Cancer Chemother Pharmacol (2003) 52 (Suppl 1): S29–S33
DOI 10.1007/s00280-003-0623-y

Chris H. Takimoto

Anticancer drug development at the US National Cancer Institute

Published online: 18 June 2003

Abstract Anticancer drug discovery and development is a rapidly evolving field. Recent advances in molecular oncology and the effort to completely sequence the human genome has led to an explosion in our understanding of the mechanisms involved in the transformation and growth of malignant cells. This in turn has led to major changes in our approach to traditional drug discovery and development. A dynamic example of how genomics is affecting cancer developmental therapeutics is provided by the ongoing changes being implemented in the anticancer drug development program run by the US National Cancer Institute (NCI). This review summarizes the history of drug screening and development efforts at the NCI over the past five decades from its inception up to its current state emphasizing molecularly targeted therapies. These changes have not only had an impact on drug discovery, but they are also providing new paradigms for the design and conduct of preclinical and early clinical trials.

Keywords Drug discovery · Drug development · Screening

Introduction

From the time a promising molecule is first identified in a drug discovery and screening program to the time it enters a phase I clinical trial, an enormous amount of scientific work and evaluation must be performed. Preclinical development encompasses all the activities that must take place before a new chemical entity can be administered to humans. As such, it spans the gap between drug discovery and clinical testing. Since the 1950s, the National Cancer Institute (NCI) of the USA has devoted extensive resources to the discovery, and preclinical and clinical development of anticancer therapies. This review focuses on the current programs, resources, and concepts involved in anticancer developmental therapeutics that are in place at the NCI.

This work was presented at the 18th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "New Strategies for Novel Anticancer Drug Development," 8–9 November 2002, Nagoya, Japan.

C. H. Takimoto
Department of Medicine/Oncology,
University of Texas Health Science Center at San Antonio,
7703 Floyd Curl Drive, MSC 7884,
San Antonio, TX 78229, USA
E-mail: takimoto@uthscsa.edu
Tel.: +1-210-5674777
Fax: +1-210-5676687

Drug development programs at the NCI

History

Systematic drug screening began at the NCI in 1955 with the establishment of the Cancer Chemotherapy National Service Center (NSC) screening program [12]. Even today, all screened compounds are given an NSC number to aid their identification. However, until the 1980s, most screening was performed in vivo using murine P388 or L1210 leukemia cell lines [10]. These hematologic murine tumors were employed because they were generally inexpensive, stable, reproducible, and easily handled. However, these in vivo screening efforts also had substantial limitations. Screening with rapidly growing leukemic cells was biased towards compounds with activity against rapidly growing tumors with high growth fractions. The relative lack of success during this period in identifying agents with activity against common human solid tumors was thought to be due, at least in part, to the lack of adequate screening models.

Because of these limitations, in 1989 the NCI changed to a rationally designed "disease-oriented" screening panel incorporating 60 cell lines derived from a variety of different human solid tumors [1]. Currently, the cell-line screen is a key component of a comprehensive in vitro and in vivo preclinical screening and drug

development program that is overseen by the NCI's Developmental Therapeutics Program (DTP) in the Division of Cancer Treatment and Diagnosis. An overview of this program is available online at http://dtp.nci.nih.gov.

Three cell-line prescreen and 60 cell-line screen

Because over 85% of the compounds submitted for screening are found to have no antiproliferative activity, the NCI adopted a three cell-line prescreen in 1999. All compounds submitted to the NCI are now prescreened in vitro against a panel of three highly sensitive human tumor lines that include the MCF-7 breast, NCI-H640 lung, and the SF-268 glioma cell lines. Demonstration of growth inhibitory activity is required in this prescreen panel before a compound can undergo advanced testing in the full 60 cell-line screen.

Originally, the NCI 60 cell-line screening panel was composed of lines derived from seven different human histologic tumor types including brain, colon, leukemia, lung, melanoma, ovarian, and renal cancers [1]. Later, breast and prostate cancer cell lines were added. An automated sulforhodamine blue cytotoxicity assay is used to assess the relative potency of a compound against all 60 cell lines using five different drug concentrations incubated for a standard 48 h. Endpoint parameters that are calculated for each individual cell line include: the GI_{50}, which is defined as the drug concentration that inhibits growth by 50%; the TGI, which is the lowest drug concentration that totally inhibits cell growth; and the LC_{50}, which is the lowest concentration that kills 50% of cells.

