International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (3): 337-343
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Review Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

In vivo Methods for Preclinical Screening of Anticancer Drugs

Rani Sebastian[1], B. Jaykar[2] and V. Gomathi[3]
[1]Assistant Professor, College of Pharmaceutical Sciences, Government Medical College, Kottayam, Kerala.
[2]Principal, Vinayaka Mission's College of Pharmacy, Salem.
[3]Professor, Vinayaka Mission's College of Pharmacy, Salem

Received: 10 Mar 2020 / Accepted: 9 Apr 2020 / Published online: 1 Jul 2020
***Corresponding Author Email:** ranisalapatt@yahoo.com

Abstract
Development of an effective and safe anticancer therapy is the prime concern of anticancer research today. Both *in vitro* and *in vivo* studies play a pivotal role in the development of new anticancer agents. *In vitro* studies have the advantage that many drugs can be studied at one time in a large number of samples of cells. An absence of biokinetics is one of the significant drawbacks of *in vitro* studies. *In vivo* testing is more specific and reliable. But the researcher has to balance the benefits with the cost and length of time. *In vivo* studies are definitely a smart step in the preclinical testing of new anticancer agents. This article gives an insight into the various *in vivo* methods for the screening of anticancer agents.

Keywords
In vivo screening, Hollow fiber assay, Xenograft, Genetically Engineered Models

*****

INTRODUCTION
Cancer is one of the most life-threatening diseases and possess many health hazards in both developed and developing countries [1]. Even though many treatments are available for cancer therapy, still cancer is the 2nd leading cause of death in the globe. There are different treatment modalities for cancer like surgery, radiotherapy, immunotherapy, chemotherapy and stem cell transplantation. Anticancer drugs are the main method to fight against cancer. Currently, there are about 130 – 150 types of approved anticancer drugs in the market around the world. However, despite the continuing development of numerous chemotherapeutic drugs, their effectiveness is still limited in that the majority of patients continue to die within five years of the commencement of treatment [2]. The failure in the process of development of anticancer agents is due to the lack of proper preclinical screening methods. This may be due to imperfections o\of existing *in vitro* and *in vivo* testing models [3].

A screening process is required to identify products that will be able to perform anti-tumor effects. The biological evaluation of these newly synthesized compounds includes various *in vitro* or *in vivo* techniques. Direct screening by *in vivo* technique requires lot of expenses and approval from animal ethics committee hence the synthesized drugs are initially screened by various *in vitro* techniques which are cheaper. After screening by invitro technique, the promising compounds can be screened further by *in vivo* technique.

The screening and evaluation of the compound by *in vitro* *in vivo* animal models are important tools in cancer research which will enable the identification of carcinogens, the development of cancer therapies, drug screening and providing an insight into the molecular mechanisms of tumor growth and metastasis. Large scale screening using animal systems is highly unethical and strictly regulated. In most cases, either cellular or target-based high throughput assays will precede *in vivo* evaluation of

potential anticancer drugs. High throughput screening (HTS) plays an essential role in contemporary drug delivery processes. Presently, active compounds are selected by prescreening and screening against transplanted mouse tumors and human tumor xenografts as well as by the *in vitro* systems. Because of ethical, medical and economical limitations and constraints on the number of patients eligible for clinical trials, most of the research has been done in experimental system.

Most pre-clinical data on new anticancer drugs were obtained using transplanted tumors in mice. For practical reasons, scientists mainly use ectopically implanted, subcutaneously growing tumor models, frequently as xenografts of human origin. An enormous variety of different tumor systems for *in vivo* evaluation of new anticancer agents is available. Mostly murine host systems are used for experimental tumor therapy because of the availability of in-bred lines at relatively low costs [4].

