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***Pathology in Biomedical Research: A Mission-Critical Specialty for Reproducibility and Rigor in Translational Research***

**Brayton et al. An Introduction to Pathology in Biomedical Research: A Mission-Critical Specialty for Reproducibility and Rigor in Translational Research, pp. 1-3**

Domain 1

SUMMARY: This write up introduces the importance of pathology in the process of discovery in biomedical research.  It mentions briefly the 6 articles that are at the core of this issue of ILAR.

Pathology is the study of disease.  It is critical for researchers to understand and know when/if a treatment is helping a disease process, not helping, or potentially causing harm.  For this reason, preclinical research is done in an animal model before moving into human clinical trials.  In this way, researchers and clinicians can have some understanding of what to expect in the human trials based on information gleaned during the preclinical trial. This introduction to topic covered in this ILAR issue stresses the importance of having properly trained pathologists as integral team members during the preclinical phase of biomedical research.  Having comparative pathologists provide their input and expertise can help increase the reproducibility of an experiment as well as minimize the number of drugs/treatments that fail at the human clinical trial phase.

QUESTIONS

1.  According to the National Cancer Institute, what is the definition of a preclinical study?

a.  A study that occurs in a veterinary hospital prior to being tested in a human hospital

b.  Testing of a drug or treatment reagent *in vitro* under GLP conditions.

c.  Testing a new therapeutic agent on a small group of at risk individuals prior to a wider clinical trial.

d.   Research using animals to find out if a drug, procedure, or treatment is likely to be useful.  Preclinical studies take place before any testing in humans is done.

2. What might be some negative consequences of hiring a comparative pathologist on an as needed basis rather than partnering with them long term on a multi-year study?

a.  The pathologist might have limited familiarity with the model/project

b.   Lack of continuity

c.   Potential for different scoring methodologies

d.   All of the above

ANSWERS

1. d

2. d

**Everitt et al. Pathology Study Design, Conduct, and Reporting to Achieve Rigor and Reproducibility in Translational Research Using Animal Models, pp. 4-12**

Domain 3: Research

SUMMARY: There has been recent concern regarding the quality of pre-clinical data that has been produced from academic research as well as the reproducibility of scientific data. Utilizing comparative pathologists in conjunction with pathology best practices in planning and conducting animal research may be a beneficial way to increase the quality and reproducibility of data. Histopathology is the gold standard translational endpoint in animal studies, particularly in disease models and can help provide context to biologic findings and suggest underlying mechanisms.

The degree of collaboration needed with a comparative pathologist depends on the type of model, experience of the lab and pathology needs for the study. High quality pathology support is best supported by a centralized core of pathologists. Pathologists in the subdiscipline of toxicologic pathology are well-suited for studies related to disease models, although most work in industry and are not well-represented in academia. If access to a comparative pathologist is difficult, references for collaborators, internet searches for pathology labs/consultants and communication with pathologists from larger institutions may be helpful.

Identifying pathology support early in the study design and planning process is critical. Pathologists can train researchers to complete necropsies and collect tissue and provide insight into unexpected losses. Funding agencies are increasingly inquiring about pathology support when analyzing research proposals.

Pathology for animal studies is often not well-reported in academic studies. Study-specific protocols are beneficial and should document euthanasia methods, detailed necropsy procedures, tissue sampling, trimming and handling procedures, fixation, tissue processing, staining and molecular assay information. The Society of Toxicological Pathology  provides useful information and best practice guidance publications (<https://www.toxpath.org/best-practices.asp>).

No single optimal method or technique is ideal for all studies. Necropsy and pathology methods should be specifically tailored for the type of study, objectives and animal model. A systematic approach and impeccable documentation contributes to high-quality, reproducible data.

The ARRIVE guidelines were published to enhance transparency and reproducibility in animal studies and represent a "checklist approach". Recently, the MINPEPA guidelines have been published to aid in reporting experimental pathology data and complement the ARRIVE guidelines. The MINPEPA guidelines address tissue harvest, fixation and post-fixation techniques, processing, staining, molecular techniques, lesion scoring, data processing/review and figure presentation.

QUESTIONS

1. T/F: Histopathology is the gold standard translational end point for animal research

2. T/F: MINPEPA guidelines were published to aid in reporting experimental pathology data

3. What is the purpose of the ARRIVE guidelines ?

ANSWERS

1. True

2. True

3. To enhance transparency and reproducibility in animal studies

**Meyerholtz and Beck. Fundamental Concepts for Semiquantitative Tissue Scoring in Translational Research, pp. 13-17**

SUMMARY: Tissue evaluation is common in research. Scoring of tissue lesions aids in assessing model phenotypes, disease pathogenesis, toxicities and efficacy of therapies. However, these observations are qualitative in nature and have limitations for group comparisons. In general quantitative and semi quantitative scoring can be applied. “Quantitative” scores are derived from measuring tissue parameters often using manual techniques or software to analyze digital images, whereas “semi-quantitative” scores are assigned by an observer based on predefined morphologic criteria which can be applied to macroscopic and microscopic tissue changes. This study was done due to the concern in failure to reproduce some scientific studies. Semiquantitative scoring is a way of transforming qualitative tissue date into numerical data that allow more robust group comparisons and reproducibility can be improved by constraining bias through appropriate experimental design, randomization of tissues, effective use of multidisciplinary collaborations and valid masking procedures. Integration of semiquantitative scoring can be useful that they are relatively inexpensive, can be a quick screening method to produce pilot data for grant application, can enhance the rigor of descriptive text and can be used to guide , corroborate and validate observations or data obtained from other assays.

Fundamental Concepts:

1.  BIAS CONTROL

1. Experimental design- strong foundation and sound experimental design
2. Randomization- heterogenization to prevent bias from overly homogenized group
3. Expertise- trained statisticians and board certified pathologists must not be omitted
4. masking (aka as blinding)- blinding observer from treatment groups when assigning tissue scoring.

