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EMERGING CANCER MODELS FOR DRUGS AND NOVEL DOSAGES DEVELOPMENT

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Abstract – *Objective:* *Cancer is the primary cause of death all over the world, despite of its early detection and novel therapies available. The process of cancer drug development is growing tremendously. With respect to many limitations in human studies, there is the need to develop experimental models for screening of efficacy and toxicity of developing drugs. The purpose of this study is to investigate the cancer model for drug development.*

Materials and Methods: *In this review, articles are extracted with selected keywords from the PubMed, SID, Springer, Medlib, Web of Science (Clarivate) and ScienceDirect databases without any language restrictions.*

Results: *The efforts to reduce global cancer burden are mainly focused on developing innovative diagnostic and therapeutic tools. A plenty of* in vitro *and* in vivo *models are utilized in cancer drug discovery process. Successful bench-to-bedside translation of basic scientific findings about cancer into therapeutic interventions for patients depends on the selection of appropriate experimental models for testing.*

Conclusions: *The presented models have both advantages and disadvantages. None of them are absolutely ideal while they are chosen according to the purpose of the investigator and the advantages of each method for cancer research.*

KEYWORDS: *Animal models, Cancer,* In vitro *and* in vivo *models, Clinical translation.*

INTRODUCTION

Cancer is an utmost serious malignant disease in today's world which represents uncontrolled growth and spread of abnormal cells[1]. Due to the advancement in medical science the mortality rates related to many different illnesses have declined in current decades, but cancer-associated deaths have remained surprisingly constant[2]. Recent worldwide data from the World Health Organization (WHO) Global Cancer Observatory (GLOBOCAN) estimate that till 2018 there is an approximately 18.1 million of new case and deaths of 9.6 million occur because of cancer[3,4]. The major problem of cancer is the lack of its early detection testing which corresponds to diagnosis of disease in more complex condition that may enhance death rate. Almost 50% cases of all cancer types are diagnosed at distant stages which results meagre survival of patients. The pathway of developing advanced cancer diagnostics and therapeutics is more adventurous, it is both lengthy as well as highly priced[5]. A variety of therapies and strategies has been flourished to fight against this deadly disease such as systemic chemotherapy, radiation therapy and also more targeted approaches including immunotherapy and antibody-based therapies. But still in a petty number of new drugs have received FDA approval which shows that there may be something missing in the way of discovering new agents and testing protocol of these agents prior to clinical trials[6].

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Early detection of tumours and accurate monitoring of responses during treatment are pivotal to patient survival. For upgrading the detection and productive treatment of early cancer there is a need to discover specific molecular targets for tumours. Thus, the appropriate use and development of *in vitro* and *in vivo* cancer models is extremely desirable[7]. Drug discovery is a monotonous process and encompasses a high failure rate. The new compounds shown to be effective in laboratory *in vitro* cell culture system might not be able to reproduce their activities in animal tumor models. Albeit they are acting in animal tumor systems most of these drugs are not very effective in humans[8]. The animal models used in the preclinical research must truly reflect the microenvironment of tumour in human. Additionally, the therapeutic effectiveness and toxicity profile of the agent at the specific doses that may be rationalized to human ought to be thoroughly investigated. Often, many new small molecules are tested at doses that do not show any significant pharmacological affect. There is a need for better design of preclinical animal model studies in order to enhance the success rate of new drugs introduced into clinical trials[9].

The discovery of anti-cancer drugs continuous to develop at an alarming rate, and a large amount of recourses are affianced for drug discovery and design. The evaluation of designed drug molecule is a key step in drug discovery program. Inappropriate selection of evaluation method may result in potential drugs being excluded from further development[10]. The use of cell lines with high throughput screening is the primary screening method, but due to limitations such as low relevance to clinical condition there is an additional need to use appropriate *in vivo* model for screening. The selected animal model should reflect the human tumour microenvironment as closely as possible. At the same time, their feasibility and cost efficiency for large scale drug screening programs are also important factors[11]. The most common cancers leading to cause of death globally are depicted in Figure 1.

Animal model for cancer

Animal models are indispensable tools for investigating the cancer pathogenesis, tumor invasion and metastasis mechanisms and also for new therapeutic approaches. Various types of *in vitro* and *in vivo* tumor models have been developed to understand the biological changes associated with tumorigenesis. These models are also integral in drug development[12]. The development of *in vivo* animal models that mimic the natural history of human cancers and its clinical response to treatment is an important prerequisite for rapid translation of anticancer therapies[13].

In-vitro *models for cancer*

In vitro cancer research utilized animal and human cancer cell lines to reveal biochemical pathways associated with cancerous cells[14]. These cancer cell lines are derived from high-grade, high-stage cancers. At present, clinical trials are the eventual determinant of drug efficacy but due to limited ethical and safety considerations, preclinical studies using *in vitro* tumor models are

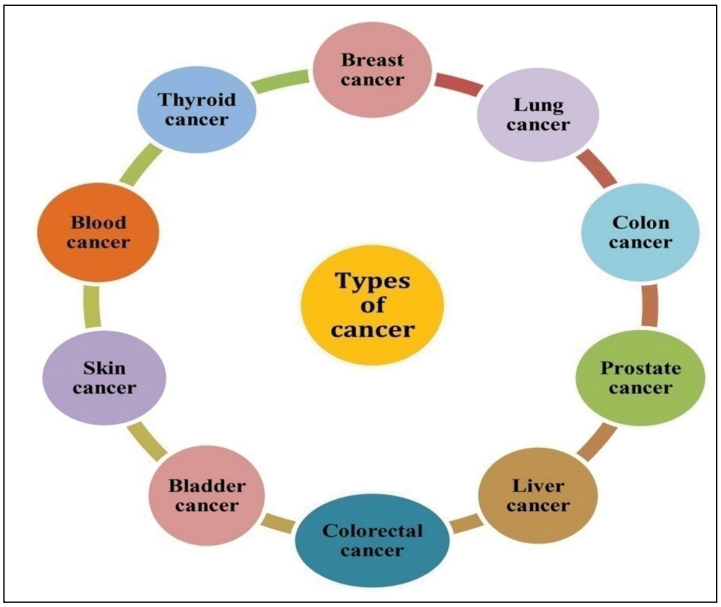

Figure 1. Types of most common cancer which lead to increasing death rate globally.

particularly important. The advantages of *in vitro* cancer models are its highly controlled conditions, homogeneity, revealing underlying molecular mechanisms, and reproducibility[15]. *In vitro* human cancer models have varying degree of complexity, ranging from 2D single-layer models to 3D tumor models. *In vitro* tumor models can be developed from patient cells, cell lines, stem cells, stromal cells, immune cells, and expose to various physical and chemical conditions according to experimental requirements[16]. With the help of *in vitro* tumor models one can explore the important cellular and molecular mechanism associated with carcinogenesis including survival, proliferation, migration, invasion, matrix remodelling, latency, and angiogenesis[17]. The limitations of two-dimensional *in vitro* model are selection of phenotypic and genotypic cells in the *in vitro* adaptation process, a homogeneous cell population, accumulation of cell mutations over time during the culture process, and separation of cells in the tumor microenvironment[18]. Therefore, in order to overcome the limitations of 2D cells, a 3D *in vitro* cancer model is proposed as an alternative method, which is able to accurately simulate some characteristics of solid tumors, such as their spatial structure, physiological responses, soluble mediators secretion, pattern of gene expression and mechanism of drug resistance[19,20]. The 3D model over 2D is depicted in Figure 2.

Here we discuss some of the models that are mostly used such as spheroids, scaffolds, organoids, and microfluidic devices. They are also used in combination with another such as scaffolds are often placed inside microfluidic devices or spheroids can be placed inside scaffolds.

a) Spheroids: Three-dimensional (3D) spheroid models contain complex structures composed by one or more cell type. When these cell types are grown and large enough, they form a necrotic cell nucleus surrounded by a layer of actively proliferating cells[21]. The complex formed also includes an extracellular matrix (ECM) composed of the produced protein[22]. These models are very similar to tumors *in vivo* because they have similar gene expression, growth kinetics, and cell heterogeneity; in addition, the presence of natural ECM allows the simulation of natural barriers for drug penetration studies, and the internal structure of the spheroids has also great *in vivo* environment mimicking ability, where the internal region appears necrotic due to lack of nutrients and oxygen[23].

Several methodologies proposed to produce 3D spheroids are as follows[24,25]:

I) Spinner flash technique: stir the cell suspension continuously to promote the formation of cell aggregates.

II) Liquid overlay technique: cells grow on an on-stick surface and promote aggregation instead of adhering to the surface of the flask.

III) Hanging drop technique: the cells grow in the form of droplets of culture medium on the lid of the petri dish, the lid is turned upside down and placed on the petri dish below, and then the cells are cultured until the sphere reaches the desired size.

b) Scaffold based model: The three-dimensional scaffold-based model is based on the growth of cells in a three-dimensional structure, and in some respects mimics the extracellular matrix[26]. The structures used in the model can be made of natural (e.g., collagen), semi-synthetic (e.g., chitosan), or synthetic (e.g., polycaprolactone) biomaterials. In addition, cells can be cultured on prefabricated/synthesized structures, or 3D bio-printing[27]. The use of structural elements makes it possible to simulate tiny and complex shapes, and at the same time, the simulation of ECM produces a certain resistance to drug penetration, similar to tumors in the body[28]. In addition, these models usually have the following

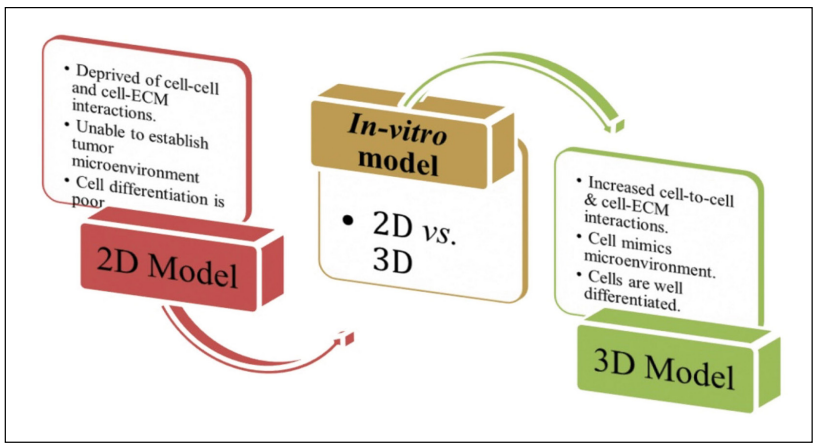

Figure 2. *In-vitro* 2D *vs.* 3D cell culture. 3D models have several advantages over 2D models. Thus, 3D models are preferable for cancer drug screening.

characteristics: spontaneous cell organization, possible heterogeneity, gene expression, and tumor-like cell phenotype *in vivo*. The main obstacle of this model is the use of artificial ECM structure. In addition, it also requires the use of highly biocompatible cell-containing building materials. Because of the bioprinting structure, it must be printed efficiently[29].

c) **Organoids based model:** Organoid tumor model is a new method of tumor research that has emerged in recent years. Organoids are derived from stem cells and can mimic all aspects of organ structure and function, including differentiation into different cell types[30]. Organelles can be cultured for a long time (especially for patient-specific tumor cells and establishment of in vitro cell banks) and transferred under three-dimensional conditions. In addition, the organelles can retain the histology, immunohistochemistry, and genetic heterogeneity of the original tumor tissue, which makes it suitable for high-throughput drug screening[31]. Although this is a relatively new approach, these two organelles derived from normal tissue stem cells, and those derived from cancer stem cells, have made significant contributions to cancer biology and personalized medicine[32]. Organoid models have been developed for many different normal tissue types (for example, gut, salivary glands, mammary glands and liver), and an increasing number of cancer types (including for example breast, prostate, pancreatic, colon, bladder and gastrointestinal cancers)[33]. The only disadvantage of this model is the lack of interstitial cell components and the absence of interactions between cells[34].

d) **Microfluidic models:** Microfluidics has become a powerful platform for cancer metastasis research and drug discovery[35]. Microfluidics technology has completely changed our ability to simulate the natural biophysical/chemical conditions of cells in *in vitro* models. The goal of microfluidics is to create a platform that can simulate the pathophysiological functions of tissues and organs, so-called "organ-in-chips." These platforms are composed of a network of microfluidic channels that can cultivate continuously perfused cells[36]. The main advantage of microfluidics is the ability to create complex three-dimensional culture systems in which various parameters can be changed and controlled. A variety of cell types can be grown on microfluidic chips to analyze specific interactions, which is particularly interesting when studying the relationship between cancer and stromal cells[37]. Microfluidic devices are usually made of polydimethylsiloxane (PDMS), which has many outstanding properties such as, due to its transparent nature *in vivo,* real time imaging, but requires new materials to fabricate the chips because PDMS can absorb small molecules in a non-specific manner[38,39]. There are several challenges in using this method to translate results into the clinic, including validating *in vitro* function in *in vivo* tumor models and correlating the results with patient samples[40].

Some advantages and disadvantages of *in-vitro* model are discussed in Table 1.

In-vitro *evaluation analysis*

A great number of *in vitro* methods has been developed to measure the efficiency of anticancer compounds. The different parameters analyzed by different assay/detection methods are described below.

I. **Cell viability/cytotoxicity determination**: Different methods used for detection of cell viability are:

a) ***MTT assay*:** MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. MTT assay is based on the conversion of the yellow tetrazolium salt-MTT to purple-formazan crystals by metabolically active cells and quantitative determinations of viable cells. Cells are seeded in 96 well plates at 5000 cells/well and allowed to grow in incubator at 37°C with 5% CO_2 for 24 h. The medium is then removed and replaced by fresh medium containing different concentrations of sample and then they are incubated at same

TABLE 1. Advantages and disadvantages of *in vitro* model in cancer research.

Model	*Advantages*	*Disadvantages*
In vitro model	– Cheap, relatively easy, and fast maintenance – Wide range of cell lines available – Highly controlled experiment – High degree of similarity with initial tumor – Less ethical containments	– Less mimic tumor environment – Different expression of genes and markers – Less sensitivity to same environmental features – Inevitable mutations of certain cellular features – Genomic instability

condition for 24-48 h. Then, 20 µL MTT dye stock solution (5 mg/ml in PBS) is added to each well and incubated for 2-3 h. The medium is removed and 200 µL dimethyl sulfoxide (DMSO) is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560 nm. Untreated cells (basal) are used as a control of viability (100%) and the results are expressed as % viability (log) relative to the control[41,42].

b) ***LDH (Lactate dehydrogenase) Assay*:** Another method for determining cytotoxicity is based on measuring the activity of cytoplasmic enzymes released by damaged cells. LDH is a stable cytoplasmic enzyme found in all cells and it is rapidly released when the plasma membrane is damaged, a key feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage. LDH activity can be easily quantified by using the NADH (nicotinamide adenine dinucleotide + hydrogen) produced during the conversion of lactate to pyruvate. The protocol includes: the cell cultures suspension is centrifuged for 4 min, and then transfer 50 µL of the supernatant into a 96-well plate. Add 50 µL of LDH assay substrate to the medium. Cover the plate with foil or a small opaque box to protect it from light and incubate at 37°C for 15-30 min. Then add 100 µL of Stop solution (50% dimethylformamide and 20% Sodium dodecyl sulphate at pH 4.7). Measure the absorbance at 490 nm within 1 h of adding the Stop solution. Set the background absorbance at 690 nm and subtract this value from the primary wavelength measurement (490 nm). The percentage of LDH released is calculated as percentage of the total amount, considered as the sum of the enzymatic activity present in the cellular lysate and that in the culture medium[43,44].

c) ***XTT assay*:** In order to measure the proliferation response, the (2,3-bis[2-Methoxy-4-nitro-5-sulfophenyl]-2Htetrazolium-5-carboxyanilide inner salt (XTT) assay is used. The tetrazolium salt, XTT is especially useful in quantifying viable cells. This assay is based on the cleavage of yellow tetrazolium salt, XTT, to orange formazan dye by metabolically active cells. XTT cleavages into an orange formazan dye by the mitochondrial enzyme, dehydrogenase, occurs exclusively in living cells. Cells are grown in growth medium containing 10% fetal bovine serum (FBS) in 96-well plates until 70-80% confluence and then treated with drug sample. 50 mL of XTT labelling mixture solution is added to each well and the cells are incubated at 37°C for 4 h. The formazan dye formed is soluble in aqueous solutions and quantified optical density at 450 nm, compared with that of control wells with a screening multiwell spectrophotometer enzyme-linked immunosorbent assay (ELISA) reader. The reference wavelength should be more than 650 nm[45,46].

d) ***Sulforhodamine B (SRB) assay*:** Sulforhodamine B assay is a bright pink aminoxanthine dye that binds to basic amino acids in mild acidic conditions and dissociates under basic conditions. Cells are plated in 96-well-flat bottom plates at 5000-10000 cell/well. The difference in cell numbers plated adjusts for differences in the growth rates of the various cell lines. Cells are allowed to adhere to the wells overnight, then the samples are added to triplicate wells in serial 3-fold dilutions. Water is added to the control wells at a 1:10 dilution in medium. Plates are incubated in humidified atmosphere (37°C, 5% CO_2) for 3 days, and then assayed for growth inhibition using a Sulforhodamine B (SRB) assay. The cells are fixed with 10% (w/v) trichloroacetic acid and then stained with 0.4% SRB dissolved in 1% acetic acid for 15-30 min and subsequently washed with 1% acetic acid to remove excess stain. After the plates are air dried at room temperature, the bound dye is solubilized with 10 mM Tris base and the plates are analysed for optical density at 595 nm using a microplate reader[47,48].

e) ***WST-1 Assay*:** This assay is designed for evaluation of intracellular metabolic activity of cells. The reduction of the WST-1 into a water soluble formazan occurs by the NADH dehydrogenase and plasma membrane electron transport. Here, 10 uL of WST-1 reagent is added to 100 uL of media in a 96-well plate. Seeded cells are incubated overnight at 37°C with 5% CO_2 in a humidified atmosphere for 24 h. Then cells are treated with apoptotic inducers, cell proliferation triggers, or cytotoxic reagents and then 10 uL/well of WST-1 is added and incubation is continued for an additional 1-2 h at same humidified atmospheric condition. The absorbance is measured at 450 nm *vs.* a 650 nm reference by using a plate reader[49,50].

f) ***Acid Phosphatase assay*:** It is the quantification of cystolic acid phosphatase activity on the cells. The assay is based on hydrolysis of p-nitrophenyl phosphate to p-nitrophenol by the intracellular acid phosphatase enzyme. The absorbance is measured at 405 nm and absorbance at this wavelength is monitored as a measure of cell number[51,52].

