Preclinical Screening for New Anticancer Agents

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Abstract Preclinical screening procedures for anticancer agents have evolved from empirical to target-oriented screens and have contributed to the approval of a number of molecularly targeted drugs over the past decade. This chapter reviews historical in vitro and in vivo screens, the currently used cell-based as well as cell-free high-throughput screens. Tailored, secondary predictive screening procedures employing primary patient tumors and clonogenic or nude mouse xenograft assays are also described. Examples of approved drugs that have been developed based on a particular screening approach and future perspectives for finding novel and more potent drugs are discussed.

Keywords Tumor models • NCJ 60 cell line • Marine models • Cell based screen • HTS • Xenografts • Patient derived xenografts • Hollow fiber assay • Clonogenic assay

1 Introduction

Cancer chemotherapy is a relatively young discipline of oncology. It has only been pursued with scientific vigor and multinational collaborations since the mid-twentieth century. To date, over 100 monographs of drugs used for the treatment of more than 200 different tumor types exist [1, 2]. Over the past decade, cancer has become a large therapeutic market, third only after central nervous system and cardiovascular drugs, and it is continuously growing. The number of blockbuster anticancer drugs with sales of $1 billion or more increased from 19 in 2007 to 24 in 2008. Nonetheless, the cure rate of 4% for cancers that require systemic treatment remains very low [2].

Thus, the need for novel drugs is still pressing. Public institutions, the pharmaceutical industry, small business, and biotech companies create hundreds of thousands of compounds with potential anticancer activity. Only a certain number of drugs and concepts, however, can be evaluated clinically because of cost and ethical considerations. A preselection, called the screening process, is therefore required. The aim of screening efforts is to identify products that will produce antitumor effects...
matching the activity criteria used to define which compounds can progress to the next stage in the preclinical development program. Anticancer drug screening can be performed using various types of in vitro and in vivo tumor models. The ideal screening system, however, should combine speed, simplicity, and low costs with optimal predictability of pharmacodynamic activity.

2 History of Anticancer Drug Screens

Initial screening and drug development programs were small in scale and directed toward the evaluation of antitumor activity of small numbers and specific types of potential drugs [3]. Stimulated by the approaches of Ehrlich and Warburg, studies were conducted on the effects of dyes or respiratory poisons on tumor growth [4, 5]. In the 1930s, several researchers engaged in systematic studies of certain classes of compounds such as Boyland in the United Kingdom, who tested aldehydes in spontaneous tumors in mice, and Lettre in Germany, who studied colchicine derivatives and other mitotic poisons in tissue culture and ascites tumors [6]. In the United States, Shear, first at Harvard and then at the National Cancer Institute (NCI), inaugurated a screening program for testing and isolation of bacterial polysaccharides employing mice bearing sarcoma 37 as test systems for necrosis and hemorrhage. The program was quickly extended to plant extracts and synthetic compounds. In the early 1950s, the program had evaluated more than 300 chemicals and several hundreds of plant extracts. Two of these materials were tested clinically [7].

Larger-scale screens emerged around 1955, stimulated by the discovery that chemical agents, such as nitrogen mustard and folic acid antagonists, were capable of producing remissions of malignant lymphomas [8, 9]. As a result, the program of Shear at the NCI was extended to incorporate the evaluation of synthetic agents and natural products for antitumor activity. Further institutions that engaged in screening programs were Sloan–Kettering in New York, the Chester Beatty Research Institute in London, and the Southern Research Institute in Alabama [3]. In addition, screening, evaluation, and development programs were established at chemical and pharmaceutical companies, research institutions, medical schools, and universities in various countries in the world. As a result of these efforts, several agents were found with clinical activity, particularly against leukemias and lymphomas. Currently they still provide the mainstay of available drugs for systemic treatment of cancer and encompass alkylating agents (cyclophosphamide, bis(chloroethyl)nitrosourea [BCNU], 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea [CCNU], antimetabolites (methotrexate, 5-fluorouracil [5-FU], 6-mercaptopurine), antitumor antibiotics (mitomycin C, adriamycin), and mitotic spindle poisons (Vinca alkaloids, taxanes) [3].

