Review article

Natural product drug discovery and development at the United States National Cancer Institute

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In 1937, the United States National Cancer Institute (NCI) was established with its mission being "to provide for, foster and aid in coordinating research related to cancer." In 1955, NCI set up the Cancer Chemotherapy National Service Center (CCNSC) to promote a cancer chemotherapy program, involving the procurement, screening, preclinical development, and clinical evaluation of new agents. All aspects of drug discovery and preclinical development are now the responsibility of the Developmental Therapeutics Program (DTP), a major component of the Division of Cancer Treatment (DCT). During the past 39 years, over 400,000 chemicals submitted by investigators and organizations worldwide, have been screened for antitumor activity, and NCI has played a major role in the discovery and development of almost all of the available commercial and investigational anticancer agents (1).

Prior to 1960, the screening of natural products was concerned mainly with the testing of fermentation products, but the establishment of an interagency agreement with the United States Department of Agriculture (USDA) in 1960 for the collection and screening of plants marked the start of a systematic program for the discovery and development of anticancer agents from plant sources. Between 1960 and 1982 35,000 plant samples were collected by the USDA, mainly from temperate regions. Over 114,000 extracts of these plants were tested, and a large number of agents belonging to a wide variety of chemical classes were isolated and characterized (2). Few of these new agents, however, satisfied the stringent preclinical development requirements and advanced to human clinical trials. Clinically-active agents to emerge from this program include taxol from *Taxus brevifolia* Nutt. and other *Taxus* species (3), topotecan (4) and CPT-11 (5), semisynthetic derivatives of camptothecin from *Camptotheca acuminata* Decne, and homoharringtonine from *Cephalotaxus harringtonia* var. *drupacea* (Sieb. & Zucc.) Koidzumi (6). Taxol was approved by the United States Food and Drug Administration (FDA) for general use in the treatment of advanced ovarian cancer in December, 1992, and for treatment of breast cancer in April, 1994. Thus, it joins vinblastine and vincristine, and etoposide and teniposide, semisynthetic derivatives of epipodophyllotoxin (an epimer of podophyllotoxin) (7), as the only plant-derived anticancer drugs to receive full FDA approval to date

The plant collections, started in 1960 in collaboration with the USDA, were discontinued in 1982 since it was perceived that few novel agents were being isolated from plants and other natural sources. Of particular concern was the failure to yield agents effective against the resistant solid tumor disease-types. This apparent failure may have been due more to the limitations of the *in vivo* primary mouse leukemia screens rather than a deficiency in Nature. With the development of a new *in vitro* screening strategy, involving the use of 60 human tumor cell lines (1), in 1985, however, the NCI once again turned to Nature as a potential source of novel anticancer agents, and a new natural products acquisition program was implemented in 1986. The initiation, in 1987, of a major new program within the NCI for the discovery and development of agents for the treatment of acquired immunodeficiency syndrome (AIDS) (8) provided yet further impetus for the revitalization of the NCI's focus upon natural products.

The National Cancer Institute Plant Aquisition Program: 1986-Present

The NCI places a major emphasis on natural products as a source of potential new anticancer and anti-HIV agents, and, in addition to terrestrial plants, is investigating marine invertebrates and plants, as well as unusual microorganisms, such as fungi, marine anaerobes and protists. For the purposes of this presentation, however, the discussion will be limited to the terrestrial plant collection program.

In September of 1986, three five-year contracts were awarded for the collection of plants in tropical and sub-tropical regions worldwide. In selecting these regions as the new focus for plant collections, the NCI recognized that tropical forests contain well over 50% of the estimated 250,000 plant species found on earth; only a very small percentage have been investigated for their chemotherapeutic potential, and relatively few had been screened in the earlier NCI program. In addition, the rapid destruction of tropical forests and the disappearance of indigenous knowledge associated with the use of many of the plants lent an urgency to the initiation of a collection program before these valuable resources had been too severely depleted. While investigating the potential of these plants in drug discovery and development, the NCI also wishes to promote the conservation of biological diversity, and recognizes the need to compensate source country organizations and peoples in the event of commercialization of a drug developed from a plant collected within their borders.

