

Guidelines

Guidelines for the welfare and use of animals in cancer research

P Workman^{*1}, EO Aboagye², F Balkwill³, A Balmain⁴, G Bruder⁵, DJ Chaplin⁶, JA Double⁷, J Everitt⁸, DAH Farningham^{9,18}, MJ Glennie¹⁰, LR Kelland¹¹, V Robinson¹², IJ Stratford¹³, GM Tozer¹⁴, S Watson¹⁵, SR Wedge¹⁶, SA Eccles^{*1}, An *ad hoc* committee of the National Cancer Research Institute¹⁹,
Observers: **V Navaratnam¹⁷ and S Ryder¹⁷**

¹Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, UK; ²Comprehensive Cancer Imaging Centre, Imperial College London Faculty of Medicine, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK; ³Centre for Cancer & Inflammation, Barts and The London School of Medicine and Dentistry, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK; ⁴Helen Diller Family Comprehensive Cancer Center, University of California San Francisco 1450 3rd Street, San Francisco, CA 94158, USA; ⁵Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Manchester M20 4BX, UK; ⁶OXiGENE Inc., 701 Gateway Boulevard, San Francisco, CA 94080, USA; ⁷University of Bradford, Richmond Road, Bradford BD7 1DP, UK; ⁸GlaxoSmithKline Pharmaceutical R&D, PO Box 13398, Five Moore Drive, N2.2210.2B, Research Triangle Park, NC 27709-3398, USA; ⁹Cancer Research UK, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts EN6 3LD, UK; ¹⁰Tenovus Laboratory, Cancer Sciences Division, Southampton University School of Medicine, General Hospital, Southampton SO16 6YD, UK; ¹¹Cancer Research Technology Development Laboratories, Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, UK; ¹²National Centre for the Replacement, Refinement and Reduction of Animals in Research 20, Park Crescent, London W1B 1AL, UK; ¹³School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK; ¹⁴Department of Oncology, K Floor, School of Medicine, University of Sheffield, Beech Hill Road, Sheffield S10 2RX, UK; ¹⁵Division of Pre-Clinical Oncology & PRECOS, D Floor West Block, Queen's Medical Centre, University Hospital, Nottingham NG7 2UH, UK; ¹⁶Cancer Bioscience, AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK; ¹⁷The Home Office, ASPD (mail point 1B), 1st floor Seacole Building, 2 Marsham Street, London W1P 4DF, UK; ¹⁸Current address: Medical Research Council, 20 Park Crescent London W1B 1AL, UK

British Journal of Cancer (2010) 102, 1555–1577. doi:10.1038/sj.bjc.6605642 www.bjcancer.com
© 2010 Cancer Research UK

Keywords: animal welfare; cancer research; fundamental and translational research; replacement, reduction and refinement (3Rs); pilot studies; tumour models; genetically engineered mouse models; human tumour xenografts; orthotopic models; metastatic models; therapy; imaging; pharmacokinetic, pharmacodynamic and efficacy studies; drugs; radiation therapy; imaging techniques; anaesthesia; restraint; humane endpoints; tumour burden; clinical signs; publication; best practice

Abstract: Animal experiments remain essential to understand the fundamental mechanisms underpinning malignancy and to discover improved methods to prevent, diagnose and treat cancer. Excellent standards of animal care are fully consistent with the conduct of high quality cancer research. Here we provide updated guidelines on the welfare and use of animals in cancer research. All experiments should incorporate the 3Rs: replacement, reduction and refinement. Focusing on animal welfare, we present recommendations on all aspects of cancer research, including: study design, statistics and pilot studies; choice of tumour models (e.g., genetically engineered, orthotopic and metastatic); therapy (including drugs and radiation); imaging (covering techniques, anaesthesia and restraint); humane endpoints (including tumour burden and site); and publication of best practice.

*Correspondence: Professor P Workman and Dr SA Eccles;
E-mail: Paul.Workman@icr.ac.uk and Sue.Eccles@icr.ac.uk

We dedicate these Guidelines to Professor Lloyd Kelland and Dr Peter Twentyman, who made important contributions to these Guidelines and/or previous published versions, and who have now sadly passed away.

¹⁹The National Cancer Research Institute (NCRI) is a partnership of 21 organisations from the government, charity and commercial sectors who support cancer research in the UK. Further information about NCRI can be found at <http://www.ncri.org.uk>

Received 5 March 2010; accepted 15 March 2010

LAY SUMMARY

In order for scientists to understand how cancers develop and spread throughout the body and to discover new and more effective ways to diagnose and treat cancer, it is necessary to carry out research on live animals. Animal studies (over 95% of which are conducted in mice) are essential to understand the complexities of the fundamental processes that underpin cancer within living organisms. They are also required by regulatory authorities before any trials of new drugs can be tested in humans. Animal studies are only performed after every feasible test has been conducted on cancer cells in the laboratory and where no alternative exists. Adverse effects on the animals are minimised as far as possible. However, it is a source of concern for society and research scientists alike that, as we cannot replace all animal experiments in the immediate future, the highest standards of welfare are upheld. This publication builds on two previous sets of guidelines to provide updated and enhanced recommendations for the care and use of animals in cancer research; to develop procedures that reduce, replace or refine animal studies; and to communicate best practice throughout the world. In all cases, however, experimental designs and procedures should be tailored to the needs of the specific studies.

BACKGROUND AND SCOPE

Over the last decade there has been an extraordinary increase in our knowledge of the fundamental molecular processes that are involved in the development of cancer and its response to treatment (Hanahan and Weinberg, 2000; Vogelstein and Kinzler, 2004; Stratton *et al*, 2009). The public rightfully expect this explosion in basic research understanding to be translated into rapid improvements in prevention, diagnosis and treatment, particularly for the more common cancers and indeed for any malignant disease where there is still clearly an unmet need for more effective therapies. In recent years the identification of the genes and pathways that give rise to cancer dependencies and vulnerabilities has taken us further towards the development of individualised, molecularly targeted therapies (Sawyers, 2004; Collins and Workman, 2006; Workman and de Bono, 2008).

Along with growth in fundamental knowledge and greater translational insight has come the development of new *in vitro* and *ex vivo* methodologies and research techniques that should further extend our still incomplete genetic, molecular and holistic understanding of cancer, and in addition should help to ensure that improved methods for diagnosis, therapy and prevention will be developed more effectively for patient benefit. Nevertheless, we are still some way from the point where all of the necessary information that is required to introduce a new drug into the clinic in terms of safety and efficacy could be gained without the use of animals in research. Moreover, animals remain essential to extend our understanding of the mechanisms responsible for cancer and to identify, for example, new targets and biomarkers.

It is clearly important that the welfare of animals in cancer research is protected, both from an ethical point of view and also because it is widely acknowledged to be entirely consistent with good science (Osborne *et al*, 2009). Under the earlier sponsorship of the former United Kingdom Coordinating Committee for Cancer Research (UKCCCR), two sets of guidelines have been published previously (Workman, 1988; Workman *et al*, 1998). Although these guidelines were well received, and are still widely used and cited, it is over 10 years since they were last revised, in which time the science has moved on appreciably. The main aim of this article is to provide new guidelines for the cancer research community concerning the use of experimental animals in oncology, with a major emphasis on their welfare. We focus on rodents as these are predominantly used for cancer research: in 2008, for example, the UK government Home Office statistics showed that 96.8% of animals used in cancer research were mice (<http://scienceandresearch.homeoffice.gov.uk/animal-research/publications-and-reference/statistics/index.html>). While development of medicines may require testing in other species, use of animals in regulatory toxicology is outside the scope of this review.

The present guidelines should be applied to studies focused on all aspects of cancer research, including experiments aimed at understanding fundamental cancer biology as well more translational work, and should be used in conjunction with more general guidelines for the care and welfare of animals (see examples below and Additional information). It is expected that animal housing will be maintained according to the highest standards, including environmental enrichment (Tsai *et al*, 2006), and that local ethical review will precede any experimental animal studies. In addition, these guidelines should be used in conjunction with appropriate national legislation: UK Animals (Scientific Procedures) Act 1986; USA Institute for Laboratory Animal Research (ILAR) Guide for the Care and Use of Laboratory Animals (<http://dels.nas.edu/Laboratory>); EU webpage on laboratory animals (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm); Public Health Service Policy on Humane Care and Use of Laboratory Animals (Office of Laboratory Animal Welfare, National Institutes of Health,

2002); <http://grants.nih.gov/grants/olaw/references/phspol.htm>. A complementary key recent publication, coordinated by the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R), is also recommended (Biotechnology and Biological Sciences Research Council; Department for Environment, Food and Rural Affairs; Medical Research Council; Natural Centre for the Replacement, Refinement and Reduction of Animals in Research; Natural Environment Research Council; Wellcome Trust, 2008). We also feel it is important that the public is made fully aware of the current justification for the use of animals in cancer research and the genuine concern for their welfare by researchers involved with their use. To help with this, a lay summary of the guidelines is also provided on page 1555. A glossary of terms can be found at the end of this article. Finally, it is important to emphasise that high standards of animal care and welfare should be fully consistent with, and helpful to, the conduct of high-quality cancer research (Osborne *et al*, 2009).

GENERAL RECOMMENDATIONS

The use of animals raises scientific and ethical challenges. In 1959, Russell and Burch published *The Principles of Humane Experimental Technique* in which they stated that all animal experiments should incorporate, as far as possible, the 3Rs: replacement (of animals with alternative methods), reduction (in the numbers of animals used to achieve scientific objectives) and refinement (of methods to minimise animal suffering) (Russell and Burch, 1959). These principles underpin the legislation, guidelines and working practices concerning the use of animals in scientific procedures. Consideration of the 3Rs must be an integral part of planning cancer research using animals and the 3Rs need to be implemented throughout the lifetime of the study. Funding bodies and scientific journals (Osborne *et al*, 2009) should encourage scientists to use humane methods, to supply information on how the principles of the 3Rs are implemented and to publish improvements in experimental design and animal models for the benefit of the research community (www.nc3rs.org.uk/reportingguidelines). Details on the application of the 3Rs in cancer research are provided in Box 1 for ease of reference, together with information on implementation and monitoring in Box 2. Examples of tumour models, experimental design and procedures are provided throughout these guidelines. However, it is emphasised that these are intended to act as a guide only, and each study should be tailored to the specific experimental objectives.

TUMOUR MODELS

Preclinical cancer studies fall into two broad categories: those using tumour cell transplantation (Tables 1A and B), and those in which tumours arise or are induced in the host (Tables 2A and B). The choice of animal model depends on the scientific question being investigated, but the mildest possible procedure should always be used. An example of the type of illustrative aid that can be used to facilitate the rational choice of appropriate models is shown in Figure 1. Cellular interactions and immune responses require immunocompetent animals and syngeneic systems, whereas cancer development or chemoprevention studies may use transgenic models or chemically induced tumours. In the case of translational studies designed, for example, to discover and develop therapies to exploit oncogenic abnormalities, the tumours should have the appropriate molecular genetic defect. Furthermore, real-time optical imaging will require engineered bioluminescent/fluorescent tumour models.

Transplantation tumour models

These normally involve the transplantation of mouse or rat tumour cells into a host of the same (syngeneic) species and strain. Growth

**Box 1 THE 3Rs:
REPLACEMENT, REDUCTION AND REFINEMENT****Replacement**

Absolute replacement techniques avoid the use of animals; relative replacement techniques include substituting non-vertebrate species

1. Investigate the potential of novel and existing alternative approaches to animals
2. Use *in silico* and/or *in vitro* pre-screens before commencing animal studies

Reduction

Minimise the number of animals used to achieve specific scientific objectives

1. Ensure that all studies are scientifically robust and apply appropriate statistical methods to experimental design
2. Reduce experimental variability by conducting studies of animals of defined health status and, wherever possible, using inbred strains
3. Minimise surplus breeding by avoiding unnecessarily narrow specifications for animal sex, age and weight
4. Freeze rodent embryos, sperm and cancer cell lines not immediately required for scientific studies
5. Prevent duplication by making specific strains and genetically modified lines available throughout the research community
6. Consider use of serial sampling or longitudinal imaging in which each animal acts as its own control to reduce study group sizes

Refinement

Continual review of improvements in experimental design, techniques and husbandry to minimise adverse effects and improve welfare

1. Apply all available knowledge to predict adverse effects and ensure that appropriate humane endpoints are developed and specialist care is provided, especially when using genetically modified animals (e.g., immune deficient or tumour-prone strains)
2. Provide animals with an appropriate environment (e.g., nesting material, shelter for rodents), including sufficient space and complexity to satisfy their normal species-typical behaviours
3. Undertake pilot studies of unfamiliar tumour cell lines or novel procedures to establish experimental and humane endpoints
4. Perform post-mortem examinations as a routine part of all pilot studies and to investigate any unexpected deaths
5. Include appropriate controls to understand individual and combined effects of tumours and treatments
6. Use anaesthesia and analgesia whenever appropriate. This should be regularly reviewed by a vet to ensure that contemporary best practice is followed
7. Consider imaging methods to monitor non-superficial tumour burden and to aid the timely implementation of humane endpoints
8. Do not allow animals to become moribund: death as an intentional endpoint is unacceptable
9. Maintain and share detailed information, on all experimental procedures, including behaviour of the tumour and host animals under various conditions
10. Include principal details relating to the use of animals, such as study design, adverse effects, specialist care and the 3Rs, in all scientific publications

of human (xenogeneic) tumour cells can be achieved using immunodeficient (e.g., nude or SCID) mice to prevent rejection (Table 1A). Most transplantable tumours are established subcutaneously. These subcutaneous (s.c.) tumours are simple to initiate but may lack relevance in terms of stromal/vascular interactions and metastasis. More complex models may involve orthotopic transplantation at appropriate primary sites, or inoculation of tumour cells through routes which maximise the chance of

Box 2 IMPLEMENTATION AND MONITORING

For assurance that best practice is implemented and rigorously applied, practical guidelines should be clear and readily available, with staff fully engaged and educated in their use

1. Establish a clear chain of responsibility to ensure that prompt action is taken where necessary: for example, if the condition of an animal deteriorates unexpectedly
2. Establish humane endpoints, specialist care, monitoring methods and criteria for intervention between researchers, veterinary and animal care staff before initiating studies
3. Ensure that all staff involved in the use and care of animals are aware of their personal and legal responsibilities, and are trained to recognise adverse effects and apply humane endpoints
4. Seek additional external expertise where unfamiliar models or techniques are being introduced
5. Ensure adequate staffing levels are available for the duration of the study, particularly during crucial periods where additional observations are needed
6. Inspect animals at a frequency determined by the known biology of the tumour, the effects of any interventions and the clinical status of the animals
7. Monitor adverse effects, including general signs of welfare and more detailed indicators appropriate to the specific model and procedures
8. Give all staff appropriate training and professional development opportunities
9. Provide appropriate supervision of staff and confirm, review and document competence on a regular basis

metastatic spread (Table 1B). There is an increasing trend to establish xenograft tumours directly from human cancers, to avoid artificial selection of cells in tissue culture and changes in gene expression and phenotype, which this may induce. Such transplants may better model the principal facets of clinical cancer, for example, maintenance of tumour architecture, heterogeneity, expression of certain targets and response to therapy (Dong *et al*, 2010), but can be less reproducible (especially as primary grafts) and slower growing than well-established models (Neale *et al*, 2008; Rubio-Viqueira and Hidalgo, 2009). Detailed molecular and genetic characterisation, facilitated by modern high-throughput technologies (e.g., see <http://www.sanger.ac.uk/genetics/CGP>), is now available for human cancer cell lines used for xenografts (Masters *et al*, 2001; Park *et al*, 2010) and is important to understand the biology of these models and to select the most appropriate for each study.

Autochthonous tumour models

There are two broad categories: those arising in outbred or inbred rodents (Table 2A), or those from animals harbouring genetic changes that alter tumour susceptibility (Table 2B). Certain mouse or rat strains are susceptible to spontaneous development of tumours. More commonly, tumours are induced by chemical carcinogens, radiation, viruses or bacteria. Such models may mimic some of the aetiological events in human cancer development; exposure to such agents may induce systemic effects that are difficult to replicate in genetically engineered models.

Major advances have been made in the development of sophisticated mouse models of cancer that mimic many of the genetic and biological characteristics of human malignancies, although the host genetic background may affect tumour incidence and/or malignant potential (Lifsted *et al*, 1998; Winter and Hunter, 2008). A range of technologies now allows the inducible expression of oncogenes or inactivation of tumour-suppressor genes *in vivo* in a precisely controlled manner in virtually any tissue or cell type. (Chen *et al*, 2004; Christophroru *et al*, 2006; Sharpless and DePinho, 2006). Such genetically engineered mouse models

(GEMMs) provide excellent experimental systems to develop a deeper understanding of cancer biology *in vivo* and are increasingly being used for preclinical testing of molecularly targeted therapies, as they depend on or are 'addicted' to the specific molecular abnormalities and biochemical pathways engineered to drive the malignant process.

Routine use of GEMMs for preclinical testing of anticancer therapies can be hampered by variable tumour latency, incomplete penetrance and complicated breeding schemes. The full potential of such mouse models is yet to be realised and further work is required to derive maximum benefit for cancer patients from these initiatives (Frese and Tuveson, 2007). Newer models (e.g., exploiting double or multiple genetic abnormalities) have resulted in enhanced tumorigenicity and metastatic capacity, and some studies have shown that mouse cancer models with relevant human gene mutations respond to appropriate targeted therapies (Politi *et al*, 2006), and also may develop common secondary mutations associated with acquired resistance (Politi *et al*, 2010). As an example of target validation, reversible, systemic expression of a dominant-negative mutant Myc oncogene in transgenic Ras-induced lung carcinoma model caused the tumours to regress, whereas effects on normal regenerating tissue were well tolerated and reversible (Soucek *et al*, 2008). To overcome heterogeneity issues, transplantation of transgenic tumours can provide higher throughput models, for example, for testing therapeutics (Varticovski *et al*, 2007). Commonly used GEMMs include mammary carcinomas induced by the viral oncogene polyoma virus middle T (Guy *et al*, 1992; Fluck and Haslam, 1996; Marcotte and Muller, 2008) or by the human or rat *Her2/neu* oncogene (Chan *et al*, 1999; Quaglini *et al*, 2008), or colon adenomas and carcinomas induced by inactivation of the adenomatous polyposis coli (APC) tumour-suppressor gene (Taketo, 2006). Space constraint does not allow a full description or listing of the many more sophisticated, patient-like models now available, examples of which are shown in Table 2B. The reader is referred to the more complete information available at http://emice.nci.nih.gov/mouse_models.

