“Special Considerations for Laboratory Research”

2014 RSNA - WRITING A COMPETITIVE GRANT PROPOSAL
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Special Considerations for Wet Labs

• With the cut backs in NIH funds and Imaging Center Grants (i.e. ICMIC P50, P41s, etc.) getting the funds to purchase needed equipment to fully create a functional free standing laboratory for an individual PI/group is exceedingly difficult and requires multiple individual grants over a period of several years.

• Need now more than ever to develop multi-disciplinary intramural collaborations.

• “It is better to be a slave in heaven than the master of hell.” (a deliberate misquote from Dante’s Inferno)
Special Considerations for Wet Labs

• It is clear the most core imaging facilities and surgical suites, GMP labeling / chemical processing, gene or RNA chip scanning, FACS, HPLCs, lyophilizing equipment, cold high speed centrifuges, etc. will need to be supported as in the old days by individual investigator R01s, R21, shared imaging and equipment grants for both the purchase and maintenance contracts.

• This puts a premium in developing collaborations and being part of a larger center and will make your chances of getting a grant greater than doing it on your own as in the days of old.

• Wet labs, small animal imaging, surgery contrast and radiotracer development, etc. require the consideration a number of unique factors when writing an NIH or other type of government grant including:

  • Wet labs- need actual assigned space at your place of work. Need some access to sinks, storage space, freezer space and work benches that you can mention in your resource and facilities pages of the proposal.

  • For wet labs- need to determine if you need a fume hood to store and use volatile chemicals and solvents for these such as H & E and special staining
• For wet labs – working with radio-isotopes need space zoned and approved by the institution and state regulatory agencies before you apply for a grant. Also need to be sure you can have a leaded pit for handling and radiolabeling along with a dosimeter. Access to a cryo-microtome (or purchase) that is zoned for animal use and radiotracers is helpful even one does not plan on doing autoradiography.

• For wet labs – working with DNA, RNA, viruses, human samples, blood need to be approved by your local IRB and for the machines and equipment that is planned to be used before you apply for a grant.

General Considerations

Need to think about what specific pieces of equipment that are essential to have totally under your control and therefore can be justified as part of your NIH budget (i.e. at least an individual investigator R01).

Examples - a cryo-microstat that can be used to cut sections for autoradiography.

Freezers to store decaying samples

Biohazards including infectious agents that can only be studied in a limited number instruments
General Considerations

• Costs per hour for use of facilities equipment

• Usually senior investigators will cut young PIs some slack on price and scheduling time on the equipment.

• NIH is far more likely to fund use of key facilities or equipment rather than purchasing big ticket items that usually will go under utilized if used by just one lab. (more the merrier approach).

General Considerations

• Unique expensive instruments or equipment (ie small bore MR, microPET, SPECT/CT) are usually funded by equipment centered grants submitted by a group of investigators or collaborators.

• Center grants in general are on the way out and most imaging centers or core imaging facilities will increasingly rely on individual R01s on member faculty to fund not only the purchase but the service contracts (frequently 10 to 15% of the purchase price annually and needs to be included in the costs of you NIH budget).
• The days of small or larger companies loaning or giving the use of specialized equipment to a University with little or no costs, contracts, or other strings attached are essentially over because of the general new awareness of conflicts of interest and kick-backs etc, by academic institutions (not the companies themselves. They are predictable as the just want to make money any legal way possible.......)

General Considerations

The setting up of a successful wet lab etc. really requires multiple overlapping collaborators usually within the same institution especially for your PIs without national connections.

If there is a core facility or access to someone else’s lab for things such as; sterile fume hoods, cell culture (CO₂ for incubation), nitrogen storage units (freezing of cells) small animal imaging (IVUS, SPECT, CT, PET, US) which are too expensive for one investigator to buy on their own (>150 K).
General Considerations

The setting up of a successful wet lab etc. really also requires ideally an experienced or at least very talented full time post-Doc or better an RA or research line faculty to make the trains run on time and to keep consistency in both protocols, procedures, surgeries etc, especially when there a frequent change (ie every 1 to 3 years) depending on the post-Doc, trainee.

General Considerations

Need enough space for storage of samples etc (usually chemicals and freezers) and pits dosimeters.

If SPECT/CT does radiation effect other pieces of equipment (usually institutional architects will pick up zoning requirements)

Need to work with others but have to prevent open access as even the best post-Doc can really mess up your equipment
• For Wet labs – need all planned animal use experiments, surgeries, imaging protocols, pre-approval with an IRB animal subjects certification number (s). Can attach the finer details of your specific animal protocol(s) as separate files that don’t count against your page limits.

• Fill out the five points for animal use and justification of requested number of mice

• An example.

• Ready access to core facilities needed for the grant proposal based on actual proximity, membership in the controlling consortium group running the core facility (i.e. MIPS for Stanford).

• Institution certification of use of radioactivity biohazards etc. especially infectious agents when considering larger animal studies in clinical sites (i.e. imaging monkeys, pigs, rabbits) with MR, CT, PET equipment (usually after hours)
• The five points are as follows: If an award is made, prior to the involvement of animals the grantee must submit to the NIH awarding office detailed information as required in points 1-5 above and verification of IACUC approval. If the grantee does not have an Animal Welfare Assurance then an appropriate Assurance will be required (See Part III, Section 2.2 Vertebrate Animals for more information).

• 1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

• 2. Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

• 3. Provide information on the veterinary care of the animals involved.

• 4. Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

• 5. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.
• **Vertebrate Animals (example)**

• **1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

• Murine/tumor models for mammary fat pad (MFP, orthotopic) tumor experiments in Specific Aims 1 & 2.

• ….have used the …..models extensively …. for radiotherapy with scVEGF/177Lu, VEGF receptor imaging and testing of anti-angiogenic drug therapy....

