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National Cooperative Drug Discovery Groups (NCDDGs): A Successful Model for Public Private Partnerships in Cancer Drug Discovery

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Abstract

The United States National Cancer Institute (NCI) has been investigating Nature as a source of novel agents for the treatment of cancer for over 45 years, and has contributed to the development of many of the currently available anticancer drugs. The National Cooperative Drug Discovery Group (NCDDG) program was started in 1983 as mechanism to promote multidisciplinary, multi-institutional approaches to drug discovery, and has led to the development of a number of successful clinical agents. The program is reviewed in terms of achievements and challenges facing further research in the exploration of natural resources as a source of new drugs.

Background

The National Cancer Institute (NCI) was established in 1937, 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 coordinate a national voluntary cooperative cancer chemotherapy program, involving the procurement of drugs, screening, pre-clinical studies, and clinical evaluation of new agents. By 1958, the initial service nature of the organization had evolved into a drug research and development program with input from academic sources and substantial participation of the pharmaceutical industry. The responsibility for drug discovery and pre-clinical development at NCI now rests with the Developmental Therapeutics Program (DTP), a major component of the Division of Cancer Treatment and Diagnosis (DCTD). Thus, NCI has for the past 40 years provided a resource for the pre-clinical screening of compounds and materials submitted by grantees, contractors, pharmaceutical and chemical companies, and other scientists and institutions, public and private, worldwide, and has played a major role in the discovery and development of many of the available commercial and investigational anticancer agents. During this period, more than 500,000 chemicals, both synthetic and natural, have been screened for antitumor activity.

Initially, most of the materials screened were pure compounds of synthetic origin, but the program also recognized that natural products were an excellent source of complex chemical structures with a wide variety of biological activities. From 1960 to 1982 over 180,000 microbial-derived, some 16,000 marine organism-derived, and over 114,000 plant-derived extracts were screened for antitumor activity, mainly by the NCI, and, as mentioned above, a number of clinically effective chemotherapeutic agents have been developed (Cragg & Newman, 1999).

Contracts for the cultivation and extraction of fungi and cyanobacteria, and for the collection of marine invertebrates and terrestrial plants, were initiated in 1986, and, with the exception of fungi and cyanobacteria, these programs continue to operate. Marine organism collections originally focused in the Caribbean and Australasia, but have now

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expanded to the Central and Southern Pacific and to the Indian Ocean (off East and Southern Africa) through a contract with the Coral Reef Research Foundation, which is based in Palau in Micronesia. Terrestrial plant collections have been carried out in over 25 countries in tropical and subtropical regions worldwide through contracts with the Missouri Botanical Garden (Africa and Madagascar; 1986–present), the New York Botanical Garden (Central and South America; 1986–1996), and the University of Illinois at Chicago (Southeast Asia; 1986–present), and have been expanded to the territorial United States through contracts with the Morton Arboretum (1996–2001) and World Botanical Associates (2001–present) .

In carrying out these collections, the NCI contractors work closely with qualified organizations in each of the source countries. Botanists and marine biologists from source country organizations collaborate in field collection activities and taxonomic identifications, and their knowledge of local species and conditions is indispensable to the success of the NCI collection operations. Source country organizations provide facilities for the preparation, packaging, and shipment of the samples to the NCI's Natural Products Repository (NPR) in Frederick, Maryland. The collaboration between the source country organizations and the NCI collection contractors, in turn, provides support for expanded research activities by source country biologists, and the deposition of a voucher specimen of each species collected in the national herbarium or repository is expanding source country holdings of their biota. When requested, NCI contractors also provide training opportunities for local personnel through conducting workshops and presentation of lectures. In addition, through its Letter of Collection (LOC; Appendix A) and agreements based upon it, the NCI invites scientists nominated by Source Country Organizations to visit its facilities, or equivalent facilities in other approved U.S. organizations for 1–12 months to participate in collaborative natural products research. Representatives of many of the source countries have visited the NCI and contractor facilities for shorter periods to discuss collaboration. The LOC also dictates terms of benefit-sharing and use of source country resources in the event of the licensing and development of a promising drug candidate. It should be noted that the formulation of the NCI policies for collaboration and compensation embodied in the Letter of Collection predated the drafting of the United Nations Convention on Biological Diversity in Rio De Janeiro by some four years. Contract collections of plants are now being de-emphasized in favor of establishing direct collaborations with qualified organizations in the source countries.

Dried plant samples (0.3–1 kg dry weight) and frozen marine organism samples (~1 kg wet weight) are shipped to the NPR in Frederick where they are stored at −20 °C prior to extraction with a 1 : 1 mixture of methanol: dichloromethane and water to give organic solvent and aqueous extracts. All extracts are assigned discreet NCI numbers and returned to the NPR for storage at −20 °C. In carrying out the collection and extraction of thousands of plant and marine organism samples worldwide, the NCI has established a Natural Products Repository which is a unique and valuable resource for the discovery of potential new drugs and other bioactive agents. The NCI has developed policies for the distribution of extracts from the NPR to qualified organizations for testing in screens related to all human diseases, subject to the signing of a legally-binding Material Transfer Agreement (MTA) which protects the rights of all parties (see http://dtp.nci.nih.gov/branches/npb/repository.html). One of the key terms of the MTA is the requirement that the recipient organization negotiate suitable terms of collaboration and compensation with the source country(ies) of any extracts which yield agents which are developed towards clinical trials and possible commercialization.

With the increased awareness of genetically-rich source countries to the value of their natural resources and the confirmation of source country sovereign rights over these resources by the U.N. Convention on Biological Diversity, organizations involved in drug discovery and development are increasingly adopting policies of equitable collaboration and compensation in interacting with these countries. Particularly in the area of plant-related studies, source country scientists and governments are committed to performing more of the operations in-country, as opposed to the export of raw materials. The NCI has recognized this fact for several years, and has negotiated Memoranda of Understanding (see http://dtp.nci.nih.gov/branches/npb/agreements.html) with a number of source country organizations suitably qualified to perform in-country processing. In considering the continuation of its plant-derived drug discovery program, the NCI has de-emphasized its contract collection projects in favor of expanding closer collaboration with qualified source country scientists and organizations. In establishing these collaborations, the NCI undertakes to abide by the same policies of collaboration and compensation as specified in the LOC. Through this mechanism collaborations have been established with organizations in Australia, Bangladesh, Brazil, China, Costa Rica, Fiji, Iceland, South Korea, Mexico, New Zealand, Nicaragua, Pakistan, Panama, Papua New Guinea, South Africa, and Zimbabwe.

In the case of organizations wishing to have pure compounds tested in the NCI drug screening program, such as pharmaceutical and chemical companies or academic research groups, the DTP/NCI has formulated a screening agreement which includes terms stipulating confidentiality, patent rights, routine and non-proprietary screening and testing versus non-routine, and levels of collaboration in the drug development process (see http://dtp.nci.nih.gov/docs/misc/common_files/canagr.html). Individual scientists and research organizations wishing to submit pure compounds for testing generally consider entering into this agreement with the NCI DCTD. Should a compound show promising anticancer activity in the routine screening operations, the NCI will propose the establishment of a more formal

collaboration, such as a Cooperative Research and Development Agreement (CRADA) or a Clinical Trial Agreement (CTA).