II. Cell death/ Apoptosis: Apoptosis or programmed cell death is the process of cell death that occurs as a normal and controlled part of an organism's growth or development. Apoptosis takes place through a complex signalling mechanism and is dedicatedly balanced or regulated in a physiological context[53]. Apoptosis can be detected by the following assay:

a) ***Trypan blue dry exclusion assay*:** In this assay, cell suspension is mixed with trypan blue and visually examined to determine whether cells take up or exclude the dye. Viable cells have clear cytoplasm and non-viable cell have blue cytoplasm. The cell suspension is washed with buffered Salt Solution and centrifuged for 5 min at 10,000 rpm. Then resuspend the cell pellets in 1 mL PBS. The cells are exposed to drug dilutions and incubated at 37°C for 3 h. After 3 h, equal quality of the drug treated cells are mixed with trypan blue (0.4%) and left for 3 min at room temperature. Then a drop of trypan blue/cell mixture is loaded in a haemocytometer and viable and non-viable count are recorded within 2 min. Viable cells do not take up colour, whereas dead cells take up colour[53]. The percentage of growth inhibition is calculated using the following formula:

$$\text{Growth inhibition (\%)} = 100 - \frac{(\text{Totalcells-Deadcells})}{\text{Totalcells}} \times 100$$

b) ***Live/Death staining*:** It is done for identification and quantification of live and dead cells. The cells are stained with fluorescent dyes such as calcein-acetoxymethyl (calcein-AM), intercalating agent- propidium iodide (PI) and ethidiumhomodimer (EthD-1)). Live cells are stained in green following intracellular cleavage of the acetomethoxy group of calcein-AM. Dead cells are stained in red following penetration of the intercalating agents through their permeable membrane[54].

c) ***Annexin V-FITC staining*:** It is used to detect apoptosis marker phosphatidylserine molecules which have translocated to the outside of the cell membrane. In normal cells, phosphatidylserine (PS, membrane phospholipids) is held on the inner layer of the cell membrane, so Annexin V does not attach to the cells. During early apoptosis, the PS is exposed on the outer layer, where they attach to the FITC (Fluorescein isothiocyanate)-labelled Annexin V and stain the cell surface green. During late apoptosis, propidium iodide (PI) enters the cell and stains the contents red. Analysis is done by flow cytometry or fluorescence microscopy[55].

In-vivo *model of cancer*

The development of an *in vivo* animal model that simulates the natural history of human cancers and its clinical response to treatment is an important prerequisite for laboratory to clinical transformation in which investigational cancer treatment and imaging technologies have shown promise in *in-vitro* models. There are different animal species which are used as an *in vivo* model for cancer screening. It is important to select the appropriate animal model to evaluate a specific cancer. Animal model should reflect the human disease as much as possible. At the same time its feasibility and expenses to be used in large drug screening programs is also important factors[56]. Commonly animal models are divided in to two types (I) Spontaneous tumor model and (II) Induced tumor model. The models are depicted in Figure 3 and briefly discussed below:

I) Spontaneous tumor model

It includes the selection and use of animals that naturally occurs cancer. For example, some innate mouse and rat strains are particularly susceptible to various cancers, especially leukemia, breast cancer, adenoma and lung hepatoma[57]. In DA/Han rats more than 60% of female animals die from endometrial adenocarcinoma. In BDII/Han rats, 87 to 90% of animals die of endometrial adenocarcinoma[58]. In DA/Han inbred rats, 53.9% of males and 14.4% of females developed spontaneous urinary bladder tumors. These models mimic the clinical situation most closely. They are similar to human cancers in kinetics and antigenicity, but these systems have many limitations. It is impossible to obtain a sufficient number of tumors of the same size for screening at a time. Tumors can usually only be measured at the end of their development, but the mode of metastasis is not, and it is difficult to accurately determine homogeneity at this stage. These models are usually not replicable, and most of them are viral origin[59].

II) Induced tumor model: Cancer can be induced by various agents such as some chemicals and viruses can act as a cancer inducing agent. Different induced cancer models are discussed below in this section.

a) Chemically induced tumor model

Tumors caused by chemical carcinogens originate from the host's own cells and therefore resemble human clinical cancer more closely than transplantable tumors. The limitation of chemically induced tumors is that carcinogens may have an impact on tumor behaviour, and the excretion of carcinogens and their metabolites in animal feces and urine may cause danger to other animals and personels[60,61].

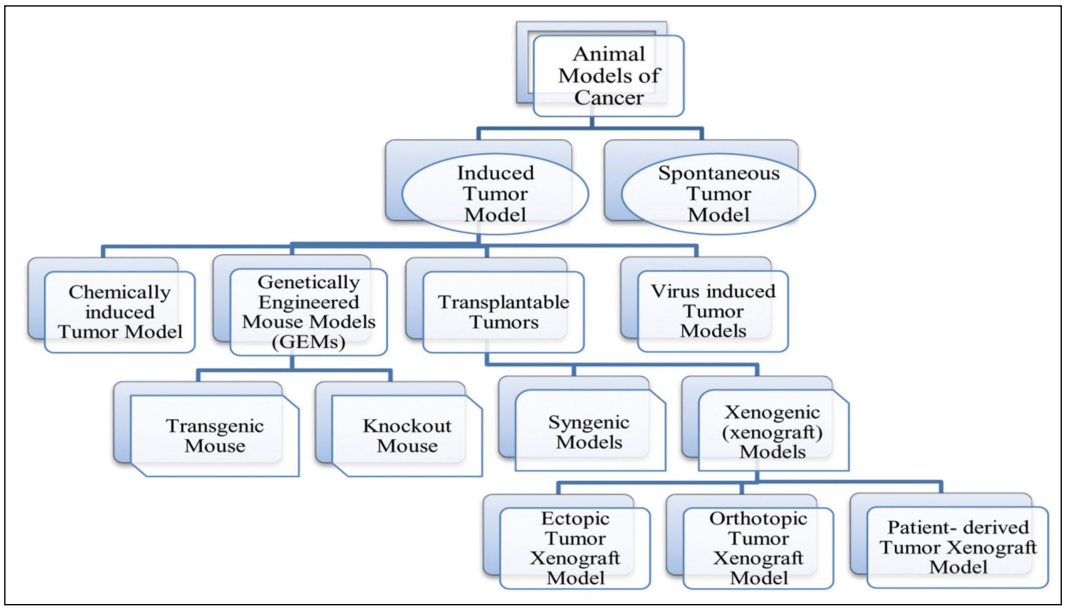

Figure 3. Commonly used animal models in cancer. The models portrayed here are mostly used to determine safety and efficacy in drug and dosage development process.

Chemical carcinogens can be divided into two categories:

i. Direct acting agents: require no chemical transformation to induce carcinogenicity.
ii. Indirect acting agents: become active only after metabolic conversion. Also known as procarcinogens and their active end products are called ultimate carcinogens.

Although any gene may be the target of chemical carcinogens, RAS gene mutations and TP53 genes are the important targets. Few examples of chemical carcinogenesis are mentioned below:

Urinary bladder cancer induced using N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)

Mice are treated with BBN at 500 ppm in drinking water, after 2-6 weeks mutagenesis in urothelial cells are greater than the spontaneous mutation background and that in the smooth muscle cells of the urinary bladder; after 20 weeks of BBN treatment at the same concentration, bladder cancer developed in all treated mice. The detailed histopathological and immunohistochemistry examination of tumour is valuable in determining the drug action[62].

Colon cancers induced using DMAB (3, 2-dimethyl-4-aminobiphenyl)

These tumours occurred after subcutaneous injection of the carcinogens for 6 consecutive weeks. It can cause multiple colon tumours in approximately 26-30% of animals fed on modified fatty diet. Induced tumors are adenoma (benign tumours) and adenocarcinoma (malignant tumours) in the large bowel. This model provides good information about tumour induction and the shape, size and histological properties of tumour tissue. The only drawbacks of this model is that it requires multiple injections of carcinogens and form secondary tumours in other areas such as skin, mammary glands, stomach, salivary glands, lymph nodes, ear canal and urinary bladder, which will affect the effectiveness of the drug and survival rate and make it difficult to compare[63].

Fibrosarcoma Model in Mouse Using 3,4,9,10-dibenzopyrene

Single subcutaneous injection of carcinogen is sufficient to form uniform subcutaneous tumours at the site of injection within 30-40 days post injection. The most important advantage of this model is induction of tumour with single dose of carcinogen and no excretion of carcinogen through faeces or urine.

b) Genetically Engineered mouse model (GEM)

Cancer originates from genetic changes, and hence the animals genetically engineered depict cancer scenario in humans better than other animal models[64]. Compared with tumor models with subcutaneous xenograft, these animals showed spontaneous development of cancer in their natural anatomical site. In GEM, tumours

show natural progression and metastatic biology similar to human counterpart. Importantly these tumours are originated within self; hence, there is no need for immunosuppressed animal to grow them[65]. GEM animals include (i) transgenic and (ii) knockout mice.

(i) **Transgenic mouse model**: These animals are the result of introducing foreign genes into the pronucleus after the eggs are fertilized. Later offspring developed from this modified egg vector and expresses the foreign gene which was inserted and passes to further offspring. The gene to be studied can be introduced into the pronuclei by microinjection, retroviral vector or embryonic stem cell (ESC) transfer, which has proven to be an excellent animal model system for studying oncogenic phenotype, resulted from the dysregulation of a known gene[66]. One can study the characteristics of oncogenes such as TP53, C-myc, E2F1, retinoblastoma (RB), neurofibromin 1 (NF1) and others in these animals. Due to known pathway defects, these animals expressing oncogene will develop spontaneous tumours, so it is most suitable to evaluate drugs/molecules specifically targeting these molecular signalling pathways in transgenic mouse models[67]. Some examples of transgenic mice are as follows:

1. **The TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) Mice:** This consists of a minimal rat probasin promoter that drives expression of simian virus40 (SV40) tumor antigens. These mice develop prostate cancer within 12 weeks of age and ultimately develop metastasis by 30 weeks. The TRAMP mice recapitulate many salient aspects of human prostate cancer[68].
2. **p53$^{+/-}$ Wnt-1transgenic mice:** p53$^{+/-}$mice have been crossed with mWnt-1 transgenic mice, which express Wnt-1 transgene in the mammary epithelial cells (MECs) under the control of the mouse mammary tumor virus (MMTV). These combined transgenic mice develop a model of mammary tumorigenesis[69].
3. **Apc deficient mice:** These mice spontaneously develop preneoplastic intestinal polyps due to a dominant mutation of an *Apc (*adenomatous polyposis coli) gene. Mutation of this gene is common to most human colon cancers[70].

(ii) **Knockout mouse model:** In these animals, the gene is deleted or inactivated artificially using synthetic DNA segment. These knockout mice are the other type of genetically engineered animals generated by deleting both the alleles of a gene of interest[71]. Some examples of knockout mice are as follows:

1. **The *Nkx 3.1* knockout mice:** *Nkx 3.1* is a prostate specific tumor suppressor gene. It is important for differentiation and function of prostate. Loss of function of this gene leads to histopathological defects that resemble human prostate cancer. This model also provides a model for studying mechanism of prostate cancer initiation as well as to explore the tissue specific features of the disease[72].
2. **Homozygous p53 knockout mice:** Mutation of *p53* tumor suppressor gene is the most frequently observed genetic lesion in human cancer. Over 50% of all human tumors have identifiable *p53* gene point mutation or deletions. These mice are highly susceptible to spontaneous tumorigenesis particularly lymphomas[73].
3. **Brca1 conditional knockout model:** Breast cancer suppressor gene 1 (Brca1) mutation is the main reason for 45% of breast cancer cases. Animal develop mammary tumor by the age of 10 to 15 months[74].

c) Transplantable tumors: These models are the most used model systems utilizing cancer cell lines or tissues of human or mouse origin. These models are divided into two types depending on methods of transplantation[75].

i) **Heterotopic tumor transplantation**
In this case, implantation of tumour cells or tissues is done at a site other than its anatomical site. For example, breast cancer cells are transplanted subcutaneously or intraperitoneally. This method generally involves subcutaneous or intraperitoneal transplantation of tumour cells; the tumour proliferates in that site to form solid nodules or as cites, respectively. The procedure of inoculation is simple, quick, and precise and allows inoculation in a large number of animals for screening of anticanceragents[76].

ii) **Orthotopic tumor transplantation**
It refers to the transplantation of cancer cells into the anatomical site or tissue where the tumor appears. For example, transplanting lung tumors into the lungs. Utilization of this method leads to the development of tumor models which more closely resemble tumor histology, vas-

cularity, gene expression, sensitivity to chemotherapy and metastatic biology of human cancers. In the orthotopic tumour model, the interaction of the host microenvironment in tumorigenesis, progression, and metastasis behaviour is more strongly perceived, so it can be considered superior over conventional flank mouse models[77].

These models are developed by inoculation of cancer cells by direct injection or by surgical procedure in anatomical site, i.e. by implanting cancer cells or intact tumor fragments orthotopically by invasive procedures. Surgical implantation is invasive as it improves the reproducibility and metastatic behaviour of the model[76].

Transplantable tumour models are further categorized into two categories depending upon the origin of the tumour and the host used.

I) **Syngeneic model:**

In these animal models, the cancer cell lines or tissues of murine (mouse or rat) origin are used for transplantation. Syngeneic models are obtained by injecting a recipient of a specific genetic background with cell lines previously established through isolation of tumor cells from a mouse of the same genetic background. The advantage of this model is that the transplanted tissues, the tumor microenvironment, and the host are from the same species[78]. Some of the examples of syngeneic tumor models are given below[79,80]:

- B16-F10 melanoma cell line obtained from C57BL/6J mouse is used to develop cancer models in animals of same species.
- L1210 leukemic cell line obtained from DBA/2 mouse grown in animals of same species.
- Lewis lung carcinoma model was developed by isolation from a spontaneous epidermoid carcinoma of the lung in C57BL/6 mouse.

II) **Xenogenic (xenograft) models:**

This tumor model closely resembles the clinical scenario because they are designed with transplanted tumor of human origin. Since the transplanted cancer cells are from different host origin such as human tumor in mice, which may result in severe immune rejection. To overcome this problem, mice immunosuppressed by thymectomy or radiation are used. But there are several limitations associated with this method. Therefore, athymic (nude) mice or mice with severe combined immunodeficiency (SCID) are used. These animals lack an immune response to the transplanted foreign material[81].