A key question that continues to be debated is whether human cancer xenografts or murine transgenic models best reflect the human disease in terms of biology and predictions of

efficacy of therapeutic agents (Becher *et al*, 2006; Dennis, 2006; Garber, 2006; Sausville *et al*, 2006; Sharpless and DePinho, 2006). Some GEMMs have shown patterns of sensitivity to chemotherapeutic agents and development of resistance that are similar to their human tumour counterparts (Rottenberg and Jonkers, 2008). The predictive value of neither type of model has been fully established; however, there is agreement that molecular characterisation of all tumours is required to underpin the choice of model.

Selection and optimisation of experimental systems

As mentioned, selection of tumour models should be based on both molecular characteristics, for example, expression or mutation of a target of interest or other relevant molecular pathology, either endogenously or through transfection/transgenic technology, together with desired properties such as the rate and reproducibility of growth, metastatic potential and chemosensitivity.

Cell line verification and molecular characterisation

Given the frequency of misidentification and cross-contamination (Nardone, 2007; Lacroix, 2008) it is essential that all cell lines are rigorously checked for their provenance and genetic identity (Parodi *et al*, 2002; Yoshino *et al*, 2006). It is also important that cell lines are free from contamination with infectious agents such as mycoplasma, which can influence their biological behaviour and present a risk to handlers and animals (Ishikawa *et al*, 2006; Sung *et al*, 2006; Harlin and Gajewski, 2008). Regardless of origin, detailed characterisation of tumours should be performed and checked periodically to ensure that desired properties are maintained and are commensurate with the molecular pathology of the corresponding human malignancy (Santarius *et al*, 2010). A thorough literature review should establish their reported tumorigenic and immunogenic properties, with special attention paid to the selection of the correct host animal strain and sub-strain. Residual immune responses to xenografted tumours in nude/SCID mice may occur and the sex of the host should be

Table 1A Transplantable tumour models

	Examples of models	Advantages	Disadvantages
Syngeneic	MC26 colon cancer in BALB/c mice (Alsheikhly <i>et al</i> , 2004)	Conventional rodents and normal housing	Tend to be aggressive and grow over a short time frame
	BI6 melanoma in C57/Bl mice (Rusciano <i>et al</i> , 1994)	Covers wide range of tumour types	Not applicable if investigating human-specific parameters
	4T1 mammary carcinoma in BALB/c mice (Kim <i>et al</i> , 2009)	Models immune and stromal interactions	The genetics and histology of tumours may not reflect the human situation
Xenogeneic	HCT116 colon cancer in athymic mice (Huxham <i>et al</i> , 2004)	Allows direct investigation of human cells	Requires genetically immunodeficient (nu/nu or SCID) animals
	PC3 prostate cancer in athymic mice (Patel <i>et al</i> , 2002)	Human cancer cell lines are increasingly being characterised by genetic and other molecular techniques (Ihle <i>et al</i> , 2009; Bignell <i>et al</i> , 2010)	Requires sterile isolation and ACDP containment level-II
	Systemic leukaemias in irradiated NOD/SCID mice (Liem <i>et al</i> , 2004; Lock <i>et al</i> , 2005)	Can be an established cell line or human primary tissue (Neale <i>et al</i> , 2008; Rubio-Viqueira and Hidalgo, 2009)	Genetically modified cells will require ACGM containment
	GFP transgenic mice to enhance visualisation of tumour–host interactions (Yang <i>et al</i> , 2004)	Amenable for immune reconstitution	May not be suitable for use with agents modifying the immune system or where cellular interactions are being investigated
	Luciferase-expressing cancer cells for bioluminescent imaging (Dickson <i>et al</i> , 2007; Comstock <i>et al</i> , 2009; Shibata <i>et al</i> , 2009)	Can be grown s.c. or orthotopically	

Abbreviations: ACDP = Advisory Committee on Dangerous Pathogens; ACGM = Advisory Committee on Genetic Modification; GFP = green fluorescent protein; NOD = non-obese diabetic; s.c. = subcutaneous; SCID = severe combined immunodeficient.

Table 1B Transplantation models used for therapeutic studies

Transplantation site	Notes and examples of models	Advantages	Disadvantages
Subcutaneous tumour transplantation	A high % of established lines will grow at this site	Simplest model for routine evaluation of anticancer agents Uses cancer cells or tissue Simple administration with anaesthetic only required for tissue transplantation Allows continuous monitoring Facilitates the use of bilateral transplantation after pilot studies	Ectopic site for most tumours Metastases rarely develop from s.c. tumour xenografts May be poorly vascularised with necrotic centres
Tumour cells within hollow fibres	Amenable for use with a wide range of cell lines (Decker <i>et al</i> , 2004)	Allows multiple cell lines to be implanted for simultaneous assessment Useful for human cell lines that fail to grow as xenografts Short-term assay requiring ex vivo end-stage analysis Provides a quick assessment of drug efficacy and may predict xenograft response	Careful scoring system required Lack of vasculature may miss 'indirect' effects or access issues Inflammation around fibres can affect responses Variation in properties of fibres Possibility of paracrine effects if multiple cell lines implanted in same animal
Experimental metastasis (site of spread)			
Intravenous (primarily lung)	3DC13B lymphoma (Golay <i>et al</i> , 2006) M24met melanoma (Becker <i>et al</i> , 1996) A549 NSCLC (Kennel <i>et al</i> , 1999) Lewis lung 3LL (Li <i>et al</i> , 2001)	Most cells injected i.v. colonise lung Some (notably lymphoid tumours) may colonise other organs Models end-stage of metastasis in a controlled manner Simple model to establish Cells may be tagged for imaging	Bolus injection of cells does not mimic natural dissemination and the 'conditioning' effect of primary tumour growth Does not model the full spectrum of metastatic cascade Difficult to monitor and quantify accurately without imaging Pilot studies required to establish incidence and growth kinetics
Intra-peritoneal (ascites and peritoneal/omentum-associated tumours)	GW-39 colon (Sharkey <i>et al</i> , 1991) MGLVA1 gastric (Watson <i>et al</i> , 1999b) OVCAR3 ovarian (Zavaleta <i>et al</i> , 2008)	Mimics late-stage ovarian cancer and carcinomatosis associated with some abdominal cancers	Difficult to quantify
Intra-tibial ^a (bone)	ARCaP prostate (Zhau <i>et al</i> , 2000)	Mimics bone tumour growth seen with myeloma or prostate and breast cancer metastases	Does not mimic natural tumour dissemination Risk of infection and pain Requires imaging
Intra-cardiac ^a (bone)	MDA MB 231 breast (Serganova <i>et al</i> , 2009) A375 melanoma (Nakai <i>et al</i> , 1992) Mat Ly-lu prostate (Blouin <i>et al</i> , 2008)	To some extent mimics the dissemination phase of metastasis	Requires advanced surgical technique Risky procedure requiring particular care Only suitable for a limited number of cell lines
Intra-portal vein (liver, nodes)	LS174T colon (Mahteme <i>et al</i> , 1998)	Localises tumour cells to liver Appropriate for colon, pancreatic and breast cancers	Requires advanced surgical technique to avoid cell leakage into peritoneal cavity
Intrasplenic (liver)	WiDR colon (Miyazaki <i>et al</i> , 1999)	Easier procedure than mesenteric vein or intraportal cell inoculation	Local growth in spleen may complicate the interpretation of results unless splenectomy is performed
Orthotopic and spontaneous metastasis models (sites of metastasis)			
Intra-caecal/lymphoid follicle (liver)	TK-3,-4,-9 colon (Tanaka <i>et al</i> , 1995)	Relevant stromal/vascular cell interactions Models the full metastatic cascade	Most require delicate surgical procedures Risk of local tumour spread
Intra-dermal (lung/nodes/liver)	NC65 renal (Nakatsugawa <i>et al</i> , 1999) A-07melanoma (Graff <i>et al</i> , 2005)	Certain models may involve lymph node spread	Risk of infection
Mammary fat pad (lungs/nodes/brain)	MDA-MB-231 breast (Serganova <i>et al</i> , 2009) 4T1 breast (murine) (Mitra <i>et al</i> , 2006)	Cells may be genetically tagged to allow real-time imaging Chemosensitivity more relevant	Pilot studies must be used to determine the time frame of disease events

Table 1B (Continued)

Transplantation site	Notes and examples of models	Advantages	Disadvantages
Muscle layer of peritoneal cavity (lymph nodes/lungs/liver)	AP5LV colon (Watson <i>et al</i> , 1999a) UCRU-BL-17 clone 28B bladder (Russell <i>et al</i> , 1991)	Human tumour micro-environment modelled by co-transplantation with human stromal cells	End-stage quantification may be difficult if real-time imaging is not used
Bladder wall ^a (lung)	HepG2 Hepatocellular (Han <i>et al</i> , 2005)	Spontaneous metastasis models may facilitate simultaneous investigation of primary and secondary lesions	
Liver	Morris hepatoma (rat) (Hirayama <i>et al</i> , 2006)		
Pancreas	PANC-1 (Harris <i>et al</i> , 2004)		
Stomach	SGC-7901 gastric (Zhu <i>et al</i> , 2007)		
Brain ^a	C6 glioma (rat) (Takeda and Diksic, 1999) T9 glioma (rat) (Graf <i>et al</i> , 2005) U87MG, U251 gliomas (Radaelli <i>et al</i> , 2009)		
Kidney capsule (lung/liver)	RCC renal (Zisman <i>et al</i> , 2003) RENCA (mouse) (Verheul <i>et al</i> , 2007)		
Prostate (nodes, bone)	PC3 prostate (Patel <i>et al</i> , 2002)		
Immune reconstitution	Primary leukaemic cells (Nijmeijer <i>et al</i> , 2001) A20 B-lymphocytic (murine) (Glass <i>et al</i> , 1996)	Allows relevant stromal/immune interactions	Immune reconstitution requires whole-body irradiation
Matrigel plug assays for testing antiangiogenic agents	Amenable for use with a range of endothelial or tumour cell lines (Kragh <i>et al</i> , 2003)	Short-term assay allowing <i>ex vivo</i> analysis Requires small doses of compounds Allows quantitative (endpoint) analysis of angiogenesis	Subcutaneous system End-stage analysis May not fully mimic tumour angiogenesis
Dorsal skin-fold chamber	Amenable for use with a range of endothelial or tumour cell lines (Koehl <i>et al</i> , 2009)	Enables real-time visualisation of angiogenesis	Highly invasive procedure. Risk of inflammation/infection

Abbreviation: i.v. = intravenously. ^aThese models are specialised and not recommended for routine use. All examples involve human cell lines unless indicated as rodent.

considered, particularly for hormone-responsive tumours such as breast and prostate.

Pilot studies and optimisation

Pilot tumour growth studies using small numbers of animals (5–10) are recommended to establish that patterns of local and metastatic growth are reproducible. They also show any adverse effects associated with tumour progression and enable humane endpoints to be identified. The data derived should feed into group numbers used for definitive studies (e.g., therapy experiments) in order for experimental time frames and statistically significant endpoints to be established. Use of a relevant positive control treatment may be useful at this stage to ensure that tumour growth/responsiveness is as expected. This can be dictated by a variety of factors, including the site of growth. Subcutaneous tumours may grow rapidly and some are prone to developing haemorrhagic areas, which can cause rapid expansion and ulceration (e.g., human A2780 ovarian carcinoma and AR42J pancreatic carcinoma xenografts).

For tumours growing as a suspension in the peritoneal cavity, it is important to establish clear criteria to ensure that studies are terminated before animal welfare is compromised. This site is only appropriate for models where ascites is a feature of the natural progression of the human cancer (e.g., ovarian carcinoma, peritoneal mesothelioma, gastrointestinal tumour carcinomatosis). Similar criteria apply to other sensitive specialised sites such as muscle or brain. For metastatic models, pilot experiments should define the extent and time course of dissemination to internal organs.

Pilot studies should include sequential analysis of animals to determine the time course required to achieve scientific goals. Termination of studies at the earliest possible point will minimise adverse effects on the animal. Where possible, use of biomarkers (e.g., serum levels of prostate-specific antigen, PSA) and real-time

imaging are highly recommended. It is also possible to measure circulating tumour cells using fluorescence and PCR-based techniques (Glinskii *et al*, 2003; Komatsubara *et al*, 2005). For spontaneously arising tumours, including those in transgenic animals, particular attention should be paid to the time course of tumour development and issues relating to the development of multiple tumours. Progression may be unpredictable and involve rapid dissemination and subsequent deterioration in clinical condition, in which case careful and frequent monitoring is required.

Refinement and welfare issues

Subcutaneous implantation of tumour material should use a trocar or surgical formation of a small s.c. pocket. Appropriate anaesthetics must be used and post-implantation analgesia is also strongly recommended. Veterinary advice should be sought to ensure that the agents selected reflect contemporary best practice. Anaesthesia/analgesia is also required for implantation of 'hollow fibres' or slow release devices such as osmotic mini-pumps. Hormone pellets (oestrogen/testosterone) may be required to support hormone-dependent tumours, but first-time use in a particular strain will require pilot experiments with different doses/exposures to assess tolerance, especially with oestrogen pellets where urinary tract side effects may be encountered (Pearse *et al*, 2009).

For injection of cell suspensions, the minimum number of cells in the smallest volume should be used, consistent with the properties of the tumour. For s.c. sites, 1–5 million cells in 100 μ l is typical. For orthotopic sites, this should be reduced to avoid excessive tissue damage or leakage (e.g., 50 000 cells in 30 μ l into the prostate, or 10–50 000 cells in 5 μ l into the brain). Intramuscular tumours in the leg can affect mobility, and this site should only be used if there is special justification (e.g., for tumours which naturally develop in this tissue). Similarly, footpad

injection, which has been traditionally used to potentiate lymphatic dissemination, is unacceptable without exceptional scientific justification and should then only involve a single paw.

Surgical removal of a primary tumour may be justified, for example, from s.c. sites, mammary fat pad or removal of the spleen following intrasplenic injection, to allow time for outgrowth of any secondary deposits. Surgery must be performed using sterile techniques with appropriate post-operative monitoring and control of any pain and inflammation/infection.

Cell lines should be checked regularly for contaminating microorganisms to avoid infection of host animals. This is especially important if tumours are routinely passaged between animals, which may be justified for those that are difficult to establish from cell cultures. Asymptomatic infection of experimental animals may affect tumour properties, for example, metastasis (Rodriguez-Cuesta *et al*, 2005). Procedures can be used to improve tumour take rate. For example, moderate doses of whole-body irradiation may further enhance engraftment of tumour cells in athymic mice (Baersch *et al*, 1997; Nijmeijer *et al*, 2001; Li *et al*, 2006), although the added stress and risk to the animal must be considered. Co-administration of human tumour cells with allogeneic bone marrow transplantation may reduce graft-vs-host activity but preserve graft-vs-tumour effects in allogeneic leukaemia models (Prigozhina *et al*, 2002; Giver *et al*, 2004).

Transplanted tumours (especially xenografts) may not develop with an incidence, growth rate or malignant potential required; however this can often be enhanced by selection of tumorigenic/metastatic variants (Bruns *et al*, 1999; Nguyen *et al*, 2009a). In addition, co-injection of tumour cells with extracellular matrix proteins and/or angiogenic factors (Collado *et al*, 2007), cancer-associated fibroblasts (Noel *et al*, 1993; Orimo *et al*, 2005) or mesenchymal stem cells (Karnoub *et al*, 2007; Spaeth *et al*, 2009) can increase tumorigenicity, better recapitulate the human tumour microenvironment and enhance metastatic potential. Cells may be transfected with fluorescent or bioluminescent markers allowing serial imaging of internal tumours/metastatic spread. However, such tagged cell lines should be profiled to establish that their biological characteristics are unchanged and consideration should be given to the dependence of luminescence/fluorescence on factors in the tumour microenvironment, for example, molecular oxygenation, necrosis, or ascites fluid from peritoneal tumours (Condeelis and Segall, 2003).