The NCI DTP offers access to a considerable body of data and background information through its WWW Homepage: http://dtp.nci.nih.gov/

The National Cooperative Drug Discovery Program Group (NCDDG)

Past history

Under the NCDDG funding mechanism, support is provided for investigator-initiated research consortia, including significant participation of the funding agency, involved in the performance of research related to the discovery and development of novel cancer treatments. The program was initiated in 1982 when the NCI, acting on the advice of the former Division of Cancer Treatment's Board of Scientific Counselors, issued the first NCDDG Request for Applications in 1983. The intent of this program was to combine the talents and expertise of researchers in cancer biology, pharmacology and chemistry in the formation of teams possessing a high degree of synergy for the development of systematic approaches for drug discovery. Since then the NCDDG program has been competed several times, most recently in 2004. During its 20-year history from 1983 to 2003, a total of 42 awards have been made, with 16 groups successfully receiving competitive renewal of funds. During these years, the Program has evolved into a highly successful model for the implementation of multi-institutional and multidisciplinary approaches for drug discovery. Through the participation of academia, industry and government, the program has created a team approach, and has been able to leverage considerable resources and talents from these participating components. Compared to other programs of this size, such as the program project grants funded by the NIH, the NCDDG's focus is on the discovery of drugs, and not just restricted to mechanistic studies; it emphasizes the development and use of assays incorporating novel targets and pathways, and industry cost shares in terms of the provision of many such assays. There has been significant cross-fertilization of knowledge and expertise among the consortium members, and recently, with the explosion of knowledge in cancer genetics and the availability of high throughput assays, as well as imaging in studying drug uptake and distribution, chemists continue to learn about new developments in assays, and preclinical models. Similarly biologists are gaining greater appreciation on the chemistry front, especially related to the intricacies associated with natural product sourcing and compound availability.

The NCDDGs are cooperative agreement awards funded by the NCI and managed by the Grants and Contracts Operations Branch (GCOB) of the Developmental Therapeutics Program (DTP) in the Division of Cancer Treatment and Diagnosis. DTP supports all aspects of preclinical anticancer drug discovery and treatment strategies, including drug design, selective targeting of therapeutic agents, development of new preclinical models for drug discovery, and understanding, preventing and overcoming drug resistance. GCOB's grants and cooperative agreement portfolio covers synthesis, medicinal and natural products chemistry, screening and experimental therapeutics, pharmacology, toxicology, biochemistry and mechanism of action studies. GCOB functions in a unique position in relation to the extramural grant community and the drug discovery and development activities of DTP. This organizational integration allows GCOB staff to maintain extensive interactions with the extramural research community and provide advice to foster the drug discovery mandate of DTP. These activities include assisting investigators to access the DTP's resources and expertise in preclinical research. The Natural Products Branch, with its history of managing and coordinating NCI's natural products sample collection contracts in the international arena, has been invaluable in assisting the management of natural products-related grants and cooperative agreements, such as the NCDDGs. Various components of DTP are working in concert to help grantees to access the NCI synthetic and natural products repositories.

Since the inception of the NCDDG concept, the program has grown from the first funding cycle of two grants to today's 13 funded groups. Among them, five NCDDGs are natural products-based (Table 1), one is on biologicals, and the remaining seven groups are focused on mechanism of action studies (see Table 2).

Major NCDDG accomplishments

Throughout the 20-year period of NCDDG funding, hundreds of thousands of natural products extracts and synthetic compounds have been tested for their potential as anticancer drug leads, and a number of agents have progressed through various stages of preclinical development. Some agents derived from NCDDG programs are currently in advanced preclinical and clinical development. The following are examples of investigative agents that have had substantial involvement from the NCDDG program, and some have successfully advanced through clinical trials and gained market approval by the Food and Drug Administration (FDA) as treatments for cancer.

Topotecan

Approved in 1996. Topotecan is a semi-synthetic derivative of camptothecin, an alkaloid discovered by Wall and Wani from *Camptotheca acuminata*. The natural product itself had undesirable pharmaceutical properties, such as poor water solubility. The water-soluble topotecan, like camptothecin, is a topoisomerase I inhibitor. The agent was developed by the NCDDG headed by Warren Ross at the University of Florida at Gainesville. Much of the preclinical development work was supported by SmithKline Beecham (now

Table 1. Currently Funded Natural Products NCDDG Groups.

PI	Institution	Collaborating Institutions	Project
Crews, Phillip	University of California at Santa Cruz	Oregon State University, Harbor Branch Oceanographic Institute, Novartis Biomedical Research Institute	Marine Natural Products
Fenical, William	Scripps Institution of Oceanography	University of Rhode Island, Bristol-Myers Squibb Pharmaceutical Research Institute	Marine Microorganisms and Invertebrates
Hecht, Sidney	University of Virginia	Virginia Polytechnic Institute and State University, University of Pittsburgh, Galileo Laboratories	Plants for Nuclear and Signaling Targets
Ireland, Chris	University of Utah	Harvard Medical School, University of British Columbia, Wyeth Research	Marine Microorganisms and Invertebrates, Terrestrial Plants
Kinghorn, A. Douglas	University of Illinois at Chicago	Research Triangle Institute, Bristol-Myers Squibb Pharmaceutical Research Institute	Plants from Temperate, Subtropical and Tropical Environments

Table 2. Currently Funded non-Natural Products NCDDGs.

PI	Institution	Collaborating Institutions	Project
Brem, Henry	Johns Hopkins University School of Medicine	Duke University, Yale University	Controlled Release Polymers for Brain Tumors
Chin, Allison	Geron Corporation	Memorial Sloan-Kettering	Telomerase
Halperin, Jose	Harvard Medical School	Harvard	Translation Initiation Inhibitors
Merrill, Alfred	Georgia Institute of Technology	Emory University	Sphingolipids
Parker, William	Southern Research Institute	Cornell University, University of Alabama	Gene Therapy/Purine Analogs
Powis, Garth	Arizona Cancer Center	Burnham Institute, University of Pittsburg	Cell Cycle and Signaling Pathways
Sebti, Said	Lee Moffitt Cancer Center	Yale University, University of North Carolina	Geranylgeranyltransferase Inhibition
Jaffee, Elizabeth	Johns Hopkins University School of Medicine	University of Pennsylvania	Antigen-specific Vaccines

GlaxoSmithKline), the industrial partner in the project. Since the marketing approval of topotecan, irinotecan, another semi-synthetic derivative of camptothecin, has been approved. Currently there are several other camptothecin analogs in advanced preclinical development or clinical trials, such as 9-aminocamptothecin, polymer-camptothecin conjugates and liposomal camptothecins.

Gliadel

Approved in 1996. This controlled release system was developed by the NCDDG headed by Henry Brem at Johns Hopkins University for the post-surgical treatment of brain tumors, especially glioblastoma multiforme, a malignancy with poor prognosis. The product consists of BCNU, an

alkylating agent, impregnated in a wafer composed of polyanhydride, a biodegradable polymer invented by Robert Langer of MIT. Wafers are implanted at the time of surgical resection of brain tumors. The product is manufactured by Guilford Pharmaceuticals, an exclusive licensee of MIT. It received market approval in 1996 in the United States and later was approved in Brazil, Canada and several European countries.