Transplantation of tumor cell lines into nude mice can be accomplished via multiple routes like subcutaneous, intraperitoneal, intravenous, intracranial, intrasplenic, and renal subcapsular, or through a new orthotopic model by site-specific organ inoculation. Each site has specific advantages and limitations[82].

When human tumor cells are transplanted in nude mice, they undergo kinetic changes. The doubling time is usually shorter than that of the original tumor and decreases with subsequent passages. Despite this, many xenografted human tumors maintain original morphologic and biochemical characteristics. Therefore, human tumor xenografts are mainstay of cancer drug discovery programs[83].

Xenograft tumor models are further classified based on the transplant site, which are as follows:

1. *Ectopic tumor xenograft model*

Generally, human cancer cells are subcutaneously injected into the hind leg or back of mice. In an ectopic tumor xenograft model (ectopic model), the transplanted site is different from the origin of the cultured cells. The ectopic model is considered as the standard model for evaluation of anticancer efficacy because it can be used to monitor tumorigenicity and tumor growth easily. Furthermore, the ectopic model is very reproducible, homogenous, and amenable to use. But they have some limitations such as limited histological and phenotypic similarities to primary cancer, low metastatic rates and lack of native tumor microenvironment[84,85].

2. *Orthotopic tumor xenograft model:*

In orthotopic tumor xenograft model (orthotopic model), variety of human tumor cells are transplanted into the same origin site of the tumor, so they develop the same anatomical microenvironment. For instance, lung cancer cells are directly injected into the mouse lung for the orthotopic model. Orthotopic tumors show faster early-stage tumor growth, angiogenesis and hyper-permeability of blood vessels compared to ectopic tumors. But the limitation associated with this model is determination of drug efficacy therapies endpoints is more complex unlike subcutaneous ectopic models[86,87].

3. *Patient derived tumor xenograft model:* Although the xenograft model has advantages, it has limited ability to show the response of cancer patients to specific treatments. Clinical trials require reliable prognosis of drug response, and existing models are insufficient[88]. To overcome the shortcomings of these models, patient-derived tumor xenografts (PDTX) were developed and utilized[89]. Because PDTX involves directly transplanting cancer patients' tissue into immunosuppressed mice; genetic information and immunohistological markers are relevant to patient and can be used to evaluate new anticancer drugs and personalized cancer therapies[90]. The several advantages of PDTX can be summarized as follows: 1) it maintains and stabilizes genetic, histological and phenotypic characteristics of tumors, 2) maintains stromal and stem cell components of the tumor, 3) facilitates the evaluation of biomarker, and 4) can be used to predict the response to an anticancer drug[91].
However, the PDTX model has technical constraints, and is expensive and time-consuming. In this scenario the freshly excised human primary tumors must be transfer from operating room to the laboratory within a few hours. At the same time, samples of the primary human tumors should be examined through immunohistological analysis[92]. Therefore, cooperation between surgeons, histologist, scientist and laboratory staff is required to collect specimens correctly and subsequently obtain approval. In addition, approval from institutional review board (IRB) is required because utilization of patient-derived tumor tissue entails clinical and ethical considerations. Notwithstanding these efforts, the take rate of PDTX is about 25%, and establishment of PDTX takes approximately three months before the first passage[93]. Like xenotransplantation, it is necessary to transplant to SCID mice for the first time to avoid acute immunorejection, which is expensive. Additionally, the volume of tumor tissue obtained from patients is very limited so, the size of the PDTX population must increase with the passage of tumor tissue. Simultaneously, each passage of tumor tissues should be histopathologically analysed and compared with the original tissue. From the second passage, nude mice can be utilized. The tumor tissue fragments can be frozen and preserved in liquid nitrogen. PDX has been explored as a promising model for personalized cancer therapy[94]. In Table 2 we report different tumor types and anatomical sites utilizing various mouse strain and engraftment rate in PDX model.

d) Virus induced tumor model

The carcinogenic effects of viruses are related to miscarriage and non-productive infections. The infectious nature of oncogenic viruses sets them apart from other carcinogenic agents. Therefore, a comprehensive study of the pathogenesis and host response of viral infection is crucial to a full understanding of the resulting cancers[105]. This knowledge in turn expands our understanding of cellular pathways involved in growth and disease, differentiation and neoplasia. Human oncogenic viruses belong to different virus families and utilize diverse strategies to promote cancer development, but they have many common characteristics. A key feature is that it can infect but not kill their host cell[106]. Unlike many other

TABLE 2. Different tumor types and anatomical sites utilizing various mouse strain and engraftment rate in PDX model.

Tumor type	*Mouse strain*	*Implantation site*	*Engraftment rate (%)*	*References*
Colorectal cancer	SCID	s.c	87	[95]
Colorectal cancer	Nude	s.c	64	[96]
Colorectal cancer	Nude	orthotropic	89	[97]
Breast cancer	Nude	s.c	13	[98]
Prostate cancer	NOD/SCID	Subrenal capsule	95	[99]
Pancreatic ductal carcinoma	Nude	orthotopic	62	[100]
Pancreatic ductal carcinoma	Nude	orthotopic	62	[101]
Gastric cancer	Nude and NOG	i.p	31	[102]
SCCHN/SCC	Nude	s.c	54	[103]
Renal cell carcinoma	Nude	s.c	8.9	[104]

SCCHN = Squamous cell carcinoma of head and neck; SCC = Squamous cell carcinoma; NOD = Non-obese diabetic; SCID = Severe combined immunodeficiency; s.c = Subcutaneous; i.p = Intraperitoneal

TABLE 3. Viruses associated with human malignancies.

Name of Virus	*Type of cancer*
Human T-cell leukemia virus (HTLV-1)	Adult T-cell leukemia (ATL)
Human Papillomavirus (HPV)	Cervical cancer, skin cancer in patients with epidermodysplasia verruciformis (EV), head and neck cancers, and other anogenital cancers
Human herpes virus 8 (HHV8)	Kaposi's sarcoma (KS), primary effusion lymphoma
Epstein-Barr Virus (EBV)	Burkitt's Lymphoma (BL), nasopharyngeal carcinoma (NPC), post-transplant lymphomas, and Hodgkin's disease
Hepatitis B virus (HBV) and Hepatitis B virus (HBV)	Hepatocellular carcinoma
Simian vacuolating virus 40 (SV40)	Brain cancer, Bone cancer, and mesothelioma
BK virus (BKV)	Prostate cancer
JC virus (JCV)	Brain cancer
Human endogenous retroviruses (HERVs)	Breast cancer, Ovarian cancer, melanoma
Human mammary tumor virus (HMTV)	Breast cancer
Torque teno virus (TTV)	Gastrointestinal cancer, lung cancer, breast cancer, and myeloma

disease causing viruses, cancer causing viruses tend to establish long-term persistent infections. The long-term interactions between virus and host are key features of the oncogenic viruses, as they set the stage for a variety of molecular events that may contribute to eventual virus-mediated tumorigenesis[107].Viruses that are compellingly associated with human malignancies are depicted in Table 3.

There is no more perfect animal model than a human virus. All animal models, in terms of their nature and design represent an aspect of the disease rather than the entire life cycle of human tumor virus. Different rodent models are used to stimulate virus induced human cancer. Rabbit serves as a useful animal model to study HTLV-1 induced cancer. Immunodeficient and genetically engineered mouse models are used to study Epstein - Barr virus (EBV) induced cancer[108].

The methods for development of different *in vivo* anticancer models are pictorially represented in Figure 4.

Zebrafish as a novel in vivo *model in cancer research*

Zebrafish (*Danio rerio*) is a small vertebrate tropical fish that has emerged as a powerful model to study cancer biology, process for cancer drug discovery and also for toxicity screening[109]. The growing interest of this model leads to the existence of a bunch of advantages that make it a good model for cancer drug development and its safety assessment[110]. Zebrafish (ZF) can be used at any stage of development to conduct experiments whether it's in a larval stage (3 to 29 day after fertilization, dpf) or the adult stage (90 dpf-2 years)[111]. The husbandry cost of zebrafish is less expensive and due to smaller size of the adult fish, its breeding can be done in a small freshwater tank hence less space is required. It can absorb compounds, small molecules, or drugs directly from the water environment which make drug administration easy and feasible[112]. Zebrafish contribute a high level of physiological and genetic resemblance with human, almost 70% of human genes are functional analogous to zebrafish[113]. Furthermore, zebrafish gestation period is so fast, it is capable of fertilizing 200-300 eggs in a week thus enough number of animals are available to utilize in the experiment although the zebrafish are fully developed by 24 hours post fertilization (hpf), and they are ready for use in larvae experiments by 3 days post fertilization[114]. Their extrauterine development is rapid and require less time for development of zebrafish organ system[115]. Moreover, other benefit of utilizing zebrafish model is transparency of its embryos and larvae, which allows visualization of organ and tumor growth inside the fish body and dynamic evaluation at the stages of drug development[116]. Embryos of ZF stay transparent to 60 hpf, their transparency period can be extended by treating embryos with 1-phenyl 2-thiourea (PTU), which inhibits the pigmentation process[117]. The transparency of ZF embryo also allows investigating anticancer nanomedicines biological effects, therapeutic efficacy, and safety by simply utilizing *in vivo* imaging[118]. A diversiform of cancer models have been developed in zebrafish which pathophysiologically or in molecular level also resemble human counterparts. Scrutinizing these features of zebrafish it is considered as a devoted model for cancer drug discovery and toxicity screening.

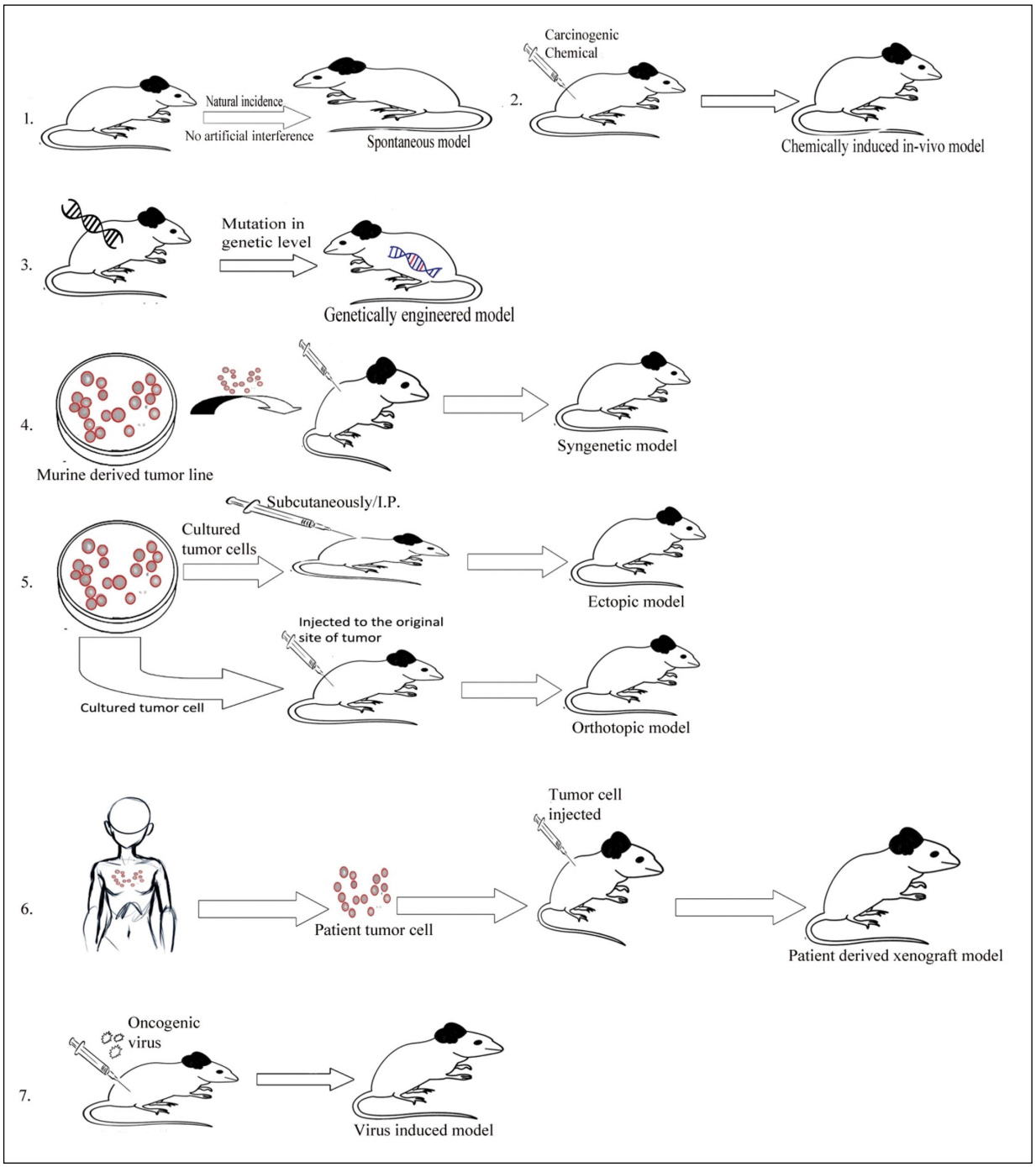

Figure 4. Pictorial presentation of different procedures for the development of *in-vivo* anticancer models. (1) *spontaneous model* are naturally cancer developing model i.e no artificial treatment required for cancer induction (2) *chemically induced model* is develop by introducing chemical carcinogens to the animal (3) *genetically engineered model* is develop by altering the targeted genetic makeup of the animal which induce cancer susceptibility (4) In *syngeneic model* tumour cell line of a murine species is injected to another animal of same species to produce cancer (5) *ectopic model*- human cancer cell lines subjected to murine model s.c/i.p and in *orthotopic model*- cancer cells of human specific cancer is injected to original site of tumour (6) *patient derived xenograft model* develop by collecting tumor tissue of patient and inject the same to the animal (7) In *virus induced model* oncogenic viruses are introduced to animal which interfere with the DNA synthesis process and produce cancer.

Zebrafish is a convenient model for induction of tumor by the utilization of different methods such as (a) chemical exposure (b) genetically engineered and (c) xenotransplantation. Different approaches are used to generate these zebrafish models which investigate cancer progression, molecular mechanism involved and study novel anticancer drugs efficacy[119]. Methods for induction of zebrafish tumor model are briefly explained in the following and schematically represented in Figure 5.

