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From serendipity to design: the evolution of drug development in oncology

EDITOR'S NOTE: This article describes the process by which cancer chemotherapeutic agents are developed, tested in patients, and incorporated into standard management. This process is quite elaborate, because of the potential toxicity of the drugs and their delicate balance between risk and benefit. — M. MARKMAN

ABSTRACT: Although screening of natural products remains the major method of discovering new anticancer drugs, newer techniques of rational drug design, computer-aided drug design, and combinatorial synthesis promise to broaden the scope of compounds available for screening. Recent changes in Food and Drug Administration rules allow for accelerated approval of drugs for treating cancer and other life-threatening illnesses, although the three-phase process of clinical trials remains largely unchanged.

One of the first anticancer drugs came to clinical use largely by accident. In 1941, an explosion in Bari Harbor, Italy during military exercises released nitrogen mustard gas. Army physicians reported that soldiers exposed to the gas experienced suppression of bone marrow and lymphoid tissue.[1] Noting these effects, researchers organized the first clinical trial of anticancer therapy 2 years later, using nitrogen mustard to treat lymphoma.[2] One patient achieved a transient complete response, a unique event at the time.

Since then, the process of developing drugs for the treatment of cancer has become highly structured to ensure patient safety and antitumor efficacy in the use of potentially highly toxic drugs. The National Cancer Institute (NCI) formerly performed most of the tasks of drug development, but pharmaceutical firms now play a major role.

For the past 40 years, drug development in oncology has been a slow process: researchers acquire large numbers of compounds (mostly from natural sources), screen them for anticancer activity in vitro, devise formulations, perform preclinical pharmacologic and toxicologic studies, perform clinical trials, and negotiate with the Food and Drug

TABLE 1

EXAMPLES OF ANTICANCER DRUGS DERIVED FROM NATURAL PRODUCTS

Drug class	Drug	Source
Vinca alkaloids	Vincristine Vinblastine	Madagascar periwinkle plant
Taxanes	Paclitaxel Docetaxel	Pacific yew tree
Bryostatins	Bryostatin 1	Marine bryozoan *Bugula neritina*
Antitumor antibiotics	Bleomycin	Fungus *Streptomyces verticullus*
Epipodophyllotoxins	Etoposide Tenoposide	Mandrake plant
Camptothecins	Irinotecan Topotecan	Chinese tree *Camptotheca accuminata*

TABLE 2

EXAMPLES OF NEW ANTICANCER DRUGS DERIVED FROM PARENT DRUGS

Modified drug	Parent drug	Advantage
Ifosphamide	Cyclophosphamide	More potent
Cyclophosphamide	Mechlorethamine	Oral bioavailability
Mitoxantrone	Doxorubicin	Less cardiotoxic
Carboplatin	Cisplatin	Less nephrotoxic
Docetaxel	Paclitaxel	Easier to produce

Administration (FDA) for approval. The process takes an average of 10 to 12 years[3] and costs more than $100 million per drug approved.

As basic research uncovers the molecular defects and genetic aberrations that underlie cancer, more drugs will be designed rather than discovered, and powerful new techniques of analytic chemistry will speed the process. Meanwhile, streamlined clinical trials and an expedited FDA approval process may allow faster approval of new drugs without compromising patient safety.

HOW DRUGS ARE DISCOVERED (OR DESIGNED)

Finding natural products

Up to now, researchers discovered most new drugs by screening natural compounds isolated from crude extracts from plants, marine organisms, and microbes. The NCI receives these raw materials from several agencies that procure them from all over the world, stores them in its Natural Product Repository, subjects them to aqueous and organic extraction, and screens approximately 50 000 extracts per year for antitumor activity.[4] Currently, about 30% of anticancer drugs are natural products or derivatives thereof, discovered through this empiric process; examples are shown in **TABLE 1**.[5]

This approach takes advantage of the biological diversity of the species of the world—a renewable source of molecules that generally are far too complex for cost-effective large-scale synthesis. Its disadvantage is its randomness. In addition, scarcity of a natural product can limit drug production. For example, the development of paclitaxel (Taxol) was delayed by the need for a large quantity of bark from the Pacific yew tree, of which a limited supply was available.

Modifying existing drugs

Another method of drug creation is to alter an existing drug slightly to produce an analogue that is more effective, less toxic, more easily produced or more bioavailable (**TABLE 2**). Development of an analogue requires knowledge of the relationship between the structure of the drug to be modified and its activity.

