine IVDD remains a compelling model of acute or chronic SCI in humans given the similarities of the disease to humans and anatomical similarities between the human and canine spinal column. Mechanistic data concerning neuroprotective effects of OEC and MSC will be crucial to advance these therapies. Alternative cell sources sought by investigators include epidermal neural crest cells [134], umbilical tissue-derived MSC [135, 136], and induced pluripotent stem cell-derived MSC [131] or neural progenitor cells. In addition to their added accessibility for manufacturing, these novel cell types might improve neuroprotection, neuronal regeneration, and more effectively reduce inflammation.

Atopic Dermatitis

Atopic dermatitis is a condition that afflicts ~8.7% dogs [71] similar to children (10%-20%) and adults (3%-4%) [137], that is associated with breed predilections, polymorphisms at specific gene loci (e.g., by genome-wide association study - GWAS), altered gene expression, and specific allergens (Table 1). The concept behind employing MSC for immunomodulation of atopic dermatitis, led Hall et al. [121] to implement an open label baseline controlled clinical trial employing a single dose of autologous AD-MSC (1×10^6 cells IV) in five canine patients, using established clinical scores to record the effects (Table 2). While the injections were found to be safe, no benefits of AD-MSC treatment were observed in this trial. The dosage of AD-MSC was lower than employed in other studies reviewed herein, and lower than dosages typically employed in human studies ($\geq 2 \times 10^6$/kg bwt). The selection of dosages for companion animals has generally been modeled after human studies, rather than formulated from rodents which employ significantly higher dosages per kilogram bodyweight. It is unclear if any preclinical studies were performed to establish the immune modulatory capacity of the AD-MSC used in this study. In future studies to increase rigor, it will be important to establish whether the specific cell lines employed in veterinary trials have these attributes given the known variability in MSC quality based on donor and manufacturing factors [138].

Perianal Fistulas

In approximately one third of Crohn's patients, cutaneous or rectocutaneous fistulas develop which are often relapsing, unremitting, or resistant to immunosuppressive therapies [139, 140]. The canine disease "perianal fistulas" (i.e., "anal furunculosis") resembles Crohn's fistulitis with respect to clinical signs, immunopathology, association with certain gene regions, and therapeutic responses to immunosuppressive agents (Table 1). As such, the disease serves as a potentially important model of Crohn's fistulitis, in particular for the study of novel intralesional and systemic therapies.

In an open-label, baseline controlled study by Ferrer et al. [123], dogs with perianal fistulas were treated intralesionally with human embryonic stem cell-derived MSC (hESC-MSC) that were extensively characterized, for example, by immunophenotype, immune modulatory capacity by mixed lymphocyte assays, cytokine production, and in vivo suppression of auto-immune diseases in rodent models of lupus and experimental autoimmune encephalitis [141]. The study was an open label baseline controlled design involving six dogs with cyclosporine refractory perianal fistulas. Fistulas received a total of 2×10^7 hESC-MSC divided over two to four sites, with sealant placed over the fistula opening to prevent leakage of the hESC-MSC dosages. All dogs showed marked progression toward remission, although one dog relapsed by 6 months. Cyclosporine dosage needed to maintain the dogs in remission was reduced by ~50%. This study demonstrates

that xenogeneic delivery of MSC can exert potent biological effects in canine patients, providing feasibility and proof of principle. The lack of vehicle controls confounds the interpretation of this study, but the patients were refractory to SOC for a prolonged period prior to MSC administration, so the paper provides compelling initial data. The results support recent studies in humans that similarly show the benefits of intralesional MSC for perianal fistulas [142]. The canine model will be useful to advance novel medical therapies for perianal fistulas, including cell sources, formulations, dosages, schedules, and interactions with surgical interventions. A deeper understanding of the molecular phenotype of canine perianal fistula will aid in these translational efforts.