THERAPY

Preclinical discovery and development of therapeutics

There is a concerted effort to identify and develop small-molecule drugs or biopharmaceuticals (e.g., antibodies, protein therapeutics,

Table 2A Primary tumour models

Model type	Examples of models	Advantages	Disadvantages
Chemically-induced tumours	Dimethyl hydrazine – gastric cancer (Watanabe <i>et al</i> , 1999)	Model the full spectrum of carcinogenic events	Low incidence and heterogeneous tumour development
	Azoxymethane – colon cancer (Hirose <i>et al</i> , 2004)	Useful in chemoprevention studies	Safety aspects associated with use of carcinogens – may need to house animals in isolator
	Diethylnitrosamine – hepatocellular carcinoma (Ha <i>et al</i> , 2001)		Long time frame for tumour development
	Dimethyl benzantracene – breast cancer (Hawariah and Stanslas, 1998)		Continuous monitoring not feasible
	N-acetylcysteine – squamous oesophageal carcinoma (Balansky <i>et al</i> , 2002)		Often highly immunogenic
	Dimethylbenzantracene/tetradecanoyl phorbol acetate (TPA) – skin cancer (Johansen <i>et al</i> , 2009)		
Radiation-induced tumours	Ultraviolet light (Ahsan <i>et al</i> , 2005; De Fabo, 2006; El-Abaseri and Hansen, 2007)	Models non-melanoma (using UVA) and melanoma (UVB) skin cancer Useful for prevention (e.g. sunscreen) studies	Requires hairless mice
Inflammation-induced tumours	<i>Helicobacter pylori</i> -induced gastric cancer in gerbils (Zheng <i>et al</i> , 2004)	Use of conventional rodents to facilitate the involvement of the full spectrum of immune mediators Models malignant progression and amenable for use of chemopreventive agents	Limited availability of models Long time frame and variability in tumour development
Surgically-induced tumours	Oesophago-gastroduodenal anastomosis model of oesophageal carcinogenesis (Chen <i>et al</i> , 1999)	Can model malignant progression or metastatic spread	High level of skill required for initiation Incidence may not be 100% Accurate quantification can be difficult unless using real-time imaging
Spontaneous tumours, sometimes with viral/genetic component	T138 mice and mammary carcinoma (Wood <i>et al</i> , 1992; Nordsmark <i>et al</i> , 1996) Cotton rats and neuroendocrine gastrointestinal tumours (Martinsen <i>et al</i> , 2003) Eker rat model of tuberous sclerosis (Kenerson <i>et al</i> , 2005)	Develop cancer without any intervention Conventional rodents, therefore fully immunocompetent	Limited tumour types and strains Variability in the time frame of tumour development

Table 2B Genetically engineered mouse models (GEMMs)

Examples of models	Spectrum of tumours	Spectrum of tumours in humans	Recent genetic modifications	Spectrum of tumours in modified models	References
Rb	Brain, pituitary	Retinoblastoma, osteosarcoma, medulloblastoma	Additional loss of p107, p130	Retinoblastoma	(Robanus-Maandag <i>et al</i> , 1998)
Trp53	Osteosarcoma, lymphoma, soft-tissue sarcoma, germ cell tumours	Breast carcinoma, brain, sarcomas, leukaemia, endocrine	Additional loss of Terc Trp53(ER)TAM	Breast and other carcinomas, germ-cell tumours	(Artandi <i>et al</i> , 2000) (Christophorou <i>et al</i> , 2005)
Apc (Apc ^{MIN} , Apc ^{D716} , Apc ^{D580})	Multiple polyps in small intestine	Polyps in colon progressing to carcinomas	Conditional colon-specific inactivation	Polyps in colon Mammary carcinoma	(Shibata <i>et al</i> , 1997)
Ink4a	Fibrosarcoma, lymphoma, squamous-cell carcinoma	Familial melanoma, sporadic pancreatic, brain tumours	Crossed with Arf ^{+/–} mice	Metastatic melanoma, sarcoma, carcinoma, lymphoma	(Krimpenfort <i>et al</i> , 2001)
Brca1	No tumour susceptibility	Breast ovary	Conditional mammary-specific inactivation of Brca1	Mammary tumours	(Xu <i>et al</i> , 1999)
Brca2	No tumour susceptibility	Breast, ovary	Conditional mammary-specific inactivation of Brca2 and Trp53	Mammary tumours	(Jonkers <i>et al</i> , 2001)
Nf1	Pheochromocytoma, myeloid leukaemia	Neural-crest-derived benign neurofibroma and malignant fibrosarcoma	Additional loss of Trp53	Neural-crest-derived malignant glioblastoma	(Reilly <i>et al</i> , 2000)
Nf2	Osteosarcoma, fibrosarcoma, lung adenocarcinoma, hepatocellular carcinoma	Schwannomas, meningiomas, ependymomas, gliomas	Schwann-cell precursor-specific ablation of Nf2	Schwannomas	(Giovannini <i>et al</i> , 2000)
K-ras ^{G12D}	Lung adenoma, adenocarcinoma PanIN	NA	Mutant Trp53 Pancreas-specific K-ras ^{G12D} + <i>Ink4a/Arf</i> deficiency or combined with Trp53 ^{R172H}	Metastatic lung cancer Metastatic pancreatic cancer	(Johnson <i>et al</i> , 2001) (Aguirre <i>et al</i> , 2003; Hingorani <i>et al</i> , 2005)
AML1/ETO or MLL fusion proteins	AML	AML			(Zuber <i>et al</i> , 2009)
MYCN	Neuroblastoma, Rhabdomyosarcoma, medulloblastoma	Neuroblastoma, Rhabdomyosarcoma, medulloblastoma	TH-driven overexpression in neural crest		(Weiss <i>et al</i> , 1997)
MYCC	Pancreatic adenoma, melanoma, lymphoma, AML, breast	Pancreatic adenoma, melanoma, lymphoma, AML, breast	BCL-XL crosses Lck overexpression Eu-Tta-c-myc MMTV-c-myc		(Pelengaris <i>et al</i> , 1999) (Felsher and Bishop, 1999) (Sinn <i>et al</i> , 1987)
Ptc1	Medulloblastoma		Trp53-knockout crosses		(Wetmore <i>et al</i> , 2001)
T-antigen	Pancreatic adenocarcinoma		RIP1-Tag2 driven overexpression in pancreatic islets		(Bergers <i>et al</i> , 1999)
PTEN	Breast, endometrial, glioblastoma, prostate, and thyroid carcinoma, Cowden syndrome, hamartomas, urothelial tumours of renal pelvis	Breast, endometrial, glioblastoma, prostate, and thyroid carcinoma, Cowden syndrome, hamartomas, urothelial tumours of renal pelvis			(Stambolic, 2000; Qian, 2009)

Abbreviations: AML = acute myeloid leukaemia; NA = not available; PanIN, pancreatic intraepithelial neoplasia.

vaccines, gene therapy) targeted against cancer cells or associated host cells (Sawyers, 2004; Collins and Workman, 2006; Workman and de Bono, 2008). A representative 'test cascade' for discovering new small-molecule inhibitors of cancer targets is shown in Figure 2. As a consequence of extensive *in vitro* testing, comparatively small numbers of prioritised compounds progress to examination *in vivo* (Collins and Workman, 2006). *In vivo* studies use sequential,

discriminatory tests to prioritise compounds at each stage. Different tests may need to be applied to biopharmaceuticals, such as antibodies and vaccines, as they may work by recruiting host effectors (e.g., cytotoxic leukocytes). Epitope specificity can also require the development of an antibody or vaccine initially using anti-rodent reagents (before switching to the clinical form) or use of a genetically modified mouse model. In addition, agents directed

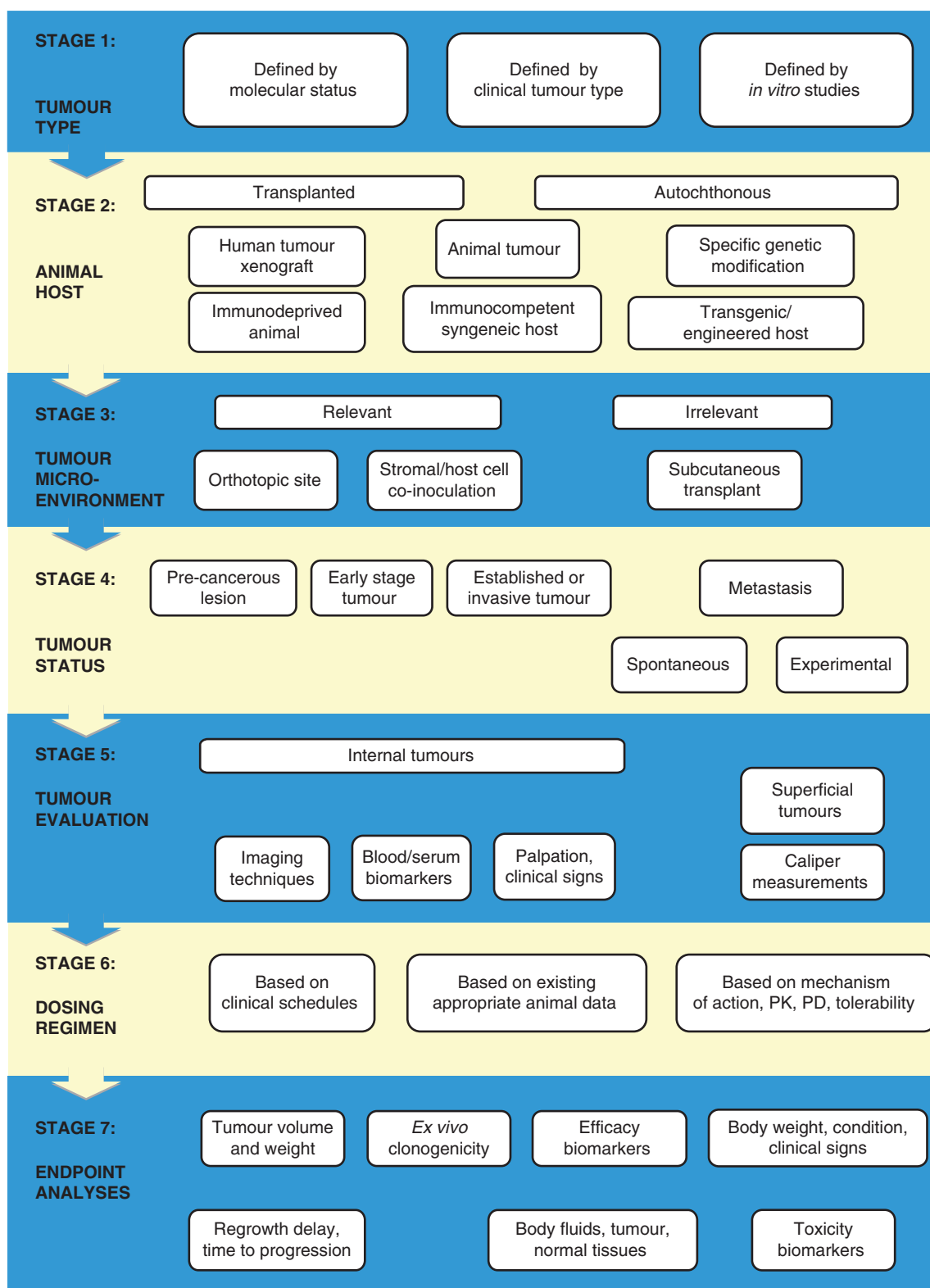


Figure 1 An illustrative process for tumour model selection and use. This representative schema provides an illustration of factors to be considered when designing an animal study. In this particular example, all the factors listed at a given stage (and potentially others) should be considered before moving down, stepwise, to the next stage. Here, an initial consideration is that the choice of model may be based on the relevant molecular status, clinical tumour type or *in vitro* studies. At the next stage, the animal host will be dictated by the need for, say, a human tumour xenograft versus a genetically engineered mouse model, which have advantages discussed in the text. Considerations of tumour environment and site then follow, after which, in therapy studies, are dosing and endpoint aspects. Note that this schema is illustrative and not prescriptive and that each study must be tailored to the specific scientific question and experimental objectives, with appropriate humane endpoints always applied and pilot studies carried out as needed.

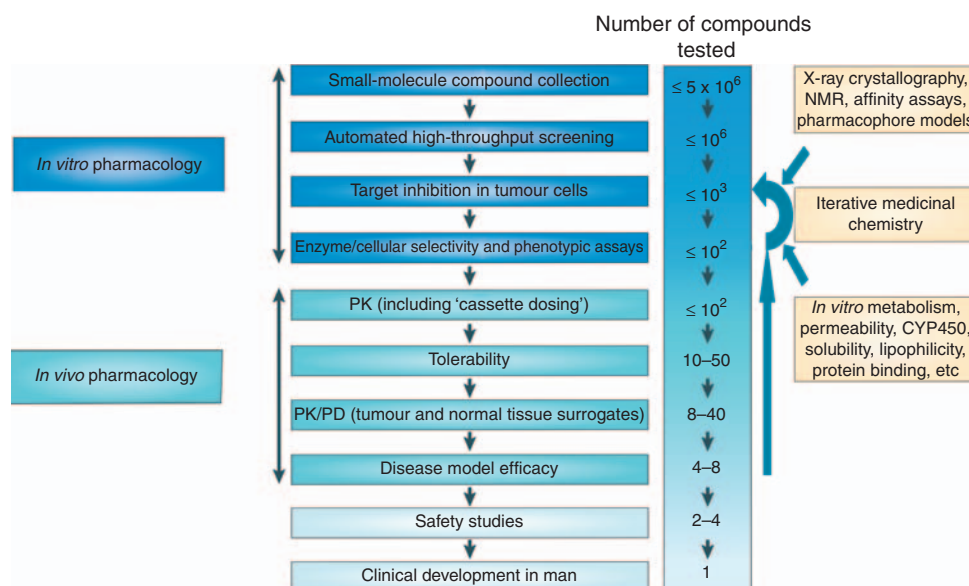


Figure 2 Example of a drug discovery test cascade for identifying small-molecule antitumour drugs. A representative test cascade for identifying a potential small-molecule drug against a given target is shown. A subset of a compound library is initially screened vs the target *in vitro*, in recombinant protein or cellular assays, using high-throughput automation to identify 'hits'. Subsequent leads are examined in more detail by assessing their effect on downstream molecular events in cells and their selectivity vs other proteins. A battery of additional *in vitro* tests is also used for measurement or prediction of physical properties and pharmacokinetic parameters. Only compounds with a promising balance of features are progressed to *in vivo* testing, usually in mice. Pharmacokinetic (PK) studies, used to understand drug exposure, may initially involve co-inoculation of low doses of compounds ('cassette dosing') to minimise animal usage. The tolerability of leads with favourable PK is then assessed at higher doses, before evaluating their pharmacodynamic (PD) effect on tumour and normal tissues at well-tolerated doses. Compounds that do not meet the anticipated level of performance at any stage may result in subsequent rounds of iterative medicinal chemistry to generate improved leads. Selected leads are progressed to efficacy testing to determine the link between target inhibition and the effect on tumour growth or spread (metastasis). Safety studies on late-stage leads are also required before a candidate drug can be selected for examination in cancer patients (not covered here). The application of the test cascade means that compounds are filtered by the earlier stage assays so that a smaller number of compounds, and only those of higher quality, are taken into later stage *in vivo* assays in animals.

against the tumour microenvironment (e.g., angiogenesis, tumour-promoting stromal or inflammatory cells) will require appropriate specialised assays. A range of technical platforms are used preclinically to define responses to therapy, the most informative of which are adopted for use in patients (Figure 3). Careful assessment of a therapy's safety profile (outside the scope of this review) is also required for regulatory submission.

Defining tolerable doses for efficacy studies

An investigational treatment should be examined at a potential therapeutic dose level and using a relevant dosing regimen that covers the longest duration anticipated. These parameters can, for example, be estimated from consideration of mechanism of action, *in vitro* potency, pharmacokinetics, protein binding and pharmacodynamic biomarker data. Studies typically use two mice per dose level with a doubling dose-escalation or dose-halving de-escalation design. For studies involving a single dosing event, an interval of 24 h should be used before an alternative dose level is examined, to allow any acute adverse effects to be seen. For more chronic administration schedules (e.g., daily for 21–28 days) this interval should be at least 5 days. Animals should be examined at least twice daily (see humane endpoints below). Note that presence of a tumour may reduce host tolerance to therapy. Studies of mice may be used to predict dose requirements in other species through allometric scaling of pharmacokinetic parameters (Freireich *et al*, 1966).

Combination studies

There is a strong rationale to study combinations of agents *in vivo* to guide clinical studies. Relevant prior *in vitro* studies such as Combination Index or isobologram analyses to discriminate

additive, synergistic or antagonistic interactions should be completed to guide the selection of combinations and schedules. Compounds are added to tumour cells in culture over a range of concentrations, alone or in combination, and the changes in sensitivity are observed. Compounds may also be added sequentially as the order of administration may significantly influence responses (Chou, 2006). Care needs to be taken with *in vivo* studies in addressing the choice of individual drug doses and scheduling, particularly if overlapping toxicities are likely. Pilot experiments must assess tolerability (see above), and pharmacokinetic data (see below) should also be generated to determine whether interpretation of efficacy data is affected by pharmacokinetic interactions (Siim *et al*, 2003).

Pharmacokinetic studies

In vitro and *in silico* methods are useful to predict absorption, distribution, metabolism and elimination (ADME) properties and to help prioritise compounds for evaluation in animals (Table 3; Singh, 2006). However, at present such methods are unable to predict accurately the full pharmacokinetic profile of an agent. Pharmacokinetic studies should use a validated and sufficiently sensitive detection method, ideally avoiding the need to pool separate blood samples, thereby minimising animal usage. Typical experiments on mice use a single dose and 5–8 time points (2–3 mice per point) over 24–48 h with small molecules (usually administered p.o., i.v. or i.p. at doses of 0.5–100 mg kg⁻¹) and over 1–21 days with biopharmaceuticals (administered i.v., i.p. or s.c. at doses ranging from 10 to 1000 µg per mouse).