• …..tumor bearing mice with signs of significant lethargy, weight loss (> 20% of baseline) or distress or a measured tumor volume of 2000 mm$^3$ or more will be euthanized via CO$_2$ inhalation and record as a tumor related death....

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**Statistical Justification**

…….expect tumor uptake of scVEGF/Tc (for SPECT in Specific Aims 1 & 2) to vary by a factor of 2 to 3 fold (or higher).........

…….wish to have a standardized effect size ..............

..........of 1.5 ( = \(\mu_1-\mu_0\)/\(\sigma\); (now can get with standard statistical software around $1000 to $1500, worth buying).

........\(\mu_1\) = mean of angiogenic rim,.... \(\mu_0\) = mean, surrounding soft tissue uptake....\(\sigma\) = variance

..... with a power of at least 80% for pair-wise one sided two sample t-test............... 

..........assuming a 25% variance for both tumor and adjacent soft tissue and a p-value of 0.05 ...

...need a minimum of 5 animals for each subgroup.
Specific Aim #1 (Year 1) “Optimize the tolerability and effectiveness of scVEGF/177Lu therapy in orthotopic breast tumor mouse models ... with repeat dosing delivered at the time of re-emergence of VEGFR-2 expression, as seen by serial scVEGF/99mTc SPECT imaging.”

(Total = 220, ... tumor bearing SCID mice & 150, ... tumor bearing Balb/c mice)

I) Time Course Experiments for Dose Timing

Group 1 (slower growing tumor).... mice
a) After 1st dose...(n=5) re-imaged and euthanized @ 3 ... 28 Days; n=30
b) After 2nd dose...(n=5) re-imaged and euthanized @ 3 ... 28 Days; n=30
c) After 3rd dose....(n=5) re-imaged and euthanized @ 3 ... 28 Days; n=30
d) Untreated controls; (n=5) re-imaged and euthanized @ 3....28 Days; n=30

Group 2 (faster growing tumor).... mice
a) After 1st dose....(n=5) re-imaged and euthanized @ 3 ... 28 Days; n=25
b) After 2nd dose....(n=5) re-imaged and euthanized @ 3 ... 28 Days; n=25
c) Untreated controls; (n=5) re-imaged and euthanized @ 3 ... 28 Days; n=25

Subtotals = 120 - .../SCID mice + 75 - ..../Balb/c mice

• 2. “Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.”

• Animal studies are required ....to extensive study the biology of scVEGF/177Lu, its relationship to scVEGF receptor expression...... ......over time; experiments that cannot be done in human subjects. ...

• Factors controlling radiotherapy with cVEGF/177Lu and their relationship to VEGF receptors and tumor margins can only be determined by in vivo studies

• ......effects of drugs and their combination with systemic radiotherapy with scVEGF/177Lu on tumor growth rate, etc......overall survival can only be done in tumor bearing mice as outlined in our survival studies.
• 2. Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

• Mice are the most appropriate species for these studies because they are relatively inexpensive to purchase and house and can be readily dissected for biodistribution, multi-modality imaging and other ex vivo imaging modalities. ………

• The number of mice to be used for each set of experiments is the minimum necessary to achieve statistically valid results (n = 5 per group for biodistribution, IHC and imaging assays, n = 25 per group for survival studies in which there may be a anesthesia related death rate of 20 to 30 % due to serial weekly imaging experiments).

• 3. Provide information on the veterinary care of the animals involved.

• Experienced research-oriented veterinarian support is available through the Stanford Department of Laboratory Animal Medicine (DLAM), including focused veterinary medical questions and assistance in designing and carrying out research protocols. ………

• ………AAALAC-approved Research Animal Facility, where the chronic animals will be housed, is staffed 24 hr daily by experienced research animal technicians in the ICU, is equipped with individual examination and treatment rooms, and is fully supported by a complete veterinary laboratory and x-ray facility...

• … All invasive procedures as well as imaging sequences will be performed under general anesthesia……..
• 3. Provide information on the veterinary care of the animals involved.

• All animals will receive humane care in compliance with the policies of Stanford’s Division of Laboratory Animal Medicine (DLAM), and “Principals of Laboratory Animal Care” formulated by the National Society for Medical Research, The Guide for Care and Use of Laboratory Animals” prepared for the National Academy of Sciences and published by the National Institutes of Health DHEW (NIH) Publication 85-23, revised 1985)

• Each of the multiple experimental protocols requiring the use of animals is reviewed annually and approved by the Stanford Laboratory Animal Review Committee and will be conducted according to University regulations.

• 4. Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

• Any animal that experience signs of distress (i.e. weight loss, lethargy, pain associated behaviors such as hunching, limited motion from bulky tumor, tremor, or primary tumor size of 2000 mm³ or greater) will be euthanized via CO₂ inhalation with the death recorded as due to tumor....

• Mice with implanted primary mammary tumor will undergo every other day measurements of primary tumor (three times per week) with calipers and recorded.
• 5. **Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia. If not, include a scientific justification for not following the recommendations.**

• 5. After completion of an experiment or if animal is found in a moribund state (with a tumor of 2000 mm³ size or greater), animals will be euthanized using inhalation of CO₂ (approved by Stanford Comparative Medicine). This method provides a quick painless euthanasia that will not interfere with the validity of biodistribution measurements or length of survival in treated tumor bearing mice. This method of euthanasia is also consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

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**Summary**

• **Be part of a group prior to and after getting an award. You need to be planning your next grant at least one year prior to the end of any current grants.**

• While in the past one could was expected to create their own lab and stock with their own equipment and facilities with the falling off of funding for center grants for imaging is no longer realistic.

• **Get all the approvals and certifications and completely work through all animal, biohazard protocols, prior to grant submission.**