2.   METHODS

a. Lesions – size, shape, distribution, presence or absence

b. Stains- distribution, intensity

c. Scoring methods- progressive numerical score must have well defined descriptor (% )

3.   EVALUATION

a. Biological validation – semiquantitative scoring must be tested for a correlation with biologically relevant data

b. Validation of repeatability – repeatability by observer both intra-observer (same person) and inter-observer (diff people)

c. Group comparisons – statistical tests

Conclusion: Semiquantitative scoring must be validated to demonstrate relevance to biological data and to demonstrate reproducibility. Statistical analysis should make use of appropriate tests to give robust confidence in the result interpretations and by following key principles of semiquantitative scoring will not only enhance descriptive tissue evaluation but also improve quality, reproducibility and rigor of tissue studies.

Semiquantitative scoring is simple and relatively inexpensive approach to enhance descriptive/ qualitative tissue date. And by understanding common applications of scoring and key concepts will enhance scientific studies in translational research.

QUESTIONS

1. Is a term applied to areas of subjectivity that can skew data and contribute to lack of scientific reproducibility
2. How to prevent the bias that happens for example when harvesting tissue from a large cohort of animals that produces wide range of times from experimental day until necropsy
3. T or F: when studies uses merged scoring (average or sum of scores) approach in which multiple parameters are combined to form one final “composite” score, biological relevance is not required.
4. T or F: Descriptors that are vague and subjective such as 0-3 (normal-severe) scoring must be avoided
5. T or F: in ordinal scoring system, range of level up to 6 is recommended

ANSWERS

1. Bias
2. Randomization (heterogenization)
3. False. biological relevance is required
4. True. Ordinal scoring system recommendation is up to 4-5 and must have defined descriptors such as percentage of tissue affected.
5. False. 4-5 is recommended, fewer than this decreases sensitivity and more than this reduces repeatability

**Bolon et al. Good Laboratory Practice in the Academic Setting: Fundamental Principles for Nonclinical Safety Assessment and GLP-Compliant Pathology Support When Developing Innovative Biomedical Products, pp. 18-28**

Domain 3:Research

SUMMARY: A well-defined problem in animal based research over the last decade has been the reproducibility of studies. This paper proposes that the indoctrination of Good Laboratory Practice (GLP) or at least conducting studies in the spirit of GLP in the academic setting will help to address this problem. Large academic institutions are capable of producing large amounts of compounds and medical devices but are typically unable to complete the “bench to bedside” cycle because of the need to conduct the nonclinical safety studies offsite typically at a contract research organization (CRO). Safety studies conducted for acceptance to a regulatory agency must be conducted in a very controlled way. The manner in which these controlled studies are structured is the basis of the guidelines that form the basis of GLP. Academic centers tend to focus on basic research and rarely focus on the development of therapeutic products.

Guidelines that govern GLP principles were originally develop in the USA in 1978. Since that time other countries have developed similar guidelines. An attempt to globally harmonize GLP principles was created by the Organization for Economic Co-operation and Development (OECD). The OECD is not a regulatory agency that approves and registers new products. Mutual acceptance of data is a concept where any member country of the OECD will accept test data from any other member country. Studies conducted under the GLP’s are very controlled giving assurance of the quality and integrity of test data. This is not unlike “good research practice” already present is quality research labs. A major difference between these two processes is that under the GLP’s studies must be audited by independent Quality Assurance (QA). This ensures that the data was generated under controlled conditions.

An advantage of universities conducting in-house GLP studies is the ability to maintain control over intellectual property. There may also be the opportunity to control costs. There is also the opportunity to create a revenue source by offering the service to external stakeholders. A hybrid approach that generates the benefits of data integrity but controls costs is to do studies in the “spirit” of GLP. In these studies the quality management system is followed but the studies are not audited.

GLP studies are conducted in a test facility and are led by facility management. Facility management should be agent of the university that has the authority to control personnel and financial resources. The Study Director is responsible for the overall conduct of a GLP-compliant nonclinical study. The Study Director writes the Study Plan which describes the study in detail and the final study called the Study Report. All personnel in a study must have documentation demonstrating adequate training and experience for the role they are assigned. A defining part of a GLP study is that they must be audited. QA staff must be independent of the study and offer no scientific input but rather confirm the study reliability and rigor and thus data integrity. QA inspects critical phases of a study. In addition QA inspects the facility and processes that run that facility. Facilities and equipment must be maintained according to manufactures recommendations and be regularly validated. A major corner stone of running GLP studies is a quality management system comprised of extensive SOP’s that are carefully written, reviewed and under strict version control.

Facility Management maintains a master study schedule that keeps track of all studies in the test facility. All records must be archived in a way that protects them and ensures accurate recovery on demand. GLP studies at their core are safety studies and the outcome of the study relies heavily on the qualitative and semi-quantitative results of a pathologist. To increase the reliability of the data GLP studies should include pathology peer review. The second pathologist should review target tissues and all tissues from a subset of normal animals.

QUESTIONS

1. When and where were GLP guidelines first developed?
2. What is the name of the group that has developed globally harmonized guidelines for GLP?
3. What is the role of a Study Director.
4. T/F QA provides important scientific contributions to studies.
5. What is the role of a second pathologist during pathology peer review?

