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***Naturally Occurring Diseases in Animals: Contributions to Translational Medicine***

**Lairmore and Khanna. Naturally Occurring Diseases in Animals: Contributions to Translational Medicine, pp. 1-3**

SUMMARY: This issue of ILAR presents natural animal diseases and their usefulness as animal models for intervention strategies of human diseases.

In general, the natural occurring canine cancers have numerous advantages such as sharing same environment as their owners; recent knowledge of the canine genome, selective breeding and reduced generation time of dogs. At the molecular level, the canine osteosarcoma parallels what is known about pediatric osteosarcoma, making the dog model useful for development of new targeted drugs against the cancer. Naturally occurring bladder cancer in dogs has many similarities to invasive bladder cancer in humans at the cellular, molecular, and biological levels. Bladder cancer studies in dogs have the potential to define heritable and environmental risks for the human disease. The knowledge obtained from comparative imaging studies in cancer-bearing animals has improved assessment of human cancers. Adoptive immunotherapy including use of cytokines or T-cell therapy in canine lymphoma can overcome immune tolerance and elimination of cancer cells. Nonhuman primate models were critical to the discovery of hepatitis E virus (HEV) and pathogenesis of HEV infection. Recent advances in genetic identification and molecular characterization of naturally occurring HEV infections in swine, chicken, and rabbits have contributed to develop new interventions to prevent HEV infection. The studies of bartonellosis in multiple animal species showed the diverse animal reservoir hosts and evolving arthropod vectors which may transmit disease to humans. The lamb model of respiratory syncytial virus bronchiolitis has contributed to therapeutic strategies (e.g., cytokines, compounds) to block damaging effects of the infection in preterm infants. The naturally occurring epilepsy in dogs is superior to rodent model as an alternative approach to understanding the disease and investigating new treatments to block seizure development. The equine athletes are good model for Achilles tendon injury in people. These tendons share similar functional and clinical features that are not reproduced in other animal models. Dog breeds such as boxers that spontaneously develop spondylosis and intervertebral disk disease may help to define the disorder in humans at genetic level.

QUESTIONS:

1. Which of the following dog breed can be used as an animal model for spondylosis?
   1. Boxer
   2. Cocker Spaniel
   3. Staffordshire Bull Terrier
   4. Border Collie
2. T/F. HEVis the only hepatitis virus that infects animalsother than primates.
3. Which of the following animal is considered a suitable model for preterm infant respiratory syncytial virus bronchiolitis?
   1. Calf
   2. Kid
   3. Lamb
   4. Kitten
4. Which of the following is untrue about bartonellosis?
   1. Caused by a gram negative bacteria
   2. It is a zoonotic disease
   3. Can be transmitted by cat scratch only
   4. Has diverse animal reservoir hosts

ANSWERS

1. A.
2. T
3. c. Can be transmitted by insect vectors
4. b.

**Ackermann. Lamb Model of Respiratory Syncytial Virus-Associated Lung Disease: Insights to Pathogenesis and Novel Treatments, pp. 4-15**

Domain 3: Research

Secondary Species: Sheep (Ovis aries)

SUMMARY: This article discusses various aspects of the lamb as a model for human infant respiratory syncytial virus (RSV) studies.  RSV is an enveloped, negative strand RNA virus of the Paramyxoviridae family.  It is a lower respiratory tract pathogen of children in their first year of life.  Numerous therapeutic compounds against RSV are being developed and assessed.

Neonatal lambs are used as an animal model for human RSV because ovine lungs, especially lambs, have multiple features consistent with infant lungs.  These features include alveologenesis, airway branching patterns, percentage of Club (Clara) cells, and the presence of submucosal glands.  Similarities of RSV lung lesions in lambs and preterm infants include enhanced bronchiolitis, neutrophil infiltration, syncytial cell formation, myeloperoxidase (MPO) production, and enhanced RSV replication levels.  Neonatal lambs can be deprived of colostrum, eliminating the effects of maternal antibody on clearance of RSV and other pathogens.  Features of severe RSV disease in lambs and infants is due, at least in part, to 1) the tropism of RSV to the bronchiolar epithelium, 2) the narrow lumen of the bronchioles and distal airway, 3) low airflow pressure in the bronchioles and distal airways, and 4) alterations in airway dilation due to inflammation.

The similarities outlined above allow for assessment of RSV therapies using a lamb model.  The author described several RSV therapies used in the lamb model.  Pretreatment of lambs with vascular endothelial growth factor (VEGF) reduced RSV disease severity.  The dual oxidases(Duox)/lactoperoxidase (LPO) oxidative system has activity against both bacteria and multiple respiratory viruses, including RSV.  KI administration daily to RSV challenged lambs significantly reduced RSV disease severity, gross lesions, mRNA levels, antigen, and titers through enhanced oxidative responses.  Club (Clara) cell secretory protein (also known as CC10 and CC16) has immunomodulatory properties.  A pilot study used twice daily treatment with recombinant human CC10 prior to and after RSV challenge, resulting in reduced gross RSV lesions and decreased RSV mRNA levels.  ALX-0171 is a therapeutic protein developed with a strong binding affinity to the F (fusion) protein of the RSV virus, thereby inhibiting RSV fusion to target cells.  ALX-0171 delivery through nebulization is currently being studied in the lamb model.  Newborn lambs develop enhanced RSV lesions after vaccination with a formalin inactivated RSV (FI-RSV) vaccine, similar to infants, setting the stage for lamb studies to understand FI-RSV pathogenesis and for assessment of vaccines.

QUESTIONS

1.  Which one of the following statements about RSV treatment and prevention is true?

a.   Ribivirin is currently used in hospitals but has availability and cost issues.

b.   Palivizumab has set criteria due to toxicity issues.

c. Formalin-inactivated RSV vaccine has less vaccine-enhanced RSV response compared to live virus, vectored virus, or subunit vaccines.

d.  ALX-0171 inhibits RSV fusion to target cells and has demonstrated antiviral efficacy in the lamb model.

2.   Which statement is false?  The lamb lung is a good model for infant lungs because?

a.  Lesions are especially intense in the distal bronchi, bronchioles, and terminal airways/alveoli.

b.  Submucosal glands express surfactant proteins (SP) A and SP-D, which bind and aggregate RSV.

c.  Of the presence of airway submucosal glands which express lactoperoxidase (LPO).

d.  The percentage of Club (Clara) cells in bronchioles is 18-22% in lamb and infants.

3.   Which of the following statements is false?

a.  The lamb model has several features consistent with RSV in infants.

b.  VEGF and CC10 can enhance a wide variety of immunomodulators that mediate resistance to RSV infection.

c. The ability to study lambs preterm is one of the unique features of the lamb model.

d. Maternal ethanol consumption has no impact on development but alters the innate immunity of the lung in utero.

ANSWERS

1.  d.

2.  b. Club (Clara) (and type II) cells produce substances with known anti-RSV activity, including SP-A and SP-D.

3.   d. Maternal ethanol consumption alters development and innate immunity of the lung in utero, potentially explaining a reason why ethanol predisposes the infant lung to increased RSV disease severity.

**Alvarez. Naturally Occurring Cancers in Dogs: Insights for Translational Genetics and Medicine, pp. 16-45**

SUMMARY

Naturally Occurring Cancers in Dogs: Insights for Translational Genetics and Medicine: Because dogs are genetically more similar to humans, share environmental exposures with their owners, suffer from the same diseases as humans, and receive a high level of health care, dogs can make a good model for human cancer. The authors propose that humans are most ideal for the study of somatic cancer genetics, whereas dogs are the most ideal for germ line genetics. That proposition is supported by comparison of genome-wide association studies (GWASs) in human and canine cancer.  One of the advantages of dog cancer GWASs is the ability to rapidly map complex traits, conduct fine mapping and identification of causative variation, and thus be in a position to move on to functional studies.

