Cancer Drug Discovery and Development

Beverly A. Teicher
Editor

Tumor Models in Cancer Research

Second Edition

Humana Press

Cancer Drug Discovery and Development

Series editor

Beverly A. Teicher
Genzyme Corporation, Framington, MA, USA

For other titles published in this series, go to
www.springer.com/series/7625

Beverly A. Teicher
Editor

Tumor Models in Cancer Research

Second Edition

Humana Press

Editor
Beverly A. Teicher
Genzyme Corporation
Boston, MA
USA
beverlyteicher@aol.com

ISBN 978-1-60761-967-3 e-ISBN 978-1-60761-968-0
DOI 10.1007/978-1-60761-968-0
Springer New York Dordrecht Heidelberg London

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Preface

Progress in a given field is often dependent upon the development of appropriate, accurate models. In modern times, cancer research has been engaged in a focused search for such models for more than 50 years. The foremost problem in developing such models is that cancer is many, many diseases arising from nearly every tissue and metastasizing to many. A major breakthrough for model in cancer research was the development of transplantable rodent tumors. Many of the early tumor lines were carcinogen-induced, but other arose naturally in elderly animals from inbred strains of mice. These syngeneic tumors grown in the inbred host of origin allowed reproducible tumor growth and reproducible response to anticancer agents to be achieved. These tumor lines also frequently allowed the analysis of tumor metastasis in the host.

The mutual needs for as large an array as possible of tumor types and expansion of true inbred strains of mice to carry these tumors lead to the identification of mutant mice with characteristics of deficient immunity suitable for the growth of human tumors as xenografts. The most frequently used of these mutant mouse strains are nude mice and SCID mice. Human tumor xenograft models were established from the many human tumor cell lines developed in the 1970s and 1980s and from fresh tumor explants. Since techniques for genetic manipulation have become more routine, animals expressing "oncogenes" or missing "tumor suppressor" genes have been developed, allowing a new level of understanding of the process of malignancy and new models for testing anticancer agent efficacy. Through the use of these techniques for some diseases and targets, it has been possible to establish specific animal models.

Therapeutic index continues to be a critical variable for anticancer agents directed toward any cellular target related to proliferation. Animal models developed to determine potential normal tissue toxicities of new agents as well as the potential of normal tissue protectors have focused on proliferating normal tissues such as mucosa, gut, skin, and bone marrow although cardiac, renal, and lung toxicity can also be modeled. Still, it is the determination of meaningful experimental endpoints that defines the usefulness of models to a field. Increase-in-lifespan (survival) was an endpoint used by Dr. Howard Skipper and colleagues in their groundbreaking murine leukemia studies. Many current models, especially solid tumor models, are not amenable to a survival endpoint; therefore, other measures of tumor

response, usually involving tumor volume measurements are applied. Endpoints such as tumor growth delay and tumor growth inhibition closely mimic clinical endpoints, such as response time and time to recurrence. Other endpoints, such as ratio of treated group to control group, log kill, percent apoptosis, and tumor cell survival, depend upon the availability of an untreated or vehicle-treated control group in the experiment.

The past 6 years since the first edition of this book have seen great progress in the development of genetically engineered mouse (GEM) models of cancer. These models are finding an important role in furthering our understanding of the biology of malignant disease. A comfortable position for GEM models in the routine conduct of screening for potential new therapeutics is slowly but surely coming. Increasing numbers of genetically engineered mice are available, some with conditional activation of oncogenes, some with multiple genetic changes providing mouse models that are moving closer to the human disease.

While we wait for the perfection of the GEMs, the transplantable tumor remains the main resource for drug discovery and efficacy modeling. Though often maligned as models of human disease, antitumor activity in syngeneic mouse tumors and human tumor xenografts is a requirement for most therapeutics prior to entry into development. The criticism directed at these models is frequently a result of the differences between mice and humans. Drug pharmacokinetics in the mouse can be markedly different from pharmacokinetics for the same molecule in other species. The mouse is a remarkably resilient host often able to tolerate much higher doses of experimental therapeutics than human patients, thus allowing blood levels to be reached in mice that cannot be attained in humans frequently leading to disappointing clinical findings. These limitations of the host cannot readily be solved but are limitations which are recognized and are increasingly taken into account in decision making in selecting development candidates.

An ideal tumor model would imitate in scale and mirror in response to the human disease. Though no such ideal models exist for the diseases that are cancer, the models described herein represent the efforts of many investigators for many years and approach with closer and closer precision examples that can serve as guides for the selection of agents and combinations for the treatment of human malignancy.

Beverly A. Teicher

Contents

Contributors

Enrique Alvarez
Biomodels, 313 Pleasant street, Watertown, MA 02472, USA

K.K. Ang
The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Nabeel Bardeesy
Massachusetts General Hospital Cancer Center, Boston, MA, USA

Powel H. Brown
University of Texas at Austin, Austin, TX, USA

Angelika M. Burger
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Michele Carbone
New York University Medical Center, New York, NY, USA

William E. Carson, III
The Ohio State University, Columbus, OH 43210, USA

Thomas H. Corbett
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Shannon Decker
University of Maryland Marlene & Stewart Greenebaum Cancer Center, Baltimore, MD, USA

Mark W. Dewhirst
Duke University Medical Center, Durham, NC, USA

Suzanne A. Eccles
McElwain Laboratories, CRC Center for Cancer, Institute of Cancer, Surrey, UK

Ene T. Fairchild
The Ohio State University, Columbus, OH, USA

Heinz-Herbert Fiebig
Oncotest GmbH Institute for Experimental Oncology 12. D-79108, Freiburg, Germany

Dai Fukumura
Department of Radiation Oncology, Massachusetts General Hospital Cancer Center, Boston, MA, USA

Stuart Hazeldine
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Aram F. Hezel
Massachusetts General Hospital Cancer Center, Boston, MA, USA

Daniel Hicklin
Schering-Plough Research Institute, Kenilworth, NJ, USA

Gilda G. Hillman
Department of Radiation Oncology, School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI 48201, USA

Robert M. Hoffman
AntiCancer, Inc., and Department of Surgery, University of California, San Diego, CA, USA

Jerome P. Horwitz
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Peter J. Houghton
Center for Childhood Cancer, Nationwide Children's Hospital, Columbus, OH 43205, USA

Stephen D. Hursting
University of Texas at Austin, Austin, TX, USA

Rakesh K. Jain
Department of Radiation Oncology, Massachusetts General Hospital Cancer Center, Boston, MA, USA

Vinod Kaimal
Charles River Laboratories, Ann Arbor, MI, USA

Juiwanna Kushner
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Laura M. Lashinger
University of Texas at Austin, Austin, TX, USA

Alfred J. Lawson
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI 48108, USA

