Chapter 2 / Drug Screening

Handbook of Anticancer Pharmacokinetics and Pharmacodynamics
Edited by: W. D. Figg and H. L. McLeod © Humana Press Inc., Totowa, NJ

1. INTRODUCTION

Cancer chemotherapy is a rather young discipline. It has been pursued with scientific vigor and multinational collaborations only since the mid-20th century. Although 92 approved anticancer drugs are available today for the treatment of more than 200 different tumor entities, effective therapies for most of these tumors are lacking (1). Out of the 92 registered drugs, 17 are considered by oncologists to be more broadly applicable and 12 additional agents are perceived as having certain advantages in some clinical settings (2). They are mostly cytotoxic in nature and act by a very limited number of molecular mechanisms. Thus, the need for novel drugs to treat malignant disease requiring systemic therapy is still pressing. Public institutions, the pharmaceutical industry, and, more recently, 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 on tumor growth of dyes or respiratory poisons, respectively (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 instituted 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 provide the battery 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), antibiotics (mitomycin C, adriamycin), and hormones (androgens, estrogens, corticoids) (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 used were always adopted 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/branches/btb). 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 three-cell-line 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. This prescreen (MCF-7 breast, H460 lung, and SF268 brain cancer lines) would test for the presence of toxicity at a drug concentration of 10⁻⁴ M and could eliminate a large proportion of the inactive agents, but preserve “active” agents for multidose 60-cell-line testing. Computer modeling indicated that approx 50% of drugs could be eliminated without a significant decrease in ability to identify active agents, and should be able to increase the throughput and efficiency of the main cancer screen with limited loss of information (http://dtp.nci.nih.gov/branches/btb)/.

Thus, the current 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 were adopted for clinical use have been identified based on their unique patterns of activity in the in vitro screen such as spicamycin (NSC 650425), a glycoprotein synthesis inhibitor; the protein kinase C inhibitor UCN-01; or depsipeptide (NSC 630176), a histone deacetylase (HDAC) inhibitory agent (17).

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).

4. STRENGTHS 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 that emerged recently (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 1536-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 (21,22). 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 (23).
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 (24) or use of a luciferase reporter (25,26). 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 d) may select for cytotoxic drugs.
2. Tumor cell growth can continue despite the fact that clonogenic cells are reduced, missing certain classes of cytostatic agents (e.g., inducers of cellular senescence).
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 (27).
5. Drug penetration barriers occur only in multilayered solid tumors.

Drugs that are encompassed by this list include signal transduction inhibitors, antibodies, bioreductive drugs, anti-angiogenic peptides, small molecules, or antitelomerases. 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

Examples of successful in vitro models of a tumor environment using multicellular spheroids or postconfluent multicell layers for screening of bioreductive agents have been reported by Phillips et al. (27,28). The latter showed that agents requiring bioactivation by the microenvironment such as mitomycin C or EO9 were differentially chemosensitive in plateau-phase multilayered cells as compared to exponentially growing monolayers. The novel structures RSU 1069 and SR4233 were found by this procedure (27,28).

Over the past couple of years, the enzyme telomerase has been causally linked to immortalization and cancer and thus has arisen as a promising anticancer target (29,30). Telomerase acts at the end of chromosomes termed telomeres by synthesizing telomeric repeat sequences. Telomeres are important noncoding sequences that protect chromosomes from end-to-end fusions and maintain chromosomal stability. In each round of cell division telomeric sequences (approx 30–100 bp) are lost owing to the end replication problem. As a result, cells expressing telomerase have an unlimited proliferation capacity, for example, cancer or renewal stem cells, while telomerase-negative cells will undergo replicative senescence when their telomeres become critically short (30). Specific telomerase inhibition has been shown to require a certain number of population doublings or in vitro passages of tumor cells, a so-called lag time, to induce cessation of cell growth (29,31). Such agents would be missed in, for example, the NCI screen based on a 48-h drug incubation period and most other conventional cellular screens.