Inflammatory Bowel Diseases

Inflammatory bowel disease (IBD) in companion animals (i.e., dogs, cats) includes several histopathologic variants, including lymphocytic-plasmocytic colitis, histiocytic ulcerative colitis (Boxer dog colitis), eosinophilic colitis, and regional granulomatous colitis [65]. Canine spontaneous lymphocytic-plasmocytic colitis which is the most common form of this enteropathy has several histopathologic and cellular-molecular features that strongly resemble human IBD, including increases in the number of mast cells, infiltration of lamina propria with $CD4^{pos}$ T cells and intraepithelial zones with $CD3^{pos}$ T cells, upregulation of NF-κB [143], decrease in the density of Tregs ($FoxP3^{pos}$) in duodenal villi [144], and gene associations including NOD2 [66], TLR4 and TLR5 [67–69]. Canine and feline companion animal models of IBD have the potential to overcome some of the major obstacles to laboratory animal modeling of human IBD, namely the challenges of simulating the multifactorial pathogenesis of IBD which is less compelling in rodent models (e.g., dioctyl sodium sulfosuccinate-induced colitis) [145], understanding stem cell (i.e., MSC) immune modulation mechanisms, determining dose equivalence, and the biological effects of stem cell therapies in *refractory* IBD (i.e., refractory to corticosteroids or cyclosporine A in veterinary patients).

In an open label baseline controlled study by Perez-Merino et al. [124, 125], 12 dogs that were partially tolerant to SOC with histologically confirmed lymphocytic-plasmocytic IBD, received a single intravenous injection of 2×10^6 per kg body weight (bwt) allogeneic, single donor sourced AD-MSC [101, 102]. These patients were monitored for 42 days after transplantation using two different clinical scoring systems which incorporated laboratory and clinical observations (including owner observations of attitude, appetite, stool consistency and frequency, vomiting, pruritus) along with ascites, peripheral edema, body weight, and serum albumin as well as biomarkers folate, cobalamin, and C-reactive protein (CRP). Treatment significantly improved clinical scores, serum albumin, and biomarkers (although not CRP) compared to baseline values. The absence of a control group obscures our understanding of the magnitude of effects achieved with AD-MSC, and the open label design may contribute to observer (owner, veterinarian) bias. However, these data support the safety and therapeutic activity of allogeneic AD-MSC in partially refractory canine IBD at the selected dosage, one that mirrors the dosage range employed in past human studies (1-2 $\times 10^6$/kg bwt). It is noteworthy that recent ongoing Phase III human trials employing BM-MSC (Prochymal) by Osiris (NCT00482092) employ 600-1200 $\times 10^6$ per patient (for 70 kg patient, this equates to 8.6-17.1 $\times 10^6$/kg bwt), delivered 4 times over 2 weeks [146]. For future studies, it will be critical to evaluate a dosage and schedules, especially in refractory IBD to understand the therapeutic potential and safety of MSC in companion animal disease models.

Questions remain about how IV transplantation of MSC imparts a local effect on bowel inflammation in this model. Furthermore, the benefits of allogeneic source which eliminates the potential confounding effect of donor disease on cell quality, may play an important role in success of clinical trials. The short duration of study (42 days) leaves unanswered the duration of the observed effects. The impact of single versus multiple injections of allogeneic MSC on the recipient immune system also needs to be explored. Further, interpretations of these data established in partly refractory canine IBD patients *after washout of SOC* (corticosteroids, immunosuppressive agents) cannot be generalized to more refractory patients that are concurrently receiving MSC and SOC, that is, the effects may be greater or lesser in those patients.

In feline patients with lymphocytic-plasmocytic enteritis, Webb and Webb [126] conducted a single blinded (i.e., owner blinded) randomized placebo controlled study of AD-MSC. Groups were carefully matched with respect to age, body weight, body condition score, and fecal consistency score. Patients continued to receive SOC. Allogeneic AD-MSC (2×10^6/kg bwt, two biweekly injections) were observed to be safely administered, and improved clinical signs in 5/7 animals, versus 0/4 treated with placebo were recorded at the 2-month follow up time point. These data support the feasibility, and immune modulatory effects of allogeneic AD-MSC therapy in feline enteritis at the dosages employed. Further study in larger numbers of patients will be useful to understand the reproducibility of these findings, dosages, regimens, and the importance of allogeneic cell source to the outcome.

The studies in IBD thus far have utilized native MSC. Future studies are warranted which involve cytokine (IFNγ, TNFα, or IL-17) preconditioning of MSC, which is known to enhance immune modulatory capacity of MSC [147].