Wilbur R. Leopold III
Charles River Laboratories, Ann Arbor, MI, USA

Ming Liu
Schering-Plough Research Institute, Kenilworth, NJ 07033, USA

Gregory Lyng
Biomodels, Watertown, MA, USA

Katherine A. Mason
The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Patrick McConville
Charles River Laboratories, Ann Arbor, MI, USA

Raymond E. Meyn
The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Luka Milas
The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

Richard Moore
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Sharon D. Morgenbesser
Genzyme Corporation, Framington, MA, USA

Christopher L. Morton
St. Jude Children's research Hospital, Memphis, TN, USA

Lance L. Munn
Department of Radiation Oncology, Massachusetts General Hospital Cancer Center, Boston, MA, USA

Ralph E. Parchment
Laboratory of Human Toxicology and Pharmacology, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD 21702, USA

Harvey I. Pass
New York University Medical Center, New York, NY, USA

Susan N. Perkins
University of Texas at Austin, Austin, TX, USA

Joseph B. Pincus
New York University Medical Center, New York, NY, USA

Magdalena Plasilova
New York University Medical Center, New York, NY, USA

Lisa Polin
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI 48201, USA

Kimberly Pouliot
Biomodels, Watertown, MA, USA

James Rake
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Bill J. Roberts
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Sara Rockwell
Yale Cancer Center, New Haven, CT, USA

Edward Sausville
University of Maryland Marlene & Stewart Greenebaum Cancer Center, Baltimore, MD, USA

Thies Schroeder
Duke University Medical Center, Durham, NC, USA

Siqing Shan
Duke University Medical Center, Durham, NC, USA

William Siders
Genzyme Corporation, Framington, MA, USA

Stephen T. Sonis
Harvard-Farber Cancer Center, Boston, MA, USA; Biomodels, Watertown, MA, USA

L.C. Stephens
The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Beverly A. Teicher
Genzyme Corporation, Framington, MA, USA

Douglas H. Thamm
The Animal Cancer Center, Colorado State University, Fort Collins, CO, USA

Henry J. Thompson
Cancer Prevention Laboratory, Colorado State University, Fort Collins, CO, USA

David M. Vail
University of Wisconsin, School of Veterinary Medicine, Madison, WI, USA

William R. Waud
Southern Research Institute, Birmingham, AL 35205, USA

Kathryn White
School of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Part I
Introduction

Chapter 1
Perspectives on the History and Evolution of Tumor Models

Shannon Decker and Edward Sausville

Abstract Modern cancer therapeutic research is at crossroads in evolving our approaches to discovering, developing, and entering novel therapeutics into early-stage clinical trials. This chapter endeavors to summarize the customary use and interpretation of animal models used for prioritization of cancer treatments for entry into clinical trials through the end of the last century. We then consider the novel screening paradigms currently in use which exemplify the diverse types of challenging lead compounds for in vivo evaluation. Finally, we offer a strategic overview of steps to maximize utility of the animal model information in selecting agents for clinical study in the twenty-first century.

Keywords Targeted in vivo models • Cancer drug development

1.1 Introduction and Statement of the Problem

Modern cancer therapeutic research is at crossroads in evolving our approaches to discovering, developing, and entering novel therapeutics into early-stage clinical trials. The sequencing of the human genome [1] and the increasing awareness of the detailed sequence of numerous cancer cell genomes raises the possibility that the empiricism so characteristic of past cancer drug development will give rise to an approach more analogous to current AIDS or cardiovascular disease-related paradigms, where a precise knowledge of the structure of a putative target guides all aspects of a drug's conceptualization, development, and clinical testing. Yet we have not arrived there yet, as it is currently not feasible in most diseases to employ clinically applicable testing to predict the value of novel agents, outside of fairly specific

E. Sausville (✉)
University of Maryland Marlene & Stewart Greenebaum Cancer Center,
22 S. Greene St, Baltimore, MD 21201, USA
e-mail: esausville@umm.edu

B.A. Teicher (ed.), *Tumor Models in Cancer Research*, Cancer Drug Discovery and Development, DOI 10.1007/978-1-60761-968-0_1,

examples relevant to antibody-based therapeutics. Indeed, as an example, while the recently observed lack of value of anti-epidermal growth factor antibody therapy in patients with colon cancer with mutated K-ras alleles is understandable post hoc, an appreciation of that reality was apparent only in retrospective analysis [2]. Had it been possible to model reliably that circumstance, it is conceivable that a more efficient and focused development strategy could have been designed. Thus, the challenges facing the use and interpretation of animal models for human cancer drug development center on the predictability of the models in forecasting effects on tumor cells as well as in predicting tolerability of the agent by the host. The model use should occur within a product lifecycle that usually offers no more than 2 or 3 years in most industrial development paradigms after a lead has been identified, and ideally has a direct relevance to how the agent will be studied in early clinical trials.

A related issue that will always inject an element of empiricism into the use and interpretation of animal models used in prioritizing human therapeutics for clinical study is the intrinsic unpredictability of animal (usually rodent) vs. human pharmacology and metabolism. While algorithms exist to predict susceptibility to, for example, cytochrome p450 metabolism features [3] or bioavailability [4], since molecules for cancer treatment, at least the classical cytotoxics, are usually employed at close to their maximum tolerated dose (MTD), even minor differences from the human in rodent compound handling parameters (absorption, plasma protein binding, clearance mechanisms, intrinsic susceptibility of host tissues) can translate into decreased relevance of murine dosing and efficacy information as predicting clinical value. Table 1.1 lists points of model departure from rodent vs. human behavior. In contrast, it is interesting to consider that certain classes of agents, particularly monoclonal antibodies with intrinsic anti-signaling of tumor cell tropism properties have for the most part rather reliably defined useful effects that were eventually borne out in humans [5] perhaps in part because of the bland interaction of human antibodies with both mouse and ultimately human physiology,

Table 1.1 Potential points of divergence between rodent and human drug features

Property	Example
Plasma protein binding	Camptothecins [61]; result in stabilization of lactone in mice and therefore increased perception of activity
	7-OH staurosporine [62]; much more avid protein binding in humans prolong half-life and diminish potential for activity
Half-life	MS-275 [63]; human clearance much slower than mouse; correlates with mice tolerating more frequent dosing schemes while humans do not
Intrinsic drug target susceptibility	Neriifolin and cardiac glycosides [64]; murine Na/K ATPase intrinsically less susceptible to agents therefore mouse model over-predict capacity for anti-tumor activity
	H-ras farnesylation intrinsically more sensitive to certain farnesyltransferase inhibitors and therefore not appropriate model for human Ki-ras associated tumors [65]
Differing end-organ susceptibility	Bizelisin [66] murine marrow cells intrinsically less susceptible to antiproliferative effects than humans therefore under-predict human toxicity

and the capacity of certain antibodies such as trastuzumab to down-regulate the action of a target to whose action the relevant cell type is "addicted."