DAB389IL-2

Approved in 1998. DAB389IL-2 is a fusion protein containing the catalytic and transmembrane domains of diphtheria toxin and interleukin-2. It was generated by the NCDDG headed by John Murphy, the University Hospital, Boston, in collaboration with Seragen, Inc., a company based in MA. This product has been shown to be well tolerated and effective in hematologic malignancies that are characterized by the expression of high affinity IL-2 receptors. Also known as ONTAK, IL-2 Fusion Protein, Denileukin and Difitox, this product was approved for treating adult patients with recurrent or persistent cutaneous T-cell lymphomas. Ligand Pharmaceuticals Inc., San Diego, CA acquired the marketing rights from Seragen, Inc.

Erbitux (C225)

Approved in 2004. This anti-epidermal growth factor receptor (EGFR) antibody was developed by John Mendelson, when he was at Memorial Sloan-Kettering Cancer Center prior to his move to M.D. Anderson Cancer Center, with support from an NCDDG and other sources. This human-mouse chimeric fusion protein is used in combination with chemotherapeutic agents, such as cisplatin, and with radiation. Mendelson hypothesized in the early 1980s that blocking EGF receptors with a monoclonal antibody prevents receptor activation. Erbitux is an IgG1 monoclonal antibody designed to selectively target and block the epidermal growth factor receptor (EGFR), which is expressed on the cell surfaces of a number of tumor types. Erbitux binds to EGFR and prevents natural ligands from binding and inducing phosphorylation. Clinical trials for colon cancer and refractory advanced squamous cell head and neck carcinoma have shown promising results. Phase III trials of Erbitux were initiated in 1999 by ImClone Systems, Inc. in collaboration with Merck KGaA of Darmstadt, Germany. In 2001, in what has become one of the largest deals for a cancer agent in the pharmaceutical industry, Bristol-Myers Squibb agreed to co-develop Erbitux (cetuximab) for marketing rights in the United States. Although initial submission of a Biological License Application (BLA) in 2001 was not approved by the FDA, and the drug gained a lot more press coverage for reasons unrelated to the performance of the agent, subsequent clinical trial results in Europe and the United States showed Erbitux's effectiveness in colorectal cancer. In June 2003, Merck KGaA filed marketing applications with the European Agency for the Evaluation of Medicinal Products and with Swissmedic for the treatment of metastatic colorectal cancer. Erbitux gained approval in Switzerland in December 2003. In February 2004, Erbitux gained the FDA approval for the treatment of colorectal cancer. The agent is to be used in combination with irinotecan for patients with EGFR expressing tumors that are irinotecan-refractory. Erbitux is also being studied in other types of tumors that express EGFR, including lung, pancreatic, ovarian and head and neck cancers. Ruling by the European Agency is anticipated in 2004. (from *R&D Directions*, 2003, Oct issue: 29; Nov–Dec issue: 52).

N^1,N^{14}-diethylhomospermine (SunPharm) and N1, N14-diethylnorspermine (Park-Davis, a division of Warner-Lambert)

Investigational New Agents. These spermine derivatives are polyamine analogs synthesized by the NCDDG headed by Carl Porter, Roswell Park Memorial Institute. These compounds, prepared by Raymond Bergeron, disrupt polyamine homeostasis. Diethylhomospermine (DEHOP) is in Phase II clinical trials for HIV positive patients with uncontrolled, refractory diarrhea. Diethyl-norspermine has entered Phase II clinical trials for treatment of solid tumors.

O^6-Benzylguanine (OBG)

Investigational New Agent. OBG was identified and evaluated by the NCDDG headed by Anthony Pegg, The Milton S. Hershey Medical Center. It was selected from a series of compounds synthesized by Robert Moschel at NCI-Frederick. OBG is used in combination with BCNU, an alkylating agent, as a means to block the activity of alkylguanine transferase, a DNA repair protein responsible for drug resistance to BCNU and similar agents. The OBG/BCNU combination is in NCI-sponsored clinical trials.

Provenge (a vaccine for prostate cancer)

Investigational New Agent. This vaccine was developed by Riner Laus, Dendrion Corp., a collaborator in Ronald Levy's NCDDG, and has now completed Phase II clinical evaluation under the direction of Eric Small at the University of California at San Francisco. The approach involves isolating a patient's dendritic cells, then pulsing the cells with a peptide derived from prostatic acid phosphatase, and re-administering the modified dendritic cells back to the patient (Small et al., 2000). Provenge has obtained fast-track designation from the FDA for treatment of androgen-independent prostate cancer. Currently, it is in a pivotal, double-blinded, placebo-controlled Phase III trial to confirm previous results that indicate the immunotherapy may delay progression of prostate cancer and development of disease-related pain. (from *R&D Directions*, Sept, 2003).

Cordycepin and deoxycoformycin

Investigational New Agent. These compounds entered Phase I clinical trials for the eventual treatment of lymphoblastic leukemias and lymphomas which contain terminal deoxynucleotidyl transferase (TdT), a unique DNA polymerase which catalyzes the polymerization of deoxyribonucleotides on the 3′-hydroxyl ends of preformed oligo- or polydeoxynucleotide initiators, without the need of a template. Cordycepin inhibits TdT if its own metabolism is blocked by deoxy-coformycin, an inhibitor of adenosine deaminase. This project was conceived and brought to trial by the NCDDG headed by Ronald McCaffrey at the University Hospital, Boston.

HTI 286

Investigational New Agent. Hemiasterlins are linear peptide-like molecules isolated from the marine sponge *Cymbastela* sp. collected in Papua New Guinea. Raymond Andersen at the University of British Columbia at Vancouver, a Project Leader in the Chris Ireland NCDDG, discovered these compounds and their ability to induce microtubule depolymerization and mitotic arrests in cells. The hemiasterlins are also potent inhibitors of cell proliferation with nM levels of activity. Andersen's group completed the total synthesis of hemiasterlin A and did extensive structure-activity relationship work (Andersen, 1997). The Group's pharmaceutical partner, Wyeth Research, licensed the technology and started development work on some of the analogs, one of which, HTI 286, emerged as a clinical candidate. One of the notable features of the HTI compounds is their ability to overcome P-glycoprotein mediated chemo-resistance. As reported in the Ireland NCDDG report in this issue, HTI-286 is shown to have remarkable activity against paclitaxel- and vincristine-resistant solid tumors *in vitro* and *in vivo*, and is orally available. Wyeth scientists through detailed binding kinetics work with photoaffinity analogs of HTI 286, showed that HTI 286 may interact with alpha-tubulin, consistent with mutations observed in alpha tubulin in HTI-286 resistant cells (Nunes 2002). A Phase I clinical trial, sponsored by Wyeth using HTI286 as a single agent, started in March 2002 in the United States. A Phase I clinical trial in combination with carboplatin has also started in the U.S. for patients with solid tumors. Another Phase I trial in Japan is planned. A Phase II clinical trial with HTI286 as single agent began in October 2003 for patients with refractory non-small cell lung carcinoma.