3 The NCI Screen

The NCI Developmental Therapeutics Program (DTP) anticancer drug screen has undergone several changes since its inception in 1955 [10]. It has become the foremost public screening effort worldwide in the area of cancer drug discovery, not the least because the experimental screening models were always adapted to novel emerging knowledge and technologies. The early philosophy from which the NCI endeavor proceeded was that the elucidation of empirically defined antitumor activity in a model would translate into activity in human cancers. The choice of specific screening models was guided by sensitivity to already identified clinically active agents and in the early period was exclusively focused on in vivo testing procedures [11]. Initially, three transplantable murine tumors were employed, namely, the sarcoma 180, the carcinoma 755, and the leukemia L1210. The latter was
found to be the most predictive rodent model among the available panel and was retained in 1975, when the NCI screening process was changed in that the P388 murine leukemia model was utilized as a prescreen and followed by a panel of tumors now also including human xenografts (breast MX-1, lung LX-1, colon CX-1) [12]. The human xenografts were utilized with the intent to achieve a better prediction for clinical response against solid human malignancies as compared to hematological malignancies.

For the same reason, starting in 1985, the human tumor cell line panel comprised of 60 different cell types, including mainly solid malignancies, was introduced and replaced the P388 in vivo leukemia prescreen in the 1990s (Fig. 1; see also http://dtp.nci.nih.gov/screening.html). This project has been designed to screen up to 20,000 compounds per year for potential anticancer activity. Selection criteria for preclinical drug candidates are cytotoxic potency and differential activity against particular tumor types and/or a few specific cell lines [13]. The screen is unique in that the complexity of a 60-cell-line dose response produced by a given compound results in a biological response pattern that can be utilized in pattern recognition algorithms [14]. Using these algorithms, it is possible to assign a putative mechanism of action to a test compound or to determine that the response pattern is unique and not similar to that of any of the standard prototype compounds included in the NCI database. Such agents are then tested against the sensitive cell line grown as subcutaneous xenografts in nude mice in vivo [15]. Because of the vast number of molecules emerging from the in vitro screen for nude mouse testing, in 1995 the preclinical development cascade was amended to include the hollow fiber (HF) assay [16]. The HF assay is a short-term in vivo assay combined with in vitro culture methods. It has been proven as a rapid and efficient means of selecting compounds with the potential for in vivo activity in conventional xenografts [10, 16].

In parallel with the implementation of the HF “in vivo filter system,” a prescreen preceding the 60-cell-line screen was established in early 1995 as it became obvious that many agents were completely inactive under the conditions of the assay. Initially, the prescreen comprised three cell lines (MCF-7 breast, H460 lung, and SF268 brain cancer lines) tested against a range of drug concentrations. Currently, the prescreen assesses a new drug at one concentration of $10^{-5}$ M in all 60 cell lines. Only compounds which satisfy predetermined threshold inhibition criteria will progress to the five-dose screen. The threshold inhibition criteria for progression to the 5-dose 60-cell-line screen were designed to efficiently capture compounds with antiproliferative activity and are based on careful analysis of historical DTP screening data (http://dtp.nci.nih.gov/announcements/chg_to_screen.html). The actual NCI preclinical anticancer drug screening process is summarized in Fig. 2. Although the NCI drug development scheme is still empirical as it is based on selection of in vitro and in vivo antiproliferative activity, a number of new agents that are now in clinical use have been identified based on their unique patterns of and/or activity in the in vitro screen such as bortezomib (Velcade®, NSC 681239), romidepsin (depsipeptide, NSC 630176), a histone deacetylase (HDAC) inhibitory agent, and tanespimycin (17-AAG, NSC 330507) [17–19].

Recent insights into the molecular basis of human cancer and high-throughput profiling of the genome and proteome of the NCI 60-cell-line panel initiated a transition to rational molecular targeted discovery and development of anticancer agents in vitro and also in vivo [18, 19]. New programs such as the NCI Chemical Biological Consortium (CBC) have therefore been implemented. The CBC will select targets, actively screen for agents that affect these targets, and optimize the “drug-like” properties of hits, rather than focus on developing new agents submitted by outside investigators. The CBC drug discovery process is divided into four distinct stages including Exploratory Screen Development (ESD), Screening/Designed Synthesis (SDS), Lead Development, and Candidate Seeking with the goal to test the latter in phase 0/I trials. The CBC will mobilize a cancer drug discovery group on the scale of a small biotechnology concern, with an R&D pipeline linked to the academic community (http://dtp.nci.nih.gov/docs/CBC/cbc_index.html).
Fig. 1 Example of NCI 60-cell-line screening data. Shown is the sensitivity profile of bortezomib (681239) in 9 different tumor histologies on the basis of the 50% growth inhibition (GI50). Bars to the left indicate more resistant and bars to the right, more sensitive cell lines.
**DTP Anti-Cancer Screening Paradigm**

