ONKOLOŠKI INŠTITUT LJUBLJANA

INSTITUTE OF ONCOLOGY LJUBLJANA

Severity classification of procedures used in experiments on live animals in cancer research

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The choice of experimental models

- The experimental material (model) should be selected with the purpose of solving the case being studies in the simplest possible way.

- Together with the scientific considerations, there are also legal, ethical matters which have to be taken into account when selecting experimental model.

Definition of „animal model“

- Most of our knowledge regarding general cell biology, biochemistry, physiology and endocrinology stems from an animal experiments, which ideally should be extrapolated to man.
- Animal models – „modelling of humans“
- Animal model is a living organism in which normative biology or behaviour can be studied, or in which spontaneous or induced pathological processes can be investigated and in which the phenomenon in one or more respects resemble the same phenomenon in humans or other species of animal. The choice of the most appropriate model in based on the possibility to extrapolate the results on humans.

Extrapolation from animals to humans

- *Extrapolation* of the results form one animal species to another relies on homology
- *Homology* refers to the evolutionary similarity between morphological structures and physiological processes amongst different species but also animal and man.
- Extrapolation is qualitative and quantitative
- *Qualitative extrapolation* deals with an animal‘s pathophysiological processes and its reaction to stimuli extrapolated to other animals or man.
- *Quantitative extrapolation* involves assessing, on the basis of animal tests, the dose of a certain compound which would be beneficial or harmful to a man or the target animals.

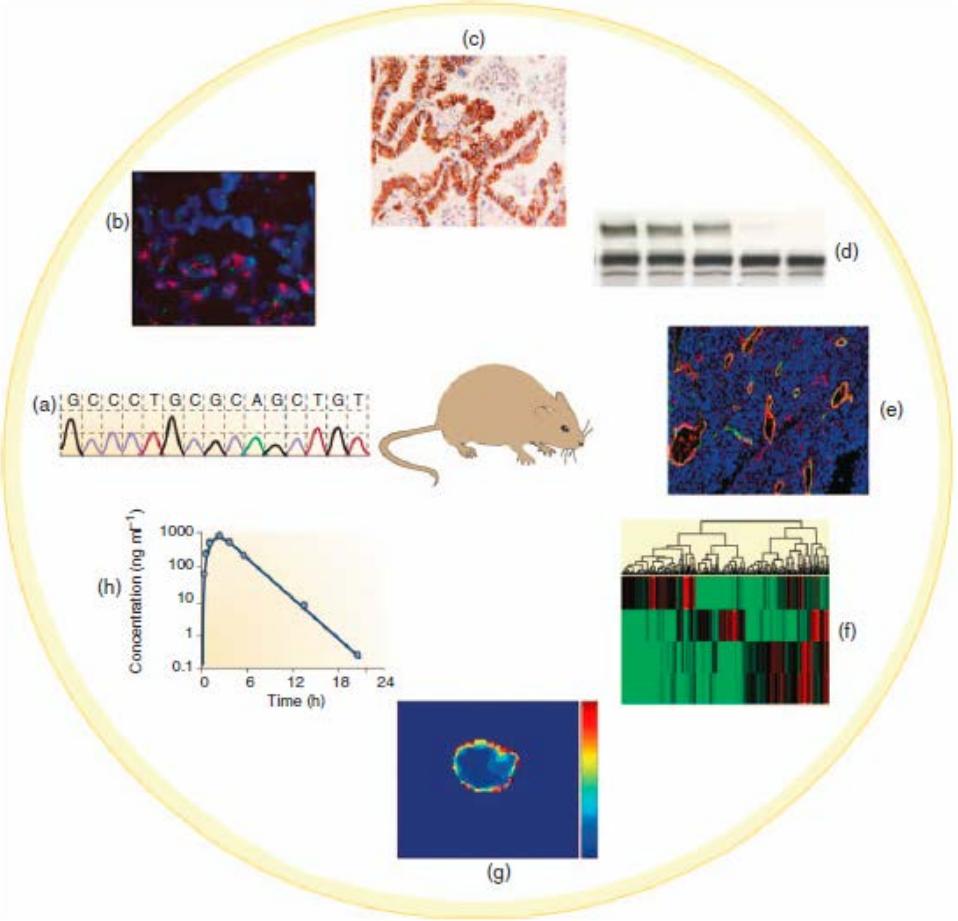
The selection of animal model

Steps involved

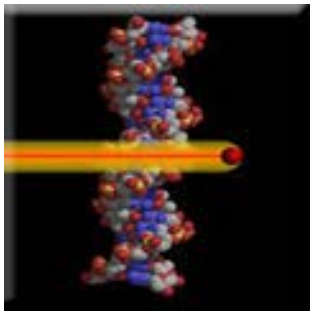
1. Define the key question
2. Decide on the key substrate – cell, organ, whole organism..
3. Determine in which animal species/strains this key substrate is found
4. Establish which animal species/strains possessing the key substrate are the most advantageous fro the technical point of view and which cause the minimum discomfort in the animals
5. Establish which practical factors should be decisive i.e. Availability, accommodation, care, tractability, published information, expertise, expense….
6. Select the animal models on the basis of scientific, practical and ethical considerations

Work in cancer research involves

- Human tumor specimens and DNA
  - Genomics, proteomics, metabolomics...
- In vitro models:
  - Cell cultures
- Mathematical models
- In vivo models:
  - Carcinogenesis
  - Evaluation of new treatment modalities
  - Test of strategies for cancer prevention
  - Development of procedures for early detection and diagnosis of cancer



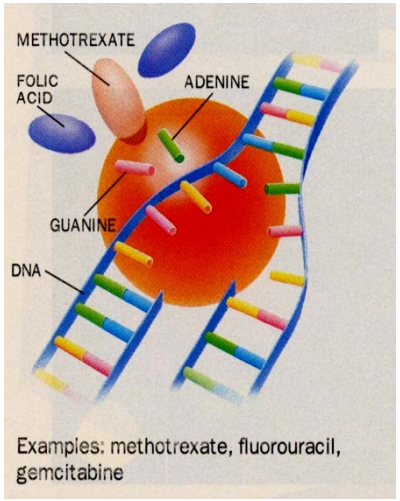
Tumor models in radiobiology



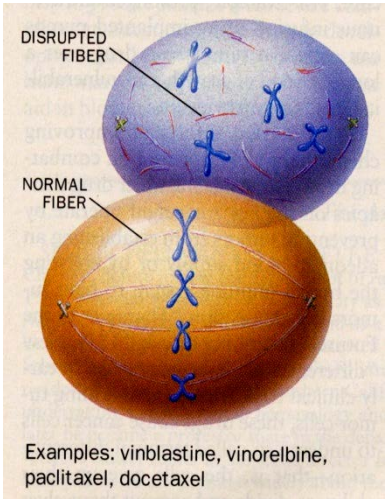
- Evaluation of tretment effectiveness of radiotherapy combined with other tretaments (chemotherapy, antiangiogenic therapy, immunogene therapy...)
- Basic mechanisms of action
- Normal tissue reaction (acute and long-term)

Tumor models and laboratory animals in chemotherapy

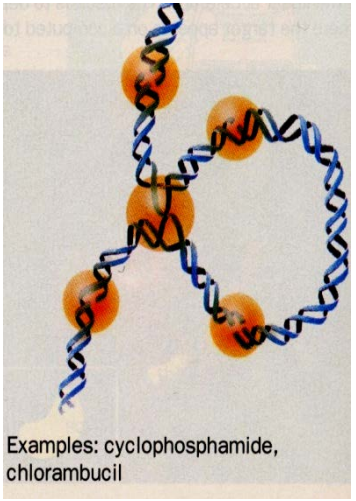
- Effectiveness
- Toxicity
- Metabolism
- Dictribution
- Tumor microenvironent
- Clinical study phase I/II



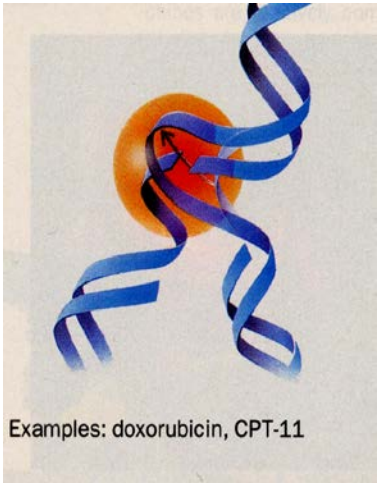
Antimetabolites



Plant alkaloides



Alkylating agents



Topoisomerase inhibitors

Chain of research in translational studies

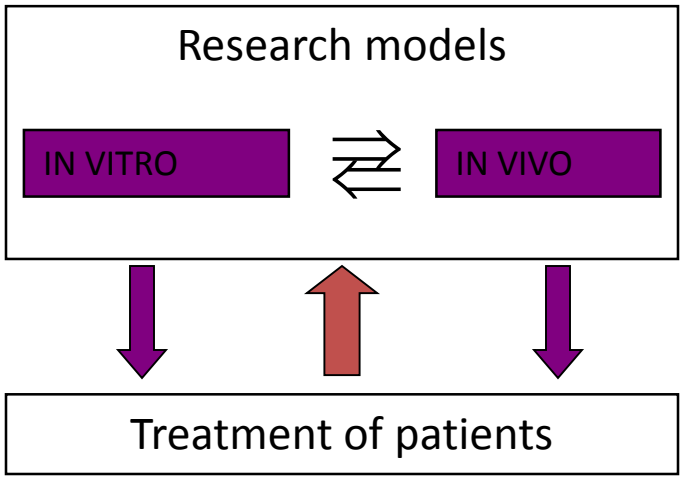
In vitro studies

In vivo studies on laboratory animals

Clinical studies phase I/II

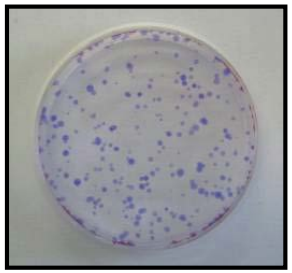
Clinical study phase III

Standard treatment



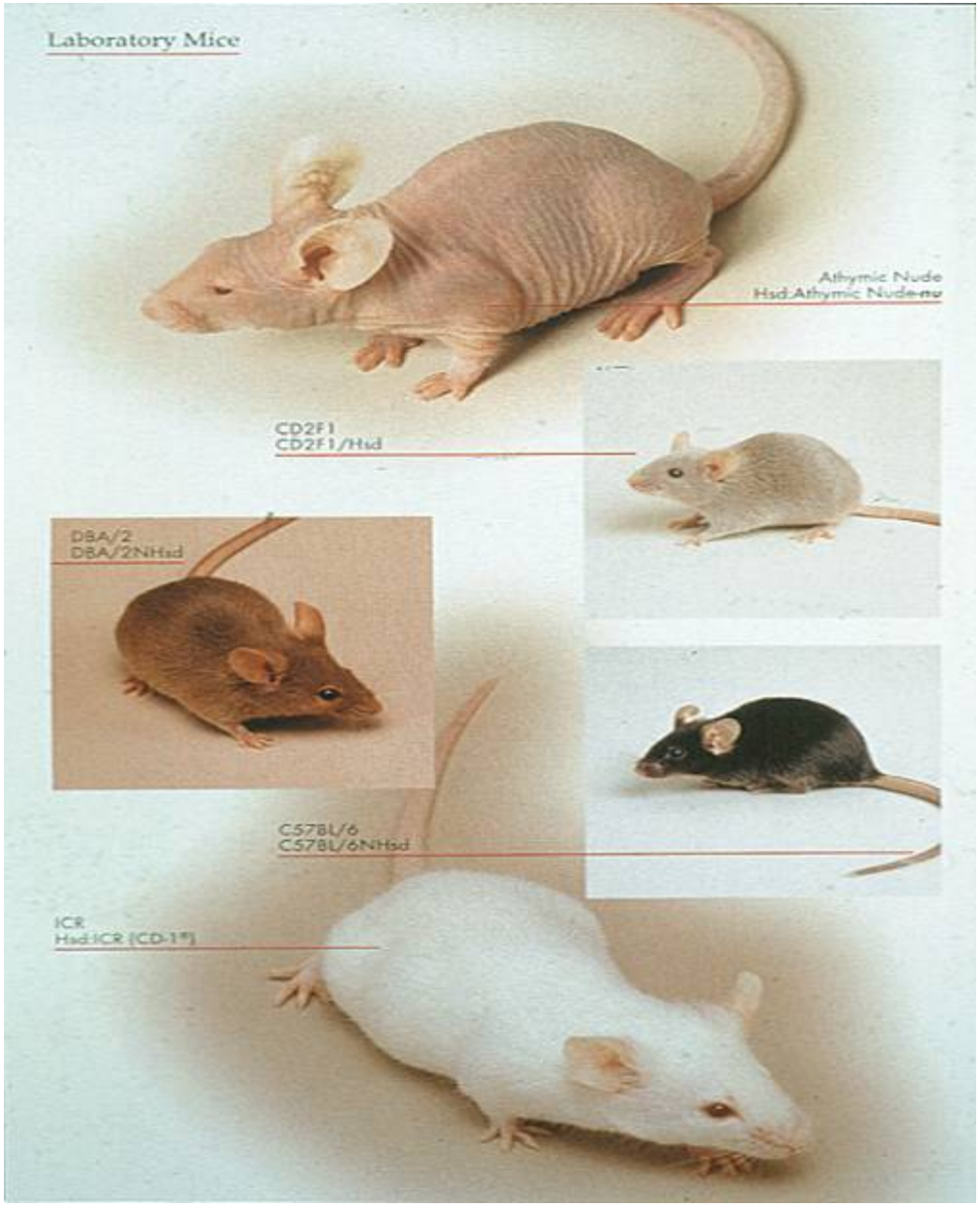
Models used in preclinical oncology

- Cell cultures (including spheroides)
- Murine tumor models
- Human tumor xenografts