Dilated Cardiomyopathy

Nonischemic cardiac diseases are a relatively underserved area of investigation in regenerative medicine, with significantly more attention given to myocardial infarction. While myocardial infarction is rarely observed as a primary lesion in companion animals, dogs and cats display a high prevalence of various nonischemic cardiac diseases also found in humans, including feline hypertrophic cardiomyopathy (HCM), and canine dilated cardiomyopathy (DCM), mitral valve disease-prolapse (MVP), and arrhythmogenic right ventricular dysplasia/cardiomyopathy (Table 1). These canine nonischemic cardiac diseases offer unique opportunities to develop therapeutic interventions that mitigate cardiac remodeling, progression to heart failure, fatal arrhythmias, and biomarkers that improve diagnosis and prognostication in these diseases. The only published study to date employing stem cells in nonischemic heart disease in companion animals was performed in DCM [120]. Spontaneous DCM has a similar progression and phenotype in dogs and humans [38]. The study in dogs ($n = 15$) with DCM employed an open label design. The investigators delivered a single retrograde coronary venous treatment of allogeneic AD-MSC which were transduced using adenoviral associated virus (AAV subtype 2) to overexpress

stromal derived factor-1, with the purpose to enhance homing and engraftment of endogenous MSC to myocardium. While 14 out of 15 dogs were discharged within 24 hours of cell delivery, 1 dog developed malignant ventricular arrhythmias before, during, and after the intracoronary treatment and died from cardiac arrest. With a 2-year follow up, there was no difference in median survival, echocardiographic progression to congestive heart failure, ECG, or hematologic indices between treated dogs and historical controls. Interestingly, dogs did not develop anti-AAV2 antibodies. Challenges addressed in this study include safe and effective route of administration, genetic augmentation of homing and survival mechanisms, and application of specific AAV. Canine nonischemic heart disease models may be underutilized for studies in the field of regenerative medicine given their high prevalence and striking similarities in pathology with human DCM, HCM, and MVP. It is important at this time to improve our understanding of the molecular pathology associated with cardiac remodeling in each model. This will open doors to improved specificity of therapies based on stem cells (by way of genetic enhancements), RNA (e.g., miRNA mimics, RNAi, antagomiR), and DNA (gene therapies).

Keratoconjunctivitis Sicca

Dry eyes and mouth are local manifestations of Sjogren's syndrome in humans, which results from IL-17 mediated immunologic injury to lacrimal and salivary glands and subsequent loss of exocrine secretory function [148]. Similarly, canine KCS manifests dry eye which stems from infiltration of lacrimal and third eyelid glands with B lymphocytes and CD4 and CD8 expressing T helper cells as well as mast cells, cell types whose infiltration diminishes after cyclosporine A ophthalmic therapy [82]. One published investigation utilized an open label protocol to test the effects of a single injection of allogeneic AD-MSC transplanted around the lacrimal gland (5×10^6 cells) and gland of the third eyelid (3×10^6 cells) in 12 KCS patients in each eye (totaling 24 eyes) [129]. Patients were monitored using scores for Schirmer's tear test, ocular discharge, hyperemia, and corneal opacity. The average scores improved significantly compared to baseline, and nadir (best) scores persisted for $\sim$9 months after treatment. It is unlikely that AD-MSC persisted in the area of the transplantations for more than 2-3 weeks based on an earlier cell tracking study [133]. Therefore, it is plausible that MSC exerted a paracrine effect on the lacrimal gland, comprising immunomodulatory or trophic (regenerative) effects on the glandular cells, to improve volume, composition, or rheology of the secretions. In Sjogren's syndrome, umbilical cord MSC were recently found to suppress T cytotoxic cells [149, 150], suggesting that AD-MSC may be immune modulatory by similar mechanisms in canine lacrimal and third eyelid glands. Canine KCS may serve as a useful model for studying novel stem cell-based approaches to Sjogren's syndrome given similarities of disease phenotype, and availability of longitudinal access to ocular data and biofluids (tears, saliva, blood).