Abbreviations: NFT = No further testing; BEC = Biological Evaluation Committee; MTD = Maximum tolerated dose; HFA = Hollow fiber assay

*Fig. 2* Developmental Therapeutics Program (DTP) anticancer drug screening and decision-making process as of May 2008
4 Strength and Pitfalls of Cell-Based Screens vs. Cell-Free HTS on Isolated Targets

Large-scale screening using animal systems as practiced in the past (the P388 model; see above) is highly unethical and, particularly in Europe, strictly regulated. In the majority of cases, either cellular or target-based high-throughput assays will precede in vivo evaluation of potential anticancer drugs. High-throughput screening (HTS) plays an essential role in contemporary drug discovery processes. Miniaturization, robot-aided automatization, and data management by novel information technologies have provided the means of testing large compound libraries comprising several hundreds of thousands of molecules either from collections or combinatorial chemistry approaches [2]. Estimates of HTS screening capacity range from 100,000 to 1 million compounds per week. Whereas cell-based assay formats can be performed in 96- to 384-well plates, high-density formats such as 1,536-well plates with an assay volume of only 10 μL are suitable only for a cell-free isolated target-based screening setup [20].

4.1 Cell-Based Screening Assays

4.1.1 Conventional Cellular Screens

Cellular screens in cancer research employ mainly permanent human tumor cell lines; their immortal nature and hence manageable, reproducible growth behavior make them suitable test systems. Of critical importance, however, is the detection method, the choice of which depends on the cell number used and thus the desired sensitivity. Various procedures to determine cell growth are employed in screening laboratories. The earliest broadly used growth inhibition assays were developed by Mosmann and the NCI screening staff, namely, the methylthiazoldiphenyl tetrazolium (MTT) assay. The yellow MTT dye is reduced by mitochondria into a purple formazan, which can be read with ultraviolet/visible light scanners [20, 21, 49]. Its limitations are the use of large quantities of a hazardous solvent, dimethyl sulfoxide, which is required to dissolve the resulting formazan crystals and the varying number of mitochondria in cells. Currently employed in the NCI 60-cell-line screen is the sulforhodamine B (SRB) assay; SRB is a dye that stains protein [22].

Most industrial-scale cellular screens prefer the use of fluorescence or luminescence detection systems. The latter include, for example, the propidium iodide (PI) assay staining for DNA content [23] or use of a luciferase reporter [23–25]. They appear to offer the most advantages, such as high sensitivity and easy handling. The use of one-dimensional or monolayer cultures to measure cell growth is the most convenient and frequently applied method. Owing to tumor heterogeneity and three-dimensional in vivo growth, however, currently employed monolayer assays of human tumor (epithelial) cells are oversimplistic and have some disadvantages for the in vitro evaluation of certain anticancer agents:

1. Short-term culture conditions (2–6 days) may select for cytotoxic drugs.
2. Tumor cell growth can continue despite of the fact that clonogenic cells are reduced, missing certain classes of cytostatic agents (e.g., stem cell-targeted agents, differentiating agents).
3. Extracellular matrix and blood vessel targets (angiogenesis) are absent.
4. Gradients of oxygen tension, extracellular pH, nutrients, catabolites, and cell proliferation rate are a function of distance in solid tumors from blood vessels and are also not possible to mimic by monolayers.
5. Drug penetration barriers occur only in multilayered solid tumors.
Drugs that are affected by this list include signal transduction inhibitors, drugs targeting protein/protein interactions, antibodies, bioreductive drugs, antiangiogenic compounds, cancer stem cell-targeting agents, or telomerase inhibitors. These classes of drugs therefore might best be examined in either specially designed cell systems and tailored screens or biochemical assays.