Cancer cells require the following: self-sufficiency in growth signals, insensitivity to antigrowth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evasion of apoptosis.

By sequencing tumor and non-tumor DNA in the same subjects, it is possible to determine which variation is inherited and which is due to somatic changes associated with cancer. A striking aspect of cancer is that is predominately driven by one type of evidence- somatic. The reason for this is that somatic mutations are extremely heterogeneous even within one tumor type or subtype. Detection of driver variants in a vast excess of noise has been nearly overwhelming until the emergence of high throughput sequencing

Model relevance is often considered at the level of phylogeny. For example, the study of breast biology and disease is limited to mammals.  The choice of rodent models generally requires major trade-offs between ase and speed versus disease relevance. In addition to not being naturally induced, rodent cancer models also frequently lack key aspects of human cancer. Among the most important of those are that mouse studies almost never involve old olge and very often do not feature metastatic disease.  Also the use of extremely inbred lines lack the genetic diversity that is present in humans.

The main focus on canine models has been on theory validation and the development of genetic and clinical trials resources. The following are areas that would greatly improve veterinary translation: veterinary registries with longitudinal data for epidemiological and translational studies, which can be enhanced with breed specific data: SNP haplotypes, CNV, RNA expression. The development of multiple areas (metabolism/ obesity, thyroid biology, etc) should be developed quickly.

QUESTIONS

Match the breed to naturally occurring cancer or cancer to genes

a.  KIT oncogene

b. BRCA1 and BRCA2 SNP markers

c. Testicular seminoma

d. Nasal cavity carcinoma

e.  Osteosarcoma

1.  Canine mast cells

2. Mammary tumors in English Springer Spaniels

3. Norwegian Elkhound

4.  Golden retriever

5.  Greyhound

ANSWERS

a.  1

b. 2

c. 3

d. 4

e.  5

**Bealmear Breitschwerdt. Bartonellosis: One Health Perspectives for an Emerging Infectious Disease, pp. 46-58**

**SUMMARY:** In recent years, an increasing number of Bartonella species have been identified as zoonotic pathogens, transmitted by animal bites, scratches, arthropods and even by needle sticks. Considering the diversity of newly discovered Bartonella species and subspecies and the large number and ecologically diverse animal reservoir hosts and the evolving spectrum of arthropod vectors that can transmit these bacteria among animals and humans, the clinical and diagnostic challenges posed by Bartonella transmission in nature are presumably much more complex than is currently appreciated by diagnosticians, vector biologists, ecologists, physicians, or veterinarians. Historically the term "bartonellosis" was attributed to infections with *Bartonella bacilliformis*, transmitted by sand flies in the Peruvian Andes. Currently, however, bartonellosis now includes infections caused by any Bartonella sp. anywhere in the world. Potentially, because Bartonella spp. can infect erythrocytes, endothelial cells, pericytes, CD34+ progenitor cells, and various macrophage-type cells, including microglial cells, dendritic cells, and circulating monocytes in vitro, the clinical and pathological manifestations of bartonellosis appear to be very divers in both sick animals and human patients. Because 75% of emerging infectious diseases are zoonoses, many of which are vector-transmitted by an arthropod, a one health approach to bartonellosis and other zoonotic infections is needed to properly address animal health, public health, and environmental factors that influence the distribution and transmission of these bacteria. The one health concept encourages a spirit of cooperation among animal, environmental, and human health professionals and promotes developing integrated solutions for complex problems that impact the health of animals, humans, and the planet. Importantly, substantial this article supports the need for more research to define the medical importance of this genus as a cause of animal and human illnesses.

***Table 1 (page 47)-see attachment***

QUESTIONS

1.  T or F. Because conventional microbiological techniques lack sensitivity, bartonellosis is usually diagnosed by PCR amplification of organism-specific DNA sequences and/or through serological testing.

2. Match the following Bartonella spp with the corresponding primary reservoir/vector:

\_\_\_B. henselae

\_\_\_B. bacilliformis

\_\_\_B. quintana

\_\_\_B. elizabethae

a. Human/ Body louse (*Pediculus humanis*)

b. Human/ Sandfly (*Lutzomia verrucarum*)

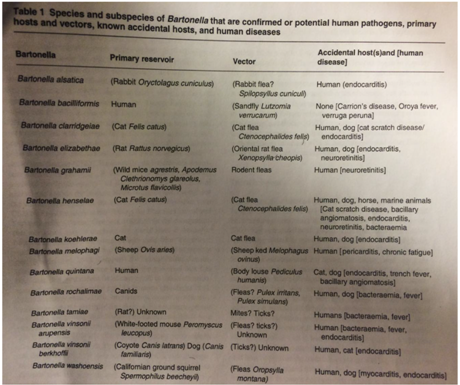
c. Rat (*Rattus norvegicus*)/ Oriental rat flea (*Xenopsylla cheopis*)

d.  Cat (*Felis catus*)/ Cat flea (*Ctenocephalides felis*)

ANSWERS

1.  True

2. d, b, a, c

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**Davis and Ostrander. Domestic Dogs and Cancer Research: A Breed-Based Genomics Approach, pp. 59-68**

Domain 3

Primary Species: Dog (*Canis familiaris*).

SUMMARY: Dogs are diagnosed with many of the same cancers as humans suggesting that canines may be an informative system for the study of cancer genetics. Improved health care and diagnostic test for pets are tools that provide a more effective therapy. Breeds with specific phenotypic traits allow circumventing the outbred population structure that characterizes human cancer gene mapping and allows scientists to avoid setting up breeding colonies.

The National Veterinary Cancer Registry connects researchers with oncologists and informs owners about ongoing clinical trials. However, they provide little available data on breed-specific incidence of cancers. The best resources on that matter are targeted academic studies and veterinary school studies. These were also the first studies to suggest that spontaneous dog tumors could be informative for learning about human cancers.

It is probable that different dog breeds with high risk for the same type of cancer share underlying genetic predisposition. Comparing whole genomes as a predetermined set of single nucleotide polymorphisms (SNPs) is called genome-wide association study (GWAS). Scientists are skipping GWAS and proceeding to whole-genome sequencing in order to characterize single nucleotide polymorphisms (SNPs), structural variants such as insertion-deletion events, translocations, and copy number changes for a cost that continues to drop. Obtaining samples from the same breed from breeders to use as control is important to minimize false positives. The older the control, the better the choice. The required number of samples for a canine GWAS is smaller than for a human study, especially if we sample unrelated dogs from the same breed and a hypothetic trait with dominant inheritance like squamous cell carcinoma.

Histiocytic sarcoma (HS) is a rare cancer that have arisen independently in distinct breeds. High-throughput sequencing (HTS) provides the ability to detect all variants within the genome. This is one of the examples where canine studies preceded human studies. Gene expression studies have revealed in the case of HS changes in genes from 24 distinct pathways involved in tumor development. However, the information is not as exhaustive as RNA-Seq which is unbiased.

Colony-based studies are a poor choice for those cancers that environment play a role on their onset, like transitional carcinoma of the bladder (TCC), or factors such as hormone levels are important, like mammary cancer. Another example is prostate cancer which, even being extremely rare in dogs, is in the only animal that has a spontaneous presentation.

The combination of chromatin immunoprecipitation sequencing and RNA-Seq has been used to identify the activation of hormonal binding sites and the resulting changes to the total transcriptional landscape in hormone-responsive cancers.

The rapid advances in genomic information provides a powerful tool towards human and animal health alongside improving animal research and their welfare.