ANSWERS

1. Guidelines that govern GLP principles were originally develop in the USA in 1978
2. Organization for Economic Co-operation and Development (OECD)
3. The Study Director is responsible for the overall conduct of a GLP-compliant nonclinical study
4. QA staff must be independent of the study and offer no scientific input but rather confirms study reliability and rigor and thus data integrity
5. The second pathologist should review target tissues and all tissues from a subset of normal animals

**Elmore et al. A Review of Current Standards and the Evolution of Histopathology Nomenclature for Laboratory Animals, pp. 29-39**

SUMMARY: History of International Laboratory Animal Pathology Nomenclature

INHAND for Use in Toxicology Safety Assessment:

INHAND = International Harmonization of Nomenclature and Diagnostic criteria  – international collaborative effort to codify and publish uniform nomenclature for both proliferative and nonproliferative lesions in laboratory rodents and non-rodent species; nomenclature is primarily descriptive and denotes findings that can be documented from review of routine histologic specimens

Participating organizations –

* Strategic and Regulatory Policy Committee of the STP (Society of Toxicologic Pathology)
* Members of major Societies of Toxicologic Pathology (European STP, Japanese STP, British STP)
* Registry of Industrial Toxicology Animal-data (RITA)
* Oversight provided by Global Editorial and Steering Committee (GESC) consisting of members from each major STP

Unique features –

* International scope
* Implementation of open comment period
* Inclusion of neoplastic and nonneoplastic terminology
* Available in web-based format and published in society journals
* Includes a formal change control process to implement addition of lesions, changes to terminology, or correct inaccuracies

Standard for Exchange of Nonclinical Data (SEND) – standardized procedure for submitting data from nonclinical studies to the Food and Drug Administration (FDA) electronically and in standardized format

* Provides definitions for processes and modifiers associated with INHAND published terminology (continues to grow as INHAND publishes updated information)
* Current terminology found at the National Cancer Institute Enterprise Vocabulary Services

National Toxicology Program Nonneoplastic Lesion Atlas (NTP NNLA) – online guide for diagnosis and recording of nonneoplastic lesions in studies conducted by the NTP to standardize nomenclature and diagnostic strategy and create a more consistent database of nonneoplastic lesions, allowing for comparison across studies, facilitate data mining, and allow for generation of historical control data

* Online document updated as the field of toxicologic pathology changes; available for free as a public guide through NIEHS NIH website

NCI Mouse Models of Human Cancer Consortium (MMHCC Nomenclature) – nine organ system working groups organized under NCI Division of Cancer Biology’s extramural grant program tasked with developing comparable human and mouse consensus pathology meetings in coordination with NCI vocabulary informatics experts

* Merged with NCI vocabularies and no longer exists as an independent taxonomy
* Results of each pathology committee group were published in refereed journals to report comparative pathology for organ-specific carcinogenesis for the purpose of developing mouse models of cancer

Computable Terminology: Development and Implementation of the Mouse Pathology Ontology (MPATH) for Use in Mouse Research:

MPATH – constructed according to “good practice” rules consistent with Open Biological and Biomedical Ontology Foundry principles, including separation of physical pathological entities and pathological processes

* Consists of 2 major branches: 1) pathologic processes, and 2) pathologic structures, both constructed as a taxonomy of classes linked by relational terms, allowing computational work of data and bridging different groups
* Utilizes Phenotype and Trait Ontology (PATO, ontology of qualities that qualify or provide formal attributes to an entity or process, such as color, texture, or malignancy)
* May be obtained from an online repository for use by trained histopathologists
* Possible to combine and semantically integrate different datasets using MPATH for coding pathology even if performed at different institutions or different levels of granularity

Standardized Histopathology Terminology Implemented by the IMPC – established as global consortium of large-scale mouse production and phenotyping (19 research institutions, 5 national funders, 11 countries)

* 10yr goal = generate a “knockout” mutant for every protein coding gene in the mouse genome in an effort to characterize the phenotype(s) that each gene confers
* Freely available to public providing a unified single point of access to production and phenotype data, including all negative results with an automated statistical analysis tool
* Majority of tests are standardized or mandatory with histopathology objectives including: 1) identify abnormalities correlated with clinical phenotype, 2) identify abnormalities not directly correlated with clinical phenotype, 3) identify abnormalities that are novel findings in strains with no clinical phenotype, and 4) classify findings as “not significant” or “significant”
* Utilizes MPATH, PATO, and MA

QUESTIONS

1. Which system focuses on genetic phenotype characterization?
2. IMPC
3. MPATH
4. MMHCC
5. NTP NNLA
6. SEND
7. Which organization is a closed US funded group with independent terminology?
8. MPATH
9. MMHCC
10. NTP NNLA
11. IMPC
12. None of the above

ANSWERS

1. a. IMPC
2. e. None of the above – all systems are a combined effort of multiple organizations or depend on utilization of joint/combined terminology from global organizations/systems

**Knoblaugh et al. Pathology Principles and Practices for Analysis of Animals Models, pp. 40-50**

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Animal models are invaluable for elucidating the mechanisms of human disease and for the discovery of novel treatments. It is therefore quintessential to translational research that animal models are accurately characterized and validated as models of human disease. Phenotypic analysis of genetically engineered mice (GEM) and detailed evaluations of mouse models of disease have become increasingly important. Pathology including histopathology and immunohistochemistry based analysis is an important endpoint and verification of these models. The authors discussed that a comparative pathologist is an indispensable member of the research team who can not only help in pathology but also will be very valuable in experimental design, animal model development, sample collection, and data interpretation. The authors further discussed pathology considerations including histopathology and a detailed outline for consideration of necropsy, fixation and trimming for mouse models.