More recently, repeat sampling of small volumes of blood from a superficial vein in mice over a series of time points has been established to reduce animal numbers. This can be employed either for isolation of plasma and analysis by sensitive liquid

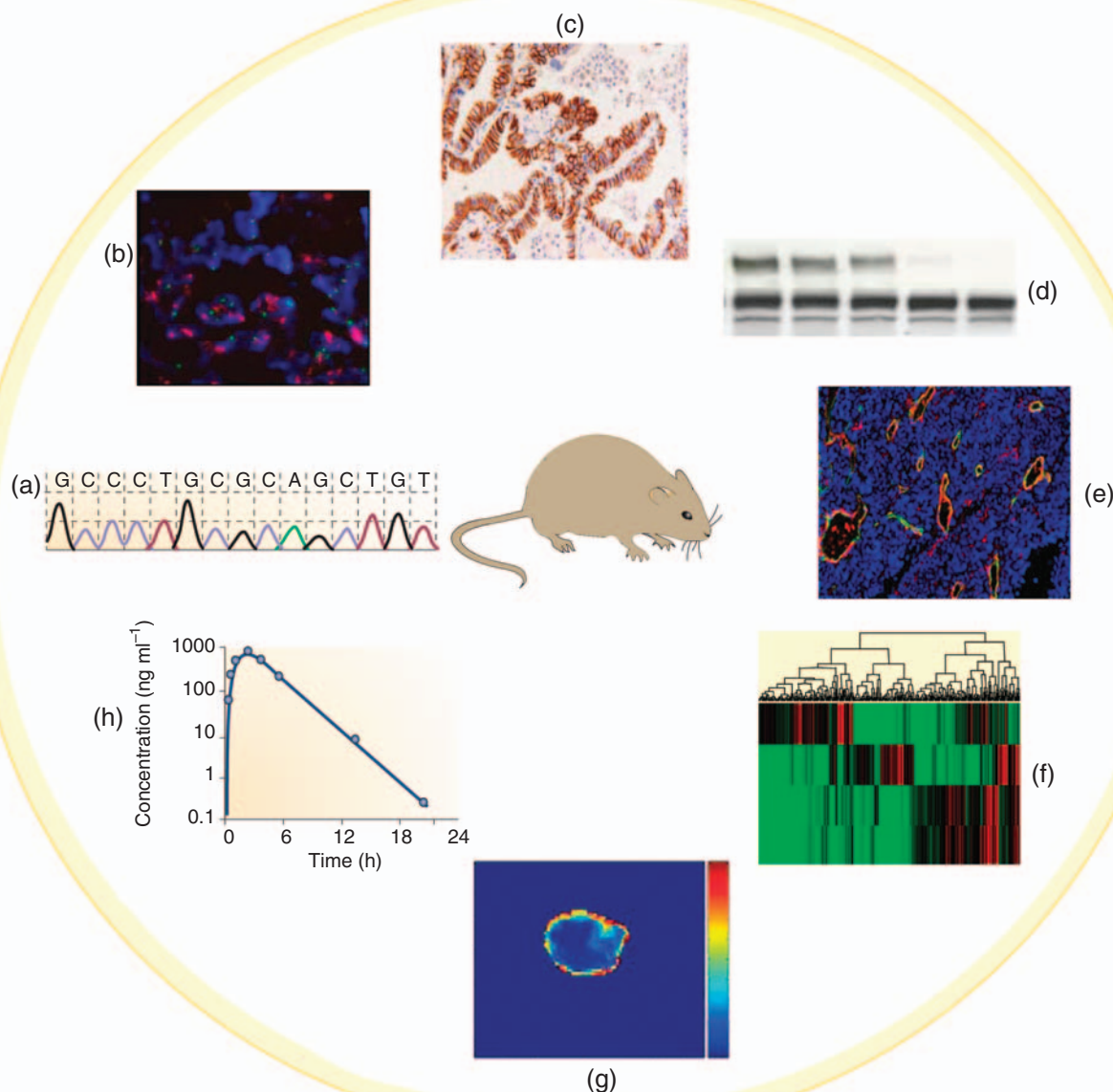


Figure 3 Examples of technologies used in animals for therapeutic cancer research. *In vivo* tumour models have an essential role in the development of new cancer medicines, enabling the temporal and quantitative effects of treatment to be examined on tumour and normal tissues in the intact organism. Methods used include those to examine (clockwise from far left) molecular determinants of sensitivity to treatment (initially *in vitro*, corroborated *in vivo*) such as (a) gene mutations by sequencing, or (b) gene amplification by fluorescent *in situ* hybridisation; detection of target phospho-epitopes and their inhibition in tumour tissue as determined by: (c) immunohistochemistry or (d) western blotting of cell lysates; (e) tumour vascular density and maturation by fluorescent immunohistochemistry; (f) tumour mRNA expression by gene array analysis with hierarchical clustering of information; (g) imaging techniques such as dynamic contrast-enhanced MRI to measure tumour haemodynamics; and (h) pharmacokinetic analysis of drug concentrations in plasma by mass spectrometry.

chromatography–mass spectrometry/mass spectrometry (LC/MS–MS or tandem MS) instrumentation (Abatan *et al*, 2008), or by spotting microlitre volumes of whole blood onto specialised paper cards, which are then dried and extracted before analysis (Barfield *et al*, 2008). In rats, a 5–8 time-point pharmacokinetic profile may be generated using 2–6 animals in total, through repeated blood sampling. ‘Cassette dosing’, which involves administration of low doses of compound mixtures, should also be considered initially as this can reduce animal usage (Watanabe *et al*, 2006; Smith *et al*, 2007). Wherever possible, computational compartmental kinetic modelling should be used to predict optimal doses or multiple dosing protocols, to facilitate more limited sampling (Rowland and

Tozer, 1995). It is noteworthy that the plasma half-life of monoclonal antibodies is frequently extended in immunocompromised mice, which are deficient in IgG production (Bazin *et al*, 1994).

Pharmacodynamic biomarkers

Initial studies of investigational therapies using tumour-bearing animals should aim to determine whether the target, or an appropriate downstream pathway or phenotype, is modulated by using suitably validated pharmacodynamic biomarkers (Collins and Workman, 2006). Typically, animals are humanely killed at intervals to determine the extent and duration of

pharmacodynamic changes and to investigate biomarkers in tumour and normal tissues (e.g., blood or skin) that may be relevant to clinical development (Banerji *et al*, 2005). In vaccine studies, responses are assessed by changes in immune status,

Table 3 Some *in vitro* assays for pharmacokinetic/ADME properties

Property	Assays
Chemical and physical properties	<i>In silico</i> predictions or <i>in vitro</i> measurements of log <i>P</i> (Mannhold <i>et al</i> , 2009), log <i>D</i> (Bruneau and McElroy, 2006; Dohda <i>et al</i> , 2007), p <i>K_a</i> (Lee <i>et al</i> , 2007) and solubility (Colclough <i>et al</i> , 2008; Du-Cuny <i>et al</i> , 2008)
Metabolism	Mouse, rat, human liver microsomes, hepatocytes, S9 incubations, UGT assays (Houston and Carlile, 1997; Riley <i>et al</i> , 2002)
Passive diffusion/cell uptake	PAMPA (Ottaviani <i>et al</i> , 2006)
Cell and gut permeability predictions	Caco-2 cells (Artursson <i>et al</i> , 2001)
Blood–brain barrier permeability predictions	hCMEC/D3 cells (Poller <i>et al</i> , 2008)
Drug–drug interactions (e.g., CYP450)	Human liver microsomes, hepatocytes, CYP enzyme screens (Masimirembwa <i>et al</i> , 2001)
Protein binding	Measurement by dialysis or ultrafiltration (Howard <i>et al</i> , 2010)

Abbreviations: ADME = absorption, distribution, metabolism and elimination; CYP450 = cytochrome-P450; PAMPA = parallel artificial membrane permeability assay; UGT = uridine diphospho (UDP)-glucuronosyltransferase.

including evidence of tumour-infiltrating leukocytes by immuno-histochemistry, and specific cellular or humoral immunity (Gajewski, 2000). It should be possible to use much smaller group sizes of 3–5 in pharmacodynamic studies in comparison to those in efficacy studies (see below). Simultaneous measurement of drug concentrations and mechanistic biomarkers is recommended to reduce animal numbers and establish a pharmacokinetic–pharmacodynamic relationship. Judicious application of such studies in a drug discovery test cascade should be used to prioritise agents before entry into efficacy studies.

Efficacy determinations

All relevant information should be used to guide the design of tumour efficacy studies. Such studies generally involve examination of treatment effects over a 2- to 4-week period and establish how the therapeutic response relates to pharmacokinetic and pharmacodynamic parameters. Typically, with treatments delivered by an appropriate route of administration (Table 4), response is determined in 6–10 animals per study group (*vs* a control group) either by direct twice-weekly calliper measurement of superficial tumours (Kelland, 2004), counting lung or liver metastases *ex vivo*, or using imaging methodologies (Edinger *et al*, 2002; Hoffman and Yang, 2005; Brindle, 2008; McCann *et al*, 2009; Yang *et al*, 2009). Alternatively, post-treatment excision of tumours for *in vitro* determination of clonogenic survival, or determination of the dose required to inhibit tumour growth by 50% (tumour control dose-TCD₅₀) may be appropriate (see Radiation therapy section below). Methods are available to determine sample sizes for single- and combination-agent studies and to allow for incomplete data sets (Tan *et al*, 2005). For certain targets, alternative, surrogate *in vivo* efficacy models in non-tumour-bearing animals may be used, such as assessment of anti-oestrogenic activity by determining the effect on hypothalamic function (Kato *et al*, 1968).

Table 4 Maximum volumes to be administered on each occasion

Route	Mouse	Rat
Intravenous, bolus injection	10 ml kg ⁻¹	5 ml kg ⁻¹
Intra-arterial, bolus injection	NA	0.1 ml
Intraperitoneal, bolus injection	20 ml kg ^{-1a}	10 ml kg ⁻¹
Subcutaneous, bolus injection	20 ml kg ^{-1ab}	10 ml kg ⁻¹
Intramuscular, bolus injection	0.05 ml per site, using contralateral limbs for sequential doses or 0.1 ml on one occasion only,	0.1 ml per site, using contralateral limbs for sequential doses or 0.2 ml on one occasion only
Intradermal, bolus injection	0.05 ml per site ^b	0.1 ml per site ^b
Oral, by gavage	20 ml kg ⁻¹ , or 50 ml kg ⁻¹ on one occasion only	20 ml kg ⁻¹ , or 30 ml kg ⁻¹ on one occasion only
Slow intravenous/arterial injection	0.8 ml over 2 min	5 ml over 2 min
Constant intravenous infusion	0.04 ml per min	0.2 ml per min ^c
Constant intra-arterial infusion	NA	0.1 ml per min
Constant intraperitoneal infusion	0.04 ml per min ^d	0.2 ml per min ^d
Intratumour, bolus injection	0.1 ml	0.1 ml

Abbreviation: NA = not applicable. The volumes used should be the minimum practicable depending on the solubility of the agent and accuracy of administration, and should be adjusted according to individual animal body weights at each dosing. Multiple administrations should typically be separated by a period of 6–8 h. The frequency of dosing should be balanced against the duration of the treatment schedule such that the total number of doses administered is not excessive (Diehl *et al*, 2001). ^aExceptionally, compounds, which are poorly soluble in water may be administered as a weak solution in a volume of up to 50 ml kg⁻¹ in the mouse, to avoid the use of organic solvents or detergents. Additionally, 5% dextrose/saline may be injected in volumes up to 50 ml kg⁻¹ for rehydration of mice after surgery. Similar increases in volume can be applied to rats. ^bMaximum of two sites per animal. ^cMaximum rate of 2.5 ml min⁻¹ should be used for total infusion times < 1 min. ^dWhere agents need to be administered over several days or weeks, s.c. or i.p. osmotic pumps may be used, which may be left in position for up to 4 weeks.

Box 3 EXPERIMENTAL STUDY DESIGN

1. Power analysis calculations should be applied to determine sample sizes. There are many commercially available statistical packages to support such calculations.

A number of variables need to be specified to perform the analysis, including the effect size of biological interest (specified by the experimenter), the standard deviation, the significance level (normally set to 5%) and the desired power of the experiment. The desired power should be set to a minimum of 80% (i.e., at least an 80% chance of declaring the defined 'meaningful biological change' as being statistically significant).

Estimates of biological variability should be used in sample size and power calculations. These estimates are established from accrued historical databases, pilot studies or published data. Biological databases must be continually updated and monitored with a regular review of group sizes. It is helpful to plot the variance estimates for control groups from a given type of test with time, and constant attempts made to identify any underlying cause of variation, which may ultimately lead to a reduction in group size.

2. Multiple treated groups will often be compared against one control to reduce the number of studies performed. As the control group is involved in every comparison, i.e., to all treated groups, it is often appropriate to set the control group size to be higher in comparison with the treated groups.
3. When optimising animal model conditions, factorial design provides a set of tools for efficiently exploring multiple parameters simultaneously. Factorial design is a more efficient approach in comparison with the common one-variable-at-a-time approach, and leads to a more reliable understanding of the effects of parameters and their interactions. This in turn can lead to a better animal model.
4. The power analysis calculation described above provides fixed sample sizes for each compound. An alternative, applicable in appropriate situations, would be to adopt a sequential design. Compounds are tested on more than one occasion and stopping rules are devised, so that extreme compounds (either highly effective or not effective at all) are dropped early from the study. The advantage to a sequential design is that on average fewer animals will be used per compound in comparison with the fixed-sample-size case.

Administration of experimental agents

Various sources are available for advice on well-tolerated injection volumes and recommended administration schedules. It is important to note that, from an animal welfare point of view, frequency and duration of dosing are as important as the volume and composition of the injected solution. Some commonly used examples are given in Table 4 and the following references: Diehl *et al* (2001); Morton *et al* (2001). More frequent dosing would need to be justified by pharmacokinetic or pharmacodynamic data. As an illustration of standard procedures, for oral/i.p. or i.v. dosing in mice, volumes of 10 and 5 ml kg⁻¹, respectively (equating to 200 and 100 µl for a 20 g mouse), are widely accepted. However, the smallest volume that can be accurately and safely administered must always be used.

Where possible, compounds should be administered in an aqueous solution (sterile water for injections, 0.9% saline or 5% dextrose/saline) that is as close to physiological pH as possible, as highly acidic or basic solutions can be an irritant. If organic solvents (like dimethylsulphoxide, DMSO) are necessary, these should not exceed 5 ml kg⁻¹ or 10% of the injected volume. Detergents (such as Tween), solubilisers or emulsifiers should not exceed 20% of the injected volume. Cyclodextrins should not exceed 2 ml kg⁻¹ or 45% of the injected volume, and where used at >20% of the injected volume, animals need to be rehydrated within 2–4 h.

Experimental design including statistics

To maximise the scientific integrity of data generated while at the same time using the minimum number of animals, statistical

Box 4 DATA ANALYSIS

1. Sometimes data need to be transformed before data analysis. The justification for transforming data should be given. A pertinent example is determining the percentage inhibition of tumour growth from comparative tumour volume data. As the variance of tumour measurements increases with the mean, data should ideally be analysed on a logarithmic scale, with each animal exhibiting a difference in log₁₀ (tumour volume) from initial.
2. Meaningful biological change, measurable endpoints and intended statistical analyses should be pre-defined. For the percentage inhibition of tumour growth example, a suitable endpoint would be a comparison of the change in tumour volume: i.e., log₁₀ (final volume)–log₁₀ (initial volume) between the control and the treatment groups.
3. When examining changes in means in one direction only (e.g., when identifying inhibition rather than change) then *one-sided* (rather than *two-sided*) statistical tests will be used. For a comparison of two groups a *t*-test is adequate, whereas experimental data with multiple groups (vs a control) should be analysed by one-way ANOVA.

Abbreviation: ANOVA = analysis of variance

expertise should be applied to all experimental design and analyses (Festing, 2002; Festing and Altman, 2002; Festing *et al*, 2002; see Boxes 3 and 4).

Chemoprevention

These studies routinely use either carcinogen-induced rat tumours (e.g., azoxymethane-induced colorectal cancer) or mouse genetic models of carcinogenesis (e.g., *Apc*^{Min} colorectal; Corpet and Pierre, 2003; Cai *et al*, 2009). Generally, animals receive the putative chemopreventive agent in the diet or drinking water over an extended period at innocuous doses. Tumour development is measured at the end of the study and compared with animals on a relevant control diet. Relatively large numbers of rodents (e.g.; ≥14 per group; Cai *et al*, 2009) may be required for the observed differences between the intervention and control groups to be robust. Mechanistic and pharmacodynamic endpoints should also be included (Yang *et al*, 2001; Corpet and Pierre, 2003).

Radiation therapy

External beam radiotherapy is primarily used for local tumour irradiation, which requires lead shielding to minimise normal tissue exposure. Typically, s.c. tumours are used and combination treatment with a novel therapy is tested. Endpoints include local control, growth delay and *in vivo*–*in vitro* clonogenic survival (TCD₅₀). Time to re-growth is preferred to a single time point analysis. Local tissue toxicity is usually manifest as skin erythema but should be minimised by restricting localised doses to less than 30 Gy (single dose). Exploration of better tolerated, clinically relevant fractionated doses (e.g., 2–5 Gy per fraction over 1–2 weeks) is encouraged. Should moist desquamation occur, this should not be allowed to persist for more than 24 h. Irradiated s.c. tumours can show ulceration, which may reflect tumour response. However, if there is evidence of infection and/or no signs of tissue repair the animal should be humanely killed. The acute and late effects of radiation treatment may also be examined in a relevant organ, particularly when studying new combination paradigms. A common endpoint has been the development of fibrosis in lung tissue, although more recently measurement of breathing rate has been implemented to detect symptoms before they become distressful to the animal (Jackson *et al*, 2010).

Radiotherapy can also be delivered in the form of targeted radionuclides (normally attached to antibodies; e.g., Martenson *et al*, 2005). Normal tissue toxicity will depend on antigen

expression on tissues relative to the tumour and the nature of the emitter. Whole-body irradiation can also be used to suppress the immune response of an animal, for example, or to treat disseminated disease. Selected doses should not manifest toxicity over the duration of the experiment, for example, gut toxicity within 5 days or haematological toxicity within 30 days.

UV radiation (UVR)

The response of mouse skin to UVR may be used, for example, to study the aetiology of non-melanoma skin cancer (van Kranen and de Gruijl, 1999; Hedelund *et al*, 2006). Generally, experiments are performed with hairless (Skh-hr2) mice. As mouse skin does not show signs of burning, it is important to use a biologically relevant, non-burning dose of 0.2–0.3 MED (minimal erythema dose; 50% skin thickening = 0.5 MED). Skin thickness should be measured 2–3 times weekly after increasing the dose of UVR until 20–30% thickening has occurred. If hyperplasia is maintained over 12–15 weeks skin tumours may form. A protective mouse restrainer should be used as UV radiation is damaging to eyes and ears.

IMAGING

General considerations

Imaging techniques now have a principal role in translational cancer research, enabling sequential analysis of biological endpoints in the same animal, with obvious welfare benefits. The main utility of small-animal imaging is for monitoring deep-seated tumours and metastases with or without treatment. Applications include studies of basic biological processes and of tissue pharmacokinetics and pharmacodynamic responses to treatment (Paulmurugan *et al*, 2002; Galbraith *et al*, 2003; Pillai *et al*, 2008; Tennant *et al*, 2009; Nguyen *et al*, 2009b). However, animal numbers may not be reduced if, for example, full endpoint analysis requires surgical intervention such as cannulation of blood vessels or when contrast agents have a long half-life. Here, sequential imaging may not be possible and alternative techniques involving tissue excision may provide more information (usually at higher spatial resolution) from the same number of animals.

There is an increasing clinical need for pharmacodynamic imaging with molecularly targeted cancer therapeutics. However, interpretation of imaging signals is often difficult and animal models have an important role in rigorous validation of new techniques. This needs to be accompanied by consideration of unique animal welfare issues. Use of external imaging techniques on small animals is not completely non-invasive as some form of anaesthesia or physical restraint is necessary and surgery or administration of contrast agents may be required.

Imaging techniques

The applications, advantages and disadvantages of commonly used imaging technologies are summarised in Table 5 and have also been reviewed recently (Workman *et al*, 2006; Brindle, 2008; Weissleder and Pittet, 2008). Whole-body optical imaging is relatively simple and cost-effective (Edinger *et al*, 2002). Tumour cells are genetically modified to constitutively or inducibly express a fluorescent protein (e.g., eGFP, dsRed) or an enzyme that activates an exogenously administered substrate to a bioluminescent molecule (usually luciferase for activation of a luciferin). The whole animal is imaged using sensitive optical detectors, which may or may not incorporate a tomographic facility (Figure 4). The potential influences of genetic modification and/or substrate administration on immunogenicity and response to treatment, as well as animal welfare, must be considered

(Tuchin, 1993; Dennis, 2002; Condeelis and Segall, 2003; Wells *et al*, 2006).