QUESTIONS

1. Cancer in dogs has had a tendency to increase in the last decade.
2. The number of SNPs needed for a canine GWAS is much smaller than that required for a comparable human study.
3. The best way of studying a single cancer is maintaining dog colonies at veterinary school.
4. The best source for establishing breed-specific trends in cancer research are highly targeted academic studies and selected veterinary school studies.
5. The data obtained with whole-genome sequencing is of greater efficiency than comparing whole genomes of several dogs simultaneously as a predetermined set of SNPs.
6. The best control to minimize false positive are closely related breeds and the same age individuals.

ANSWERS

1. False
2. True
3. False
4. True
5. True
6. False

**Fenger et al. Canine Osteosarcoma: A Naturally Occurring Disease to Inform Pediatric Oncology, pp. 69-85**

Domains 1 (Management of Diseases) and 3 (Research/Models)

Primary Species: Dog (Canis familiaris)

SUMMARY: There are many advantages to using naturally occurring canine cancers to model human disease including similar environmental factors, relative ease of genetic mapping using dog breeds, and larger size than the laboratory rodent. Osteosarcoma (OSA) is the most common primary malignancy of bone in dogs and children. Genetically engineered mouse models, while they have some value, typically develop primary tumors of the flat bones unlike the primary tumors of the long bones seen in dogs and humans. Unfortunately no improvement in survival times has been achieved in the past 15 years and the ability to develop therapeutics is hindered by the low incidence of this cancer in humans. OSA is 10 times more common in dogs and their relatively short lifespan and survival times after diagnosis make it possible to conduct clinical trials faster.

For both species OSA has a bimodal age distribution with humans getting OSA most common during adolescence (10-14 years) and in dogs it commonly occurs in middle age or older animals (median 7 years). Growth factors appear to play an important role in this disease as taller individuals of both species are at greater risk and studies have demonstrated IGF-1 receptor and its ligand in canine OSA cells. OSA is typically seen in the weight bearing region of long bones. In dogs the distal radius and the proximal humerus are the most common site, while in humans the distal femur, proximal tibia, and proximal humerus are most common.

Risk factors and molecular mechanisms: Radiation can be a risk factor in dogs and humans, but radiation-induced OSA is rare. Physical risk factors such as heavy weight bearing and malignant transformation around metallic implants or previous fractures have been reported. In humans Paget’s disease, a premalignant condition involving excessive bone production, is a risk factor for a biologically aggressive high grade form of OSA. Several genes have been implicated including p53, RB1, and PTEN.  In dogs, several breed specific genetic associations have also been documented: loss of WT1 and a novel germline mutation in the receptor tyrosine kinase MET in Rottweilers, the CFA34 locus in Scottish deerhounds, and a locus in greyhounds that is upstream of CDKN2A/B. Constitutive activation of STAT3 is seen in some tumors and cell lines, and human tumors expressing high levels of STAT3 have a worse prognosis. RTK MET, EGFR, and Ron receptor cross talk has also been implicated and are promising targets for therapeutics. Ezrin, a membrane-cytoskeleton linker, is involved in early metastatic survival and high ezrin expression is associated with poor outcome in human and canine OSA patients. Metastatic disease is the most significant cause of death for OSA patients, so making therapeutics that target metastasis especially important.

Pathophysiology and Natural Behavior: OSA arises from a mesenchymal stem cell with the capability to produce osteoid. The tumors are classified by location, cell type, and histologic grade. High histologic grade as determined by cell pleomorphism, mitotic index, and necrosis is associated with a worse prognosis. Most human and canine patients initially present for lameness and the primary tumors have a very characteristic radiographic appearance. Long-term survival rates in dogs with OSA are only 10-15% compared to a 60-70% 5 year survival rate if there are no metastases at the time of diagnosis in humans. This suggests that the biological behavior of OSA may be more aggressive in the dog.

Prognostic factors: Prognostic factors in the two species are remarkably similar. Proximal humeral location has a worse prognosis than other locations in both dogs and humans. Other prognostic factors both species have in common are presence of metastasis, use of adjuvant or neoadjuvant chemotherapy, and postoperative infection at limb-sparing surgical sites. In human OSA response to neoadjuvant chemotherapy as evidenced by the degree of necrosis of the resected tumor correlates with disease free interval. However, neoadjuvant chemotherapy is uncommonly used in dogs and there is no comparable histologic grading scheme in veterinary medicine. In addition, comparative genome wide expression profiling has allowed prognostic molecular classification of human tumors.

Current treatment options for canine OSA: Interventions for dogs and humans are similar in terms of techniques, devices, allografts, clinical healing and complications. Interestingly, dogs with allograft infections of the limb spare surgery site had a longer overall survival time than dogs without allograft infection. The mechanisms are still under study but proposed mechanisms include nonspecific immunologic stimulation and the antiangiogenic properties of some antibiotics. Other evidence for the role of the systemic immune response in OSA include the correlation between CD8+ T cells:Treg ratios and survival time of dogs and shorter DFI associated with relative lymphocytosis and monocytosis on initial blood work.  Platinum based chemotherapeutics and doxorubicin are standard therapeutics for both dogs and humans, although even with amputation and chemotherapy >50% of dogs do not live beyond 1 year post-amputation and 90% die before 2 years. Immune modulating novel therapies, a synthetic analog of a mycobacterium cell wall component (muramyl dipeptide) and nebulized IL-2, have been tested in dogs. Tyrosine kinases and IGF-1 signaling pathways have also been targeted in several investigations.

RT and palliative treatments: Radiation therapy is considered the most effective treatment modality for osteolytic bone pain in people and has been extensively applied to bone cancer pain dogs. Other strategies to mitigate osteolytic bone pain include systemic administration of conjugated radiopharmaceuticals targeted to areas of increased proliferation (Samarium-153-EDTMP) and aminobisphosphates. Intraoperative radiation therapy for limb sparing has been investigated to preserve limb function in challenging anatomical sites, but the use of this technique has been limited by the high rate of complications. Stereotactic RT has the potential to deliver high-dose RT while relatively sparing nearby tissue and merits further study.

Dog model for development of novel therapies for OSA: Dogs have been used to establish pharmacokinetic and pharmacodynamics relationships in developing new drugs and drugs with novel routes of delivery (such as aerosolized gemcitabine). Canine OSA models have been used to inform preclinical drug development for mTOR inhibitors, Heat shock protein 90 inhibitors, antiangiogenic agents, and matrix metalloprotease inhibitors.

QUESTIONS

1. Genetically engineered mouse models are poor models of human OSA because:

a. They preferentially develop chondromas, not osteosarcomas

b. They develop OSA primarily in the flat bones not the long bones

c. The bone tumors they develop are benign

d. OSA in mice typically metastasizes to the spleen not the lungs

2.  Which tumor location carries the worst prognosis in canine and human OSA?

a. Proximal humerus

b. Distal radius

c. Distal femur

d. Proximal tibia

3. Ezrin is…

a. A membrane-cytoskeleton linker

b. Involved in early metastatic survival of OSA

c. Associated with poor outcome is in human and canine OSA patients when highly expressed

d. All of the above

4. The most effective modality for treating osteolytic bone pain is…

a. Platinum based chemotherapeutics

b. Doxorubicin

c. Radiation therapy

d. Aminobisphosphates

ANSWERS

1. b

2. a

3. d

4. c

**Patterson-Kane and Rich. Achilles Tendon Injuries in Elite Athletes: Lessons in Pathophysiology from Their Equine Counterparts, pp. 86-99**

Domains 1 (Management of Diseases) and 3 (Research/Models)

Tertiary Species: Other mammals

SUMMARY: The equine superficial digital flexor tendon (SDFT) injury is a well-accepted, scientifically supported companion animal model of Achilles Tendon (AT) Injury in humans. The SDFT and AT are functionally and clinically equivalent energy-storing structures, for which no equally appropriate rodent or rabbit models exists. Study of equine tissues has facilitated significant advances in knowledge of tendon maturation, ageing and definition of some of the earliest stages of subclinical pathology.

