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We use this protocol and it's working

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Initiating and Performing in vivo experiments of anti-cancer treatments in mice (General)

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ABSTRACT

This protocol describes our approach to setting up and designing experiments to study the effects of a cancer treatment in mice.

A. Design

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- What question(s) will this experiment answer?
- Before initiating a large experiment, consider performing a pilot study with fewer animals to prove/test concept.
- Consider working with a statistician using power and sample size calculations.
- Determine:

1.1 Species and strain of animal that will answer research question e.g mouse - BALB/c Nude, SCID etc. Mouse, rat and other animals are bred specifically to answer different research questions.

1.2 The number of study groups or arms as well as what each will be e.g 4 groups: Control, Drug, Radiation, Drug + Radiation.

1.3 What anatomical/regional sites are of interest to the study e.g bilateral flank.

1.4 Number of animals needed per group e.g 12 athymic mice/treatment group. This would include the number of mice to be monitored during the entire study plus mice needed to be euthanized at one or multiple time points (usually 8 hours, 24 hours, 48 hours) for tissue collection.

1.5 Will this experiment require an early sacrifice of mice, what samples need to be collected and how many? e.g early sac done after first 48 hours of treatment, 4 tumors collected/group – collect samples to make paraffin blocks and fast frozen samples to extract DNA.

1.6 The duration of the experiment e.g 7, 14 or 21 days of treatment.

1.7 How many times a day each treatment will be delivered and how many days a week e.g Drug delivery is 2x/day/5 days/week.

1.8 Route of drug or treatment delivery e.g Cetuximab administered via intraperitoneal injection, most of the other drugs given as oral gavage.

1.9 For drug or radiation – determine dose/volume to be given. Radiation is usually given at least an hour after drug treatment.

1.10 What metrics will be evaluated to determine success or failure of treatment and how will they be measured? E.g drug treatment in mice with bilateral flank tumors will be evaluated based on size of tumors throughout the treatment period. Size will be measured with calipers.

1.11 **Once all of the above questions have been answered, set up for the experiment can begin**

B. Set Up

2 All experiments must be performed under an approved IRB protocol by research staff trained to handle animals and perform experimental procedures. Academic institutions will require and provide training programs to meet animal training requirements.

3 Order animal model in number required for experiment (note: it is prudent to order a few more animals (3-4) than what is required in case of poor growth/uptake or animal death)

4 Ensure that experimenter has access to required animal facilities and is trained on how to reserve work areas in these facilities and how to use any equipment in these areas e.g irradiator, anesthesia equipment, etc.

5 Ensure that there is enough starting material (animal tissue, cell lines or other) to initiate experiment. e.g bilateral flank injections into 40 mice would be 80 injections total and would require, in the case of a PDX experiment, about 2-3g of tissue to inject all experimental mice.

6 In addition to starting material – ensure enough media and supplies are available e.g Matrigel and culture media, in the case of PDX or cell line injections.

7 If drug is to be administered to the mice for treatment – determine amount of drug needed in advance – order drug in appropriate quantity and allow for shipping time. Also, confirm the vehicle solution to be used to make the working stock of the drug. This solution will be administered in mice under the Control arm.

8 Inject/implant PDX/cell line/other into animal model to initiate experiment.

9 While waiting for the animals to become ready for treatment – set up experiment documentation that will be used to track and document all work done for experiment. This should include:

a.Treatment schedule
b.Table displaying treatment, treatment routes, treatment dose, treatment volume, dose calculations
c.Randomization
d.Tumor measurement (or the metric of interest in the study)
e.Tumor volume
f.Animal weights
g.Animal identifier (example- ear tag number)

10 **Another experimenter should be able to view the document and data and recreate the experiment based on the information provided**.

C. Experiment

11 Once the animals have been injected or implanted, monitor at least twice a week until they are

ready to be treated – this varies by experiment but in the case of experiments involving bilateral tumors on mouse flanks, treatment is often begun when the tumor average is 150-200mm^3.

12 On reaching at the initiation point (established during design phase) randomize animals into groups based on a final (pre-treatment) tumor measurement. In the case of bilateral flank tumors – the tumor volume of each tumor on a single mouse is averaged. This average is used to move the mouse into a treatment group. The goal is to have a similar tumor average/group.

13 Separate mice into their respective groups by whatever identifier has been chosen e.g ear tag, ear punch etc. (Note: this process can take time – especially with many animals – allow two hours for moving animals into respective cages and marking the cages).

14 Once the mice have been randomized, begin treatment of animals based on the schedule created in the design phase.

15 Perform an early sacrifice – if decided in the design phase. Collect appropriate tissue samples.

16 Take metrics of growth at least twice a week (if that is the interest of the study) e.g measure tumors and weigh mice 2x a week – Monday and Thursday

17 1. Once treatment is complete, decide if it would benefit the experiment to sacrifice the animals immediately and take samples or delay sacrifice and continue to take measurements.

D. Euthanasia and Sample Collection

18 Euthanize animals according to approved protocols at experimenters institution. e.g CO2 euthanasia followed by cervical dislocation.

19 Collect samples as determined by the design phase of experiment.

20 It is important to properly label and record every sample collected and their banked location.