

Use of Animal Models for Imaging Studies: The CRO Perspective

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A contract research organization (CRO) is hired to obtain quantitative data according to a Client's specifications. The CRO is an extension of the Client's research and development capabilities. CRO scientists must be able to work closely with the Client to ensure an accurate implementation of the experimental protocol. The physical separation between the CRO and the Client's project team can usually be overcome with modern communication tools. Client confidentiality is a cornerstone of CRO interactions. Concerns around confidentiality and proprietary information can usually be overcome with appropriate legal agreements. The ability to openly share and discuss information around the molecule of interest is critical not only to ensure that the scientific protocol is robust but also to ensure compliance with animal care and use policies and regulatory requirements governing animal use. Finally, the phase of compound development may impact the decision or need to conduct the work in accordance with Good Laboratory Practices.

Working with a CRO

There are several stages to the process of working with a CRO. Below, these are considered for a pre-clinical drug efficacy study that has image-based measurements.

Inquiry. The Client describes his/her requirements at a broad level, providing sufficient information to allow the CRO to evaluate the feasibility of the requested experiments. The CRO discusses the available imaging equipment and expertise with the requested project needs. Validation studies may be presented. It is helpful if the Client has a well thought out draft study design as a framework for discussion or a specific set of questions that the Client wishes to have addressed. Previous study findings, therapeutic target, and other information provided by the Sponsor help to provide context for the study and ensure that specific critical needs for the proposed study are addressed. In this phase, the Client and CRO work together to identify the appropriate imaging modalities and any additional non-imaging endpoints that might be needed.

Proposal. The Client and CRO work together to flesh out the details of the proposed experiments. The goal is to describe the scope of the work so that the CRO can determine study cost, the feasibility of the proposal can be assessed, and required resources can be identified. Various experts within the CRO may be consulted (e.g. veterinary services, animal care, etc.) to ensure all aspects of the proposal have been discussed and addressed. The proposal will include specifics of the animal model, test article formulations and dosing paradigms, image acquisition protocols, and image analysis needs. If requested, additional services (non-imaging endpoints) such as tissue preparation for histopathology are included. The Client's timing needs should be discussed.

Quotation and Award. The CRO provides a formal quote for the proposal, along with certain contractual information. The Client is asked to review the proposal and cost and to authorize proceeding with the work.

Final Protocol. The language of the proposal is made more specific, and the CRO assigns a study number. A study calendar is created. Each of the CRO support functions is notified of the study requirements. A request for approval is submitted to the CRO Institutional Animal Care and Use Committee (IACUC).

Study Conduct. The Client is apprised of study progress. Animal health is monitored, and veterinary evaluation provided. Modifications to the study may be suggested by the Client or the CRO dependent on clinical signs or other interim data, and the study protocol may be amended if authorized by the Client.

Data Transfer / Draft Report. The study protocol dictates how specimens and data are to be either returned to the Sponsor or archived at the CRO. Such data may include animal observations, formulations and dosing information, images, image analysis results and other data such as histopathology findings. A draft report including the data (summary or individual animal data tables) methods and results is written. The data and report are reviewed (quality control) and submitted to the Client for review and approval.

Final Report. The final report has the approval of the Client. For regulated studies (e.g. GLP studies), Quality Assurance reviews the report prior to the report being signed and finalized. Subsequent to final report approval and signature, all information and data are archived at the CRO unless otherwise directed in the protocol.

CRO qualifications

The CRO must be critically evaluated for its ability to provide all of the services required for the proposed study. The Client may visit the CRO site to make this assessment.

Services will likely include the following.

- Animal housing
- Animal care and handling
- Veterinary services
- Drug formulations
- Dosing
- Services to create animal models of disease, such as cell culture and implantation, chemical exposure, diet modification, immunization, or surgery
- Imaging capabilities
- Adjunct services, such as tumor volume measurement, behavioral assessment, measurement of physiological functions, and clinical pathology
- Necropsy
- Tissue preparation and pathology

It is important to review the skills of the personnel who will be performing the aspects of the study. The CRO should be able to provide training records and documentation describing the experience of these persons.

Important considerations for animal imaging laboratories

It can be appreciated that imaging is just one of the services that may be needed for the conduct of a drug efficacy study. Some questions to consider include:

What is the availability of the appropriate modality? Is the available instrumentation capable of addressing the protocol?

Are the imaging systems routinely used for animal studies? Are the imaging systems housed in the vivarium?

Are multiple modalities needed for the study? Are these in proximity to one another, simplifying animal transfer between modalities?

Are the imaging systems equipped for animal maintenance and monitoring during imaging? At a minimum, the imaging system must have a heating system and a means of monitoring animal respiration. Additional options may include monitors for body temperature, blood oxygenation, and heart rate.

Are imaging protocols optimized to allow examination of sufficient numbers of animals for the study within a reasonable time period? Is the acquisition protocol optimized to reduce stress to the animal?

Has the image acquisition and analysis protocol been validated for the pathology? At a minimum, the ability to detect the pathology must be demonstrated. Preferably, the ability to detect a change in the pathology with a benchmark drug should be shown as well.

IACUC issues

In the United States, the Animal Welfare Act of 1966 and its amendments mandated that each institution conducting animal research must create an Institutional Animal Care and Use Committee (IACUC). The IACUC of each institution must review each application for animal use. The specific process is defined by each institution. Therefore, the Client must be made aware of the IACUC requirements at the CRO. Furthermore, it is the responsibility of the CRO study leader to represent the Client during IACUC review. Since it is advisable to review any intended animal procedure with the site veterinary staff, it is incumbent upon the CRO scientist to start this interaction no later than the proposal stages and possibly as early as the inquiry.