METHODS OF IN-VIVO SCREENING

A number of different tumor systems for *in vivo* evaluation of new anticancer agents is available. Mostly murine host systems are used for experimental tumor therapy because of the availability of in-bred lines at relatively low costs, the ease of obtaining tumor models and established, widely accepted experimental endpoints. Spontaneous or transplanted murine tumors can be studied in immunocompetent mice whereas investigation of human tumors requires an immunodeficient host, e.g. nude mice, to avoid tissue rejection. Spontaneous tumor models offer some advantages over transplanted tumor cell lines, e.g. genetic diversity, growth in the original environment and angiogenesis. Genetically engineered mice may help to improve the situation [5]. Experimental data show that tumor characteristics such as growth rate and potential to metastasize depend on implantation site. Tumors injected orthotopically, i.e. into the organ of origin, apparently behave more similarly to the clinical situation. Also, the response to anticancer drugs may depend on the implantation site. For practical reasons, scientists mainly use ectopically implanted, subcutaneously growing tumor models. Most pre-clinical data on new anticancer drugs were obtained using transplanted tumors in mice, frequently as xenografts of human origin. Animal tumor systems have to meet several requirements to be suitable for experimental tumor therapy. It is very important that the tumor precisely reflects treatment response, and that the natural history of the host allows the study of the experimental endpoint, e.g. a sufficient lifespan for follow-up to assess local tumor control. Stable biological characteristics of the tumor system such as expression of the molecular target, growth rate, differentiation and immune response are also required to assure the high quality of experiments [6].

The *in- vivo* methods include:

1. The Hollow Fiber Assay
2. Human Tumor Mouse Xenotransplant Models
3. Tumor Xenograft Model
4. Autochthonous Tumor Models
5. Genetically Engineered Mouse Models

The Hollow Fiber Assay

The hollow fiber assay (HFA) is a fast-in vivo assay to determine the cytotoxic effect of drugs, as well as their pharmacodynamic effects on human tumor cell lines grown in hollow fibers that are implanted subcutaneously or intraperitoneally in mice or rats. Currently, the most commonly used models for *in vivo* anticancer drug screening are xenotransplantation of human tumor to mice and the hollow fiber assay (HFA). Both models utilize the transplantation of tumor cells into immunodeficient mice.

The xenotransplantation model has some serious drawbacks including the time required to screen prospective anticancer agents, the number of animals required, and the cost involved. These issues led to the development of the hollow fiber method at the NCI. Actually, it bridges the gap between *in vitro* and xenograft screening of anticancer compound [7]. The purpose of this assay is to predict which compounds, that showed promise during the course of NCI60 human tumor cell line anticancer drug screening protocol, will show promise during invivo xenograft screening [8]. HFA was developed as a heterogeneous solid tumor model. The assay is based on the tumor cells ability to form tumors in hollow tubes consisting of polyvinylidene fluoride (PVDF). Within the tube a central core of necrotic cells is surrounded by a thin layer of living cells that are in contact with the wall of the hollow tube. HFA screening is carried out using a standard panel of 12 cancer cell lines (Table 1).

Cell line	Description
NCI-H23	non-small cell lung cancer
NCI-H522	non-small cell lung cancer
MDA-MB-231	breast cancer
SW-620	colon cancer
COLO 205	colon cancer
OVCAR-3	ovarian cancer
OVCAR-5	ovarian cancer
U251	Encephaloma
SF-295	Encephaloma
MDA-MB-435	Melanoma
LOX	Melanoma
UACC-62	Melanoma

Table 1: Tumor cell lines used in HFA

Screening of specific compounds is also possible in other cell lines. Cells to be used are cultured until they reach log phase growth (approximately 2 x 10^6 cells /ml), then the cell suspension is introduced into the tubes and incubated for 24-48 hours. The hollow tubes, which have an internal diameter of 1 mm and a length of 2 cm are permeable to molecules with a molecular weight up to 500 kDa and allow nutrients and potential anticancer drugs to enter the tubes and come into direct contact with the tumor.

In a typical experiment, each animal receives three different implants, each containing a single tumor cell line [9]. This reduces the number of animals necessary for the analysis, thus reducing the cost. After 3- or 4-days post implantation, the drug to be tested is introduced into the animal through an intraperitoneal injection and is continually delivered for the next 4 days. On the 6^{th} day of treatment, the tube is removed and cell viability is determined by a modified MTT-test, which takes into account such *in vivo* parameters as pharmacokinetics, pH, and oxygen content within the tumor.