Neuroinflammation: Granulomatous Meningomyeloencephalitis

Meningoencephalomyelitis of unknown origin encompasses several noninfectious neuroinflammatory processes in dogs which appear to have an auto-immune basis. Granulomatous meningomyeloencephalitis (GME) in particular is characterized by perivascular infiltration by $CD3^{pos}$ and IL-17 expressing T lymphocytes and $CD163^{pos}$ glial/macrophages consistent with a delayed type hypersensitivity [63], and increased C-C motif ligand 19 (CCL19/MIP3β) levels in cerebrospinal fluid (CSF) [151] resembling neuroinflammatory changes observed in people with multiple sclerosis. Neuroinflammation in GME affects the forebrain, brainstem, and spinal cord, including white and gray matter (unless chronic, whereby white matter is affected predominantly) resulting in either diffuse, multifocal, or focal signs, including an ocular form [64] (Table 1). The acute inflammatory process can be controlled in some patients by aggressive high dose corticosteroids and immunosuppressive agents, but failure of this SOC is common. One group of investigators explored the use of a single injection of autologous BM-MSC in dogs with steroid refractory GME delivered by the intrathecal (IT, 4×10^6 cells) plus intravenous (IV, 2×10^6 cells), or intrathecal (IT, 4×10^6 cells) plus intracarotid artery (IA, 2×10^6 cells) routes of administration [119] (Table 2). Follow-up ranged from 6 to 24 months. There were no adverse events reported other than one transient increase in body temperature. The authors describe that seven of the eight dogs survived for the full (2 years) monitoring period, with progressive improvements in neurologic signs, and disappearance in CSF inflammation (mononuclear pleocytosis) and MRI lesions. Only two dogs required antiseizure medication, while the other dogs were free from any medication. While this was an open label study without placebo controls, the fact that dogs did not relapse in this study is an important finding, given the typical refractory and relapsing-remitting presentation of GME. Furthermore, it demonstrates that BM-MSC can be transplanted intrathecally and intra-arterially in dogs with GME without clinically adverse effects; therefore, the safety of intrathecal injections is consistent with findings in mice with experimental allergic encephalomyelitis [152] and in humans with multiple sclerosis [153, 154]. The canine model of GME is a compelling disease model that can assist in preclinical evaluation of novel routes of administration and cell therapy strategies for neuroinflammatory disorders of humans.

Feline Chronic Kidney Disease (End-Stage Renal Disease)

CKD is very prevalent in older cats, with estimates that as many as 85% of cats over the age of 15 have some degree of renal functional impairment. Pathologically, CKD in cats is characterized by widespread tubulointerstitial nephritis, with progressive infiltrates of lymphocytes, plasma cells, and macrophages. Although the etiology of CKD in cats is still poorly understood, feline CKD resembles in many respects the inflammatory pathology present in humans with end stage renal disease (ESRD) from diverse causes, including diabetes mellitus and tubular nephropathies. Thus, the final common pathways for renal functional decline appear to converge in both feline and human ESRD.

Stem cell therapy for feline CKD has been investigated using both autologous and allogeneic MSC [155]. In the original feline CKD study, six cats (two healthy cats and four with CKD) were injected once by the intrarenal route, with approximately 1×10^5 autologous bone marrow or adipose tissue-derived MSC per injection [156]. In this study, adverse effects from MSC injection were not noted, and a modest

improvement in renal function was detected by nuclear scintigraphy in two treated cats with CKD. However, the stress associated with multiple anesthetic episodes for cell collection and injection made the intrarenal approach unfeasible and unsafe option for management of CKD in cats.

A second study of MSC therapy in cats with CKD involved IV administration of allogeneic AD-MSC. A total of 11 cats with CKD received three infusions at 2-week intervals of either 2×10^6 cells per kg bwt (five cats) or 4×10^6 cells per kg bwt (six cats) of cryopreserved AD-MSC [127]. Study cats in the lower dose group did not experience adverse effects from the repeated IV administration of MSC, whereas the majority of cats in the high-dose group experienced rapid adverse effects, including vomiting, salivation, and dyspnea which required in some cases supportive therapy. Renal functional parameters were not improved. A third study investigated IV administration of 4×10^6 AD-MSC per kg bwt (five cats), but in this case cryopreserved cells that had been thawed and cultured in vitro for 24 hours prior to administration. None of these cats developed adverse effects from repeated IV allogeneic MSC administration. However, they also did not exhibit signs of improvement in renal function. These studies were very informative in terms of identifying an acute reaction syndrome to freshly thawed MSC administered by the IV route in cats. It is not known if this response is unique to cats, or a problem that might occur in other species as well. Nonetheless, this particular toxicity warrants particular caution with regards to IV administration of cryopreserved cells. Interestingly, a relatively brief in vitro culture period (24 hours) completely eliminated the response, and it has not been observed in cats repeatedly treated by IV administered allogeneic MSC for up to nine infusions in cats with experimental asthma [96].

In a final CKD study in cats, a randomized clinical trial was conducted in six cats with advanced CKD (four treated, two placebo-treated, with cross-over)c [128]. Each treated cat received 4×10^6 AD-MSC IV every 2 weeks for a total of three treatments, and effects on renal function were assessed by routine blood work and glomerular filtration rate by scintigraphy. The 6-week study did not detect any significant differences in renal functional parameters between MSC-treated and placebo-treated cats in the study. Taken together, these two allogeneic feline AD-MSC studies suggest that IV administration of MSC may not be particularly effective for management of relatively advanced CKD in cats or humans.