An example of this process is camptothecin, tested in the 1960s and found to have antitumor activity, but abandoned because it caused severe hemorrhagic cystitis

and colitis. In the 1970s, water-soluble camptothecin analogues proved to be more potent and thus to produce only mild nonhematologic toxic effects. Several of these compounds may have activity in refractory tumors such as non-small cell lung cancer.[6]

The supply issue and environmental concerns that threatened the development of paclitaxel stimulated synthesis of its analogue, docetaxel (Taxotere), which is produced from yew needles, an abundant and renewable portion of the tree.[7] Moreover, chemists can now semisynthesize paclitaxel from Pacific yew needles, which is expected to allow its large-scale production.[8]

TABLE 3

EXAMPLES OF ANTIMETABOLITE ANTICANCER DRUGS

Drug	Natural analogue	Metabolic pathway inhibited	Tumor types used against
5-Fluorouracil	Uracil	Pyrimidine synthesis	Colon, head and neck, breast
6-Thioguanine	Guanine	Purine synthesis	Childhood acute myelogenous leukemia
Cytosine arabinoside	DeoxyCytidine	Pyrimidine synthesis	Acute myelogenous leukemia Acute lymphocytic leukemia Non-Hodgkin's lymphoma
Cladribine	Adenosine	DNA synthesis	Hairy cell leukemia Chronic lymphocytic leukemia Non-Hodgkin's lymphoma
Fludarabine	Adenosine	DNA synthesis	Chronic lymphocytic leukemia
Gemcitabine	DeoxyCytidine	Pyrimidine synthesis	Non-small cell lung cancer Small cell lung cancer Ovarian, bladder, pancreatic cancer

An example of a drug analogue with improved oral bioavailability is cyclophosphamide. This congener of mechlorethamine is metabolized in the liver to its active form, 4-hydroxycyclophosphamide, allowing oral administration.

Rational drug design

Rational drug design is the synthesis of a compound to block a specific molecular target or process. Often, these drugs are analogues of relatively simple ligands such as nucleic acids. Examples of such drugs include 5-fluorouracil (5-FU), a fluorinated uracil analogue first synthesized by Heidelberger and colleagues.[9] Another is methotrexate, an analogue of folic acid, responsible for the first reported remissions in acute leukemia (in 1948),[10] and the first cure of a cancer (choriocarcinoma) with chemotherapy.[11] Several other antimetabolites have become important anticancer drugs (**TABLE 3**).

With increased understanding of the molecular and genetic abnormalities of cancer, researchers have begun to target metabolic pathways and mutations to try to add more specificity to anticancer therapy. Examples of such targets and the drugs designed to interact with them include growth factor receptors (blocked by suramin),[12] protein kinase C (blocked by bryostatin 1),[13] *bcr-abl* oncogene (blocked by antisense oligonucleotides),[14] and tumor angiogenesis (blocked by carboxyamido-imidazole, CAI).[15]

Computer-aided drug design

Computers can create a molecular model of a substrate of interest (often an enzyme) on the basis of its X-ray crystallographic structure,[16] determine the size, shape, and electrical charge of its binding site or receptor site,[17,18] and create templates of molecules expected to bind to this receptor.[19,20] The computer then can match this array of putative ligands to the receptor site, evaluating the "quality" of their fit on the basis of steric complementarity and intermolecular forces,[16,19,20] and estimating their binding energies.[16] This procedure can screen up to 100 000 compounds per week.[21]

Because the substrates are usually enzymes and the ligands frequently peptides, delivery of intact drug to the site of action remains a major challenge in the further development of drugs designed by computer.[22] In addition, computer-aided drug design requires detailed knowledge of the structure of the target, and drugs so designed can lack specificity because of redundant domains on other proteins.[16]

Several novel inhibitors of thymidylate synthase are the first such anticancer drugs to undergo clinical trials.[23,24]

Combinatorial synthesis

The newest technique for drug creation is combinatorial synthesis, in which biochemists build and screen large numbers of compounds to bind a given receptor.

This powerful method allows biochemists to screen all permutations of the desired molecule, and to synthesize ligands which bind most effectively.