Access to human surgical biopsies has provided complementary information on more advanced phases of disease. Currently equine SDFT injuries are a model for acute ruptures in athletes, and not the entire spectrum of human tendonopathy because human tendonopathy (tendonopathy) is a term better used to refer to a syndrome of chronic tendon disease rather than one specific acute situation.

Evidence from both horses and humans however indicates that the multiple concurrent stresses experienced by tenocytes in these energy-storing tendons during exercise result in cellular dysfunction and this is likely to play an active role in the development of and failure to repair microdamage. Genetic profiling has resulted in the identification of a number of genetic polymorphisms in matrix protein-encoding genes (nonfibrillar collagens and tenascin C) that have association with higher risk of AT injury in humans and SDFT injury in horses.

Poor conservation of genomic responses to inflammatory disease has been noted between humans and mice.  Data supportive that organisms of comparative longevity and with tissues that have experienced similar stresses may be better models for human disease. Early investigation of equine promyelocytic leukemia protein (PML) protein as a stress response factor, but other key proteins, including p53, require further analysis in this species.

 Mechanistic research is now required after a protracted period of time during which therapies have been tested, often initially in horses, without scientific justification, and there has additionally been a failure to suggest any possible means of prevention of these common and devastating athletic injuries. Mechanistic studies, including tendon cell responses to combinations of exercise-associated stresses, require a more thorough investigation of cross-species conservation of key stress pathway determinants.

Finally equine models may be additionally justifiable based on comparable species longevity, lifestyle factors, and selection pressure by similar infectious agents (e.g., herpesviruses) on general cell stress pathway evolution.

Key Words:  Achilles tendon; aging; athletes; horse; superficial digital flexor tendon tendonopathy; tendon; tenocyte

QUESTIONS

1.  True or False: The equine SDFT is a good research model for chronic Achilles tendon injury in humans

2.  True or False: Rabbit models have been suggested to be a good model of AT injury in humans

ANSWERS:

1.  False; the equine SDFT model represents a good model of acute AT injury in humans but not AT chronic injury

2.  False: Both rabbits and rodents have been demonstrated to be a suboptimal model of human AT tendon injury

**Knapp et al. Urinary Bladder Cancer in Dogs, a Naturally Occurring Model for Cancer Biology and Drug Development, pp. 100-118.**

Domain 2

Primary Species: Dog (Canis familiaris)

Bladder cancer in humans

* + Affects 65,000 people every year leading to approximately 14,000 deaths per year just in the US
  + Transition cell carcinoma (TCC) aka urothelial carcinoma
  + Associated with smoking/chemical in the workplace and older males are at higher risk than females
  + Two forms: low-grade (superficial disease) and high-grade (invasive cancer)

o   In humans low-grade is more common (more than 80% of cases)

  Alteration of Ras-mitogen-activated pathway

  Located in different areas within the bladder

  Treat w transurethral resection and intravesical therapy

o   High-grade are about 20% of total human cases

  Deregulation in the p53 and retinoblastoma pathways

  Cystectomy for primary tumor and chemotherapy for metastasis (methotrexate, vinblastine, doxorubicin, and cisplatin)

   Metastasis occurs in 50% of high-grade TCC (especially to regional lymph nodes and lungs)

  Most deaths are due to metastasis

Naturally occurring bladder cancer in dogs

* Like in humans, TCC correspond to 2% of all naturally occurring cancers in dogs with more than 20,000 cases diagnoses each year
* Diagnosis is made by histologic exam of tissue biopsies (surgery, cystoscopy, or catheterization)
* High-grade TCC correspond to more than 90% of total canine cases

o   It is believed that specific pathway that leads to low-grade TCC in not active in dogs

o   Located in the trigone region of the bladder (males and females) or can spread to urethra and prostate (males)

  Metastasize to lungs and regional lymph nodes

  Spread to the abdominal wall through instruments and needles used in surgical and nonsurgical procedures and naturally along ligaments that support the bladder

   In this location, the cancer typically grows aggressively and is poorly responsive to medical therapy

* Risk factors include: age (older animals), gender (more common in females), history of spay or neuter, obesity, strong breed-related risk (Scottish terrier, West highland white terrier, Shetland sheepdog, Beagle, Dalmatian are more common), and exposure to chemicals (herbicides and insecticides)
* World Health Organization and WHO/International Society of Urological Pathology have useful clinical staging system and classification for canine TCC
* Treatment include surgery, radiation therapy, chemotherapy and other drugs (cyclooxygenase inhibitors)

o   Most TCC in dogs are not in a location where complete surgical excision is possible

o   Platinum agents appear to be the most active in canine TCC, especially when combined with a cox inhibitor (deracoxib, firocoxib, piroxicam)

* Even though TCC is rarely curable in dogs, it is considered highly treatable with median survival time with multimodal treatment of more than 250 days
* Similarities between bladder cancer in humans and dogs
* The microscopic anatomy of canine TCC are similar to humans
* The clinical presentation is also similar and is composed primary of older individuals with hematuria and stranguria being the most common clinical signs. A history of urinary tract infection might also be observed in more advanced cases.
* In the cellular and molecular levels, different lipid patterns related to glycerophospholipids, free fatty acids, and other lipid species were found between TCC and normal bladders. Also, expression of cox-2 is overexpressed in TCC compared to normal urothelium. Moreover, an apoptosis-inhibiting protein call “survivin” has been found overexpressed in many TCC cases and is a target for TCC detection in humans in a urine-based detection test. Finally, telomerase activity has been detected in 90% of human TCCs, and is also used as a target in TCC detection assays.
* Models of high-trade TCC in dogs have the most translational value to humans

QUESTIONS

1.   Dogs usually develop high-grade transitional cell cancer instead of the less invasive form (low-grade) due to the lack in activity of what pathway

a. P53

b.  Retinoblastoma

c.  Ras-mitogen

d.   All of the above

2.   \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ and \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ in \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ individuals are the most common clinical signs observed in both humans and dogs with TCC.

3.  Differences between canine and human TCC include:

a. Low-grade, superficial TCC comprises the majority of TCC in humans but is very uncommon in dogs

b.  The majority of dogs have trigonal disease, and extension down the urethra while humans, TCC distributes more evenly across the areas of the bladder

c.  In humans, TCC is more common in males while in dogs the disease is more common in females

d. All of the above

ANSWERS

1. c. Ras-mitogen

2. Hematuria, stranguria, older

3. d. All of the above

**Kornegay et al. Pharmacologic Management of Duchenne Muscular Dystrophy: Target Identification and Preclinical Trials, pp. 119-149**

Primary Species – mice (*Mus musculus*) and dogs (*Canis familiaris*)

Domain 3 – Research; K3 – animal models

**Summary**

(Abstract) Duchenne muscular dystrophy (DMD) is an **X-linked human disorder** in which **absence of the protein dystrophin** causes degeneration of skeletal and cardiac muscle. For the sake of treatment development, over and above definitive genetic and cell-based therapies, there is considerable interest in drugs that target downstream disease mechanisms. Drug candidates have typically been chosen based on the nature of pathologic lesions and presumed underlying mechanisms and then tested in animal models. Mammalian dystrophinopathies have been characterized in mice (**mdx mouse**) and dogs (**golden retriever muscular dystrophy [GRMD]**). Despite promising results in the mdx mouse, some therapies have not shown efficacy in DMD. Although the GRMD model offers a higher hurdle for translation, dogs have primarily been used to test genetic and cellular therapies where there is greater risk. Failed translation of animal studies to DMD raises questions about the propriety of methods and models used to identify drug targets and test efficacy of pharmacologic intervention. The **mdx mouse and GRMD dog are genetically homologous to DMD but not necessarily analogous**. Subcellular species differences are undoubtedly magnified at the whole-body level in clinical trials. This problem is compounded by disparate cultures in clinical trials and preclinical studies, pointing to a need for greater rigor and transparency in animal experiments. Molecular assays such as mRNA arrays and genome-wide association studies allow identification of genetic drug targets more closely tied to disease pathogenesis. Genes in which polymorphisms have been directly linked to DMD disease progression, as with osteopontin, are particularly attractive targets.