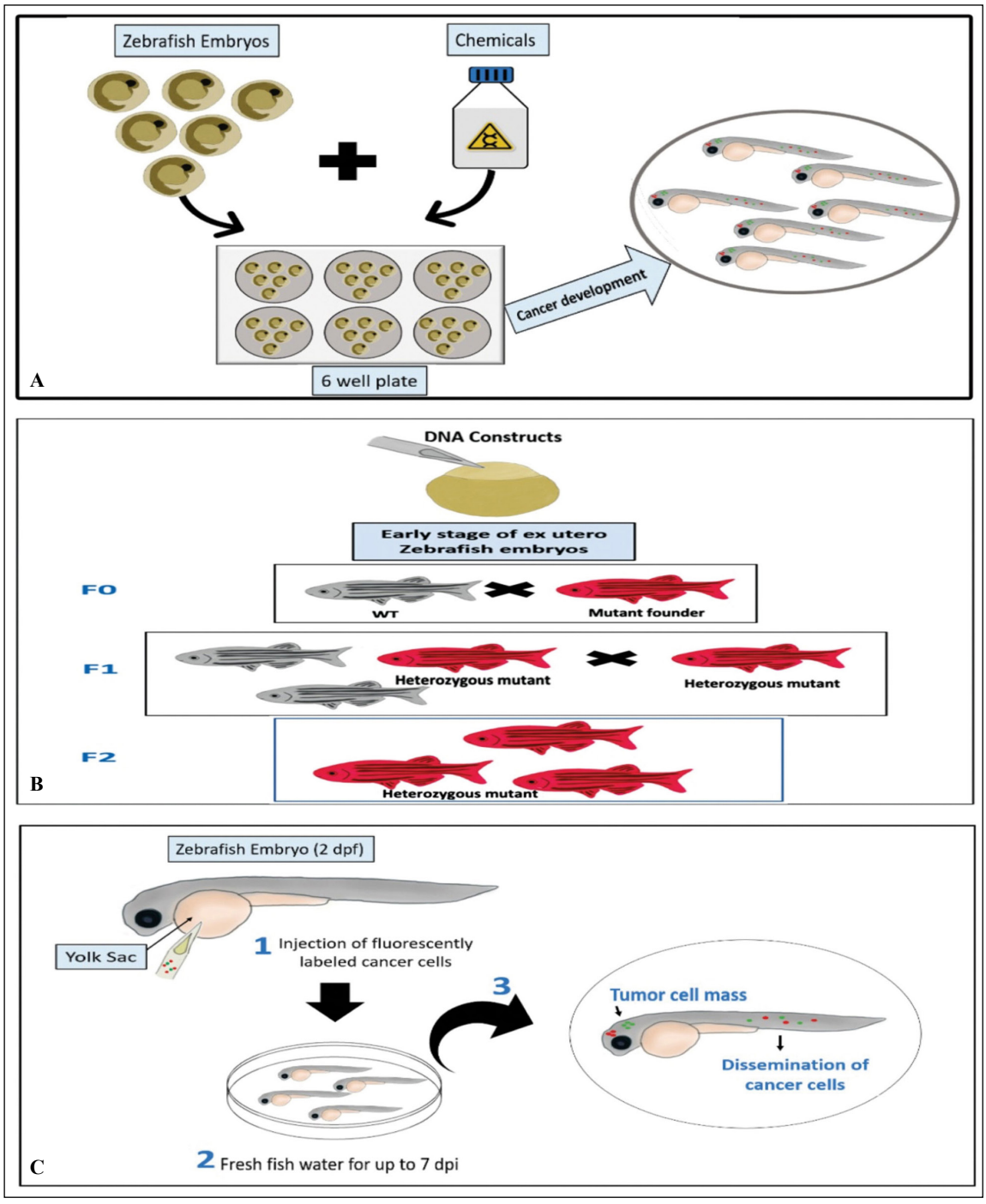

Figure 5. Different zebrafish model for cancer screening. *A, Zebrafish chemical carcinogenesis model;* Zebrafish embryos are collected, and healthy embryos are arrayed into a multi-well plate. Chemical carcinogens are then added to the multi-well plate following dissolving or suspending them in the fish water. After appropriate incubation period, embryos are screened for cancer growth. *B, Transgenic cancer zebrafish lines;* DNA constructs of cancer gene are microinjected into the cell of fertilized egg which is an early stage of zebrafish embryos. The adult mutant founder fish (Red) that have the germ cells, in which the construct was integrated into the genome, is mated with a wild-type (WT) zebrafish (Gray) at F0 to produce the F1 generation of fish, in which some of the progenies are heterozygous (Red) for the constructs and these heterozygous fish are then mated with each other to produce the F2 generation of fish that are homozygous to the construct (Red). *C, Xenotransplantation assay in zebrafish embryos;* Human cancer cells stained with red- or green- fluorescent dye and injected alone or in combination using a glass micropipette or a glass capillary needle in the yolk sac of zebrafish embryos at 2 dpf. The xenografted embryos are then maintained at a specific temperature between 28°C to 37°C. After 2 to 7 days post-injection (dpi), the number of tumor cells increases, and cancer cells disseminate at distance sites such as head and tail[118].

(a) Chemically induced zebrafish cancer model

Zebrafish animal model has shown terrific diversity in mutation of neoplasm as compared to other fish species[120]. Induction of tumor by chemical exposure was the initial approach to develop zebrafish as a cancer model. Zebrafish treated with carcinogen can provide a robust cancer induced model of any type and are substantially less difficult to carry out as compared to murine mouse model[121]. The spontaneous development of almost any type of cancer by these carcinogens resembles similar morphology and mechanical pathway to human. The most prevalent process for chemical induction of cancer is dissolving the carcinogen in the water and then soaked the fish for varied period of time. Carcinogen with different concentration for a changeable duration is utilized such as smaller doses soaked up to 24 h and large doses for 8 h or less. Additional method for induction is injection of carcinogen directly to embryo and topical application[122]. Allied numbers of carcinogens are used to develop cancer in mammals and zebrafish. For example, N-methyl-N-nitro-N-nitrosoguanidine (MNNG) dimethylbenzanthracene (DMBA), N-nitrosodiethyl- amine (DEN), N-nitrosodimethylamine (NDMA) when exposed to zebrafish, can induce a variety of cancers such as rhabdomyosarcomas, leiomyosarcoma, chondromas, seminomas, hemangiosarcomas, papillomas, and hepatic cancer. These chemically induced zebrafish exhibit molecular and histologic similarities with human cancer[123].

N-nitrosodiethyl- amine (DEN) induced pancreatic cancer: zebrafish was immersed in DEN solution (100 ppm) in an acrylic tank for a period of 8 weeks. The water temperature maintained at 26 ± 0.5°C and the water containing the carcinogen was exchanged every 2 weeks. After exposure to 8 weeks, the fish was transferred to fresh water tank and the formation of tumor was observed[124].

N-nitrosodimethylamine (NDMA) induced hepatocellular carcinoma: 50 ppm NDMA was dissolved in dechlorinated tap water and then transferred to 20 L acrylic tank and immersed 50-60 fish in a tank. The tank was thermo-regulated and attached with mechanical filtration and water exchanged carried out every week. Then after exposure of fish to NDMA for 8 weeks, it was transferred to a fresh carcinogen water until observe the carcinoma produced. During the experiments diet, temperature, and light/dark cycle remained at the standard conditions[125].

7,12-dimethylbenzanthracene (DMBA) induced intestinal carcinoma: DMBA was initially dissolved in DMSO and then mixed into the oil component and then finally mixed to a purified casein diet. Gelatin (2%) added to this modified diet (MPC). Diet was prepared in moist form by mixing 65% hot (55°C) distilled water with 35% dry mix. Then juvenile fish (2 months of age) were fed MPC diet containing 0, 100, 500, or 1,000 ppm DMBA for 4 months. Fish were then fed basal MPC diet for an additional 3 months, for a total of 7 month. After initiation of DMBA exposure at different interval of 4, 8, 12, and 16 weeks, histopathological study was carried out to evaluate intestinal tumour[126].

(b) Genetically engineered zebrafish cancer model:

Genetic manipulation is another significant method for developing zebrafish as a cancer model. Different mutant and transgenic zebrafish models are utilized as potent model for cancer research which are generated by using different techniques such as forward or reverse genetic approaches which directly assess the role of various genes in cancer related phenotypes[127].

The reverse genetic approach in zebrafish through the specific knockdown or knockout genes of interest has previously been limited but with the advancement of new techniques this area is progressed[128]. Furthermore, another reverse genetic approach to induce gene knockout is antisense morpholino oligonucleotides technique, the microinjection of the antisense morpholino oligonucleotides (MO) into the zebrafish embryos' blastomeres or yolks in early stages binds to endogenous mRNAs and would prevent the targeted gene translation[129].

In forward genetic screening mutation in the zebrafish genome has been done by introducing chemical (ethylnitrosourea) and viral mutagenesis strategies[130]. Mutagenized zebrafish are then screened for identifying mutation in respective gene by gene mapping and high-throughput sequence analysis[131]. Targeting induced local lesions in genomes (TILLING) by using chemical mutagens such as N-ethyl-N-nitrosourea (ENU) techniques have been used for the target-selected mutagenesis in the zebrafish embryos' genome[132]. Another targeted genome modification technology includes ZFNs (zinc finger nucleases), CRISPR (clustered regularly interspaced short palindromic repeats) system, and TALENs (transcription activator-like effector nucleases)[133-135]. As a result of utilizing this mutation techniques, a numerous number of stable mutant zebrafish line established that harbour mutation in a ribosomal protein (RP) or specific tumor suppressor genes such as p53, adenomatous polyposis coli (APC), PTEN, Brca2, and neurofibromatosis type 2 (NF2) genes[136]. This genetically mutated

model can de distinguish from chemical induced mutations because higher rates of cancer incidence observed in genetic model such as 28% forp53 (17% for Apc, up to 100% for ribosomal proteins mutants) and that each of these mutant lines shows predisposition for a defined set of cancer types[137].

Transgenic zebrafish model: Zebrafish can also be used as convenient model for human cancer by targeting misexpression of a known oncogene under a zebrafish tissue-specific promoter. Microinjection of foreign DNA constructs in early zebrafish embryos' driven by zebrafish tissue-specific promoter accomplish the spatial and temporal control of the expression of some transgenes such as the GAL4-UAS and Cre-LoxP and Tol2 transposon and LexPR binary systems had led to the development of several transgenic zebrafish cancer models. Modeling human cancers in zebrafish through transgenesis becomes much easier[138].

A few examples of genetically engineered zebrafish cancer model are given below:

Myc and TEL-AML1 transgenic zebrafish leukemia models: T cell acute lymphoblastic leukemia in transgenic zebrafish expressing mouse *c-myc* and chimeric *EGFP-mMyc* transgenes under control of the zebrafish *Rag2* promoter are established. The resulting transgenic *zRag2 EGFP-cMyc* fish line showed rapid (21-42 days) onset of T-cell acute myeloid leukemia (AML)[139].

Apc mutated zebrafish colon cancer model: The *APC* gene mutant in zebrafish leads to colon adenoma initiation and progression, suggesting an association with the activation of the *Wnt* signaling pathway[140].

$BRAF^{V600E}$ transgenic zebrafish melanoma model: $BRAF^{V600E}$ oncogene under the control of melanocyte-specific mitf promoter injected into wt fish, this transgene led to an increased formation of nevi. However, when injected into *p53*-homozygous mutants, 7% of fish developed melanoma by 4 months of age, indicating cooperation between *BRAF* and *p53* pathways in the pathogenesis of these neoplasms[141].

HBx (hepatitis B virus X antigen) transgenic Hepatocellular carcinoma model: Overexpression of *HBx-mCherry* under the control of the liver-specific *fabp10* promoter takes place. This transgenic zebrafish develops hepatocellular carcinoma in *TP53−/−* background at 11 month post fertilization via Src (non-receptor tyrosine kinase) activation[142].

P53 knockdown malignant peripheral nerve sheath tumors (MPNSTs) model: Mutation in the wild-type p53 DNA-binding domain of the $tp53^{M214K}$ mutant zebrafish model line could spontaneously develop the malignant peripheral nerve sheath tumors at eight and half months and 16.5 months with an incident of 28%[143].

(c) Xenograft zebrafish model:

A foremost method for establishment of cancer model involves transplantation of human cell lines into zebrafish embryos. The primary advantage of using zebrafish for xenotransplantation is its lack of an innate immune system until 72 hpf and a mature adaptive immune response until 4 weeks of life which overcome the limitation of immune rejection and also the transparency of embryo allow detailed *in vivo* environment imaging for real-time visualization of cell-cell interactions[144]. A numerous types of human cancer cell lines are transplanted into different zebrafish embryonal stages mainly 48 hpf stage and blastula stage of the embryo are considered[145]. Xenotransplantation of patient-derived tumor cell into Zebrafish can be utilized as a pre-clinical screening model for personalized cancer therapy. Different sites such as yolk sac, vein, duct of Cuvier and hindbrain are utilized for transplantation and established a good model for studying cancer pathogenesis as well as screening of novel drug and therapeutics[146].

Zebrafish melanoma xenograft model: Melanoma cell line WM-266-4 labeled with CM-DiI, a lipophilic fluorescent tracking dye inject on 2 dpf, albino zebrafish maintaining at 28°C into the yolk sac, hindbrain ventricle or circulation and then transferred to fresh fish water and maintained at 35°C for up to 7 dpi and activated xenograft zebrafish formed melanoma[147].

Zebrafish glioblastoma xenograft model: Stellettin B, a naturally occurring marine triterpenoid, was tested in a zebrafish xenograft model of glioblastoma and it shown significant inhibition of angiogenesis *in vitro* as well as *in vivo* in zebrafish[148].

Zebrafish breast cancer xenograft model: The zebrafish breast cancer xenograft model is pertinent for revealing underlying mechanism, screening and development of anti-transforming growth factor-β drugs for the treatment of metastatic breast cancer[149].

Zebrafish prostate cancer (PCa) xenograft model: PC3-CTR cell line injected subcutaneously to zebrafish larvae at 48 hpf and then migration and proliferation of cells evaluated. This model serves as a best model for drug screening[150].

Some examples of the *In vitro* and *In vivo* models already used for evaluation of several cancers are depicted in the Table 4.

TABLE 4. Application of *in vitro-in vivo* model in anticancer drug development.

Name of model	Type of cancer	Key findings	References
In vitro *model used for cancer research*			
Spheroids model	Non-small cell lung cancer (NSCLC)	Establishment of an expandable model for drug screening and drug resistance	[151]
	Colorectal cancer	Mimicked different features of the tumor, such as ECM production, spatial organization, formation of a necrotic core, and ability to differentiate and polarize monocytes to M2-like macrophages	[152]
	Colorectal adenocarcinoma	Represents patient to patient differences in response to chemotherapy	[153]
	Breast cancer	Developed advanced model of breast cancer provides a possibility to study fibrosis and optimize antifibrotic therapies in cancer treatment.	[154]
	Prostate cancer	Spheroid formation from human prostate tumor cells exhibits tissue-like features.	[155]
	Hepatocellular carcinoma	Tumor spheroids not only mirror the 3D cellular context of the tumors but also exhibit therapeutically relevant pathophysiological gradients and heterogeneity of *in vivo* tumors	[156]
	Squamous cell carcinoma	Present a unique opportunity to evaluate growth properties that may not be realized when studying tumor cell growth as a monolayer.	[157]
Organoids model	Breast cancer	Predict the therapeutic response of anti-tumor drug in individual patients	[158]
	Liver cancer	Establishment of hepatocellular carcinoma organoids from needle biopsies, and cancer organoids maintain the genomic features of the original tumors for up to 32 weeks	[159]
	Gastric cancer	First reveal the potential metastatic role of TGFBR2 loss-of-function in diffuse gastric cancer	[160]
	Colorectal cancer	Verify the deficient of key DNA repair gene MLH1 role in drives tumorigenesis	[161]
	Prostate cancer	Show the role of nucleoporins in the progression of pancreatic cancer	[161]
	Pancreatic cancer	Evaluate cancer-stroma cell interactions	[163]
	Glioblastoma	Patient-derived organoids display histological features and recapitulate the hypoxic gradients	[164]
Scaffold model	Breast cancer	Cancer cell-laden polymeric scaffolds support consistent tumor formation in animals and biomarker expression as seen in human native tumors	[165]
	Osteosarcoma	Improved the osteosarcoma stem cell niche micro-environment simulation, providing precise inputs supporting cell-cell and cell-ECM interactions and tumour signalling pathways in vitro.	[166]
	Pancreatic ductal adenocarcinoma	Developed hybrid zonal model was able to support long-term growth and proliferation of cancer (PANC-1), endothelial (HMEC), and stellate (PS-1) cells for up to 35 days	[167]
	Prostate cancer (PCa)	Chitosan-alginate scaffold cultures could be used to identify PCa phenotypic characteristics, with potential applications for in vitro anti-cancer drug screening.	[168]
	Lung cancer	PLGA microparticles show better responses in the prepared model.	[169]
Microfluidic model	Cervical cancer	Study the efficacy and mechanism of action of tannin from Spatholobi Caulis as anticancer agent	[170]
	Lung cancer	Mimic the *in vivo* microenvironment of cancer metastasis and to investigate cell-cell interactions during metastasis	[171]
	Colon cancer	Developed tumor-on-chip platform used for high-content image-based screenings or gene expression analysis to study drug-dose responses	[172]
	Breast cancer	Mimic the Ductal carcinoma in situ (DCIS) structure, identifying multiple cellular adaptations to endure the hypoxia and nutrient starvation generated within the mammary duct	[173]

Continued

TABLE 4 CONTINUED. Application of *in vitro-in vivo* model in anticancer drug development.