Alzheimer's Disease, Amyotropic Lateral Sclerosis, and Epilepsy

Three other spontaneous conditions of the CNS found in dogs strongly resemble human neurologic diseases including ALS (canine degenerative myelopathy), epilepsy (canine epilepsy), and Alzheimer's Disease (Canine Cognitive Dysfunction Syndrome) (Table 1). MSC have been shown to mitigate the progression of human ALS and status epilepticus or chronic epilepsy [157] and ALS [158]. Therefore, ALS and epilepsy remain as compelling targets for stem cell based therapies, and canine models are underutilized for this purpose. Similarly, in Canine Cognitive Dysfunction Syndrome, novel biomarkers, or interventions (dietary, pharmacologic, cell-based) to slow the progression of Alzheimer's disease could be evaluated prior to testing in humans.

Participant Engagement in Companion Animal Studies

The majority of these trials reported here were performed in companion animal disease groups with protracted, refractory, or incurable conditions. For these animals, veterinarians and owners are eager to find relief and clinical trials offer a potential option. While owners may be motivated to participate in clinical trials, they can be concerned about the risk of companion animals serving as "guinea pigs." Education about preclinical safety data and the benefits of trial participation are critical. A major disruptor to enrollment in veterinary patient trials is the owner's concern about their animal receiving a placebo instead of stem cell therapy. This is perhaps more evident in stem cell trials where the public perception exists that they will exert benefits, and in disease models with life-threatening disease. These concerns can be partially alleviated by conducting trials with asymmetric over-assignment to the active treatment arm, by means of cross-over trials that allow the placebo-treated groups access to treatment later (i.e., treatment extensions), and by financial compensation for trial participation. In the future, it will be important to understand the factors that lead owners to participate in clinical trials, and how to improve education and communication about clinical trials, clinical trial progress, and the scientific information that is gained from them.

Conclusions About Companion Animal Disease Model Applications

Based on the above review of stem cell trials in companion animal disease models, there is good evidence that the study protocols, including enrollment, treatments, dosage, and measured endpoints, were feasible. This is relevant because the protocols closely simulate the features of human clinical trials. Also, treatments with stem cells (including MSC, OEC, or MSC derived neural lineage cells) reviewed in the companion animal literature were not associated with significant adverse events, an observation consistent with laboratory animal and human studies at equivalent dosages. Only 4 of 19 studies reviewed were randomized controlled clinical trials (RCT), so it is premature to make broad comparisons between efficacy results in companion animals versus human or laboratory animal studies or to conclude what impact companion animal studies will have on decision making for human trials. In two larger scale RCT concerning IVDD reviewed herein, benefits were observed for autologous intraspinal OCE as a sole treatment [112], and for allogeneic intraspinal AD-MSC as an adjunctive treatment to decompressive surgery [116]. These trials suggest that a strategy of intraspinal MSC warrants further investigation in compressive lesions of the spinal cord in humans. In other non-RCT trials, novel applications and protocols of experimentation were advanced, for example, the use of SDF1 overexpression in AD-MSC for DCM, combined AD-MSC and platelet rich plasma or HA (chondroprotective agent) for OA, percutaneous intraspinal injections of fetal BM-MSC for IVDD, neural lineage cells derived from MSC (NLBM-MSC) for intraspinal treatment of IVDD, intrathecal injections of AD-MSC for neuroinflammation (GME), the use of embryonic stem cell-derived MSC for intralesional treatment of perianal fistulas (model of Crohn's fistulas), and perilacrimal injections of AD-MSC for KCS (model of Sjogren's

syndrome). These studies could not be readily performed in rodent models due to the complexity of the natural disease modeled, and the routes of administration.

Given that these protocol were successful at engaging participants (including owner observations) and show preliminary evidence of safety and benefits (acknowledging limitations of baseline or historical controls), they should be advanced to more rigorous, larger scale, double blinded RCT to investigate these novel strategies. Finally, it is important to note that companion animal diseases cannot be manipulated from the perspective of injury severity, onset, time course, survival endpoints, uncontrolled variables (e.g., comorbidities), and refractoriness to therapies. Therefore, treatment failures may be more effective in predicting risk of failure in human trials (e.g., IV administration of MSC for end-stage kidney disease in cats) [128]. Given that stem cell trials in rodents often fail to predict human outcomes, this is a crucial role for companion animal studies in regenerative medicine.