**Key points:**

* Introduction
  + Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that affects 1 in 5000 newborn human males
    - Absence of the protein dystrophin causes degeneration of skeletal and cardiac muscle
    - Less severe form is termed Becker muscular dystrophy (BMD)
      * BMD has in-frame *DMD* gene mutations
  + Spontaneous mammalian forms of X-linked muscular dystrophy due to dystrophin deficiency have been identified in mice, cats, pigs, and multiple dog breeds
    - Golden retrievers, Pembroke Welsh Corgi, Labrador, Tibetan terrier, Cavalier King Charles Spaniel, Rottweiler, Cocker spaniel
  + DMD phenotype in dogs is more severe than in mice
    - Canine studies might translate better to humans
    - Dogs with golden retriever muscular dystrophy (GRMD) exhibit marked phenotypic variation at the level of individual animals and different muscles
      * Confounds statistical analysis in preclinical trials
      * Provides a platform to define disease pathogenesis
  + Frequent failure of animal experiments to translate to human trials
    - Potential for genetic assays to better identify drug targets
    - Genome wide association studies (GWAS) – used to identify genetic markers (typically single nucleotide polymorphisms [SNPs]) that may be associated with complex traits
* Current status and therapeutic goals
  + Glucocorticoids are the standard of care for DMD
    - Show beneficial effects such as prolonged ambulation and improved cardiopulmonary function
    - Often discontinued due to side effects ranging from weight gain to pathologic bone fractures
* Drug development regulatory process
  + Food and Drug Administration (FDA) is governed by the Federal Food, Drug, and Cosmetic Act (FDCA)
    - FDCA defines drugs as “articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals,” and as:
      * “Articles (other than food) intended to affect the structure or any function of the body of man or other animals
      * Therapeutic biologics are included in the definition of drugs
  + FDA becomes involved in the drug discovery process after the drug’s sponsor has screen the new molecule for pharmacologic activity and acute toxicity potential in animals and wishes to test its diagnostic or therapeutic potential in humans
    - Process includes filling out an Investigational New Drug (IND) application and testing through a series of clinical trials (Phases 1-4)
      * Phase I - Researchers test a new drug or treatment in a small group of people for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.
      * Phase II - The drug or treatment is given to a larger group of people to see if it is effective and to further evaluate its safety.
      * Phase III - The drug or treatment is given to large groups of people to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely.
      * Phase IV - Studies are done after the drug or treatment has been marketed to gather information on the drug's effect in various populations and any side effects associated with long-term use.
  + European Medicines Agency regulates drug development in Europe
  + Top two reasons for failed drug development in pharmaceutical companies in the UK were pharmacokinetics (PK) and efficacy
    - With PK data, it is very important to pay close attention to differences among species and even breeds (e.g., in dogs)
    - Problems with drug targets and efficacy have persisted
    - Phase II failures often occur due to insufficient efficacy
      * This is despite the fact that most drugs proceeded through standard validation methods
        + Including assessment in animal models
        + Animal efficacy studies included dose-response gradients, clinically relevant outcomes, and long-term endpoints

Few incorporated random animal allocation, adjustment for multiple hypothesis testing, or blinded outcome assessment

* + Recommendations to improve translation of preclinical research include:
    - Power calculation to determine sample size
    - Randomized treatment allocation
    - Characterization of disease phenotype in the animal model prior to experimentation
    - Blinding of data assessment
    - Increased stringency in data handling
  + The FDA’s “Animal Rule”
    - Guides drug approval in instances of chemical, biologic, radiologic, and nuclear threats “when adequate and well-controlled clinical studies in humans cannot be ethically conducted and field efficacy studies are not feasible”
      * Drugs may be approved for human use when 4 criteria are met:
        + Data define pathophysiological mechanisms for the product
        + The product effect is demonstrated in more than one species
        + Animal study endpoint is clearly related to the desired benefit in humans
        + PK data in animals and humans are sufficiently well understood to allow selection of an effective dose in humans
* Animal models
  + General information
    - National Research Council (NRC) definition of a biomedical model:
      * “A surrogate for a human being, or a human biologic system, that can be used to understand normal and abnormal function from gene to phenotype and to provide a basis for preventive or therapeutic intervention in human diseases”
      * “A model need not be an exact replica of a human disease or condition”
        + E.g., the mdx mouse model for DMD/BMD
    - Biological models can be divided into two broad classes
      * Modeling by analogy – point-by-point relationship between one structure or process to another
        + Requires that there are similarities between the structures and processes being compared in the modeling relationship
      * Modeling by homology – implies a shared evolutionary history and matching DNA makeup between the two structures or processes
        + For models by homology to be functionally useful, must also be good models by analogy for the phenomenon being studies
        + Homolog animal models are not always good analog models because of physiologic adaptations that may have occurred over time
    - Surrogate models may be further classified as:
      * One-to-one – disease state in humans and a particular species that share the same clinical features
      * Many-to-many – findings from more than one species or organ system may model particular features of the underlying process or state
    - Animal studies play a critical role in all phases of preclinical drug development:
      * Original target identification
      * Determination of the drug’s PK, absorption, distribution, metabolism, and excretion
      * Potential toxicity to the treated animal or person
      * Ultimate efficacy against the intended disease
        + Efficacy studies often completed in symptomatic, experimental animal models

Drug’s efficacy tested on a clinical effect versus the underlying disease itself

* + - Spontaneous and genetically modified animal models
      * Nonmammalian models – zebrafish, *Drosophila* fly, *Caenorhabditis elegans* nematode
      * Spontaneous mammalian models – mouse, cat, pig, multiple dog breeds
        + Cats with *DMD* gene mutations have a debilitating hypertrophic myopathy

Predisposed to a malignant hyperthermia-like syndrome during stress or anesthesia

