WIS SOP for Rodents with Ulcerated Subcutaneous Tumors: Protocol Requirements, Monitoring, Managing and Humane Endpoints

*This protocol was adapted to WIS from UBC

Background and Purpose

National regulations currently recommends that ulceration of subcutaneous tumors should be considered a humane endpoint (2005). Traditional concerns for keeping research animals with ulcers are related to the impact on animal welfare and data validity (e.g. changes to tumor growth patterns once ulcers form).

The Weizmann institute of Science follows this guideline but recognizes that scientific understanding of tumor models has progressed in recent years and there are exceptional circumstances where ulcers could be scientifically justified.
These guidelines are intended to provide general guidance to WIS reviewers, veterinarians, researchers and animal care staff in cases of a specific approval of applications with ulcerated tumors.
The guidelines will provide information on:
1) How to plan and evaluate protocols that request permission to keep animals with ulcerated tumors.
2) Pathology of ulcers.
3) Appropriate monitoring and care of animals with ulcerated tumors.
4) Appropriate humane endpoints.

Evaluation and Planning of Protocols

Justification for keeping animals with ulcerated tumors must be evaluated on a model-dependent basis, according to the submitted IACUC application.
Reviewers should look for evidence that every effort has been made to choose models that are not prone to ulceration. Where possible, when ulceration is characteristic of the tumor line, the aim of the study should be to complete the experiment in the latent period before ulceration.

If ulcers are justified then researchers must make every effort to analyze data in a timely manner to ensure that experimental endpoints for animals with ulcerated tumors are reached as early as possible.

See “Monitoring”, “Management” and “Humane Endpoints” (below) for specific criteria that must be included in the protocol.

Potential Scientific Rationale and Justification for Keeping Animals with Ulcerated Tumors

1. In order to better model human cancer, allowing ulcerated tumors may be important.

2. Some research areas e.g. immunotherapy, target cancer in its later stages which often present with ulcerated tumors.

3. Ulcerated tumors may heal with drug therapy and therefore if animals are pre-maturely euthanized one cannot know these results.

4. Some models are prone to ulcerate prior to completion of data collection.

When submitting an application to the WIS IACUC, details on monitoring and record sheets are required. Justification for keeping animals with ulcers must be included.

Progress Reports

When protocols are renewed, information should be provided regarding the welfare of animals with ulcerated tumors, as well as whether keeping animals with ulcerated tumors turned out to be scientifically warranted. For example, was the success of the study dependent on keeping animals with ulcerated tumors, what welfare issues arose, what percentages of animals had ulcers etc.?

Monitoring and Welfare

Major animal welfare concerns related to using research animals with ulcers include concerns for:
1. Loss of body fluid
2. Infection
3. Pain and discomfort experienced by the animal

Criteria for Monitoring:
Monitoring ulcers will depend on: a) what is already known about the tumor model and ulcer development in the species under study and b) the type of experimental treatment.
These criteria must be defined and approved in the protocol.

See example of monitoring sheet (Table 1) for information that should be collected and Grading Sheet (Table 2) for examples of how to grade animal with ulcers.

Features Important to Monitoring:

• **Size of ulcer** – Ulcerated tumors should be monitored on a daily basis, for size and general condition (wellbeing). This must be documented. Changes in ulcer size (area and depth) should be documented as well. Ulcers that enlarge fast (e.g. daily) should be monitored more frequently. Larger ulcers will increase the risk of fluid loss, infection and pain. Thus, increased monitoring is critical.

• **Evidence of healing** – Extensive ulcers that persist without evidence of healing should be considered grounds for humane endpoint.