These data are then analyzed by the COMPARE algorithm, which is a program that categorizes and compares different groups of agents based on their patterns of cytotoxic activity in the 60 cell-line screen (Fig. 1) [5]. This powerful program can identify similar classes of anticancer agents, such as platinum analogs, microtubule agents, or topoisomerase I inhibitors, based purely on their cytotoxicity patterns [6]. Thus hypotheses can be generated about the potential mechanisms of action of completely new anticancer agents using data generated in the 60 cell-line screening panel. New and exciting agents with novel mechanisms of action may be identified by the screen if they demonstrate a unique pattern of antitumor activity. Thus the COMPARE program has converted a relatively simple test of growth inhibition into a sensitive probe for studying drug pharmacology.

Recent efforts have made the 60 cell-line screen an even more powerful tool for analyzing drug effects at the molecular level. This new approach involves the characterization of the relative expression of specific molecular targets important for drug sensitivity in each of the cell lines contained in the 60 cell-line screen. For example, this diverse group of targets can include oncogenic proteins such as RAS, N-MYC, P53, RB protein, or key metabolic enzymes such as thymidylate synthase, dihydrofolate reductase, or topoisomerase I and II. Relative expression of drug-resistance proteins, such as P-glycoprotein or multidrug resistance-associated protein, are other examples. Characterization of these molecular targets in each of the 60 cell lines allows the screening data to be analyzed from an entirely new perspective. The relative pattern of drug sensitivity in each cell line can now be correlated with the relative expression of hundreds or more different specific molecular targets. This generates a much more data-rich screening tool for analyzing new compounds. Because of the complexity and wealth of information generated, it also creates major challenges in the field of bioinformatics. However, pioneering work by Weinstein et al. in analyzing this type of data-rich information has allowed correlations to be made between the patterns of drug sensitivity in the cell lines and the relative expression of these molecular targets [11]. This approach offers an extremely powerful method for identifying novel new anticancer agents based on their activity in cell lines expressing different molecular targets relevant to drug action. In addition, it is rapidly becoming possible to characterize the relative expression of literally thousands of different specific molecular targets at the mRNA level in the 60 cell lines using cDNA microarray chip technology [8]. This flood of additional information will further increase the power of this approach.

Although the utility of this method as an anticancer drug screening and discovery tool must still be proven, its potential is great. Conceptually, it is important because it extends the disease-oriented approach originally envisioned in the 1980s when the 60 cell-line screen was created to a more molecular targeted-based approach. Thus the 60 cell lines no longer represent a simple collection of cell lines arising from nine different human histologic tumor types; instead, they have been transformed into a panel of thousands of different molecular targets, each of which is expressed at 60 different levels. Each individual target can then be individually correlated with drug sensitivity for any new or novel anticancer agent that is subjected to the screen [9]. This correlation has enormous potential for identifying new targeted-based agents for further clinical development.

NCI drug development programs

At the NCI, two separate programs have been established by the DTP to develop novel therapeutic agents. The first is the Rapid Access to Intervention Development (RAID) program. This novel program is designed to assist academic investigators with the early steps required to initiate clinical trials with their own discoveries. Under the RAID program, the clinical agent will be developed under an Investigational New Drug (IND) application that is held by the investigator or the investigator's designee. Successful application to the RAID program allows academic and other not-for-profit investigators to have access to the extensive range of