NVP-LAQ824

Investigational New Agent. Acetylation and deacetylation of histones play an important role in the regulation of gene expression. In addition to histone deacetylases (HDACs), histone acetyltransferases (HATs) are also involved in the regulation of histone acetylation. At least two subfamilies of HDACs with eight members have been found in mammalian cells so far. Disruption of the balance between the activity of HATs and HDACs has been associated with cancer. HDAC inhibitors can arrest tumor cell growth, and HDAC is considered as a promising cancer target. The Novartis Oncology Group, the industrial partner in the NCDDG consortium headed by Phillip Crews of the University of California at Santa Cruz, discovered that the psammaplins, a series of related compounds isolated from the marine sponge, *Pseudoceratina purpurea*, by Francis Schmitz at the University of Oklahoma, a Project Leader in this NCDDG, potently inhibit HDAC in their high throughput screens (Remiszewski, 2003; see also the Crews report later in this issue). Through a combination of structural biology work, and extensive synthetic efforts, LAQ824, which bears minimal structural resemblance to the psammaplins, was developed. LAQ824, which inhibits HDAC with an IC_{50} of 32 nM and proliferation of cancer cell lines with IC50s of 10–150 nM, was selected for preclinical development (Remiszewski, 2002). The compound is more than 200 times more potent *in vitro* than SAHA, another HDAC inhibitor currently in the clinic. LAQ824 is efficacious in a number of solid tumor xenograft models, and tumor stasis was observed at the tolerated dose. Phase I clinical trials, using LAQ824 as a single agent, were initiated in Europe and the US in 2002 in patients with solid tumors or leukemia. HDAC isoform specificity of NVP-LAQ824 is being studied. Recently, it was reported that LAQ824 down regulates Her-2 and attenuates the levels c-Raf-1, pAKT, and AKT, and enhances apoptosis of breast cancer cells induced by chemotherapeutic agents that have diverse mechanisms of action, including an antimetabolite, gemicitabine, and antimicrotubule agents, taxotere and epothilone B (Fuino, 2003). Theses results suggest possible combination strategies in the clinic. At the AACR/NCI/EORTC meeting in Boston in 2003, a number of academic researchers presented posters on preclinical combination studies of LAQ824 with established chemotherapeutics.

Cryptophycin

Phase II Trials Closed. This novel depsipeptide was isolated by Richard Moore, University of Hawaii, from a blue-green algae, *Nostoc* sp., and was found to have potent antiproliferative and antimitotic activities that are different from those of paclitaxel. A number of related natural analogs was also found by Moore and co-workers. The development of the cryptophycins was coordinated by the NCDDG headed by Frederick Valeriote, previously at Wayne State University and now at Henry Ford Hospital, with Eli Lilly as the industrial partner. Lilly chemists developed a total synthetic process and launched a significant SAR effort. Several candidate drugs showed remarkable preclinical efficacy in solid tumor models. Cryptophycin 52, one of the more than 200 analogs made by Eli Lilly, entered Phase II clinical trials for treatment of solid tumors, but in 2001, the company decided to

halt further evaluation due to untoward toxicities. Despite disappointing clinical results, the successful collaboration between academia and industry to move a lead to preclinical and eventual clinical development, demonstrated the power of the NCDDG as a good model for facilitating the sharing of resources in a multidisciplinary and multi-institutional setting. Currently, several groups are in the process of delineating the biosynthetic pathways of cryptophycins through cloning of biosynthetic gene clusters. Hopefully, in the not too distant future, the potential of this series of compounds will be reexamined.

LAF389

Phase I Clinical Trial Closed in 2002. LAF389 is a synthetic analog of the natural products, bengamides, isolated from a Fijian sponge, *Jaspis* cf *coriacea*, by Phillip Crews (see report later in this issue). When tested in the NCI 60 cell tumor panel, the bengamides showed novel mechanisms of action. In 1997, Novartis, the Crews NCDDG industrial partner, tested and found the bengamides were active in xenograft tumor models. Although bengamide B is more active than others in the series, a number of undesirable pharmaceutical properties, such as poor water solubility, limited stability in plasma, and short half-life, together with the supply issue, led to an extensive synthetic medicinal chemistry effort at Novartis (Kinder, Versace et al., 2001; Kinder, Wattanasin et al., 2001; Thale et al., 2001). One of the synthetic analogs, LAF389, with an improved pharmaceutical profile, was selected as a clinical candidate. The compound entered a Phase I clinical trial in 2000. Novartis scientists and collaborators conducted a number of experiments aimed at identifying the molecular target of LAF389, and found that LAF389 inhibits MetAp1(methionine aminopeptidase 1) and MetAp2 (Towbin et al., 2003). Novartis scientists successfully co-crystalized LAF389 with hMetAp2. However, further studies revealed that MetAp may not be responsible for LAF389 pharmacology. LAF389 showed cardiotoxicity in the clinic and Novartis terminated clinical development in June, 2002.

Murine anti-transferrin receptor monoclonal antibodies (A27.15/E2.3)

IND closed in 2001. These antibodies were discovered by Ian Trowbridge, Salk Institute, through the NCDDG headed by Mendelson (C225, Erbitux). The products entered Phase IA clinical trials at the University of Arizona, and were administered as a combination in a 1 : 1 ratio based on preclinical data which showed that the most effective inhibition of the transferrin receptor occurred when the products were given as a pair, presumably by cross-linking the transferrin receptor. The clinical studies were conducted without an industrial partner. Any future clinical studies would require considerable investment to re-engineer the antibodies as human-mouse chimeras to meet current FDA guidelines.

FTIs

Investigational New Agent, Phase I Trial Stopped. This agent was developed by the NCDDG headed by Said Sebti at the Moffit Cancer Center, University of South Florida. The CAAX-motif is present in all Ras proteins and CAAX-signal modifications are critical for the function of oncogenic Ras proteins. Based on the peptide structure of the CAAX box, this Group synthesized and evaluated a series of peptidomimetics. This Group was the first to publish the antitumor properties of this class of compounds in xenograft models and to demonstrate, contrary to earlier expectations, that the Ras protein is probably not the target nor the determinant of antitumor activity. One derivative entered Phase I clinical trials but was later dropped when the orally administered drug did not achieve required blood levels. The Sebti group is currently developing plans to reformulate the drug for intravenous administration and is also examining close analogs for possible development.

New challenges and directions

As we continue to advance our understanding of cancer biology and the pathways that promote tumor cell progression and proliferation, there is great anticipation that new and better therapeutics which target tumor cells much more selectively will become available. The success of Gleevec has demonstrated that selective targeting of transformation processes in tumors is possible. Through the continuing innovation of biotechnological tools in drug screening and model systems for preclinical testing, extracts and compound libraries derived from natural products will be tested in more sensitive assays that will give more informative outcomes for their potential as drug leads.

Natural product chemical libraries

In the era of high- and ultra high-throughput assays with targets discovered through proteomics and genomics, discovering meaningful potent ligands for the target of interest, which can serve as starting points for further evaluation, is a challenging process despite the availability of commercial compound libraries. Natural products, through evolution, have incorporated complex stereochemical features and pharmacophores which often are not readily accessible by combinatorial syntheses. They continue to serve as remarkable probes for defining protein functions in biological systems. A number of researchers have long lists of cumulative discoveries of natural products. Each of the NCDDG Groups has a reasonable size collection of natural products discovered through the years. This is a valuable resource, and this natural compound library should be systematically catalogued and plated in a format amenable to high through screening. Given the highly competitive environment of drug discovery, NCDDG industrial partners frequently change their screening targets. Once new targets become available,

a screening campaign will begin. Thus, the natural compounds already isolated should be plated and ready for testing. Storing compounds in this format may seem laborious to the natural products chemists interested in pursuing the next extract for novel structures and making new discoveries, but for drug discovery purposes, these compounds are a very valuable resource. The NCDDG groups are highly encouraged to store their natural products collections in formats that can readily be made available for new screens.