Only tools that are used to answer specific questions pertinent to human tumors

Laboratory mice

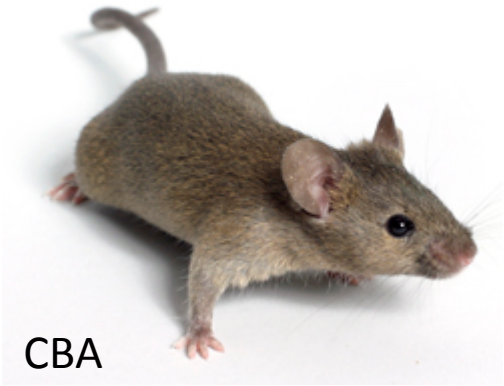


Mice used in cancer research

- Inbred immunocompetent mice
- Nude mice (w/o T lymphocytes)
- SCID mice(w/o B and T lymphocytes)
- Transgenic mice
- Knockout mice

Inbred immuno competent strains of mice

C57BL/6 mouse
1920 Clarence Cook Little
(Jackson laboratory)
2002 genome sequenced



CBA

- Advantages
  - High number of available tumor models
  - Many different istes for tumor induction

Balb C

- Drawbacks
  - The presence of active immune system
  - Genetic variability

A/J

Nude mice

- Advantages
  - Good characterization
  - Absence of hair enables good visualization of subcutaneous tumors
  - Immuno compromised – available many human tumor xenografts
  - Available on many different genetic backgrounds
- Drawbacks
  - Some extrathymus T cell function is still present
  - Presence of B lymphocytes
  - Normal number of macrophages, NK cells, normal function of APC and normal function of complement

SCID mice

Fox Chase SCID® Beige Mouse

A congenic mouse that possesses both autosomal recessive mutations SCID (*Prkdc^{scid}) and beige (Lyst^{bg}).* The SCID mutation results in severe combined immunodeficiency affecting both the B and T lymphocytes. The beige mutation results in defective natural killer (NK) cells.

Advantages

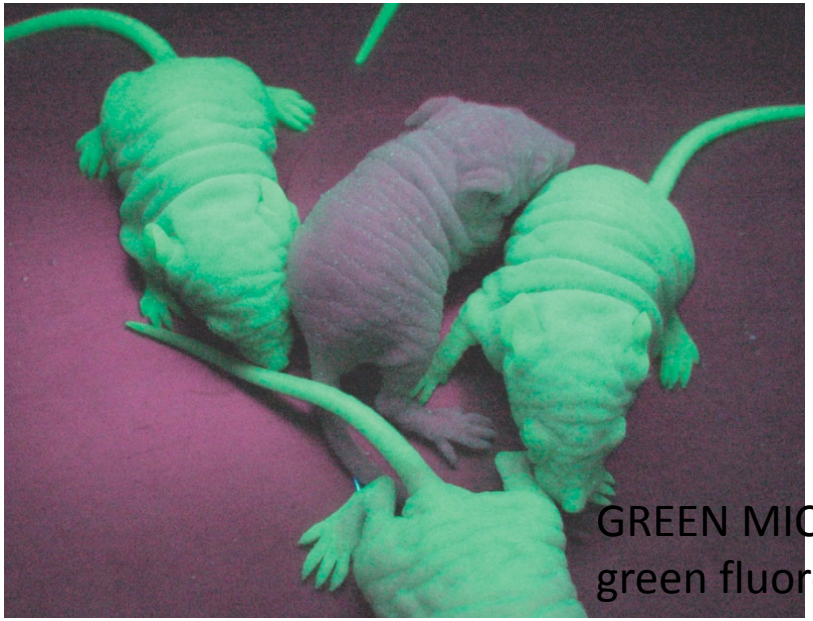
- Very suitbale for growing of human tumors
- Available on different genetic backgrounds

Drawbacks

- Some production of T and B lymphocytes after 12 weeks of age
- Normal number of macrophages, NK cells, normal action of APC cells, in some strains the function of complement is increased
- High incidence of lymphoma of thymus
- Shorter life span
- Radiosensitive due to the mutations in gene encoding DNA repair proteins

Transgenic mice

- Advantages
  - Tumors develop spontaneously and in a „natural“ organ
  - Tumors have natural growth rate and metastatic potential
  - Nonimmunogenic



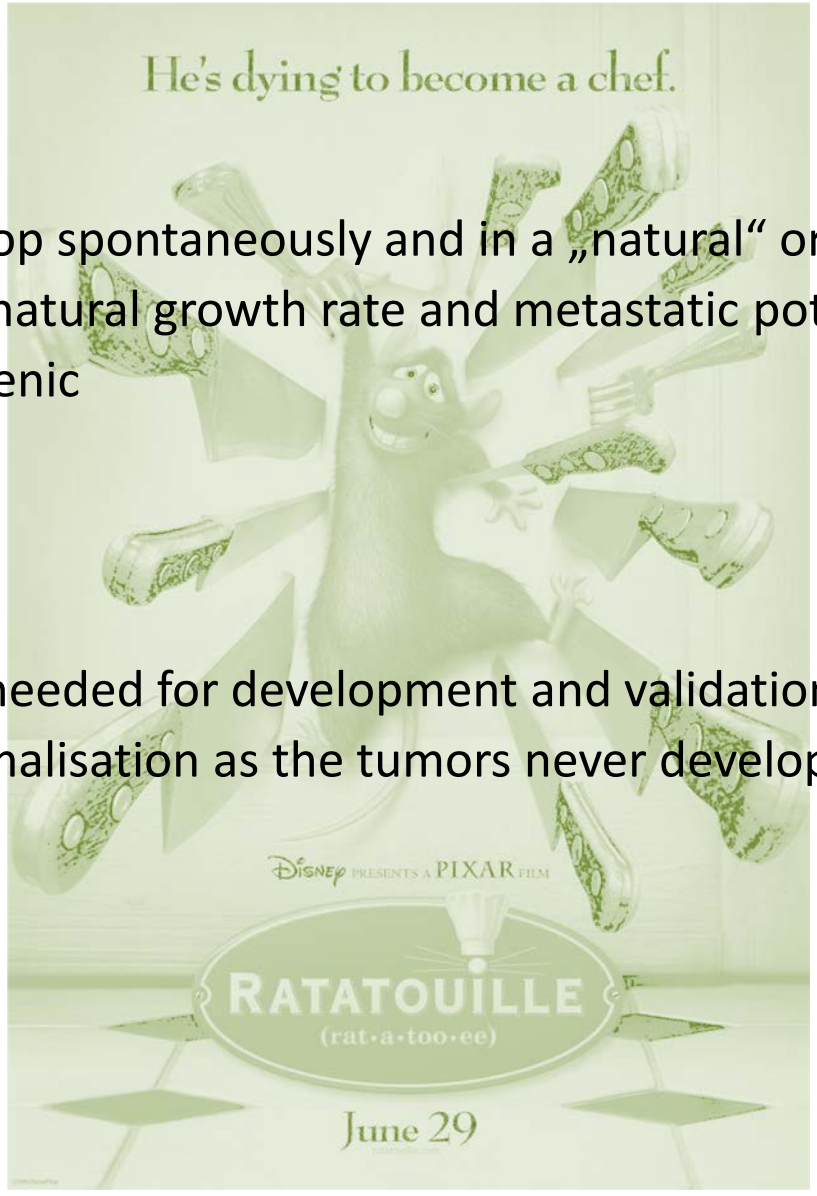
GREEN MICE – expressing green fluorescence protein

- Drawbacks
  - Expensive
  - A lot of time needed for development and validation

Knockout mice

- Advantages
  - Tumors develop spontaneously and in a „natural“ organ
  - Tumors have natural growth rate and metastatic potential
  - nonimmunogenic

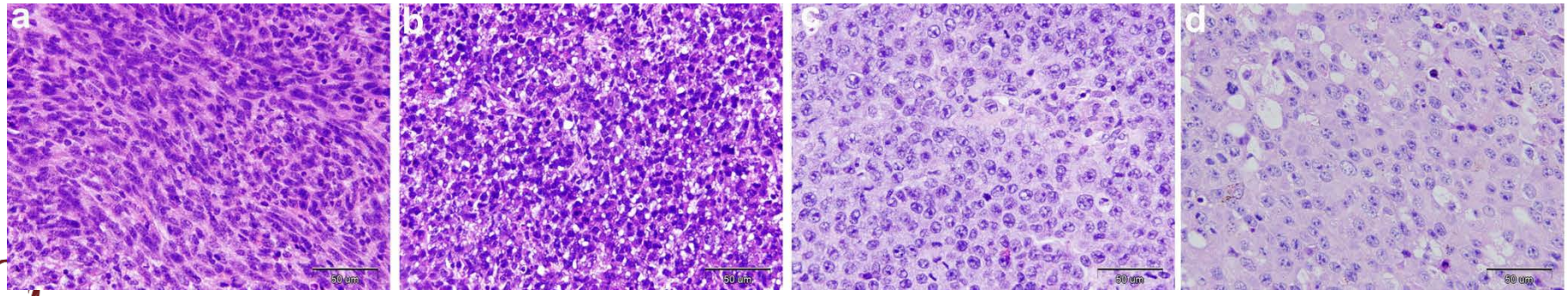
- Drawbacks
  - Expensive
  - A lot of time needed for development and validation
  - Need for normalisation as the tumors never develop in all animals



Mouse tumor models

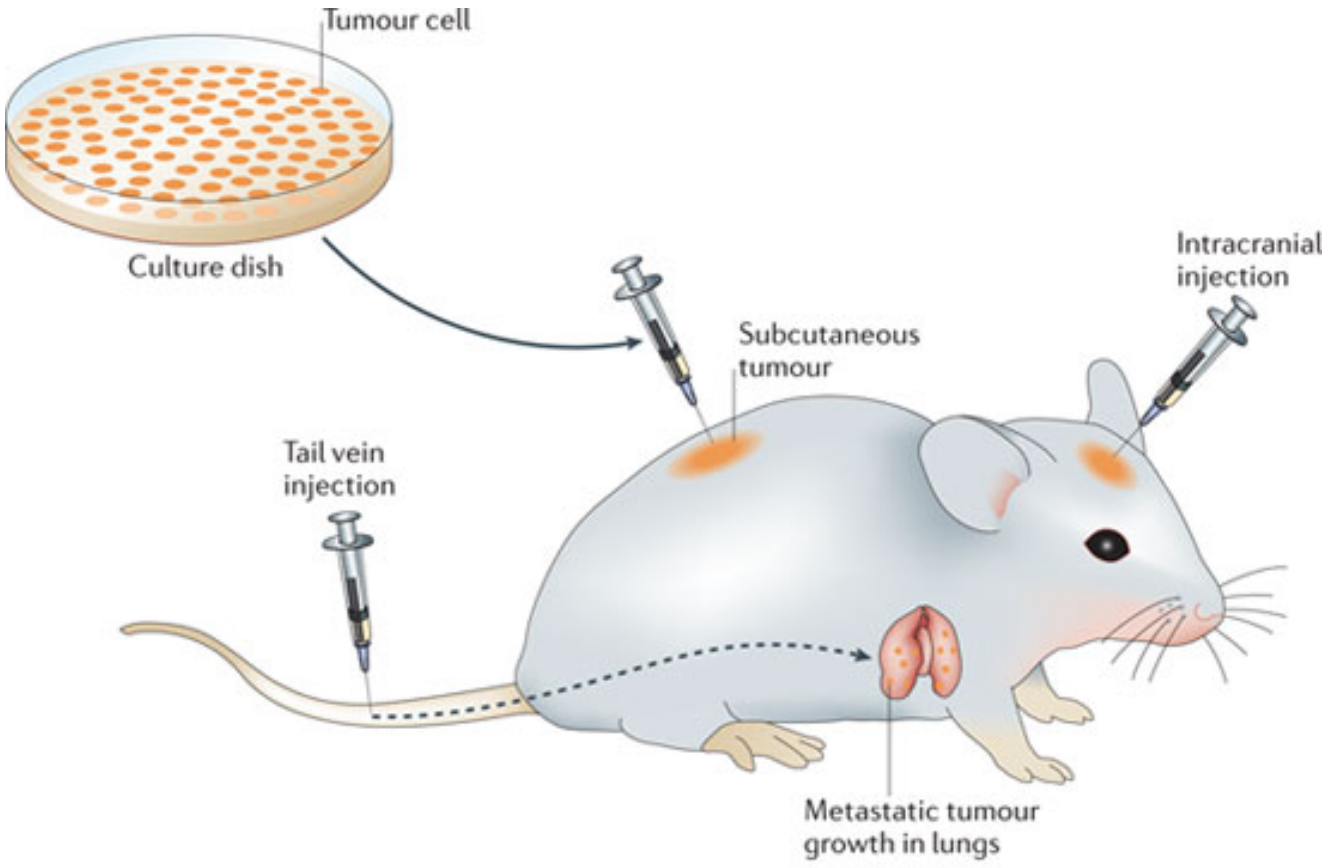
- ❑ Spontaneous tumors
- ❑ Early generation transplanted tumors
- ❑ Established transplanted tumors