Similar syndrome also occurs in DMD and occasionally GRMD dogs

* + - Treatments should ideally first be tested in the mdx mouse then moved to GRMD dogs before initiating DMD (human clinical) trials
  + DMD
    - DMD patients have delayed milestones, typically not walking until approximately 18 months of age or older
    - Early symptoms include frequent falls and difficulty in running and climbing stairs
    - Variation among DMD patients in other outcome parameters
    - 6-minute walk test (6MWT) has become the principal outcome measure used in DMD clinical trials
      * Measures the distance a patient can quickly walk on a flat, hard surface in a period of 6 minutes
        + Walking course must be 30 m in length, with the length of the corridor measured in 3 m increments
      * Evaluates the global and integrated response of all systems involved during exercise:
        + Pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism
        + Does not provide specific information on the function of each of the different organs and systems
      * Results correlate with wheelchair status and knee extensor strength
    - DMD is a proximal myopathy
      * Muscles like the quadriceps are selectively affected
      * Certain other muscles, like those of the eye, are relatively spared
      * Some muscles may undergo hypertrophy
        + “Classical calf pseudohypertrophy”
      * Fast twitch (type IIb) myofibers are selectively affected
        + Leads to a characteristic slow twitch (type I) performance
    - Differential muscle involvement is a prominent feature of both the GRMD and mdx models
      * Cranial sartorius (CS) muscles undergo initial enlargement due to increased muscle mass, followed by deposition of connective tissue and fat (pseudohypertrophy)
  + Mdx mouse
    - Spontaneous nonsense point mutation in exon 23
    - Genetically homologous to DMD
    - Animals have a relatively mild phenotype – model is not analogous
      * Acute wave of muscle necrosis at 3 to 5 wk of age
      * Mice largely recover and do not experience clinical dysfunction until ~18 mo of age
      * Affected mice do have progressive histopathologic changes and a 20% reduction in life span
    - Ages in mice and humans have been compared
      * 3, 4, 6, and 8 wks of age in mice roughly correspond to 6 mo, 10 yr, 16 to 18 yr, and 20 yr for humans
    - Mdx phenotype is exaggerated when the utrophin gene is knocked-out to produce a double knock-out (dko) mice
      * Double mutants typically have a more severe phenotype that better models symptoms of DMD
      * Second mutation introduces a biochemical or biologic difference that could affect the disease course independent of the absence of dystrophin
    - Relatively mild mdx phenotype has caused concerns about the degree to which findings will translate to humans
      * International consensus has established the mdx mouse as the model of choice for preclinical and proof-of-concept studies
        + Have the exact monogenic biochemical defect present in DMD
    - Two major recommendations to ensure more consistent results:
      * Employ basic standard experimental regimes for preclinical testing
      * Employ basic methods to analyze the preclinical experiments
    - Body weight, normalized grip strength, horizontal activity, rest time, cardiac function measurements, blood pressure, total central/peripheral nuclei per fiber, and serum creatine kinase were the most effective for detecting drug-induced changes
      * Due to the mild phenotype of mdx mice, eccentric contraction and exercise are often used to exaggerate clinical signs
        + Forced treadmill protocol to accentuate the phenotype and increase likelihood of detecting drug effects over a 3- to 6-month trial period has been recommended
      * Mdx and dko mice develop cardiomyopathy and respiratory disease
        + Allows for preclinical testing of drugs that target the heart and improve diaphragmatic function
  + GRMD
    - Dystrophin-deficient form of muscular dystrophy originally characterized in golden retrievers (GRMD)
      * mRNA processing error in GRMD dogs results from a single base change
    - Dystrophic dogs more closely mimic the DMD phenotype compared to mdx mice
      * Dystrophic pups are often ineffectual sucklers and exhibit stunted growth
      * By 6 wks of age the pelvic limbs may be simultaneously advanced (“bunny-hopping gait”) and trismus is noted
      * Other developments include:
        + Stilted gait; atrophy of particularly the truncal temporalis, and certain extensor muscles; plantigrade stance due to hyperextension of the carpal joints and flexion at the tibiotarsal joints; excessive drooling, suggesting pharyngeal muscle involvement; initial lumbar kyphosis that progresses to lordosis
      * While most muscles atrophy, some, such as the cranial sartorius and tongue, hypertrophy
      * Respiratory and cardiac involvement occurs and can be objectively assessed
    - Clinical signs of GRMD progress particularly rapidly between the ages of 3 and 6 months
      * Natural history of GRMD provides a relatively short window over which therapies can be assessed
      * The first year of a golden retriever’s life roughly equates to 20 years of a human’s life
        + 3- to 6-month age in a GRMD dog would correspond to the 5-10 yrs of a DMD boy

Symptoms progress very rapidly at this point in the human patient

* + - Loss of ambulation is the defining clinical feature of DMD
      * Although occasionally severely affected dogs lose the ability to walk, most remain ambulatory
      * Affected dogs sill walk shorter distances than normal/carrier dogs
    - GRMD cardiomyopathy has a later clinical onset than the skeletal muscle phenotype
      * Echocardiographic changes are not seen until at least 6 mo of age, and often considerably later
      * For determining drug effects on the dystrophic heart, dogs should be older when entered into preclinical trials, or the cardiac phenotype should be exaggerated by stressing the heart, as with dobutamine
  + Optimization of animal model use for DMD preclinical trials
    - Design powered studies with sample sizes sufficient to detect drug effects with the outcome parameters used
    - Follow randomization and blinding procedures, including who is blinded and when
    - Provide details of the statistical methods used for data analysis and report all the results for each analysis
    - Develop reliable and sensitive primary and secondary endpoints for the animal model used
    - Independently validate drug efficacy results in another laboratory
    - Validate drug efficacy in two species whenever possible and especially with treatments that carry substantial risk
* Conclusions
  + Failure of translation between animal models and human clinical trials likely occurs due to both physiologic differences between animals and humans and the lack of sufficient rigor in preclinical studies

QUESTIONS

1. Describe the genetic abnormality of Duchenne muscular dystrophy and in what it results (i.e., name the missing protein).

2. True or False: the mdx mouse model of DMD is an analogous model.

3. Describe the test used as the principal outcome measure in DMD clinical trials.

4. True or False: all dogs affected with GRMD lose the ability to ambulate.

ANSWERS

1. X-linked recessive disorder that results in the loss of the protein dystrophin.

2. False – the mdx mouse model is homologous (genetically) but is not analogous in terms of phenotype.

3. The 6-minute walk test (6MWT) has patients walk as far as they can in a 6-minute interval.

4. False – while some severely affected dogs will occasionally lose the ability to ambulate, most retain it.

**Kranenburg et al. Naturally Occurring Spinal Hyperostosis in Dogs as a Model for Human Spinal Disorders, pp. 150-163**

Domain 3: Research

Primary Species: Dog (Canis familiaris)

SUMMARY: This article describes the potential utility of certain breeds of dogs as models for human spinal disorders. Both spondylosis and diffuse idiopathic skeletal hyperostosis (DISH) are prevalent in humans, and can co-occur in dogs as well. Boxers may serve as translational disease models for the elucidation of the gene(s) involved in the etiology/pathogenesis of spondylosis and DISH. Similarities and differences in spinal anatomy of humans and dogs is described - both have 7 cervical vertebrae, humans have 12 thoracic, dogs have 13 thoracic, humans have 5 lumbar, dogs have 7 lumbar, humans have 5 sacral, dogs have 3 fused sacral, humans have 4 fused coccygeal vertebrae, and dogs have 20 caudal vertebrae. Both humans and dogs have IVDs made up of two cartilaginous endplates, an inner gel-like nucleus pulposus and annulus fibrosus. Spondylosis deformans in humans as defined as a degenerative process of the spine involving essentially the annulus fibrosis and characterized by anterior and lateral marginal osteophytes arising from the vertebral body apophyses. Diagnosis, prevalence, skeletal distribution, clinical symptoms, and treatment of spondylosis deformans in humans and dogs is discussed in detail in this article. DISH in humans is a common, systemic disorder of the axial and abaxial skeleton in middle-aged and elderly humans that results in ossification of soft tissues (ex. longitudinal spinal ligaments) and sites of attachment of tendons or muscles and capsules to bone. Diagnosis, prevalence, skeletal distribution, clinical symptoms, and treatment of DISH in humans and dogs is described. Animal models of spondylosis and DISH may be useful to study the currently unknown etiology of both disorders, specifically the involvement of specific genes.