High throughput isolation and structure determination

The increases in screening capacity and the subsequent data analysis of high throughput assays place higher demands on investigators for better quality natural products matrices that are devoid of interfering nuisance compounds which often exhibit nonselective modulating enzymatic activities. Although de-replication processes, such as approaches using LC-MS and database-searching, are important in gaining more knowledge on the extracts, other procedures, such as pre-fractionation and the rapid generation of semi-purified samples, are being developed and have shown promising results in generating higher hit rates. Strategies for pre-fractionation should be incorporated in bioassay-guided isolation processes. The introduction of robotics and computational tools some 20 years ago has revolutionized the biology field and has fueled the proliferation of new tools for drug discovery. High throughput and ultrahigh throughput systems that can screen hundreds and thousands of samples per day are now available in a large number of laboratories. This automation in the biological screens has made chemistry the rate limiting step in the drug discovery process. There are many reasons why some pharmaceutical companies have reduced or altogether eliminated their efforts or interest in natural products, not the least of which is the slow pace of generating high numbers of molecules. New proteins and genes are routinely discovered and become new targets for ligand discovery. Although natural products have the structural diversity and functional complexity, and have a long history of being the power house of clinical drugs, the pace of isolation and characterization in a traditional sense, can no longer be in step with that of the screens with their constant demand for large numbers of samples. Most screening laboratories use a "campaign" strategy to perform high throughput screens on a particular set of molecular targets of interest, and this does not allow much time for the isolation of a minor component from a complex natural matrix. Although LC/MS analysis has gained increased use by natural products laboratories, application of other informative and efficient tools such as LC/NMR is less common, especially in academic laboratories. If natural products laboratories are equipped with half of the tools that are available to high throughput screening laboratories, the process of getting purified compounds from a whole organism (plants, or sponge or culture) will be greatly shortened and the output will increase accordingly. The concept and adaptation of automated isolation, such as with SEPBOX or similar systems, and high throughput structural elucidation, such as with LC-MS/MS and LC/NMR, coupled with chemical profiling and data analysis with a vast number of known compounds, could change the face of natural products research. However, the automated separation equipment is in an early stage of development and is cost prohibitive for most academic laboratories. Chemical engineers, instrument companies and chemists can work together to make these integrated systems and make them less cost prohibitive.

Combinatorial biosynthesis

Harnessing the biosynthetic pathways of natural products toward the production of structural diversity through genetic engineering has gained momentum in recent years. Research in this area is driven by the unparalleled commercial success of antibiotics derived from microbial sources. Many widely used clinical drugs, such as erythromycin, rapamycin, and daunorubicin are of polyketide origin whose biosyntheses are catalyzed by polyketide synthases (PKS). Equally valuable are a smaller number of peptidic compounds, such as cyclosporin A and vancomycin, whose syntheses are catalyzed by non-ribosomal peptide synthases (NRPS). A number of PKS and NRPS gene clusters have been cloned and characterized (Du, 2003). In addition, an increasing number of natural products with hybrid PKS-NRPS gene clusters have been investigated. Genetic manipulation of biosynthetic pathways has been shown to be a viable strategy for generating novel structural diversity and resolving supply issues, such as in the cases of erythomycin, epothilone, and rapamycin.

Currently increasing attention is being paid to the biosynthetic studies of PKS, NRPS and PKS/NRPS natural products from marine environments. Successful gene cloning and characterization and heterologous expression would not only define the true bio-origin of these compounds, which are generally obtained from a symbiotic source, but also solve the common supply problem. A team based approach that often incorporates the expertise of marine natural products chemists, ecologists and microbiologists has generated some remarkable successes to date; however, it is still challenging to study, for example, natural products isolated from sponge or other invertebrates with a rich associated bacterial population. Nonetheless, the potential for generating diversity through combinatorial biosynthesis has now been demonstrated.

The unexplored potential of microbial diversity

In a report released by the American Academy of Microbiology entitled "The Microbial World: Foundation of the Biosphere", it is estimated that "less than 1% of bacterial species and less than 5% of fungal species are currently known", and recent evidence indicates that millions of microbial species

remain undiscovered (Young, 1997). Until recently, microbiologists were greatly limited in their study of natural microbial ecosystems due to an inability to cultivate most naturally occurring microorganisms, but procedures for the isolation, cultivation and identification of microorganisms are being developed which will aid microbiologists in their assessment of the earth's full range of microbial diversity. The application of a technique for the massive parallel cultivation of gel-encapsulated single cells (gel micro-droplets; GMDs) derived from microbes separated from environmental samples (sea water and soil) has resulted in the identification of previously undetected species (using 16S rRNA gene sequencing), and the culturing and scale-up cultivation of previously uncultivated microbes (Zengler et al., 2002).

Work reported by the Fenical group in this issue has revealed that deep ocean sediments are a valuable source of new actinomycete bacteria that are unique to the marine environment. Based on combined culture and phylogenetic approaches, the first truly marine actinomycete genus named *Salinospora* has been described (Mincer et al., 2002). They can be cultured using the appropriate selective isolation techniques, and significant antibiotic and cytotoxic activity has been observed, leading to the isolation of a very potent cytotoxin, salinosporamide A, a very potent proteasome inhibitor (IC_{50} = 1.3 nM) (Fehling et al., 2003).

While plants have received extensive study as sources of bioactive metabolites, the endophytic microbes which reside in the tissues between living plant cells have received scant attention. The relationship established between the endophytes and their host plants may vary from symbiotic to pathogenic, and limited studies have revealed an interesting realm of novel chemistry (Strobel et al., 2004).

Extreme habitats harbor a host of extremophilic microbes (extremophiles), such as acidophiles (acidic sulfurous hot springs), alkalophiles (alkaline lakes), halophiles (salt lakes), piezo (baro)- and thermophiles (deep-sea vents) (Persidis, 1998), and psychrophiles (arctic and antarctic waters, alpine lakes) (Psenner & Sattler, 1998). While investigations thus far have focused on the isolation of thermophilic and hyperthermophilic enzymes (Adams & Kelly, 1998), these extreme environments will also undoubtedly yield novel bioactive chemotypes.

In addition, procedures based on the extraction of nucleic acids (the metagenome) from environmental samples permit the identification of uncultured microorganisms through the isolation and sequencing of ribosomal RNA or rDNA (genes encoding for rRNA); samples from soils are currently being investigated, and the methods may be applied to other habitats, such as the microflora of insects and marine animals (Handelsman et al., 1998). Valuable products and information are certain to result from the cloning and understanding of the novel genes which will be discovered through these processes. Heterologous expression of gene clusters encoding the enzymes involved in biosynthetic pathways in viable host organisms, such as *Escherichia coli*, should permit the production of novel metabolites produced from as yet uncultured microbes.