  - Availability of inbreed strains
  - Well-defined end points
  - Fast growth of the tumors
  - Fast and reproducible results



LPB fibrosarcoma SA-1 fibrosarcoma EAT carcinoma B16 melanoma

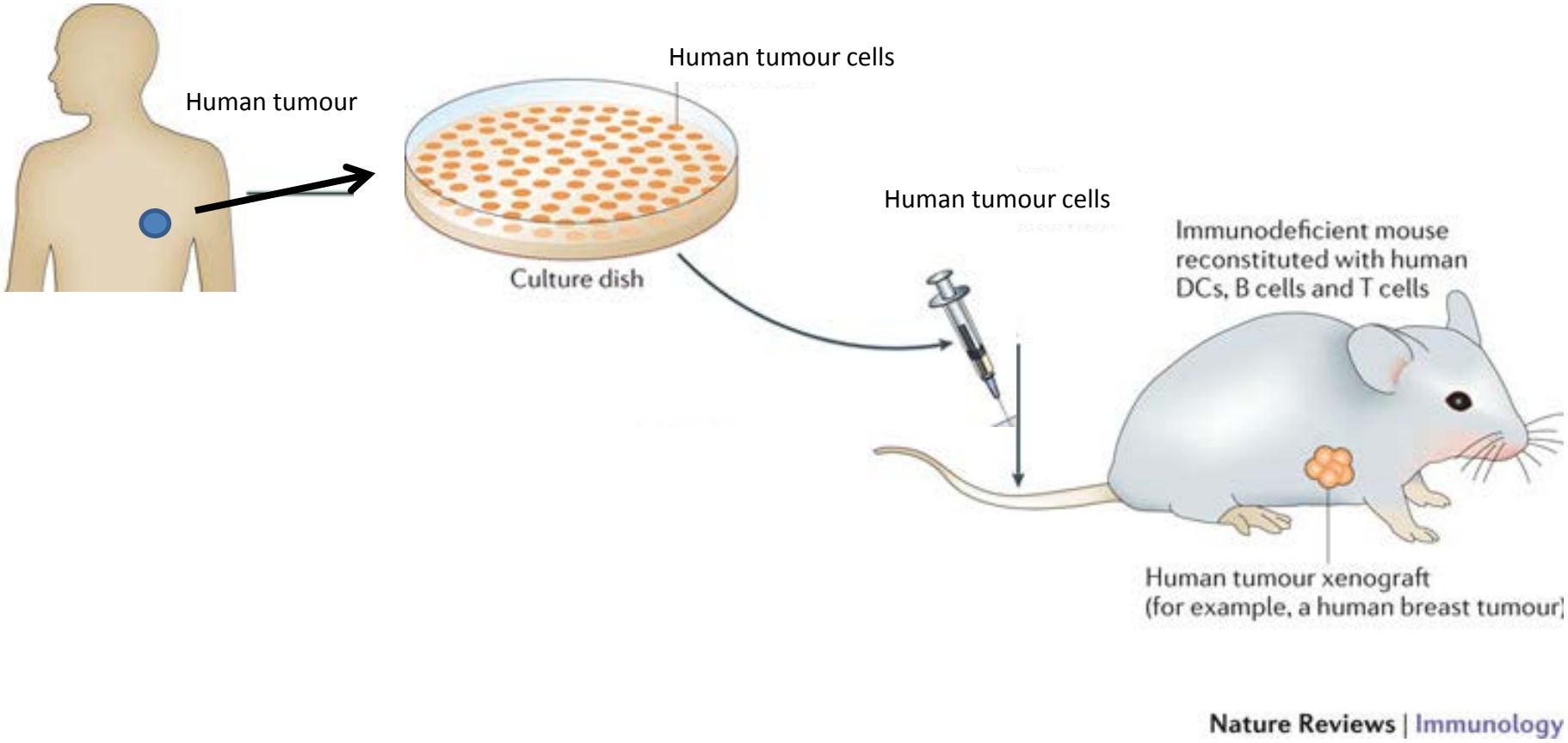
Transplantable tumor models



Cellular interactions

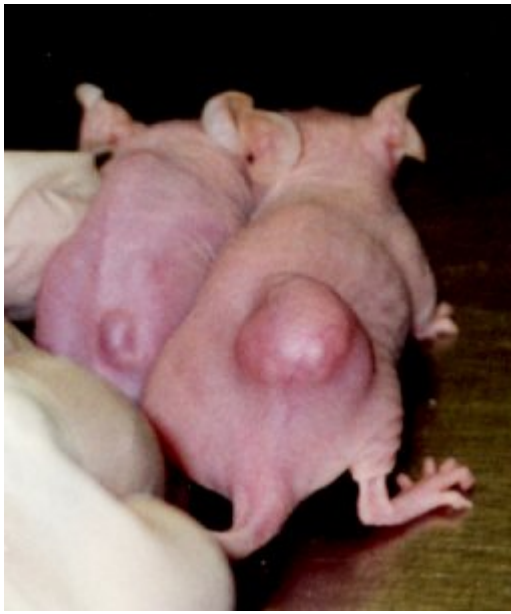
Immune responses

Human tumour xenografts

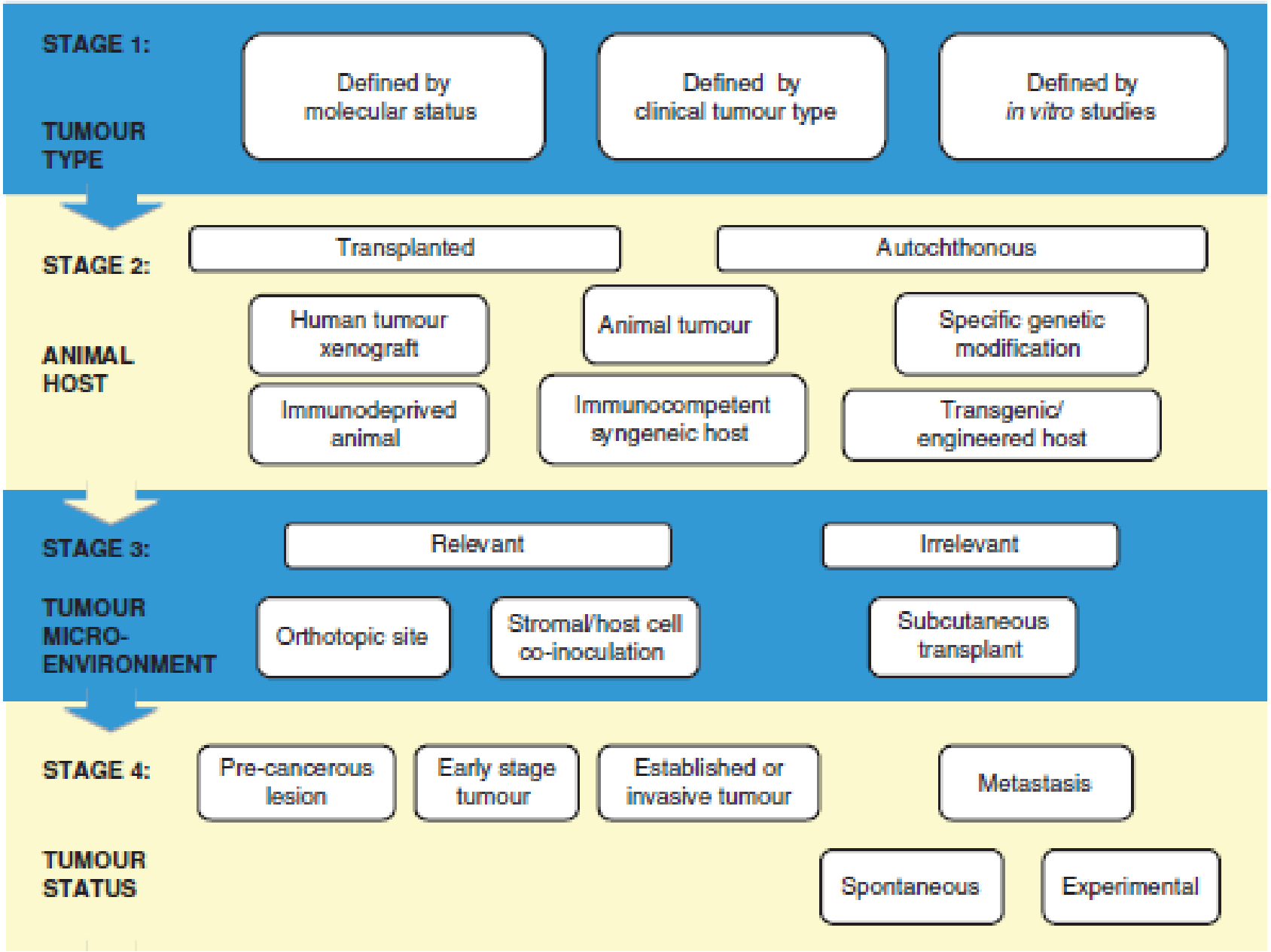


Human tumor xenografts

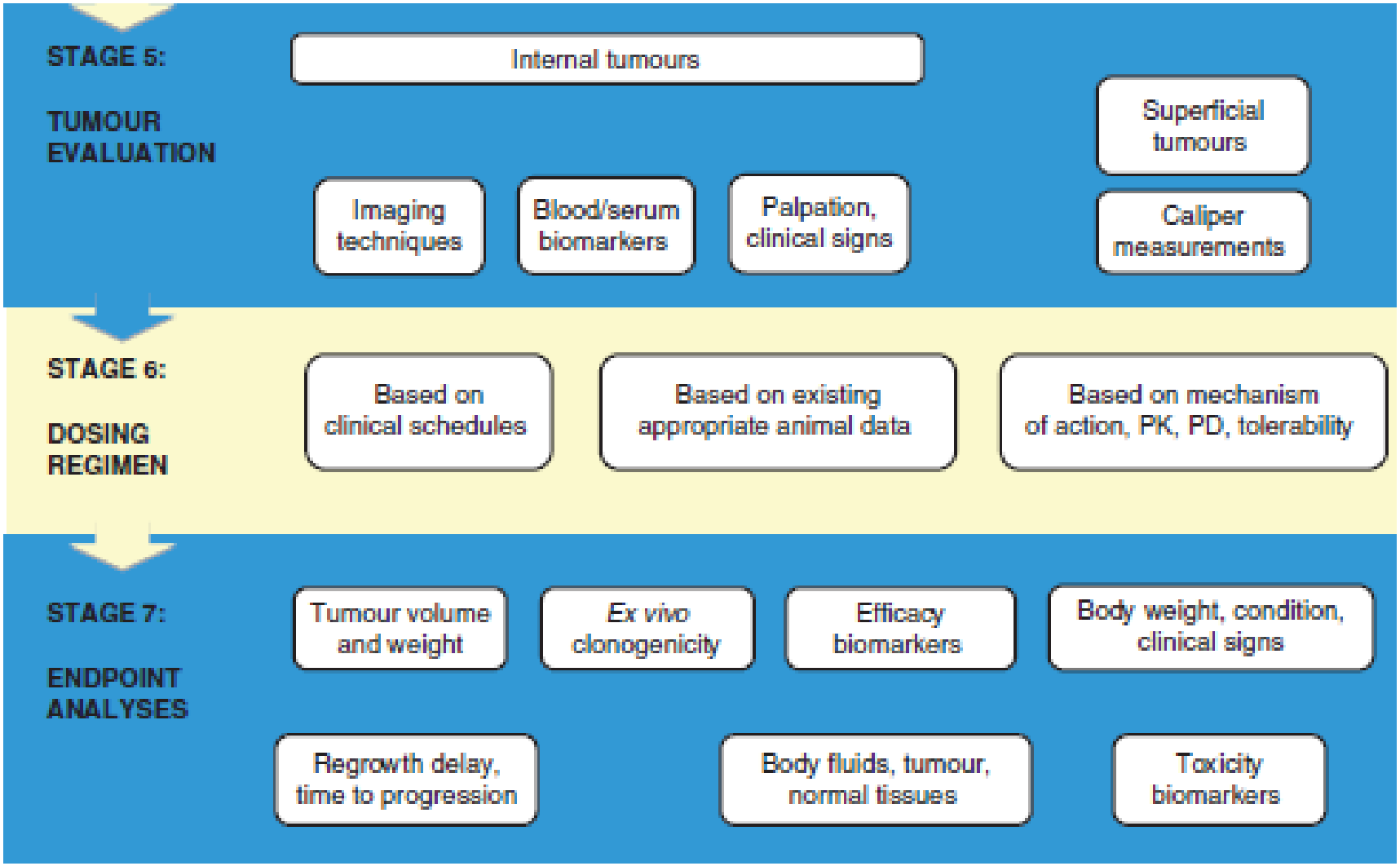
- Advantages
  - morphological and biological characteristics of original tumors are preserved
  - The response to treatment correlates with clinical response
- Drawbacks
  - Vascularisation is specific for host, not tumor
  - Tumor doubling time is shorter
  - Immune response of the host



