

APPLICATION OF HUMANE ENDPOINTS IN CANCER RESEARCH



Berlin 2005

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

Passage #	Tumour Engraft	Latency/ Months	Lymph Node Metastases	Lung Metastases	Systemic Metastases	Time of Necropsy
1	2/2	1	1/2	1/2	None	6 months
2	5/5	1	5/5	5/5	None	3 months
3	15/15	0.5	15/15	15/15	15/15*	2 months

(* 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

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- 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

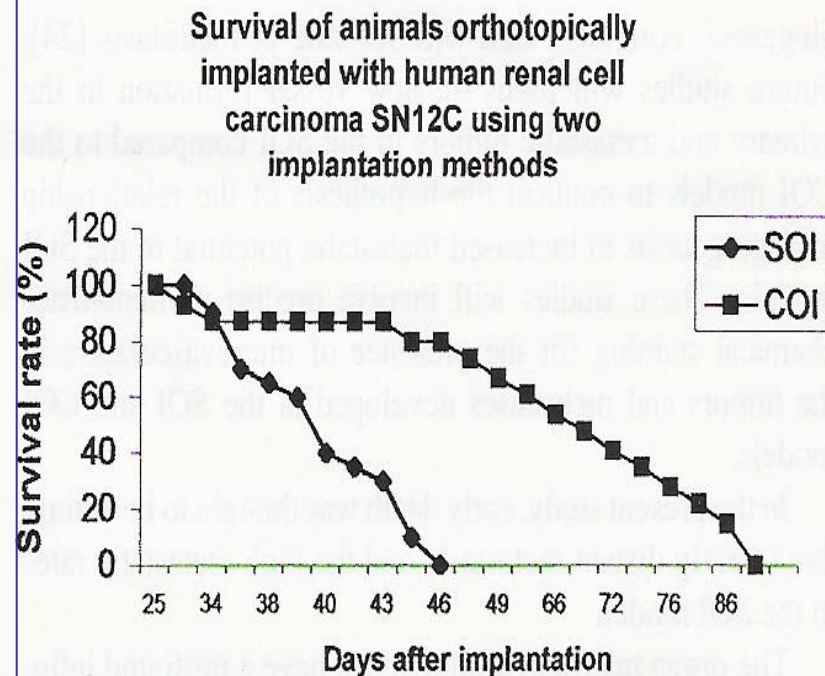


Figure 8. Survival curve of SOI and COI models of human renal cell carcinoma SN12C.

Zili AN et al Clin J Exp Met 1999

CHANGES IN TUMOUR INDUCTION METHODS OR ANIMAL MODEL MAY AFFECT TUMOUR GROWTH PATTERN AND PATHOLOGY

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

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

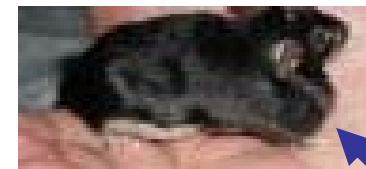
Tumour Development

- 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

Subcutaneous tumour with few adverse effects



Large tumours may inhibit normal behaviours and “drag” on tissues



Spontaneous Mammary Tumour. Usually benign but will eventually inhibit behaviour and feeding

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

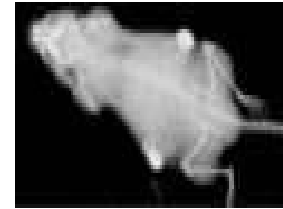
Special Cases :

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?
- 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



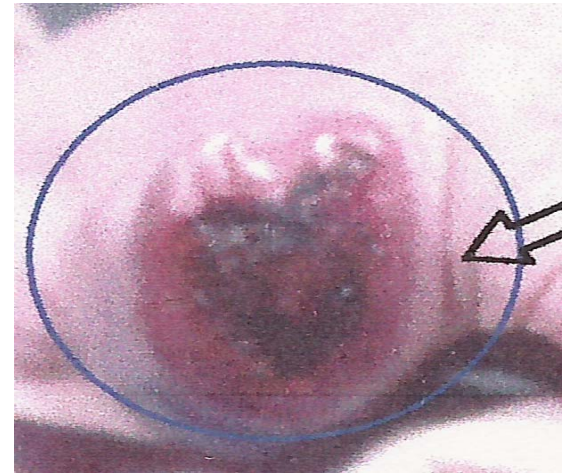
Animal with ascites may gain weight but may lose condition

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

UNTREATED



TREATED



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/Immunosuppression
- Monitoring- Anaesthetics/ Blood Samples/Surgery / Imaging/ Biopsy



Cumulative
Challenge

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

Analytical Assessment

Tumour Biomarkers

Haematological Values

Biochemical Biomarkers

Non-invasive Imaging

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



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

Scientific Journals & Humane Endpoints- Applying Standards

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.