Regulatory Pathway Forward for Stem Cell Clinical Trials in Companion Animals

In accordance with FDA guidance for industry (GFI 218 June 4, 2015), legal marketing of stem cells in companion animals in the U.S. will require premarket evaluation of safety, effectiveness, and manufacturing using the New Animal Drug Application (NADA) mechanism of approval. Specifically, this will require FDA evaluation of stem cell tumorigenicity, formation of ectopic tissue, immunogenicity, donor selection criteria, transmission of adventitious agents, survival, toxicity, and biodistribution. While there may be different requirements for documentation and reporting between industry and nonindustry (noncommercial) sponsors, the FDA guidance is equally applicable to all individuals and institutions involved in development of a stem cell product. This includes academic centers, private industries, processing and manufacturing facilities, and veterinary practices. Investigators can file an investigational exemption through the Investigational New Drug Application (INAD) mechanism for *bona fide* research studies. In this scenario, veterinarians cannot charge for harvesting tissues or cells because this activity is considered by the FDA to be part of the manufacturing process; however, they can charge for professional services related to diagnosis, sedation, or delivery of an investigational product. This is a major paradigm shift for the veterinary profession, which has operated without FDA guidance up to this point. This has significant implications for stem cell manufacturing employed for stem cell trials. Given that there is no FDA approved product at the time of this writing, all participants in manufacturing chain must be working under FDA guidance. It is unclear which if any facilities for commercial production of stem cells are operating under FDA guidance. It is plausible that investigators can file an INAD in conjunction with a manufacturing site to advance a stem cell trial. More transparency from veterinary stem cell manufacturers (including commercial laboratories) is important at this time.

Based on the information in the new FDA guidance and with respect to scientific diligence, we propose for design of studies of cell-based therapies in companion animals a comprehensive "menu" that includes FDA recommendations and related studies to address scientific questions beyond those strictly recommended by the FDA to verify safety (Fig. 1). This menu of options is meant to serve as an exhaustive framework, rather than a one-size-fits-all protocol. In some instances, published data may satisfy preclinical requirements while in others, new data will need to be generated. In addition to discussions between sponsors and investigators to settle on which studies are appropriate for the specific cell source, species, and application to be tested, the current recommendation is to contact the FDA early in the process to discuss details of clinical studies and file (INAD or preclinical IND). In addition to receiving timely advice, filing will permit the FDA to keep records of trial activity (shipments) and adverse events, ultimately in an effort to inform and safeguard consumers.

Reflecting on the published clinical trials reviewed here, (Table 2), all of those studies were performed prior to the issuance of the new FDA guidance. As the cells employed in those veterinary trials were almost exclusively involved Type 1 autologous, allogeneic, or xenogeneic cells, rather than Type 2 minimally manipulated autologous cells as defined in the FDA guidance document, further investigations using such stem cells, for example, would necessitate FDA filing according to the published guidance. Only one study [123] summarized in Table 2, sponsored by industry, was conducted in the spirit of the current FDA guidance. In that study, it was stated that parent cells (human embryonic stem cells) passed GMP sterility and mycoplasma testing, karyotype by fluorescent in situ hybridization (FISH), flow cytometry (to exclude residual hESC and standard MSC immunophenotype), and in vivo tumorigenicity (xenotransplant into NOD/SCID mice). In addition, biological plausibility was supported by demonstrating immune modulation in mixed lymphocyte assays, and in cytokine responses to hESC-MSC in vitro.

In the future, additional iterations of the regulatory guidance can be expected; however, transition to FDA (INAD) filing of clinical trials of stem cells in companion animals is expected to persist. It is unclear at present whether these new regulations will have a significant negative impact on the advancement of stem cell trials.