Tumor model selection and use

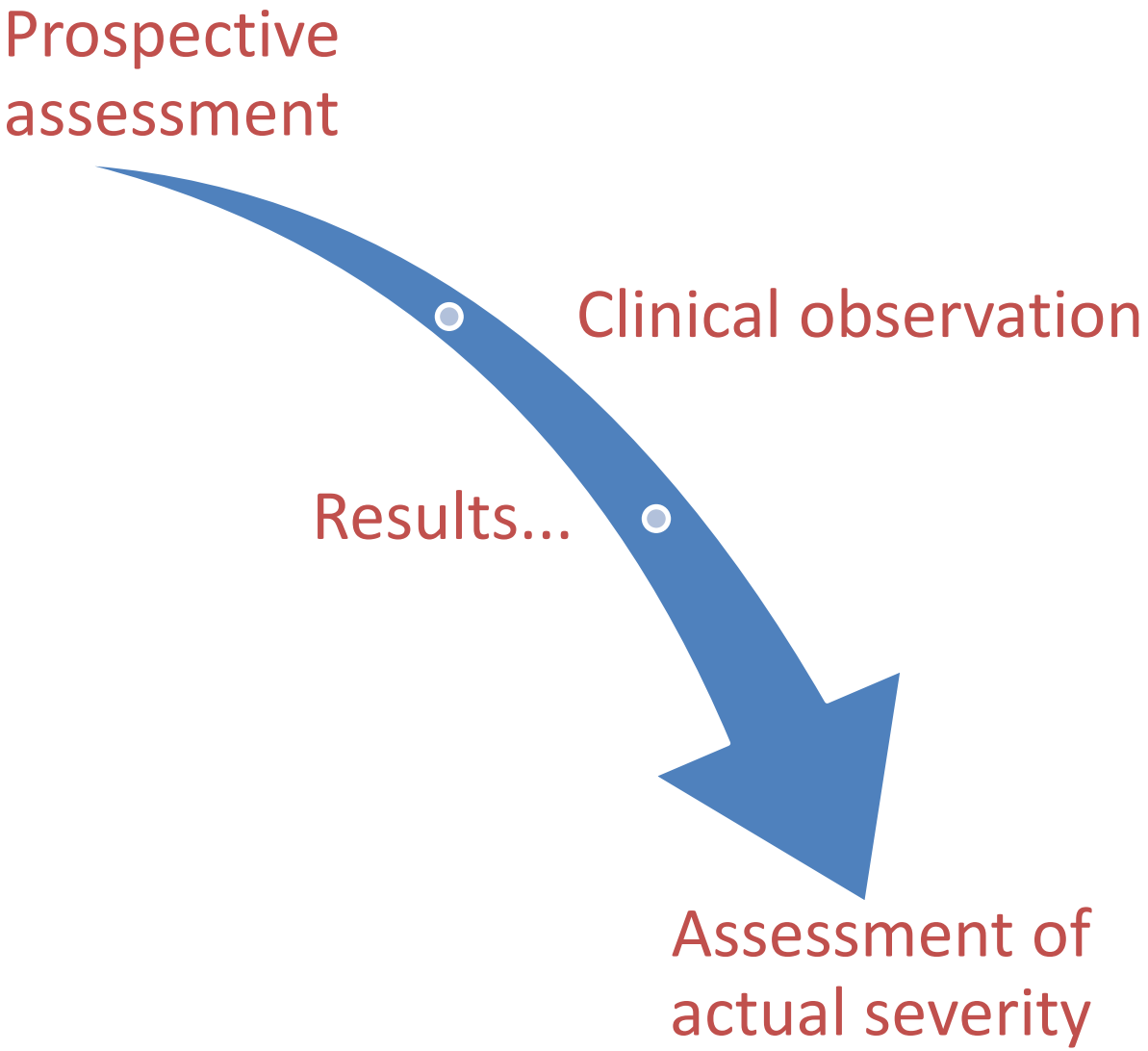


Tumor model selection and use



Workman et al BJC 2010

Assesment of severity: for each procedure and for the whole project



Severity of procedures; classification of severity

- ***Non-recovery***

All procedures are performed under general anaesthesia and the animal will not recover consciousness.

- ***Mild***

Procedures cause an animal to experience short-term mild pain, suffering or distress. This category also includes procedures which cause no significant impairment to the animal‘s wellbeing or general condition.

Procedures that could produce greater suffering may be classified as mild if there are effective safeguards in place to treat the animal or stop the procedure before the animal shows more than adverse minor effects.

Severity categories

- ***Moderate***

Procedures where animals are likely to experience short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. This category also includes procedures that are likely to cause moderate impairment of the animal‘s wellbeing or general condition.

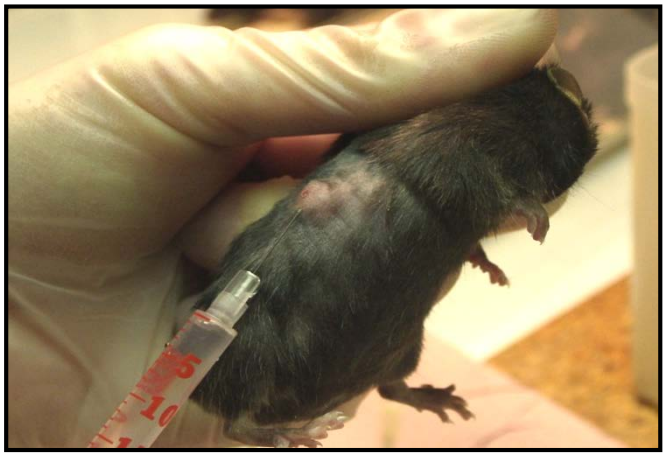
- ***Severe***

Procedures where the animal is likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. This category also includes procedures that are likely to cause severe impairment of the animal‘s wellbeing or general condition.

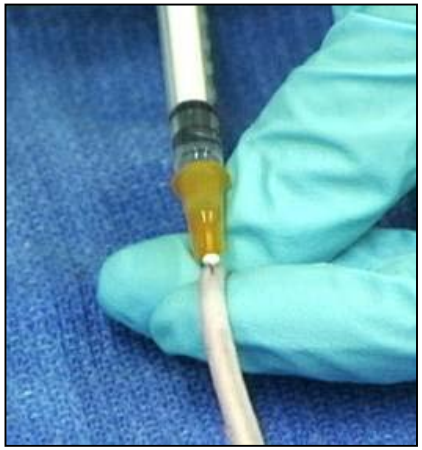
Examples – **mild** severity procedures

- administering anesthesia except for the sole purpose of killing;
- pharmacokinetic study where a single dose is administered and a limited number of blood samples are taken (totaling < 10 % of circulating volume) and the substance is not expected to cause any detectable adverse effect;
- non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anaesthesia;
- superficial procedures, e.g. ear and tail biopsies, non-surgical subcutaneous implantation of mini-pumps and transponders;
- applying external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behaviour;
- administering substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than a mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal;
- inducing tumours, or spontaneous tumours, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, non-invasive nodules);
- breeding of genetically altered animals, which is expected to result in a phenotype with mild effects;
- feeding of modified diets, that do not meet all of the animal‘s nutritional needs and are expected to cause mild clinical abnormality within the timescale of the study;
- short-term (<24h) restraint in metabolic cages;
- studies involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains;
- models which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid;

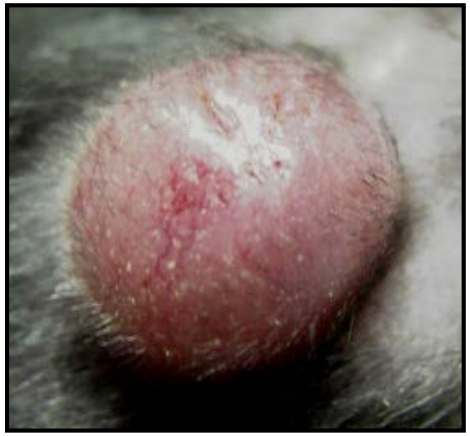
Methods: tumor implantation



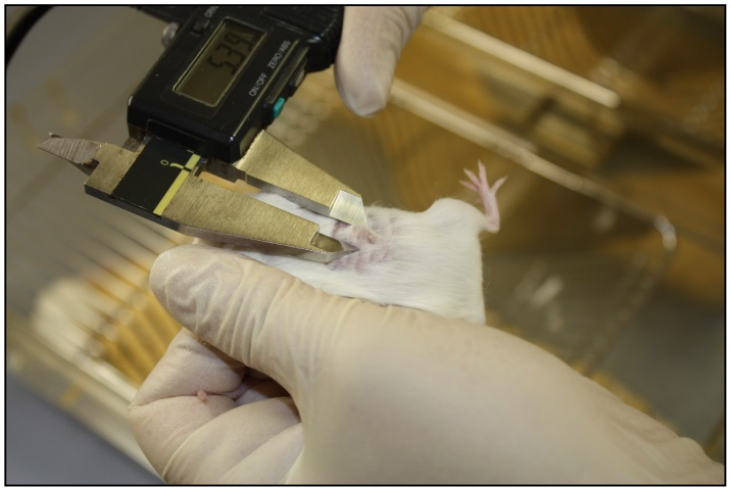
Subcutaneous tumors

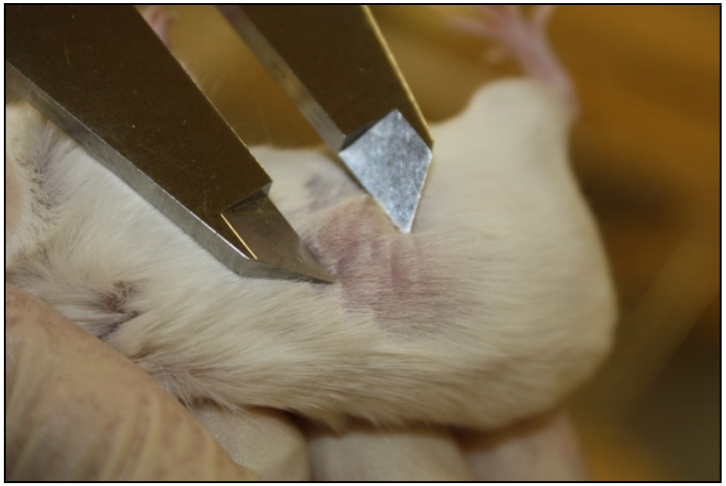


Induced lung metastases



Subcutaneous tumor measurements





Mouse

CALIPER 1.3 File name: KONTROLA 01.xls

NEW GROUP SAVE SAVE AS CLOSE QUIT

Time - days

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0100	48,9		68,9			249,7			306,7
0101	34,3		108,8			201,2			252,4
0110	40,3		102,5			272,5			381,4
0111	42,0		90,2			171,0			347,1
0102	48,3		92,1			210,6			264,4
0120	46,7		105,4			359,7			449,9
0112									
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a	5,75		7,67			8,60			12,74
b	6,64		8,32			9,52			11,00
c	2,10		2,70			3,99			4,73
mass	0		25,9			25,7			25,3
St									

a b c mass St

a * b * c * Pi / 6

Measurement of three perpendicular diameters

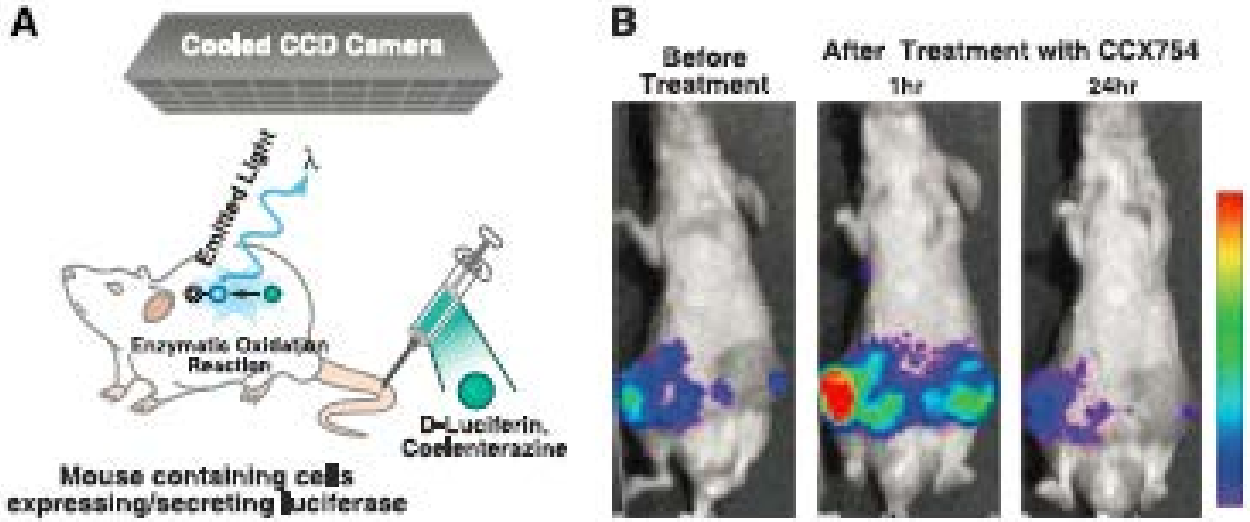
Calculation of volume by the formulae for elipsoid