For example, suppose we wish to find a hexapeptide (a peptide six amino acids long) that will bind to a particular receptor. With 20 naturally occurring amino acids and six amino-acid positions, there are 20^6 (64 million) possible hexapeptides.

Testing all 64 million compounds is impossible, but an iterative process makes the task easier.[29–30]

The first step is to synthesize and test 20 different mixtures of hexapeptides, each one of which has a particular amino acid in the first position and a random sequence of amino acids in the remaining five positions denoted by DXXXXX, LXXXXX, etc, where D and L represent specific amino acids and X represents any of 20 possible amino acids. Of the 20 initial mixtures, the mixture with the greatest binding affinity (eg, DXXXXX) is selected.

Computer-aided drug design can create a molecular model of a receptor, then determine templates of molecules expected to bind to this site. Such techniques can screen up to 100 000 compounds per week

In the second step, 20 mixtures are created each with D in the first position and a specific amino acid in the second position (eg, DFXXXX, DKXXXX, etc). The most avid binding mixture (eg, DKXXXX) dictates synthesis of the third combination of mixtures, designated DKEXXX, DKLXXX, and so on.

By repeating this process, researchers can quickly zero in on the hexapeptide with the greatest binding affinity. Similar iterative synthesis and selection can be applied to molecules with variable sidegroups.

This method can produce large numbers of ligands. Current methods (ie, principally collection of natural products) add approximately 30 000 new compounds each year to the pool of compounds available for screening.[25] In contrast, combinatorial synthesis can generate up to 100 000 new compounds each month.[25]

Although combinatorial synthesis has not yet produced any cytotoxic anticancer agents, it has yielded novel agents for other diseases, and it holds extraordinary potential.[28,31–33] Small peptides that bind the opioid receptor might be useful as opioid antagonists[26]; those that bind the human immunodeficiency virus [HIV] protease could be useful as anti-HIV drugs.[27]

SCREENING NEW DRUGS

Natural products or synthesized compounds referred to the NCI enter the development pipeline by being screened for antitumor activity in vitro. The NCI tests the new drugs against an array of 60 human tumor cell lines representing the most common solid and hematologic malignancies,34 using an automated assay of cytotoxicity.

Knowing which cell lines a new drug is active against, researchers make a preliminary guess about which malignancies might respond to the drug. They also compare the drug's pattern ("fingerprint") of antitumor activity with those of existing drugs. This comparison can suggest a mechanism of action for a compound if its activity pattern matches that of known drugs. More important, a unique pattern of activity may suggest a novel mechanism of action. Such a compound is given high priority in the drug development queue.

Multidrug resistance. Some of the cell lines in the NCI screening panel on their cell surface a transport protein (P-glycoprotein) that expels foreign compounds from the cell, rendering them resistant to a wide variety of drugs.[35,36] Because many refractory tumors have multidrug resistance, agents active against several such cell lines are of considerable interest.

PRIORITIZING DRUG DEVELOPMENT

During its first 2 years of using the screening panel described above, the NCI screened more than 27 000 compounds, and referred more than 1000 (about 4%) of them for further evaluation.[4] With this many potential drugs, the NCI must prioritize. Criteria established in 1992 give priority to drugs with activity in a

disease-specific group of cell lines, a unique chemical structure, a unique cell-line activity profile suggesting a novel mechanism of action, and an adequate supply.[4]

FORMULATING DRUGS

Because most anticancer drugs are given by vein, formulation of an insoluble compound into a clinically useful drug that can be given intravenously can present an obstacle.[4] For example, paclitaxel was formulated in a mixture of polyoxyethylated castor oil (Cremophor EL) and ethanol. This castor oil vehicle may be the cause of the hypersensitivity reactions associated with this drug.[7]

In some cases, synthesis of a prodrug can solve the problem of solubility. This approach was applied to fludarabine, an insoluble nucleoside formulated as fludarabine monophosphate.[37] This molecule is dephosphorylated after administration and rephosphorylated inside the cell.[38]

PRECLINICAL TESTING

Compounds with promising in vitro activity enter animal testing, first in mice to test the drug's anticancer activity. Further tests, primarily in mice, rats, and dogs, give preliminary data about pharmacokinetics, metabolism, oral bioavailability, and toxicity.[4] The LD10—the dose of the drug that kills 10% of the animals—is used to estimate safe initial doses for human trials.