Nonetheless, Nassani’s and colleagues’ approach to the identification of telomerase inhibitory agents employed parts of the NCI screening procedure, namely, the COMPARE algorithm (14), which is an bioinformatic analysis of sensitivity patterns generated in preliminary random screens (32). Nassani had carefully characterized a panel of tumor cell lines for telomerase and its components and used a “seed” compound with telomerase inhibitory activity, but low cytotoxic potency, berberine (IC$_{50}$ ~ 35 µM), for search of better analogs.
FJ5002 was found as a genuine and effective antitelomerase agent (IC\(_{50}\) ~ 2 µM) and is currently in preclinical development (33).

A third example for tailored screens is the NCI angiogenesis screen, which was established after recommendations that NCI should facilitate research into mechanisms of tumor angiogenesis and the development of drugs that target the essential tumor vasculature (http://dtp.nci.nih.gov./aa-resources/aa_index.html). This program is unique inasmuch as it not only offers the screening of potential antiangiogenic drugs, but also distributes validated endothelial cells and the required reagents, controls (TNP470 [IC\(_{50}\) = 0.5–1.5 nM]), and reproducible angiogenesis bioassay procedures. A 72-h proliferation at passage 1–6 of primary endothelial cells is guaranteed to retain TNP470 sensitivity and endothelial markers in terms of proliferation, growth inhibition, cord formation, and migration. Because the screen has been inaugurated only recently, the discovery of new agents has not been reported so far.

### 4.2. Biochemical Screening Assays

Biochemical assays are compared to cellular assays “target-driven” and provide the means for evaluating high numbers of compounds (2). 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 hundred 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 (34).

Suitable platforms have been proven to be enzyme linked immunoadsorbent assays (ELISA) and 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).

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, Gleevec™ (STI571) was found in an effort to develop bcr-abl kinase inhibitors. Bcr-abl is a chromosomal translocation product causing chronic myeloid leukemia, Gleevec has proven to be able to produce complete hematological and cytogenetic responses in this disease in patients (35). Only careful testing of STI571 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, STI571 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 IC₉₀ of target and growth inhibition of approx 20 µM (36,37).

### 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 such as, for example, specific telomerase inhibitors owing to lack of cytotoxic potency in short-term assays. 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 the spicamycin analog KRN5500. The compound was found potently active in the 48-h SRB assay over 68 cell lines, however, showing differential cytotoxicity in that six cell lines were exquisitely sensitive (IC₅₀ = 5 nM), and 17 were resistant (IC₅₀ > 10 µM), the pattern of activity in the screen was unique, indicating a novel mechanism of action (14,38). Careful studies in the molecular mechanism of KRN5500 using sensitive and resistant cell lines revealed that the agent might target the enzymatic machinery or organelles important for proper glycoprotein processing, representing novel targets in cancer treatment. Thus, KRN5500-resistant cells had elevated levels of enzymes associated with the endoplasmatic reticulum (ER), namely, glucose-6-phosphatase and...
GalNAc transferase, while KRN5500-sensitive cells in contrast had high levels of ceramidase, an enzyme that is localized in the ER and Golgi apparatus and has been linked to carcinogenesis (38–41).

Another advantage of compounds identified in cellular screens are 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 would therefore be the most optimal methodology in new cancer drug discovery.