Graphical representation of growth curve

Molecular imaging – main fields of usage – implementation of 3Rs‘

- Monitoring deep seated tumors with or without therapy
- Study of basic biological processes
- Tissue pharmacokinetics and pharmacodynamic responses to treatment
- Pharmacodynamic imaging of molecular targeted therapeutics

Small animal optical bioluminescence imaging



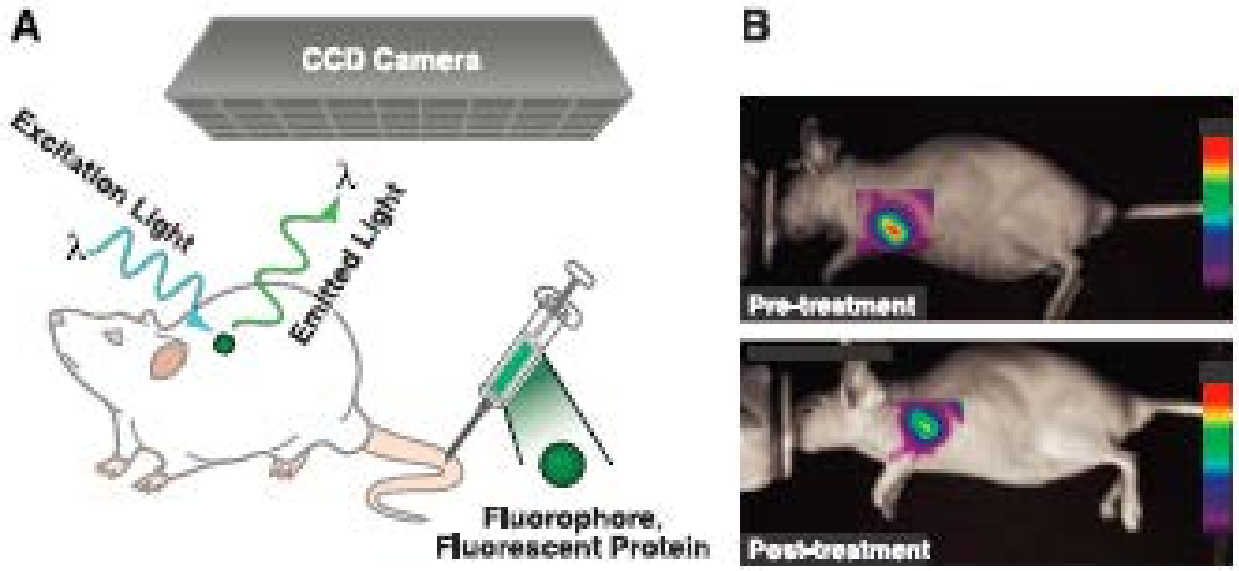
Advantages

- Relatively inexpensive
- Excellent sensitivity
- Good temporal resolution
- User friendly
- Multiplexing capabilities
- No endogenous bioluminescence

Drawbacks

- Limited depth of penetration
- Poor spatial resolution at greater depths
- Images are relatively surface-weighted
- Substrates and enzymatic co-factors required
- Tomography challenging
- Clinical translation very limited

Small animal optical fluorescence imaging



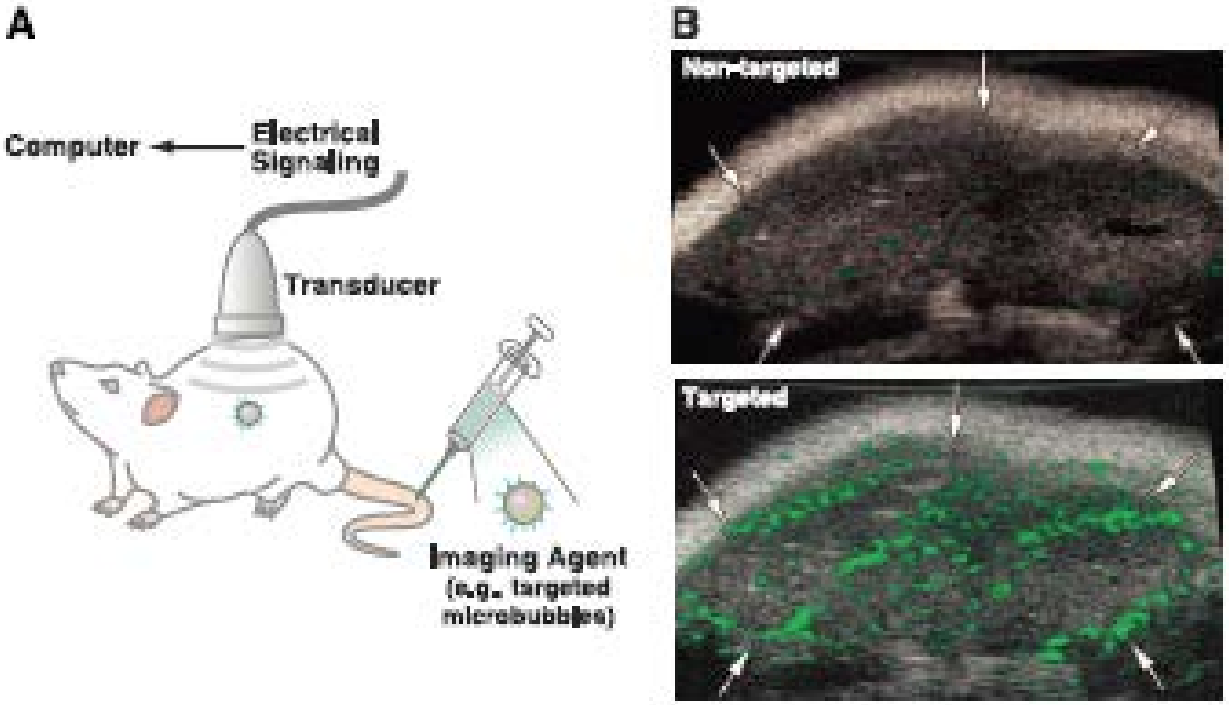
Advantages

- Relatively inexpensive
- User friendly
- Multiplexing capabilities

Drawbacks

- Limited depth of penetration
- Poor spatial resolution at greater depths
- Surface weighted images
- Autofluorescence

Small animal ultrasound - US



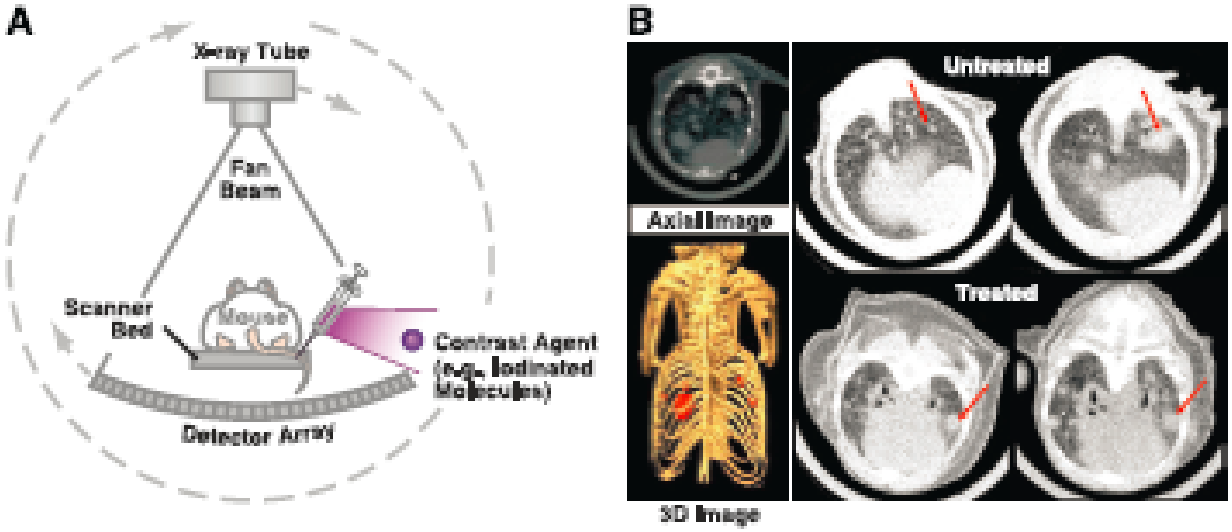
Advantages

- Relatively inexpensive
- No ionizing radiation
- Good temporal resolution
- Quantitative data
- Excellent sensitivity with microbubbles
- Clinical utility

Drawbacks

- Limited depth of penetration
- Primarily anatomical information
- Limited molecular imaging applications
- Limited to imaging of soft-tissues only

Smal animal computed tomography - CT



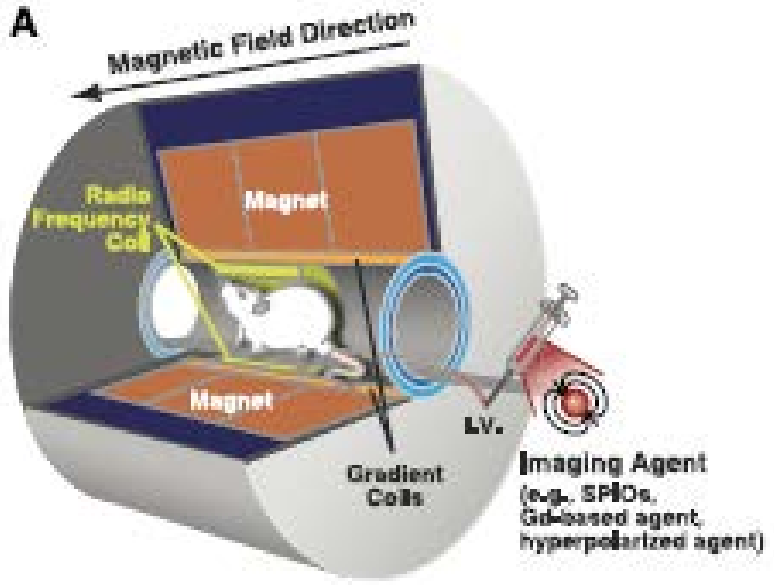
Advantages

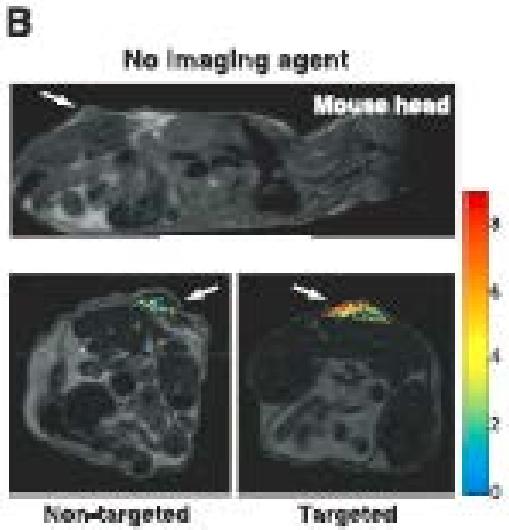
- Limitless depth of penetration
- High spatial resolution
- Good temporal resolution
- Clinical utility

Drawbacks

- Poor sensitivity (requires large mass of imaging agent)
- Primarly anatomic information
- Limited soft tissue resolution
- Limited molecular imaging applications
- Ionizing radiation