Significance

Analysis of cell cycle, DNA damage and apoptosis induction can also be determined [10]. One disadvantage of HFA is the spatial limitations of the model. Tumor growth is inhibited by the inside diameter of the tube and, therefore, to ensure credible experimentation, it is necessary to maintain tumor growth within the fiber close to its maximum [11]. Another drawback is that the fiber wall is an artificial barrier between the tumor and its environment. This hampers the diffusion of large biomolecules, such as DNA and antibodies, which implies that the model is not suitable to studies using macromolecular agents or nanoparticles [12].

Human tumor mouse xenotransplantant models

Human tumor cells, cultured *in vitro*, can be implanted into immunodeficient mice [13]. The normal adaptive immune responses associated with foreign tissue rejection is suppressed and the tumor is not rejected. When the tumor reaches a given size, introduction of a potential anticancer drug is made, and the efficacy of the drug is determined by changes in the tumor size. If a potential candidate shows promising results, a series of experiments can be conducted out to optimize the drug dosing and determine the efficiency of the substance in order to reduce its toxicity by adjusting the dose and mode of application. The simplest model of xenotransplantation is achieved by the subcutaneous introduction of tumor cells. This model allows the rapid quantification of a compound's anticancer properties and its toxicity [14].

Subcutaneous xenotransplantants have been used successfully to predict clinical outcomes of substances such as cyclophosphamide [15]. This model is efficacious in treating rhabdomyosarcoma and adenocarcinoma human colon cancers in clinical trials [16]. As the xenotransplantation model is to be conducted using immunodeficient mice, the tumor microenvironment afforded by this system is not necessarily a precise representation of the naturally occurring microenvironment [17]. In addition, immunodeficient mice are not suitable for testing substances that interact with or modulate the immune system. However, the xenotransplantation model is a pillar of preclinical anticancer drug testing. An obvious limitation to the subcutaneous transplantation system is that the tumor cells are grown in a tissue microenvironment that may be substantially different when compared to the environment they experience when naturally occurring in a human subject [18]. To address this limitation, the orthotopic xenotransplantation model was developed which more closely simulates the morphology and growth properties a tumor experiences in its natural microenvironment [19]. In this model, a subject`s tumor cells are transplanted into the orthotopic area of a mouse; for example,

colon cancer cells are transplanted into the intestinal wall of an immunodeficient mouse [20]. Moreover, it is necessary to sacrifice the animal in order to investigate the tumor, further adding to the cost of an already expensive experiment [21]. Figure: 1.