Intravital microscopy uses a wide variety of optical imaging techniques, often incorporating fluorescent or bioluminescent genetic reporters or markers, including nano-particles (Hoffman, 2005). It has particular animal welfare issues because it involves surgery to provide optical clarity and visualisation on a microscope stage or using fibre-optic light guides (Weissleder and Pittet, 2008). Some intravital microscopy techniques (e.g., tumours growing in the intestinal mesentery) require laparotomy with deep anaesthesia, so that imaging is only possible for a few hours under terminal anaesthesia. Surgical implantation of 'window' chambers for tumour implantation enables imaging to be performed over days to weeks (Dewhirst *et al*, 1987; Lehr *et al*, 1993; Brown *et al*, 2001; Reyes-Aldasoro *et al*, 2008). Here, general anaesthesia is only essential for the initial surgery and imaging may be performed with restrained animals. Strict aseptic technique and good post-operative care and analgesia are essential (Richardson and Flecknell, 2005; Flecknell, 2008).

Most physical imaging techniques require use of exogenous contrast agents and only positron emission tomography (PET) and single photon emission computed tomography (SPECT) are sufficiently sensitive to allow use at true tracer levels; so possible pharmacological effects of contrast agents need to be carefully considered. The same procedures for tolerability testing should apply to imaging agents as for new drugs. Some magnetic resonance imaging (MRI) techniques use inherent properties of tissues to provide endogenous imaging contrast. For instance, BOLD (blood-oxygen-level-dependent) MRI allows assessment of tissue oxygenation. These techniques avoid the use of pharmacological agents but results may be difficult to interpret.

Contrast-enhanced CT has the highest spatial resolution of all clinically applicable imaging techniques and is amenable to rapid kinetics. However, depending on the operating parameters and scan length, this may involve considerable ionising radiation dose per scan (0.02–0.6 Gy; typically 0.1–0.3 Gy) (Boone *et al*, 2004; Carlson *et al*, 2007; Brindle, 2008). Doses should be minimised to avoid compromising experimental results through interaction of ionising events with the biological processes of interest, as well as welfare issues; as a guide, total radiation dose >1 Gy can affect tumour growth and whole-body doses >6 Gy are generally lethal to small rodents. Users of fused PET–CT or SPECT–CT systems should note that the radiation dose from the PET or SPECT can be as large as the CT dose. In addition, iodine-based contrast agents are nephrotoxic and, if required for repeat studies, well-tolerated doses should be established.

Anaesthesia and restraint for imaging

Physical restraint and/or general anaesthesia are required for small-animal imaging. Both procedures can affect animal well-being and introduce experimental artefacts. Body temperature must be maintained and monitored during general anaesthesia using thermostatically controlled heating pads, microwaveable gels or warm air blowers. Light general anaesthesia using an inhalational anaesthetic such as isoflurane or a short-lived i.v. injectable such as propofol should be used for pharmacological restraint, wherever possible. Deleterious effects of physical restraint can be minimised by appropriate design of restrainers, provision of black-outs and acclimatisation (Warden *et al*, 2000; Narciso *et al*, 2003; King *et al*, 2005). Preferred methods will depend on the species, imaging modality and device used. Where general anaesthesia is not appropriate, sedation with use of gentle physical restraint is encouraged, taking account of veterinary advice. Acclimatisation needs to be thorough, as a short period of training can induce more stress (Warden *et al*, 2000; Narciso *et al*, 2003).

Table 5 Examples of imaging techniques

Imaging method	Imaging time	Spatial resolution ^a	Main purpose	Advantages	Disadvantages
Optical: Bioluminescence and fluorescence	Sec–min	Organ: 50 μ m Whole body: 1–5 mm	Monitoring tumour response to treatment in deep-seated/ orthotopic sites and metastatic spread; imaging gene expression and protein– protein interactions	Relatively non-invasive (requires restraint), high sensitivity (amol–nmol); many fluorescent and near-infrared probes available; amenable to use of gene reporters; relatively cheap	In most cases, these methods require genetic modification of tumour cells for detection; quantitation relies on 2D images in current imaging systems; nude or shaved animals are required
Three-dimensional high-frequency ultrasonography	Sec–min	100 μ m in-plane resolution	Relative measure of tissue blood flow; three-dimensional measurement of tumour size	Relatively non-invasive (requires restraint), high sensitivity (single particle); absolute measurements are possible with suitable contrast agents, e.g. microbubbles	Specialized application
Magnetic resonance imaging (MRI), spectroscopy (MRS) or spectroscopy imaging (MRSI)	Min–hours	100 μ m in-plane resolution (7 T); much higher for spectroscopy without imaging	Pharmacodynamics, pathophysiology, pharmacokinetics, anatomy	Some techniques use endogenous contrast; good spatial resolution (imaging)	Poor sensitivity (mmol), so that exogenous contrast agents and drugs need to be given at high concentration
Standard or contrast- enhanced computed tomography (CT)	Sec–min	100 μ m	Pharmacodynamics, anatomy	Very good spatial resolution combined with relatively good sensitivity	High radiation dose (for standard CT, 2 cGy for most currently available systems), limited number of contrast agents, which may be nephrotoxic
Single photon emission computed tomography (SPECT)	Min	1–2 mm	Pharmacodynamics, pathophysiology, pharmacokinetics	High sensitivity (pmol) Radiochemistry can be performed in nuclear medicine department	Limited radiochemicals available compared with PET, less quantitative
Positron emission tomography (PET)	Min	1–2 mm	Pharmacodynamics, pathophysiology, pharmacokinetics	High sensitivity (pmol) allowing true tracer kinetics; unlimited range of radiochemicals making it very flexible; fully quantitative	Poor spatial resolution; full quantitation requires cannulation (for kinetic studies); requires specialized radiochemistry in most situations
Optical intravital microscopy	Sec	1–5 μ m	Real-time imaging of tumour microcirculation	Microscopic spatial resolution, good sensitivity (single cell), many fluorescent contrast agents readily available, amenable to use of gene reporters; relatively cheap	Requires surgical intervention

^aOptimum spatial resolution is usually achieved at the expense of ability to obtain fast kinetics.

Length of imaging sessions

If applicable, animals should be transported to imaging facilities in suitable transport boxes, with food and water provided before imaging. The length, total number of imaging sessions and intervals between them depend on factors such as time required to acquire images, tolerance to restraint or general anaesthesia, half-life of the contrast agent and whether cannulation is required. Consideration also needs to be given to exposure of immune-deprived animals to a non-pathogen-free environment, as well as monitoring and control of animal physiology during imaging. If animals have no access to water, an imaging session should typically last no more than 2 h and total imaging time should not exceed 2–3 h in a 24-h period. Use of un-anaesthetised animals restrained for more than 2 h must be avoided except where there is exceptional justification, for example, for animals recovering from general anaesthesia after cannulation of superficial vessels before imaging. In this case, use of local analgesia around the cannulation site is essential. Animals anaesthetised for more than 2 h should be rehydrated if recovery is prolonged, for example, by injection of

dextrose/saline. If animals need to be anaesthetised more than once per day, they must be fully recovered, eating and drinking before being re-anaesthetised. On completion of a session, animals should either be killed or kept warm until full recovery from anaesthesia or until the next analysis session. Analysis may be repeated on the same animal but typically this should not exceed five sessions within a 1- to 2-week period and typically no more than one imaging session per day.

HUMANE ENDPOINTS

There are ethical, scientific and legal reasons for ensuring that adverse effects are minimised. Choice of appropriate humane endpoints provides significant opportunities for refinement, and should be developed in tandem with the requirements for a valid scientific outcome. Early endpoints reduce non-specific systemic effects and so may increase the precision of the results obtained. Pilot studies, including autopsy to determine the full extent of tumour growth, will facilitate the definition of robust and refined

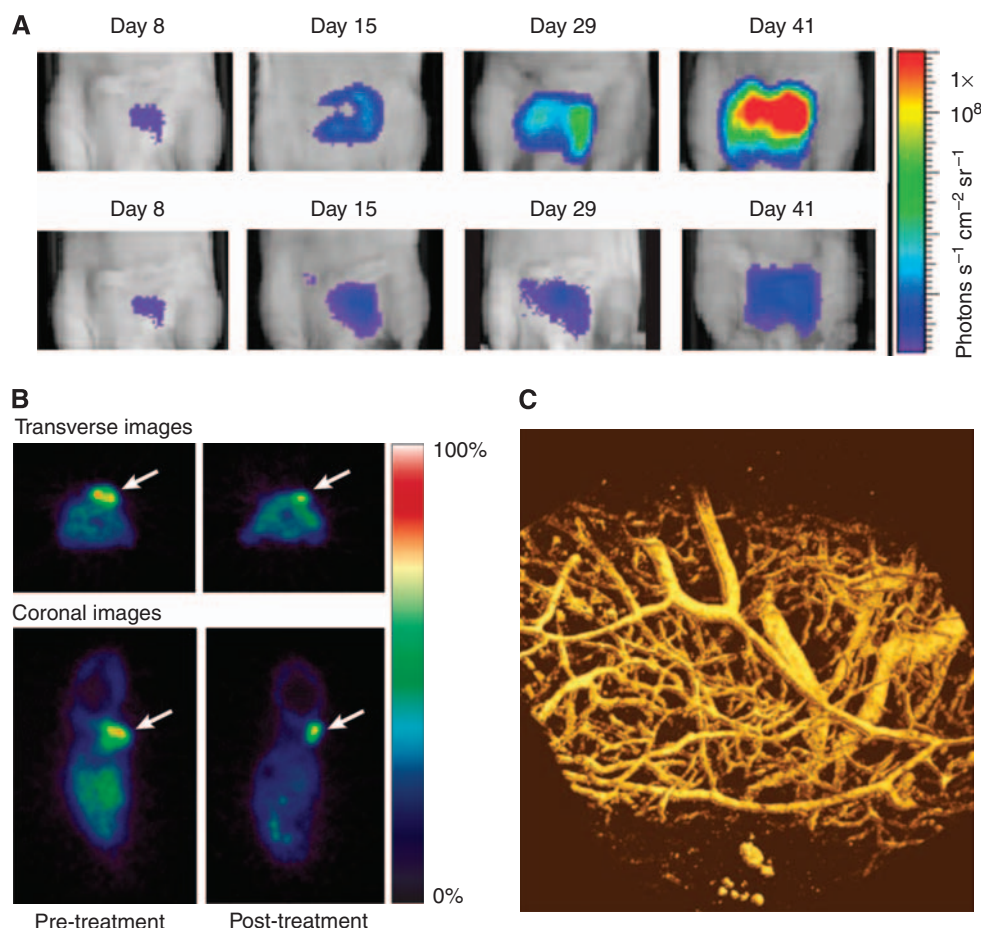


Figure 4 Examples of *in vivo* imaging in pre-clinical cancer research. **(A)** Optical surface bioluminescence imaging of orthotopically xenografted human PC3 prostate carcinoma cells transfected with luciferase (PC3luc2a). Mice were imaged using a Charged Coupled Device (CCD) camera, which is super-cooled to enhance detection sensitivity and image resolution. The images shown were taken after systemic administration of luciferin, with 'intensity of luminescence' shown as 'heat' maps and red as maximum intensity. The scale shows the number of photons detected. Top panel: Untreated mice at day 8–41 after transplantation; bottom panel: before and after treatment with 5 mg kg^{-1} taxotere on day 10. This technique is useful for monitoring treatment effects in deep-seated tumour sites. Light scattering through tissues makes precise quantitation difficult. **(B)** PET imaging of tumour cell proliferation using ^{18}F -3'-fluoro-3'-deoxy-L-thymidine (FLT). Transverse and coronal (0.5 mm) images of HCT116 tumour-bearing mice 24 h before treatment and after 4 daily treatments with the histone deacetylase inhibitor LAQ842 at 25 mg kg^{-1} . 30- to 60-min summed images from a dynamic scan are presented. Numerous radiotracers are available for investigating specific biochemical pathways *in vivo*, if specialised facilities are available. The scale shows the intensity of radiotracer uptake. **(C)** Intravital imaging of tumour vasculature of the P22 rat sarcoma growing in a dorsal skin flap window chamber. The image was obtained by multi-photon fluorescence microscopy after i.v. administration of 70 kDa FITC-dextran. High spatial resolution is obtained but surgical intervention is required.

endpoints. Endpoints for particular models must also take account of the known pathogenesis of the particular tumour model in question and should be regularly reviewed in the light of experience.

The endpoints proposed are based on animal models in widespread use (for examples see Tables 1 and 2); however, each study should be considered on its own merits. For example, tumorigenicity studies can be terminated as soon as progressive tumour growth is evident. By contrast, carcinogen-induced skin papillomas, for example, undergo malignant transformation late in their development and may require later endpoints. Imaging techniques facilitate the development of more defined endpoints for some tumour models. Every effort should be made to identify factors allowing scientific decisions to be made at the earliest stage possible, while taking into account the total burden of procedures on animal welfare. The intentional use of death as an endpoint is unacceptable and animals should not be allowed to become moribund.

The choice of site for solid tumours will influence the maximum acceptable tumour load and the appropriate humane endpoints. Sites such as the footpad, tail, eye or bone are likely to be painful or distressing and require special justification

and earlier endpoints. Similarly, tumours that metastasise to sensitive sites need great care. If brain tumours can be justified (e.g., to increase understanding of their biology and to develop therapies for this area of unmet clinical need), body weight loss is reportedly a sensitive endpoint (Redgate *et al*, 1991) and MRI or bioluminescent imaging (BLI) techniques can be very useful (van Furth *et al*, 2003; Ragel *et al*, 2008; McCann *et al*, 2009). Intramuscular tumours are painful and only justified where there is a strong case for orthotopic studies, for example, for sarcomas.

In genetically modified animals, particular care is needed to ensure detection of unexpected sites of tumour development. As with all internal tumour sites, this includes clinical examination, measurement of body weight, abdominal palpation and loss of condition. Humane endpoints, specialist care and interventions should reflect best practice and be discussed and agreed between researchers, veterinarians and animal care staff before commencement of the experiment. Development and publication of appropriate experimental analyses (e.g., pharmacodynamic determinations, functional imaging) to capture detailed phenotypic information assists rational determination of endpoints.

Tumour burden

Tumour burden should always be limited to the minimum required for a valid scientific outcome. For example, efficacy studies should be terminated once durable, statistically significant therapeutic effects can be shown. Therapeutic studies should be designed to avoid the need for control tumours to become excessively large. The size of any tumours should be limited when they are used simply for routine transplantation or as a source of tumour tissue. In all cases the general health and condition of an animal remains the overriding determinant. Adverse effects on the animal will depend on the biology, site, mode of growth of the tumour and any additional procedures or treatments. Despite the caveats, estimation of tumour size and burden is an important consideration in determining endpoints.

Assessment of the size of superficial tumours using callipers (usually of two diameters at right angles) is an easy and definable method. Measurement variations can be minimised by ensuring that the same well-trained technician is involved for the duration of the study. Response to therapy may be measured by changes in tumour growth rate, re-growth delay, cell survival (measured by clonogenic assay) or an appropriate surrogate marker. Excising and weighing tumours at the end of a study can provide an additional objective endpoint, which avoids errors due to variations in tumour shapes and estimations of volume or mass. For an animal carrying a single tumour, the mean diameter should not normally exceed 1.2 cm in mice or 2.5 cm in rats, or 1.5 and 2.8 cm, respectively, for therapeutic studies. Where two tumours per animal are grown, for example, in contralateral flanks, the size should be correspondingly less and should not exceed the maximum burden of a single tumour. Multiple tumours may develop in genetically modified animals (e.g., mammary tumours in polyoma virus middle T transgenic mice; Guy *et al*, 1992) or in the skin of animals subjected to UVR (El-Abaseri and Hansen, 2007) or chemical carcinogens (Johansen *et al*, 2009), for which similar limits should be observed. Exceptions to these advised size limits would require rigorous scientific justification.

Determining the tumour burden of internal orthotopic cancers, systemic lymphoreticular tumours or metastatic disease is challenging. Pilot experiments using small numbers of animals are important to allow characterisation of the kinetics and patterns of spread, to predict clinical signs and to define humane endpoints. Biomarkers or circulating cancer cells may be used as surrogates for assessing the burden of lymphomas and leukaemias, and real-time imaging is a valuable adjunct. Appropriate biochemical and pathological indicators or use of engineered reporter systems or imaging techniques should be used to determine the onset of disease. Reliance must also be placed on the general condition of the animal, together with assessment of palpable tumours and specific signs such as hind-limb weakness or paralysis.

Clinical signs

In general the clinical signs shown in Box 5 are principal indicators of rare but severe symptoms of potential adverse effects, which should be avoided. Where any one sign is present the animal should immediately be humanely culled and vigilance increased for the remainder of the cohort.

With solid tumours, scoring of ulceration, distension of covering tissues and cachexia (severe body weight loss) should be incorporated into the endpoints. Ulceration is a lesion typified by necrosis of superficial tissues, which may be dry, suppurating or exudative. Necrosis resulting in skin breakdown or exudation persisting beyond 48 h is grounds for termination. Some tumours, such as those grown in sensitive sites or that develop extensive necrosis, may be painful, although objective criteria are lacking for mice. Further research is required to enable better assessment of pain and to assist in formulating the most appropriate endpoints.

Box 5 CLINICAL SIGNS NECESSITATING IMMEDIATE INTERVENTION

1. Failure to eat or drink over a 24- to 48-h period resulting in emaciation or dehydration
2. Consistent or rapid body weight loss reaching 20% at any time or 15% maintained for 72 h compared with the pre-treatment weight of adult mice or age-matched, vehicle-treated controls. With some tumours body weight is a very poor indicator and muscle atrophy or emaciation is more useful. Body condition scoring provides a very useful indication of muscle loss (Ullman-Cullere and Foltz, 1999)
3. Persistent hypothermia
4. Bloodstained or mucopurulent discharge from any orifice
5. Laboured respiration, particularly if accompanied by nasal discharge and/or cyanosis
6. Enlarged lymph nodes or spleen
7. Hind-limb paralysis or weakness
8. Anaemia as indicated by symptoms such as pale feet, or haematological measures
9. Significant abdominal distension or where ascites burden exceeds 10% of the bodyweight of age-matched controls. Accurate determination is difficult but body girth is useful and a 20% increase should be the maximum normally allowed; similar to the appearance of a pregnant mouse
10. Incontinence or diarrhoea over a 48-h period
11. Tumours that interfere with locomotion or cause abnormal vocalisation, animal behaviour or function

Such severe symptoms are likely to occur very rarely in well-designed experimental studies, should be avoided and require immediate humane termination.