QUESTIONS

#### 1. Most commonly used mouse models utilized in research are

a. Immunodeficient

b. Chemically Induced

c. Genetically Engineered

d. Surgically Induced

2. Authors in this manuscript argued involvement of which member in the research team as quintessential in terms of use of animal models?

a. Clinical Veterinarian

b. Comparative Pathologist

c. Biostatistician

d. Molecular Biologist

3.  Popular immunocompromised mouse model includes except?

a. NSG (NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ)

b. NOG(NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac)

c. NRG (NOD.Cg-Rag1tm1MomIl2rgtm1Wjl/SzJ)

d. MISTRG (C;129S4-*Rag2tm1.1Flv Csf1tm1(CSF1)Flv Csf2*/*Il3tm1.1(CSF2,IL3)Flv Thpotm1.1(TPO)Flv Il2rgtm1.1Flv*Tg(SIRPA)1Flv/J)

4. As a general guideline, how many mice per group recommended?

a. 5-10

b. 1-5

c. 3-6

d. 8-12

5.  Which guidelines provide recommendations on proper necropsy, trimming, Orientation and the number of sections per organ for rodent models?

a. ARRIVE guidelines

b. INHAND guidelines

c. MINPEPA guidelines

d. RENI guidelines

6.  For study consistency and reproducibility which guidelines were developed by NC3Rs of animals in research to provide guidance and an online experimental design assistant tool for the design, analysis and publication reporting of research utilizing animal models?

a. ARRIVE guidelines

b. INHAND guidelines

c. MINPEPA guidelines

d. RENI guidelines

7.  True/False. Inbred mouse strains are genetically identical and often vary by source?

8.  True/False. C57BL/6J mice obtained from the Jackson lab are different from C57BL/6N mice obtained from the NIH.

9. True/False. Subcutaneous xenograft tumor models are easy to monitor and provide easy access for treatment

10. True/False. For rodent models, essential model detail includes species, age, background strain, sex, immune status, diet, and housing.

ANSWERS

1.  c

2.  b

3.  d

4.  a

5.  d

6.  a

7.  False

8.  True

9.  True

10. True

**Himmel et al. Beyond the H&E: Advanced Technologies for in situ Tissue Biomarker Imaging, pp. 51-65**

Domain 1: Management of spontaneous and experimentally induced diseases

SUMMARY: Light microscopy-based tissue evaluation, specifically H&E staining, is the gold standard for pathology data. Advanced tissue microscopy, such as immunofluorescence (IF), in situ hybridization (ISH), laser capture microdissection (LCM), matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) and spectroscopic/optical techniques offer additional means for analysis.

Immunofluorescence (IF): This technique has allowed for advanced quantitative analysis from whole tissues and single cells, and is an alternative for immunohistochemistry (IHC). Both IF and IHC use antibody-mediated antigen detection, but IF binds through fluorescence rather than chromogenic stains. The advantages of IF are the ability to capture targeted molecules in separate fluorescent channels, quantify data, use multiplexing through multiple distinct fluorophores and directly compare two or more stains in the same physical location. Limitations include photosensitivity of fluorophores, which may limit their stability and longevity.

*In Situ* Hybridization (ISH): This technique can detect coding and noncoding RNA transcripts in tissue sections, cell culture, viral particles and stem cells. Multiple techniques are available. RNAscope has been recently developed and allows for detection of single mRNA transcripts of up to four target sequences. A similar derivative, BaseScope, detects shorter sequences. RNAscope is fast, easy to use and requires minimal preparation and equipment. RNAscope and  BaseScope can be used on cells, fresh frozen, fixed and formalin-fixed paraffin-embedded (FFPE) sections as well as whole mounts and free-floating sections of tissue. Positive and negative controls are useful when using these assays. Advantages include high sensitivity and specificity, availability of ready-to-use kits and assays that can be completed in 1-2 days with minimal expertise and training. Cost is a limitation, but the need for limited labor can help offset the costs. Another emerging imaging technique is fluorescent in situ sequencing, which builds off of FISH technology. It provides deeper, ultra-sensitive analysis for quantitative and visual detection of RNA expression in FFPE tissue, whole mounts and organoids.

Laser Capture Microdissection (LCM): This technique uses an inverted light microscope with a laser for precise collection of cells within a heterogeneous tissue. LCM can be used on FFPE, frozen sections, cell culture and cytology slides. These tissues allow for down-steam analysis of DNA, RNA and protein, although frozen sections are optimal for RNA and proteomic analysis (RNA degrades quickly in the presence of RNAses in the environment).  Tissues used for LCM can also be stained with many popular histology stains. The major limitation of this technique is that the user needs to be knowledgeable in microscopic anatomy and be able to identify and collect the cells of interest. Processing is also labor intensive. Collaboration with a pathologist or histotechnologist is often necessary and access to equipment may be challenging.

Matrix-Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI IMS): This technique is used for direct analysis of tissue. It combines mass spectrometry with histology to provide analytical power and spatial analysis. MALDI IMS can be used on fresh frozen and formalin-fixed tissue. Benefits include the ability to use this technique without specialized reagents or prior knowledge of tissue composition, which is ideal for discovery-based research. Additionally, it is minimally destructive to tissue and can analyze a variety of analytes including metals, pharmaceuticals, metabolites, proteins and peptides. Limitations include the need for specialized equipment. Specialized software is available to display data as heat maps.

Optical Imaging and Vibrational Spectroscopy: Advancements in this field have recently begun to simplify and augment standard histopathological tissue analysis. Some techniques can be applied directly to unfixed tissue for the development of a computer-based H&E style imaging without labor-intensive tissue preparation. These technologies can provide additional tissue contrast and structural details that are observed with traditional histology as well. Many specific techniques are available, each with its benefits and limitations.

Emerging Technologies: Multi-, super- and hyperplex antibody-based labeling assays allow for analysis of parallel biomarkers on a single slide. These technologies generally consist of fluorophore-, hapten- or metal-coupled antibodies. Confocal laser endomicroscopy allows for real-time micron-level imaging using a miniprobe and has been used to diagnose neoplastic and inflammatory disease in conjunction with histopathology. Microscopy with ultraviolet surface excitation (MUSE)  is another technology that generates in vivo histology-like images. MUSE is a simple, nondestructive and does not require a slide. It provides high-resolution images from fresh or frozen tissue in only a few minutes.