CLINICAL TRIALS

Clinical evaluation proceeds through three phases, each having specific goals and study designs. In general, eligible patients must have a good performance status (and thus be more likely to tolerate therapy and to benefit from it). In addition, they usually must have normal hepatic, renal, and bone marrow function. Finally, most patients entering phase I trials have received prior chemotherapy, whereas phase II and III trials are generally restricted to untreated patients.

Phase I

Phase I trials assess a drug's toxic effects, determine its maximum tolerated dose, and study its pharmacokinetic profile. Dosage schedules in phase I trials are based mainly on efficacy in animal studies but also on convenience. For example, a single dose every 3 weeks is likely to be more acceptable to patients than a 5-day-per-week schedule.

Most phase I trials are sponsored by the pharmaceutical firms that did the preclinical testing, and are carried out at several hospitals under contracts with this firm. If the NCI developed the drug, it sponsors phase I trials at institutions with contracts to conduct such trials. These hospitals generally have experience in conducting clinical trials and have laboratories for performing detailed pharmacokinetic and pharmacodynamic analyses.

In a typical phase I trial, cohorts of three to six patients receive increasing doses of a drug according to a predetermined dose-escalation scheme. The initial dose level is commonly a fraction, usually 10%, of the LD10 in the most sensitive animal species tested. The likelihood that this initial dose level will exceed the maximum tolerated dose is only 1%.[39] The dose escalation usually follows a modified Fibonacci series in which early dose increases are large fractions of the initial dose level.[40]

The primary goal in phase I trials is to find the maximum tolerated dose; once this dose is reached, the trial is stopped. Researchers try to reach the maximum tolerated dose with a minimum number of patients treated, but without starting at a dose higher than the maximum tolerated dose.

Phase I trials find the maximum tolerated dose

The secondary goal is to obtain accurate and detailed pharmacokinetic data, which can provide important pharmacodynamic correlates of toxicity and efficacy, and can suggest rational dosage schedules for a given drug, especially in combination regimens.

Phase I trials give little information about efficacy, for several reasons. Because these trials usually include patients with a variety of malignant diseases, they cannot provide efficacy data for a particular disease. Further, because of the dose escalation format, most patients receive less than the optimal dose. Patients who enter a phase I trial usually do so because of lack of effective standard therapy or after having exhausted standard therapies. Thus, many have refractory diseases. For these reasons, and because most drugs that reach phase I trials ultimately prove ineffective, the overall response rate in phase I trials is probably less than 10%.

Therefore, lack of activity in a phase I study does not rule out further evaluation. Conversely, any antitumor activity observed in a phase I trial, even if modest, almost always

warrants a phase II trial in that tumor type. Several proposed modifications of the phase I study design would minimize the study size and expedite the dose escalation.[41,42]

Phase II

After phase I trials determine the phase II dose, an array of phase II trials begins. The drug is tested in several diseases, including those in which responses occurred in phase I trials and those for which adequate chemotherapy is lacking (eg, pancreatic cancer, non-small cell lung cancer). A specified dose is given to patients with a specific malignancy to assess the activity of the drug against that malignancy. This small, single-arm trial will suggest whether the drug represents an advance in the therapy of the disease in question and whether a larger, randomized phase III trial is warranted. A phase III trial is indicated if the drug appears more effective than available drugs, or if it is equally effective but less toxic or more convenient (eg, can be given on an outpatient basis).

Approval of drugs for treating serious illnesses can be based on surrogate endpoints

The results of phase II trials determine whether further development of a drug should proceed,[3] as drugs with insignificant anticancer activity are usually discarded. Phase II studies also provide important additional data about toxicity.

Phase III

Once a drug has demonstrated activity in a phase II study, a randomized phase III trial compares it with a control treatment, usually the standard chemotherapy for the disease under study. For poorly responsive tumors such as non-small cell lung cancer or pancreatic cancer, the control treatment may be placebo or "best supportive care."[43] Because the difference in efficacy or toxicity between the treatments may be small, phase III trials need large numbers of patients to have sufficient power to detect such a difference.[40] Thus a phase III trial may require hundreds of patients and may take several years to complete. Careful design of such a large trial is critical to its success and usefulness.

A phase III trial can establish a new standard of care. For example, in a phase III trial in patients with advanced epithelial ovarian cancer,[44] the combination of paclitaxel and cisplatin induced a better response rate than did the standard regimen of cyclophosphamide and cisplatin (77% vs 64%); it also produced a 34% lower risk of recurrence, a longer progression-free interval (18 vs 13 months), and a longer overall median survival (37 vs 24 months).