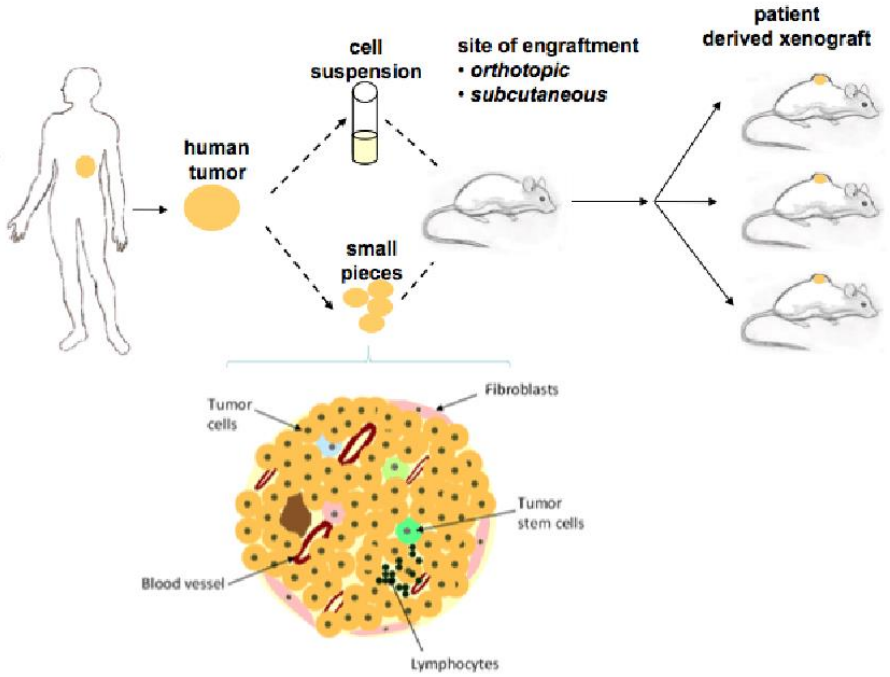

Figure 1: Human tumor mouse xenotransplant method

For tumors growing as murine xenotransplantants, it has been shown that a clinically relevant dose in mice is often similar to that observed for primary human tumors [22].

Tumor Xenograft Model

Growth of human tumor in an immunodeficient "nude" (athymic) mouse was reported in 1969 by Ritard and Povelsen. A localized, well-defined tumor process was obtained which was easily accessible for observation, measurement, and biopsy procedures after simple transplant of human tumor tissue to nude mice. The human tumor tissue could be transplanted either in intact form or in form of suspension of cells obtained from the human tumor. The tumor tissue could be surgically transplanted or injected to subcutaneous tissue of trunk, muscles of flank, or orthotopically to any organ (generally similar to human organ from which tumor tissue is obtained) of nude mice [23].

Subcutaneous implantations are much easier to perform than orthotopic implantations and have been shown to closely maintain the histopathological, cytological, and biochemical characteristics typical of original tumor. However, they do not reproduce the primary site of the common human cancers and lack the invasive and metastatic potential. It was shown that metastasis after implantation of human tumor in nude mice depends on various factors such as site of implantation, blood supply to the implanted site, presence of fibrous capsule (mice origin) surrounding the human tumor, and the cell types of implanted human tumor. Orthotopically growing tumors have the advantage that metastases occur in much higher frequency and the invasion seems to be more pronounced when compared with subcutaneously growing models. Human tumor xenografts grown subcutaneously or orthotopically in nude or in severe combined immunodeficiency mice are available for all the major tumor types and have become the major model for preclinical *in vivo* anticancer screening and drug development. Tumors growing in internal organs (orthotopic model) are usually not accessible for serial size measurements; therefore, the mice have to be sacrificed to measure tumor volume that allows only a one-point measurement [24]. This drawback can be overcome by using imaging techniques now available. While the subcutaneous human tumor xenograft model is good for evaluating cytotoxic or cytostatic drugs, the orthotopic model provides the most appropriate evaluation of specific inhibitors of metastases or invasion [25].

One of the main contributions of this model is that the efficacy of an anticancer drug in patient can be compared with the effects in the xenograft model *(in vivo*) obtained with the tumor of patient and with the well-established parallel cell lines (*in vitro*) [26]. The

human tumor xenograft model is a good predictor of clinical activity for anticancer drugs and is useful in assessing the drug's pharmacokinetics and pharmacodynamics and it also provides a renewable and readily accessible source of target human tumor cells.

Significance

These models are used to investigate the factors involved in malignant transformation, invasion and metastasis, as well as to examine response to therapy. Human tumor xenografts can be used to aid in the development of individualized molecular therapeutic approaches [27].

Autochthonous tumor models

Autochthonous mouse models for human cancers are obtained by initiating tumors in a normal cell de novo and within the intact organism [28]. The main advantage of these models is the pathophysiological relevance of the tumor initiation. Autochthonous tumors either arise spontaneously or can be induced by carcinogens or other chemical, viral, bacterial, or physical triggers [29]. Their histological characteristics are more like those of human tumors than of xenotransplantants. Autochthonous tumor models allow one to investigate new molecular targets for preventive chemotherapy by studying processes of mutations, oxidative stress and inflammation, which occur during tumor formation [30].

Significance

These models may be used to identify new molecular targets and are rarely used to directly test the antitumor agents.