Name of model	Type of cancer	Key findings	References
In vivo *models used for cancer research*			
Spontaneous tumor model	Breast cancer	Loss of estrogen and progesterone receptors and low expression of Her2/neu and overexpression of c-Myc, cyclin D1 and VEGF were observed	[174]
Chemically induced tumor model			
7,12-dimethylbenz [a]anthracene (DMBA) induced cancer	Squamous cell carcinoma	Skin lesions were induced by twice-weekly topical application of DMBA (100 nmol/100 µL acetone) for 8 weeks	[175]
Diethylnitrosamine (DEN) induced	Lung adenocarcinoma	A single dose (15 µg/g body weight) of DEN injecte intraperitoneally& serve as a useful model for targeting KRAS and EGFR negative tumors	[176]
7,12-dimethylbenz [a]anthracene (DMBA) induced cancer	Breast cancer	50 mg/kg of DMBA once a week for 4 weeks to induced cancer	[177]
Benzo[a]pyrene induced cancer	Liver cancer	Twice a week for a period of 60-90 days (50mg/kg body weight). There is a marked elevation in lipid peroxidation, diminished level of glutathione peroxidase (GPx)	[178]
2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP)	Breast cancer	PhIP dissolved in 5% DMSO (75 mg/kg body wt.) for 1-5 and 8-12 days. Mechanism of metformin in PhIP induced breast cancer was observed.	[179]
N-nitroso-N-methylurea (MNU)	Prostate cancer	Single i.v. dose (50 mg/kg body weight) of MNU (dissolved in saline at 10 mg/ml) and after induction observe the chemopreventive effect of quercitin.	[180]
Genetically Engineered mouse model			
MT/ret transgenic mouse model	Skin melanoma	Development of cancer appears to resemble that of the malignant transformation of a human giant congenital melanocytic nevus.	[181]
p53val135/wt transgenic mice	Lung cancer	p53perhaps, the most important cancer genes in cancer development and progression	[182]
MMTV-PyMT transgenic mice	Breast cancer	Increased metastatic potential depend on colony-stimulating factor-1 (CSF-1) and EGF ligands between macrophages and tumor cells	[183]
BRCA1 knockout mice	Breast cancer	Disruption of Brca1 causes genetic instability and triggers further alterations, including inactivation of p53, that leads to tumour formation	[184]
p53 knockout mice	Skin cancer	Absence of p53 does not augment the frequency of initiation or the rate of promotion but greatly enhances malignant progression.	[185]
PTEN (Phosphatase and tensin homolog) knockout mice	Prostate cancer	PTEN-deficient mouse model represents useful tool for the preclinical evaluation and characterization of chemopreventive agents.	[186]
Syngeneic tumor model	Lung carcinoma	$Kras^{G12D}$.p53−/− line derived in a C57BL/6 background that forms lung tumors in C57BL/6 mice	[187]
	Triple negative breast cancer	Mvt-1and 4T1 TNBC mouse cell lines were injected into the mammary ducts via nipples of FVB/N mice and BALB/c mice	[188]
	Ovarian cancer	ID8 murine ovarian cancer cells transduced with pFB-neo-Luciferase (ID8-luc cells) to induce intra-peritoneal tumors in mice	[189]
Orthotopic mouse xenograft models (HT-29 and HCT-8)	Colorectal cancer (CRC)	The human HT29 cell line and HCT8 cell line are phenotypically similar to human CRC	[190]
Patient-derived xenograft model	Triple negative breast cancer	Phosphatidylinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathways were observed	[191]
	Lung cancer	PDX model is sensitive with EGFR mutations to EGFR-TKI and induced acquired resistance to EGFR-TKI	[192]
	Melanoma skin cancer	PDTX can help guide vemurafenib treatment for metastatic melanoma	[193]
	Cervical cancer	Ability to detect cervical dysplasia and normal cervical tissue cells is novel and provides models for the study of tumour initiation and progression	[194]

Continued

TABLE 4 CONTINUED. Application of *in vitro-in vivo* model in anticancer drug development.

Name of model	Type of cancer	Key findings	References
Zebrafish *in vivo* model			
Chemically induced zebrafish model			
Ethylnitrosoure (ENU) induced cancer	Skin cancer	Immersion of zebrafish in 2.5-3 mM ENU for 8 weeks develop skin tumor	[195]
N-methyl-N-nitro-N-nitrosoguanidine (MNNG) induced	Liver cancer	Zebrafish embryo (80 hpf) immersed for 1 hr containing 10 ppm (mg/L) MNNG, transferred to fresh water. After 3 months produce liver carcinoma.	[196]
Genetically engineered zebrafish model			
Twist1-ERT2 transgenic zebrafish	Hepatocellular carcinoma	80% of Twist1-ERT2/xmrk double-transgenic zebrafish spontaneously show metastatic dissemination of hepatic cells	[197]
zRag2-EGFP-mMyc transgenic zebrafish	T cell acute lymphoblastic leukemia (T-ALL)	Screening efficacy of cyclophosphamide (CY), vincristine (VCR) and prednisolone (PRE) in leukemia therapy	[198]
eGFP-KRAS(G12V) transgenic zebrafish	Pancreatic carcinoma	Cells expressing the transgene undergo minimal differentiation during embryogenesis and subsequently instigate several types of malignant pancreatic neoplasia in adult zebrafish	[199]
rag2–KRAS^{G12D} transgenic zebrafish	Rhabdomyosarcoma	Molecular pathway of disease analysed, identification of tumour-initiating cell populations	[200]
Neurofibromin 1 (NF1) knockout zebrafish	Malignant peripheral nerve sheath tumors (MPNSTs)	Identifying the regulatory pathway of tumorigenesis	[201]
Xenograft zebrafish model			
Cell line derived Zebrafish xeno-transplantation	Lung cancer	Inhibition of proliferation of tumor cells with EGFR mutation and T790M resistance mutation by Osimertinib was observed	[202]
	Ovarian cancer	Suggested that targeting extracellular signal-regulated kinase (ERK2) in the presence of cisplatin may reduce the burden of residual tumor	[203]
	Triple negative breast cancer	Inhibition of CXCR4 (chemokine receptor) signalling with IT1t (CXCR4 antagonist) led to a 39-60% decrease in tumor burden at 4 dpi.	[204]
Patient-derived xenograft (PDX) model	Breast cancer	PDX zebrafish gives better understanding of drug sensitivity and identify both prognostic markers and markers that are predictive of response to therapy	[205]
	Gastric cancer	PDX model showed potential proliferating, angiogenic and metastatic activity	[206]
	Pancreatic cancer	Develop same tumor environment to the original tumor, efficient model	[207]
	T-ALL	Elucidate molecular pathogenesis and targetable gene mutation	[208]

Parameter evaluated for in vivo *anticancer efficacy analysis*

Various parameters are evaluated after induction of carcinogenesis in different tumor models and evaluate the anticancer efficacy[209, 210]. For example,

i) Tumor volume: it is measured by the following equation:
Tumor volume= length x width^2/2 where length represents the largest tumor diameter and width represents the perpendicular tumor diameter.

Relative tumor volume is calculated by using the formula:

$$\text{Relative tumor volume} = \frac{T_x \times 100}{T_0}$$

T_x = absolute tumor volume of the respective tumor on day x
T_0 absolute tumor volume of same tumor on day 0, when the treatment started.

ii) Tumor weight: the weight of each tumor is measured at the end of the experiment.

iii) Body weight: the weight of the animal is measured each week and percent increase in body weight is calculated by the following formula: % increase in body weight = [(animal weight on respective day/animal weight on day 0)-1]× 100.

iv) Haematological parameters: in order to assess the influence of treatment on the haematological status of animals is observed by determining the following parameters:
- White blood cell total count
- Red blood cell total count
- Haemoglobin contents

v) Biochemical estimation: different set of methods, assays, and procedures are utilized to determine the substances present the serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphate level (ALP), blood urea, serum creatinine, total protein, albumin.

Evaluation of in vivo *pharmacokinetic parameters*

Pharmacokinetic (PK) understanding is an essential component of drug discovery process because it directly associated with the efficacy and safety profile of the drug. Different techniques have been developed to measure PK in both preclinical and clinical developmental stages of new drug discovery process. Pharmacokinetics can be determined by conducting two methods- either invasively, i.e., by collecting blood, tumor or tissue samples, or non-invasively, i.e., by using various imaging techniques[211].

Bioanalytical method

PK profiles can also be obtained by collecting plasma, urine and tissue samples at various time points from the dosed (via different route) animal models through either serial or composite sampling and by analyzing the samples for the drug using analytical methods such as high-performance liquid chromatography coupled with mass spectrometry[212]. The drug concentration against the time curve performed using non-compartmental analysis. Pharmacokinetic parameters such as the time taken to reach the maximum plasma concentration (t_{max}), half-life time ($t_{1/2}$), peak plasma concentration (C_{max}), the area under the curve ($AUC_{0-\alpha}$ h), volume of distribution (V_d) and the mean residence time (MRT) were determined. The drug concentration is usually expressed as ng/mL for plasma and ng/g for various tissues[213].

Molecular imaging method

Various non-invasive imaging methodologies are available for assessing pharmacokinetic. Molecular imaging can give information about biodistribution and presence of drug to the site. Different imaging techniques for PK assessment are:

Magnetic resonance spectroscopy (MRS) and Magnetic resonance imaging (MRI): MRS is the non-invasive *in vivo* method for measuring the concentrations of biochemical compounds (or drugs and their metabolites). MRI is used to define tumor location and detecting drug accumulation, but MRS can be used to measure the concentration of drugs in that tumour in real time. For PK studies, drug concentration in the tumor and also accumulation of drug in other organs can also be measured by this technique. Thus, drug retention, metabolism, and elimination can be studied in the target tissue by using MRI techniques followed by MRS[214].

Positron emission tomography (PET): The PK profiles can be obtained by radiolabeling the drug of interest with different radioisotopes. Many positron emitting isotopes of carbon, oxygen, nitrogen and fluorine are available which has half-lives of few seconds to several days. When absorption, distribution, metabolism and excretion of the analyte take place, the concentration of these radiolabeled compounds in different compartments such as blood, plasma, urine, bile, faeces and tissues correspond to the amount drug and its metabolites. PET can provide valuable information on drug pharmacokinetics in tumour and normal tissue by mathematical modelling of data. Rapid plasma radioactive profiling during the PET scan can be used for calculation of plasma input function and to provide evidence of specific metabolic processes in preclinical and clinical drug screening. PET/MRI scans will yield sensitive images of probe uptake with excellent soft-tissue contrast[215,216].

Computed tomography: X-ray computed tomography (CT) is used for visualizing three-dimensional tomographic imaging and diagnosis of soft-tissue and bone cancer treatment response. In PD studies, CT is used mainly to measure changes in the volume of disease with treatment[217].

Ultrasound imaging (US): US have high sensitivity for measuring tumor vasculature and blood flow. Tumor perfusion rates can be measured within a field of view by acoustically bursting the bubbles and then calculating the time spent to repopulate and regain contrast[218].

Fluorescence imaging (FRI): Fluorescence imaging is the most commonly used powerful imaging tool for pharmacokinetic assessment. A plentiful of suitable fluorescent dyes are available that are excitable in the visible to near-infrared

(NIR) wavelengths of the spectrum. FRI gives information on biodistribution of drug to the tumor environment and also accumulation of drug to the other organ parts. It is also accessible for gathering information on elimination or clearance of drug from kidney. Laser scanning confocal microscopy (LSCM) is a commonly used technique in preclinical research to obtain high-resolution optical images at controllable depths. By measuring the intensity of the fluorescent, the quantitative determination is monitored, higher intensity accomplish accumulation of more drug. Fluorescence molecular tomography (FMT) is an advanced tool for quantification of drug into the deeper tissues[219].

Translation of animal models to clinic

Due to practical and ethical issues associated with human experimentation, animal models play an essential role in cancer research. Animal models are needed to bridge the translational gap between preclinical and clinical research. However, the average rate of successful implementation of animal models to clinical cancer trials is less than 8%[220]. Preclinical testing in animal models is an integral component of the drug discovery and development process, which can predict the clinical efficacy and safety profile of the drug. The failure to translate from animals to humans is likely due in part to poor methodology and failure of the models to accurately mimic the human disease condition. A major obstacle to adequate prediction of clinical outcome based on preclinical animal studies may be potentially attributed to insufficient external and internal validity within the study design[221]. Table 5 depicts some reported clinical trials on the model used for anticancer screening.

Patents and other forms of intellectual property protection are generally thought to play essential roles in encouraging innovation in biopharmaceuticals. Patenting is the most effective mean of excluding market competitions[222]. Standard essential patents and patent pooling are seldom seen in the biopharma industry. The key to success of a biopharmaceutical product is to prove its safety and therapeutic benefits in clinical trials, which are stringently regulated and the outcomes are unpredictable. Such stringency and unpredictability have consumed enormous human and financial resources, and greatly increased the risk in product development. To protect potentially massive returns, drug makers have raced to build strong patent portfolios. Patent analysis can provide a snapshot of patenting activities of the industry[223]. Some of patented *in vitro-in vivo* model used in cancer research are depicted in Table 6.

CONCLUSIONS

In vitro and *in vivo* models are important tools in cancer research because they allow the identification of genetic manipulation culpable for cancer, the development of cancer therapies, drug screening, and provide information on the molecular mechanisms of tumor growth and metastasis. Although conventional human tumor models, including two-dimensional (2D) monolayer culture, are widely used in experimental models, they come with a variety of limitations. Therefore, the three-dimensional (3D) *in vitro* tumor models are most widely used as a bridge between

TABLE 5. Clinical trial on cancer management using different in vitro-*in vivo* model.

Type of Study design	*Clinical phase*	*Investigated application*	*CT Identifier*
Developing mouse model	Completed	Breast cancer	NCT00897468
Constitution of ex-vivo model	Recruiting	Ovarian cancer	NCT03831230
Establishment of Patient Derived Cancer Cell Models	Recruiting	Metastatic cancer	NCT02646228
Patient-derived Organoid Model	Recruiting	Lung cancer	NCT03655015
Patient-derived xenograft model	Recruiting	Head and neck cancer	NCT02572778
Three-dimensional tumor model	Recruiting	Skin cancer	NCT03136783
3D Organoid model	Recruiting	Vaginal cancer	NCT04278326
Patient-derived organoid model	Recruiting	Breast cancer	NCT03544047
Patient-derived organoid model	Recruiting	Esophageal cancer	NCT03283527
Organoid model	Recruiting	Kidney cancer	NCT04342286
Develop *in-vitro* organoid model	Recruiting	Liver and Pancreatic cancer	NCT02436564
Xenograft model	Phase I	Liver cancer	NCT04690972
Patient-derived xenograft model	Completed	Triple negative breast cancer	NCT02247037
Patient-derived xenograft model	Recruiting	Prostate cancer	NCT03786848
Patient-derived xenograft model	Completed	Breast cancer	NCT02752893

TABLE 6. Some patents on use of different in vitro-*in vivo* models for cancer research.

Title	*Year*	*Patent number*	*Inventors/Assignee*
Mice models of human prostate cancer progression	2002	US6365797B1	The Regents of the University of California (USA)
In-vitro model for a tumor Microenvironment	2017	US9617521B2	HemoShear, LLC, Charlottesville (VA, USA)
Rodent mammary window for Intravital microscopy of orthotopic breast cancer and related method	2004	US20040151666A1	Dewhirst MW, Shan S
Method and a kit for the *in-vitro* diagnosis of Pancreatic ductal Adenocarcinoma or for determining the predisposition to pancreatic ductal adenocarcinoma	2015	US20150177249A1	Natimab Therapeutics, SRL, Colleretto Giacosa, Torino
PIK3CA H1047R knock-in non-human animal breast cancer model	2013	WO2013015833A3	Seshagiri S (USA)
Spontaneously immortalized prostate cancer cell line	2014	US20140017721 A1	The Research Foundation of State University of New York
In-vitro tumor metastasis model	2012	WO2013017282A1	Roche diagnostics GMBH
Model of colorectal cancer	2014	WO2015013432A1	Erica J, Kevin L (USA)
An *in-vitro* 3d cell culture model-based tumor relapse assay	2016	WO2017029414A1	Insphero AG
Methods for the *in-vivo* detection and treatment of patient-centric tumor dependencies	2017	WO2017197290A1	Board of Regents, The University of Texas System (USA)
Animal models of prostate cancer and methods for their use	2003	WO2004000010A2	The Regents of the University of California (USA)
Mice model of human prostate cancer	2002	US6828471B2	The Regents of the University of California (USA)
Genetically modified mice expressing humanized PD-1	2017	US20190387724	Biocytogen Pharmaceuticals (Beijing) Co., Ltd
MMTV-SV40-spy1A and spy1A-pTRE transgenic mouse models	2013	US20140109244	University of Windsor
Animal model of human cancer and methods of use	2012	US20140047570	Altiok S
Transgenic Non-human animal model of lung tumorigenesis	2006	US20080313759A1	Dartmouth college
Knock-in mouse prostate cancer model	2003	WO2004044197	Procyon Biopharm Inc.
Three-dimensional bioprinted pancreatic tumor model	2015	WO2016022830A1	Oregon Health and Science University, US
Patient-derived xenograft model in gastric cancer and a use thereof	2015	KR101743340B1	Jeong J, Moon-Hee S, Lee J, Yoon-young C
Xenograft model of human bone metastatic prostate cancer	2012	US20140304844A1	The Regents of the University of California, (US)
A liver cancer PDX standardization model base	2016	CN105684989A	West China Hospital Sichuan University

in vitro and *in vivo* systems because they reflect similar cellular, molecular, and phenotypic features that closely generalize the characteristics of human malignant neoplasm. Varied animal (rodent) models have been developed for the cancer drug discovery process, which closely mimics human cancer environment and comfort to study the etiology of many types of cancer**,** assessment of drug toxicity, and the therapeutic potential of drugs. The zebrafish model can be used as an alternative *in vivo* model for investigating the cancer biology due to its cost-effectiveness, resembling a high homology to humans, rapid development, optical transparency, and the ease of genetic manipulation. The choice of a preclinical model is crucial to the drug discovery process. Therefore, there is no single model that fits all drug discovery programs, and no single type of model is universally the best. There is still a lot that needs to be done to fill the gap in translating the results of the animal model into the clinic. The development of animal models that fully epitomize the evolution, complex and heterogeneous biological process dynamics, including molecular, cellular, and histological changes, as well as the development of tumors in humans, are a prerequisite for the effective progress of translational research.