Small animal magnetic resonance imaging - MRI





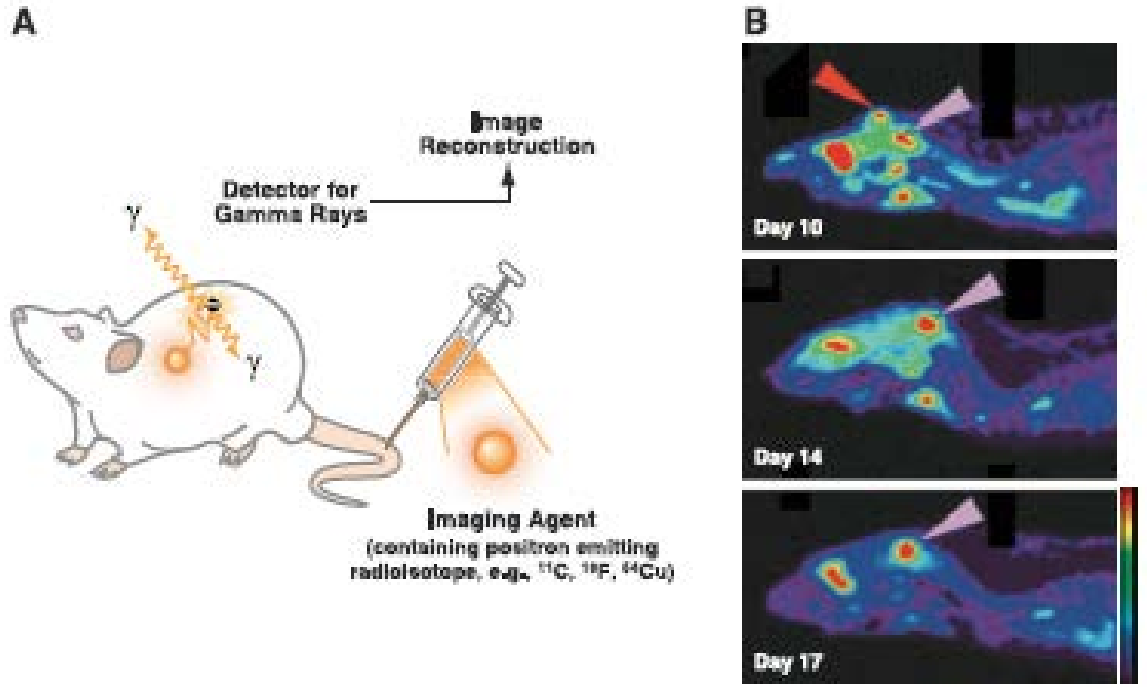
Advantages

- Limitless depth of penetration
- High spatial resolution
- Quantitative data no ionizing radiation
- Clinical utility

Drawbacks

- Poor sensitivity (requires large mass of imaging agent)
- Relatively expensive
- Relatively poor temporal resolution

Small animal positron emission tomography -PET



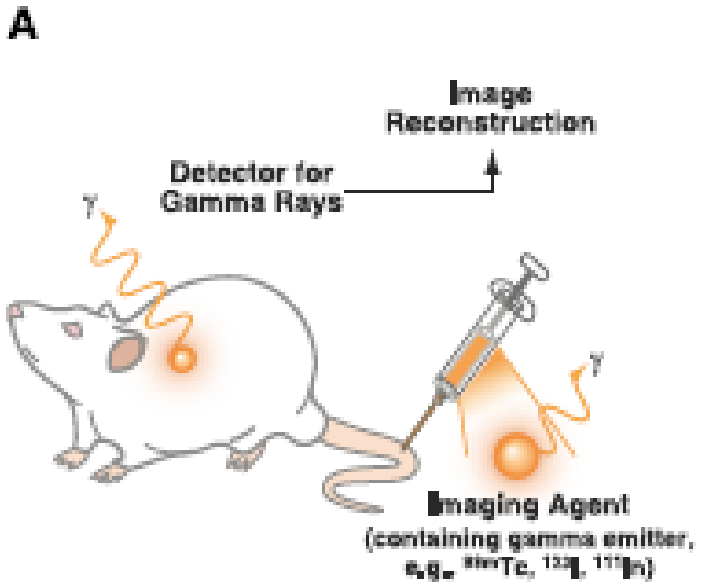
Advantages

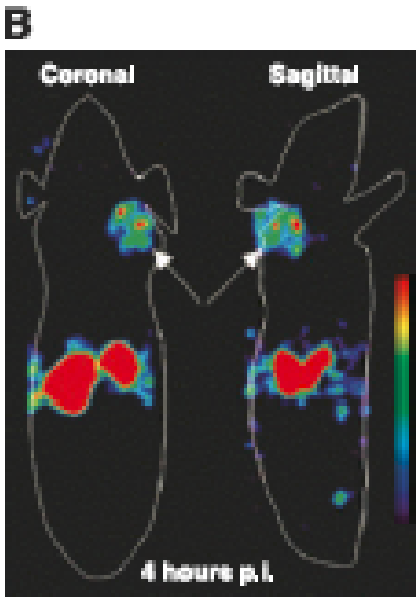
- Limitless depth of penetration
- Excellent sensitivity
- Quantitative data
- Clinical utility

Drawbacks

- Relatively expensive
- Requires cyclotron/generator
- Limited spatial resolution
- Ionizing radiation

Small animal single photon emission computed tomography - SPECT





Advantages

- Limitless depth of penetration
- Excellent sensitivity
- Clinical utility

Drawbacks

- Relatively expensive
- Requires cyclotron/generator
- Limited spatial resolution
- Lack of attenuation corrections
- (therefore only semi-quantitative)

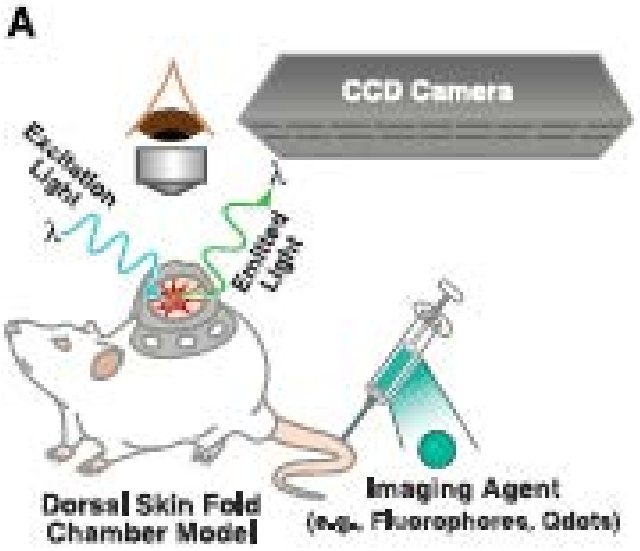
A combination or accumulation of the following examples may result in a „mild“ classification:

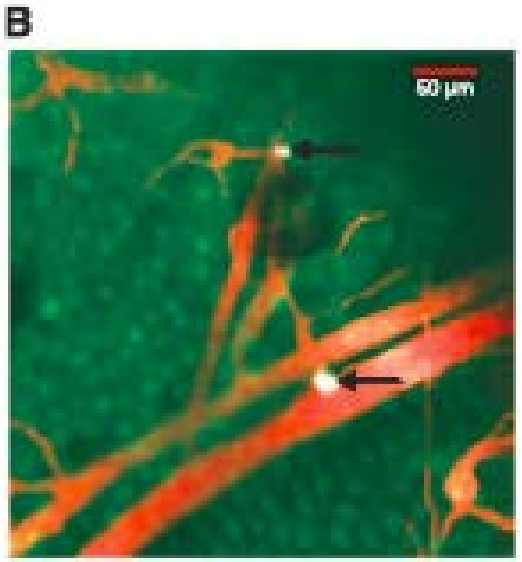
- assessing body composition by non-invasive measures and with minimal restraint;
- monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals;
- applying external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behaviour;
- breeding genetically altered animals which are expected to have no clinically detectable adverse phenotype;
- adding inert markers in the diet to follow passage of digesta;
- withdrawal of food for <24h in adult rats;
- open field testing.

Examples – **moderate** severity procedure

- frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (>10 % of circulating volume) in a conscious animal within a few days without volume replacement;
- acute dose-range finding studies, chronic toxicity/carcinogenicity tests, with non-lethal end-points;
- surgery under general anaesthesia and appropriate analgesia, associated with post-surgical pain, suffering or impairment of general condition. Some examples are: thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy, thyroidectomy, orthopaedic surgery with effective stabilisation and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, minipumps etc.);
- models of induction of tumours, or spontaneous tumours, that are expected to cause moderate pain or distress or moderate interference with normal behaviour;

Small animal intravital microscopy (IVM)





Advantages

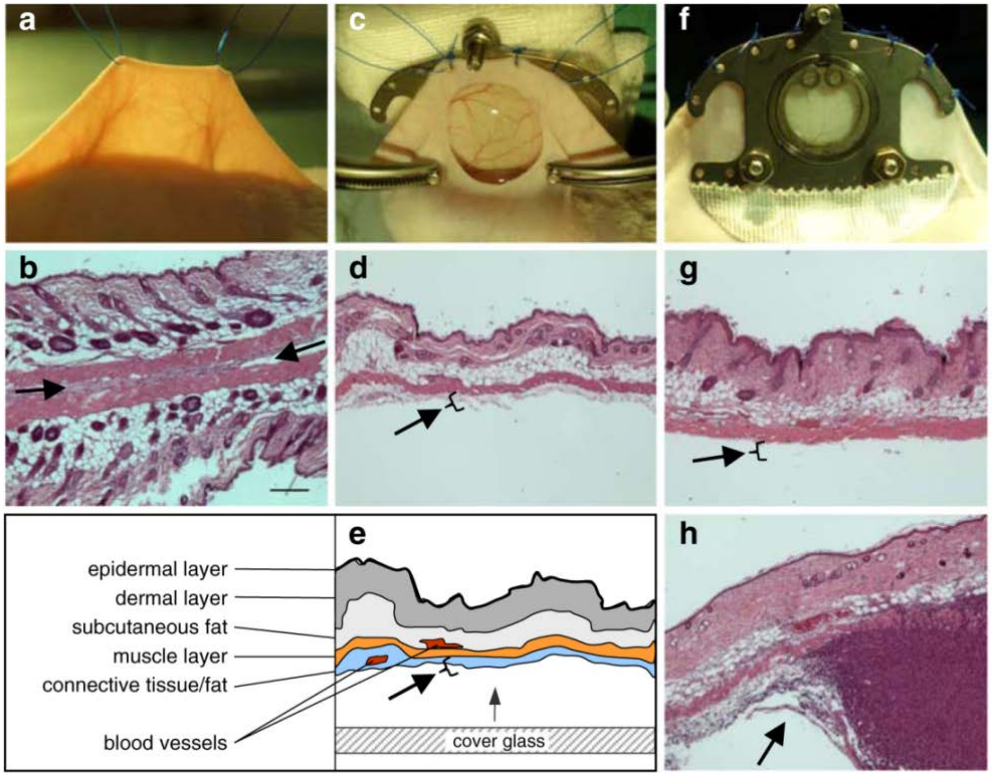
- Excellent spatial distribution
- Multiplexing capabilities
- Yields quantitative measures of cell size and motility
- Dynamic information about microscopic cellular events

Drawbacks

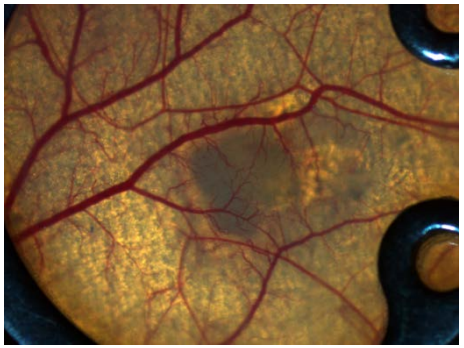
- Poor depth of penetraton
- Small field of view
- Can require multiple laser excitations
- Animal models are limited

Intravital microscopy

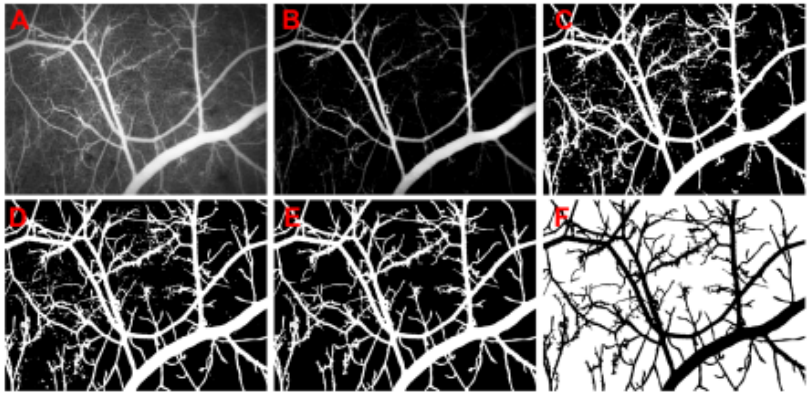
- Direct visual access to the blood vessels and surrounding tissue
- Repetitive observations of the same animal
- In combination with modern microscopy techniques it enables high spatial and time resolution of imaging