### 4.1.2 Tailored Cellular Screens

Cancer stem cell-targeted drugs and inhibitors of pathways that regulate stem cell growth, such as Hedgehog and Notch inhibitors, are an emerging class of novel anticancer agents [26]. Examples of successful in vitro models that can be used to assess drug effects on cancer stem cells have been reported by Chang et al. [27] and Nakanishi et al. [28]. Cancer stem cells are a rare fraction of cells within a tumor which retain self-renewal properties. They also have self-protection mechanisms owing to the expression of high levels of drug efflux pumps [26]. Self-protection properties allow cancer stem cells to survive cytotoxic chemotherapy and their self-renewal capacity leads to the repopulation of tumors and, thus, recurrence [26]. Tumor recurrence is usually associated with development of resistance to the agents to which the patient initially responded. Conventional cellular screens are not suitable to evaluate stem cell-targeted treatments because they are aimed to measure tumor cell inhibition or kill the bulk cell mass.

Chang and coworkers have established an in vitro screen for the identification of drugs that can be used against treatment-resistant breast cancers. Their concept is based on growing mammospheres (tumorspheres) that can self-renew and grow in an anchorage-independent manner from tumor tissue biopsies. The mammospheres are then analyzed for breast cancer stem cell markers such as CD44<sup>Hi</sup>/CD24<sup>−/Low</sup>/Lin<sup>−</sup> or ALDH 1 (aldehyde dehydrogenase 1) and treated with drugs. While cytotoxic drugs such as adriamycin induced the fraction of CD44<sup>Hi</sup>/CD24<sup>−/Low</sup>/Lin<sup>−</sup> breast cancer stem cells, the HER2/EGFR tyrosine kinase inhibitor lapatinib was found to prevent the expansion of stem cells and led to a slight decrease [29]. Our laboratory also reported on an assay that can be used to identify drugs aimed to treat drug-resistant breast cancer stem cells [28]. The method is based on measuring the side population (SP). SP cells are characterized by having a high density of drug efflux pumps such as breast cancer-resistant protein (BCRP) or P-glycoprotein (Pgp), which causes these cells to efflux a fluorescence dye (Hoechst 33342), while mature bulk tumor cells take up the dye and can therefore be distinguished by fluorescence-activated cell sorting. The percent of side population cells in a given breast cancer cell line, including those resistant to tamoxifen, letrozole or trastuzumab, correlates with its ability to form colonies in soft agar [28]. We also demonstrated that inhibition of the HER2 family of growth factor receptors, particularly HER2 and HER3, by, e.g., trastuzumab can eradicate drug-resistant breast cancer stem cells. These examples show that cancer stem cell-targeted agents must be evaluated in a setting combining a stem cell-specific marker/characteristic with a growth assay format that allows only anchorage-independent and hence pluripotent or cells with self-renewal capacity to survive.

### 4.2 Biochemical Screening Assays

Biochemical assays are compared to “target-driven” cellular assays and provide the means for evaluating high numbers of compounds [30]. These screens are primarily employed in the pharmaceutical industry and institutions that harbor large compound libraries for systematic search of novel agents. Figure 3 summarizes the procedure for such an approach. An important advantage of biochemical screens is that they can be fully automated; thus, most steps can be performed by robot or computer...
systems such as dispensing of targets, addition of drugs and detection reagents, as well as compound library storage and management. Key requirements for target-oriented screening are:

1. The molecular target must be validated, shown to be causally linked to disease initiation or progression.
2. The target required for in vitro assays must be made available in large quantities, for example, by recombinant DNA techniques.
3. Defined, pure compound libraries comprising hundreds of thousands of structures derived from combinatorial approaches or collections of natural substances should be available.
4. Simple, cost-effective, highly reproducible assay and detection systems, which can be performed in microplate formats.

Suitable platforms have been proven to be enzyme-linked immunoadsorbent assays (ELISA) or other enzyme-based colorimetric methods. Further technologies that are frequently used are (1) radiometric assays dependent on scintillation proximity counting by employing scintillant-coated beads in microtiter plates, (2) time-resolved fluorescence based on highly fluorescing rare-earth metal–ligand chelates (europium, samarium, terbium), (3) fluorescence polarization, and (4) luminescence detection including chemiluminescence or electrochemiluminescence [2].