Conflict of Interest:
The Authors declare that they have no conflict of interests.

REFERENCES

1. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemol Glob Health 2019; 9: 217-222.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2018; 68: 394-424.
3. Arruebo M, Vilaboa N, Saez-Gutierrez B, Lambea J, Tres A, Valladeres M, Gonzalez-Fernandez A. Assessment of the evolution of cancer treatment therapies. Cancers 2011; 3: 3279-3330.
4. Chen H, Zhang W, Zhu G, Xie J, Chen X. Rethinking cancer nanotheranostics. Nat Rev Mater 2017; 2: e17024.
5. Schachtschneider KM, Schwind RM, Newson J, Kinachtchouk N, Rizko M, Mendoza-Elias N, Grippo P, Principe DR, Park A, Overgaard NH, Jungersen G, Garcia KD, Maker AV, Rund LA, Ozer H, Gaba RC, Schook LB. The oncopig cancer model: an innovative large animal translational oncology platform. Front Oncol 2017; 7: e190.
6. Nabil G, Bhise K, Sau S, Atef M, El-Banna HA. Nano-engineered delivery systems for cancer imaging and therapy: recent advances, future directions and patent evaluation. Drug Discov Today 2019; 24: 462-491.
7. Cekanova M, Rathore K. Animal models and therapeutic molecular targets of cancer: utility and limitations. Drug Des Devel Ther 2014; 8: 1911-1922.
8. Waring MJ, Arrowsmith J, Leach AR, Leeson PD, Mandrell S, Owen RM, Pairaudeau G, Pennie WD, Pickett SD, Wang J, Wallace O, Weir A. An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat Rev Drug Discov 2015; 14: 475-486.
9. Moreno L, Pearson AD. How can attrition rates be reduced in cancer drug discovery? Expert Opin Drug Discov 2013; 8: 363-368.
10. Archana M. Navale. Animal models of cancer: a review. Int J Pharm Sci Res 2013; 4: 19-28.
11. Namekawa T, Ikeda K, Inoue KH, Inoue S. Application of prostate cancer models for preclinical study: Advantages and limitations of cell lines, patient-derived xenografts, and three-dimensional culture of patient-derived cells. Cells 2019; 8: e74.
12. Costa E, Ferreira-Goncalves T, Chasqueira G, Cabrita AS, Figueiredo IV, Reis CP. Experimental models as refined translational tools for breast cancer research. Sci Pharm 2020; 88: e32.
13. Knapp DW, Waters DJ. Naturally occurring cancer in pet dogs: important models for developing improved cancer therapy for humans. Mol Med Today 1997; 3: 8-11.
14. Masters JR. HeLa cells 50 years on: the good, the bad and the ugly. Nat Rev Cancer 2002; 2: 315-319.
15. Mao S, Pang Y, Liu T, Shao Y, He J, Yang H, Mao Y, Sun W. Bioprinting of in vitro tumor models for personalized cancer treatment: a review. Biofabrication 2020; 12: e042001.
16. Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. Front Bioeng Biotechnol 2016; 4: 1-14.
17. Jensen C, Teng Y. Is it time to start transitioning from 2D to 3D cell culture? Front Mol Biosci 2020; 7: 1-17.
18. Carvalho MR, Lima D, Reis RL, Oliveira JM, Correlo VM. Anti-cancer drug validation: the contribution of tissue engineered models. Stem Cell Rev 2017; 13: 347-363.
19. Smalley KSM, Lioni M, Noma K, Haass NK, Herlyn M. In vitro three-dimensional tumor microenvironment models for anticancer drug discovery. Expert Opin Drug Discov 2008; 3: 1-10.
20. Rodrigues J, Heinrich MA, Teixeira LM, Prakash J. 3D in vitro model (R) evolution: unveiling tumor-stroma interactions. Trends Cancer 2021; 7: 249-264.
21. Lazzari G, Couvreur P, Mura S. Multicellular tumor spheroids: a relevant 3D model for in vitro preclinical investigation of polymer nanomedicines. Polym Chem 2017; 8: 4947-4969.
22. Sant S, Johnston PA. The production of 3D tumor spheroids for cancer drug discovery. Drug Discov Today Technol 2017; 23: 27-36.
23. Penfornis P, Fernandes JD, Abraham A, Gurumurthy B, Janorkar AV, Pasco D, Pochampally RR. Three-dimensional spheroid model using cancer and stromal cells for in vitro drug screening assays. J Stem Cell Res Med 2017; 2: 1-5.
24. Nunes AS, Barros AS, Costa EC, Moreira AF, Correia IJ. 3D tumor spheroids as in vitro models to mimic in vivo human solid tumors resistance to therapeutic drugs. Biotechnol Bioeng 2019; 116: 206-226.
25. Pinto B, Henriques AC, Silva PMA, Bousbaa H. Three-dimensional spheroids as in vitro preclinical models for cancer research. Pharmaceutics 2020; 12: 1186-1224.
26. Zhang M, Boughton P, Rose B, Lee CS, Hong AM. The use of porous scaffold as a tumor model. Int J Biometer 2013; e396056.
27. Alaribe FN, Manoto SL, Motaung SCKM. Scaffolds from biomaterials: advantages and limitations in bone and tissue engineering. Biologia 2016; 71: 353-366.
28. Rijal G, Li W. A versatile 3D tissue matrix scaffold system for tumor modeling and drug screening. Sci Adv 2017; 3: e1700764.
29. Dunne LW, Huang Z, Meng W, Fan X, Zhang N, Zhang Q, An Z. Human decellularized adipose tissue scaffold as a model for breast cancer cell growth and drug treatments. Biomaterials 2014; 35: 4940-4949.
30. Fan H, Demirci U, Chen P. Emerging organoid models: leaping forward in cancer research. J Hematol Oncol 2019; 12: 1-10.
31. Clevers H, Tuveson DA. Organoid models for cancer research. Annu Rev Cancer Biol 2019; 3: 223-234.
32. Nagle PW, Plukker JTM, Muijs CT, Luijk PV, Coppes RP. Patient-derived tumor organoids for prediction of cancer treatment response. Semin Cancer Biol 2018; 53: 258-264.
33. Lau HCH, Kranenburg O, Xiao H, Yu J. Organoid models of gastrointestinal cancers in basic and translational research. Nat Rev Gastroenterol Hepatol 2017; 17: 203-222.
34. Kretzschmar K. Cancer research using organoid technology. J Mol Med 2020; 1-15.
35. Bersini S, Jeon JS, Dubini G, Arrigoni C, Chung S, Charest JL, Moretti M, Kam RD. A microfluidic 3D in vitro model for specificity of breast cancer metastasis to bone. Biomaterials 2014; 35: 2454-2461.
36. Phelps AS, Hassell BA, Ingber DE. Modelling cancer in microfluidic human organs-on-chips. Cancer 2019; 19: 65-81.
37. Ruzycka M, Cimpan MR, Mondragon IR, Grudzinski IP. Microfluidics for studying metastatic patterns of lung cancer. J Nanobiotechnol 2019; 17: e71.

38. Shang M, Soon RH, Lim CT, Khoo BL, Han J. Microfluidic modelling of the tumor microenvironment for anti-cancer drug development. Lab Chip 2019; 19: 369-386.
39. Zhang H, Whalley RD, Ferreira AM, Dalgarno K. High throughput physiological micro-models for in vitro pre-clinical drug testing: a review of engineering systems approaches. Prog Biomed Eng 2020; 2: 1-25.
40. Halldorsson S, Lucumi E, Sjoberg RG, Flemig RMT. Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. Biosens Bioelectron 2015; 63: 218-231.
41. Colley HE, Hearnden V, Avila-Olias M, Cecchin D, Canton I, Madsen J, MacNeil S, Warren N, Hu K, McKeating JA, Armes SP, Murdoch C, Thornhill MH, Battaglia G. Polymersome-mediated delivery of combination anticancer therapy to head and neck cancer cells: 2D and 3D in vitro evaluation. Mol Pharm 2014; 1: 1176-1188.
42. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55-63.
43. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harb Protoc 2018; 6: 465-468.
44. Wei X, SenanayakeTH, Warren G, Vinogradov SV. Hyaluronic acid-based nanogel-drug conjugates with enhanced anticancer activity designed for the targeting of CD44-positive and drug-resistant tumors. Bioconjug Chem 2013; 24: 658-668.
45. Uzunoglu S, Karaca B, Atmaca H, Kisim A, Sezgin C, Karabulut B, Uslu R. Comparison of XTT and Alamar blue assays in the assessment of the viability of various human cancer cell lines by AT-101 (-/- gossypol). Toxicol Mech Methods 2010; 20: 482-486.
46. Economou MA, Andersson S, Vasilcanu D, All-Ericsson C, Menu E, Girnita A, Girnita L, Axelson M, Seregard S, Larsson O. Oral picropodophyllin (PPP) is well tolerated in vivo and inhibits IGF-1R expression and growth of uveal melanoma. Invest Ophthalmol Vis Sci 2008; 49: 2337-2342.
47. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 2006; 1: 1112-1116.
48. Orellana EA, Kasinski AL. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio Protoc 2016; 6: e1984.
49. Rao W, Wang H, Han J, Zhao S, Dumbleton J, Agarwal P, Zhang W, Zhao G, Yu J, Zynger DL, Lu X, He X. Chitosan-Decorated Doxorubicin-Encapsulated Nanoparticle Targets and Eliminates Tumor Reinitiating Cancer Stem-like Cells. ACS Nano 2015; 9: 5725-5740.
50. Lu H, Blunden BM, Scarano W, Lu M, Stenzel MH, Anti-metastatic effects of RAPTA-C conjugated polymeric micelles on two-dimensional (2D) breast tumor cells and three-dimensional (3D) multicellular tumor spheroids. Acta Biomater 2016; 32: 68-76.
51. Yang TT, Sinai P, Kain SR. An Acid Phosphatase Assay for Quantifying the Growth of Adherent and Nonadherent Cells. Anal Biochem 1996; 241: 103-108.
52. Du AW, Lu H, Stenzel M. Core-cross-linking accelerates antitumor activities of Paclitaxel-conjugate micelles to prostate multicellular tumor spheroids: a comparison of 2D and 3D Models. Biomacromol 2015; 16: 1470-1479.
53. Kang A, Seo HI, Chung BG, Lee SH, Concave microwell array-mediated three-dimensional tumor model for screening anticancer drug-loaded nanoparticles. Nanomed Nanotechnol Biol Med 2015; 11: 1153-1161.
54. Hong-Jun L, Jin-Zhi D, Liu J, Xiao-Jiao D, Shen S, Yan-Hua Z, Wang X, Ye X, Nie S, Wang J. Smart superstructures with ultrahigh ph-sensitivity for targeting acidic tumor microenvironment: instantaneous size switching and improved tumor penetration. ACS Nano 2016; 10: 6753-6761.
55. Hong-Jun L, Jin-Zhi D, Xiao-Jiao D, Cong-Fei X, Chun-Yang S, Hong-Xia W, Zhi-Ting C, Xian-Zhu Y, Yan-Hua Z, Nie S, Wang J. Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. Proc Natal Acad Sci 2016; 113: 4164-4169.
56. Barre-Sinoussi F, Montagutelli X. Animal models are essential to biological research: issues and perspectives. Future Sci OA 2015; 1: 1-3.
57. Onaciu A, Munteanu R, Munteanu VC, Gulei D, Raduly L, Feder RI, Pirlog R, Atanasov AG, Korban SS, Irimie A, Berindan-Neagoe I. Spontaneous and induced animal models for cancer research. Diagnostics 2020; 10: e660.
58. Vollmer G. Endometrial Cancer: Experimental models useful for studies on molecular aspects of endometrial cancer and carcinogenesis. Endocr Relat Cancer 2003; 10: 23-42.
59. Yang Z, Shen Z, Zhang N, Bai S, Wang L, Naz F, Chang M, Chen T, Yu C, Li C. Spontaneous and Chemically-induced Bladder Cancer Animal Model. Cancer 2020; 2: 9-18.
60. McCreery MQ, Balmain A. Chemical carcinogenesis models of cancer: back to the future. Annu Rev Cancer Biol 2017; 1: 295-312.
61. Kemp CJ. Animal models of chemical carcinogenesis: driving breakthroughs in cancer research for 100 years. Cold Spring Harb Protoc 2015; 10: 865-874.
62. He Z, Kosinska W, Zhao ZL, Wu XR, Guttenplan JB. Tissue-specific mutagenesis by N-butyl-N-(4-hydroxybutyl) nitrosamine as the basis for urothelial carcinogenesis. Mutat Res 2012; 742: 92-95.
63. Williams GM, Iatropoulos MJ, Jeffrey AM, Shirai T. Protective effect of acetaminophen against colon cancer initiation effects of 3,2'-dimethyl-4-aminobiphenyl in rats. Eur J Cancer Prev 2002; 11: 39-48.
64. Kersten K, Visser KE, Miltenburg MHV, Jonkers J. Genetically engineered mouse models in oncology research and cancer medicine. Mol Med 2017; 9: 137-153.
65. Walrath JC, Hawes JJ, Dyke TV, Reilly KM. Genetically engineered mouse models in cancer research. Adv Cancer Res 2010; 106: 113-164.
66. Tratar US, Horvat S, Cemazar M. Transgenic Mouse Models in Cancer Research. Front Oncol 2018; 8: 1-18.
67. Abate-Shen C, Shen MM. Mouse models of prostate carcinogenesis. Trends Genet 2002; 18: S1-S5.
68. Kido LA, Lamas CA, Marostica MR, Cagnon VHA. Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model: a good alternative to study PCa progression and chemoprevention approaches. Life Sci 2019; 217: 141-147.
69. Hursting SD, Nunez NP, Patel AC, Perkins SN, Lubet RA, Barrett JC. The utility of genetically altered mouse models for nutrition and cancer chemoprevention research. Mutat Res 2005; 576: 80-92.
70. Washington K, Zemper AED. Apc-related models of intestinal neoplasia: a brief review for pathologists. Surg Exp Pathol 2019; 2: 1-9.
71. Hall B, Limaye A, Kulkarni AB. Overview: generation of gene knockout mice. Curr Protoc Cell Biol 2009; 19: 1-47.
72. Wu X, Gong S, Roy-Burman P, Lee P, Culig Z. Current mouse and cell models in prostate cancer research. Endocr Relat Cancer 2013; 20: 155-170.