This chapter endeavors to summarize the customary use and interpretation of animal models used for prioritization of cancer treatments for entry into clinical trials through the end of the last century. We then consider the novel screening paradigms currently in use which exemplify the diverse types of challenging lead compounds for in vivo evaluation. Finally we will offer a strategic overview of steps to maximize utility of the animal model information in selecting agents for clinical study in the twenty-first century.

1.2 Tumor Models for Cancer Drug Development: Where We Were

1.2.1 Historical Basis

Drug treatments for cancer arose from three distinct philosophical points of view. Classically, Ehrlich's concept of "magic bullets" [6] that would cause toxicity to tumor cells while sparing normal cells arose from the observation that different dyestuffs had obvious affinity for different parts of the cell or different cell types. By this logic, therefore, screening for chemicals that might have a differential effect on tumor cells in comparison to normal cells might be a basis for deriving useful therapeutics related to cancer. A second potential direction was suggested by the observation of profound leucopenia as part of the symptom complex imparted by exposure to mustard gas during World War I. This suggested to some that in lower doses such chemicals might be useful in controlling tumors of (in that case) the hematopoietic system while not ablating all normal marrow elements [7]. Finally the observation that hormonal manipulation could cause useful regression of tumors derived from endocrine responsive organs [8] suggested that an understanding of the biological bases of tumor growth could impart strategies for treatment. This latter point of view, when coupled to the then emerging knowledge of the biochemistry of nucleic acids and the increase particularly in RNA content of tumor cells, led naturally to the efforts to develop what we now call anti-metabolites such as folate antagonists by Farber et al. [9] and purine and pyrimidine analogs by Elion, Hitchings, Heidelberger and a large number of colleagues [10]. Ironically, although such agents are now "lumped" into the category of "cytotoxics," anti-metabolites were the rationally "targeted" therapeutics of the middle of the last century.

The plethora of new chemicals potentially available for cancer treatment, along with relative indifference for cancer as a focus of opportunity by corporate pharmaceutical entities of the time created the perceived need to develop common platforms for evaluation of new molecules available for cancer treatment. This resulted in the evolution of tumor models that were geared for high throughput and mostly employed serially propagated tumor cells in syngeneic hosts. As recounted

elsewhere by Zubrod et al. [11], such screening efforts in academia exemplified by Memorial Sloan Kettering were helpful but to keep up with demand for compound evaluation, Congress in 1955 directed the U.S. National Cancer Institute (NCI) to develop a publicly funded and publicly accessible resource that would promote both clinical testing and pre-clinical evaluation of novel anti-cancer agents. The former initiative was the precursor of the current national Cooperative Group approach to clinical trials. The latter initiative resulted in the formation of the Cancer Chemotherapy National Service Center (CC-NSC), whose "NSC" accession catalog of compounds continues to the present day at NCI's Developmental Therapeutics Program as the successor to the CC-NSC. Compounds studied by NCI for the most part were synthesized by contractors or solicited from academic or commercial parties by an active compound acquisition program [12]. Encouragement to industry as well as academic participants was provided by confidentiality agreements that assured protection of the submitting party's intellectual property. Results generated by the NCI screening effort could then be the basis for development of the compound to clinical trials sponsored either by the NCI through its Cooperative Groups or privately funded ventures.

1.2.2 Early Screening Models

The models employed in efforts at NCI and at academic screening centers included and were exemplified by the L1210 and P388 mouse leukemias serially transplanted by the peritoneal route and treated by intraperitoneal injection of drug. The endpoint of the screening assay was survival of the treated vs. untreated or vehicle-treated groups of animals. A compound was considered to show preliminary evidence of activity if the mean or median lifespan of the treated animals was increased by 125%, with the control group survival set at 100%, and with the important caveat that "positive" compounds had to have acceptable therapeutic index with evidence of maintained or increasing body mass in treated animals and no untoward short-term toxic phenomena. Among the advantages of this model as a screening tool were its relative speed, with experiment evaluation generally complete by 2–3 weeks; capacity for high throughput allowing many compounds to be evaluated; and reproducibility of the model owing to high take rate and uniform growth rate. Using these and related models, important clinically relevant principles of cancer chemotherapeutic development were elucidated and formed the basis for construction of human chemotherapy regimens and practices. These principles include the demonstration that active agents produced with each dose increment reduction in the tumor cell population a reduction in tumor cell mass by logarithmically increasing increments. This led to the concept that valuable agents had to be applied in successive "cycles" to cause tumor-free animals to emerge. The inverse relationship of tumor cell inoculum to curability at a constant dose led likewise to the theoretical underpinnings of "adjuvant" treatment programs [13, 14].

Potential pitfalls of such models were numerous. Most obviously was the "same site" nature of the treated space, without a physiological barrier between the administration site and the locus of drug action. A second concern was the potential lack of relevance of such models to solid tumors. Both of these concerns were partially addressed by the use of syngeneic metastasizing murine models such as Lewis lung carcinoma or B16 melanoma. These models could be run either as solid allografts, with treatment intravenously or orally in a way that mimicked human treatment, or following a period of residence in a body part, generally an extremity, removal of the "primary" tumor could allow for observation of compound activity against the establishment or formation of metastases. Although such models were valuable adjuncts to evaluating positive compounds in the murine leukemia studies, an emerging concern throughout the later 1970s was that the paucity of agents emerging through such murine leukemia-based screens that ultimately had robust activity in human solid tumors.

The limited value of agents detected in murine leukemia screening models when applied to human solid tumors resulted in enormous interest in the use of immunocompromised animals to study xenografts of human tumors through technology that was first applied on a large scale commencing ~1980 using athymic "nude" mice [15]. One initial hope was that agents thereby revealed to be active would be intrinsically more suitable for use in human solid tumors. An immediate problem in the use of these models, however, is their intrinsically less efficient throughput owing to a variety of factors including the mechanics of implanting and sizing tumors in a subcutaneous site; the fact that different human tumor cell lines had intrinsically different "take rates" and variable growth rates. This encouraged the development of prioritization criteria often after in vitro screening to assure that compounds entering into in vivo study already had evidence of cytotoxic potential. The "NCI 60" cell line panel is representative of one such large-scale effort of this type whose historical basis and output has been described elsewhere [16, 17]. Moreover, criteria for value of an agent in athymic mouse xenografts are problematic in that tumor growth delay is more frequently encountered than actual responses of established tumors, and the meaning of this to the clinical setting remains undefined in a precise way to this day.

Looking at the performance of predominantly classical cytotoxic agents studied at the NCI in a variety of murine syngeneic and prototypic human xenograft systems, one can conclude that agents irrespective of their level of in vitro activity which have activity in less than 33% of the models tested had no "positive" phase 2 clinical trials. In contrasts, agents with activity in at least 33% of such models had an approximately 50% likelihood of positivity in phase 2 clinical trials [18]. Noteworthily, there was little histology-specific correlation of activity in models with activity in the clinic. As described above, the reason for this disconnect between animal and human experiences when ultimately understood has in the examples cited in Table 1.1 largely related to differences in animal and human pharmacological features or target susceptibility or importance to the host organism. This and related experiences [19] has reinforced that from a purely stochastic viewpoint there is value in prioritizing compounds for entry into the clinic by their behavior in some number of animal models.