More recently, fluorescence resonance energy transfer (FRET) techniques have become a preferred method in high-throughput screens. FRET biosensors can readily be engineered and are suitable for cell-free and cellular systems [31].
Prominent targets for which these strategies have been employed and led to drugs that have progressed to advanced clinical development or even FDA approval are the protein kinases. For example, imatinib was found in an effort to develop bcr–abl kinase inhibitors after going through a biochemical screen using a panel of recombinant kinases. Bcr–abl is a chromosomal translocation product causing chronic myeloid leukemia. Imatinib has proven to be able to produce complete hematological and cytogenetic responses in this disease in patients [32]. Only careful testing of imatinib and its analogs in in vitro kinase assays and structural optimization of pharmacologic properties led to its success. If the agent would have been evaluated in a conventional cellular screen, it would have failed common activity criteria. In the NCI 60-cell-line screen, for example, only one cell line, namely, K562, possesses the bcr–abl abnormality; in addition, imatinib antiproliferative activity as a means of IC50 concentration is rather low. Mow et al. found, even in the K562 cell line, values for colony formation in the order of 12 μM and IC90s of target and growth inhibition of approx 20 μM [33, 34].

4.3 Combination of Target and Cell Screens

Both cell- and target-based screening procedures have clear advantages and disadvantages. While cell-based approaches will miss agents with certain defined modes of action owing, e.g., to lack of cytotoxic potency in short-term assays or the targeting of a rare subpopulation of cells in a bulk tumor mass, they might, on the other hand, identify compounds as active with previously unknown targets and hence allow for identification of novel mechanisms of action as well as the elucidation of their interplay in certain pathways. An example of this from the NCI 60-cell-line screen is bortezomib (Fig. 1).

Adams and colleagues synthesized a series of boronic acids as potential potent and selective inhibitors of the proteasome [35]. They submitted the compounds to the NCI 60-cell-line screen for evaluation. The average growth inhibition of 50 % (GI50) value for bortezomib across the entire NCI cell panel was 7 nM. Moreover, when 13 dipeptide proteasome inhibitors from the boronate series were examined, a strong correlation (Pearson coefficient, $r^2 = 0.92$) was noted after plotting $K_i$ vs. GI50 values. Using the NCI’s algorithm COMPARE, the bortezomib 60 cell line “fingerprint” was compared to the historical file of 60,000 compounds and found it to be unique, with little correlation to other “standard” or investigational agents, prompting further exploration of its activity in cell culture and in murine and human xenograft models. In these models, bortezomib exhibited many of the properties seen in preclinical studies of proteasome inhibitors such as lactacystin: activity as a single agent. They included enhancement of apoptosis induced by chemotherapy or radiation and specificity for transformed cells [17]. Subsequently, fluorogenic kinetic assays for measuring the inhibition of both chymotryptic and tryptic activities of the proteasome were developed [36] and found that bortezomib was a reversible, selective proteasome inhibitor [35, 36]. Phase I clinical studies found significant activity in multiple myeloma, with patients showing reductions in myeloma-related immunoglobulins and marrow plasmacytosis, which led to the development of bortezomib as an agent for the treatment of multiple myeloma and its approval by the FDA [17]. The mechanism(s) of action of bortezomib were identified retrospectively. One mechanism that is believed to contribute most to myeloma sensitivity to bortezomib is the dependence of this tumor type on the constitutive activation of NF-kappa B. If the proteasome is inhibited, I-kappa B, which binds to NF-kappa B, is not degraded and prevents the release of free NF-kappa-B which then can induce transcription and myeloma cell growth [37].