"Letting Out the Leash" to See where Companion Animal Research can Lead Us

In the 20th century, a "one molecule, one target, one drug" strategy of drug discovery was born and remains a prevalent approach to discovery of cures. Bunnage et al. [159] pointed out that a high rate of attrition of therapies at Phase II is due to our failure to comprehensively define the biology of these singular molecular targets. One might ask: are we "barking up the wrong tree?" For complex diseases, stripping away the dependence on the "one molecule, one target" paradigm, that is, the acknowledgement of the multiplicity and complexity of molecular targets and their interaction with other molecules, would lead to a better understanding of the static and dynamic nature of molecular targets and open a window to more comprehensive and personalized approaches. Indeed, the drive to develop new therapeutic approaches to address multiple targets simultaneously has led to the discipline of theranostics, the exploitation of one's individual pharmacogenetic, proteomic, and biomarker repertoire in the design of a specific therapeutic strategy [160]. It follows that companion animal research offers a preclinical window into the feasibility, safety, and effectiveness of therapies in the context of

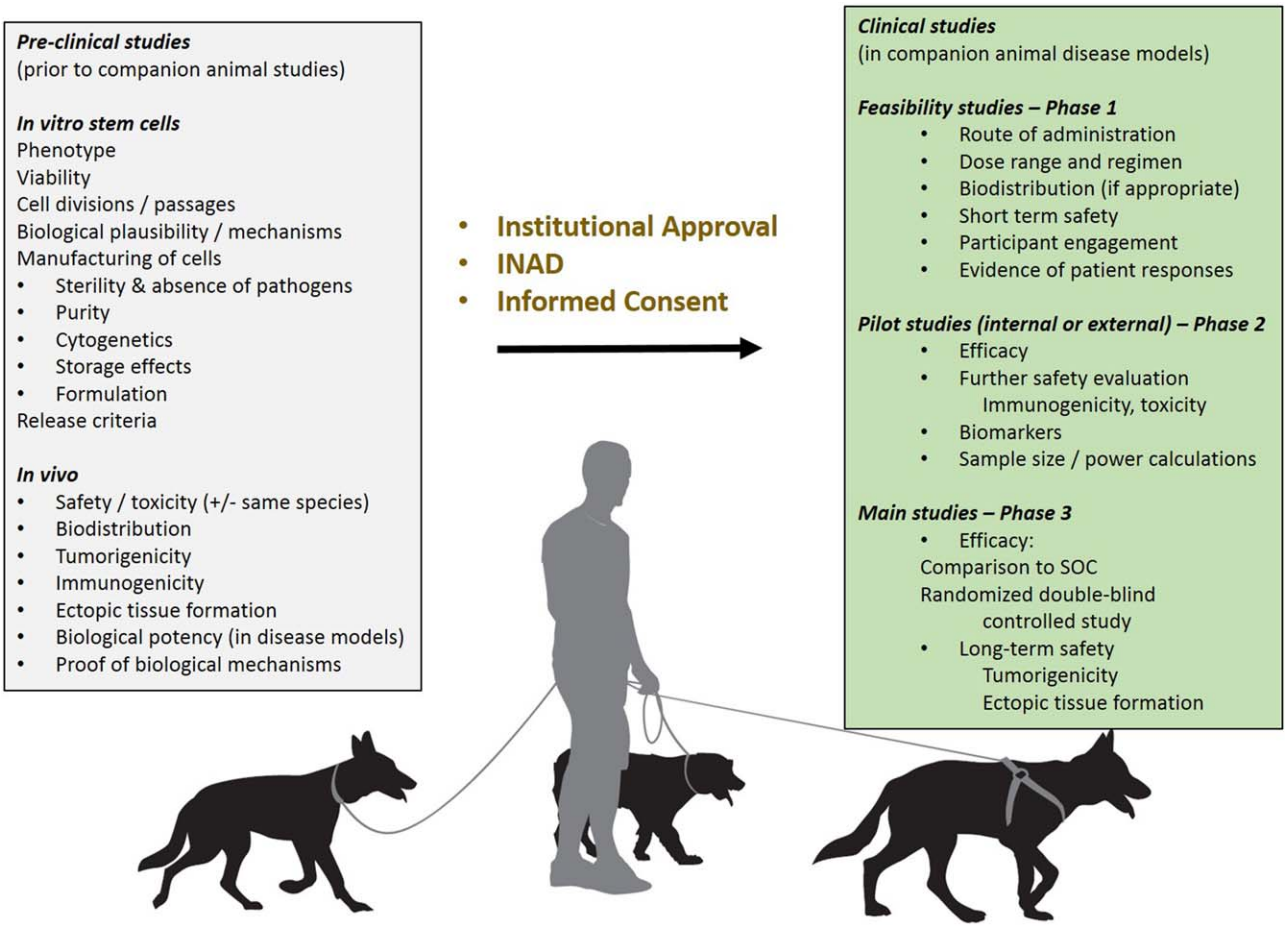

Figure 1. A comprehensive menu of proposed preclinical and clinical studies based on current FDA guidance and scientific standards to address feasibility, safety, and efficacy of stem cell products. Investigators considering clinical trials in companion animals may need to address any or all of the preclinical studies, depending on cell source, species, application, scope of scientific information sought, and intent to commercialize. Abbreviations: INAD, investigational new drug application; SOC, standard of care.

a naturally complex, if not hostile environment that more accurately reflects the human condition.