Drug lead development

Although there are many examples of natural products that have advanced through the pre-clinical evaluation for toxicity and efficacy, and ended up as marketed cancer chemotherapeutics, more often than not, natural products serve as the initial leads from which derivatives with better pharmacological and pharmaceutical profiles may be obtained through chemical synthesis. Most of the natural products groups in the NCDDGs rely on their industrial partners to take on extensive structure activity studies. While some companies have been able to contribute in this area, the efforts in general have been quite uneven as the decision to commit manpower to optimize a natural product lead from the NCDDG may compete for resources being assigned to the company's internal pipeline or priorities. Perhaps the NCDDGs should incorporate academic or small business partners as components or subcontracts that would respond more readily to NCDDG's natural product leads. The availability of the NCI compound sets (Challenge, Diversity, Mechanistic, Natural Products; http://dtp.nci.nih.gov/branches/dscb/repo_open.html), as well as the NCI natural products extracts repository (http://dtp.nci.nih.gov/branches/npb/repository.html), means that more extramural investigators, including the NCDDGs, are able to use these resources for screening for potential leads in their assays. However, the leads in most cases only serve as a framework or starting point for development of a more robust lead that may survive secondary and *in vivo* evaluations. The development of a robust lead often needs strong commitment from collaborating synthetic or medicinal chemists in the consortia partner organizations (academic and/or industrial) to perform specific synthetic manipulations of the initial hit. In the future, biosynthetic manipulations also may play a greater role in lead discovery and enhancement. Again, NCI resources, such as the Rapid Access to Intervention Development (RAID; http://dtp.nci.nih.gov/docs/raid/raid_index.html) and Rapid Access to NCI Discovery Resources (RAND; http://dtp.nci.nih.gov/docs/rand/rand_index.html) programs, are available to develop promising leads, and have been used in the case of the MDR-reversing agents, the pervilleines, discovered by the Kinghorn NCDDG group (see Kinghorn report later in this issue). These resources, however, have limited capacity, and it is important that consortium partners be prepared to devote their own resources to leads meriting further development.

Source country access

One of the important issues facing the NCDDGs as well as other natural products researchers is the sourcing of material. Acquisition of samples from geographic locations outside of the jurisdiction of the United States must be compliant

with, and respectful of, national and international laws governing the collection and export of the natural material. Some source countries with the world's highest biodiversity currently may not have clear permit granting processes. Since the signing of the Rio Convention on Biological Diversity (CBD) Treaty in 1993, investigators from the United States and elsewhere have been challenged with issues such as prior informed consent, ownership of natural resources, equitable benefit-sharing and intellectual property rights. Although the general idea of respecting indigenous people's rights over their natural resources is well understood, the terms and conditions which lead to a collaboration are often vague and subject to change dependent on the political situations of the source countries. Some investigators from the NCDDGs at times have been frustrated in their attempts to establish collaborations, despite the best of intentions and several rounds of negotiations. Sometimes, it is unclear which government entity or official really represents the people. In some cases it is necessary to negotiate, not only with the national government, but also with regional governments, collaborating research institutions and non-governmental organizations (NGOs), as well as with local communities. Sourcing of terrestrial plant material, especially if used ethnobotanically or linked with traditional knowledge, may at times require more stakeholders to be involved than sourcing of marine samples (Gollin, 2002). Needless to say, in either case a lot of time and resources are consumed in this process. On the positive side, it is worthy of note that, despite the complexities and difficulties, as will be seen in the publications in the following pages of this issue, all of our NCDDG investigators have been able to successfully execute collaborative agreements and/or MOUs with some source countries around the world. The US State Department, after getting input from several other government agencies, including the NIH and NSF, has published a set of guidelines (www.State.gov/g/oes/rls/or/25962.htm) for US Government-funded researchers collecting samples outside of the US. The International Cooperative Biodiversity Group (ICBG) program, funded by several US government agencies, including NIH and NSF, also offers good examples of successful collaborations between academia, industry and source country organizations (http://www.fic.nih.gov/programs/icbg.html).

It is the NCI's standing policy that all NCDDGs, as well as other NCI funded researchers involved in foreign sample collections, must have a fully signed research or commercial agreement in which the source country is informed clearly of the intended use, and the potential for benefits which may result from the investigation of these samples. Plans with regard to compensation of the appropriate people, monetary and non-monetary, need to be addressed. Some NCDDGs have conducted workshops, trained source country researchers in the US, and, in the Ireland NCDDG, shared benefits with the source country when a clinical candidate is identified. Because of the dynamic nature and length of the process of forming collaborative relationships with source countries, sometimes the natural products groups may request a change of collection site from what was originally proposed, to a site where they can more readily execute an agreement. This situation could arise after an initial two to three years of sampling during a 5-year grant cycle, when sufficient samples representative of the diversity of a locality have been obtained, or may be due to difficulties in securing a permit for collections from the proposed site(s). In these instances, the NCI will engage in discussions with the investigators, and if the proposed new areas have not been extensively and systematically collected for cancer drug discovery, grantees may be granted a change of venue. Since the beginning of the NCDDG program, many of the rules governing the collections have changed and these rules are still evolving. NCDDGs, as well as other natural product chemists funded by NCI, must provide an executed agreement with the foreign countries from where collections are to be made. NCI, working with the NIH, will consult the State Department for clearance. Once the clearance is obtained, investigators will be notified and work can commence. Natural products researchers and drug companies interested in bioprospecting must stay abreast with these developments, and if necessary, should provide leadership and input in formulating policies and strategies with the mutual interests of source country and the drug researcher in mind (Gollin, 2002). It must be emphasized that the ultimate success of these multi-institutional consortia rests on mutual trust, a collaborative spirit, and a willingness to adhere to the terms and conditions of the agreement.

The Academic Public Private Partnership Program (AP4)

The NCDDG program has proved to be a highly successful process for coordinating the discovery and development of novel drug leads, and has served as a model for another successful initiative, the multi-agency International Cooperative Biodiversity Group program. Building on this positive experience, the NCI Developmental Therapeutics Program (DTP) has launched the AP4 initiative to provide support for the formation of new partnerships, or significant expansion of existing partnerships, among academia, industry, non-profit institutions, and government entities. The partnerships will conduct research related to cancer therapy, prevention, diagnostics and imaging, with the goal being to speed the translation of newly discovered cancer interventions to clinical trials (http://grants.nih.gov/grants/guide/rfa-files/rfa-ca-04-005.html).

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References

Adams MW, Kelly RM (1998). Finding and using hyperthermophilic enzymes. *Trends in Biotech 16*: 329–332.

Anderson HJ, Coleman JE, Andersen RJ, Roberge M (1997): Cytotoxic peptides hemiasterlin, Hemiasterlin A and Hemiasterlin B induce mitotic arrest and abnormal spindle formation. *Cancer Chemotherapy and Pharmacology 39*: 223–226.

Andersen RJ, Coleman JE, Piers E, Wallace D (1997): Total synthesis of (−) Hemiasterlin, a structurally novel tripeptide that exhibits potent cytotoxic activity. *Tetrahedron Lett 38*: 317–320.

Cragg GM, Newman DJ (1999): Discovery and development of antineoplastic agents from natural sources. *Cancer Investigation 17*: 153–163.

Du L, Cheng Y, Ingenhorst G, Tang G, Huang Y, Shen B (2003) Hybrid peptide-polyketide natural products: biosynthesis and prospects towards engineering novel molecules. *Genetic Engineering 25*: 227–267.

Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W (2003): Salinosporamide A: A highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora. Angew Chem Int Ed 42*: 355–357.

Fuino L, Bali P, Wittmann S, Donapaty S, Guo F, Yamaguchi Y, Wang H, Atadja P, Bhalla K (2003): Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. *Mol Cancer Ther 3*: 971–984.

Gollin MA (2002): Linking intellectual property rights with traditional medicine. In: Iwu MM, Wootton JC, Eds, *Ethnomedicine and Drug Discovery*. Amsterdam Elsevier, pp. 201–209.