Another advantage of compounds identified in cellular screens is their proven cell-permeable properties, which might be missing in cell-free systems. In addition, ligand interactions might be more appropriate in the biological environment. Considering these facts, a combination of rational biochemical and “more” empirical cellular screening systems seems therefore the most optimal methodology in new cancer drug discovery.
5 Using Model Organisms for Screening

Nonmammalian organisms as systems for anticancer drug screening arose in the late 1990s as a potential alternative to human models in the light of advances in genomic research. A group at the Fred Hutchinson Cancer Research Center in Seattle headed by Steven Friend proposed to use yeast (Saccharomyces cerevisiae), the nematode Caenorhabditis elegans, or the fruit fly Drosophila melanogaster, because they share similar signaling and growth regulatory pathways with humans [38]. The advantage, particularly of yeast, is that the complete genome comprises only 6,250 defined genes, and, most importantly, many genes that are altered in human tumors have homologs in this model organism. For example, the p53 tumor suppressor gene has its structural homolog in RAD9, the mismatch repair genes MSH2 and MSH1 in MSH2Sc and MLH1Sc or the cyclins D and E in cyclin DDm and cyclin EDm, respectively [38]. These models are therefore thought to provide a valuable resource to achieve a greater understanding about human cancer and hopefully give insights into new approaches for therapy. Friend and coworkers have chosen to employ DNA damage response elements/pathways to delineate mechanisms of actions of known, very effective anticancer agents (e.g., cisplatin in germ cell tumors) and to find novel targets for therapy by defining molecular changes underlying genetic instability of cancers, which they believe are mainly defects in DNA repair pathways, cell cycle checkpoints, and cell cycle transition. The group has determined the effects of cancer mutations on sensitivity or resistance to various chemotherapeutic agents in a panel of isogenic yeast strains, each defective in a particular DNA repair or cell cycle checkpoint function. Widely different toxicity profiles were observed for 23 standard anticancer agents and X-ray treatment, indicating that the type of DNA repair and cell cycle checkpoint mutations in individual tumors could strongly influence the outcome of a particular chemotherapeutic regimen [39]. While cisplatin was specifically toxic to yeast strains defective for the Rad6/Rad18-controlled pathway of damage tolerance during the S-phase, sensitivity to the ribonucleotide reductase inhibitor hydroxyurea was seen in the intra-S-phase checkpoint-deficient mecl and mec2 strains. Hence, some of the commonly used anticancer agents showed significant specificity in their killing in yeast, and this provides strong evidence that new molecular diagnostics could improve the utility of the standard therapies [39]. However, screening and predicting activity of anticancer agents in yeast is limited by some differences in biology of yeast and mammalian cells such as tubulin. Spindle poisons are not toxic to S. cerevisiae and are therefore not active against yeast tubulin. Hormones, growth factors, and prodrugs requiring metabolic activation also cannot be modeled in yeast [38, 39].

Nonetheless, the yeast studies have brought about new useful anticancer agents based on the concept of synthetic lethality: two genes are synthetic lethal if mutation of either alone is compatible with viability but mutation of both leads to death. Targeting a gene that is synthetic lethal to a cancer-relevant mutation should kill only cancer cells and spare normal cells [40]. This paradigm arising from yeast led to the chemical synthetic lethality approach of total cancer cell kill and to the development of poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of cancers that have BRCA1 and BRCA2 mutations. The PARP inhibitor olaparib, which was the first drug to reach the clinic and blocks nucleotide excision repair, has shown responses in patients with BRCA1 or BRCA2, genes important in homologous recombination repair [41]. Phase II studies in BRCA mutant breast cancers are currently ongoing and reported very promising preliminary activity in this tumor type that has an overall poor survival outcome. When tumors with a genetic defect in the double-strand repair pathway are treated with a DNA single-strand repair inhibitor such as olaparib, chemical synthetic lethality occurs.
6  Predictivity of Screening Data

One of the key criteria for the strength/power of screening programs is their predictiveness of clinical response. Unfortunately, these analyses are very time consuming, as the process of preclinical and clinical development requires several years, so that outcomes of screens employing novel strategies are not yet foreseeable.

6.1  NCI Analysis of Activity in Preclinical Models and Early Clinical Trials

6.1.1  Xenografts

The NCI has conducted a retrospective review of the predictivity of their in vitro and in vivo screening efforts based on the 60 human cell line panel and xenograft testing in the 1990s. At the time of the review, the NCI procedures were mainly empirical and disease rather than target based [10, 42]. Data were available on 39 agents with both xenograft data and phase II trial results. The analysts found that histology of a particular preclinical model showing in vivo activity did not correlate with activity in the same human cancer histology. However, drugs with in vivo activity in a third of the tested xenograft models did correlate with ultimate activity in some phase II trials. This and the fact that none of the currently registered anticancer drugs was devoid of activity in preclinical tumor models, but showed activity in the clinic, led to the conclusion that activity in in vivo models of compounds demonstrating in vitro activity remains desirable [10, 43]. The hollow fiber assay has proven a valuable interface for selecting development candidates from large pools of compounds with in vitro antiproliferative activity for expensive and time-consuming subcutaneous xenograft testing (Fig. 2).