At our institution, the IACUC requires that one address several issues. These requests are so specific that it is more likely that the Client scientist can provide the most accurate information. These issues include:

- Rationale for animal use
- Justification of species
- Justification of number of animals to be used

Examples of appropriate responses to each of these are provided in the Appendix.

Appropriate categorization of pain / distress is also necessary for IACUC review. These categories include:

USDA C - No pain or distress is anticipated (other than possible slight / momentary pain or distress). If undue pain or distress is observed, then appropriate veterinary services will be rendered. It is suggested that some explanation be provided as to why this category has been chosen, particularly if this is a range-finding study.

USDA D - More than slight or momentary pain or distress is anticipated which will be managed and/or treated. No alternatives were found to be acceptable. Describe why this category has been chosen, the possible (anticipated) effects, and procedure(s) that will be used to provide relief from pain/distress. If the use of a drug is planned to provide pain/distress relief, then indicate the route, dose level, and dose schedule to be used. If the pain/distress is anticipated to occur as a result of the treatment / test article, provide a reference for that information.

USDA E - More than slight or momentary pain or distress is anticipated which will not be managed or treated due to scientific reasons. Describe why this category has been chosen, the possible or anticipated effects, and clearly indicate the scientific rationale as to why no pain/distress relief will be provided.

Although seemingly innocuous to many imaging scientists, the image acquisition process may be considered USDA D due to the anesthesia procedure. USDA D may be indicated if the duration of anesthesia is long (> 30 minutes for a rodent) or if anesthesia is frequent (weekly). Injectable contrast agent administration may also be considered USDA D if administered frequently (2 times per week) or via a cannula / catheter during the imaging procedure, for example, as for DCE-MRI studies.

Animal model considerations

Open and interactive discussion between the Client and CRO should facilitate a decision on the appropriate animal model. In addition to the variety of transgenic animal models there are a number of disease models leveraging surgical and/or pharmacologic manipulations of standard laboratory animals. Experience of the CRO may provide multiple potential options.

The interaction with a CRO can be streamlined by addressing the following.

Prior knowledge about the animal model. It may be possible that the CRO can obtain the animal model but may have limited experience with it. For example, a certain mouse strain may spontaneously develop tumors. Are these animals sensitive to anesthesia? When and where will the tumors appear? What is the typical structure of these tumors? What is their growth rate?

Licensing agreements. The purchase of a certain animal strain may be limited by licensing agreements. It is possible that a CRO will not obtain a licensing agreement for one-off studies, and a multi-study commitment is needed. An

alternative is for the Client to own a licensing agreement and transfer animals to the CRO.

Transferring animals from the Client to the CRO. Each institution has its own policies regarding receipt of animals from other institutions. Receipt of animals from a Client's vivarium requires that procedures are in place at the CRO vivarium to reduce or eliminate the chance of contamination. These include the use of personal protective equipment (PPE) and adherence to PPE disposal policies.

Decontamination. Imaging systems are shared across multiple animal species.

Procedures must be defined to de-contaminate equipment between studies or even between individual examinations.

Impact of disease progression on timing for image acquisition. A CRO attempts to be flexible, but it is not always possible to schedule imaging sessions at the desired time. For a tumor study, for example, the Client may consider MRI at day 13 post-implant instead of day 14.

Client sponsorship of pilot studies

The CRO may not have a working imaging application for the animal model of interest. However, this should not *a priori* dissuade a Client, because all of the expertise and tools needed to build the application are present. This situation is especially true for studies that include imaging. To address the infrastructure needs, the Client may wish to sponsor a pilot study. The pilot study may include the implementation of an animal model, preparation of an image acquisition protocol, and/or the development of image analysis tools. Following this, a validation study, in which the image-based findings are compared to a benchmark, such as histopathology is warranted. Finally, an efficacy study with a benchmark control compound will demonstrate the power of the imaging application for detection of disease modification.

Appendix. Examples of responses to IACUC requests

Appropriate responses are indicated by the following example. The goal is to conduct a DCE-MRI study of a mouse subcutaneous pancreatic carcinoma xenograft model.

Rationale for animal use:

There is a need for non-invasive measurements that can quantify the efficacy of a drug based on its mechanism. Assays that are translatable from animals to humans are particularly desirable. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is such an assay. It measures parameters that describe the extravasation of a contrast agent in a tumor. An extensive and growing scientific literature, encompassing animal and human studies, indicates that DCE-MRI parameters may be important predictors of response to anti-tumor therapies, especially those that work via anti-angiogenesis and/or vascular disruption mechanisms. Animals are required to allow a controlled evaluation of the *in vivo* DCE-MRI parameters with comparison to tumor structure *ex vivo*.

Justification of species:

The MRI/S Laboratory has implemented the DCE-MRI methodology for several rat and mouse cancer models, but DCE-MRI has not been used to study the mouse BxPC-3 pancreatic carcinoma xenograft model. The Client specifically requested the mouse BxPC-3 xenograft model because 1) it has been used by the Client for their in-house studies and 2) relative to other models examined with DCE-MRI, such as the U87MG flank xenograft, the BxPC-3 xenograft has a slower growth curve which may allow the opportunity to obtain pre- and post-treatment measurements before necrosis occurs.

Justification for number of animals to be used:

Growth rates of individual pancreatic carcinomas are not anticipated to be equivalent. The study requested by the Client aims to have 8 tumors with volumes of $\sim 200 \text{ mm}^3$ for imaging; 16 animals should guarantee this.