In all cases endpoints must provide for action to be taken to terminate animals humanely when the degree of suffering cannot be justified by the scientific objective, when the objective has been achieved or cannot be realised, or when the quality of the results has been compromised.

SUMMARY AND CONCLUDING REMARKS

This set of guidelines is designed to update and enhance the second edition (Workman *et al*, 1998). Information is provided on the more complex, molecularly defined and biologically relevant models now available, including genetically engineered, orthotopic and metastatic tumour systems. These more 'patient-like' models require sophisticated methods of evaluation; hence a detailed section on the different imaging modalities that are now used has been added. Tables 1 and 2 provide examples of some widely used experimental models. Figure 1 offers an example of the type of illustrative aid that can be used to facilitate the rational choice of appropriate models in a given study. Examples of tumour models, experimental design and procedures are provided throughout. However, it is emphasised that these are intended to act as a guide only, and each study should be tailored to the specific experimental objectives. There is renewed emphasis on continuing applications of the 3Rs – replacement (of animals with alternative methods), reduction (in the numbers of animals used to achieve scientific objectives) and refinement (in experimental design, techniques and husbandry to minimise adverse effects and improve welfare). There is an expectation that the highest animal welfare standards will be demanded from grant-awarding bodies and scientific journals. It is also emphasised that there is a responsibility for researchers to publish improved models and methodology for the benefit of the research community worldwide. A comprehensive bibliography is included to cover all of the principal topics and links to other, online resources are also provided. It is to be stressed that animal welfare considerations are not only important for ethical and legal reasons, but also should be fully consistent with the highest standards of scientific investigation. It is anticipated that the appropriate use of animal models will make an important contribution to increasing further our fundamental understanding of cancer and will enhance our growing ability to diagnose, treat and prevent it.

ACKNOWLEDGEMENTS

We thank authors Professor Fran Balkwill, Dr David Farningham, Professor Gill Tozer, Professor Sue Watson and Dr Steve Wedge for leading and coordinating subgroups of the committee dealing with specific topics.

REFERENCES

- Abatan OI, Welch KB, Nemzek JA (2008) Evaluation of saphenous venipuncture and modified tail-clip blood collection in mice. *J Am Assoc Lab Anim Sci* **47**: 8–15
- Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, DePinho RA (2003) Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev* **17**: 3112–3126
- Ahsan H, Aziz MH, Ahmad N (2005) Ultraviolet B exposure activates Stat3 signaling via phosphorylation at tyrosine705 in skin of SKH1 hairless mouse: a target for the management of skin cancer? *Biochem Biophys Res Commun* **333**: 241–246
- Alsheikhly AR, Zweiri J, Walmesley AJ, Watson AJ, Christmas SE (2004) Both soluble and membrane-bound forms of Flt3 ligand enhance tumor immunity following 'suicide' gene therapy in a murine colon carcinoma model. *Cancer Immunol Immunother* **53**: 946–954
- Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA (2000) Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* **406**: 641–645
- Artursson P, Palm K, Luthman K (2001) Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv Drug Deliv Rev* **46**: 27–43
- Baersch G, Möllers T, Hotte A, Dockhorn-Dworniczak B, Rube C, Ritter J, Jurgens H, Vormoor J (1997) Good engraftment of B-cell precursor ALL in NOD-SCID mice. *Klin Padiatr* **209**: 178–185
- Balansky RM, Ganchev G, D'Agostini F, De Flora S (2002) Effects of N-acetylcysteine in an esophageal carcinogenesis model in rats treated with diethylnitrosamine and diethyldithiocarbamate. *Int J Cancer* **98**: 493–497
- Banerji U, Walton M, Raynaud F, Grimshaw R, Kelland L, Valenti M, Judson I, Workman P (2005) Pharmacokinetic–pharmacodynamic relationships for the heat shock protein 90 molecular chaperone inhibitor 17-allylamine, 17-demethoxygeldanamycin in human ovarian cancer xenograft models. *Clin Cancer Res* **11**: 7023–7032
- Barfield M, Spooner N, Lad R, Parry S, Fowles S (2008) Application of dried blood spots combined with HPLC–MS/MS for the quantification of acetaminophen in toxicokinetic studies. *J Chromatogr B Analyt Technol Biomed Life Sci* **870**: 32–37
- Bazin R, Boucher G, Monier G, Chevrier MC, Verrette S, Broly H, Lemieux R (1994) Use of hu-IgG-SCID mice to evaluate the *in vivo* stability of human monoclonal IgG antibodies. *J Immunol Methods* **172**: 209–217
- Biotechnology and Biological Sciences Research Council; Department for Environment, Food and Rural Affairs; Medical Research Council; National Centre for the Replacement, Refinement and Reduction of Animals in Research; Natural Environment Research Council; Wellcome Trust (2008) Responsibility in the use of animals in bioscience research: expectations of the major research council and charitable funding bodies. National Centre for the Replacement, Refinement and Reduction of Animals in Research, London. <http://www.nc3rs.org.uk/downloaddoc.asp?id=719> (Accessed date 30 March 2010)
- Becher OJ, Holland EC, Sausville EA, Burger AM (2006) Genetically engineered models have advantages over xenografts for preclinical studies. *Cancer Res* **66**: 3355–3359
- Becker JC, Pancook JD, Gillies SD, Mendelsohn J, Reisfeld RA (1996) Eradication of human hepatic and pulmonary melanoma metastases in SCID mice by antibody-interleukin 2 fusion proteins. *Proc Natl Acad Sci USA* **93**: 2702–2707
- Bergers G, Javaherian K, Lo KM, Folkman J, Hanahan D (1999) Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science* **284**: 808–812
- Bignell GR, Greenman CD, Davies H, Butler AP, Edkins S, Andrews JM, Buck G, Chen L, Beare D, Latimer C, Widaa S, Hinton J, Fahey C, Fu B, Swamy S, Dalglish GL, Teh BT, Deloukas P, Yang F, Campbell PJ, Futreal PA, Stratton MR (2010) Signatures of mutation and selection in the cancer genome. *Nature* **463**: 893–898
- Blouin S, Basle MF, Chappard D (2008) Interactions between micro-environment and cancer cells in two animal models of bone metastasis. *Br J Cancer* **98**: 809–815
- Boone JM, Velazquez O, Cherry SR (2004) Small-animal X-ray dose from micro-CT. *Mol Imaging* **3**: 149–158
- Brindle K (2008) New approaches for imaging tumour responses to treatment. *Nat Rev Cancer* **8**: 94–107
- Brown EB, Campbell RB, Tsuzuki Y, Xu L, Carmeliet P, Fukumura D, Jain RK (2001) *In vivo* measurement of gene expression, angiogenesis and physiological function in tumors using multiphoton laser scanning microscopy. *Nat Med* **7**: 864–868
- Bruneau P, McElroy NR (2006) logD7.4 modeling using Bayesian Regularized Neural Networks. Assessment and correction of the errors of prediction. *J Chem Inf Model* **46**: 1379–1387
- Bruns CJ, Harbison MT, Kuniyasu H, Eue I, Fidler IJ (1999) *In vivo* selection and characterization of metastatic variants from human pancreatic adenocarcinoma by using orthotopic implantation in nude mice. *Neoplasia* **1**: 50–62
- Cai H, Sale S, Schmid R, Britton RG, Brown K, Steward WP, Gescher AJ (2009) Flavones as colorectal cancer chemopreventive agents – phenol-o-methylation enhances efficacy. *Cancer Prev Res (Phila PA)* **2**: 743–750
- Carlson SK, Classic KL, Bender CE, Russell SJ (2007) Small animal absorbed radiation dose from serial micro-computed tomography imaging. *Mol Imaging Biol* **9**: 78–82
- Chan R, Muller WJ, Siegel PM (1999) Oncogenic activating mutations in the neu/erbB-2 oncogene are involved in the induction of mammary tumors. *Ann N Y Acad Sci* **889**: 45–51
- Chen D, Livne-bar I, Vanderluit JL, Slack RS, Agochiya M, Bremner R (2004) Cell-specific effects of RB or RB/p107 loss on retinal development implicate an intrinsically death-resistant cell-of-origin in retinoblastoma. *Cancer Cell* **5**: 539–551
- Chen X, Yang G, Ding WY, Bondoc F, Curtis SK, Yang CS (1999) An esophagogastrroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. *Carcinogenesis* **20**: 1801–1808
- Chou TC (2006) Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev* **58**: 621–681
- Christophorou MA, Martin-Zanca D, Soucek L, Lawlor ER, Brown-Swigart L, Verschuren EW, Evan GI (2005) Temporal dissection of p53 function *in vitro* and *in vivo*. *Nat Genet* **37**: 718–726
- Christophorou MA, Ringshausen I, Finch AJ, Swigart LB, Evan GI (2006) The pathological response to DNA damage does not contribute to p53-mediated tumour suppression. *Nature* **443**: 214–217
- Colclough N, Hunter A, Kenny PW, Kittlety RS, Lobedan L, Tam KY, Timms MA (2008) High throughput solubility determination with application to selection of compounds for fragment screening. *Bioorg Med Chem* **16**: 6611–6616
- Collado B, Carmena MJ, Clemente C, Prieto JC, Bajo AM (2007) Vasoactive intestinal peptide enhances growth and angiogenesis of human experimental prostate cancer in a xenograft model. *Peptides* **28**: 1896–1901
- Collins I, Workman P (2006) New approaches to molecular cancer therapeutics. *Nat Chem Biol* **2**: 689–700
- Comstock KE, Hall CL, Daignault S, Mandlebaum SA, Yu C, Keller ET (2009) A bioluminescent orthotopic mouse model of human osteosarcoma that allows sensitive and rapid evaluation of new therapeutic agents *in vivo*. *In Vivo* **23**: 661–668
- Condeelis J, Segall JE (2003) Intravital imaging of cell movement in tumours. *Nat Rev Cancer* **3**: 921–930
- Corpet DE, Pierre F (2003) Point: from animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice

- and choice of the model system. *Cancer Epidemiol Biomarkers Prev* 12: 391–400
- De Fabo EC (2006) Initial studies on an *in vivo* action spectrum for melanoma induction. *Prog Biophys Mol Biol* 92: 97–104
- Decker S, Hollingshead M, Bonomi CA, Carter JP, Sausville EA (2004) The hollow fibre model in cancer drug screening: the NCI experience. *Eur J Cancer* 40: 821–826
- Dennis C (2006) Cancer: off by a whisker. *Nature* 17: 739–741
- Dennis Jr MB (2002) Welfare issues of genetically modified animals. *ILAR J* 43: 100–109
- Dewhirst MW, Gustafson C, Gross JF, Tso CY (1987) Temporal effects of 5.0 Gy radiation in healing subcutaneous microvasculature of a dorsal flap window chamber. *Radiat Res* 112: 581–591
- Dickson PV, Hamner B, Ng CY, Hall MM, Zhou J, Hargrove PW, McCarville MB, Davidoff AM (2007) *In vivo* bioluminescence imaging for early detection and monitoring of disease progression in a murine model of neuroblastoma. *J Pediatr Surg* 42: 1172–1179
- Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van der Vorstenbosch C (2001) A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 21: 15–23
- Dohta Y, Yamashita T, Horiike S, Nakamura T, Fukami T (2007) A system for LogD screening of 96-well plates using a water-plug aspiration/injection method combined with high-performance liquid chromatography-mass spectrometry. *Anal Chem* 79: 8312–8315
- Dong X, Guan J, English JC, Flint J, Yee J, Evans K, Murray N, Macaulay C, Ng RT, Gout PW, Lam WL, Laskin J, Ling V, Lam S, Wang Y (2010) Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. *Clin Cancer Res* 16: 1442–1451
- Du-Cuny L, Huwyler J, Wiese M, Kansy M (2008) Computational aqueous solubility prediction for drug-like compounds in congeneric series. *Eur J Med Chem* 43: 501–512
- Edinger M, Cao YA, Hornig YS, Jenkins DE, Verneris MR, Bachmann MH, Negrin RS, Contag CH (2002) Advancing animal models of neoplasia through *in vivo* bioluminescence imaging. *Eur J Cancer* 38: 2128–2136
- El-Abaseri TB, Hansen LA (2007) EGFR activation and ultraviolet light-induced skin carcinogenesis. *J Biomed Biotechnol* 2007: 97939
- Felsher DW, Bishop JM (1999) Reversible tumorigenesis by MYC in hematopoietic lineages. *Mol Cell* 4: 199–207
- Festing M, Overend P, Gaine Das R, Cortina Borja M, Berdoy M (2002) *The design of Animal Experiments: Reducing the Use of Animals in Research Through Better Experimental Design*. Royal Society of Medicine Press: London
- Festing MF (2002) The design and statistical analysis of animal experiments. *ILAR J* 43: 191–193
- Festing MF, Altman DG (2002) Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR J* 43: 244–258
- Flecknell P (2008) Analgesia from a veterinary perspective. *Br J Anaesth* 101: 121–124
- Fluck MM, Haslam SZ (1996) Mammary tumors induced by polyomavirus. *Breast Cancer Res Treat* 39: 45–56
- Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother Rep* 50: 219–244
- Frese KK, Tuveson DA (2007) Maximizing mouse cancer models. *Nat Rev Cancer* 7: 645–658
- Gajewski TF (2000) Monitoring specific T-cell responses to melanoma vaccines: ELISPOT, tetramers, and beyond. *Clin Diagn Lab Immunol* 7: 141–144
- Galbraith SM, Maxwell RJ, Lodge MA, Tozer GM, Wilson J, Taylor NJ, Stirling JJ, Sena L, Padhani AR, Rustin GJ (2003) Combretastatin A4 phosphate has tumor antivascular activity in rat and man as demonstrated by dynamic magnetic resonance imaging. *J Clin Oncol* 21: 2831–2842
- Garber K (2006) Realistic rodents? Debate grows over new mouse models of cancer. *J Natl Cancer Inst* 98: 1176–1178
- Giovannini M, Robanus-Maandag E, van der Valk M, Niwa-Kawakita M, Abramowski V, Goutebroze L, Woodruff JM, Berns A, Thomas G (2000) Conditional biallelic Nf2 mutation in the mouse promotes manifestations of human neurofibromatosis type 2. *Genes Dev* 14: 1617–1630
- Giver CR, Li JM, Hossain MS, Lonial S, Waller EK (2004) Reconstructing immunity after allogeneic transplantation. *Immunol Res* 29: 269–282
- Glass B, Uharek L, Zeis M, Loeffler H, Mueller-Ruchholtz W, Gassmann W (1996) Graft-versus-leukaemia activity can be predicted by natural cytotoxicity against leukaemia cells. *Br J Haematol* 93: 412–420
- Glinskii AB, Smith BA, Jiang P, Li X-M, Yang M, Hoffman RM, Glinsky GV (2003) Viable circulating metastatic cells produced in orthotopic but not ectopic prostate cancer models. *Cancer Res* 63: 4239–4243
- Golay J, Cittera E, Di Gaetano N, Manganini M, Mosca M, Nebuloni M, van Rooijen N, Vago L, Introna M (2006) The role of complement in the therapeutic activity of rituximab in a murine B lymphoma model homing in lymph nodes. *Haematologica* 91: 176–183
- Graf MR, Sauer JT, Merchant RE (2005) Tumor infiltration by myeloid suppressor cells in response to T cell activation in rat gliomas. *J Neurooncol* 73: 29–36
- Graff BA, Benjaminsen IC, Melas EA, Brurberg KG, Rofstad EK (2005) Changes in intratumor heterogeneity in blood perfusion in intradermal human melanoma xenografts during tumor growth assessed by DCE-MRI. *Magn Reson Imaging* 23: 961–966
- Guy CT, Cardiff RD, Muller WJ (1992) Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. *Mol Cell Biol* 12: 954–961
- Ha WS, Kim CK, Song SH, Kang CB (2001) Study on mechanism of multistep hepatotumorigenesis in rat: development of hepatotumorigenesis. *J Vet Sci* 2: 53–58
- Han Y, Chen XP, Huang ZY, Zhu H (2005) Nude mice multi-drug resistance model of orthotopic transplantation of liver neoplasm and Tc-99m MIBI SPECT on p-glycoprotein. *World J Gastroenterol* 11: 3335–3338
- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100: 57–70
- Harlin H, Gajewski TF (2008) Diagnosis and treatment of mycoplasma-contaminated cell cultures. *Curr Protoc Cytom Appendix 3: Appendix 3C*
- Harris JC, Gilliam AD, McKenzie AJ, Evans SA, Grabowska AM, Clarke PA, McWilliams DF, Watson SA (2004) The biological and therapeutic importance of gastrin gene expression in pancreatic adenocarcinomas. *Cancer Res* 64: 5624–5631
- Hawariah A, Stanslas J (1998) Antagonistic effects of styrylpyrone derivative (SPD) on 7,12-dimethylbenzanthracene-induced rat mammary tumors. *In Vivo* 12: 403–410
- Hedelund L, Lerche C, Wulf HC, Haedersdal M (2006) Carcinogenesis related to intense pulsed light and UV exposure: an experimental animal study. *Lasers Med Sci* 21: 198–201
- Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, Rustgi AK, Chang S, Tuveson DA (2005) Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* 7: 469–483
- Hirayama T, Honda A, Matsuzaki Y, Miyazaki T, Ikegami T, Doy M, Xu G, Lea M, Salen G (2006) Hypercholesterolemia in rats with hepatomas: increased oxysterols accelerate efflux but do not inhibit biosynthesis of cholesterol. *Hepatology* 44: 602–611
- Hirose Y, Hata K, Kuno T, Yoshida K, Sakata K, Yamada Y, Tanaka T, Reddy BS, Mori H (2004) Enhancement of development of azoxymethane-induced colonic premalignant lesions in C57BL/KsJ-db/db mice. *Carcinogenesis* 25: 821–825
- Hoffman RM (2005) The multiple uses of fluorescent proteins to visualize cancer *in vivo*. *Nat Rev Cancer* 5: 796–806
- Hoffman RM, Yang M (2005) Dual-color, whole-body imaging in mice. *Nat Biotechnol* 23: 790; author reply 791
- Houston JB, Carlile DJ (1997) Prediction of hepatic clearance from microsomes, hepatocytes, and liver slices. *Drug Metab Rev* 29: 891–922
- Howard ML, Hill JJ, Galluppi GR, McLean MA (2010) Plasma protein binding in drug discovery and development. *Comb Chem High Throughput Screen* 13: 170–187
- Huxham LA, Kyle AH, Baker JH, Nykilchuk LK, Minchinton AI (2004) Microregional effects of gemcitabine in HCT-116 xenografts. *Cancer Res* 64: 6537–6541
- Ihle NT, Lemos Jr R, Wipf P, Yacoub A, Mitchell C, Siwak D, Mills GB, Dent P, Kirkpatrick DL, Powis G (2009) Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer Res* 69: 143–150
- Ishikawa Y, Kozakai T, Morita H, Saida K, Oka S, Masuo Y (2006) Rapid detection of mycoplasma contamination in cell cultures using SYBR Green-based real-time polymerase chain reaction. *In Vitro Cell Dev Biol Anim* 42: 63–69
- Jackson IL, Vujaskovic Z, Down JD (2010) Revisiting strain-related differences in radiation sensitivity of the mouse lung: recognizing and avoiding the confounding effects of pleural effusions. *Radiat Res* 173: 10–20
- Johansen C, Vestergaard C, Kragballe K, Kollias G, Gaestel M, Iversen L (2009) MK2 regulates the early stages of skin tumor promotion. *Carcinogenesis* 30: 2100–2108