6.1.2  Hollow Fiber Assay

The HF assay was developed by Hollingshead et al. [16] at the NCI and is composed of 2 cm tubes filled with tumor cell lines. These fibers are implanted into mice at two sites (intraperitoneal and subcutaneous). The fibers are removed after 4–6 days in the animal and processed in vitro for quantification of tumor cell growth. By determining net cell kill, one can examine whether drugs administered via different routes are bioavailable and can reach the tumor sites [16, 42]. Of 564 compounds tested in the HF model and that were also tested in in vivo xenografts, 20 % showing HF activity also responded in xenograft models. This response was most likely if the intraperitoneal fiber activity was found in more than six intraperitoneal fibers. While a positive HF result could correctly predict in vivo xenograft response in one-fourth of the cases, 60-cell-line screening activity was able to predict correctly HF response in the order of 50 %. Significant HF activity in more than six intraperitoneal fibers was likely if the mean IC50 for in vitro growth inhibition of a compound was below 10–7.5 M. These analyses showed that the HF assay is a very valuable, rapid model system with predictive value.

6.2  Predictive Value of the Colony-Forming Assay

Another combined in vitro/in vivo testing procedure is the soft agar colony-forming assay, also termed tumor clonogenic assay (TCA). The TCA can either be used for sensitivity screening of patient tumor material in vitro predicting direct clinical response or with fresh xenograft tissue for selecting the
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**Fig. 4** Activity of trabectedin in 74 human tumor models in the clonogenic assay in vitro. The tumor types are listed on the y-axis; the numbers behind the tumor designation indicate the actual number of different patient-derived xenografts tested against trabectedin. The x-axis shows the IC70 values in nanomolar for each individual tumor in relation to the mean IC70 of all tumors (dotted vertical line). Data points to the left represent more sensitive and those to the right more resistant tumors.

most appropriate in vivo model [44–47]. However, its high-throughput application is limited by lack of reproducibility (unique sample material) and the elaborative assay procedure.

A correlation between in vitro human tumor sensitivities and clinical responses of the same patients was first established by Salmon and coworkers. Their results demonstrated a highly significant correlation of in vitro tumor resistance to specific drugs with failure of the patient to respond to the same drugs clinically. Although the prediction for resistance was very high, that for sensitivity was less precise. Although in vitro tumor sensitivity was noted in every case where the patient responded, there was a significant fraction of false-positive tests resulting in clinical therapy failure [45]. Similar results were found in our laboratories when the response of xenograft tissue derived from patient tumors was compared to that of the patient. The TCA predicted correctly for tumor response in 62 % and for resistance in 92 % of the examined cases [45, 46]. The latter is mirrored by the even better response prediction of the Freiburg nude mouse xenografts if used in vivo. Figure 4 shows an example of the novel agent trabectedin (Yondelis®, eceatinascidin 743) and its activity in a panel of 16 tumor types in the TCA. Trabectedin is approved in Europe for the treatment of advanced soft tissue sarcoma. The European Commission and the US Food and Drug Administration (FDA) have granted orphan drug status to trabectedin and a registration dossier has been submitted to the European Medicines Agency (EMA) and the FDA for trabectedin when administered in combination with pegylated liposomal doxorubicin (Doxil, Caelyx) for the treatment of women with relapsed ovarian cancer.