QUESTIONS

1.  Which breed may serve as a translational disease model for elucidation of the gene(s) involved in the etiology/pathogenesis of spondylosis and diffuse idiopathic skeletal hyperostosis (DISH)?

a.  Dachshund

b.  German Sheppard

c.  Boxer

d.  Cairn Terrier

2.  The human spine consists of \_\_ cervical, \_\_\_ thoracic, \_\_\_ lumbar, \_\_\_ sacral and \_\_\_fused coccygeal vertebrae, while the canine spine consists of \_\_ cervical, \_\_\_ thoracic, \_\_\_ lumbar, \_\_\_ (fused) sacral and up to \_\_\_ caudal vertebrae,

a.  9, 12, 5, 5, 4; 9, 12, 5, 5, 4

b.  7, 12, 5, 5, 4; 7, 13, 7, 3, 20

c.  7, 13, 3, 3, 2; 9, 12, 3, 3, 5

d.  6, 13, 5, 5, 4; 7, 12, 7, 3, 20

3.  Spondylosis in humans is defined as:

a. A degenerative process of the spine involving essentially the annulus fibrosus and characterized by anterior and lateral marginal osteophytes arising from the vertebral body apophyses, while the IVD height is normal or only slightly decreased.

b.  A common, systemic disorder of the axial and abaxial skeleton in middle-aged and elderly humans, resulting from ossification of soft tissues such as ligaments and sites of attachment of tendons or muscles and capsules to bones (enthuses).

4.  What is one way that spondylosis can be distinguished from DISH radiographically?

a.  The two conditions are indistinguishable radiographically.

b.  Enthesophytes are seen in spinal DISH, osteophytes are seen in cases of spondylosis.

c.  The difference in amount and appearance of new bone formation; focal osteophytes are formed with spondylosis, while contiguous/flowing new bone formation is formed in DISH.

d.  Osteophytes are seen in cases of spondylosis, whereas enthesophytes are seen in spinal DISH.

5. Which human population has the highest prevalence of DISH?

a. Elderly, male

b.  Elderly, female

c.  Young, male

d.  Young, female

ANSWERS:

1. c

2. b

3.  b

4. c & d

5. a

**LeBlanc. Cancer and Comparative Imaging, pp. 164-168**

Domain 3: Research; K2. Research methods and equipment (e.g., antibody production; adjuvants; tumor induction; imaging; data collection techniques such as telemetry; observation; behavioral assessment methods).

Primary Species: Mouse (*Mus musculus*), Rat (*Rattus norvegicus*), Dog (*Canis familiaris*), Pig (*Sus scrofa*)

SUMMARY: Comparative oncology research is gaining traction as a method for streamlining the drug discovery and development strategies currently in place worldwide. This approach uses the tumor-bearing pet dog as a relevant and complementary model alongside the traditional use of rodents, no-human primates, and other large mammalian species such as purpose bred dogs or pigs.

To date, most comparative oncology studies have been designed and executed to evaluate new anticancer drugs using tumor-bearing dogs with specific naturally occurring cancers as models for humans. These studies have proved extremely valuable for modeling pharmacokinetic-pharmacodynamic relationships, refining drug doses and schedules, and validating an individual drug’s target in vivo. The National Cancer Institute’s Comparative Oncology Trials Consortium (<http://ccr.cancer.gov/> resources/cop/COTC.asp) is a cooperative effort that provides infrastructure and resources to support this effort.

To complement ongoing efforts in this field, we propose expansion of comparative cancer imaging as a component to drug discovery and development. Diagnostic imaging is critical to diagnosis and management of malignancy in both humans and animals. Molecular imaging techniques allow for detection of disease-specific signals that provide individualized data to aid in patient selection, response to therapy, and prognostication. In this review, we will highlight the comparative oncology studies that have used molecular imaging techniques, demonstrating the value of spontaneous canine cancers as a research tool in drug and imaging agent development.

Comparative oncology’s contributions to the field of cancer biology and anticancer drug development are now fully recognized. Noninvasive molecular imaging techniques are a key component of these areas of research. Inclusion of the tumor-bearing dog in comparative imaging studies, both in imaging agent development and in the drug development pathway, are critical to the forward progress of this field.

QUESTION

1.  Which agency provides resources for comparative oncology studies and methodologies>

a. The National Institutes of Health

b.   The National Institute on Aging

c.  The National Cancer Institute’s Comparative Oncology Trials Consortium

ANSWER

* 1. c. The National Cancer Institute’s Comparative Oncology Trials Consortium

**O’Connor and Wilson-Robles. Developing T Cell Cancer Immunotherapy in the Dog with Lymphoma, pp. 169-181**

Domain 3: Research

Primary Species: Dog (Canis familiaris)

SUMMARY: Immunotherapy is not a new concept for veterinary medicine; however, adoptive T cell therapy is a new area of research in humans and canines alike. In humans, T cell therapy has been used against many different tumor histologies, including lymphoma, melanoma, and colon cancer. Although in dogs this approach has currently only been applied to lymphoma, other tumor types are under investigation. There are many different strategies used to take advantage of cell-mediated antitumor properties of T cells. This review will discuss many of the current strategies used in both humans and canines in regards to adoptive T cell therapy. Although the adoptive cellular treatment arm of immunotherapy is new to veterinary medicine, the successes and advances of knowledge into the intricacies of the immune system have renewed interest in clinical use for humans and dogs alike. Comparative oncology and immunology research has better classified canine T cells and their role in tumor prevention, progression and treatment. Clinical trials are currently being developed to implement CAR-specific T cell therapy in dogs with LSA and other neoplasms. Because of their ability to infiltrate and destroy solid tumor masses, nonspecific and tumor-specific T cell therapies may be able to improve survival outcomes, whether used alone or in combination with chemotherapies and radiotherapies. Additionally, using these strategies to program T cells to specifically target B cells may allow for treatment of immune-mediated or allergic diseases. This type of approach has been used with rituximab in humans with much success for many different diseases ranging from immune-mediated hemolytic anemia to asthma. Adoptive T cell therapy provides a new and exciting approach to cancer therapy in humans and pet animals. This new technique, although cumbersome to manufacture, may provide the necessary therapeutic approach to push progression-free survival in our patients beyond 1 year and possibly even provide long-term immune monitoring through immunologic memory in successfully treated patients.

QUESTION

1.   The most common malignancy in the dog is?

a.   Canine Lipoma

b. Canine Hemangiosarcoma

c.  Canine Lymphoma

d.  Canine Transmissible Venereal Tumor

ANSWER

1.   c. Canine Lymphoma: accounts for up to 24% of all reported neoplasms and 85% of diagnosed hematological malignancies. Similar to humans, approximately 60–80% of canine LSA arises from malignant B cells.

**Patterson. Canine Epilepsy: An Underutilized Model, pp. 182-186**

Domain 1. Management of Spontaneous and Experimentally Induced Diseases

Domain 3. Research

Primary Species: Dog (Canis familiaris)

SUMMARY: Dogs and humans share commonalities regarding clinical and electroencephalographic manifestations of epilepsy as well as response to pharmacologic treatments. Due to these similarities, dogs are an excellent animal for studying this disorder and canine epilepsy research supplements the findings from rodent models of induced epilepsy.

Epileptic seizures occur due to abnormal neuronal activity in the brain. There are structural, metabolic, genetic, or unknown causes of epilepsy in dogs. Antiepileptic drugs, ketogenic diets, surgery, and medical devices are used in the treatment of epilepsy in humans. Many of the same medications used to treat epilepsy in humans are used in dogs. Some drugs and diets are metabolized differently in canines but it may still be beneficial to develop new therapeutics. Research on surgical treatment has not been reported in dogs. Vagal stimulators, implanted electrodes, and other devices have been trialed in dogs. Actual devices to be used in humans may be implanted in dogs due to their larger size. Overall, dogs can be useful to study epilepsy for the benefit of both humans and dogs affected by this disorder.

