Guidance for Justification of Animal Numbers

Paragraph 14C - Justification for Animal Numbers

With reference to the examples below, please select the most appropriate line diagram to present and justify the number of animals used for the experiments. Always include a power calculation, or at least an estimate, or indicate whether this is a pilot study where one study objective is to assess the variation readouts/endpoints.
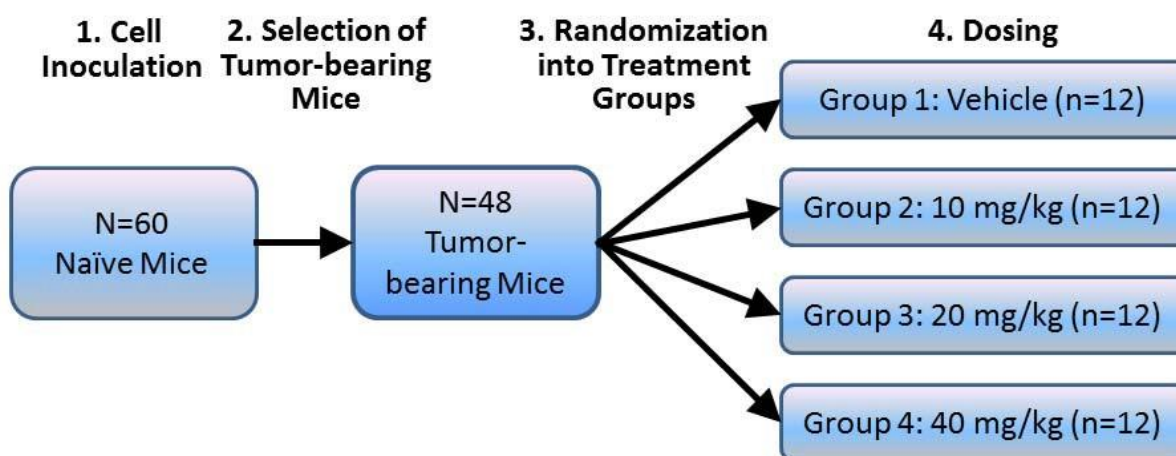
Example 1: Establish the dose dependency of anti-tumor activity of compound X in a standard subcutaneous xenograft model.

Power Calculation:
We want to pick up a 20% decrease of tumor size ("effect size"), with a 95% confidence interval and 90% power. Therefore, we need a group size of n=12 animals/group (calculated using a simple online power calculation tool, see resources below).

Required animal number per study:
To establish a dose-effect relationship, a minimum of 3 dose levels is required, plus a vehicle control group. Therefore, we need 4 groups, or a total of 48 tumor-bearing mice. From previous studies, we can estimate that the take rate of the tumor model is >80%, the required number of animals will be 48/08 = 60, we need to inoculate 60 mice per study (schematic below).

Online tools & applications for power calculations:

- https://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx
- http://hedwig.mgh.harvard.edu/sample_size/size.html

- http://www.softpedia.com/get/Science-CAD/G-Power.shtml

Free publications on statistical aspects of animal studies:

- http://www.researchgate.net/publication/224889585_In_Vivo_Assay_Guidelines
- http://www.3rs-reduction.co.uk/assets/applets/Dell_et_al_v4304Dell.pdf
- http://www.bu.edu/orccommittees/iacuc/policies-and-guidelines/sample-size-calculations/
- http://www.jpharmacol.com/temp/JPharmacolPharmacother44303-8564016_234720.pdf

Example 2: Inter-breeding of heterozygotes to generate homozygote animals

Production of experimental cohorts of transgenic animals often requires a lot of breeding to generate enough animals with the desired genotype. All of the animals produced during breeding, not just the animals used for experiments, must be included in the IACUC application. The below is an example of a crossing between HET and HET to generate HOMO animals for experiment and using WT animals as control:

HET x HET = WT (25%) + HET (50%) + HOMO (25%)

Assume 12 HOMO animals and the same number of WT littermates are needed for the experiment and each litter produces 8 pups on average:

HET (6) x HET (6) = WT (12) + HET (24) + HOMO (12)

While the WT pups can be used as control animals, the HET is unwanted genotype for the experiment. Nonetheless, **all the HETs must be counted in the total animal number** though they will be euthanized immediately after genotype is determined. In addition, when dealing with small numbers, Mendelian distribution ratio may not be precise, therefore, a correction factor of 20-30% should be included.

Therefore, the total number: [12 (WT) + 24 (HET) + 12 (HOMO)] x 125% + 12 (Parents) = 72 animals (if multiple litters to be produced from the same pair of parents, the number of parents should be calculated accordingly).