Koehl GE. et al, Clin Ex Metastasis, 2009



Tumor grown in dorsal window chamber

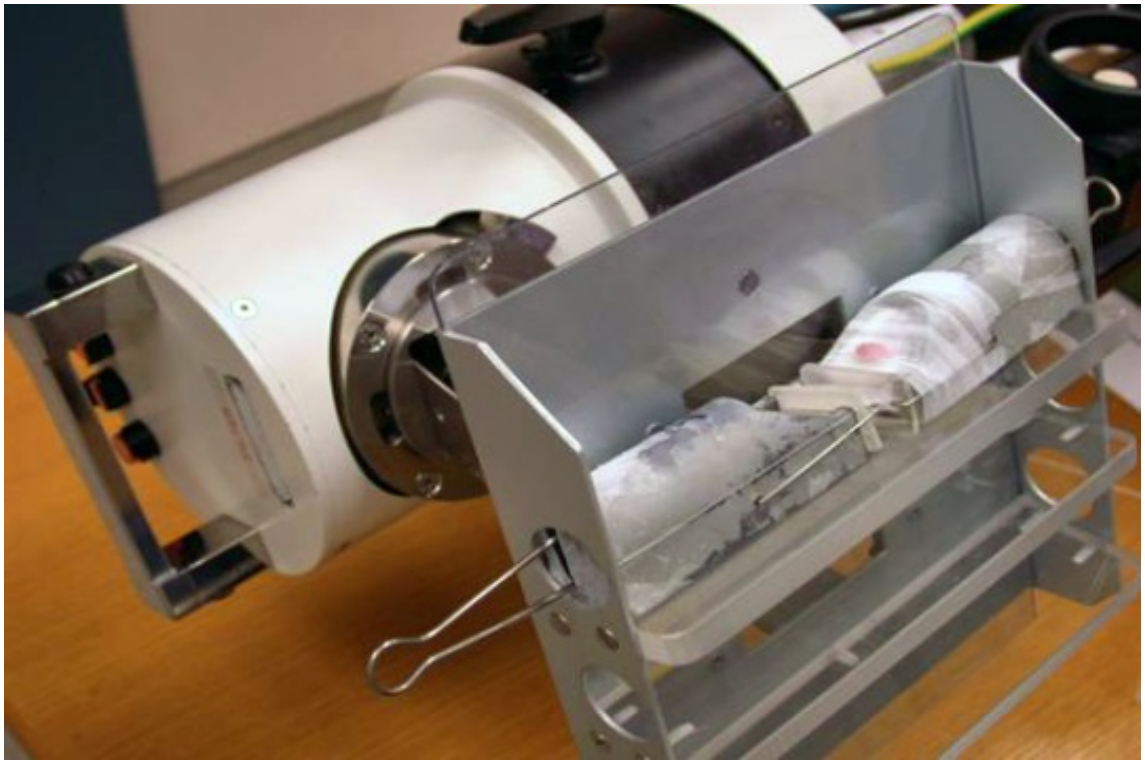


Masks of blod vessels: calculation of fluorescence intensity inside the vessles and in extravascular space

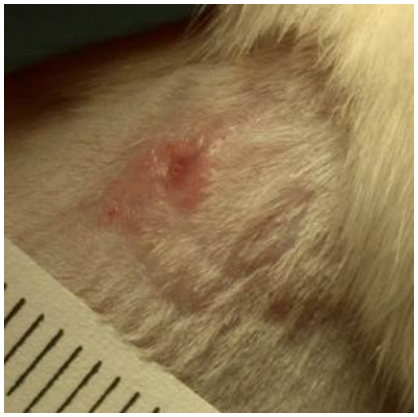
Examples – moderate severity procedures

- irradiation or chemotherapy with a sub-lethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects would be expected to be mild or moderate and would be short-lived (<5 days);
- breeding of genetically altered animals which are expected to result in a phenotype with moderate effects;
- creation of genetically altered animals through surgical procedures;
- use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days);
- studies with modified diets that do not meet all of the animal‘s nutritional needs and are expected to cause moderate clinical abnormality within the time-scale of the study;
- withdrawal of food for 48 hours in adult rats;
- evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus, and are expected to result in moderate distress.

Irradiation - moderate procedure



Setup of tumour irradiation – shielding of the body, while only tumour is exposed to irradiation



Dry desquamation < 20 % of irradiated area

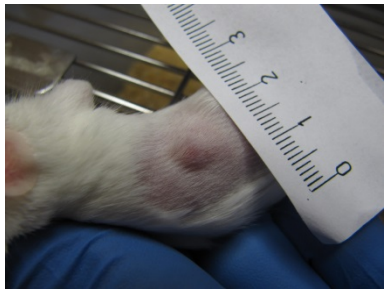
Examples – severe severity procedures

- toxicity testing where death is the end-point, or fatalities are to be expected and severe pathophysiological states are induced – e.g. single dose acute toxicity testing (see OECD testing guidelines);
- testing of a device where failure may cause severe pain, distress or death of the animal (e.g. cardiac assist devices);
- vaccine potency testing characterised by persistent impairment of the animal‘s condition, progressive disease leading to death, associated with long-lasting moderate pain, distress or suffering;
- irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease;
- models with induction of tumours, or with spontaneous tumours, that are expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering – for example tumours causing cachexia, invasive bone tumours, tumours resulting in metastatic spread, and tumours that are allowed to ulcerate;

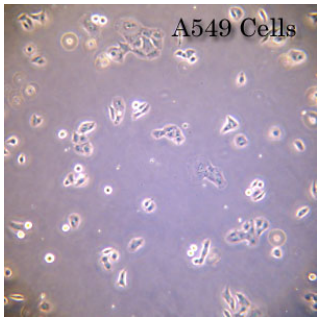
Examples – severe severity classification

- surgical and other interventions in animals under general anaesthesia which are expected to result in severe or persistent moderate postoperative pain, suffering or distress or severe and persistent impairment of the general condition of the animals. Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure;
- organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (e.g. xenotransplantation);
- breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, e.g. Huntington‘s disease, muscular dystrophy, chronic relapsing neuritis models;
- use of metabolic cages involving severe restriction of movement over a prolonged period;
- inescapable electric shock (e.g. to produce learned helplessness);
- complete isolation for prolonged periods of social species e.g. dogs and non-human primates;
- immobilisation stress to induce gastric ulcers or cardiac failure in rats;
- forced swim or exercise tests with exhaustion as the end-point.

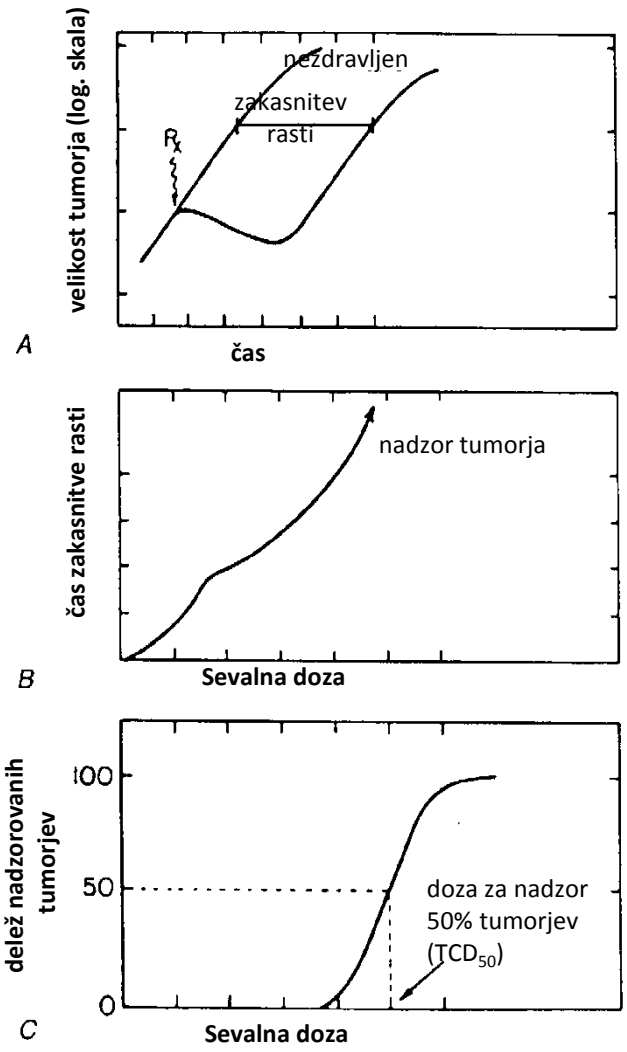
Real example – experiment to assess the effectiveness of new compound on tumour growth



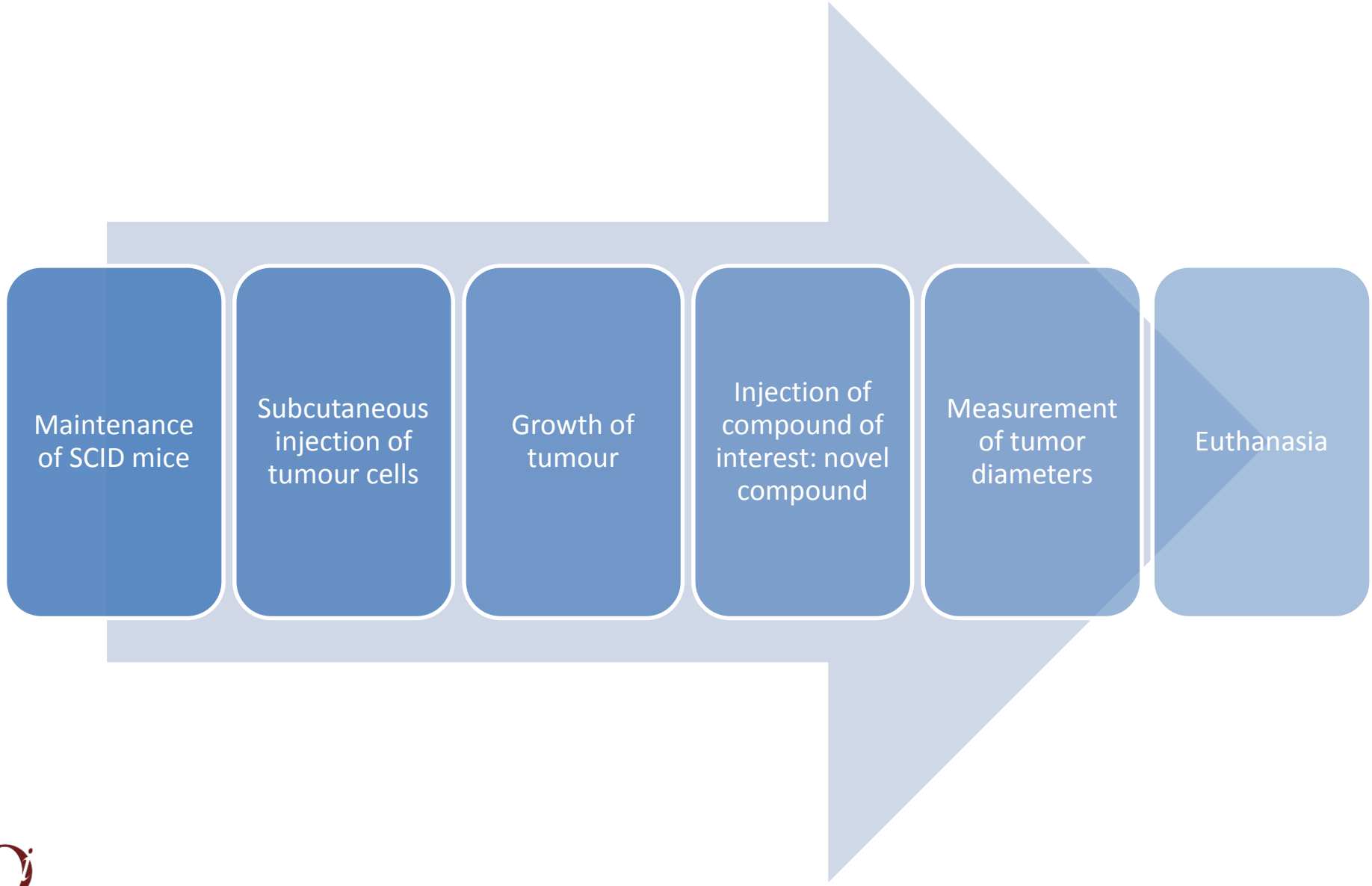
- Mice: SCID mice
- Tumor: human lung adenocarcinoma A549
- Novel compund: C8



- Treatment evaluation: tumor growth delay



Procedures involved in experiment



Real example – experiment to assess the effectiveness of new compound on tumour growth

procedures	Adverse effects	Methodology and intervention	End-points
Maintainance of SCID mice	Mice are susceptible to infection	Housed in IVCs and husbandry practices tailored to minimise risk of contamination Animals group housed and environmental enrichment provided to reduce stress Husbandry and care will be reviewed if any signs of distress, aggression or abnormal behaviours observed	Any animal showing signs of intercurrent disease will be killed

