Draft UBC ACC Guidelines for Cancer Researchers Using Animal Tumour Models

Preface: In our quest to develop better strategies for the prevention and treatment of cancer, small animal models are frequently used to collect information about tumour cell biology, tumour physiology as well as treatment responses. Animal models have been criticized due their limited ability to predict tumour behaviour in patients. However, when used in a manner that allows researchers to link molecular genetics and biochemistry information with information about tumour pathology/physiology and, in cases where anticancer drugs are being studied, information about the fate of the drug (s) after administration, these models are very powerful and important predictive tools. One of the best predictors of therapeutic effects in humans is demonstrable activity in multiple tumour models. In addition to standard models based on subcutaneous injection of tumour cells to initiate development of a solid tumour, more sophisticated models are also being developed. Tumour cells can be injected in a manner that allows the cells to grow in their natural host tissue (e.g. breast cancer cells grown in breast tissue; lung cancer cells grown in lung tissue) and these are referred to as orthotopic models. Other models assess metastatic disease where the tumour cells grow in various sites such as the brain, liver and bone and this can be achieved by use of special routes of tumour cell inoculation (intracardiac, intrafemur, foot pad) as well as by allowing a solid tumour to progress to a size where metastases have occurred. Finally, investigators are developing transgenic mouse strains, where the genetic manipulation results in an increased potential for the mouse to develop cancer at some time during their lifetime. These new and better models are difficult to monitor, hence there is a need to continually reassess methods used to evaluate monitoring and endpoints used in these animal models so that the animals suffer as little as possible and that the basic tenants of the Canadian Council on Animal Care are met. These tenants would include replacement (the more we understand about the relationship between in vitro models of cancer and in vivo models the more likely that in vitro models will replace some in vivo models), refinement (ensure that researchers are collecting as much information as possible from the animal models being used) and reduction (ensure that the least number of animals are used to achieve the experimental objective).

CCAC Guidelines: The current CCAC guidelines indicate that for cancer research the experiment must be designed with endpoints that minimize the potential for pain and/or distress. In general terms the CCAC *recommends* that tumour mass or tumour progression should not interfere with normal body functions, cause pain, or distress. These parameters are further defined by:

- Weight loss should not exceed 20%, a value determined based on the animals weight prior to study initiation minus the weight of the tumour bearing animal less the weight of the tumour.

- A calibration curve should be provided to link the investigators method for calculating tumour size (typically calibre methods measuring width and length) to actual measured weights (calibrated scale).
- Tumour burden should not exceed 5% of the animal's normal weight for studies not involved in therapeutic assessments. This would be a 1 gm tumour for most murine experiments.
- Tumour burden should not exceed 10% of the animal's normal weight when the study involves a therapeutic endpoint. This would be a 2 gm (length 17mm, width 17mm) tumour for mice or a 25 gm (length 17mm, width 17mm) tumour for rats.
- Animals with ulceration or infected tumours must be terminated.
- Animals with local solid tumours that have invaded surrounding tissues should be terminated.
- Animals that persistently cause self-induced trauma to the tumours should be terminated.
- Survival can not be used as an endpoint to define tumour progression and alternative endpoints (tumour induced weight loss, tumour growth delay, assessment of log cell kill, clonogenic assay following tumour excision, histopathological endpoints)

UBC Animal Care Committee guidelines: In an effort to address the changing needs of cancer care researchers that are using and developing better predictive animal models[1] of human disease, the committee *recommends*:

- All staff involved in studies involving animal tumour models should be familiar with the model and the experimental objectives. The decision-making process should be clearly defined so that under all circumstances appropriate actions are taken promptly in order to minimize pain and distress which are a natural consequence of tumour progression and/or treatment.
- A comprehensive assessment of the animal's health status should be completed daily for all animals inoculated with tumour cells[2], regardless of route of administration. This should include but is not necessarily limited to, measurements of weight loss, activity,

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[1] *Definitions: The model descriptions are designed to be general and not necessarily scientifically accurate. For instance, the metastatic model examples are not true metastatic models, rather they refer to models where the tumour is not visible by eye.*

[2] *Tumour cell lines being used must be specified in the protocol and the characteristics of the tumours arising from each specific cell line should be described by the investigator. If growth attributes and tumour characteristics are not known then the investigator should submit a pilot study to define this as well as the tumour growth attributes and calibration curves recommended below. Contamination of tumour cell lines with viruses or other microorganisms may compromise experimental results, cause illness in other animals and may be a health risk to animal care staff. Screening of cell lines for specified viruses is recommended for this reason.*

appearance and hydration. A health status score that would result in the immediate termination of the animal must be provided. A health status score indicative of very poor health, but not poor enough to result in termination of the animal must be available.[3] Unless otherwise indicated animals should be euthanized prior to predictable death occurs, before the animal is in poor condition or the tumour mass is too large or ulcerated (see below).