QUESTIONS

1. What technology is the gold standard for histopathology analysis?

2. Why are frozen sections preferred for RNA analysis?

3. T/F:  Immunofluorescence and immunohistochemistry use antibody-mediated antigen detection for staining

4. A benefit of laser capture microdissection (LCM) is the ability to stain the tissue with other common histology stains

ANSWERS

1. H&E staining

2. RNA degrades quickly in the environment when exposed to RNAses

3. True

4. True

**Aeffner et al. 2018. Digital Microscopy, Image Analysis, and Virtual Slide Repository, pp. 66-79**

Domain 3

SUMMARY:  Starting around the year 2000 Whole Slide Imaging (WSI) has been becoming more accessible and mainstream.  Digitization of slides has made it faster and easier to share slides thus allowing distance examination and teaching.  Furthermore, the digital WSI now allows for objective analysis of a whole slide, rather than a random section of the slide.  The FDA recently approved a digital pathology solution which has helped establish the standard for equipment, workflows and analysis.

Scanning slides is broken into 4 parts (image acquisition, storage, editing, and display of images).  Images are captured at different magnifications and then each of the overlapping titles is digitally stitched together to give the illusion that it is one giant single image.  20x and 40x are the most common, though higher magnifications are available with oil on some slide scanners.  The complete slide at a high resolution is very large in terms of storage on a computer.  To help with the difficulties of working with such a large file, the portions of the slide that are of interest to the viewer are loaded in as needed.  This is a similar approach to what is done with Google Maps, the whole country is not downloaded to a computer, only the areas that the viewer wants to see.  The monitor that is used to view digital slides needs to be of sufficient resolution and correct color to ensure accuracy to the viewer.  A controlled and consistent viewing environment is also helpful in viewing slides.

The existence of digital slides does mean that there is not a pathologist looking at glass slides any longer.  But, a pathologist should still be consulted in study design, tissue collection, staining, scan quality, analysis, etc.  So called “do it yourself” pathology is still just as problematic as with glass slides (e.g. A mouse nipple was identified by a non-pathologist as a premalignant papilloma).  Digital slides should be seen as a way to expand the relationship between researchers and pathologists.

Great steps are now being made in image analysis of digital slides.  This is broken down into three major parts (area-based measurements, cell based measurements, and measurement of other items other than cells).  These may be based on color or color intensity.  Software has to be trained how to recognize the features of interest in the digital slides.  The depth and accuracy of the training will permit for more reliable results by the software that analyses the digital images.  Automated scoring of images still should be validated by a pathologist.

Another benefit to the use of digital slides is that physical space is not take with their storage.  Furthermore, the databases of these slides can contain metadata that is able to be searched long after a study has concluded.  It is incredibly important to have backups of all your digital slides, both on and offsite.

QUESTIONS

1.  Image file sizes for 40X scanned slides are approximately how much larger than files created using 20X?

a.   2 times larger

b.   4 times larger

c.  8 times larger

d.   16 times larger

2. What is the preferred compression method for image files used for digital slides?

a.   TIFF

b.   JPEG

c.  JPEG2000

d.   GIF

3.   What is stereology?

a.   3D interpretation of 2D pictures

b.  Specialized staining in digital slides

c.   Ultra-high resolution microscopy without oil

d.   Digital slides of wet tissues

ANSWERS

1.  b

2. c

3.  a

**Gabrielson et al. In Vivo Imaging With Confirmation by Histopathology for Increased Rigor and Reproducibility in Translational Research: A Review of Examples, Options, and Resources, pp. 80-98**

Domain 3: Research

SUMMARY:  Currently, 2 decades of advancements in clinical imaging have allowed a wide range of possibilities for preclinical imaging methodologies to be applied in practically all smaller animal species. The increased resolution of the equipment accommodates the needs of researches to captures images in zebrafish, rodents and rabbits, for example: 11.2 Tesla MRI or ultrasound transducer at 50-70MHz. These advance have enabled an increase in the refinement of animal experimentation, due to with the application for serial imaging over time, researcher could obtain accurate data of diseases progression with a minimum number of animals. Besides, imaging methods that offer molecular imaging (by the use of bioactive molecules or radioactive tracers) are an advantage to perform functional dynamic studies in just an animal, recording minute-to-minute changes induced by an experimental drug, for example. The purpose of the present review is to draw parallels and give examples of imaging-to-pathology correlations in multiple species, where in vivo imaging was used to document anatomical, functional, and molecular features in an animal model of disease.

*ULTRASOUND IMAGING*: Ultrasound imaging is one of the earlies developed, safest, and least expensive modalities used in clinically in human and veterinary medicine. This technology allows the evaluations of anatomy, endothelial molecular changes and blood flow through organs. Advances methods have been used for optical imaging, intravascular imaging or living mouse embryo. The use of microbubbles facilitates the blood flow visualization or quantification of molecular targets if these bubbles are conjugated with antibodies. Ultrasound combined with photoacoustic imaging modality could determine functional data of tissue as oxygen saturation.

Advantages:

* Less expensive
* Portable and require less maintenance
* Normally, no tracers or injectable agents are required
* Safe, good resolution and real-time acquisition
* Easy to operate
* Allows user to determine the physiologic state of an animal

Limitations:

* Poor visualization of bone-obscure organs (brain)
* Limited depth penetration with high frequency

Ultrasound developed for human patients are easily used for large animals, but small rodents require high-resolution systems. Awake mice are easily monitored with high frame rates, up to 1000. In anaesthetized rodents ECG monitoring can be synchronized with echocardiography data, allowing speckle-tracking-based strain analysis.