- Johnson L, Mercer K, Greenbaum D, Bronson RT, Crowley D, Tuveson DA, Jacks T (2001) Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature* **410**: 1111–1116
- Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* **29**: 418–425
- Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA (2007) Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* **449**: 557–563
- Kato J, Kobayashi T, Villec CA (1968) Effect of clomiphene on the uptake of estradiol by the anterior hypothalamus and hypophysis. *Endocrinology* **82**: 1049–1052
- Kelland LR (2004) Of mice and men: values and liabilities of the athymic nude mouse model in anticancer drug development. *Eur J Cancer* **40**: 827–836
- Kenerson H, Dundon TA, Yeung RS (2005) Effects of rapamycin in the Eker rat model of tuberous sclerosis complex. *Pediatr Res* **57**: 67–75
- Kennel SJ, Boll R, Stabin M, Schuller HM, Mirzadeh S (1999) Radioimmunotherapy of micrometastases in lung with vascular targeted 213Bi. *Br J Cancer* **80**: 175–184
- Kim EJ, Shin M, Park H, Hong JE, Shin HK, Kim J, Kwon DY, Park JH (2009) Oral administration of 3,3'-diindolylmethane inhibits lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice. *J Nutr* **139**: 2373–2379
- King JA, Garelick TS, Brevard ME, Chen W, Messenger TL, Duong TQ, Ferris CF (2005) Procedure for minimizing stress for fMRI studies in conscious rats. *J Neurosci Methods* **148**: 154–160
- Koehl GE, Gaumann A, Geissler EK (2009) Intravital microscopy of tumor angiogenesis and regression in the dorsal skin fold chamber: mechanistic insights and preclinical testing of therapeutic strategies. *Clin Exp Metastasis* **26**: 329–344
- Komatsubara H, Umeda M, Ojima Y, Minamikawa T, Komori T (2005) Detection of cancer cells in the peripheral blood and lung of mice after transplantation of human adenoid cystic carcinoma. *Kobe J Med Sci* **51**: 67–72
- Kragh M, Hjarnaa PJ, Bramm E, Kristjansen PE, Rygaard J, Binderup L (2003) *In vivo* chamber angiogenesis assay: an optimized Matrigel plug assay for fast assessment of antiangiogenic activity. *Int J Oncol* **22**: 305–311
- Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A (2001) Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature* **413**: 83–86
- Lacroix M (2008) Persistent use of 'false' cell lines. *Int J Cancer* **122**: 1–4
- Lee PH, Ayyampalayam SN, Carreira LA, Shalaeva M, Bhattachar S, Coselmon R, Poole S, Gifford E, Lombardo F (2007) *In silico* prediction of ionization constants of drugs. *Mol Pharm* **4**: 498–512
- Lehr HA, Leunig M, Menger MD, Nolte D, Messmer K (1993) Dorsal skinfold chamber technique for intravital microscopy in nude mice. *Am J Pathol* **143**: 1055–1062
- Li M, Huang X, Zhu Z, Wong M, Watkins S, Zhao Q, Herberman R, Gorelik E (2001) Immune response against 3LL Lewis lung carcinoma potentiates the therapeutic efficacy of endostatin. *J Immunother* **24**: 472–481
- Li Z, Chen Z, Lu J, Cen J, He J, Chen S, Xue Y, Guo L (2006) Establishment of a nude mice model of human monocytic leukemia with CNS and multiorgan extramedullary infiltration. *Eur J Haematol* **77**: 128–133
- Liem NL, Papa RA, Milross CG, Schmid MA, Tajbakhsh M, Choi S, Ramirez CD, Rice AM, Haber M, Norris MD, MacKenzie KL, Lock RB (2004) Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. *Blood* **103**: 3905–3914
- Lifsted T, Le Voyer T, Williams M, Muller W, Klein-Szanto A, Buetow KH, Hunter KW (1998) Identification of inbred mouse strains harboring genetic modifiers of mammary tumor age of onset and metastatic progression. *Int J Cancer* **77**: 640–644
- Lock RB, Liem NL, Papa RA (2005) Preclinical testing of antileukemic drugs using an *in vivo* model of systemic disease. *Methods Mol Med* **111**: 323–334
- Mahteme H, Lovqvist A, Graf W, Lundqvist H, Carlsson J, Sundin A (1998) Adjuvant 131I-anti-CEA-antibody radioimmunotherapy inhibits the development of experimental colonic carcinoma liver metastases. *Anticancer Res* **18**: 843–848
- Mannhold R, Poda GI, Ostermann C, Tetko IV (2009) Calculation of molecular lipophilicity: state-of-the-art and comparison of log P methods on more than 96,000 compounds. *J Pharm Sci* **98**: 861–893
- Marcotte R, Muller WJ (2008) Signal transduction in transgenic mouse models of human breast cancer – implications for human breast cancer. *J Mammary Gland Biol Neoplasia* **13**: 323–335
- Martensson L, Wang Z, Nilsson R, Ohlsson T, Senter P, Sjogren HO, Strand SE, Tennvall J (2005) Determining maximal tolerable dose of the monoclonal antibody BR96 labeled with 90Y or 177Lu in rats: establishment of a syngeneic tumor model to evaluate means to improve radioimmunotherapy. *Clin Cancer Res* **11**: 7104s–7108s
- Martinsen TC, Kawase S, Hakanson R, Torp SH, Fossmark R, Qvigstad G, Sandvik AK, Waldum HL (2003) Spontaneous ECL cell carcinomas in cotton rats: natural course and prevention by a gastrin receptor antagonist. *Carcinogenesis* **24**: 1887–1896
- Masimirembwa CM, Thompson R, Andersson TB (2001) *In vitro* high throughput screening of compounds for favorable metabolic properties in drug discovery. *Comb Chem High Throughput Screen* **4**: 245–263
- Masters JR et al (2001) Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc Natl Acad Sci USA* **98**: 8012–8017
- McCann CM, Waterman P, Figueiredo JL, Aikawa E, Weissleder R, Chen JW (2009) Combined magnetic resonance and fluorescence imaging of the living mouse brain reveals glioma response to chemotherapy. *Neuroimage* **45**: 360–369
- Mitra SK, Lim ST, Chi A, Schlaepfer DD (2006) Intrinsic focal adhesion kinase activity controls orthotopic breast carcinoma metastasis via the regulation of urokinase plasminogen activator expression in a syngeneic tumor model. *Oncogene* **25**: 4429–4440
- Miyazaki K, Koshikawa N, Hasegawa S, Momiyama N, Nagashima Y, Moriyama K, Ichikawa Y, Ishikawa T, Mitsuhashi M, Shimada H (1999) Matrilysin as a target for chemotherapy for colon cancer: use of antisense oligonucleotides as antimetastatic agents. *Cancer Chemother Pharmacol* **43**Suppl: S52–S55
- Morton DB, Jennings M, Buckwell A, Ewbank R, Godfrey C, Holgate B, Inglis I, James R, Page C, Sharman I, Verschoyle R, Westall L, Wilson AB (2001) Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. British Veterinary Association Animal Welfare Foundation/Fund for the Replacement of Animals in Medical Experiments/Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare. *Lab Anim* **35**: 1–41
- Nakai M, Mundy GR, Williams PJ, Boyce B, Yoneda T (1992) A synthetic antagonist to laminin inhibits the formation of osteolytic metastases by human melanoma cells in nude mice. *Cancer Res* **52**: 5395–5399
- Nakatsugawa S, Okuda T, Muramoto H, Koyama K, Ishigaki T, Tsuruoka T, Hosokawa M, Kobayashi H (1999) Inhibitory effect of ND2001 on spontaneous multiple metastasis of NC 65 tumors derived from human renal cancer cells intradermally transplanted into nude mice. *Anticancer Drugs* **10**: 229–233
- Narciso SP, Nadziejko E, Chen LC, Gordon T, Nadziejko C (2003) Adaptation to stress induced by restraining rats and mice in nose-only inhalation holders. *Inhal Toxicol* **15**: 1133–1143
- Nardone RM (2007) Eradication of cross-contaminated cell lines: a call for action. *Cell Biol Toxicol* **23**: 367–372
- Neale G, Su X, Morton CL, Phelps D, Gorlick R, Lock RB, Reynolds CP, Maris JM, Friedman HS, Dome J, Khoury J, Triche TJ, Seeger RC, Gilbertson R, Khan J, Smith MA, Houghton PJ (2008) Molecular characterization of the pediatric preclinical testing panel. *Clin Cancer Res* **14**: 4572–4583
- Nguyen DX, Bos PD, Massague J (2009a) Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* **9**: 274–284
- Nguyen QD, Smith G, Glaser M, Perumal M, Arstad E, Aboagye EO (2009b) Positron emission tomography imaging of drug-induced tumor apoptosis with a caspase-3/7 specific [18F]-labeled isatin sulfonamide. *Proc Natl Acad Sci USA* **106**: 16375–16380
- Nijmeijer BA, Mollevanger P, van Zelderen-Bhola SL, Kluin-Nelemans HC, Willemze R, Falkenburg JH (2001) Monitoring of engraftment and progression of acute lymphoblastic leukemia in individual NOD/SCID mice. *Exp Hematol* **29**: 322–329
- Noel A, De Pauw-Gillet MC, Purnell G, Nusgens B, Lapiere CM, Foidart JM (1993) Enhancement of tumorigenicity of human breast adenocarcinoma cells in nude mice by Matrigel and fibroblasts. *Br J Cancer* **68**: 909–915
- Nordsmark M, Maxwell RJ, Wood PJ, Stratford IJ, Adams GE, Overgaard J, Horsman MR (1996) Effect of hyalalazine in spontaneous tumours assessed by oxygen electrodes and 31P-magnetic resonance spectroscopy. *Br J Cancer Suppl* **27**: S232–S235
- Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth

- and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 121: 335–348
- Osborne NJ, Payne D, Newman ML (2009) Journal editorial policies, animal welfare, and the 3Rs. *Am J Bioeth* 9: 55–59
- Ottaviani G, Martel S, Carrupt PA (2006) Parallel artificial membrane permeability assay: a new membrane for the fast prediction of passive human skin permeability. *J Med Chem* 49: 3948–3954
- Park ES, Rabinovsky R, Carey M, Hennessy BT, Agarwal R, Liu W, Ju Z, Deng W, Lu Y, Woo HG, Kim SB, Cheong JH, Garraway LA, Weinstein JN, Mills GB, Lee JS, Davies MA (2010) Integrative analysis of proteomic signatures, mutations, drug responsiveness in the NCI 60 cancer cell line set. *Mol Cancer Ther* 9: 257–267
- Parodi B, Aresu O, Bini D, Lorenzini R, Schena F, Visconti P, Cesaro M, Ferrera D, Andreotti V, Ruzzon T (2002) Species identification and confirmation of human and animal cell lines: a PCR-based method. *Biotechniques* 32: 432–434, 436, 438–40
- Patel S, Turner PR, Stubberfield C, Barry E, Rohlf CR, Stamps A, McKenzie E, Young K, Tyson K, Terrett J, Box G, Eccles S, Page MJ (2002) Hyaluronidase gene profiling and role of hyal-1 overexpression in an orthotopic model of prostate cancer. *Int J Cancer* 97: 416–424
- Paulmurugan R, Umezawa Y, Gambhir SS (2002) Noninvasive imaging of protein–protein interactions in living subjects by using reporter protein complementation and reconstitution strategies. *Proc Natl Acad Sci USA* 99: 15608–15613
- Pearse G, Frith J, Randall KJ, Klinowska T (2009) Urinary retention and cystitis associated with subcutaneous estradiol pellets in female nude mice. *Toxicol Pathol* 37: 227–234
- Pelengaris S, Littlewood T, Khan M, Elia G, Evan G (1999) Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. *Mol Cell* 3: 565–577
- Pillai RG, Forster M, Perumal M, Mitchell F, Leyton J, Aibgirhio FI, Golovko O, Jackman AL, Aboagye EO (2008) Imaging pharmacodynamics of the alpha-folate receptor-targeted thymidylate synthase inhibitor BGC 945. *Cancer Res* 68: 3827–3834
- Politi K, Fan PD, Shen R, Zakowski M, Varmus H (2010) Erlotinib resistance in mouse models of epidermal growth factor receptor-induced lung adenocarcinoma. *Dis Model Mech* 3: 111–119
- Politi K, Zakowski MF, Fan PD, Schonfeld EA, Pao W, Varmus HE (2006) Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to downregulation of the receptors. *Genes Dev* 20: 1496–1510
- Poller B, Gutmann H, Krahenbuhl S, Weksler B, Romero I, Couraud PO, Tuffin G, Drewe J, Huwyler J (2008) The human brain endothelial cell line hCMEC/D3 as a human blood–brain barrier model for drug transport studies. *J Neurochem* 107: 1358–1368
- Prigozhina TB, Gurevitch O, Morecki S, Yakovlev E, Elkin G, Slavin S (2002) Nonmyeloablative allogeneic bone marrow transplantation as immunotherapy for hematologic malignancies and metastatic solid tumors in preclinical models. *Exp Hematol* 30: 89–96
- Qian CN, Furge KA, Knol J, Huang D, Chen J, Dykema KJ, Kort EJ, Massie A, Khoo SK, Vanden Beldt K, Resau JH, Anema J, Kahnoski RJ, Morreau H, Camparo P, Comperat E, Sibony M, Denoux Y, Molinie V, Vieillefond A, Eng C, Williams BO, Teh BT (2009) Activation of the PI3K/AKT pathway induces urothelial carcinoma of the renal pelvis: identification in human tumors and confirmation in animal models. *Cancer Res* 69: 8256–8264
- Quagliano E, Mastini C, Forni G, Cavallo F (2008) ErbB2 transgenic mice: a tool for investigation of the immune prevention and treatment of mammary carcinomas. *Curr Protoc Immunol* Chapter 20: Unit 20 9 1–Unit 20 9–10
- Radaelli E, Ceruti R, Patton V, Russo M, Degraffi A, Croci V, Caprera F, Stortini G, Scanziani E, Pesenti E, Alzani R (2009) Immunohistopathological and neuroimaging characterization of murine orthotopic xenograft models of glioblastoma multiforme recapitulating the most salient features of human disease. *Histol Histopathol* 24: 879–891
- Ragel BT, Elam IL, Gillespie DL, Flynn JR, Kelly DA, Mabey D, Feng H, Couldwell WT, Jensen RL (2008) A novel model of intracranial meningioma in mice using luciferase-expressing meningioma cells. Laboratory investigation. *J Neurosurg* 108: 304–310
- Redgate ES, Deutsch M, Boggs SS (1991) Time of death of CNS tumor-bearing rats can be reliably predicted by body weight-loss patterns. *Lab Anim Sci* 41: 269–273
- Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T (2000) Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. *Nat Genet* 26: 109–113
- Reyes-Aldasoro CC, Wilson I, Prise VE, Barber PR, Ameer-Beg M, Vojnovic B, Cunningham VJ, Tozer GM (2008) Estimation of apparent tumor vascular permeability from multiphoton fluorescence microscopic images of P22 rat sarcomas *in vivo*. *Microcirculation* 15: 65–79
- Richardson CA, Flecknell PA (2005) Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress? *Altern Lab Anim* 33: 119–127
- Riley RJ, Martin IJ, Cooper AE (2002) The influence of DMPK as an integrated partner in modern drug discovery. *Curr Drug Metab* 3: 527–550
- Robanus-Maandag E, Dekker M, van der Valk M, Carrozza ML, Jeanny JC, Dannenberg JH, Berns A, te Riele H (1998) p107 is a suppressor of retinoblastoma development in pRb-deficient mice. *Genes Dev* 12: 1599–1609
- Rodriguez-Cuesta J, Vidal-Vanaclocha F, Mendoza L, Valcarcel M, Gallot N, Martinez de Tejada G (2005) Effect of asymptomatic natural infections due to common mouse pathogens on the metastatic progression of B16 murine melanoma in C57BL/6 mice. *Clin Exp Metastasis* 22: 549–558
- Rottenberg S, Jonkers J (2008) Modeling therapy resistance in genetically engineered mouse cancer models. *Drug Resist Updat* 11: 51–60
- Roughan JV, Flecknell PA, Davies BR (2004) Behavioural assessment of the effects of tumour growth in rats and the influence of the analgesics carprofen and meloxicam. *Lab Anim* 38: 286–296
- Rowland M, Tozer TN (1995) *Clinical Pharmacokinetics: Concepts and Applications*. 3rd edn., Chapter 7, pp 83–105. Williams and Wilkins (now Lippincott, Williams and Wilkins): Philadelphia, USA
- Rubio-Viqueira B, Hidalgo M (2009) Direct *in vivo* xenograft tumor model for predicting chemotherapeutic drug response in cancer patients. *Clin Pharmacol Ther* 85: 217–221
- Rusciano D, Lorenzoni P, Burger M (1994) Murine models of liver metastasis. *Invasion Metastasis* 14: 349–361
- Russell PJ, Ho Shon I, Boniface GR, Izard ME, Philips J, Raghavan D, Walker KZ (1991) Growth and metastasis of human bladder cancer xenografts in the bladder of nude rats. A model for intravesical radioimmunotherapy. *Urol Res* 19: 207–213
- Russell WMS, Burch RL (1959) *The Principles of Humane Experimental Technique*. Methuen: London
- Santarius T, Shipley J, Brewer D, Stratton MR, Cooper CS (2010) A census of amplified and overexpressed human cancer genes. *Nat Rev Cancer* 10: 59–64
- Sausville EA, Burger AM, Becher OJ, Holland EC (2006) Contributions of human tumor xenografts to anticancer drug development 10.1158/0008-5472.CAN-05-3627. *Cancer Res* 66: 3351–3354
- Sawyers C (2004) Targeted cancer therapy. *Nature* 432: 294–297
- Serganova I, Moroz E, Vider J, Gogiberidze G, Moroz M, Pillarsetty N, Doubrovina M, Minn A, Thaler HT, Massague J, Gelovani J, Blasberg R (2009) Multimodality imaging of TGFβ signaling in breast cancer metastases. *FASEB J* 23: 2662–2672
- Sharkey RM, Weadock KS, Natale A, Haywood L, Aninipot R, Blumenthal RD, Goldenberg DM (1991) Successful radioimmunotherapy for lung metastasis of human colonic cancer in nude mice. *J Natl Cancer Inst* 83: 627–632
- Sharpless NE, DePinho RA (2006) The mighty mouse: genetically engineered mouse models in cancer drug development. *Nat Rev Drug Discov* 5: 741–754
- Shibata H, Toyama K, Shioya H, Ito M, Hirota M, Hasegawa S, Matsumoto H, Takano H, Akiyama T, Toyoshima K, Kanamaru R, Kanegae Y, Saito I, Nakamura Y, Shiba K, Noda T (1997) Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. *Science* 278: 120–123
- Shibata MA, Shibata E, Morimoto J, Eid NA, Tanaka Y, Watanabe M, Otsuki Y (2009) An immunocompetent murine model of metastatic mammary cancer accessible to bioluminescence imaging. *Anticancer Res* 29: 4389–4395
- Siim BG, Lee AE, Shalal-Zwain S, Puij FB, McKeage MJ, Wilson WR (2003) Marked potentiation of the antitumour activity of chemotherapeutic drugs by the antivascular agent 5,6-dimethylxanthone-4-acetic acid (DMXAA). *Cancer Chemother Pharmacol* 51: 43–52
- Singh SS (2006) Preclinical pharmacokinetics: an approach towards safer and efficacious drugs. *Curr Drug Metab* 7: 165–182
- Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P (1987) Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes *in vivo*. *Cell* 49: 465–475
- Smith NF, Raynaud FI, Workman P (2007) The application of cassette dosing for pharmacokinetic screening in small-molecule cancer drug discovery. *Mol Cancer Ther* 6: 428–440