Gollin MA (2002): Elements of commercial biodiversity prospecting agreements. In: Laird SA, Ed. *Biodiversity and Traditional Knowledge. Equitable Partnerships in Practice*. London Earthscan Publications Ltd. pp. 310–332.

Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM (1998): Molecular biological access to the chemistry of unknown soil microbes: a new friontier fir natural products. *Chem Biol 5*: R245–R249.

Kinder FR Jr, Versace RW, Bair KW, Bontempo JM, Cesarz D, Chen S, Crews P, Czuchta AM, Jagoe CT, Mou Y, Nemzek R, Phillips PE, Tran LD, Wang RM, Weltchek S, Zabludoff S (2001): Synthesis and Antitumor Activity of Ester-Modified Analogues of Bengamide B. *J Med Chem 44*: 3692–3699.

Kinder FR Jr, Wattanasin S, Versace RW, Bair KW, Bontempo J, Green MA, Lu YJ, Marepalli HR, Phillips PE, Roche D, Tran L, Wang RM, Waykole L, Xu DD, Zabludoff S (2001): Total Syntheses of Bengamides B and E. *J Org Chem 66*: 2118–2122.

Loganzo, F, Discafani C, Annable T., Beyer C, Musto S, Hardy C, Hernandez R, Baxrer M, Ayral-Kaloustian S, Zask A, Singanallore T, Khafizova G, Asselin A, Poruchynsky M, Fojo T, Nieman J, Andersen R, Greenberg L (2002): HTI286, a synthetic analog of the anti-microtubule tripeptide hemiasterlin, potently inhibit growth of cultured tumor cells, overcomes resistance to paclitaxel mediated by various mechanism, and demonstrates intravenous and oral in vivo efficacy. *Proceedings of the AACR March 2002*: 265.

Mincer TJ, Jensen PR, Kauffman CA, Fenical W (2002): Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. *Appl Envir Microbiol 68*: 5005–5011.

Nunes M, Kaplan J, Loganzo JF, Zask A, Ayral-Kaloustian S, Greenberg L (2002): Two photoaffinity analogs of HTI-286, a synthetic analog of hemiasterlin, interacts with alpha-tubulin. *European J Cancer 38*(Suppl.) (7): S119.

Persidis A (1998): Extremophiles. *Nature Biotechnology 16*: 593–594.

Pina IC, Gautschi JT, Wang G-Y-S, Sanders ML, Schmitz FJ, France, D, Cornell-Kennon S, Sambucetti LC, Remiszewski SW, Perez LB, Bair KW, Crews P (2003): Psammaplins from the sponge Pseudoceratina purpurea: Inhibition of both histone deacetylase and DNA methyltransferase. *J Org Chem 68*: 3866–3873.

Psenner R, Sattler B (1998): Life at the freezing point. *Science 280*: 2073–2074.

Remiszewski S, Bontempo J, Cohen P, Cramer J, Lassota P, Hug M, Perez LB, Thomas H, Versace R, Atadja P (2002): Preclinical efficacy, toxicology and pharmacokinetics of NVP-LAQ824, a novel synthetic histone deacetylase inhibitor. *European J Cancer 38*(Suppl) (7): S98.

Remiszewski SW, Sambucetti LC, Bair KW, Bontempo J, Cesarz D, Chandramouli N, Chen R, Cheung M, Cornell-Kennon S, Dean K, Diamantidis G, France D, Green MA, Howell KL, Kashi R, Kwon P, Lassota P, Martin, MS, Mou Y, Perez LB, Sharma S, Smith T, Sorensen E, Taplin F, Trogani N, Versace R, Walker H, Weltchek-Engler S, Wood A, Wu A, Atadja P (2003): N-hydroxy-3-phenyl-2-propenamides as novel inhibitors of human histone deacetylase with *in vivo* antitumor activity: discovery of (2E)-N-hydroxy-3-[4-[[(2-hydroxyethyl)[2-(1H-indol-3-yl)ethyl]amino] methyl] phenyl]-2-propenamide (NVP-LAQ824). *J Med Chem 46*: 4609–4624.

Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, Valone FH (2000): Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol 18*: 3894–3903.

Strobel G, Daisy B, Castillo U, Harper J (2004): Natural Products from Endophytic Microorganisms. *J Nat Prod 67*: 257–268.

Thale Zia, Kinder FR, Bair KW, Bontempo J, Czuchta AM,

Versace RW, Phillips PE, Sanders ML, Wattanasin S, Crews P (2001): Bengamides revisited: new structures and antitumor studies. *J Org Chem 66*: 1733–1741.

Towbin H, Bair K, DeCaprio J, Eck M, Kim S, Kinder F, Muller D, Schindler P, van Oostrum J, Versace R, Voshol H, Wood J, Zabludoff S, Phillips PE (2003): Proteomics based target identification: Bengamides as a new class of methionine aminopeptidase inhibitors. *J Biol Chem 278*: 52964–52971.

Young P (1997): Major microbial diversity initiative recommended. *ASM News 63*: 417–421.

Zengler K, Toledo P, Rappe M, Elkins J, Mathur EJ, Short JM, Keller M (2002): Cultivating the uncultured. *Proc N Y Acad Sci 99*: 15681–15686.

Appendix A

Letter of collection

Agreement between [Source Country Institution] and/or [Source Country Organization] and the developmental therapeutics program division of cancer treatment and diagnosis national cancer institute

The Developmental Therapeutics Program ("DTP"), Division of Cancer Treatment and Diagnosis ("DCTD"), National Cancer Institute ("NCI") is currently investigating plants, microbes, and marine macro-organisms as potential sources of novel anticancer and AIDS-antiviral drugs. The DTP is the drug discovery program of the NCI which is an Institute of the National Institutes of Health ("NIH"), an arm of the Department of Health and Human Services of the United States Government. While investigating the potential of natural products in drug discovery and development, NCI wishes to promote the conservation of biological diversity, and recognizes the need to compensate [Source Country, SC] organizations and peoples in the event of commercialization of a drug developed from an organism collected within their borders.

As part of the drug discovery program, DTP has contracts with various organizations for the collection of plants, microbes and marine macro-organisms worldwide. DTP has an interest in investigating plants, microbes and marine macro-organisms from [Source Country], and wishes to collaborate with the [Source Country Government ("SCG") or Source Country Organization(s) ("SCO")] as appropriate in this investigation. The collection of plants, microbes, and marine macro-organisms will be within the framework of the collection contract between the NCI and the NCI Contractor ("Contractor") which will collaborate with the appropriate agency in the [SCG or SCO]. The NCI will make sincere efforts to transfer knowledge, expertise, and technology related to drug discovery and development to the [appropriate Source Country Institution ("SCI")] in [Source Country] as the agent appointed by the [SCG or SCO], subject to the provision of mutually acceptable guarantees for the protection of intellectual property associated with any patented technology. The [SCG or SCO], in turn, desires to collaborate closely with the DTP/NCI in pursuit of the investigation of its plants, microbes and marine macro-organisms, subject to the conditions and stipulations of this agreement.