• **Discharge (amount, type)** – Open ulcers with discharge are at risk of infection and animals are at risk of dehydration. Typically, wet discharge indicates that damage to the skin is acute and active with minimal or no healing. The nature of the discharge can be assessed visually, however this can be challenging because rodents groom away discharge, fur may cover the area, and wet discharge will dry. Evidence of wet discharge includes: wet appearance and surrounding fur that is discolored and clumped. Assessments of discharge along with signs of general health of the animal are critical.

o Type of discharge: blood, purulent (pus), clear (serum). Blood loss may result in anemia so animals may show pale extremities and may display signs consistent with systemic illness (e.g. lethargy and poor grooming). Note that in some cancer models anemia of cancer is an expected outcome. Pus would indicate local infection with risk of systemic infection.

o Fluid loss from discharge may result in dehydration (skin tent > 2 seconds and sunken and dry eyes). The degree of dehydration should be monitored.

o Larger volumes of discharge (e.g. from cratered ulcers) with longer duration of active discharge put animals at risk of developing anemia or dehydration. These animals would be considered to be at a greater risk of declining health than animals with dry, shallower ulcers.

• **Signs of inflammation surrounding the ulcer** – Signs include redness and swelling around the tumor and ulcer.

• **Evidence of self-mutilation** – This may appear as scratches or gouges into and around the tumor. In addition you may observe the animal licking or scratching but this typically requires a longer period of observation. These signs indicate that the animal is feeling some discomfort (pain or irritation). In these cases, animals must be monitored for signs of pain.

• **Signs of Pain** – There is some consensus that ulcerated tumors are painful in humans. Some tumors, such as those grown in sensitive areas or that develop extensive necrosis, may be painful. In some studies, the treatment of pain may not be permitted because of concerns for the effect of the analgesia on data, but at this point it would be useful to monitor for pain and to consider testing the effects of analgesia. Where permitted, analgesia should be administered when behavioral signs of pain are present.

• **Overall Health and Welfare** – It is essential to take into account the overall condition of the animal (e.g. clinical health score). Clinical health should include behavior, appearance, dehydration, body weight, tumor burden and signs of pain. General health should be monitored throughout the study so that potential effect of ulceration can be tracked. If the clinical health of animal declines, the frequency of monitoring should increase or euthanasia should be considered.

Table 1. Example of monitoring record sheet specific to ulcers:

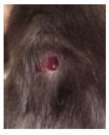
Date	Area (mm)*	Depth (mm) or What is visible	% of Tumour Surface Area Ulcerated*	Discharge	Appearance	Self-Mutilation or Scratching	Overall Health (score)	Photo	Other
Dec 10, 2016	2 x 4	1	5	Dry	Scab	No	0		
Dec 15, 2016	3 x 7	Tumour tissue visible	10	Wet, red	Cratered	No	2		

Table 2. Example of Grading Sheet:

	Score			
Signs	**2**	**3**	**4**	**5 (Humane Endpoint) ***
% Tumor surface area Red/dark area = ulcer on tumour Number = % area	< 5 	5 - 9 	10 - 15 	>20 
Discharge	None, but ulcer likely	Dry, scab	Wet: blood; signs of infection (controlled by treatment)	Wet: Pus (infection) that has not responded to treatment for 3 days. Fresh blood with signs of anemia and/or dehydration. Fresh blood in known vascular tumour. Clear discharge with signs of dehydration with no resolution in 3 days.
Depth	< 1 mm	1-2 mm	3-4 mm, cratered	> 4 mm, tumour tissue visible or loss of tumour tissue.
Signs of inflammation in surrounding area	Mild hair loss	Skin red, hair loss	Skin red and swollen	Skin severely damaged (infected, necrotic) or open to muscle or bone.
Scratching	Possible scratching but not observed	Occasional scratching	Frequent scratching	Evidence of severe self-mutilation and/or constant scratching (even when disturbed) with no resolution with treatment in 3 days.
Behaviour	Slightly slow moving; still interested in environment.	Less interested in the environment, interacts less with cage mates, disregards observer. Occasional abnormal gait e.g. limping or "tip-toed" gait.	Isolated from cage mates, minimally active; does not readily move when cage disturbed. When nudged, reluctantly moves. Frequent limping or "tip-toed" gait.	Immobile or hyperactive; not moving when nudged; animal cannot right itself.
Appearance	Piloerection/ruffled (< 10% of body).	Piloerection (25% of body e.g. base of neck) and dull fur (not shiny or smooth). Slight hunching in back.	Piloerection (50% of body), matted and un-groomed. Whiskers barbered. Severe hunching in back, even when walking.	Piloerection (>75% of body), matted and un-groomed with other severe signs of illness.
Dehydration	Mildly sunken eyes (appear >75% open).	Skin tent > 2 seconds (decrease in skin elasticity). Sunken eyes (appear half closed).	Skin tent > 5 seconds. Completely closed or severely sunken eyes; tail feels square. Cool to touch.	Animal unresponsive and cold to touch. Severe skin tent (> 10 seconds) or dehydration with no resolution after 3 days.
Pain	Facial Grimace: Narrow or closed eyes, bulge on top of nose (mice), flattening of bridge of nose (rat), cheek (between eye and whiskers) bulge (mice), cheek sunken (rat), ears back or flat, whiskers pointing back or "standing out on end". Other: Muscle twitching or flinching, staggering, back stretch (like cat), abdominal writhing or pressing.			Persistent signs of pain that interfere with normal functions or that cannot be alleviated with analgesia.

Frequency of Monitoring:

Monitoring frequency will depend on the researcher's understanding of the tumor model, characteristics and progression of the ulcer, the individual animal's general health clinical score and risk of deterioration of the animal's health and welfare. With that said, ulcerated tumors should be monitored daily, at least (in some cases more than once a day).

• For tumors where ulcers develop very rapidly (e.g. some melanoma models), animals should be monitored 2 times a day, and the monitoring should be documented on the cage card. For increased visibility of ulcers on furred animals, shaving the tumor area can help.

• For tumors with small and dry ulcers that are known to remain stable, then monitoring every second day (EOD- every other day) is likely sufficient. Researchers can justify a monitoring frequency based on their understanding and experience with their specific model.

• When models and treatments are unknown, then extra monitoring may be warranted until caregivers and researchers are confident the animals are not at risk of deterioration of their health and welfare.

Management

There are few known treatments to prevent the development of ulcers, however treatments and changes in husbandry can be implemented to reduce the potential negative impacts on animal welfare. These might include:

• If animals show signs of declining health: e.g. decrease in body weight, body condition score, and clinical signs of ill health, then consider supplemental food (e.g. bacon softies, hydrogel, diet gel). For reducing variability across treatment groups, it may be useful to provide the same supplemental food to all animals in an experiment.

• Treatment with local antibiotic or anti-inflammatory to prevent infection and inflammation.

• Shaving fur may keep the area dry, which reduces risk of bacterial infection and enables better visualization.

• Pain: Currently Metacam (NSAID) is very effective in rodents and likely would be a good analgesic choice. This could be administered initially daily for 3-5 days and then the frequency of dosing could be reduced over time. Other analgesic drugs include Buprenorphine, meloxicam, etc. Veterinarian consultation is recommended for finding a drug choice and dosing schedule that animals can tolerate over longer periods of time.

• Application of topical anesthetic (e.g. EMLA cream: 2.5% lignocaine and 2.5% prilocaine) might reduce potential discomfort and is recommended at some institutions. However, potential for oral ingestion, toxicity and effectiveness need to be considered.

Humane Endpoints Related to Ulcerated Tumors

Humane endpoints must consider a combination of signs (general health of animal and ulceration characteristics). Humane endpoints related to tumor size and burden endpoints must be followed, independent of ulcer grading. All exceptions must be justified in the protocol.

• Pus reflects infection and animals should be treated with antibiotics or be euthanized. If there is no response to an appropriate antibiotic treatment in 3 days, then animals must be euthanized.

