APPLICATION OF HUMANE ENDPOINTS IN CANCER RESEARCH

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Cancer is a global issue

- Cancer is a complex multi-stage disease process affecting man and animals.
- The incidence of cancer is increasing in the human population. In balance the survival rates are increasing.
- Many forms of cancer are associated with significant host morbidity & mortality.
- Many forms of cancer still resist therapeutic control.
- There is a major unmet need for safe, effective anti-cancer therapies.
Defining Humane Endpoints

“There is a need for less inhumane endpoints”

Michael Balls Zeist Netherlands 1998
Defining Humane Endpoints

"In experiments involving animals, any actual or potential pain, distress, or discomfort should be minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research.

Goy RW 1982 Wisconsin Regional Primate Centre

Humane endpoints should be applied as early as possible and not be the equivalent of stopping a train by letting it hit the buffers.
Humane Endpoints

DEFINE

THE

SCIENTIFIC OBJECTIVES

OF THE EXPERIMENT OR PROCEDURE
Define Scientific Objectives

- Tumour maintenance /oncogenesis
- Tumour model characterisation/biology or comparative growth techniques
- Host /tumour interactions
- Anti-tumour therapy
- Tumour diagnostic techniques
- Mechanisms of cancer development
- Avoid lethality or survival as experimental endpoints. Consider euthanasia.

CLEAR EXPERIMENTAL OBJECTIVES SHOULD BE USED TO IDENTIFY SPECIFIC SCIENTIFIC AND HUMANE ENDPOINTS BEFORE THE ONSET OF SEVERE MORBIDITY OR DEATH
Humane Endpoints

CHARACTERISE THE BIOLOGY OF THE TUMOUR MODELS AND THE POTENTIAL EFFECT ON THE ANIMALS AND THE SCIENTIFIC OUTCOME
Animal Models of Cancer

- There are three main animal tumour models
  - **ECTOPIC**- with tumours implanted or induced in superficial tissues- ear pinna / dermal / subcutaneous, mammary fatpad and footpad. The growth of the primary tumours is easily observed and they are useful for screening potential anti-cancer agents. These tumours may be benign, invasive and/or metastatic.

- **INTERNAL/ORTHOTOPIC**- where tumours are implanted or induced internally or orthotopically in the tissues of origin (brain, prostate, liver). Cancer may develop as tumours in tissues or in the haemo-lympho poetic system. These animal models are useful for studying site specific development, host/tumour interactions and therapy and also as pre-clinical models of metastasis.

- **GENETICALLY ALTERED MODELS**- derived from spontaneous mutations or generated by genetic manipulation. Useful for studying early stage events, prevention and gene directed therapies.
TUMOUR GROWTH CHARACTERISTICS MAY CHANGE
Effect of serial In-Vivo Passage on Tumourgenicity & Metastatic Behaviour of Human Breast Cancer Xenografts in SCID Mice

<table>
<thead>
<tr>
<th>Passage #</th>
<th>Tumour Engraft</th>
<th>Latency/ Months</th>
<th>Lymph Node Metastases</th>
<th>Lung Metastases</th>
<th>Systemic Metastases</th>
<th>Time of Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/2</td>
<td>1</td>
<td>1/2</td>
<td>1/2</td>
<td>None</td>
<td>6 months</td>
</tr>
<tr>
<td>2</td>
<td>5/5</td>
<td>1</td>
<td>5/5</td>
<td>5/5</td>
<td>None</td>
<td>3 months</td>
</tr>
<tr>
<td>3</td>
<td>15/15</td>
<td>0.5</td>
<td>15/15</td>
<td>15/15</td>
<td>15/15*</td>
<td>2 months</td>
</tr>
</tbody>
</table>

(* Liver, kidney, bone, brain) (Visonneau et al AJP V152 May 1998)

CHARACTERISE TUMOUR LINES AND MONITOR FOR CHANGES IN GROWTH PATTERN & EFFECTS DURING USE
Tumour Biology and Animal Welfare may be affected if the changes are made to the experimental protocol