We have recently applied this combination successfully in the identification of the novel CDK inhibitory agent E224 (5-methyl indirubin) (42,43). Figure 4 shows the discovery process followed in our laboratories. E224 emerged from a discovery program that aimed to exploit active ingredients of Chinese traditional anticancer medicines for rational drug design. The Indigo naturalis constituent indirubin had previously been shown to possess activity against leukemia cells; hence, 20 analogs were synthesized and tested for in vitro antiproliferative activity using permanent cell lines in the propidium iodide assay (24) as well as xenografts in the clonogenic assay (44). Whereas the compounds were inactive in the cell line screen (IC50 > 30 µM, Fig. 5), they showed some activity ranging from approx 10 to 50 µM in the clonogenic assay (data not shown). Had they been evaluated based only on monolayer cell line screening, these agents might have been discarded because of lack of potency. E224 and related compounds were further “rescued” by molecular mechanism based investigations into their effects on the cell cycle that revealed that they were able to block cell proliferation in the late G1 and G2/M phases. The latter effects gave an indication that cyclin-dependent kinases (CDKs) might be the target of indirubin analogs (34,42). Indeed crystal structures of CDK2 with indigoids had shown atomic interactions with the kinase’s ATP-binding pocket. Moreover, in a panel of more than 25 kinases, indirubin derivatives specifically inhibited the activity of CDK1 over CDK2, and CDK5 over CDK4 (42). Because the IC50s of kinase inhibition were similar to those of agents currently in clinical trials such as flavopiridol and roscovitine, the five most potent novel CDK inhibitors were selected for in vivo testing against the large-cell lung cancer xenograft LXFL 529, which was a very responsive line in the clonogenic assay. E224 showed marked in vivo activity against LXFL 529 lung cancer xenografts at doses of 100 mg/kg given once daily for 5 d for 3 wk intraperitoneally (Fig. 6). At a dose that was well tolerated, E224 was able to produce tumor remissions (T/C = 9%). Thus, it seems that E224 has the pharmacodynamic and pharmacokinetic properties required for further preclinical development and that target-oriented combined with empirical assays (Fig. 4) are powerful screening methods.

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 (45). The advantage, particularly of yeast, is that the complete genome comprises only 6250 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 DxDm and cyclin ExDm, respectively (45). 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 co-workers 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
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Fig. 5. Sensitivity profile of E224 over 25 human tumor cell lines comprising the Freiburg panel. The mean IC₅₀ and IC₇₀ (inhibitory concentrations 50% and 70%) of E224 was >30 µM and thus the agent was considered inactive according to our selection criteria.

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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
(45,46).

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 consum-
ing, 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 review of NCI in vitro and in vivo screening efforts based on the 60 human cell line
panel and xenograft testing in the 1990s has recently been published. The methods of the NCI
procedures were mainly empirical during this time period and disease rather than target
based (10,47). 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 anti-
cancer 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 (10,48).
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 d 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 \(47\).

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 IC\(_{50}\) 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 most appropriate in vivo model \(49–51\). 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 co-workers. 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 \(49\). 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 \(50,51\). The latter is mirrored by the even better response prediction of the Freiburg nude mouse xenografts if used in vivo.

### 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. By comparing drug efficacy 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. 59 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% \(19/21\) \(52\).
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 uncertain how the new molecular targeted agents with no prior defined clinical activity will translate into patient benefit.

It also seems further 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.

7. CONCLUSIONS AND PERSPECTIVES

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 combined with novel knowledge emerging from genome and proteome research as well as bioinformation technologies might be the most efficient way forward. The work reported recently by Scherf et al. from the NCI on a gene expression database for the molecular pharmacology of cancer is pointing in this very promising direction (19). The NCI group used cDNA microarray technology to assess the transcriptome of the 60 human cancer cell line panel. Clustering of the cell lines on the basis of their responses to drugs yielded unique gene patterns indicating mechanisms of sensitivity and resistance. For example, a negative correlation could be found between expression of the enzyme asparagine synthetase (ASNS) and L-asparaginase sensitivity in the panel lines. Certain leukemias lack ASNS and depend on exogenous L-asparagine for growth. By treating these cells with L-asparaginase, L-asparagine is depleted and they will cease growth, which was well demonstrated by employing genomics (19). If the bioinformatic and chemoinformatic analyses of the currently assembled RNA, DNA, and protein profiles of human cancer cells lead to credential/valid results such as the described ASNS sensitivity pattern, major advances will be made in new drug discovery with respect to speed of preclinical development and prediction of therapeutic usefulness.

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REFERENCES


27. Phillips RM. In vitro models of solid-tumour biology and drug delivery: implications for and applications to target-oriented screening for novel anticancer drugs. In: Fiebig HH, Burger AM (eds). Con-
47. Gura, T. Systems for identifying new drugs are often faulty. Science 1997; 278:1041–1042.
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