As examples of the accuration of ultrasound imaging and the physiologic state of an animal, ultrasound could certify mouse pregnancy at a 100% before the 7.5 day, as early as 4.5. Another one, ultrasound could be used to determine aortic enlargement or atrioventricular valve prolapse in a mouse model of Marfan disease. Ultrasound can determine the progression of the Marfan's cardiovascular phenotype, allowing to researcher a new therapeutical target observation: TFGbeta genetic dysregulation; translated to clinical trials for Marfan patients.

*NUCLEAR IMAGING*: Two main nuclear medicine imaging modalities are available for preclinical imaging: PET and SPECT. PET employs positron-emitting radioisotopes as 18F-FDG, which is used in oncology to determine the metabolic demand of tumors. PET can be used for neurodegenerative disorders or inflammation. As in ultrasound, small rodents require miniaturized high-sensitivity scanners but recently, a portable small PET scanner has been developed to determine brain function in awake rats. SPECT is similar to scintigraphy, which utilizes radioligands incorporating gamma-emitting radioisotopes. A radio-camera rotates around the subject and multiple cross-sectional images are acquired, processed later in a 3D image. When a custom-made ligand is used, it is recommended to perform a biodistribution study before planning imaging experiments to evaluate tissue and organ distribution of the ligand using a scintillation counter to quantify radioactivity in organs collected at necropsy.

Advantages :

* Allows the determination of distribution and metabolism of biomolecules
* Offers high sensitivity and can detect molecules at nanomolar and fentamolar levels

Limitations:

* Poor anatomical localization, needs CT or MRI
* Short half-life of some isotopes requires scheduled coordination of animals, treatments and personnel
* Although nuclear imaging is safe, there is a safety concerns: require special racks isolate of regular animals, precautions when a postimaging necropsy is planned
* Cyclotrons locations, risk in short-lived isotopes use
* Histological verification is required due to a bit variances from the norm, as radiotracer up-take of other not-target cells.
* If the equipment is dedicated for the usage of radioactive tissues, careful processing of tissues can be done for histology in addition to biodistribution studies.

If it is correlated the use of nuclear imaging to Pathology, one example would be the preclinical studies to determine the preventive, diagnostic and treatment option to protect the cardiotoxicity in cancer patients of anthracycline doxorubicin therapy. SPECT imaging is applied to determine the number of cell-death in cardiac tissue by detecting the exposition of Annexin V. Another example of the great value of nuclear imaging in clinical pathology is the specificity of a new tracer (124I-cRGDY-PEG-C-dots) to detect melanoma metastasis while 18FDG resulted in false positive finding caused by inflammation process.

*CT and X-RAY PLANAR RADIOGRAPHY MODALITY*: X-ray has been used for decades; CT obtains X-ray-derived images with subsequent 3D reconstruction. Many of them are built into a SPECT r PET machine and can be used independently. Planar radiography uses X-rays to produce a 2D image of single projection of the subject offering enough information less expensive but without sophistication.

Advantages:

* Imaging time is relative short and high spatial resolution
* Available at most institutions

Limitations:

* Require anesthesia
* CT x-ray exposure is higher than planar examination
* Attenuation by tissues may limit the depth of penetration and requires attenuation correction algorithms.
* Breathing movements or beating heart, require additional correction
* Poor resolution for soft tissues but contrast agents can be used to aid

Obviously, x-ray based techniques are quite suitable to determine the pathological evolution in bone tissues, as osteosarcoma. This is useful to determine the animals for a treatment group. In a rat model of osteosarcoma only tumor within the bone microenvironment will metastasize to the lung.

*OPTICAL MODALITY*: Optical imaging modalities are based on the detection of emitted light in the visible and near-infrared spectra. Bioluminescence or fluorescence imaging methods are well-established in biomedical research but newer modalities taking advantage of other physical phenomena, such as Raman spectroscopy. Tamden of luciferase and luciferin is the most common system for bioluminescence. Unlike fluorescence, no external light source is required, but luciferin substrate must be administered prior to imaging which  kinetic is affected by the injection route. Fluorescence is the phenomenon by which some molecules are excited by an appropriate wavelength light, absorb photons and emit photons of a longer wavelength.

When matter is illuminated, most of the light is elastically scattered and retains its energy and wavelength (Rayleigh scattering) but a small fraction of the light is inelastically scatters with a lower energy and longer wavelength (Raman effect). Raman spectroscopy can be performed on cells and tissues without the use of probes, revealing their molecular composition as endogenous proteins and lipids produce distinct signatures, but it can also make use of probes that can be designed to target biological structures or functions.

Advantages:

* Structures and function of interest can be precisely targeted (by molecular probes or genetic engineering)
* More safe than nuclear imaging methods
* It could reach fentomolar ranges regarding sensitivity
* Images acquisition is fast and multiple animals can be imaged at the same time
* Files are small and not needed complex postprocessing of the raw data

Limitations:

* Ability of light to travel through tissues
* Animals must be shaved before imaging
* For fluorescence -in small rodent- fluorophores with near-infrared emission spectrum have greater penetrance through tissues; but this can be applied to human, just limited to intraoperative and endoscopic applications.
* Autofluorescence of endogenous tissue components and of ingesta (alfalfa in food)

Optical imaging offers the ability to examine the entire body of a mouse and detect rare lesions such as micrometastases; but histopathology is complementary method to increase the power of the analysis. In an example of Raman spectroscopy imaging, nanoparticle were used to detect pancreatic tumors at surgery in real time. Precise correlation of imaging, macroscopic and microscopic pathologic findings, as well as immunohistochemistry for the nanoparticle, demonstrating that this technique could detect  advance neoplasms and also preneoplastic lesions.

*MRI MODALITY*: Magnetic Resonance Imaging employs a powerful and uniform magnetic field and radio frequency energy and is based on the 2 properties of charges nuclear particles: the constant angular momentum and a magnetic moment. As vector entities, both have directionality and spin features. In a magnetic field, protons align either parallel or antiparallel to the magnetic field and absorb energy when aligned by a pulse from MRI magnetic coil.