QUESTIONS

1. Canine correlates have been found for which of the human progressive epilepsy disorders?

a. Ekenstedt syndrome

b. Lafora disease

c. Sialodoses

d. Oberbauer disease

2. True or False. Metabolic causes of epilepsy include hypoglycemia and lead poisoning.

3. Which of the following human antiepileptic drugs do not work well in dogs due to shorter half-lives?

a. Felbamate

b. Zonisamide

c. Valproate

d. Levetiracetam

ANSWERS

1. b.

2. T.

3. c.

**Yugo et al. Naturally Occurring Animal Models of Human Hepatitis E Virus Infection, pp. 187-199**

SUMMARY: Historically, non-human primates such as rhesus and cynomolgus macaques, and chimpanzees have served as important animal models for HEV, however chimpanzees (and tamarins), were not consistently infected when challenged.

This paper serves as a basic introduction to hepatitis E virus, a brief history and current research considerations.

Naturally Occurring Models for HEV: In regards to naturally occurring models of human HEV, Swine, chickens and rabbits have been identified in domestic and wild populations to test positive for HEV genotypes associated with human illness (genotypes 1 – 4). Swine serve as major reservoirs for genotypes 3 and 4. Although the Avian HEV that infects chickens, and has been reported to cross species into turkeys, has not exhibited zoonotic potential, Avian HEV genotypes 1 to 3 share 60% of the nucleotide sequence identity with human HEV, and may be used to study at least some aspects of human hepatitis E disease. The rabbit HEV appears to be closely related to zoonotic genotype 3 HEV. Rabbit HEV has also been successfully transmitted to swine, and cynomolgus macaques. Additionally, rabbits are susceptible to infection with human HEV 4.

Potential Naturally Occurring Animal Models: Rats and ferrets have been identified to carry strains of HEV. A genotype 3 HEV has also been detected in some wild rats. Rats have failed to be successfully experimentally infected with HEV 1,2 and 4. Rat HEV also has failed to produce infection in rhesus macaques. Additionally, swine and avian HEV have not been shown to infect rats.

A ferret strain of HEV has been identified in the Netherlands, showing some similarity to genotypes 1 to 4, as well as rabbit and avian strains. At this time, little information is known about the ferret HEV

Vaccine Studies: Pigs have been used for HEV vaccine trials to assess cross-protective potentials for recombinant antigens.

Chickens have been used for vaccine trials and have obtained complete protective immunity against avian HEV.

Pathogenesis: Swine are of limited use in pathogenesis studies, because they develop subclinical infection, and develop only mild-moderate pathologic lesions.

Infection of chickens with avian HEV allows better characterization of pathogenesis, and the animals develop HSS, along with other pathologic changes secondary to the virus. Patterns of sero-conversion and development of clinical pathologic lesions are similar to those seen in human HEV infection. Current literature shows that other factors may play a role in the development of clinical manifestation of the disease, as clinically normal chickens reveal no significant differences in pathogenicity when compared to diseased chickens.

Rabbits inoculated with human HEV failed to produce clinical disease, and at this time, the use of rabbits as a model for HEV pathogenicity is undetermined.

Viral Replication: Hepatic expression of HEV antigens, indicating viral replication has been seen in infected rhesus macaques prior to the appearance of gross lesions.

Swine have allowed for the development of a genotype 3 infection without the need of in vitro cell culture via intrahepatic inoculation

Cross-Species Infection Studies: Rhesus monkeys are susceptible to all 4 genotypes of human HEV, which has led to them being widely used in cross-species studies.  Rhesus have also been experimentally infected with swine genotype 4.

Cynomolgus monkeys have been infected with rabbit HEV similarly to other genotype 3 HEV strains, indicating that rabbit HEV may likely infect humans.

SPF swine are also readily infected with genotypes 3 and 4 human HEV, as well as rabbit HEV, but exhibit resistance to rat HEV.

Rabbits are susceptible to genotype 4 human HEV, and are a natural host for genotype 3 HEV possibly eluding to potential for their use in cross-species studies.

QUESTIONS

1. Which genotypes of Hepatitis E virus are zoonotic?

a. 1 and 2

b. 2 and 3

c. 3 and 4

d. 1 and 4

2. Which species was the first known animal species of HEV?

a. Rabbits

b. Primates

c. Avian

d. Swine

3. Rabbits are used for all of the following HEV studies except?

a. Cross species infection

b. Molecular biology and virus replication

c. Vaccine

d. Pathogenesis

ANSWERS

1. c

2. d

3. b

**Baneux et al. Issues Related to Institutional Animal Care and Use Committees and Clinical Trials Using Privately Owned Animals, pp. 200-209**

Domain 5

SUMMARY: This ILAR paper discusses the challenges in regulatory oversight for research projects and clinical trials using client owned animals in veterinary practices. The veterinarians in private practice establishes a veterinarian client patient relationship (VCPR) during the process of providing care to the pet animals. USDA does not regulate these animals. Similarly, the clinical trials in private practices are not regulated unless funded by PHS agencies. In addition, AAALAC does not consider client owned animals as part of program of veterinary care. However, research protocol review by institutional committees becomes mandatory when data is acquired from the privately owned animals for purposes of publication and procedures performed on pets extend beyond the standard of care for the clinical condition. Besides IACUC, institutions have formed Clinical Research and Teaching Advisory Committees to review clinical studies. These committees provide support to the clinicians by drafting informed consent letters, patient recruitment, managing finances, documenting good clinical practices, providing ethical review of the studies and acting as liaison with IACUC.

The institution may choose to have research protocol review and approval done by both IACUC and the Advisory committee. The project may be exempted from IACUC review if the biological material used to analyze has been discarded after the clinical care has been completed. The journals may require proof of institutional review prior to publishing the data. The general consent for treatment can include consent for future enrollment in a clinical trial or use of discarded tissues for analysis. If the University is collaborating with a private practice to conduct a study, the operational structure of the practice needs to be included in the University’s assurance. Consent should be obtained for collection, processing and storage of any biological materials during the necropsy of privately owned animals. Clinical research committees also help to assess the safety of procedures on client owned animals for the purpose of collecting data from healthy controls. The research committee can intervene if the safety and health of the research animal becomes at risk by withholding the essential treatments to collect data for placebo control group. The off label use of a drug in the course of providing veterinary care is acceptable. However, administration of a novel therapeutic agent to judge its effectiveness requires a study design and approval by appropriate committees. Newer computer programs also allow the clinicians to perform retrospective studies on treatment modalities and outcomes. The regulatory oversight of clinical studies involving privately owned animals is vital to protect the patient, human owner and integrity of the data.

QUESTIONS

1.  A survey/questionnaire of pet owners for the purpose of analysis of data and publication of such data will require approval from

i)  IACUC

ii)  IRB

iii)  OLAW

iv) AVMA

2. The Consent form for treatment of pet animals in a Veterinary Teaching Hospital can include consent from the owner regarding collection of biological materials for research purposes provided they are collected during the process of providing standard veterinary care to the animal.

i)  True

ii)  False

3.  Animal is defined as “Any live vertebrate animal used or intended for use in research, research training, experimentation or biological testing or for related purposes” according to

i)   AWA

ii)   Guide

iii)  PHS policy

iv)  APHIS

4. Research projects with privately owned animals may require oversight of following

i)  IACUC

ii)  Clinical Veterinary Medical Research Advisory Committee

iii)  Ethical Review Committee

iv)  All of the above

ANSWERS

1. ii

2.  i

3.  iii

4.   iv