The initial contracts were awarded to the Missouri Botanical Garden, the New York Botanical Garden, and the University of Illinois at Chicago (assisted by the Arnold Arboretum of Harvard University and the Bishop Museum in Honolulu) for collections in Africa and Madagascar, Central and South America, and Southeast Asia, respectively. In carrying out these collections in over 20 countries, the NCI contractors work closely with qualified organizations in each of the source countries. Botanists from source country organizations have collaborated in field collection activities and taxonomic identifications, and their knowledge of local species and conditions has been indispensable to the success of the NCI collection operations to date. Source country organizations provide facilities for the preparation, packaging, and shipment of the samples to the NCI natural products repository in Frederick, Maryland. The collaboration between the source country organizations and the NCI collection contractors has, in turn, provided support for expanded research activities by source country botanists, and the deposition of a voucher specimen of each species collected in the national herbarium, is expanding source country holdings of their flora. When requested, NCI contractors also provide training opportunities for local personnel through conducting of workshops and presentation of lectures. In addition, through its Letter of Collection (LOC) (9) and agreements based upon it, the NCI invites scientists nominated by Source Countries Organizations to visit its facilities, or equivalent facilities in other approved

U.S. organizations, to participate in collaborative research aimed at the isolation of active agents, preferably from organisms collected in their countries.

The NCI, through its LOC, is committed to technology transfer and the sharing of expertise in order to promote greater in-country processing and evaluation of source country genetic resources. A key provision in the LOC is the requirement for the successful licensee of any drug patented by NCI to negotiate acceptable terms of compensation (e.g. a percentage of the royalties accruing from the sale of the drug) directly with the appropriate organization or Government agency in the source country of the organism yielding the drug. Such compensation is regarded as a potential long-term benefit, since the development of a drug to the stage of marketing can take from 10 to 20 years from its time of discovery.

Collections encompass a broad taxonomic diversity, but emphasis is given to the collection of medicinal plants when reliable information on their use is available. Detailed documentation of each sample is required, including taxonomy, plant part, date and location of collection, habitat, hazards (e.g., thorny) and, when available, medicinal uses and methods of preparation used by the healer. Each sample is assigned a unique NCI collection number, expressed in the form of a barcode label, which is attached to the sample bag in the field. In addition, at least five voucher specimens of each plant species are prepared. One voucher is deposited in the national herbarium in the country of collection, while another is deposited with the Botany Department of the Smithsonian Institution Museum of Natural History in Washington, D.C.; this latter collection serves as the official national and NCI collection. The dried plant samples are shipped by air freight to the NCI Natural Products Repository (NPR) in Frederick, Maryland, where they are stored at -20 degrees C for at least 48 hours in order to minimize the survival of plant pests and pathogens. This freezing period is a requirement of the import permit issued by the United States Department of Agriculture Animal and Plant Health Inspection Service, which has provided excellent support to the NCI in facilitating the importation of thousands of plant samples.

Natural product drug discovery

After freezing, the plant samples are ground and sequentially extracted with a 1:1 mixture of methanol:dichloromethane and water to give organic solvent and aqueous extracts. All the extracts are assigned discrete NCI extract numbers and returned to the NPR for storage at -20 degrees C until requested for screening or further investigation.

Extracts are tested *in vitro* for selective cytotoxicity against panels of human cancer cell lines representing major disease types, including leukemia, melanoma, lung, breast, colon. central nervous system, ovarian, prostate and renal cancers (1). *In vitro* anti-AIDS activity is determined by measuring the survival of virus-infected human lymphoblastoid cells in the presence or absence of the extracts (8). Extracts showing significant selective cytotoxicity or anti-AIDS activity are subjected to bioassay-guided fractionation by chemists and biologists to isolate the pure chemical constituents responsible for the observed activity. In bioassay-guided fractionation, all fractions produced at each stage of the separation procedure are tested for activity in the relevant bioassay, and subsequent fractionation steps are only performed on those fractions showing significant activity. This process of fractionation and testing is continued until the pure active constituent(s) is isolated. Bioassay-guided fractionation is essential since, in most instances, the active constituents are present in only small amounts in the crude extracts, and are generally isolated in yields of 0.01% or less, based on the mass of dried plant material. After the active constituent is isolated from an extract, its complete chemical structure is elucidated using modern spectroscopic techniques, and, if necessary and possible, x-ray crystallography.

This isolation and structural elucidation of a potential new agent is but the first phase in a lengthy process of development towards clinical trials, and possible general clinical use.

Drug development

Agents showing significant activity in the primary *in vitro* human cancer cell line or anti-AIDS screens are entered into various stages of preclinical development to determine their suitability for eventual advancement to clinical trials with human patients.