The nuclei return to their normal alignment (relaxation, the adsorbed energy is returned as RF pulses and an additional coil locates the X, Y, and Z orientation of the tissue magnetic resonance signal after each magnetic pulse. There are 2 main relaxation types: spin-lattice and spin-spin relaxation; and the images are produced based on tissue difference in the longitudinal (T1) and transverse (T2) relaxation times. MRI contrast agents allow blood flow dynamics, as Gadolinium which reduce T1 relaxation time. MRI is particularly advantageous in studying the soft tissue anatomy.

Advantages:

* Powerful, noninvasive method for phenotypical characterization and therapeutic efficacy evaluation
* Provide opportunities for longitudinal studies of tissue changes

Limitations:

* High cost of equipment acquisition and transportation, operation and maintenance
* Animals must be shaved before imaging

MRI is advantageous in obtaining accurate information such location, volume and number of lesions. Provide a great information to perform longitudinal assessments of changes in the target and the adjacent tissues, allowing progression, regression and therapeutic effects in a noninvasive and nondestructive manner. MRI also provide good data in ex vivo fixed organs.

*CONCLUSIONS*: Despite significant advance in technologies available to scientists for evaluation of animals models, pathology remains a gold standard for a complete analysis of the outcome of an animal model study. Imaging modalities provide longitudinal evaluation of the disease progression offering a complementary scientific data. This reduce the number of animal, reproducibility of experimental data, decipher biological questions and translate the preclinical results to human medicine.

QUESTIONS

1. Why noninvasive imaging technology is an integral part of refinements and improvement of translational values of*in vivo* models?

a. Serial imaging over time

b. Reduction the number needed for a complete study, evaluating a disease at multiple time points

c. Noninvasive imaging allows an animal to serve as a pretreatment control before treatment begins

d. Observing the animal over multiple time points is key to documenting the disease progression and determining the most accurate endpoint for organ and tissue sample collection at the interested disease stage.

e. Prevention of unexpected deaths of animals which masks disease signs

f. All of them

2. True or false: Ultrasound imaging utilizes the differences of sound wave propagation and reflection off of solid, liquid, and gaseous matter in the body. Sound waves of defined frequencies travel through the body and are reflected back as echoes. The echoes are converted in 2D-3D-4D images or movies after acquisition. The higher the difference between densities of tissue, the higher proportion of the waves reflected back. At higher frequencies (50-70MHz) the penetration depth of the sound increase, but there is a lack of resolution.

3. Which factors could affect kinetics of luciferin?

a. Route of administration

b. Organ or lesion being imaged

c. Type of luciferase

d. b and c are correct

e. All of them are correct

4. Which organism is not use for optical imaging modalities?

a. Photinus pyralis

b. Aeromonona fluorescence

c. Aequorea victoria

d. Discosoma spp

e. None of them

5. Which molecule nucleus could be used for MRI?

a. 1H

b. 31P

c. 13C

d. 23Na

e. 19F

f. 17O2

g. Helium

6. Which imaging modality offer images at real time?

a. MRI

b. Raman spectroscopy

c. PET

d. Ultrasound

e. b and d

ANSWERS

1. f

2. False: Ultrasound imaging utilizes the differences of sound wave propagation and reflection off of solid, liquid, and gaseous matter in the body. Sound waves of defined frequencies travel through the body and are reflected back as echoes. The echoes are converted in 2D-3D-4D images or movies at real time. The higher the difference between densities of tissue, the higher proportion of the waves reflected back. At higher frequencies (50-70MHz) the penetration depth of the sound decrease, but the resolution of tissue image is higher.

3. e

4. b

5. All of them

6. e

**Regan et al. Clinical, Pathological, and Ethical Considerations for the Conduct of Clinical Trials in Dogs with Naturally Occurring Cancer: A Comparative Approach to Accelerate Translational Drug Development, pp. 99-110**

Domain 3: Research

Primary Species: Dog (*Canis familiaris*)

SUMMARY: Comparative medicine has benefited significantly from studying naturally occurring disease processes in various species as the limitations of inducible and genetically engineered disease models have become apparent. These trials often involve cross-disciplinary collaborations between veterinary and human clinicians and scientists. Comparative oncology is one area of study that has benefited specifically from studying naturally occurring cancers in pet dogs. In human oncology, tumor identification via histology has given way to advanced molecular diagnostics, leading to personalized treatment of individuals and tumors. These advancements highlight the importance of pathologists in identifying accurate molecular targets in order to subtype tumors and enroll patients in clinical trials best suited to the treatment of their neoplasia. In contrast, the most common role of the veterinary pathologist in canine oncology trials is to identify and stage the tumors as well as to give guidance on tissue quality, handling, and fixation. The only canine tumor type that requires the pathologist to interpret diagnostic assays for prognostication based on molecular status is cutaneous mast cell tumor. The Veterinary Cancer Society and American College of Veterinary Pathologists has established an Oncology-Pathology Working Group (OPWG), whose primary function is to retrospectively collate per-reviewed literature. In addition, the Comparative Oncology Trials Consortium (COTC) was established by the NIH-NCI in 2004 to stimulate and advance comparative oncology drug development. The COTC PD core is a virtual laboratory composed of member investigators with expertise in pathology, immunohistochemistry, flow cytometry, and pharmacokinetics. The small size and isolation of the OPWG and COTC leave room for variability and missed opportunities. Making them into larger consortiums of multi-disciplinary pathologists and shifting their targets to histological and molecular classification of animal neoplasms would inform the design of translationally relevant canine oncology studies.