Companion animal studies can blaze new trails in regenerative medicine. These studies can lead us through novel pilot feasibility (Phase 1), safety and early efficacy (Phase 2), and major efficacy (Phase 3) studies which are too early or too expensive to attempt in human patients. These studies will inform human trials at various levels of comparable development, following the example by which canine cancer treatment trials have effectively done so for several years [161]. Specific examples in regenerative medicine, might include the evaluation of genetically enhanced stem cells, transdifferentiated (lineage specific) stem cells, induced pluripotent stem cells and derived progeny, organoids, 3D scaffolds impregnated with stem cells, extracellular vesicles and extracellular RNA, theranostics, and more personalized approaches to therapies.

Future Needs

Based on review of the literature, the utility of companion animals in stem cell trials is in the very early stages. To facilitate expanded development and application of companion animal disease model research in the future, the following areas need to be addressed with additional education, communication, and industry and government support:

- Increased collaborations between physicians and veterinarians to address specific disease conditions and models, consistent with the One Health paradigm.
- Increased understanding of the molecular pathology of specific diseases in companion animals, and detailed comparison to human samples and analogous disease processes.
- Greater characterization of companion animal stem cells and their cellular products.
- The use of more rigorous double blind (owner, investigator) randomized clinical trial designs whenever possible. Education of the public about the value placebo-controlled studies.
- Greater clinical trial infrastructure (personnel, equipment, specialized instrumentation, and imaging) to support efficient recruitment into clinical trials.
- Central registry of veterinary clinical trials (currently in progress at the American Veterinary Medical Association).
- Biorepositories and registries of companion animal disease tissue, biofluids, and nucleic acids or other samples.
- Improved availability of companion animal specific reagents, in particular probes for protein detection and quantification.
- The application of FDA guidance (NADA, INAD, pre-IND) in clinical trials involving stem cell treatments in companion animals (client owned animals).

While these issues are currently being addressed to varying degrees, there will need to be a concerted effort in the biomedical research community to drive further progress in these areas. Unleashing companion animal studies onto the field of regenerative medicine is an exciting paradigm that may increase our understanding of the complexity of molecular targets in spontaneous diseases, and bring therapies to humans in a more efficient manner, reducing the cost burden and failure of future human clinical trials using comparable cells and technologies. There may also be welcome instances where the study of companion animal disease models also reduces the need for purpose bred animals for translational studies. While the impact of companion animal disease model research on outcomes for human stem cell therapy remains untested, it is an innovative and compelling approach that deserves our attention.

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Author Contributions

A.H.: conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing. S.D.: conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing.

Conflict of Interest

The authors indicate no potential conflicts of interest.

Disclaimers

None.

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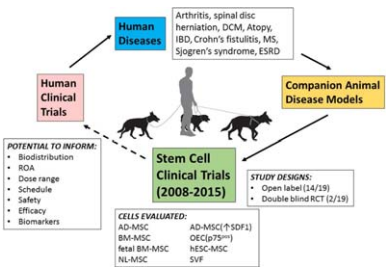

Companion animal diseases (reviewed in Table 1) have the potential to serve as realistic models of human disease, as they more closely approximate the natural history, symptoms, pathology, biomarkers, therapeutic responses, tolerance to therapies, and survival characteristics of analogous human conditions. Stem cell trials in companion animal disease models are, therefore, of interest as translational systems in regenerative medicine. We reviewed the study design, manufacturing, endpoints, safety, and efficacy data from stem cell trials in dogs and cats between the years of 2008-2015 ($n = 19$) (Table 2). Most clinical trials were open label design involving MSC (see *Cells Evaluated*), informing safety, route of administration, feasibility of protocols with modest power to evaluate efficacy. Overall safety was excellent, and patients showed responses that exceeded expectations based on baseline, historical, or interventional (placebo) controls. Companion animal disease models have potential to inform hypotheses concerning stem cell trials in humans. Improved rigor in study design and manufacturing will unmask the full potential of this approach to benefit humans and animals.