Genetically engineered mouse models

A genetically modified mouse or genetically engineered mouse model (GEMM) is a mouse (Mus musculus) that has had its genome altered through the use of genetic engineering techniques. Genetically modified mice are commonly used for research or as animal models of human diseases and are also used for research on genes. Lack of adequate therapeutic responses observed in human tumors and mouse xenografts include the fundamental differences between mice and humans (such as drug metabolism, pharmacokinetics, toxicities, and combination tolerability) [31]. Some of these issues may be resolved through the use of genetically engineered mouse models (GEMM) of cancer, which more faithfully simulate many aspects of their corresponding human disease [32]. GEMM help researchers to better test drug efficacy and model mechanisms of action and therapeutic resistance. In addition, optimum dosage regimen can be determined by using this method. In contrast to immunodeficient mice, tumors grown in genetically engineered mouse (GEM) models are affected by the immune system and interact with stroma; therefore, making it possible to test drugs with targets reside within an immunocompetent tumor microenvironment [33]. By using tissue specific promoters to regulate antigen expression, it is possible to obtain specific types of tumors. In order to activate the transgene expression in specific tissues one should also use the mouse vital regulatory elements [34]. However, the cellular introduction of transgenes does not give rise to all types of tumors; formation of some requires silencing of tumor suppressor gene expression. The process of homologous recombination can allow one to delete, move or introduce mutations into a gene in mouse embryonic stem cells and silence these genes. Tumors that arise from the spontaneously transformation of cells typically do so during the animal's juvenile or adult stage of development. By utilizing the GEM-model it is possible to change the expression of the genes of interest at the embryonic stage of development. By using methods to control recombinant genes expression, it is possible to activate a given gene in a tissue of interest and in the desired time interval. Expression activators can also be delivered as part of a genetic construct. In the last decade, by modifying genes crucial for the development of specific types of tumors, researchers have developed mouse models of lung, breast, colon, ovary, pancreas and prostate cancers [35].

An animal's life span and tumor volume are not always a reliable indicator of the efficacy of an antitumor agent. In addition, it is necessary to sacrifice an experimental animal in order to evaluate tumor growth in internal organs, making it not possible to monitor the tumor growth dynamics. GEM-models with reporter proteins enable to overcome these limitations. Such models use tumor cell lines that carry bioluminescent or fluorescent reporter proteins genes such as firefly luciferase gene (LUC) or green fluorescent protein (GFP) of jellyfish. Their *in vivo* expression in tumor cells allow one to visualize tumor progression, to monitor its response to anticancer agent, and to visualize internal metastases and tumor nodule. The advent of transgenic organisms expressing reporter protein genes has made it possible to distinguish normal cells of a host organism from implanted tumor cells. Clinical visualization tools adapted for small laboratory animals such as micro positron emission tomography, ultrasound, magnetic resonance imaging and X-ray microcomputer tomography *in vivo*, have great future potential for monitoring internal processes, including tracking the growth of tumors in these models [36]. Despite the temptation

to use GEM as a preclinical screening model, its predictive properties are still inconsistent. Genetically engineered mice are rather expensive and difficult to generate. Currently, one can quickly obtain a large number of genetically modified mice that are in one stage of development, using invitro fertilization technology. However, these mice and those obtained through traditional crossing often produce spontaneous and multifocal tumors and display variable tumor growth. Fluorescence intensity or bioluminescence reporter protein in different tissues and tumor types often varies. Moreover, the use of these mice is generally protected by patents. These issues, as well as others complicate anticancer agent testing. Although GEM-models may not replace xenotransplantants, they can play an intermediary role between xenotransplantant screening and clinical trials. Tumors obtained from genetically modified mice can be isolated and cultured *in vitro*. The advantage of these cells is that their transformation took place in a natural microenvironment and is influenced by the immune system. Such tumors when implanted subcutaneously or orthotopically into immunocompetent mice allow one to investigate the effect of anticancer agents more fully. GEM-models afford a unique opportunity to characterize differences in cell lines and natural tumors by comparing their genotypes and phenotypes. It becomes possible to restore tumor heterogeneity through *in vitro* multistep carcinogenesis. It is logical to assume that cell lines possessing changes similar to those of tumor cells *in vivo*, can be used for the initial screening of drugs.