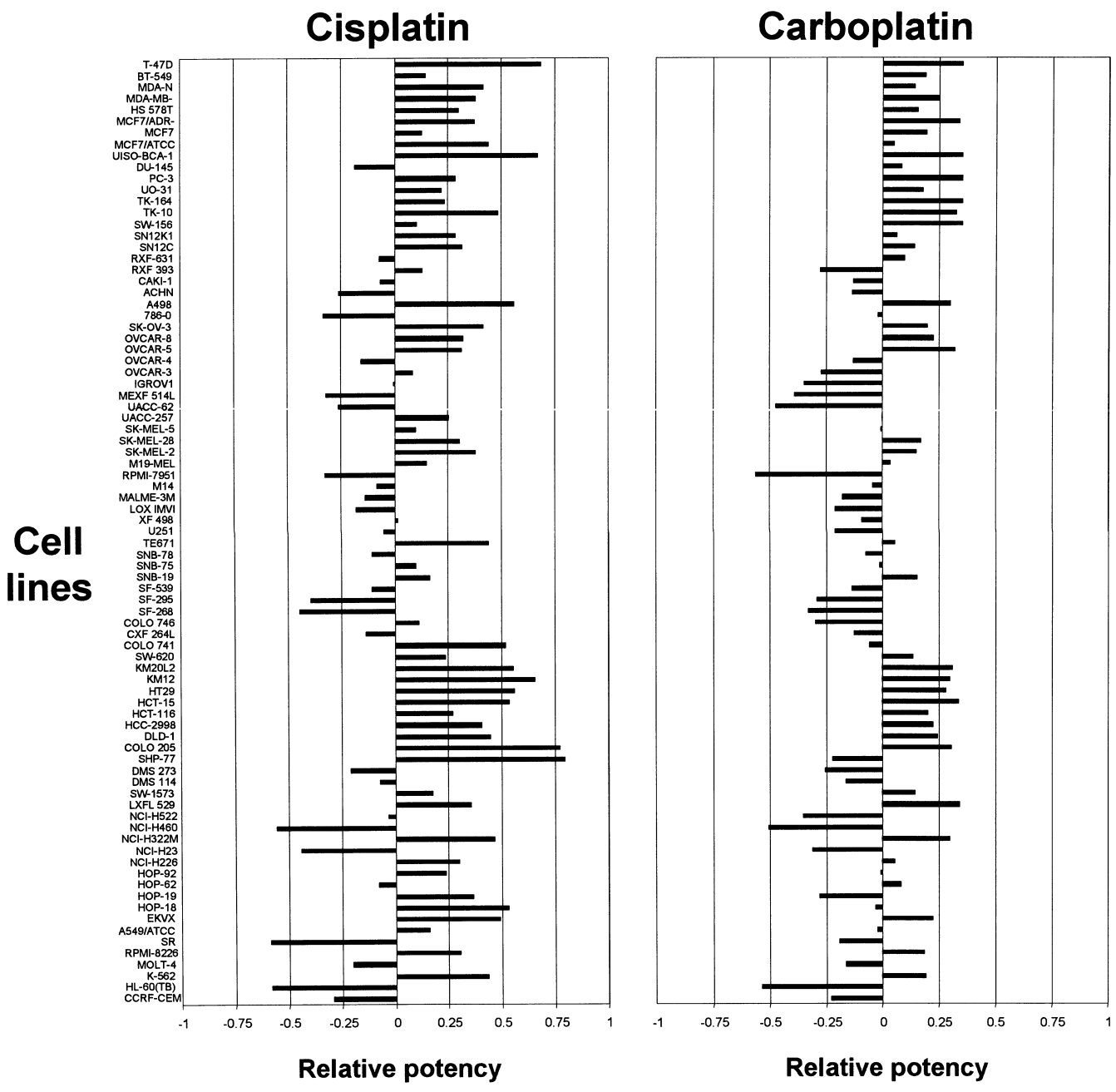

Fig. 1 COMPARE Program Algorithm Output. Plots of the mean relative sensitivity of various cell lines used in the National Cancer Institute drug-screening panel for cisplatin and carboplatin. The zero value represents the mean concentration required to inhibit 50% growth for all the cell lines (GI_{50}). The horizontal bars represent the relative difference in the GI_{50} value for a particular cell line from the mean value using a logarithmic scale. Cell lines with a bar extending to the right have a GI_{50} greater than the mean and are more resistant, while those that extend to the left have a GI_{50} lower than the mean and are more sensitive. A similar pattern of growth-inhibitory potency is demonstrated for these two platinum analogs (data obtained from the NCI website at http://dtp.nci.nih.gov). Reprinted with kind permission of Elsevier from Fig. 29.1 of Takimoto CH, Khleif SN (2001) Preclinical drug development. In: Atkinson AJ, Daniels CE, Dedrick RL, Grudzinskas CV, Markey SP (eds) Principles of clinical pharmacology. Academic Press, San Diego, p 380

NCI/DTP resources for early drug development including agents synthesized according to good manufacturing practice (GMP) procedures, clinical drug formulation studies, pharmacologic assay development, or IND-directed toxicology studies. The RAID program does not sponsor clinical trials. Information on the RAID program is available over the Internet at http://dtp.nci.nih.gov/docs/raid/raid_index.html.