Real example – experiment to assess the effectiveness of new compound on tumour growth

procedures	Adverse effects	Methodology and intervention	End-points
Subcutaneous injection of tumour cells	Transient discomfort due to injection	Injection performed once only Appropriate volume will be injected (maximum of 0.2ml)- ussualy 0.1 ml Animals will be closely monitored during immediate post injection period	Animals will be humanely killed if more than mild distress or discomfort, without rapid recovery, observed following injection (very rare)

Real example – experiment to assess the effectiveness of new compound on tumour growth

procedures	Adverse effects	Methodology and intervention	End-points
Growth of tumours	May cause discomfort or affect normal behaviour or locomotion Tumour used may become infected or ulcerate (but should not metastasise)	Daily observation of animals, regular monitoring of general health and tumour growth Monitoring scheme will include careful observation of posture, gait and tumour size and condition Pharmaceutical interventions will begin when tumour reaches 0.5 cm in diameter (measured by callipers)	Animal will be killed if tumour ulcerates, or interferes with normal behaviour, posture or locomotion, or exceeds 1.2cm in diameter (Workman et al. 2010)

Real example – experiment to assess the effectiveness of new compound on tumour growth

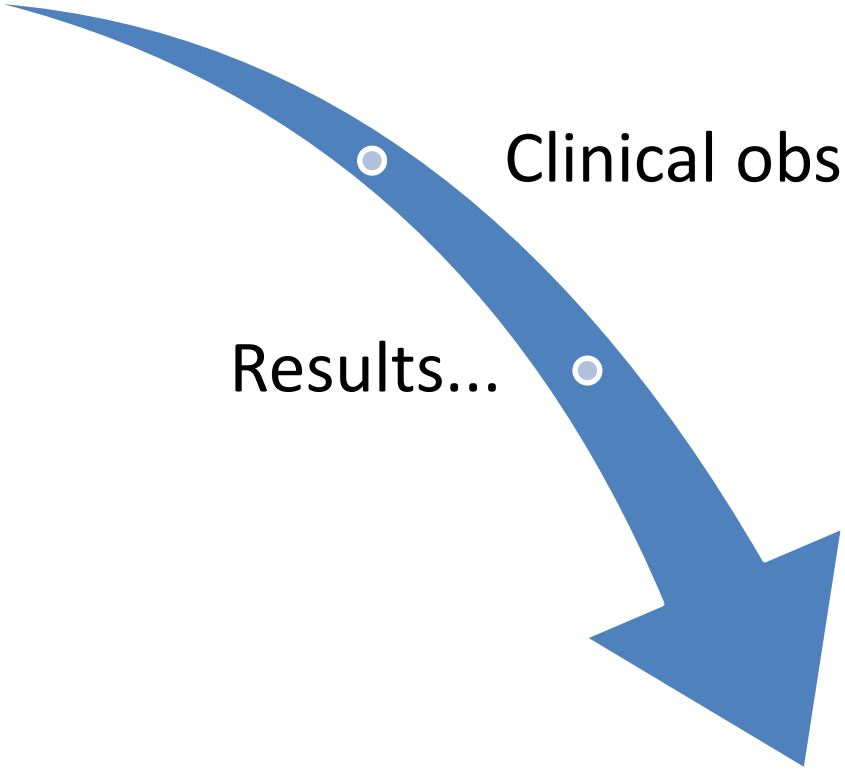
procedures	Adverse effects	Methodology and intervention	End-points
Injection of compound	Transient discomfort following injection Cytotoxic drugs may cause diarrhoea, weight loss, anorexia or lethargy	Pharmaceutical interventions will begin when tumour reaches 0.5 cm in diameter (measured by callipers) Animals will be closely monitored during immediate post injection period Volumes of injected drug will be according to the standards Clinical scoring system will be used to assess welfare	Animals will be killed if weight loss exceeds 20% of initial body weight Animals not eating or having diarrhoea for more than 48 hours will be killed An upper limit for a clinical score will be set as a humane endpoint

Real example – experiment to assess the effectiveness of new compound on tumour growth

procedures	Adverse effects	Methodology and intervention	End-points
Growth of tumours following treatment	May cause discomfort or affect normal behaviour or locomotion Tumour used may become infected or ulcerate (but should not metastasised)	Daily observation of animals, regular monitoring of general health and tumour growth – measurements with calliper. Monitoring scheme will include careful observation of posture, gait and tumour size and condition	Animal will be killed if tumour ulcerates, or interferes with normal behaviour, posture or locomotion, or exceeds 1.2cm in diameter (Workman et al. 2010)

Assesment of severity

Prospective: moderate – due to the possibility of tumour ulceration and adverse effect of drug



Clinical observation –score tabele

Appearance	**Score**
Bodyweight	
5-10% weight loss	1
11-15 % weight loss	2
16-20% weight loss	3
20% + weight loss	HEP
Coat Condition	
Coat slightly unkempt	1
Slight piloerection	2
Marked piloerection	3
Body Function	
Tachypnoea (fast breathing)	1
Dyspnoea (difficulty breathing)	3

HEP – humane endopoint

Clinical observation –score tabele

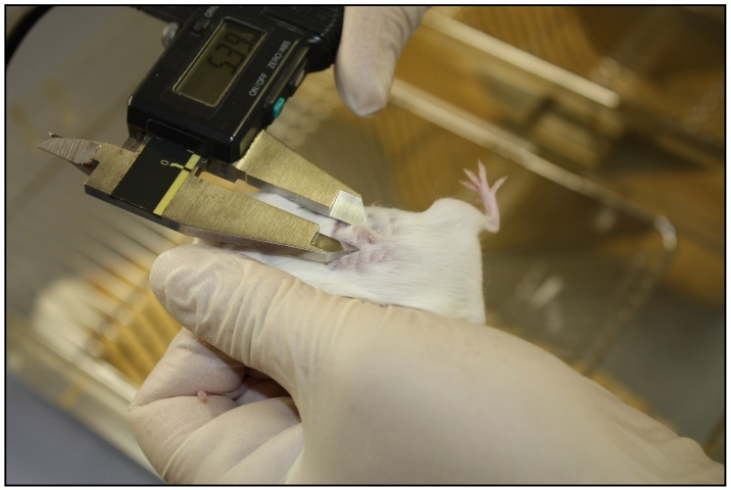
Appearance	Score
Behaviour	
Tense and nervous on handling	1
Markedly distressed on handling, e.g. shaking, vocalizing, aggressive	3
Environment	
Loose stools or diarrhoea	1
Blood in diarrhoea	HEP
Locomotion	
Slightly abnormal gait/posture	1
Markedly abnormal gait/posture	2
Significant mobility problems/reluctant to move	3
Immobility >24h	HEP
Procedure specific indicators	
Tumour size > 1.2 cm	HEP
Tumour ulceration	HEP
Tumour impending movement	HEP

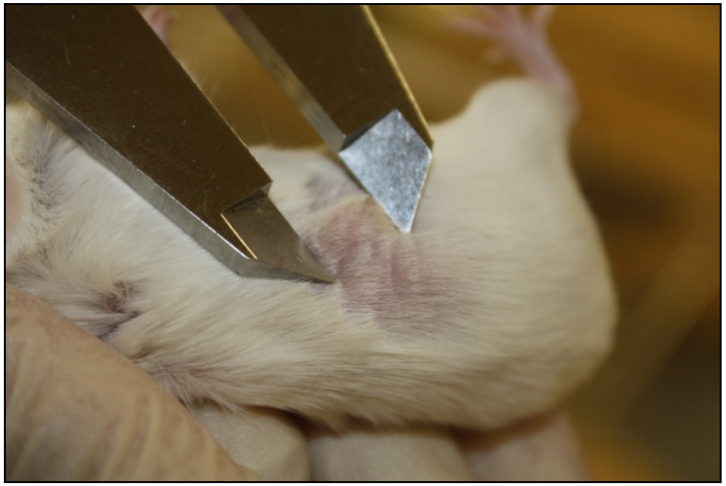
ACTION:

1 – review frequency of monitoring → 2 – consider supplementary care (extra fluids) → 4 – consult veterinarian → 6 - HEP

Score Sheet

Mouse





ALIPER 1.3 File name: KONTROLA 01.xls

NEW GROUP SAVE SAVE AS CLOSE QUIT

Time - days

	07.10.2009	08.10.2009	09.10.2009	10.10.2009	11.10.2009	12.10.2009	13.10.2009	14.10.2009	15.10.2009
0100	48,9		68,9			249,7			306,7
0101	34,3		108,8			201,2			252,4
0110	40,3		102,5			272,5			381,4
0111	42,0		90,2			171,0			347,1
0102	48,3		92,1			210,6			264,4
0120	46,7		105,4			359,7			449,9
0112									
0121									
0122									
0103									
0130									
0113									
0131									
0123									
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0133									
0104									
0140									
0114									
0141									
0124									
0142									
0134									
0143									
0144									
a	5,75		7,67			8,60			12,74
b	6,64		8,32			9,52			11,00
c	2,10		2,70			3,99			4,73
mass	0		25,9			25,7			25,3
St									

Measurement of three perpendicular diameters

Body weight

Status of mouse – other notes in LAB BOOK

a b c mass St

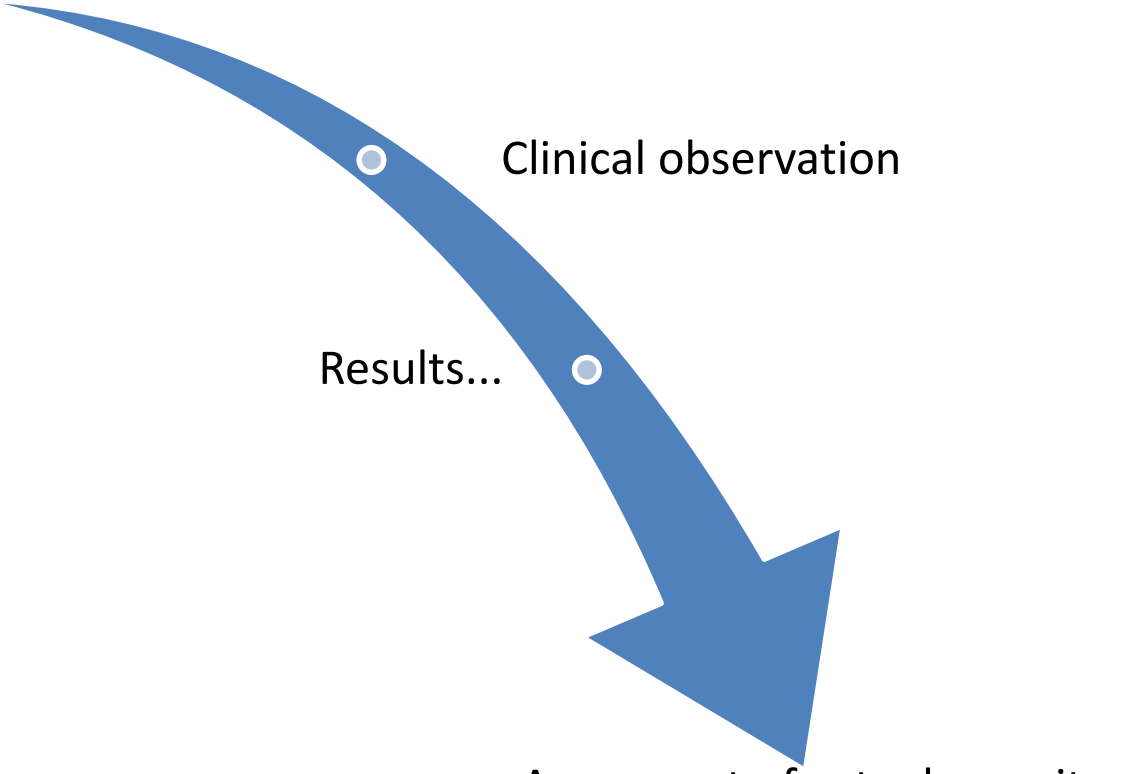
a * b * c * Pi / 6

Clinical observation – assesment of actual severity

- 3 mice did not developed tumors – euthanised as unusuable for experiment – MILD
- 2 animals developed ulceration at the tumour injection site before treatment started and were euthanised – MODERATE (rare if you are familiar with the tumor model)
- 10 mice receiving dose A had a BW loss of 15% - MODERATE
- **OVERALL CLASSIFICATION for the project: MODERATE**

Assesment of severity

Prospective: moderate – due to the possibility of tumour ulceration and adverse effect of drug



References and further reading

- Workman P et al. Guidelines for the welfare and use of animals in cancer research British Journal of Cancer 2010; 102:1555-77.
- Rodent Tumor Models in experimental cancer therapy. Kallman RF ed. 1987. Pergamon press.
- Tumor models in Cancer Research. Teicher BA. Ed. 2002. Humana press.
- James ML, Gambhir SS. A molecular imaging primer: modalities, imaging agents, and applications. Physiol Rev 2012; 92: 897-965.

Thank you for your attention!

Boy, I would love to be his pet cat!