• Frank blood (active bleeding) increases risk of infection, dehydration and anemia. If active bleeding is visible and the animal is showing signs of anemia (pale extremities such as footpads in non-pigmented mice) and dehydration (skin tent > 2 seconds, sunken and dry eyes), then the animal must be euthanized.

• If the tumor is known to be highly vascular and/or there is a known risk of excessive bleeding, then the animal should be euthanized as soon as visible active bleeding begins.

• If there is clear and wet discharge and the animal is dehydrated (skin tent > 2 seconds, sunken and dry eyes) with no resolution after 3 days of supportive care, then the animal must be euthanized.

• If there is evidence of self-mutilation such as gouges into the tumor tissue, bleeding, scratches or one can observe persistent scratching, then the animal must be euthanized or analgesia and other treatments must be implemented and monitored for success of treatment. If no improvement in 3 days, then the animal must be euthanized.

• If an animal displays signs of pain (facial grimace or other known signs) or interferes with normal functions with no resolution despite analgesia treatment, then the animal must be euthanized.

Appendix:

Anatomy and Pathology

Potential Causes of Ulceration of Tumors

1. Certain types of tumors (e.g. papillomas) or cell lines.

2. Alterations in blood supply in regions of the tumor (below to the epidermis). This results in a reduced (hypoxia) or lack of (ischemia) blood supply to the skin and subsequent cell death and sloughing of the epidermis or deeper layers.

3. Rapid tumor growth where it exceeds the skin's ability to stretch, causing rupture.

4. Tumors with hemorrhagic areas that are prone to ulceration.

5. Mechanical trauma – location of tumor results in constant friction with bedding or caging. For example tumors on the ventral surface of an animal's body.

6. Self-induced trauma – rodent over-grooms or scratches the tumor due to irritation or discomfort.

7. Technique: Tracking of tumor cells into dermal layer.

8. Experimental therapy (phototherapy, irradiation, intra-tumoral injection etc.)

Terminology

It is useful to present terminology used to describe pathology of tumors so that everyone is accurate in their descriptions of clinical signs.

Dermatitis: Inflammation of skin. Inflammation can be characterized according to the type of pathology and includes the following lesions.

Ulcer: Loss of epidermis and at least the superficial portion of the dermis (McGavin & Zachary 2011).
In tumors, ulcers typically form from cell death (necrosis). Commons terms used to describe ulcer appearance when they are sunken are "pitted" or "cratered". Both imply an ulcer that is deep, invaginated (sunken in the middle) with sharply defined walls much like a volcanic "crater". These can be called "cratered" ulcers.

Scab: Material formed by drying of exudate or secretion (fluid, cells, debris from blood vessels) on the skin surface. For scab to be present, there has to be an ulcer. The scab formation is part of the skins attempt to heal. The correct term for scab is "fibrin crust".

Necrosis: Death of cells in a living animal. The mechanism leading to cell death varies. In tumors cell death would typically be caused by "oncotic necrosis" which is death following irreversible cell injury by hypoxia, ischemia, and membrane injury. This may or may not be visible grossly. Typically when an ulcer and/or scab is visible this would be called "ulcerative dermatitis". However, sometimes the skin appears black without an ulcer and this would be called "necrotizing dermatitis". The black color indicates necrotic cells are present. Note that

necrotizing dermatitis can progress to ulcerative dermatitis so one may see a combination. If there is bleeding from the ulcer, this would be called “hemorrhagic ulcerative dermatitis”. Sometimes tumors have shiny thin skin. This likely indicates that the epidermis is separated from the dermis and in these cases there is an increased risk of ulceration.

Epithelialization: Epithelialization is an essential component of wound healing. The various cells in the skin form a lattice (granulation tissue) where the epidermal cells can migrate over top to create the normal epidermal barrier. In the absence of re-epithelialization, a wound cannot be considered healed.