The first stage of preclinical development involves the large-scale production of the active agent. Large recollections of the source raw material are carried out in the country of origin of the organism or, if supply is a limiting factor, the cultivation of the organism in the source country is studied in collaboration with source country scientists. Once sufficient supplies of the active agent are available a suitable aqueous vehicle is developed to solubilize the drug to enable administration to patients, generally by intravenous injection or infusion. Detailed pharmacological evaluation is performed to determine the availability of the drug in biological fluids (bioavailability), its rates of clearance, excretion, and metabolism. The final critical step is the toxicological evaluation in animals (rodents and dogs) to determine the types and degrees of major toxicities, and a safe starting dose in humans.

If the preclinical data is approved by the Food and Drug Administration, the drug advances to Phase I clinical trials to determine the maximum tolerated dose and the nature of toxicities in humans. If acceptable, the drug advances to Phase II trials to determine its efficacy against different cancers (or AIDS and its opportunistic infections). Advancement to Phase III trials assesses the efficacy of the drug compared to currently available chemotherapeutic agents, and it may also be tried in combination with other effective agents to determine if the efficacy of the combined regimen exceeds that of the individual drugs when used alone.

Costs and timespans of drug discovery and development

The preceding discussion illustrates the complexities of the drug discovery and development process, with particular reference to anticancer drugs. Though the percentage of natural product extracts showing preliminary activity in an *in vitro* screen might vary from less than 1% to 5%, the number of potentially valuable "leads" from plant and animal sources is more likely to be one in 5,000 to 10,000. Such "leads" will undergo extensive research and development, and probably less than 50% of those will advance to commercial drug status. Considering the NCI anticancer screening program from 1960 to 1982, of the 114,000 plant extracts screened, only taxol has advanced to final FDA approval, while camptothecin has yielded three semisynthetic derivatives which show clinical promise and might advance to commercial status. Homoharringtonine is showing efficacy against certain leukemias, and eventually might be developed as a second-line anticancer agent. Meanwhile, considerable resources were devoted to the development to clinical trials of nine other agents, including bruceantin, camptothecin, indicine N-oxide, maytansine, and thalicarpine, only to have trials terminated due to lack of efficacy or unacceptable toxicity; many other agents were entered into preclinical development but dropped for various reasons (10). The chances of

developing an effective commercial anticancer drug, such as taxol, therefore, are of the order of one in 40,000 to 50,000, based on number of plant extracts screened. The timespan for development can vary considerably, and can be from 10-20 years for anticancer drugs. Research on the isolation of taxol started in the mid-1960s and its structure was first published in 1971 (11). Its development was delayed for various reasons (12), but, once efficacy against refractory ovarian cancer was observed in late 1988, advancement to final FDA approval was relatively rapid.

In the light of the time and resources which are required for development of a commercial drug, and the resources devoted to eventual failed candidates, it is not surprising that cost estimates for drug discovery and development exceed U.S. $230 million (13). The benefits in terms of relief of human suffering that result from the development of a drug, such as taxol, however, must surely outweigh the monetary costs, and increased emphasis must be devoted to the search in Nature for other equally effective chemotherapeutic agents.

Recent developements in the NCI Drug Discovery Program

From September, 1986 to June, 1994, over 41,000 plant samples, including different parts, such as leaves, bark, wood, and fruit, had been collected. Of these, over 31,000 had been extracted with an organic solvent and water to yield over 62,000 extracts.

Over 30,000 of these extracts have been tested in the anti-AIDS screen, and over 5% have shown some *in vitro* activity. The majority of the active extracts, however, are aqueous, and, in most instances, the activity has been traced to the presence of ubiquitous chemotypes, such as polysaccharides and tannins. Such compounds are not a current NCI focus for drug development, and typically are eliminated early in the fractionation process.

A number of novel *in vitro* active anti-AIDS agents, isolated using bioassay-guided fractionation procedures, have been selected for preclinical development. The dimeric alkaloid, michellamine B has been isolated from the leaves of the liana, *Ancistrocladus korupensis*, collected in the rainforest regions of Cameroon (14). Michellamine B shows *in vitro* activity against both the HIV-1 and HIV-2 forms of the AIDS virus, as well as several resistant strains of the virus. The abundance and potential for sustainable harvest, as well the possibility of mass cultivation in Cameroon, are being investigated by the NCI contractor, Purdue University, in collaboration with Cameroon scientists, the World Wide Fund for Nature, and several U.S. organizations. The concentrations of the agent required to achieve significant *in vitro* anti-AIDS activity are relatively high, and pharmacological evaluation is currently directed at determining whether these concentrations can be achieved in animals without unacceptable toxicity being observed. The preparation of more active analogues, either using semisynthetic or total synthetic procedures, is also being investigated. Thus far, no other *Ancistrocladus* species have shown significant anti-AIDS activity. An agreement based on the LOC and including the development of michellamine B was signed with the University of Yaounde, but a new agreement with the Government of Cameroon is currently being considered.