There is interest in exploring novel cancer therapies in pets with spontaneous cancers in all 3 phases of clinical trials. The goal of phase 1 is to determine maximum tolerated dose or the biologically effective dose. Goals of phase 2 trials are to identify clinical or biological activity in well-defined patient populations and to inform decisions regarding phase 3 trials. Phase 3 trials are large, randomized, blinded studies designed to compare new drug treatment to standard of care treatments. These are uncommon in veterinary oncology due to size and expense.

Accuracy and standardization in evaluating safety and efficacy is a concern for the comparability of human cancer clinical trials and companion animal studies.  Consensus statements developed by the Veterinary Comparative Oncology Group have standardized response criteria and adverse event reporting for solid tumors and lymphomas. These nearly-universally adopted criteria have significantly improved the safety and efficacy data reported in veterinary clinical trials. Another factor increasing translatability of results from pet animals is that enrolled animals receive the same types of supportive care and symptomatic treatments as humans.

The PHS Policy, IRAC Principles, The Guide, AWA/AWRs, and IACUCs are all part of overseeing laboratory animal research. IACUCs also oversee research conducted with pet animals, but IACUC approval is not necessary for standard veterinary care of animals in context of the VCPR. The Ethical Principles and Guidelines for the Protection of Human Subjects of Research have been used as a foundation for addressing ethical concerns associated with research using client-owned animals. The 3 main tenets are 1) respect (owner is able to make informed choices), 2) beneficence (animals protected and their well-being secured), and 3) justice (study enrollment should be based reasons directly related to the problem being studied). Application of these principles means that the owner must be provided information in an easily understood way so they can give informed consent, they must be informed of alternative treatment options, they should understand how new information will be delivered to them, and the should know how adverse events will be managed and who is financially responsible for that management. Some veterinary institutions have adopted a clinical review board (CRB) to evaluate merit, feasibility, and compliance with ethical standards for clinical trials in client-owned animals in the same manner in which an IRB acts in human studies. The AVMA has also published a policy regarding studies of client-owned animals called the Establishment and Use of Veterinary Clinical Studies Committees (VCSC). Establishing a CRB that is in complete communication with the IACUC is the current “best-practice” recommendation for evaluating research involving client-owned animals.

QUESTIONS (True or False)

1. Phase 2 clinical trials are normally randomized and blinded.

2. Clinical review boards serve as the IACUC overseeing studies involving client-owned animals.

3. Clinical review boards serve the same function as IRBs do with human clinical trials.

4. IACUC approval is not required for standard veterinary care of client-owned animals enrolled in a clinical trial.

ANSWERS

1. False

2. False

3. True

4. True

**Wallace and Trundy. Animal Research Pathology: Regulatory and Safety Considerations, pp. 111-118**

Domain 5: Regulatory Responsibilities

SUMMARY:Animal research pathology includes diverse procedures, animal species, and hazards.  Pathologists have to clearly understand the laboratory procedures/activities performed in their laboratories as well as applicable regulatory and safety requirements in order to properly protect laboratory personnel while ensuring data integrity.  This article provides an overview of regulatory and safety considerations in regard to animal research pathology and emphasizes the importance of clear communication between the PI, pathologist, laboratory personnel, IACUC, and institutional safety officers/experts to mitigate potential personnel exposure to hazardous materials and collection of data that does not meet animal welfare and/or quality control standards.

QUESTIONS

1. Animal research studies that are performed in support of the development of products that will require FDA or EPA approval will require compliance with \_\_\_\_\_\_\_\_\_\_\_\_\_\_.
2. DOT Hazardous Materials Regulations
3. GLP for Nonclinical Laboratory Studies Standard
4. IATA Dangerous Goods Regulations
5. Select Agent Regulations
6. If working with sheep, which of the following agents should be a concern?
7. *Hantavirus*
8. *Macacine herpesvirus 1*
9. *Coxiella burnetti*
10. All of the above
11. If working with deer mice/other wild rodents, which of the following agents should be a concern?
12. *Hantavirus*
13. *Macacine herpesvirus 1*
14. *Coxiella burnetti*
15. None of the above
16. Which of the following requires  a study director?
17. CDC Import permit
18. DOT Hazardous Materials Regulations
19. GLP for Nonclinical Laboratory Studies Standard
20. IATA Dangerous Goods Regulations
21. Which of the following requires that shippers be trained and certified once every 3 years?
22. CDC Import Permit
23. DOT Hazardous Materials Regulations
24. GLP for Nonclinical Laboratory Studies Standard
25. IATA Dangerous Goods Regulations
26. Which of the following requires that shippers be trained and certified every 24 months?
27. CDC Import Permit
28. DOT Hazardous Materials Regulations
29. GLP for Nonclinical Laboratory Studies Standard
30. IATA Dangerous Goods Regulations
31. All general industry use of hazardous chemicals falls under the scope of which of the following?
32. OSHA Hazard Communication Standard
33. DOT Hazardous Materials Regulations
34. IATA Dangerous Goods Regulations
35. Select Agent Regulations
36. Which of the following IS an enforceable regulatory standard?
37. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
38. OSHA Bloodborne Pathogens Standard
39. Guide for the Care and Use of Laboratory Animals
40. CDC/NIH Biosafety in Microbiological and Biomedical Laboratories
41. Which of the following IS the applicable standard for transgenic animal tissues, tissues containing vectors or modified tumors, or chimeric body fluids?
42. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
43. OSHA Bloodborne Pathogens Standard
44. USDA Veterinary Service Permit
45. CDC/NIH Biosafety in Microbiological and Biomedical Laboratories
46. Which of the following IS the applicable standard for tissues and associated body fluids from animals that have been infected with an infectious agent being used under a permit?
47. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
48. OSHA Bloodborne Pathogens Standard
49. USDA Veterinary Service Permit
50. CDC/NIH Biosafety in Microbiological and Biomedical Laboratories

ANSWERS

1. b
2. c
3. a
4. c
5. b
6. d
7. a
8. b
9. a
10. c