- Soucek L, Whitfield J, Martins CP, Finch AJ, Murphy DJ, Sodor NM, Karnezis AN, Swigart LB, Nasi S, Evan GI (2008) Modelling Myc inhibition as a cancer therapy. *Nature* **455**: 679–683
- Spaeth EL, Dembinski JL, Sasser AK, Watson K, Klopp A, Hall B, Andreeff M, Marini F (2009) Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS One* **4**: e4992
- Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mark TW (2000) High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten +/– mice. *Cancer Res* **60**: 3605–3611
- Stratton MR, Campbell PJ, Futreal PA (2009) The cancer genome. *Nature* **458**: 719–724
- Sung H, Kang SH, Bae YJ, Hong JT, Chung YB, Lee CK, Song S (2006) PCR-based detection of mycoplasma species. *J Microbiol* **44**: 42–49
- Takeda N, Diksic M (1999) Relationship between drug delivery and the intra-arterial infusion rate of SarCNU in C6 rat brain tumor model. *J Neurooncol* **41**: 235–246
- Taketo MM (2006) Mouse models of gastrointestinal tumors. *Cancer Sci* **97**: 355–361
- Tan M, Fang HB, Tian GL, Houghton PJ (2005) Repeated-measures models with constrained parameters for incomplete data in tumour xenograft experiments. *Stat Med* **24**: 109–119
- Tanaka T, Konno H, Matsuda I, Nakamura S, Baba S (1995) Prevention of hepatic metastasis of human colon cancer by angiogenesis inhibitor TNP-470. *Cancer Res* **55**: 836–839
- Tennant DA, Frezza C, MacKenzie ED, Nguyen QD, Zheng L, Selak MA, Roberts DL, Dive C, Watson DG, Aboagye EO, Gottlieb E (2009) Reactivating HIF prolyl hydroxylases under hypoxia results in metabolic catastrophe and cell death. *Oncogene* **28**: 4009–4021
- Tsai PP, Stelzer HD, Schraepfer A, Hackbarth H (2006) Importance and effects of enrichment on physiology, behaviour and breeding performance in mice. *Altex* **23** (Suppl): 96–98
- Tuchin VV (1993) Laser light scattering in biomedical diagnostics and therapy. *J Laser Appl* **5**: 43–60
- Ullman-Cullere MH, Foltz CJ (1999) Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci* **49**: 319–323
- van Furth WR, Laughlin S, Taylor MD, Salhia B, Mainprize T, Henkelman M, Cusimano MD, Ackerley C, Rutka JT (2003) Imaging of murine brain tumors using a 1.5 Tesla clinical MRI system. *Can J Neurol Sci* **30**: 326–332
- van Kranen HJ, de Gruijl FR (1999) Mutations in cancer genes of UV-induced skin tumors of hairless mice. *J Epidemiol* **9**: S58–S65
- Varticovski L, Hollingshead MG, Robles AI, Wu X, Cherry J, Munroe DJ, Lukes L, Anver MR, Carter JP, Borgel SD, Stotler H, Bonomi CA, Nunez NP, Hursting SD, Qiao W, Deng CX, Green JE, Hunter KW, Merlino G, Steeg PS, Wakefield LM, Barrett JC (2007) Accelerated preclinical testing using transplanted tumors from genetically engineered mouse breast cancer models. *Clin Cancer Res* **13**: 2168–2177
- Verheul HM, Hammers H, van Erp K, Wei Y, Sanni T, Salumbides B, Qian DZ, Yancopoulos GD, Pili R (2007) Vascular endothelial growth factor trap blocks tumor growth, metastasis formation, and vascular leakage in an orthotopic murine renal cell cancer model. *Clin Cancer Res* **13**: 4201–4208
- Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. *Nat Med* **10**: 789–799
- Warden SJ, Bennell KL, McMeeken JM, Wark JD (2000) A technique for restraining rodents during hindlimb interventions. *Contemp Top Lab Anim Sci* **39**: 24–27
- Watanabe H, Uesaka T, Kido S, Ishimura Y, Shiraki K, Kuramoto K, Hirata S, Shoji S, Katoh O, Fujimoto N (1999) Gastric tumor induction by 1,2-dimethylhydrazine in Wistar rats with intestinal metaplasia caused by X-irradiation. *Jpn J Cancer Res* **90**: 1207–1211
- Watanabe T, Schulz D, Morisseau C, Hammock BD (2006) High-throughput pharmacokinetic method: cassette dosing in mice associated with minuscule serial bleedings and LC/MS/MS analysis. *Anal Chim Acta* **559**: 37–44
- Watson SA, Michaeli D, Morris TM, Clarke P, Varro A, Griffin N, Smith A, Justin T, Hardcastle JD (1999a) Antibodies raised by gastrin immune inhibit the spontaneous metastasis of a human colorectal tumour, AP5LV. *Eur J Cancer* **35**: 1286–1291
- Watson SA, Morris TM, Varro A, Michaeli D, Smith AM (1999b) A comparison of the therapeutic effectiveness of gastrin neutralisation in two human gastric cancer models: relation to endocrine and autocrine/paracrine gastrin mediated growth. *Gut* **45**: 812–817
- Weiss WA, Aldape K, Mohapatra G, Feuerstein BG, Bishop JM (1997) Targeted expression of MYCN causes neuroblastoma in transgenic mice. *EMBO J* **16**: 2985–2995
- Weissleder R, Pittet MJ (2008) Imaging in the era of molecular oncology. *Nature* **452**: 580–589
- Wells DJ, Playle LC, Enser WE, Flecknell PA, Gardiner MA, Holland J, Howard BR, Hubrecht R, Humphreys KR, Jackson IJ, Lane N, Macconochie M, Mason G, Morton DB, Raymond R, Robinson V, Smith JA, Watt N (2006) Assessing the welfare of genetically altered mice. *Lab Anim* **40**: 111–114
- Wetmore C, Eberhart DE, Curran T (2001) Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. *Cancer Res* **61**: 513–516
- Winter SF, Hunter KW (2008) Mouse modifier genes in mammary tumorigenesis and metastasis. *J Mammary Gland Biol Neoplasia* **13**: 337–342
- Wood PJ, Stratford IJ, Sansom JM, Cattanauch BM, Quinney RM, Adams GE (1992) The response of spontaneous and transplantable murine tumors to vasoactive agents measured by ³¹P magnetic resonance spectroscopy. *Int J Radiat Oncol Biol Phys* **22**: 473–476
- Workman P, Balmain A, Hickman JA, McNally NJ, Rohas AM, Mitchison NA, Pierrepont CG, Raymond R, Rowlatt C, Stephens TC, Wallace J (1988) UKCCCR guidelines for the welfare of animals in experimental neoplasia. *Br J Cancer* **58**: 109–113
- Workman P, Aboagye EO, Chung YL, Griffiths JR, Hart R, Leach MO, Maxwell RJ, McSheehy PM, Price PM, Zweit J (2006) Minimally invasive pharmacokinetic and pharmacodynamic technologies in hypothesis-testing clinical trials of innovative therapies. *J Natl Cancer Inst* **98**: 580–598
- Workman P, de Bono J (2008) Targeted therapeutics for cancer treatment: major progress towards personalised molecular medicine. *Curr Opin Pharmacol* **8**: 359–362
- Workman P, Twentymann P, Balkwill F, Balmain A, Chaplin D, Double J, Embleton J, Newell D, Raymond R, Stables J, Stephens T, Wallace J (1998) UKCCCR guidelines for the welfare of animals in experimental neoplasia (Second Edition). *Br J Cancer* **77**: 1–10
- Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX (1999) Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat Genet* **22**: 37–43
- Yang L, Mao H, Cao Z, Wang YA, Peng X, Wang X, Sajja HK, Wang L, Duan H, Ni C, Staley CA, Wood WC, Gao X, Nie S (2009) Molecular imaging of pancreatic cancer in an animal model using targeted multifunctional nanoparticles. *Gastroenterology* **136**: 1514–1525; e2
- Yang M, Reynoso J, Jiang P, Li L, Moossa AR, Hoffman RM (2004) Transgenic nude mouse with ubiquitous green fluorescent protein expression as a host for human tumors. *Cancer Res* **64**: 8651–8656
- Yang W, Velich A, Mariadason J, Nicholas C, Corner G, Houston M, Edelmann W, Kucherlapati R, Holt PR, Augenlicht LH (2001) p21(WAF1/cip1) is an important determinant of intestinal cell response to sulindac *in vitro* and *in vivo*. *Cancer Res* **61**: 6297–6302
- Yoshino K, Iimura E, Saijo K, Iwase S, Fukami K, Ohno T, Obata Y, Nakamura Y (2006) Essential role for gene profiling analysis in the authentication of human cell lines. *Hum Cell* **19**: 43–48
- Zavaleta CL, Goins BA, Bao A, McManus LM, McMahan CA, Phillips WT (2008) Imaging of 186Re-liposome therapy in ovarian cancer xenograft model of peritoneal carcinomatosis. *J Drug Target* **16**: 626–637
- Zhou HE, Li CL, Chung LW (2000) Establishment of human prostate carcinoma skeletal metastasis models. *Cancer* **88**: 2995–3001
- Zheng Q, Chen XY, Shi Y, Xiao SD (2004) Development of gastric adenocarcinoma in Mongolian gerbils after long-term infection with *Helicobacter pylori*. *J Gastroenterol Hepatol* **19**: 1192–1198
- Zhu JS, Song MQ, Chen GQ, Li Q, Sun Q, Zhang Q (2007) Molecular mechanisms of paclitaxel and NM-3 on human gastric cancer in a severe combined immune deficiency mice orthotopic implantation model. *World J Gastroenterol* **13**: 4131–4135
- Zisman A, Pantuck AJ, Bui MH, Said JW, Caliliw RR, Rao N, Shintaku P, Berger F, Gambhir SS, Beldegrun AS (2003) LABA21: a metastatic tumor model for renal cell carcinoma expressing the carbonic anhydrase type 9 tumor antigen. *Cancer Res* **63**: 4952–4959
- Zuber J, Radtke I, Pardee TS, Zhao Z, Rappaport AR, Luo W, McCurrach ME, Yang MM, Dolan ME, Kogan SC, Downing JR, Lowe SW (2009) Mouse models of human AML accurately predict chemotherapy response. *Genes Dev* **23**: 877–889

Additional information

Committee to Update Science, Medicine, and Animals, National Research Council (2004) *Sciences, Medicine, and Animals: National Research Council of The National Academies*. The National Academies Press: Washington, DC, USA

Dennis C (2006) Cancer: off by a whisker. *Nature* **17**: 739–741

Garber K (2006) Realistic rodents? Debate grows over new mouse models of cancer. *J Natl Cancer Inst* **98**: 1176–1178

Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research. Committee on Guidelines for the Use of Animals in Neuroscience and Behavioural Research, (Institute for Laboratory Animal Research, Washington, D.C. 2003). National Research Council

Flecknell P (2008) Analgesia from a veterinary perspective. *Br J Anaesth* **101**: 121–124

Richardson CA, Flecknell PA (2005) Anaesthesia and post-operative analgesia following experimental surgery in laboratory rodents: are we making progress? *Altern Lab Anim* **33**: 119–127

Roughan JV, Flecknell PA, Davies BR (2004) Behavioural assessment of the effects of tumour growth in rats and the influence of the analgesics carprofen and meloxicam. *Lab Anim* **38**: 286–296

The Royal Society (2004) *The Use of Non-human Animals in Research: a Guide for Scientists*. Science Advice Section, The Royal Society: London, UK

Glossary

Allometric scaling: Calculation of doses of drugs to be administered to animals according to their relative sizes where the relationship of a biological variable to body mass is non-linear. For example, drug dosage can be linearly related to body surface area rather than to body weight.

Ascites: Cells/fluid in the peritoneal cavity.

Autochthonous tumours: Tumours originating within the host animal, either spontaneously, or due to genetic or pharmacological intervention.

Cachexia: Severe loss of weight and muscle mass that cannot be reversed nutritionally. Can be caused by release of biologically active molecules (cytokines) from certain tumours.

Cassette dosing: Administration of multiple compounds to an individual animal followed by individual measurements in the same blood sample.

Clinically equivalent dose: A dose of a drug, which results in blood/tissue levels that reflect those that are achieved in patients.

Clonogenic assay: Measuring the effect of treatments on the ability of tumour cells to proliferate expansively. Treatment may be *in vitro* or initiated *in vivo* and the clonogenic ability of explanted cells tested *in vitro*.

Desquamation: Loss of skin integrity. Moist desquamation can be a consequence of exposure to ionising radiation (UV or X-rays) where the skin thins and then begins to weep tissue fluid as the epithelial cells lose their barrier function.

Distension: Stretched beyond normal dimensions.

Ectopic: Site of growth different from the tissue of origin, for example, s.c. transplantation of tumours derived from internal organs.

Erythema: Skin reddening and thickening in response to UV irradiation, as in mild sunburn.

Factorial design: Involves the inclusion of two or more variables and measuring the response to each variable and interactions between variables.

Genetically engineered mouse models (GEMMs): Animals in which the genetic material has been altered. For example, introduction of a mutation in cells of a particular organ may result in the development of benign or malignant tumours.

Hyperplasia: Refers to the proliferation of cells within an organ or tissue beyond that which is ordinarily seen. Microscopically

Useful weblinks

<http://scienceandresearch.homeoffice.gov.uk/animal-research/publications-and-reference/statistics/>

<http://www.sanger.ac.uk/genetics/CGP>

http://emice.nci.nih.gov/mouse_models

http://dels.nas.edu/ilar_n/ilarhome/reports.shtml

http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm

http://www.iasp-pain.org/AM/Template.cfm?Section=Animal_Research

http://ec.europa.eu/european_group_ethics/docs/opinion7_en.pdf

<http://conventions.coe.int/treaty/en/treaties/html/123.htm>

<http://www.ecopa.eu/>

<http://caat.jhsph.edu/>

<http://www.imm.ki.se/sft/pdf/OECD19.pdf>

<http://oacu.od.nih.gov/ARAC/index.htm>

<http://www.research.psu.edu/arp/health/endpoints.html>

<http://www.nc3rs.org.uk/news.asp?id=759>

<http://ddgs.utu.fi/request.php?4>

http://www.lal.org.uk/index.php?option=com_content&view=article&id=56&Itemid

<http://www.nc3rs.org.uk>

cells resemble normal cells but are increased in numbers. It is a benign condition, unlike neoplasia, which is malignant.

Intravital microscopy: A technique, which allows direct observation of small blood vessels within the organs of anaesthetised animals.

Maximum Tolerated Dose (MTD): The highest dose of a drug in which the clinical condition of the experimental animal is maintained.

Metastasis: The spread of tumour cells from a primary site to distant sites in the body, usually through the blood or lymph. The term ‘experimental metastasis’ is sometimes used to describe the colonisation of organs after injection of cells directly into the peripheral circulation.

Oncogenesis: The process of malignant transformation resulting in tumour development.

Orthotopic: Anatomically correct site (opposite of ectopic), for example, transplantation of renal tumour cells into the kidney or mammary carcinoma cells into the mammary fat pad.

Pharmacodynamics: The study of the action of and the duration of effects of agents in the body, including confirmation of mechanism of action through identification of relevant biomarkers of activity.

Pharmacokinetics: The study of the process by which agents are absorbed, distributed, metabolized and eliminated by the body, including measurement of the rate of excretion, metabolism, blood and tissue concentrations.

Syngeneic tumour models: Cells transplanted between animals of the same inbred strain.

Ulceration: An inflamed lesion on the skin or internal surface involving tissue destruction.

Xenogeneic tumour models: Cells transplanted between species (e.g., human to mouse). Requires recipients that cannot mount an immune response and reject the foreign tissue graft such as athymic mice which lack T-lymphocytes, or severe combined immunodeficient (SCID) mice.



This work is licensed under the Creative Commons Attribution-NonCommercial-Share Alike 3.0 License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>