The most profoundly dissatisfying aspect of this set of outcomes is that there is no tie on the part of the in vivo models used to evaluate the majority of screening experiences to the biology of the tumors studied. While one might argue that this is reasonable in light of the fact that most of the chemical entities selected from random screening experiences were not really designed around any key mechanism as relevant to the biology of a particular tumor, the present age has for the most part moved past the point where high enthusiasm for a compound arises solely by virtue of its behavior in a screening system. Rather novel approaches to cancer drug screening are generating lead compounds that will require distinctive approaches to further elicitation of activity in vivo, and will ideally be coupled to novel strategies to apply in early clinical trials.

1.3 Novel Screens Beget Novel In Vivo Model Challenges

Traditionally as discussed above, lead compounds were selected for study in vivo based on evidence in vitro or expectation of cytotoxicity. The molecular target era has allowed the creation of a flood of new screening models. Importantly, some screens are aimed at identifying targets or pathways of interest as an initial step in then defining the effect of a compound on the target(s) or pathway(s) of interest, but not necessarily tied to initial evidence of cytotoxicity. Whether action of a lead against the target in one of these in vitro or non-traditional assay systems is enough to justify proceeding with the lead to in vivo models discussed throughout this volume is a key strategic issue to consider. These assay systems run the gamut from non-mammalian in vivo models in an array of organisms, to information-intensive screens capitalizing on the explosion of new "data mining" technologies, to cell-based in vitro assays looking for non-classical endpoints such as angiogenesis or invasion.

1.3.1 Non-mammalian Models

In the last 10 years, efforts utilizing non-mammalian models to actually identify targets and drugs have proliferated. In some cases, such as for yeast and *Drosophila*, the organisms have been used for many decades as biological models, but have not traditionally served as a source of anti-cancer leads. Other organisms such as zebrafish have arisen relatively recently as models.

1.3.1.1 Unicellular

Yeast screens have been widely used in cell biology and genetics studies. It was the first organism to have its genome sequenced [20]. Yeast strains are easily grown

and manipulated, allowing for facile studies of DNA damage repair, cell cycle progression and checkpoint control, among other well recognized utilities. Yeast screens have provided a facile way to identify sets of genes that contribute to sensitivity or resistance to particular drugs. For instance, for the synthetic tripeptide arsenical GSAO that inhibits angiogenesis and targets actively dividing but not quiescent endothelial cells, Hogg et al. identified 88 GSAO-sensitive *Saccharomyces cerevisiae* deletion strains by screening a genome-wide set of 4,546 such strains, thus identifying potential molecular targets of GSAO and allowing for confirmatory studies in mammalian cells [21].

Classical pathways well explored in yeast actually from a biological point of view have defined strains with alterations in cell cycle and cell cycle checkpoint control, particularly in response to DNA damage [22]. This observation has been capitalized on by numerous groups to screen for compounds that interfere with cell cycle control, thus potentially enhancing sensitivity to classical DNA-damaging chemotherapy or radiation therapy. The National Cancer Institute (NCI) Yeast Anticancer Drug Screen has screened tens of thousands of compounds in selected yeast strains mutated for cell cycle control or DNA damage repair [23]. One limitation of such screens though is the possibility that larger organisms do not rely on a single mechanism for repairing DNA damage. For example, mammals in some cases appear to have checkpoint-independent mechanisms for surviving radiation [24], so a compound identified in yeast as interfering with a checkpoint may be ineffective as a radiation sensitizer in humans. Thus, yeast serve to illustrate the caveat that a mammalian relevant in vivo model may need to be carefully constructed to provide evidence that the yeast-related screen output is an accurate reflection of a human circumstance.

1.3.1.2 Multicellular

In an attempt to overcome some of the shortcomings of unicellular organism screens as predictors of in vivo activity, various groups have developed in vivo models in non-mammalian organisms ranging from zebrafish to nematodes to flies. The potential advantages of such screens generally are that they are cheaper and proceed more quickly than mammalian in vivo models, but still have the capacity to provide information about the ability of a drug lead to act in a live host.

Drosophila strains have been used for over a century for genetic studies, and have a relatively small genome, making it an attractive model for studying various biological processes. A number of different cancer-related screening campaigns have now been run in *Drosophila* models, including transgenic models. For example, extending from the observation discussed above that whole organisms have checkpoint-independent mechanisms for surviving DNA damage from chemotherapy and radiation, Tin Su et al. ran a pilot screen for radiation sensitizers using wild-type and checkpoint mutants [24]. Drug candidates were mixed into food and placed in wells with *Drosophila* larvae, and survival was determined by counting the empty pupae cases. In another screening context, by looking at phenotypic

changes from *Drosophila* developing leg imaginal discs, Phanstiel et al. screened for drug–polyamine conjugates with polyamine transporter (PAT)-selective targeting ability, deriving from the observation that PAT is elevated in many tumor types and hypothesizing that drug–polyamine conjugates may be able to selectively attack tumor cells [25]. While the limited genetic redundancy of *Drosophila* lends itself to phenotypic endpoints and is part of the basis of its attractiveness as a model, it is also potentially a limitation of the model as the hits identified in such models may fail in more complex mammalian systems where redundancy is more frequent.

The *Caenorhabditis elegans* nematode has been used as a model system for several decades. The worm goes from egg to fertile adult in 3 days, and each adult can produce 300 progeny making it a quick and inexpensive model system. Numerous knockout mutants exist and strains can be frozen for decades [26]. In one recent cancer application, Salgia et al. described a *C. elegans* nematode model in which transgenic worms were generated harboring either wild-type c-Met or mutations of c-Met commonly seen in lung cancer [27]. The worms expressing the mutant c-Mets consistently displayed the phenotypic outputs of abnormal vulval development and low fecundity. While this model can be used to investigate the role of gene mutations in a whole organism, invertebrates may not be appropriate models for certain cancer-related processes such as apoptosis due to their lesser complexity [28].

Avian embryo models have also been used in developmental biology for many years, but only more recently in cancer research with any frequency, most likely due in part to the recent sequencing of the chick genome. Advantages include the speed of the model in reproducing human tumor growth and angiogenesis. Researchers have validated that human glioblastoma grafted onto the chorioallantoic membrane (vascularized extra embryonic tissue; CAM) displays similar patterns of gene expression changes as the human disease [29]. Although they still have efficiency advantages over mouse models, CAM models also have disadvantages over other vertebrate non-mammalian systems such as a relatively lengthy assay (~10 days), higher cost than other models and difficulties in quantitation of the output [30].