- For studies involving solid tumours[4], a calibration curve must be available to link the investigators method for calculating tumour size (typically calibre methods measuring tumour width and length) to actual measured weights (calibrated scale).[5] Investigators could consider use of an alternative endpoint that does not involve tumour progression, e.g. clonogenic assay. Tumour implantation on the back or in the flank are considered to be less invasive. Implantation in the footpad, tail or eye should be avoided.
- For studies involving metastatic disease[6] or disease that can not be readily evaluated by simple observation, a calibration curve linking cell inoculation number to health status over time must be available.
- For studies involving non-invasive imaging methods (e.g. Xenogen's IVIS methods, MRI, SPEC, or PET), a calibration curve linking cell inoculation number to health status over time must be available as well as a calibration of obtained image to disease burden.
- For studies involving transgenic models that develop tumours over time, a calibration curve linking health status over time must be available.
- For studies involving tumours induced by carcinogens or viruses, the time and location of tumour development may be difficult to predict,

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[3] *All animals included within an experimental protocol must be monitored at least once per day. When animals have been inoculated with tumour cells, then they must be considered as part of an experimental protocol and daily monitoring sheets should be completed. When the animal begins to show signs of stress, whether due to tumour development or therapy, then monitoring should be done at least 2-times per day.*

[4] *Solid tumour - includes tumours that are visible by eye and would include those that arise following subcutaneous, intradermal or mammary fat pad injection of tumour cells. In addition solid tumours may arise at the site of an intraperitoneal or intravenous injection and this should be indicated in the protocol.*

[5] *Animal species: the strain of animal to be used in the study must be specified as growth attributes of tumours varying depending on animal strain used. If growth attributes and tumour characteristics are not known then the investigator should submit a pilot study to define this as well as the tumour growth attributes and calibration curves recommended below.*

[6] *Metastatic model – includes models where tumours grow in more than one site, typically in tissues that are not easily evaluated. In the context of the recommendations provided below, metastatic models would include those orthotopic models where tumour growth occurs in tissues that are not measurable by eye. In the context of the recommendations provided below, metastatic models would include transgenic models where tumour growth occurs in tissues that are not measurable by eye.*

therefore these animals must be monitored frequently for signs of tumour associated disease.

- No precise guide can be provided as to the maximum allowable tumor burden. Adverse events associated with tumour growth are dependent on many variables including the cell line used, the site of implantation and, where applied, treatment effects. For solid tumour models the tumour burden should not exceed 5% of the animals measured weight for all studies. This would be a 1 gm (13-15 mm in diameter) tumour for most murine experiments and a 12.5 gm (28-30 mm in diameter) tumour for rats. If an investigator wishes to allow tumours to progress beyond 5% of the animals measured weight then justification must be provided. This justification must indicate why alternative endpoints of tumour progression can not be used. An example of a reason that *may* be justified by the UBC ACC is where large tumour mass is required to achieve metastatic spread of the disease.

- For solid tumour models, animals with ulceration or infected tumours should be terminated. If ulceration is an important consequence of therapy and/or tumour development leading to metastasis then justification must be provided. This justification must indicate why alternative endpoints of tumour progression can not be used. In addition the investigator must define strategies that will be used to mitigate against pain, infection, self-inflicted injury or mate-inflicted injury.

- For solid tumour models, animals with local solid tumours that have invaded surrounding tissues should be terminated. If local invasion is an important consequence of therapy and or tumour development leading to metastasis then justification must be provided. This justification must indicate why alternative endpoints of tumour progression can not be used. In addition the investigator must define strategies that will be used to mitigate against pain, infection, self-inflicted injury or mate-inflicted injury.

- For metastatic models, survival can not be used as an endpoint to define tumour progression. The investigator must provide alternative endpoints (tumour induced weight loss, tumour growth delay, assessment of log cell kill, clonogenic assay following tumour excision, histopathological endpoints) that will be used to assess tumour growth and/or therapeutic response. It is recommended that, where possible, non-invasive methods are used to monitor tumour development in metastatic models.

- For transgenic models, survival can not be used as an endpoint to define tumour progression. The investigator must provide alternative endpoints (tumour induced weight loss, clonogenic assay following tumour excision, histopathological endpoints, biochemical endpoints)

that will be used to assess tumour growth and/or therapeutic response. It is recommended that, where possible, non-invasive methods are used to monitor tumour development in transgenic models.

- Animals that persistently cause self-induced trauma to the tumours should be terminated. Animals where mate-induced trauma to the tumour is observed should result in termination of the animal with the affected tumour.