- Growth characteristics and survival time were influenced by the inoculation of either tumour cell suspension or fragments using different techniques
- Reduced survival time with surgically implanted tumour fragment (SOI)
- Increased invasiveness with tumour cell suspension (COI)
- SOI - death from primary tumour. COI - death from metastatic disease

**Changes in Tumour Induction Methods or Animal Model May Affect Tumour Growth Pattern and Pathology**

Figure 8. Survival curve of SOI and COI models of human renal cell carcinoma SN12C. Zili AN et al Clin J Exp Met 1999
Humane Endpoints

USE GENERIC HUMANE ENDPOINTS AS A BASIS TO DEVELOP SPECIFIC ENDPOINTS
Determining Humane Endpoints: Potential Adverse Effects of Experimental Cancers in Research Animals

**Lethal tumour lines** - some tumour lines result in death within specific time limits

**Tumour Burden** - volume or number of tumours, tissue or organ distension, invasion of tissues, organomegally, adhesions, restricted behaviours

**Tumour Associated Disease** - paraneoplastic conditions - anaemia, cachexia, anorexia, dehydration, weight loss, respiratory difficulties, obstruction

**Disseminated & Metastatic** disease - deposits in peritoneum, lungs, brain, bone and other organs - erosion or distension of tissues

**Ulceration** - or erosion of tissues - infection, anaemia

**Pain and Discomfort** - due to tissue destruction or distension
## Generic Clinical Endpoints
### Common Cancer Endpoints in Bold Type

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Characteristics</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor growth or effects</td>
<td>Tumor exceeds 10% of normal body weight; necrosis, infection, ulceration, interference with ambulation or eating/drinking</td>
<td>Subcutaneous or intraperitoneal tumors and hybridomas</td>
</tr>
<tr>
<td>Prolonged inappetence/ Cachexia Dehydration</td>
<td>Rapid loss of weight (&gt;20% of normal body weight) and/ or condition</td>
<td>Metastatic disease, chronic infectious disease</td>
</tr>
<tr>
<td>Inability to ambulate</td>
<td>Prolonged recumbency</td>
<td>Many</td>
</tr>
<tr>
<td>Signs of severe organ or system involvement</td>
<td>Respiratory: rapid or labored breathing, Anaemia hemorrhage, anaphylaxis Gastrointestinal: severe diarrhea Peripheral Nervous System: flaccid or spastic paralysis CNS Signs: circling, blindness, dementia, convulsion</td>
<td>Toxicity testing; systemic disease</td>
</tr>
<tr>
<td>Moribund or pre-moribund state</td>
<td>Define with specific clinical signs and euthanize when reached</td>
<td>Many</td>
</tr>
</tbody>
</table>
Tumour Development

- Tumour size or weight alone are NOT reliable indicators of potential or actual animal suffering or invasion of normal tissues. Clinical condition should also be considered.
- Tumour size is a specific issue where tumours develop in restricted sites-(cranium, eye, abdomen, oral cavity, muscle, footpad) that may result in pain or discomfort.
- Wherever possible experiments should be terminated before tumour size limits behaviours or the onset of tumour associated disease.
- Endpoints related to fixed size or time period should be carefully reviewed.

TUMOUR SIZE SHOULD BE THE MINIMUM COMPATIBLE WITH EXPERIMENTAL OBJECTIVES. TUMOUR DEVELOPMENT IS BETTER CONTROLLED USING LIMITS ON SIZE AND DISTRESS SCORING RATHER THAN TUMOUR WEIGHT ALONE
Special Cases: Single or multiple tumour sites?

Single Implants
- More animals are used
- Therefore “more” potential suffering?
- Less tumour burden
- Greater experimental variables?

Double Implants
- Fewer animals used both in production & experiments
- Fewer inter-animal differences?
- Greater tumour burden?

The number of superficial tumours permitted should not be prescriptive but subject to scientific justification and the potential adverse impact on the animal.
Bodyweight & Condition
No more than 20% loss in bodyweight

- Bodyweight may increase in animals with growing tumours
- Weight loss: Relative to starting weight, control animals or normal growth curve?
- Consistent or transient weight loss?