The four xenografted soft tissue sarcomas, derived from patient explants that were tested in the TCA in our laboratories, were the second most responsive tumor type with a median IC70 of about 0.5 nM. All four sarcomas were more sensitive than the mean IC70. Sarcoma sensitivity is followed by head and neck cancers and hematological malignancies. Only the median response of small-cell lung cancers was superior to that of soft tissue sarcoma to trabectedin single-agent treatment (Fig. 4). Trabectedin was less active against 5 ovarian cancers with a median IC70 of 5 nM. However, 2 of the 5 ovarian cancers appeared to be very sensitive, whereas the others were more resistant than the mean IC70 of all tumor types combined. However, the combination of trabectedin and doxorubicin was not tested. Overall, the data in Fig. 4 demonstrate that the TCA is useful in predicting tumor response.
Owing to the small-cell lung cancer responses and the poor treatability of this tumor type with chemotherapy, it should also be considered for studies of trabectedin efficacy.

### 6.3 Relationship Between Clinical Response and Patient Explants in Nude Mice

#### 6.3.1 The Freiburg Experience

Unlike the NCI in vivo screen, the Freiburg xenograft panel is derived directly from patient explants and not established from permanent human tumor cell line material as detailed in Fig. 5. By comparing the efficacy of a standard-of-care drug or drug combinations in patients and their tumors grown in nude mice, a total of 21 patients reached a remission. The same result was observed in 19 tumors growing as xenografts. Fifty-nine patients did not respond to treatment and the same result was found in 57 cases in the nude mouse system. Overall, xenografts gave a correct prediction for resistance in 97% (57/59) and for tumor responsiveness in 90% (19/21) [46].

Although most analyses of predictivity and usefulness of in vitro and in vivo screening procedures indicate clearly a high value of anticancer drug screens, particularly if validated by employing agents that have made it to the clinic, it remains unclear how the new molecular targeted agents with no prior defined clinical activity will translate into patient benefit. It also seems to be certain that pure in vitro screening methodology will not be sufficient to delineate potential clinical activity, particularly because pharmacokinetics have a major impact on pharmacodynamic activity. Data derived from in vivo model systems deem necessary to ensure that drug concentrations inhibiting the target and in vitro cell growth to 100% or at least 50% can be reached.
Preclinical screening is necessary to prioritize compounds for further development. In the era of target-oriented molecular cancer therapeutics, screening procedures are tailored toward the desired mechanism of tumor inhibition. They require, however, careful design and validation. In the past, empirical screens designed to find highly potent cytotoxic agents produced an arsenal of clinically used drugs with low selectivity and efficacy in solid tumors. Although antiproliferative activity is generally a desirable effect, it might bias toward finding compounds poisoning DNA and the cytoskeleton in the commonly used short-term cultures rather than drugs with novel mechanisms. However, empirical screening approaches looking for compounds with novel profiles to which molecular mechanisms could be fitted retrospectively, such as the histone deacetylase inhibitor vorinostat, the proteasome inhibitor bortezomib, and the heat-shock protein inhibitor tanespimycin, led to the identification of subgroups of patients benefiting from these therapies. Thus, rational drug design or drug discovery approaches combined with novel knowledge from genome and proteome research as well as bioinformatics are the most promising ways toward individualized cancer therapy. Our drug screening and discovery pathways have evolved into an integrated approach which combines the use of cell line and tumor xenograft models that resemble very closely the patient characteristics and response (Fig. 6). They are molecularly profiled for most of the validated targets using state-of-the-art genomic and proteomic technologies as shown in Fig. 6. Drug–target interactions are assessed and tumor tissues pre- and posttreatment are used to explore and develop gene signatures or biomarkers of tumor response (Fig. 6) [48].

Target-driven drug development has led to the availability of many useful cell signal transduction inhibitors and antibodies targeting growth factor receptors. The next challenge in preclinical anticancer drug screening and development is to find the means to disrupt protein–protein interactions and to control deregulated transcription with small molecules. To accomplish the latter, molecular in vivo imaging procedures and drug delivery technologies need to be incorporated particularly into preclinical screening processes.
Acknowledgment  This work was supported by grants from the German Ministry for Education and Research (BMBF) and the US National Cancer Institute, Developmental Therapeutics Program as well as from the European Commission with a grant to A. M. B. (QLG1-CT-1999-01341).

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Handbook of Anticancer Pharmacokinetics and Pharmacodynamics
Rudek, M.A.; Chau, C.H.; Figg, W.; McLeod, H.L. (Eds.)
2014, IX, 836 p. 145 illus., 46 illus. in color., Hardcover
ISBN: 978-1-4614-9134-7
A product of Humana Press