Proponents of *Xenopus* tadpole models point to rapid extra uterine development, the transparency of developing tadpoles, permeability of the skin, and similarities to mammals in certain organ development, anatomy and physiology as advantages [31]. To identify molecules affecting angiogenesis and lymphangiogenesis, Brandli et al. screened 1,280 compounds in a *Xenopus* model looking first for edema as a phenotype and then used whole-mount in situ hybridization of *Xenopus* embryos to visualize blood and lymphatic vessel development for the 66 positive hits from the initial stage of the screen, with confirmatory endothelial cell proliferation and tube formation assays then conducted on the second level hits. The original *Xenopus* model, *Xenopus laevis* has a pseudotetraploid genome and a relatively long generation time, making the development of stable transgenic lines lengthy relative to other non-mammalian models, however work has also been done to use the diploid *Xenopus tropicalis* as a model for experimental genetics [32].

Zebrafish have been cited as having numerous advantages for screening, many of them shared with the advantages of *Xenopus* models above. The assays, while in vivo, are still relatively quick at approximately 3 days, relatively cheap, and have reasonably high throughput as they can be done on plates [9]. As demonstrated by Willett et al. using known angiogenesis inhibitors TNP470 and SU5416 [30], zebrafish, being transparent, lend themselves very well to angiogenesis-related assays as blood vessel formation can be assessed by visual inspection. Zebrafish have been used for models of drug sensitization and resistance. Transgenic models have been generated as well. Much less is known, however, about cancer-relevant issues such as DNA repair enzymes and the orthologs of human oncogenes and tumor suppressor genes in zebrafish than other model systems [33].

1.3.2 Technology-Intensive Screening

Advances in fields such as computing technology, imaging, robotics, and miniaturization among others have helped spawn a range of new screening possibilities. All of these technology-intensive methodologies produce a wealth of information much more quickly than many classical screening techniques, but the challenge is in sifting through and capitalizing on the information. In many cases in vivo models applicable to the output of such screens will need to be constructed as a dedicated effort in parallel with the design and output of the ex vivo screen.

1.3.2.1 High-Throughput Screening

High-throughput screening (HTS) methods became increasingly necessary as the number of potential molecular targets for cancer drugs grew virtually exponentially. In one possible format for an HTS assay, the activity of an enzyme is linked to an easily readable output, such as fluorescence or bioluminescence from luciferase. Cell-based HTS is also possible, many times with cell lines that have been transfected with a receptor or promoter of interest. Methodologies for HTS campaigns have been discussed extensively [34, 35] and the literature abounds with results from campaigns directed against particular enzymatic targets. In the fortunate circumstance where the role of the enzyme in a biological pathway relevant to human disease is well understood, where structural biology can show the development candidate interacting with the binding site of the enzyme, where the candidate has favorable drug-like characteristics, and where the action of the drug on the target can be tracked in cell culture and in vivo models, the path for development can be relatively straightforward. In the case where the output of a screening campaign using a cell-based assay where pathway activation or inhibition is the ultimate readout, caution must be urged in exploring activity in in vivo models prior to deconvolution of the lead compounds mechanism of action.

1.3.2.2 Chemogenomics

Unlike a single HTS assay that has the ability to screen many compounds against a single target, chemogenomics represents the integration of a study of the effects of compounds on biological targets with modern genomics technologies, attempting to comprehensively discover and describe all possible drugs to all possible drug targets [36]. For instance, for a chemical genetics application, the function of proteins is probed by small molecules by adding a library of small molecules to cells, selecting those that produce the phenotype of interest and identifying the protein bound by the molecules [37]. Many of the newer applications of yeast screens fall into the chemogenomics category, helping to identify genes that can help explain the activity of known compounds [2].

1.3.2.3 Proteome and Kinome Screens

With the success of genome-wide screens, efforts next logically extended down to the proteome and a particular target class such as protein kinases (thus a "kinome" directed virtual screen) in the search for drug targets. In one such effort, Schreiber et al. combined a chemical genetics screen that identified small molecule modifiers of rapamycin activity with a probe of a yeast proteome chip to identify proteins that bound the small molecules [38]. One potential advantage of probing of the proteome over traditional affinity chromatography is the bias of chromatography toward high-abundance proteins. Some approaches have elected to limit the probe to the kinases rather than the whole proteome. Dagorn et al. screened the human kinome for all kinases involved in pancreatic cancer cell survival and gemcitabine resistance, identifying a set of potential targets for drug discovery campaigns [39]. Comprehensive screening of the whole yeast proteome has been undertaken to systematically identify protein–protein interactions, in an effort that might eventually assist in the development of small molecules that can disrupt key interactions [40]. Analysis of such protein–protein interaction data sets however requires significant bioinformatics resources, and the complexity will only increase when multicellular organism proteomes are screened.

1.3.2.4 Nanotechnology

Considerable effort has been expended in recent years on integrating nanotechnology with more traditional biologically based methodologies. In one series of approaches nanoparticles such as quantum dots or magnetofluorescent particles are conjugated to peptides, antibodies, or small molecules to allow the targeting of the nanoparticle to specific cells, such as tumor cells. Some groups have had success in using such bioconjugates for imaging [41] and have demonstrated differential cellular uptake [42]. Others are using nanoparticles to produce formulations of compounds, ones with excellent in vitro activity but no systemic bioavailability, in an effort to make

such compounds clinically viable [43]. As a therapeutic approach however, these bioconjugates remain unproven clinically and numerous scientific, cost, and regulatory hurdles exist.

1.3.2.5 RNA Interference

Since its discovery approximately a decade ago, RNA interference (RNAi) has found application in many aspects of cancer drug discovery including target identification and validation, identification of drug resistance and sensitization mechanisms, and synthetic lethal screening. Genome-wide RNAi screens have been used successfully in *C. elegans* and *Drosophila* to understand biological processes and work toward a comprehensive characterization of gene function [44]. For example, Woo et al. identified "driver genes" in hepatocellular carcinoma, each of which can now be considered for screening to define hepatocellular carcinoma-related drugs [45]. Iorns et al. suggest the utility of conducting chemical genetics and RNAi screens in parallel to simultaneously identify small molecule inhibitors and targets, giving as an example their use of an RNAi screen to identify the PDK1 pathway as a determinant of sensitivity to tamoxifen coupled with a screen to locate chemical inhibitors of the pathway [46].

Synthetic lethal screening is another potential application of RNAi. Two genes are "synthetic lethal" when cell death results from mutation of both genes even though the cell remains viable with mutation of either alone. One recent demonstration of the relevance of siRNA-related synthetic lethal screens arose from the observation that cells deficient in BRCA-1 were highly sensitive to concomitant PARP inhibition [47], based on the inability to repair DNA lesions utilizing homologous recombination.