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- Weight may be lost due to anti-cancer therapy or other experimental techniques
- Loss of condition / emaciation / distension / ascites may be more valid limiting signs

Animal with ascites may gain weight but may lose condition

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CHANGES IN BODYWEIGHT AND CONDITION SHOULD BE BOTH CONSISTENT AND SIGNIFICANT
Humane Endpoints- Ulcerated tumours

**Ulcerated Tumours or tissue erosion** may result in anaemia, dehydration, infection in the host.

- **Tumour characteristics** - PC6, melanoma, papillomas
- **Inoculation Technique** - intradermal inoculation
- **Response to therapy** - erosion or induration of tumour
- **Tumours grow too large** - untreated control animals
- **Surface Abrasion** – tumours develop on sites in regular contact with hard surfaces

Causes of ulceration should be determined and controlled where possible. Affected area and the animal should be scored for condition.
Special Cases: Untreated Controls

- Untreated control may require special consideration if animals may suffer greater tumour burden and disease.

Mice inoculated with Ovarian Ascitic Tumour Cells

Scientific endpoints using tumour growth projections should be used in preference to fixed time points. Humane endpoints should be applied before the onset of severe and irreversible clinical signs.
Humane Endpoints

• CONSIDER THE CUMULATIVE AFFECT OF ALL THE EXPERIMENTAL CHALLENGES
Experimental adverse effects may be cumulative

- Tumour Model- Tumour burden or tumour associated disease
- Induction methods- Surgery/Irradiation/Adjuvant therapy
- Therapy- Surgery/Drugs/Drug delivery systems/Irradiation/Immunosupression
- Monitoring- Anaesthetics/Blood Samples/Surgery/Imaging/Biopsy

THE ADVERSE EFFECTS (AND ANY MODIFYING FACTORS) OF ALL THE EXPERIMENTAL CHALLENGES SHOULD BE DESCRIBED, CONSIDERED AND ASSESSED.
Humane Endpoints

• CONSIDER MEANS TO ALLEVIATE THE EFFECTS OF THE EXPERIMENTAL CHALLENGE
The animal as a patient

- Clinical signs
- Behaviour
- Tumour associated disease
- Body condition

Increasing use of modern imaging and biomarker technologies permit cancer development and potential therapies to be monitored in individual animals leading to the development of refined experimental and humane endpoints.

**Analytical Assessment**
- Tumour Biomarkers
- Haematological Values
- Biochemical Biomarkers
- Non-invasive Imaging
Controlling or Alleviating the Effects

- Refine the experimental challenge and monitoring techniques
- Determine key or limiting signs
- Define conditions for euthanasia
- Consider the use of analgesics
- Consider supportive care

Consider the impact of these measures on the experimental outcome.
Experimental Cancer & Animal- Pain and Sickness. Questions to be addressed

- Animals may be sick or uncomfortable but are they in pain?
- Are analgesics appropriate or effective?
- What may be the impact of analgesic drugs on tumour growth /experimental outcome?
- What criteria can be used to determine the presence of pain?
- Supportive therapy (hydrated feed) has been shown to reduce clinical signs but not effect experimental outcome in tumour bearing animals.

EARLY EUTHANASIA MAY BE AS EFFECTIVE IN REDUCING ANIMAL SUFFERING AS SUPPORTIVE CARE
INSTRUCTIONS TO AUTHORS

1. “This journal endorses the most humane treatment of animals in the conduct of scientific studies”

2. “Only results of those experiments, including photographic representation of data, in which proper attention has been given to ethical considerations towards animals will be published”

Materials & Methods

“Mice were sacrificed if they showed signs of distress”

In an article in an international cancer journal animals were reported as being allowed to develop 12gm metastatic hepatic tumours.

Photographs accompanying the article showed liver tumours.

DEFINE AND APPLY SPECIFIC STANDARDS. PUBLISH DATA ON DEFINED HUMANE ENDPOINTS.