The second pathway for the development of new anticancer therapeutic agents at the NCI is the Drug Development Group (DDG) pathway (Fig. 2). This program has evolved from the earlier DDG Decision Network that has screened over 70,000 compounds since it was established in 1990. The DDG program differs

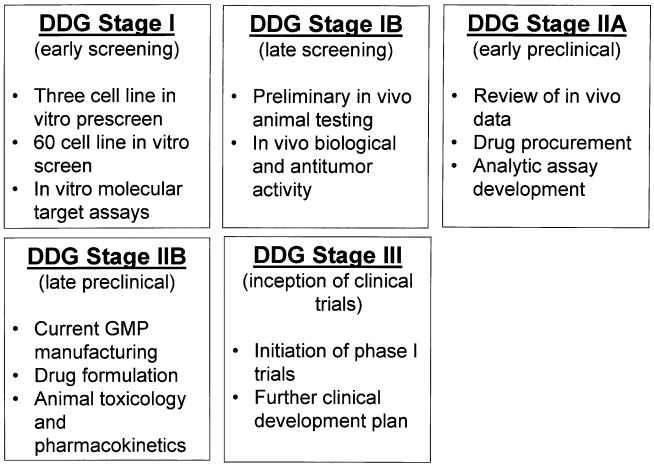

Fig. 2 National Cancer Institute Drug Development Group (DDG) program (*GMP* good manufacturing practice)

from the RAID program in that the NCI is anticipated to be the IND holder and the main sponsor of the clinical development of the agent. The DDG is the principal overseer of the agent's development, often working in close collaboration with an industry partner. The goal of the DDG pathway is the full-scale clinical evaluation of a novel therapeutic entity.

The DDG pathway involves several different committees that oversee the process. At the earliest stage, when a formal application for a new entity is submitted to the NCI, approval for preliminary in vitro screening is made by the DTP Access and Information Group (AIG). The AIG selects and prioritizes new compounds for screening and ensures that the agent has a unique chemical structure (stage I). Results of the three cell-line screen and 60 cell-line screen for a particular compound are then reviewed by an evaluation committee that selects compounds for further testing in animal in vivo studies. Specific factors used to select agents for further testing include significant potency seen in a variety of cell lines, a novel pattern of activity in the COMPARE program algorithm, or a special interest in an agent because of its chemical structure or biologic activity. Animal studies (stage IB) at the NCI are initially conducted with 12 different human cell lines using the previously described hollow-fiber assay [3]. The early in vivo data are then reviewed by the Biological Evaluation Committee and active agents are selected for further, more expensive, human xenograft studies in nude mice using tumors selected for prior sensitivity in earlier in vitro tests. At the NCI, human xenografts are implanted subcutaneously and the drugs are administered intraperitoneally. A relative difference in the tumor weight ratio of treated to control animals of less than 0.5 is considered promising for further development.

Agents with activity in the in vivo screen are then reviewed at the DDG stage IIA (early preclinical) meeting, where recommendations are made for further preclinical studies. These include determining an acceptable clinical formulation and discussing the optimal dose, route, and schedule of administration. Procurement of sufficient drug for further preclinical and clinical studies is planned, and pharmacokinetic assay development is also initiated. If no additional problems arise and the compound remains promising, then the agent progresses to a DDG stage IIB (late preclinical) meeting. At this juncture, a major commitment of research resources is made to further develop the agent. This includes contracting for current GMP drug manufacturing, the initiation of preclinical toxicology studies in two different species with histopathologic correlation, and animal pharmacokinetic and toxicokinetic studies. Toxicology, manufacturing, and formulation frequently represent the most costly steps in preclinical drug development. If a compound appears likely to be safe in humans, then a final DDG stage III (inception of clinical trials) meeting convenes to discuss the issues related to the initiation of NCI-sponsored phase I clinical trials, such as the recommendation of a safe starting dose and appropriate laboratory and clinical endpoints. The clinical development of a novel therapeutic and the IND filing is overseen by the Cancer Treatment Evaluation Program of the NCI. At this time, an NCI-sponsored IND application will also be filed with the US Food and Drug Administration. A substantial commitment is also made by the NCI to conduct an appropriate phase I and phase II clinical research program. Guidelines have been established for structuring the partnership between the NCI and drug sponsors in industry or academia that contain extensive agreements designed to protect the sponsor's intellectual property rights [7]. Further information about these programs is available on the Internet at http://dtp.nci.nih.gov.

Clearly, the NCI efforts in anticancer drug development are extensive and will continue to grow and change as the science of drug discovery and development evolves. The tremendous advances now occurring in our understanding of the molecular basis of human cancer and the identification of new molecular targets for developmental therapeutics ensures that this will be an active and exciting program for the foreseeable future.