Quality-of-life issues. Although the primary endpoints in phase III trials are response rate, response duration, and survival, quality-of-life measurements are becoming increasingly more important. Demonstration of improved quality of life is a criterion in the FDA's new drug approval process.

THE DRUG APPROVAL PROCESS

The regulatory process for investigational drugs begins when a sponsor such as a pharmaceutical firm submits an Investigational New Drug (IND) application, to notify the FDA that testing in humans is ready to begin. The FDA reviews the IND to ensure the safe conduct of clinical trials and to prevent the testing of blatantly hazardous therapies in patients.[45] Furthermore, the review process for phase II and III trials focuses on the study design to help ensure that trials, if positive, would lead to approval of the drug in question.[45] The FDA strongly encourages drug sponsors to consult it before starting phase III trials so that these large and expensive trials will have the best chance of resulting in drug approval.[46]

Once a sponsor has enough data to support the use of a drug for a given indication, a New Drug Application (NDA) is submitted.[47] This comprehensive document contains all of the data relevant to the manufacture, pharmacology, toxicology and efficacy of the drug in question. A process of negotiation between the FDA and the drug sponsor follows, in which questions regarding data in the NDA are addressed; in some cases, further clinical trials are mandated. The dialogue between the FDA and sponsor lasts months to several years.[48]

In general, to approve a new agent, the FDA requires at least two controlled studies that demonstrate improved survival or improved quality of life in comparison to standard therapy—ie, phase III studies.[46] Occasional exceptions are made if results from phase II studies are particularly striking. For example, ifosphamide was approved for salvage therapy of testicular cancer on the basis of phase II trials of ifosphamide-based combinations in which approximately 20% of patients achieved long-term remissions.[49]

Accelerated approval process. In 1992, the FDA issued criteria for accelerated approval of drugs for treating serious illnesses such as cancer or acquired immunodeficiency syndrome (AIDS). Under these guidelines, approval can be based on surrogate endpoints other than improved survival, provided the sponsors agree to perform further studies to verify benefit; failure to demonstrate clear benefit in subsequent trials would constitute grounds for the FDA to remove the drug from the market.[50]

Liposomal doxorubicin was approved for refractory AIDS-related Kaposi's sarcoma under these guidelines. The surrogate endpoints in this case were flattening of previously-raised lesions, decreased edema and pain, and improvement in lesion color.[51]

NEW DRUGS IN DEVELOPMENT

TABLE 4 lists several classes of new drugs undergoing clinical development. This list is not comprehensive but attempts to highlight cytotoxic compounds that have shown promise (eg, gemcitabine for non-small cell lung cancer), represent novel techniques of drug design (two thymidylate synthase inhibitors designed on basis of crystal structure of target molecule), have FDA approval for at least one indication (eg, paclitaxel, vinorelbine), or have made significant alterations in the management of a given disease (eg, *all-trans* retinoic acid for acute promyelocytic leukemia).

Due to the limited scope of this review, several broad categories of therapeutic agents have been excluded, such as antisense nucleotides, biologic response modifiers, and gene therapies. ■

TABLE 4

CLASSES OF ANTICANCER DRUGS CURRENTLY UNDER DEVELOPMENT

Class	Examples	Comments
Thymidylate synthase inhibitors	Tomudex LY231514 BW1843U89 AG-331 AG-337	AG-331 and AG-337 based on crystal structure of thymidylate synthase
Differentiation-inducing agents	Retinoic acid analogues	Striking activity in acute promyelocytic leukemia
Angiogenesis inhibitors	Fumagillin analogues Pentosan	
Topoisomerase I inhibitors	Topotecan Irinotecan (CPT-11) 9-Aminocamptothecin	
Antimetabolites	Gemcitabine	Analogue of cytarabine
Taxanes	Paclitaxel	Docetaxel
Vinca alkaloids	Vinorelbine	Recently FDA-approved for non-small cell lung cancer
Dihydropyrimidine dehydrogenase inhibitors	5-Ethyluracil	New class of drugs that inhibits metabolism of 5-fluorouracil
Anthracyclines	Methoxymopholino doxorubicin	Preclinical activity against multidrug resistant cell lines

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