Calanolide A is novel coumarin isolated from the leaves and twigs of the tree, *Calophyllum lanigerum* Miq. var. *austrocariaceum* (T.C. Whitmore), collected in rainforest regions of Sarawak (15). Calanolide A shows potent *in vitro* activity against HIV-1 and several resistant strains of the virus, but not against HIV-2. Recollections of plant material identified as *C. lanigerum* from the same general location have not yielded significant quantities of the active agent, and a synthesis of racemic calanolide A was recently reported. While surveying the occurrence and abundance of *C. lanigerum*, other *Calophyllum* species were screened for anti-HIV activity, and a related active agent, costatolide, has been isolated in high yield from the latex of *C. teysmanii var.* inophylloide. While costatolide does not exhibit the same degree of activity as calanolide A, its relative abundance and ease of isolation from the renewable latex source makes it an attractive candidate for consideration as an alternative to calanolide A for further research and development. An agreement between the Sarawak State Government and the NCI for the development of these agents and collaboration in the investigation of Sarawak's natural resources has been signed. A scientist from the University of Malaysia Sarawak is currently visiting the NCI to gain experience in the isolation of costatolide and the screening techniques used at the NCI.

In late 1981, a sample of a *Conospermum* species was collected in Western Australia as part of the USDA collections for the NCI screening program. This and other Western Australian samples, were not investigated at the time due to the de-emphasis of natural products in the NCI program. With the revitalization of the NCI natural products program in 1986, these samples were tested in the anticancer and anti-AIDS screens. An extract of the original collection of the *Conospermum* species showed significant activity in the anti-HIV screen, and bioassay-guided fractionation of this extract yielded conocurvone as the active agent (16). Conocurvone shows potent *in vitro* activity against HIV-1, and further development is being undertaken in collaboration with scientists of the Conservation and Land Management Agency (CALM) of the Western Australian State Government, together with other Australian organizations.

The value of traditional knowledge in guiding the discovery of new active agents is illustrated by the isolation of the potential new anti-AIDS drug, prostratin, from the Western Samoan tree, *Homalanthus nutans* (Forster) Pax (17). This plant was selected for study by Dr. Paul Cox on the basis of extensive interviews with Samoan traditional healers, who use it for the treatment of a variety of diseases, including yellow fever.

Biodiversity prospecting and drug discovery

Given the high costs and lengthy timespan required for drug development, as well as the apparently small number of commercially available natural product-derived drugs, biodiversity prospecting might seem to hold limited promise as a source of novel, effective chemotherapeutic agents. The development of new screens and the dramatic improvements in screening technology, however, are significantly expanding the potential of natural products as sources of new drugs and other bioactive agents (e.g., agrochemicals). The emergence of increasing resistance of diseases, such as malaria, tuberculosis, and pneumonia to commonly-used drugs, and the scourge of the global AIDS epidemic, make the necessity to discover new drugs all the more urgent. While it is impossible to determine how many species have been fully investigated for pharmaceutical potential, it is likely that the number is extremely small; some estimates place the number of tropical plant species that have been studied phytochemically at less than one per cent. In addition, Nature is the supreme molecular architect, producing novel complex molecules such as taxol and the *Vinca* alkaloids, vinblastine and vincristine. While some natural products constitute the actual commercial drug, many are used as models for modification by medicinal chemists in the synthesis of more effective agents. The expanding investigation of sources other than terrestrial plants, including marine invertebrates and unusual microbes, is yielding new, bioactive chemotypes which hold promise for the development of effective chemotherapeutic agents. These factors seem to suggest that "the chemical properties of biodiversity represent a potentially limitless renewable resource pool for use in the development of new pharmaceuticals" (18).

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individuals and organizations worldwide which make these programs possible. From the collection of organisms in over 25 countries to the clinical trials of new drugs, and the studies of occurrence and prevention, this is truly an international effort in the fight against the scourges of AIDS and cancer. NCI recognizes the indispensable contributions being made through the provision of valuable natural resources, expertise, knowledge, and skills; through policies of collaboration and compensation, as stated in the Letter of Collection, NCI wishes to assure participating countries of its intentions to deal with them in a fair and equitable manner.

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