Significance

GEMMs can also play a key role in elucidating the mechanisms of therapeutic response and innate resistance to both chemotherapy and targeted agents. The use of GEMMs may enable investigators to explore both the feasibility and validity of a personalized medicine approach.

CONCLUSION

Empirical screening procedure combined with novel technologies might be the most beneficial method for the determination or designing of new anti-cancer agent. Currently, there are a limited number of models designed for preclinical *in vivo* drug screening. Hollow fiber and xenotransplantant models have been utilized extensively; however, they are limited in their abilities and do not led themselves well to the high throughput screen studies necessary to evaluate antitumor drugs. Although models utilizing genetically modified mice offer an attractive and promising alternative, they fail in many respects compared to xenotransplantant models. When using mouse models, a potential anticancer drug is tested in the environment different from that in clinical subjects. For example, in contrast to humans, most murine cells have functionally active telomerase. Thus, the results of laboratory testing carried out on syngeneic or xenogeneic tumor immunized mice cannot be directly extrapolated to humans. A personalized medicine approach which uses biopsied tissue instead of tumor cell lines to screen antitumor substances may bring the researcher closer to actual clinical conditions.

REFERENCES

1. Izevbigie EB, Discovery of water-soluble anticancer agents from a vegetable found in Benin City, Nigeria, Experimental biology and Medicine, 2003; 228:293-298.
2. Wang Z, Yang HW, Wang X et al, The molecular mechanism and regulatory pathways of cancer stem cells, Cancer Translational Medicine, 2016; 2 (5): 147–53.
3. Wong KK, Qian ZR, Le Y, The role of precision medicine in pancreatic cancer: challenges for targeted therapy, immune modulating treatment, early detection, and less invasive operations. Cancer Translational Medicine, 2016; 2 (2): 41–7.
4. Edwin Sonneveld, Jacoba A. C. Riteco, Hendrina J. Jansen et al, Comparison of In Vitro and In Vivo Screening Models for Androgenic and Estrogenic Activities, Toxicological sciences, 2006; 89(1): 173–187.
5. Skehan P, Storeng R, Scudiero D et al, New colorimetric cytotoxicity assay for anticancer-drug screening, Journal of the National Cancer Institute, 1990;82:1107–12.
6. Natalia L. Blatt, Rimma N. Mingaleeva, Svetlana F, In vivo Screening Models of Anticancer Drugs. Life Science Journal 2013;10(4) :1892- 1900.
7. Shnyder SD, Hasan J, Cooper PA et al, Development of a modified hollow fibre assay for studying agents targeting the tumour neovasculature. Anticancer Research. 2005;25(3B):1889-94.
8. Damia G, D'Incalci M, Contemporary pre-clinical development of anticancer agents--what are the optimal preclinical models? European Journal of Cancer, 2009;45(16):2768-81.
9. I. Kapetanovic. Drug Discovery and Development - Present and Future. In Tech; USA;2011.
10. Temmink OH, Prins HJ, van Gelderop et al. The Hollow Fiber Assay as a model for in vivo pharmacodynamics of fluoropyrimidines in colon cancer cells, British journal of Cancer, 2007;96(1):61-6.
11. Phillips RM, Pearce J, Loadman PM, et al, Angiogenesis in the hollow fiber tumor model influences drug delivery to tumor cells: implications for anticancer drug screening programs, Cancer Res 1998;58(23):5263-6.