1.3.3 In Vitro Models

Cell-based in vitro models with cytotoxic endpoints that had the goal of identifying compounds for subsequent in vivo testing were used for several decades as primary screens (e.g., the NCI60 described above). More recently, cell-based models are being employed to either further filter hits from the high throughput and mammalian models discussed above or to look for other endpoints such as angiogenesis.

As discussed above, yeast screens have been employed to identify compounds that act against yeast strains with specific genetic mutations that are believed to be relevant to cancer. The number of hits obtained from such assays though still requires further filtering before an in vivo mammalian model can be contemplated. In vitro cell-based models, particularly those where activity in a knockout cell line can be compared to the wild-type, can act as a further filter. For instance, Lamb et al. used a three-stage screen to first identify compounds inhibiting the growth

of double-strand break repair-deficient yeast cells, producing 28 hits, which were winnowed by looking for toxicity proportional to levels of topoisomerase I or II expression [48]. They then screened the remaining eight hits in two lines of chicken pre-B-cell line DT40, one wild-type and the other defective in double-strand break repair.

Other in vitro assays assess look at endpoints other than cytotoxicity, such as endothelial cell migration or cord formation, looking for compounds that affect processes such as angiogenesis or metastasis. By themselves such assays are not necessarily sufficient to warrant pursuit of in vivo models with identified compounds. In combination with other results, however, endothelial cell assays can be a source of lead compounds. For instance, Sekhar et al. combined observations from a chemistry-driven drug discovery screen for inhibitors of endothelial cell tubule formation with biochemical pathway screening and shRNA suppression to identify compounds to pursue as drug leads, and also validate ENOX1 as a target for enhancing radiation response of tumors [49]. Other endothelial cell strategies have looked to capitalize on differences between tumor and normal endothelial cells. Camussi et al. identified cyclic peptides that showed specific binding only to tumor but not normal endothelial cells to use as a mechanism for delivering antiangiogenic agents only to the tumor [50].

The integrin inhibitors can serve as an example, however, of how action on a molecular target coupled with endothelial cell assays for angiogenesis endpoints may not be enough to guarantee a drug candidate worthy of development. Screening for inhibitors of integrins, adhesion molecules considered important in angiogenesis, has been conducted in conjunction with numerous other angiogenesis assays [51]. In this case, while data existed to support the search for integrin inhibitors, certain of the integrins are promiscuous and the biology considerably more complicated than suggested in primary screening assays, such that development of an integrin inhibitor has been thus far unsuccessful [52].

1.4 Tumor Models for Cancer Drug Development: Where We Need to Be

The above examples emerging from modern biology-driven potential cancer relevant screens illustrate the wide diversity of premises that need to be embodied in the in vivo models that might ultimately be used to further evaluate the value of such lead molecules in vivo. Given the fact that many of these leads may not be intrinsically cytotoxic but directed to particular targets, either directly in a molecular sense or as part of a pathway a readout which formed the basis of the screening efforts, how would the clinical development process be informed and fortified by knowledge gleaned from in vivo models exploring the activity of these agents? Following is a suggested series of steps that might be considered in the practice of in vivo models using such leads with the goal of pre-clinical evaluation of such a "targeted" compound. As illustrated in Table 1.2, it differs from the path that

Table 1.2 Distinction between cytotoxic and "targeted" in vivo model usage

Classical	Targeted
Maximum targeted dose driven	Biologic dose bracketing an optimized concentration
Pharmacology frequently deferrable	Early PK and PD crucial and build into correlates of clinical value
Number of models active key to prioritization	Limited number of models, but target enriched
Need to define host cell susceptibility	Need to define effect on host target in relation to toxicity observed

might have been applied to the traditional "cytotoxic" drug candidate of the last century in that the latter agents were generally developed according to an MTD model where pharmacological information could reasonably be obtained after initial confirmation of in vivo activity on a particular schedule. In contrast, most efficient and useful development steps for targeted agents would have a more early integration of pharmacological information, both kinetic and dynamic, into the early development strategy, and may actually not embark on evaluation of uncharacterized models with respect to target expression or pathway activation status. With this reasoning, the following steps might be usefully be allied to the process of in vivo model use with screening leads in the age of biologically tailored cancer drug screens.

1.4.1 "In Vitro" Area Under the Concentration × Time Curve for Target Effect

In a range of cell culture models expressing the target, definition of the time until target modulation as a function of compound addition and removal, and the relationship of this to secondary endpoints such as cytotoxicity is critical, and helps to define initial dosing strategies. Ideally controls with respect to secondary endpoints would include cell lines not expressing the relevant target or pathway. This would also provide valuable information about "off target" effects.

1.4.2 Qualification of Compound for In Vivo Study

A series of related molecules active in vitro can be further qualified for in vivo study by application of algorithms suitable for selection of oral bioavailability [53], if continuous exposure is the intended strategy. Alternatively, "cassette" type dosing schemes [54] allow preliminary assessment of pharmacological properties of a series, thereby narrowing choices of molecules for in vivo evaluation.

1.4.3 Initial Rodent Pharmacology and Model Selection

Using a realistic dosing scheme, attempts to recreate at least the area under the concentration × time curve (AUC) defined in vitro by the results of studies described above using non-tumored animals should then occur. Transition to the use of tumored animals would initially use a tumor model with cells known to be dependent on the function of the target for growth, viability, or some easily assessed biologic readout. These may express the target endogenously or heterologously; in the latter event appropriate vector alone controls are necessary. In the event the target is expressed endogenously, consideration of a cell type related to the first where the target is absent or not functional would be an additional useful control.

1.4.4 Sample Size and Randomization of Animals

Several considerations go into selecting the number of animals chosen for control and experimental groups, and consultation of a biostatistical expert in designing the experiments is useful. In part the sample size relates to the magnitude of the effect desired and the nature of the endpoint [55]. In the event that tumor is to be assessable at the initiation of the experiment, randomization of animals with different tumor sizes so that treatment groups are matched with respect to initial tumor size may be necessary.

1.4.5 Correlative Studies

Ideally evaluation of efficacy in "hitting" the putative target should accompany in vivo evaluation of the compound, as well as in a most ideal case assessment of the pharmacologic properties of the agent achieving that effect (dose–response of effect on target in association with usual parameters such as plasma maximal concentration (C_{max}), half life ($t_{1/2}$), AUC, etc.). Determination of tumor drug levels corresponding to these phenomena would be a plus. Examples of successful integration of such information obtained in early in vivo studies with value when applied to the clinic would include bortezomib anti-tumor effect correlated with effect on proteosome inhibition [56] or more recently effect of dasatinib on bcr-abl kinase substrate phosphorylation in relation to plasma concentrations in mice [57], a set of observations which assisted initial clinical development.