73. Jackson JG, Lozano G. The mutant p53 mouse as a pre-clinical model. Oncogene 2013; 32: 4325-4330.
74. Liu X, Holstege H, Gulden HV, Treur-Mulder M, Zevenhoven J, Velds A, Kerkhoven RM, Vliet MHV, Wessels LFA, Peterse JL, Berns A, Jonkers J. Somatic loss of BRCA1 and p53 in mice induces mammary tumors with features of human BRCA1-mutated basal-like breast cancer. Proc Natal Acad Sci 2007; 104: 12111-12116.
75. Chakrabarti R, Kang Y. Transplantable mouse tumor models of breast cancer metastasis. Methods Mol Biol 2015; 1267: 367-380.
76. Khanna C, Hunter K. Modeling metastasis in vivo. Carcinogenesis. 2005; 26: 513-523.
77. Kang Y, Omura M, Suzuki A, Oka T, Nakagami Y, Cheng C, Nagashima Y, Inoue T. Development of an orthotopic transplantation model in nude mice that simulates the clinical features of human lung cancer. Cancer Sci 2006; 97: 996-1001.
78. Nomikos VT, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clin Cancer Res 2003; 9: 4227-4239.
79. Nakamura K, Yoshikawa N, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo M. Characterization of mouse melanoma cell lines by their mortal malignancy using an experimental metastatic model. Life Sci 2002; 70: 791-798.
80. Kellar A, Egan C, Morris D. Preclinical murine models for lung cancer: clinical trial applications. Biomed Res Int 2015; 2015: 621324.
81. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc 2007; 2: 247-250.
82. Joohee Jung. Human tumor xenograft models for preclinical assessment of anticancer drug development. Toxicol Res 2014; 30: 1-5.
83. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, Arcaroli JJ, Messersmith WA, Eckhardt SG. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol 2012; 9: 338-349.
84. Zhang W, Fan W, Rachagani S, Zhou Z, Lele SM, Batra SK, Garrison JC. Comparative study of subcutaneous and orthotopic mouse models of prostate cancer: vascular perfusion, vasculature density, hypoxic burden and BB2r- targeting efficacy. Sci Rep 2019; 9: 1-10.
85. Jung J, Park SJ, Chung HK, Kang HW, Lee SW, Seo MH, Park HJ, Song SY, Jeong SY, Choi EK. Polymeric nanoparticles containing taxanes enhance chemoradiotherapeutic efficacy in non-small cell lung cancer. Int J Radiat Oncol Biol Phys 2012; 84: 77-83.
86. Hoffman RM. Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. Invest New Drugs 1999; 17: 343-359.
87. Bibby MC. Orthotopic models of cancer for preclinical drug evaluation: advantages and disadvantages. Eur J Cancer 2004; 40: 852-857.
88. Cook N, Jodrell DI, Tuveson DA. Predictive in vivo animal models and translation to clinical trials. Drug Discov Today 2012; 17: 253-260.
89. Bhimani J, Ball K, Stebbing J. Patient-derived xenograft models-the future of personalised cancer treatment. Br J Cancer 2020; 122: 601-602.
90. Xie J, Lin Y. Patient derived xenograft models for personalized medicine in colorectal cancer. Clin Exp Med 2020; 20: 167-172.
91. Lai Y, Wei X, Lin S, Qin L, Cheng L, Li P. Current status and perspectives of patient-derived xenograft models in cancer research. J Hematol Oncol Pharm 2017; 10: e106.
92. Zhuo Y, Wu Y, Guo A, Chen S, Su J. Establishment and its biological characteristics of patient-derived lung cancer xenograft models. Chin J Cancer 2010; 13: 568-574.
93. Jin K, He K, Han N, Li G, Wang H, Xu Z, Jiang H, Zhang J, Teng L. Establishment of a PDTT xenograft model of gastric carcinoma and its application in personalized therapeutic regimen selection. Hepato Gastroenterol 2011; 58: 1814-1822.
94. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, Clarke RB, Jong S, Jonkers J, Maelandsmo GM, Roman-Roman S, Seoane J, Trusolino L, Villanueva A. Patient derived xenograft models: an emerging platform for translational cancer research. Cancer Discov 2014; 4: 998-1013.
95. Ma H, Li X, Yang Z, Okuno S, Kawaguchi T, Yagi S, Bouvet M, Hoffman RM. High antimetastatic efficacy of MEN4901/T-0128, a novel camptothecin carboxymethyl dextran conjugate. J Surg Res 2011; 171: 684-690.
96. Nicol CJ, Yoon M, Ward JM, Yamashita M, Fukamachi K, Peters JM, Gonzalez FJ. PPAR' influences susceptibility to DMBA-induced mammary, ovarian and skin carcinogenesis. Carcinogenesis 2004; 25: 1747-1755.
97. Kinosita R. Researches on the carcinogenesis of the various chemical substances. Gann 1936; 30: 423-426.
98. Winograd B, Boven E, Lobbezoo M, Pinedo H. Human tumor xenografts in the nude mouse and their value as test models in anticancer drug development. In Vivo 1987; 1: 1-13.
99. Voskoglou-Nomikos T, Pater JL, Seymour L. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clin Cancer Res 2003; 9: 4227-4239.
100. Weisburger EK, Weisburger JH. Chemistry, carcinogenicity, and metabolism of 2-fluorenamine and related compounds. Adv Cancer Res 1958; 5: 331-431.
101. Wolf E, Schernthaner W, Zakhartchenko V, Prelle K, Stojkovic M, Brem G. Transgenic technology in farm animals-progress and perspectives. Exp Physiol 2000; 85: 615-625.
102. Hammer RE, Pursel VG, Rexroad CE, Wall RJ, Bolt DJ, Ebert KM, Palmiter RD, Brinster RL. Production of transgenic rabbits, sheep and pigs by microinjection. Nature 1985; 315: 680-683.
103. DeVita VT, Chu E. A history of cancer chemotherapy. Cancer Res 2008; 68: 8643-8653.
104. Boven E, Winograd B, Berger DP, Dumont MP, Braakhuis BJ, Fodstad O, Langdon S, Fiebig HH. Phase II preclinical drug screening in human tumor xenografts: a first European multicenter collaborative study. Cancer Res 1992; 52: 5940-5947.
105. McLaughlin-Drubin ME, Munger K. Viruses associated with human cancer. Biochim Biophys Acta 2008; 1782: 127-150.
106. Lairmore MD, Niewiesk S. Models of virus-induced carcinogenesis and oncolytic viruses. ILAR J 2016; 57: 1-2.
107. Mesri EA, Feitelson M, Munger K. Human viral oncogenesis: a cancer hallmarks analysis. Cell Host Microbe 2014; 15: 266-282.
108. Dittmer DP, Damania B, Sin SH. Animal models of tumorigenic herpes viruses -an update. Curr Opin Virol 2015; 14: 145-150.
109. MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. Nat Rev Drug Discov 2015; 14: 721-731.
110. Feitsma H, Cuppen E. Zebrafish as a cancer model. Mol Cancer Res 2008; 6: 685-694.

111. Shin JT, Fishman MC. From zebrafish to human: modular medical models. Annu Rev Genomics Hum Genet 2002; 3: 311-340.
112. Lawrence C. The husbandry of zebrafish (Danio rerio): a review. Aquaculture 2007; 269: 1-20.
113. Berger J, Currie P. The role of zebrafish in chemical genetics. Curr Med Chem 2007; 14: 2413-2420.
114. Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, Xie M, Gao C, Ye Y, Duan M, Zhou Z. The use of zebrafish (Danio rerio) as biomedical models. Anim Front 2019; 9: 68-77.
115. Hason M, Bartunek P. Zebrafish models of cancer-new insights on modeling human cancer in a non-mammalian vertebrate. Genes 2019; 10: e935.
116. White RM, Sessa A, Burke C, Bowman T, LeBlanc J, Ceol C, Bourque C, Dovvey M, Goessling W, Burns CE, Zon LI. Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell 2008; 2: 183-189.
117. McGrath, P, Li CQ. Zebrafish: a predictive model for assessing drug-induced toxicity. Drug Discov Today 2008; 13: 394-401.
118. Al-Thani HF, Shurbaji S, Yalcin HC. Zebrafish as a model for anticancer nanomedicine studies. Pharmaceuticals 2021; 14: e625.
119. Mione MC, Trede NS. The zebrafish as a model for cancer. Dis Model Mech 2010; 3: 517-523.
120. Goessling W, North TE, Zon LI. New waves of discovery: modeling cancer in zebrafish. J Clin Oncol 2007; 25: 2473-2479.
121. Xie X, Ross JL, Cowell JK, Teng Y. The promise of zebrafish as a chemical screening tool in cancer therapy. Futur Med Chem 2015; 7: 1395-1405.
122. Bunton TE. Experimental chemical carcinogenesis in fish. Toxicol Pathol 1996; 24: 603-618.
123. Dang M, Fogley R, Zon L. Cancer and zebrafish: mechanisms, techniques, and models: chemical genetics. Adv Exp Med Biol 2016; 916: 103-124.
124. Mizgireuv IV, Revskoy SY. Transplantable tumor lines generated in clonal zebrafish. Cancer Res 2006; 66: 3120-3125.
125. Mizgireuv IV, Majorova IG, Gorodinskaya V, Khudoley VV, Revskoy SY. Carcinogenic effect of N-Nitrosodimethylamine on diploid and triploid zebrafish (Danio rerio). Toxicol Pathol 2004; 32: 514-518.
126. Spitsbergen JM, Tsai HW, Reddy A, Miller T, Arbogast D, Hendricks JD, Bailey GS. Neoplasia in zebrafish (Danio rerio) treated with 7,12-dimethylbenz[a]anthracene by two exposure routes at different developmental stages. Toxicol Pathol 2000; 28: 705-715.
127. Mimeault M, Batra SK. Emergence of zebrafish models in oncology for validating novel anticancer drug targets and nanomaterials. Drug Discov Today 2013; 18: 128-140.
128. Dong M, Fu YF, Du TT, Jing CB, Fu CT, Chen Y, Jin Y, Deng M, Liu TX. Heritable and lineage-specific gene knockdown in zebrafish embryo. PLoS One 2009; 4: e6125.
129. Pluskota E, Dowling JJ, Gordon N, Golden JA, Szpak D, West XZ, Nestor C, Ma YQ, Bialkowska K, Byzova T, Plow EF. The integrin coactivator Kindlin-2 plays a critical role in angiogenesis in mice and zebrafish. Blood 2011; 117: 4978-4987.
130. Driever W, Solnica-Krezel L, Schier AF, Neuhauss SC, Malicki J, Stemple DL, Stainier DYR, Zwartkruis F, Abdelilah S, Rangini Z, Belak J, Boggs C. A genetic screen for mutations affecting embryogenesis in zebrafish. Development 1996; 123: 37-46.
131. Wiellette E, Grinblat Y, Austen M, Hirsinger E, Amsterdam A, Walker C, Westerfield, M, Sive H. Combined haploid and insertional mutation screen in the zebrafish. Genesis 2004; 40: 231-240.
132. Wienholds E, van Eeden F, Kosters M, Mudde J, Plasterk RH, Cuppen E. Efficient target-selected mutagenesis in zebrafish. Genome Res 2003; 13: 2700-2707.
133. Doyon Y, McCammon JM, Miller JC, Faraji F, Ngo C, Katibah GE, Amora R, Hocking TD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Amacher SL. Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nat Biotechnol 2008; 26: 702-708.
134. Huang P, Xiao A, Zhou M, Zhu Z, Lin S, Zhang B. Heritable gene targeting in zebrafish using customized TALENs. Nat Biotechnol 2011; 29: 699-700.
135. Ablain J, Durand EM, Yang S, Zhou Y, Zon LI. A CRISPR/Cas9 vector system for tissue-specific gene disruption in zebrafish. Dev Cell 2015; 32: 756-764.
136. Stoletov K, Klemke R. Catch of the day: zebrafish as a human cancer model. Oncogene 2008; 27: 4509-4520.
137. Lai K, Amsterdam A, Farrington S, Bronson RT, Hopkins N, Lees JA. Many ribosomal protein mutations are associated with growth impairment and tumor predisposition in zebrafish. Dev Dyn 2009; 238: 76-85.
138. Langenau DM, Feng H, Berghmans S, Kanki JP, Kutok JL, Look AT. Cre/lox-regulated transgenic zebrafish model with conditional myc-induced T cell acute lymphoblastic leukemia. Proc Natl Acad Sci 2005; 102: 6068-6073.
139. Langenau DM, Traver D, Ferrando AA, Kutok JL, Aster JC, Kanki JP, Lin S, Prochownik E, Trede NS, Zon LI, Look AT. Myc-induced T cell leukemia in transgenic zebrafish. Science 2003; 299: 887-890.
140. Phelps RA, Chidester S, Dehghanizadeh S, Phelps J, Sandoval IT, Rai K, Broadbent T, Sarkar S, Burt RW, Jones DA. A two-step model for colon adenoma initiation and progression caused by APC loss. Cell 2009; 137: 623-634.
141. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, Berghmans S, Mayhall EA, Traver D, Fletcher CDM, Aster JC, Granter SR, Look AT, Lee C, Fisher DE, Zon LI. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr Biol 2005; 15: 249-254.
142. Lu JW, Yang WY, Tsai SM, Lin YM, Chang PH, Chen JR, Wang H, Wu JL, Jin SLC, Yuh CH. Liver-specific expressions of HBx and src in the p53 mutant trigger hepatocarcinogenesis in zebrafish. PLoS One 2013; 8: e76951.
143. Berghmans S, Murphey RD, Wienholds E, Neuberg D, Kutok JL, Fletcher CDM, Morris JP, Liu TX, Schulte-Merker S, Kanki JP, Plasterk R, Zon LI, Look AT. tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors. Proc Natl Acad Sci 2005; 102: 407-412.
144. Brown HK, Schiavone K, Tazzyman S, Heymann D, Chico TJA. Zebrafish xenograft models of cancer and metastasis for drug discovery. Expert Opin Drug Discov 2017; 12: 379-389.
145. Kirchberger S, Sturtzel C, Pascoal S, Distel M. Quo natas, Danio? Recent Progress in Modeling Cancer in Zebrafish. Front Oncol 2017; 7: e186.
146. Xiao J, Glasgow E, Agarwa S. Zebrafish xenografts for drug discovery and personalized medicine. Trends Cancer 2020; 4: e00479.
147. Haldi M, Ton C, Seng WL, McGrath P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. Angiogenesis 2006; 9: 139-151.
148. Cheng SY, Chen NF, Lin PY, Su JH, Chen BH, Kuo HM, Sung CS, Sung PJ, Wen ZH, Chen WF. Anti-invasion and antiangiogenic effects of stellettin B through inhibition of the Akt/Girdin signaling pathway and VEGF in glioblastoma cells. Cancers 2019; 11: e220.