12. Benbrook D, Organotypic cultures represent tumor microenvironment for drug testing, Drug Discovery Today: Disease Models 2006;3(2):143-148.
13. Lee KH, Rhee K. Correlative Effect between in vivo Hollow Fiber Assay and Xenografts Assay in Drug Screening, Cancer Research and treatment, 2005; 37(3):196–200.
14. Elliott NT, Yuan F, A review of three-dimensional in vitro tissue models for drug discovery and transport studies, Journal of Pharmaceutical Sciences, 2010;100(1):59-74.
15. Rygaard J, Povlsen CO, Heterotransplantation of a human malignant tumour to "Nude" mice, Acta pathologica et microbiologica Scandinavica, 1969;77(4):758-60.
16. Brown C, Patenting life: genetically altered mice an invention, court declares, The Canadian Medical Association Journal ,2000;163(7):867-8.
17. Talmadge JE, Singh RK, Fidler IJ, Murine models to evaluate novel and conventional therapeutic strategies for cancer, The American Journal of Pathology, 2007;170(3):793-804.
18. Kelland LR, Of mice and men: values and liabilities of the athymic nude mouse model in anticancer drug development, European Journal of Cancer 2004; 40(6):827-36.
19. Peterson JK, Houghton PJ, Integrating pharmacology and in vivo cancer models in preclinical and clinical drug development. European Journal of Cancer, 2004;40(6):837-44.
20. Richmond A, Su Y, Mouse xenograft models vs GEM models for human cancer therapeutics, Disease models and mechanisms, 2008;1(2-3):78-82.
21. Hoffman RM, Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic, Investigational New Drugs 1999;17(4):343-59.
22. Suggitt M, Bibby MC, 50 years of preclinical anticancer drug screening: empirical to target driven approaches, Clinical Cancer Research 2005;11(3):971-81.
23. Kerbel RS, Human tumor xenografts as predictive preclinical models for anticancer drug activity in humans: better than commonly perceived-but they can be improved, Cancer Biology and Therapy, 2003;2(4):134-9.
24. Giovanella BC, Stehlin JS, Wall ME et al, DNA topoisomerase I--targeted chemotherapy of human colon cancer in xenografts, Science 1989;246(4933):1046-8.
25. Pantazis P, Hinz HR, Mendoza JT et al, Complete inhibition of growth followed by death of human malignant melanoma cells in vitro and regression of human melanoma xenografts in immunodeficient mice induced by camptothecins, Cancer Research, 1992;52(14):3980-7.
26. Takimoto CH, Why drugs fail: of mice and men revisited. Clinical Cancer Research, 2001;7(2):229-3.
27. Gordon IK, Khanna C, Modeling opportunities in comparative oncology for drug development, Institute of Laboratory Animal Resources Journal, 2010;51(3):214-20.
28. Workman P, Aboagye EO, Balkwill F et al, Guidelines for the welfare and use of animals in cancer research, British Journal of Cancer, 2010;102(11):1555-77.
29. Sugamata N, Koibuchi Y, Iino Y, Morishita Y et al. A novel aromatase inhibitor, vorozole, shows antitumor activity and a decrease of tissue insulin like growth factor-I level in 7, 12-dimethylbenz[a]anthracene-induced rat mammary tumors. International Journal of Oncology, 1999;14(2):259-63.
30. Singh M, Johnson L, using genetically engineered mouse models of cancer to aid drug development: an industry perspective, Clinical Cancer Research, 2006;12(18):5312-28.
31. Van Dyke T, Jacks T, Cancer modeling in the modern era: progress and challenges, Cell, 2002;108:135–44.
32. Sharpless NE, Depinho RA, The mighty mouse: genetically engineered mouse models in cancer drug development. Nature Reviews drug discovery, 2006;5:741–54.
33. Stewart TA, Pattengale PK, Leder P, Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes, Cell 1984;38(3):627-37.
34. Kistner A, Gossen M, Zimmermann F et al, Doxycycline mediated quantitative and tissue-specific control of gene expression in transgenic mice, Proceedings of the National Academy of Sciences of the United States of America, 1996;93(20):10933-8.
35. Heyn C, Ronald JA, Ramadan SS et al. In vivo MRI of cancer cell fate at the single-cell level in a mouse model of breast cancer metastasis to the brain, Magnetic Resonance in Medicine, 2006;56(5):1001-10.
36. Rangarajan A, Weinberg RA, Opinion: Comparative biology of mouse versus human cells: modelling human cancer in mice, Nature Reviews Cancer 2003;3(12):952-9.