1.4.6 Additional Desirable Studies

While one intensively evaluated model (with respect to pharmacodynamics and pharmacology) may be useful in setting the "boundary conditions" and expectations for benchmarking initial compound use and performance in humans, particularly

if the tumor system studied is "artificial" with respect to the anticipated state of the target or pathway of interest in the clinic (e.g., heterologously expressed or otherwise manipulated cells), enthusiasm for the compound is increased generally if a range of non-manipulated cell types are exposed to the agent at the appropriate concentration dose and range with confirmation that in that circumstance there are expected effects on target function and consequences for cellular physiology. It may not be necessary to develop stable in vivo models from each cell type; such techniques as the "hollow fiber assay" [58] can be a way of usefully assessing in vivo effect without the time and expense of deriving independent models [59].

1.5 Conclusion

The ultimate goal of in vivo model studies in the pre-clinical development of anti-cancer agents is to serve a variety of interests. First, from a strictly pragmatic standpoint, demonstration of unbiased, well understood in vivo activity serves to increase confidence in investing the considerably more time-consuming and expensive effort in developing the safety database to allow human early phase clinical testing. Valuable activity in an in vivo model should reflect pharmacological "action at a distance" across physiological and anatomical barriers in a way that has an acceptable therapeutic index on the clinical proposed dose range and schedule. Second, the in vivo model experience from a scientific standpoint becomes that which the early clinical trials would ideally seek to emulate precisely as a "mirror image" accurate reflection. Third, from an ethical standpoint, clear demonstration of in vivo activity on the part of a candidate anti-cancer agent is a basis for potentially justifying in a prospective patient's mind their participation in such a study. Although recent studies have documented that modern phase I anti-cancer drug clinical trials are extremely safe and for many of the newer molecular entities have the prospect of benefit in perhaps as much as 30% of participants [60], the initial in vivo experiences in animals can serve as a talking point in assuring potential participants that there is the possibility of benefit at doses and schedules that have a modicum of expected safety and tolerability.

The ideal for in vivo model use in therapeutics development is the assembly of a package of information that will guide in the design and ultimate interpretation of the initial human clinical trial. Conversely, it is also conceivable that once an initial appreciation of achieved human pharmacology emerges from the results of the initial early phase clinical trials in humans, a focused return to in vivo animal models with the intention of conscientiously modeling the achieved human pharmacology in the animals may allow a more realistic strategy to emerge before committing to an extensive human phase 2 program. In this way in vivo animal models can contribute not only to the initial qualification of a compound for human use but also to a more refined way of advancing it to having its best chance for positive later-stage clinical trial efforts.

References

1. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. Science. 2001;291:1304–51.
2. Liévre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66:3992–5.
3. Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, et al. Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. Pharmacol Ther. 1997;73:147–71.
4. Bachmann KA, Ghosh R. The use of in vitro methods to predict in vivo pharmacokinetics and drug interactions. Curr Drug Metab. 2001;2:299–314.
5. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, et al. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol. 1998;16:2659–71.
6. Himmelweit F. ed. The collected papers of Paul Ehrlich, Vol. I. London: Pergamon; 1956. Originally published in 1900.
7. DeVita VT Jr, Carbone PP. Treatment of Hodgkin's disease. Med Ann Dist Columbia. 1967;36:232.
8. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. Lancet. 1896;2:104–7.
9. Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. N Engl J Med. 1948;238:787–93.
10. Hitchings GH, Elion GB, Falco EA, Russell PB, Vanderwerff H. Studies on analogs of purines and pyrimidines. Ann N Y Acad Sci. 1950;52:1318–35
11. Zubrod CG, Schepartz SA, Carter SK. Historical background of the National Cancer Institute's drug development thrust. Natl Cancer Inst Monogr. 1977;45:7–11.
12. Thambi P, Sausville EA. Working with the National Cancer Institute. In: Teicher BA, Andrews PA, editors. Anticancer drug development guide: preclinical screening, clinical trials, and approval. 2nd ed. 2004. Humana press, Totowa, NJ.
13. Skipper HE, Schabel FM Jr, Wilcox WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep. 1964;35:1–111.
14. Skipper HE, Schabel FM Jr, Wilcox WS. Experimental evaluation of potential anticancer agents. XIV. Further study of certain basic concepts underlying chemotherapy of leukemia. Cancer Chemother Rep. 1965;45:5–28.
15. Plowman J, Dykes DJ, Hollingshead MG, Simpson-Herren L, Alley MC. Human tumor xenograft models in NCI drug development. In Teicher BA, editor. Anticancer drug development guide: preclinical screening, clinical trials, and approval; 1997. Totowa, NJ: Humana. p. 101–25.
16. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988;48:589–601.
17. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst.1991;83:757–66
18. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer. 2001;84:424–1431
19. 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.
20. Williams N. Genome projects: yeast genome sequence ferments new research. Science. 1996;272:481.

21. Dilda PJ, Don AS, Tanabe KM, Higgins VJ, Allen JD, Dawes IW, et al. Mechanism of selectivity of an angiogenesis inhibitor from screening a genome-wide set of *Saccharomyces cerevisiae* deletion strains**.** J Natl Cancer Inst. 2005;97:1539–47.
22. Weinert TA, Hartwell LH. The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science. 1988;241:317–22.
23. http://dtp.cancer.gov/yacds/default.html accessed 10/7/10.
24. Jaklevic B, Uyetake L, Lemstra W. Chang J, Leary W, Edwards A, et al.Contribution of Growth and Cell Cycle Checkpoints to Radiation Survival in Drosophila. Genetics 2006;174:1963–72.
25. Tsen C, Iltis M, Kaur N, Bayer C, Delcros J-G, Laurence von Kalm L, et al. A *Drosophila* model to identify polyamine–Drug conjugates that target the polyamine Transporter in an Intact Epithelium. J Med Chem. 2008;51:324–30.
26. Youds JL, Barber LJ, Boulton SJ. *C. elegans*: a model of Fanconi anemia and ICL repair. Mutat Res. 2009;668:103–16.
27. Siddiqui SS, Loganathan S, Krishnaw S, Faoro L, Jagadeeswaran R, Salgia R. *C. elegans* as a model organism for in vivo screening in cancer: effects of human c-Met in lung cancer affect C. elegans vulva phenotypes. Cancer Biol Ther. 2008;7:856–63.
28. Krumschnabel G, Podrabsky JE. Fish as model systems for the study of vertebrate apoptosis. Apoptosis. 2009;14:1–21.
29. Hagedorn M, Jave4rzat S, Gilges D, de Lafarge B, Eichmann A, Bikfalvi A. Accessing key steps of human tumor progression in vivo by using an avian embryo model. Proc Natl Acad Sci USA, 2005;102:1643–8.
30. Serbedzija GN, Flynn E, Willett CE. Zebrafish angiogenesis: a new model for drug screening. Angiogenesis. 1999;3(4):353–9.
31. Kalin RE, Banziger-Tobler NE, Detmar M, Brandli AW. An in vivo chemical library screen in Xenopus tadpoles reveals novel pathways involved in angiogenesis and lymphangiogenesis. Blood. 2009;114:1110–22.
32. Beck CW, Slack JMW. An amphibian with ambition: a new role for *Xenopus* in the 21st century. Genome Biol. 2001;2:reviews1029.1–reviews1029.5.
33. Spitsbergen JM, Kent ML. The state of the art of the zebrafish model for toxicology and toxicologic pathology research – advantages and current limitations. Toxicol Pathol. 2003; 31(Suppl):62–87.
34. Inglese J. Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries. Proc Natl Acad Sci USA, 2006;103:11473–8.
35. Shoemaker RH, Scudiero DA, Melillo G, Currens MJ, Monks AP, Rabow AA, et al. Application of high-throughput, molecular-targeted screening to anticancer drug discovery. 2002;2:229–46.
36. Caron PR, Mullican MD, Mashal RD, Wilson KP, Su MS, Murcko MA. Chemogenomic approaches to drug discovery. Curr Opin Chem Biol. 2001;5:464–70.
37. Stockwell BR. Chemical genetics: ligand-based discovery of gene function. Nat Rev Genet. 2000;1:116–25.
38. Huang J, Zhu H, Haggarty SJ, Spring DR, Hwang H, Jin F, Snyder M, Schreiber SL. Finding new components of the target of rapamycin (TOR) signaling network through chemical genetics and proteome chips. Proc Natl Acad sci USA. 2004;101:16594–9.
39. Giroux V, Iovanna J, Dagorn JC. Probing the human kinome for kinases involved in pancreatic cancer cell survival and gemcitabine resistance. FASEB J. 2006;20:1982–91.
40. Schwikowski B, Uetz P, Fields S. A network of protein–protein interactions in yeast. Nat Biotechnol. 2000;18:1257–61.
41. Gao X, Nie S. Molecular profiling of single cells and tissue specimens with quantum dots. Trends Biotech. 2003;21:371–3.
42. Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L. Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. Nat Biotechnol. 2005;23:1418–23.
43. Mukerjee A, Vishwanatha JK. Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. Anticancer Res. 2009;29:3867–75.