149. Drabsch Y, He S, Zhang L, Snaar-Jagalska BE, Dijke PT. Transforming growth factor-' signalling controls human breast cancer metastasis in a zebrafish xenograft model. Breast Cancer Res 2013; 15: e106.
150. Xu W, Foster BA, Richards M, Bondioli KR, Shah G, Green CC. Characterization of prostate cancer cell progression in zebrafish xenograft model. Int J Oncol 2017; 52: 252-260.
151. Zhang Z, Wang H, Ding Q, Xing Y, Xu Z, Lu C, Luo D, Xu L, Xia W, Zhou C, Shi M. Establishment of patient-derived tumor spheroids for non-small cell lung cancer. PLoS One 2018; 13: e0194016.
152. Ramos TB, Feijao T, Goncalves A, Shahbazi MA, Liu Z, Barrias C, Oliveira MJ, Granja P, Santos HA, Sarmento B. Colorectal cancer triple co-culture spheroid model to assess the biocompatibility and anticancer properties of polymeric nanoparticles. J Control Release 2020; 323: 398-411.
153. Jeppesen M, Hagel G, Glenthoj A, Vainer B, Ibsen P, Harling H, Thastrup O, Jorgensen LN, Thastrup J. Short-term spheroid culture of primary colorectal cancer cells as an in vitro model for personalizing cancer medicine. PLoS One 2017; 12: e0183074.
154. Yakavets I, Francois A, Benoit A, Merlin J, Bezdetnaya L, Vogin G. Advanced co culture 3D breast cancer model for investigation of fibrosis induced by external stimuli: optimization study. Sci Rep 2020; 10: 1-11.
155. Takagi A, Watanabe M, Ishii Y, Morita J, Hirokawa Y, Matsuzaki T, Shiraishi T. Three-dimensional cellular spheroid formation provides human prostate tumor cells with tissue-like features. Anticancer Res 2007; 27: 45-54.
156. Song Y, Kim J, Kim S, Park YK, Yu E, Kim KH, Seo EJ, Oh HB, Lee HC, Kim KM, Seo HR. Patient-derived multicellular tumor spheroids towards optimized treatment for patients with hepatocellular carcinoma. J Exp Clin Cancer Res 2018; 37: e109.
157. Lee C, Lee C, Atalilit A, Siu A, Ramos DM. Differential spheroid formation by oral cancer cells. Anticancer Res 2014; 34: 6945-6950.
158. Walsh AJ, Cook RS, Sanders ME, Aurisicchio L, Ciliberto G, Arteaga CL, Skala MC. Quantitative optical imaging of primary tumor organoid metabolism predicts drug response in breast cancer. Cancer Res 2014; 74: 5184-594.
159. Nuciforo S, Fofana I, Matter MS, Blumer T, Calabrese D, Boldanova T, Piscuoglio S, Wieland S, Ringnalda F, Schwank G, Terracciano LM, Ng CKY, Heim MH. Organoid models of human liver cancers derived from tumor needle biopsies. Cell Rep 2018; 24: 1363-1376.
160. Nadauld LD, Garcia S, Natsoulis G, Bell JM, Miotke L, Hopmans ES, Xu H, Pai RK, Palm C, Regan JF, Chen H, Flaherty P, Ootani A, Zhang NR, Ford JM, Kuo CJ, Ji HP. Metastatic tumor evolution and organoid modeling implicate TGFBR2 as a cancer driver in diffuse gastric cancer. Genome Biol 2014; 15: e428.
161. Drost J, Van Boxtel R, Blokzijl F, Mizutani T, Sasaki N, Sasselli V, Ligt J, Behjati S, Grolleman JE, Wezel TV, Nik-Zainal S, Kuiper RP, Cuppen E, Clevers H. Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. Science 2017; 358: 234-238.
162. Boj SF, Hwang C-I, Baker LA, Chio IIC, Engle DD, Corbo V, Jager M, Ponz-Sarvise M,Tiriac H, Spector MS,Gracanin A, Oni T, Yu KH, Boxtel RV, Huch M, Rivera KD, Wilson JP, Feigin ME, Ohlund D, Handly-Santana A, Ardito-Abraham CM, Ludwig M, Elyada E, Alagesan B, Biffi G, Yordanov GN, Delcuze B, Creighton B, Wright K, Park Y, Morsink FHM, Molenaar IQ, RinkesIHB, Cuppen E, Hao Y, Jin Y, Nijman IJ, Iacobuzio-Donahue C, Leach SD, PappinDJ, Hammell M, Klimstra DS, Basturk O, Hruban RH, Offerhaus GJ, VriesRGJ, Clevers H, Tuveson DA. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015; 160: 324-338.
163. Tsai S, McOlash L, Palen K, Johnson B, Duris C, Yang Q, Dwinell MB, Hunt B, Evans DB, Gershan J, James MA. Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. BMC Cancer 2018; 18: e335.
164. Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, Prager BC, Couce M, McLendon RE, Sloan AE, Rich JN. A three dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. Cancer Res 2016; 76: 2465-2477.
165. Rijal G, Bathula C, Li W. Application of synthetic polymeric scaffolds in breast cancer 3D tissue cultures and animal tumor models. Int J Biomater 2017; e8074890.
166. Bassi G, Panseri S, Dozio SM, Sandri M, Campodoni E, Dapporto M, Sprio S, Tampieri A, Montesi M. Scafold based 3D cellular models mimicking the heterogeneity of osteosarcoma stem cell niche. Sci Rep 2020; 10: e22294.
167. Gupta P, Mancera PP, Kocher H, Nisbet A, Schettino G, Velliou EG. A novel scaffold-based hybrid multicellular model for pancreatic ductal adenocarcinoma-toward a better mimicry of the in vivo tumor microenvironment. Front Bioeng Biotechnol 2020; 8: 1-20.
168. Xua K, Ganapathy K, Andl T, Wang Z, Copland JA, Chakrabarti R, Florczyk SJ. 3D porous chitosan-alginate scaffold stiffness promotes differential responses in prostate cancer cell lines. Biomaterials 2019; 217: e119311.
169. Kuriakose AE, Hu W, Nguyen KT, Menon JU. Scaffold-based lung tumor culture on porous PLGA microparticle substrates. PLoS One 2019; 14: e0217640.
170. Wang N, Wang J, Meng X, Bao Y, Wang S, Li T. 3D microfluidic in vitro model and bioinformatics integration to study the effects of Spatholobi Caulis tannin in cervical cancer. Sci Rep 2018; 8: e12285.
171. Xu Z, Li E, Guo Z, Yu R, Hao H, Xu Y, Sun Z, Li X, Lyu J, Wang Q. Design and construction of a multi-organ microfluidic chip mimicking the in vivo microenvironment of lung cancer metastasis. ACS Appl Mater Interfaces 2016; 8: 25840-25847.
172. Carvalho MR, Barata D, Teixeira LM, Giselbrecht S, Reis RL, Oliveira JM, Truckenmuller R, Habibovic P. Colorectal tumor-on-a-chip system: a 3D tool for precision onco-nanomedicine. Sci Adv 2019; 5: e1317.
173. Ayuso JM, Gillette A, Cintron KL, Acevedo SA, Gomez I, Morgan M. Organotypic microfluidic breast cancer model reveals starvation-induced spatial-temporal metabolic adaptations. EBioMedicine 2018; 37: 144-157.
174. Zheng L, Zhou B, Meng X, Zhu W, Zuo A, Wang X, Jiang R, Yu S. A model of spontaneous mouse mammary tumor for human estrogen receptor- and progesterone receptor-negative breast cancer. Int J Oncol 2014; 45: 2241-2249.
175. Prakash J, Gupta SK, Dinda AK. Withania somnifera root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice. Nutr Cancer 2002; 42: 91-97.
176. Mervai Z, Egedi K, Kovalszky I, Baghy K. Diethylnitrosamine induces lung adenocarcinoma in FVB/N mouse. BMC Cancer 2018; 18: e157.
177. Karimi B, Ashrafi M, Shomali T, Yektaseresht A. Therapeutic effect of simvastatin on DMBA-induced breast cancer in mice. Fund Clin Pharmacol 2019; 33: 84-93.

178. Asha S, Girija D. Benzo[a]pyrene induced liver and kidney cancer in swiss albino mice. Res Pharm 2011; 1: 22-27.
179. Sisi AE, Sokar SS, El-Sayad ME, Moussa EA, Salim EI. Anticancer effect of metformin against 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced rat mammary carcinogenesis is through AMPK pathway and modulation of oxidative stress markers. Hum Exp Toxicol 2019; 38: 703-712.
180. Sharmila G, Bhat FA, Arunkumar R, Elumalai P, Singh PR, Senthilkumar K, Arunakaran J. Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in vivo model. Clin Nutr 2013; 66: 718-726.
181. Kato M, Takahashi M, Akhand AA, Liu W, Dai Y, Shimizu S, Iwamoto T, Suzuki H, Nakashima I. Transgenic mouse model for skin malignant melanoma. Oncogene 1998; 17: 1885-1888.
182. Wang Y, Zhang Z, Lubet RA, You M. A mouse model for tumor progression of lung cancer in ras and p53 transgenic mice. Oncogene 2006; 25: 1277-1280.
183. Fantozzi A, Christofori G. Mouse models of breast cancer metastasis. Breast Cancer Res 2006; 8: e212.
184. Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. Nat Genet 1999; 22: 37-43.
185. Kemp CJ, Donehower LA, Bradley A, Balmainh A. Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. Cell 1993; 74: 813-822.
186. Koike H, Nozawa M, Velasco MA, Kura Y, Ando N, Fukushima E, Yamamoto Y, Hatanaka Y, Yoshikawa K, Nishio K, Uemura H. Conditional PTEN-deficient mice as a prostate cancer chemoprevention model. Asian Pac J Cancer Prev 2015; 16: 1827-1831.
187. Nolan K, Verzosa G, Cleaver T, Tippimanchai D, DePledge LN, Wang X, Young C, Le A, Doebele R, Li H, Malkoski SP. Development of syngeneic murine cell lines for use in immunocompetent orthotopic lung cancer models. Cancer Cell Int 2020; 20: e417.
188. Ghosh A, Sarkar S, Banerjee S, Behbod F, Tawfik O, McGregor D, Graff S, Banerjee SK. MIND model for triple-negative breast cancer in syngeneic mice for quick and sequential progression analysis of lung metastasis. PLoS One 2018; 13: e0198143.
189. Ryan IW, Pham MM, Sergent P, Tafe LJ, Berwin BL. A syngeneic mouse model of epithelial ovarian cancer port site metastases. Transl Oncol 2019; 12: 62-68.
190. Georges LMC, Wever OD, Galvan JA, Dawson H, Lugli A, Demetter P, Zlobec I. Cell line derived xenograft mouse models are a suitable in vivo model for studying tumor budding in colorectal cancer. Front Med 2019; 6: e139.
191. Zhang H, Cohen AL, Krishnakumar S, Wapnir IL, Veeriah S, Deng G, Coram MA, Piskun CM, Longacre TA, Herrler M, Frimannsson DO, Telli ML, Dirbas FM, Matin AC, Dairkee SH, Larijani B, Glinsky GV, Bind AH, Jeffry SS. Patient-derived xenografts of triple-negative breast cancer reproduce molecular features of patient tumors and respond to mTOR inhibition. Breast Cancer Res 2014; 16: e36.
192. Kita K, Fukuda K, Takahashi H, Tanimoto A, Nishiyama A, Sachiko Arai, Takeuchi S, Yamashita K, Ohtsubo K, Otani S, Yanagimura N, Suzuki C, Ikeda H, Tamura M, Matsumoto I, Yano S. Patient,Äêderived xenograft models of non,Äêsmall cell lung cancer for evaluating targeted drug sensitivity and resistance. Cancer Sci 2019; 110: 3215-3224.
193. Guerreschi P, Scalbert C, Qassemyar A, Kluza J, Ravasie L, Huglo D, Duquennoy VM, Formstecher P, Marchetti P, Mortier L. Patient-derived tumor xenograft model to guide the use of BRAF inhibitors in metastatic melanoma. Melanoma Res 2013; 23: 373-380.
194. Larmour LI, Cousins FL, Teague JA, Deane JA, Jobling TW, Gargett CE. A patient derived xenograft model of cervical cancer and cervical dysplasia. PLoS One 2018; 13: e0206539.
195. Beckwith LG, Moore JL, Tsao-Wu GS, Harshbarger JC, Cheng KC. Ethylnitrosourea Induces Neoplasia in Zebrafish (Danio rerio). Lab Investig 2000; 80: 379-385.
196. Spitsbergen JM, Tsai HW, Reddy A, Miller T, Arbogast D, Hendricks JD, Bailey GS. Neoplasia in zebrafish (Danio rerio) treated with N-methyl-N-nitro-N-nitrosoguanidine by three exposure routes at different developmental stage. Toxicol Pathol 2000; 28: 716-725.
197. Nakayama J, Lu JW, Makinoshima H, Gong Z. A novel zebrafish model of metastasis identifies the HSD11beta1 inhibitor adrenosterone as a suppressor of epithelial mesenchymal transition and metastatic dissemination. Mol Cancer Res 2020; 18: 477-487.
198. Mizgirev IV, Revskoy S. A new zebrafish model for experimental leukemia therapy. Cancer Biol Ther 2010; 9: 895-902.
199. Park SW, Davison JM, Rhee J, Hruban RH, Maitra A, Leach SD. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. Gastroenterology 2008; 134: 2080-2090.
200. Langenau, DM, Keefe MD, Storer NY, Guyon JR, Kutok JL, Le X, Goessling W, Neuberg DS, Kunkel LM, Zon LI. Effects of RAS on the genesis of embryonal rhabdomyosarcoma. Genes Dev 2007; 21: 1382-1395.
201. Shin J, Padmanabhan A, de Groh ED, Lee JS, Haidar S, Dahlberg S, Guo F, He S, Wolman MA, Granato M, Lawson ND, Wolfe SA, Kim SH, Solnica-Krezel L, Kanki JP, Ligon KL, Epstein JA, Look AT. Zebrafish neurofibromatosis type 1 genes have redundant functions in tumorigenesis and embryonic development. Dis Model Mech 2012; 5: 881-894.
202. Li XY, Huang LT, Wu JQ, He MF, Zhu SH, Zhan P, Lv TF, Song Y. Zebrafish Xenograft Model of Human Lung Cancer for Evaluating Osimertinib Resistance. Biomed Res Int 2019; 2019: 3129748.
203. Latifi A, Abubaker K, Castrechini N, Ward AC, Liongue C, Dobill, F, Kumar J, Thompson EW, Quinn MA, Findlay JK, Ahmad N. Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile. J Cell Biochem 2011; 112: 2850-2864.
204. Tulotta C, Stefanescu C, Beletkaia E, Bussmann J, Tarbashevich K, Schmidt T, Snaar-Jagalska BE. Inhibition of signaling between human CXCR4 and zebrafish ligands by the small molecule IT1t impairs the formation of triple-negative breast cancer early metastases in a zebrafish xenograft model. Dis Model Mech 2016; 9: 141-153.
205. Mercatali L, La Manna F, Groenewoud A, Casadei R, Recine F, Miserocchi G, Pieri F, Liverani C, Bongiovanni A, Spadazzi C, Vita A, Pluijm GV, Giorgini A, Biagini R, Amadori D, Ibrahim T, Snaar-Jagalska E. Development of a patient-derived xenograft (PDX) of breast cancer bone metastasis in a Zebrafish Model. Int J Mol Sci 2016; 17: e1375.
206. Wu JQ, Zhai J, Li CY, Tan AM, Wei P, Shen LZ, He MF. Patient-derived xenograft in zebrafish embryos: A new platform for translational research in gastric cancer. J Exp Clin Cancer Res 2017; 36: e160.

207. Franco GD, Usai A, Funel N, Palmeri M, Montesanti IER, Bianchini M, Gianardi D, Furbetta N, Guadagni S, Vasile E, Falcone A, Pollina LE, Raffa V, Morelli L. Use of zebrafish embryos as avatar of patients with pancreaticcancer: A new xenotransplantation model towards personalizedmedicine. World J Gastroenterol 2020; 26: 2792-2809.
208. Bentley VL, Veinotte CJ, Corkery DP, Pinder JB, LeBlanc MA, Bedard K, Weng AP, Berman JN, Dellaire G. Focused chemical genomics using zebrafish xenotransplantation as a preclinical therapeutic platform for T-cell acute lymphoblastic leukemia. Haematologica 2015; 100: 70-76.
209. Nirmala JG, Narendhirakannan RT. Vitis vinifera peel and seed gold nanoparticles exhibit chemopreventive potential, antioxidant activity and induce apoptosis through mutant p53, Bcl-2 and pan cytokeratin down-regulation in experimental animals. Biomed Pharmacother 2017; 89: 902-917.
210. Pramod SG, Jayanthi MK, Mruthunjaya K, Manjula SN. A study to evaluate the in-vivo anticancer activity of ethanolic extract of Holoptelea integrifolia leaves against Ehrlich ascites carcinoma model using Swiss albino mice. Int J Basic Clin Pharmacol 2016; 5:1739-1745.
211. Webborn PJH. The role of pharmacokinetic studies in drug discovery: where are we now, how did we get here and where are we going? Future Med Chem 2014; 6: 1233-1235.
212. Ellis SR, Bruinen AL, Heeren RMA. A critical evaluation of the current state-of-the-art in quantitative imaging mass spectrometry. Anal Bioanal Chem 2014; 406: 1275-1289.
213. Nasr AM, Elhady SS, Swidan SA, Badawi NM. Celecoxib loaded in-situ provesicular powder and its in-vitro cytotoxic effect for cancer therapy: fabrication, characterization, optimization and pharmacokinetic evaluation. Pharmaceutics 2020; 12: e1157.
214. Workman P, Aboagye EO, Chung YL, Griffi ths JR, Hart R, Leach MO, Maxwell RJ, McSheehy PMJ, Price PM, Zweit J. Minimally invasive pharmacokinetic and pharmacodynamic technologies in hypothesis-testing clinical trials of innovative therapies. J Natl Cancer Inst 2006; 98: 580-598.
215. Gupta N, Price PM, Aboagye EO. PET for in vivo pharmacokinetic and pharmacodynamic measurements. Eur J Cancer 2002; 38: 2094-2107.
216. Wang Y, Liu Y, Luehmann H, Xia X, Brown P, Jarreau C, Welch M, Xia Y. Evaluating the pharmacokinetics and in vivo cancer targeting capability of Au nanocages by positron emission tomography imaging. ACS Nano 2012; 6: 5880-5888.
217. Schambach SJ, Bag S, Schilling L, Groden C, Brockmann MA. Application of micro-CT in small animal imaging. Methods 2010; 50: 2-13.
218. Wang JW, Zheng W, Liu JB, Chen Y, Cao LH, Luo RZ, Li AH, Zhou JH. Assessment of early tumor response to cytotoxic chemotherapy with dynamic contrast-enhanced ultrasound in human breast cancer xenografts. PLoS One 2013; 8: e58274.
219. Etrych T, Janouskova O, Chytil P. Fluorescence imaging as a tool in preclinical evaluation of polymer-based nano-DDS systems intended for cancer treatment. Pharmaceutics 2019; 11: e417.
220. Denayer T, Stohr T, Roy MV. Animal models in translational medicine: validation and prediction. New Horiz Transl Med 2014; 2: 5-11.
221. Mak SWY, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 2014; 6: 114-118.
222. Grabowski HG, DiMasi JA, Long G. The roles of patents and research and development incentives in biopharmaceutical innovation. Health Aff 2015; 34: 302-310.
223. Pana CL, Chen FC. Patent trend and competitive analysis of cancer immunotherapy in the United States. Hum Vaccin Immunother 2017; 13: 2583-2593.