The challenge: intracellular pharmacodynamics and new paradigms for clinical trials

Drug development scientists involved in preclinical and early clinical drug development will face a number of new challenges in the postgenomic era. The greatest will be to determine how to best incorporate our extensive preclinical understanding of drug pharmacology and molecular targeting into the design of early clinical trials [2]. In the area of cancer chemotherapy, advances in drug screening and discovery will ensure that all new compounds entering clinical studies will have well-defined theoretical molecular mechanisms of action. Therefore new paradigms for phase I and II clinical trials will be necessary to determine if these drug

mechanisms defined in preclinical studies are relevant to the clinical use of these agents.

"Intracellular pharmacodynamics" has been defined by Martin and Kemeny as the study of the relationship between intracellular molecular drug effects and clinical outcomes [4]. Incorporation of extensive biochemical and molecular endpoints in the design of clinical trials requires a thorough understanding of the mechanism of drug action. They are also technically difficult studies to perform in cancer patients because of the requirement for sampling tumor or organ tissues targeted by the drug. These issues are particularly relevant for anticancer drugs because, although a large number of compounds enter clinical phase I trials, only a small percentage will successfully be developed into clinically useful agents. Understanding why the majority of agents fail during clinical testing due to either severe toxicity or lack of efficacy is often perplexing because by definition, all of these agents are active in preclinical models. An increased understanding of how these agents behave in actual human patients may provide important information for screening and designing more successful agents in the future.

References

1. Boyd MR (1997) The NCI in vitro anticancer drug discovery screen: concept, implementation, and operation, 1985–1995. In: Teicher B (ed) Anticancer drug development guide: preclinical screening, clinical trials, and approval. Humana Press, Totowa, p 23
2. Gelmon KA, Eisenhauer EA, Harris AL, Ratain MJ, Workman P (1999) Anticancer agents targeting signaling molecules and cancer cell environment: challenges for drug development? J Natl Cancer Inst 91:1281
3. Hollingshead MG, Alley MC, Camalier RF, Abbott BJ, Mayo JG, Malspeis L, Grever MR (1995) In vivo cultivation of tumor cells in hollow fibers. Life Sci 57:131
4. Martin DS, Kemeny NE (1992) Modulation of fluorouracil by N-(phosphonacetyl)-L-aspartate: a review. Semin Oncol 19 [Suppl 3]:49
5. Paull KD, Shoemaker RH, Hodes L, Monks A, Scudiero DA, Rubinstein L, Plowman J, Boyd MR (1989) Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. J Natl Cancer Inst 81:1088
6. Rixe O, Ortuzar W, Alvarez M, Parker R, Reed E, Paull K, Fojo T (1996) Oxaliplatin, tetraplatin, cisplatin, and carboplatin: spectrum of activity in drug-resistant cell lines and in the cell lines of the National Cancer Institute's Anticancer Drug Screen panel. Biochem Pharmacol 52:1855
7. Sausville EA (1997) Working with the National Cancer Institute. In: Teicher B (ed) Anticancer drug development guide: preclinical screening, clinical trials, and approval. Humana Press, Totowa, p 217
8. Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, Scudiero DA, Eisen MB, Sausville EA, Pommier Y, Botstein D, Brown PO, Weinstein JN (2000) A gene expression database for the molecular pharmacology of cancer. Nat Genet 24:236
9. Shi LM, Myers TG, Fan Y, O'Connor PM, Paull KD, Friend SH, Weinstein JN (1998) Mining the National Cancer Institute Anticancer Drug Discovery Database: cluster analysis of ellipticine analogs with p53-inverse and central nervous system-selective patterns of activity. Mol Pharmacol 53:241
10. Waud WR (1997) Murine L1210 and P388 leukemia. In: Teicher B (ed) Anticancer drug development guide: preclinical screening, clinical trials, and approval. Humana Press, Totowa, p 59
11. Weinstein JN, Myers TG, O'Connor PM, Friend SH, Fornace AJ Jr, Kohn KW, Fojo T, Bates SE, Rubinstein LV, Anderson NL, Buolamwini JK, van Osdol WW, Monks AP, Scudiero DA, Sausville EA, Zaharevitz DW, Bunow B, Viswanadhan VN, Johnson GS, Wittes RE, Paull KD (1997) An information-intensive approach to the molecular pharmacology of cancer. Science 275:343
12. Zubrod CG (1972) The national program for cancer chemotherapy. JAMA 222:1161