44. Kuttenkeuler D, Boutros M. Genome-wide RNAi as a route to gene function in *Drosophila*. Brief Funct Genomics Proteomics. 2004;3:168–76.
45. Woo HG, Park ES, Lee JS, Lee YH, Ishikawa T, Kim YJ, Thorgeirsson SS. Identification of potential driver genes in human liver carcinoma by genomewide screening. Cancer Res. 2009;69:4059–66.
46. Iorns E, Lord CJ, Ashworth A. Parallel RNAi and compound screens identify the PDK1 pathway as a target for tamoxifen sensitization. Biochem J. 2009;417:361–70.
47. Farmer H, McCabe, N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434:917–21.
48. Dunstan HM, Ludlow C, Goehle S, Cronk M, Szankasi P, Evans DR, et al. Cell-based assays for identification of novel double-strand break-inducing agents. J Natl Cancer Inst. 2002;94:88–94.
49. Geng L, Rachakonda G, Morré DJ, Morré DM, Crooks PA, Sonar VN, et al. Indolylquinuclidinols inhibit ENOX activity and endothelial cell morphogenesis while enhancing radiation-mediated control of tumor vasculature. FASEB J. 2009;23:2986–95.
50. Bussolati B, Grange C, Tei L, Deregibus MC, Ercolani M, Aime S, et al. Targeting of human renal tumor-derived endothelial cells with peptides obtained by phage display. J Mol Mef. 2007;85:8970906.
51. Belvisi L, Riccioni T, Marcellini M, Vesci L, Chiarucci I, Efrati D, et al. Biological and molecular properties of a new alpha(v)beta3/alpha(v)beta5 integrin antagonist. 2005;4:1670–80.
52. Hodivala-Dilke K. alphavbeta3 integrin and angiogenesis: a moody integrin in a changing environment. Curr Opin Cell Biol. 2008;20:514–9.
53. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. 1997; 23:3–25.
54. Raynaud FI, Fischer PM, Nutley BP, Goddard PM, Lane DP, Workman P. Cassette dosing pharmacokinetics of a library of 2,6,9-trisubstituted purine cyclin-dependent kinase 2 inhibitors prepared by parallel synthesis. Mol Cancer Ther. 2004;3:353–62.
55. Hollingshead MG. Antitumor efficacy testing in rodents. J Natl Cancer Inst. 2008;100:1500–10.
56. Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res. 1999;59:2615–22.
57. Luo FR, Yang Z, Camuso A, Smykla R, McGlinchey K, Fager K, et al. Dasatinib (BMS-354825) pharmacokinetics and pharmacodynamic biomarkers in animal models predict optimal clinical exposure. Clin Cancer Res. 2006;12:7180–6.
58. Decker S, Hollingshead M, Bonomi CA, Carter JP, Sausville EA. The hollow fibre model in cancer drug screening: the NCI experience. Eur J Cancer. 2004;40:821–6.
59. Zabludoff SD, Deng C, Grondine MR, Sheehy AM, Ashwell S, Caleb BL, et al. AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. Mol Cancer Ther. 2008;7:2955–66.
60. Horstmann E, McCabe MS, Grochow L, Yamamoto S, Rubinstein L, Budd T, et al.. Risks and benefits of phase 1 oncology trials, 1991 through 2002. N Engl J Med. 2005;352:895–904.
61. Supko JG, Malspeis L. Pharmacokinetics of the 9-amino and 10,11-methylenedioxy derivatives of camptothecin in mice. Cancer Res. 1993;53:3062–9.
62. Sparreboom A, Chen H, Acharya MR, Senderowicz AM, Messmann RA, Kuwabara T, et al. Effects of alpha1-acid glycoprotein on the clinical pharmacokinetics of 7-hydroxystaurosporine. Clin Cancer Res. 2004;10:6840–6.
63. Acharya MR, Sparreboom A, Sausville EA, Conley BA, Doroshow JH, Venitz J, et al. Interspecies differences in plasma protein binding of MS-275, a novel histone deacetylase inhibitor. Cancer Chemother Pharmacol. 2006;57:275–81.
64. Lin Y, Dubinsky WP, Ho DH, Felix E, Newman RA. Determinants of human and mouse melanoma cell sensitivities to oleandrin. J Exp Ther Oncol. 2008;7:195–205.
65. Stayrook KR, McKinzie JH, Barbhaiya LH, Crowell PL. Effects of the antitumor agent perillyl alcohol on H-Ras vs. K-Ras farnesylation and signal transduction in pancreatic cells. Anticancer Res. 1998;18:823–8.
66. Volpe DA, Tomaszewski JE, Parchment RE, Garg A, Flora KP, Murphy MJ, et al. Myelotoxic effects of the bifunctional alkylating agent bizelesin on human, canine and murine myeloid progenitor cells. Cancer Chemother Pharmacol. 1996;39(1–2):143–9.