A. The role of DTP, DCTD, NCI in the collaboration will include the following:

1) TP/NCI will screen the extracts of all plants, microbes and marine macro-organisms provided from [Source Country] for anticancer and AIDS-antiviral activity, and will provide the test results to [SCI] on a quarterly basis. Such results will be channeled via Contractor.
2) The test results will be kept confidential by all parties, with any publication delayed until DTP/NCI has an opportunity to file a patent application in the United States of America on any active agents isolated. Such application will be made according to the terms stated in Article 6.
3) Any extracts exhibiting significant activity will be further studied by bioassay-guided fractionation in order to isolate the pure compounds(s) responsible for the observed activity. Since the relevant bioassays are only available at DTP/NCI, such fractionation will be carried out in DTP/NCI laboratories. A suitably qualified scientist designated by [SCI] may participate in this process subject to the terms stated in Section A / Article 4. In addition, in the course of the contract period, DTP/NCI will assist the [SCO], in conjunction with [SCI], thereby assisting the [SC], to develop the capacity to undertake drug discovery and development, including capabilities for the screening and isolation of active compounds from plants, microbes and marine organisms.
4) Subject to the provision that suitable laboratory space and other necessary resources are available, DTP/NCI agrees to invite a senior technician or scientist designated by [SCI] to work in the laboratories of DTP/NCI or, if the parties agree, in laboratories using technology which would be useful in furthering work under this agreement. The duration of such a visit would not exceed one year except by prior agreement between [SCI] and DTP/NCI. The designated Guest Researcher will be subject to provisions usually governing Guest Researchers at NIH, except when carrying out research on materials provided through collections in [Source Country]. Salary and other conditions of exchange will be negotiated in good faith.
5) In the event of the isolation of a promising agent from a plant, microbe or marine macro-organism collected in

[Source Country], further development of the agent will be undertaken by DTP/NCI in collaboration with [SCI]. Once an active agent is approved by the DTP/NCI for preclinical development, [SCI] and the DTP/NCI will discuss participation by SCI scientists in the development of the specific agent.

The DTP/NCI will make a sincere effort to transfer any knowledge, expertise, and technology developed during such collaboration in the discovery and development process to [SCI], subject to the provision of mutually acceptable guarantees for the protection of intellectual property associated with any patented technology.

6) DTP/NCI will, as appropriate, seek patent protection on all inventions developed under this agreement by DTP/NCI employees alone or by DTP/NCI and [SCG or SCO or SCI] employees jointly, and will seek appropriate protection abroad, including in [Source Country], if appropriate.
7) All licenses granted on any patents arising from this collaboration shall contain a clause referring to this agreement and shall indicate that the licensee has been apprized of this agreement.
8) Should the agent eventually be licensed to a pharmaceutical company for production and marketing, DTP/NCI/NIH, will require the successful licensee to negotiate and enter into agreement(s) with the [SCG] agency(ies) or [SCO] as appropriate. This agreement(s) will address the concern on the part of the [SCG or SCO] that pertinent agencies, institutions and/or persons receive royalties and other forms of compensation, as appropriate.
9) Such terms shall apply equally to instances where an invention is directed to a direct isolate from a natural product material, a product structurally based upon an isolate from the natural product material, a synthetic material for which the natural product material provided a key development lead, or a method of synthesis or use of any aforementioned isolate, product or material; though the percentage of royalties negotiated as payment might vary depending upon the relationship of the marketed drug to the originally isolated product. It is understood that the eventual development of a drug to the stage of marketing is a long term process which may require 10–15 years.
10) In obtaining licensees, the DTP/NCI/NIH will require the license applicant to seek as its first source of supply the natural products from [Source Country]. If no appropriate licensee is found that will use natural products available from [Source Country], or if the [SCG] or [SCO] as appropriate, or its suppliers cannot provide adequate amounts of raw materials at a mutually agreeable fair price, the licensee will be required to pay the [SCG] or [SCO] as appropriate, an amount of money (to be negotiated) to be used for expenses associated with cultivation of medicinal plant, microbe or marine macro-organism species that are endangered by deforestation, or for other appropriate conservation measures. These terms will also apply in the event that the licensee begins to market a synthetic material for which a material from [Source Country] provided a key development lead.
11) Article 10 shall not apply to organisms which are freely available from different countries (i.e., common weeds, agricultural crops, ornamental plants, fouling organisms) unless information indicating a particular use of the organism (e.g., medicinal, pesticidal) was provided by local residents to guide the collection of such an organism from [Source Country], or unless other justification acceptable to both the [SCG or SCO] and the DTP/NCI/NIH is provided. In the case where an organism is freely available from different countries, but a phenotype producing an active agent is found only in [Source Country], Article 10 shall apply.
12) DTP/NCI will test any pure compounds submitted by the [SCG or SCO] and [SCI] scientists for antitumor and anti HIV/AIDS activity, provided such compounds have not been tested previously in the DTP/NCI screens. If significant antitumor or anti HIV/AIDS activity is detected, further development of the compound and investigation of patent rights will, as appropriate, be undertaken by DTP/NCI in consultation with [SCI] and the [SCG or SCO].

    Should an agent derived from the compound eventually be licensed to a pharmaceutical company for production and marketing, DTP/NCI/NIH will require the successful licensee to negotiate and enter into agreement(s) with the appropriate [SCG agency(ies) or SCO]. This agreement will address the concern on the part of the [SCG or SCO] that pertinent agencies, institutions and/or persons receive royalties and other forms of compensation, as appropriate.
13) DTP/NCI may send selected samples to other organizations for investigation of their anti-cancer, anti-HIV or other therapeutic potential. Such samples will be restricted to those collected by NCI contractors unless specifically authorized by the [SCG or SCO]. Any organization receiving samples must agree to compensate the [SCG or SCO] and individuals, as appropriate, in the same fashion as described in Articles 8–10 above, notwithstanding anything to the contrary in Article 11.

B. The role of the Source Country Government ("SCG") or Source Country Organization(s) ("SCO") in the collaboration will include the following:

1) The appropriate agency in [SCG or SCO] will collaborate with Contractor in the collection of plants, microbes and marine macro-organisms, and will work with Contractor to arrange the necessary permits to ensure the timely collection and export of materials to DTP/NCI.
2) Should the appropriate agency in [SCG or SCO] have any knowledge of the medicinal use of any plants, microbes and marine macro-organisms by the local population or

traditional healers, this information will be used to guide the collection of plants, microbes or marine macro-organisms on a priority basis where possible. Details of the methods of administration (e.g., hot infusion, etc.) used by the traditional healers will be provided where applicable to enable suitable extracts to be made. All such information will be kept confidential by DTP/NCI until both parties agree to publication.

The permission of the traditional healer or community will be sought before publication of their information, and proper acknowledgment will be made of their contribution.

3) The appropriate agency in [SCG or SCO] and Contractor will collaborate in the provision of further quantities of active raw material if required for development studies.
4) In the event of large amounts of raw material being required for production, the appropriate agency of the [SCG or SCO] and Contractor will investigate the mass propagation of the material in [Source Country]. Consideration should also be given to sustainable harvest of the material while conserving the biological diversity of the region, and involvement of the local population in the planning and implementation stages.
5) [SCG or SCG] and SCI scientists and their collaborators may screen additional samples of the same raw materials for other biological activities and develop them for such purposes independently of this agreement.

This agreement shall be valid as of the date of the final authorized signature below for an initial period of five (5) years, after which it can be renewed by mutual agreement. It may be amended at any time subject to the written agreement of both parties. Copies of such amendments will be kept on file at both of the addresses indicated below.

For the National Cancer Institute:

Andrew C. von Eschenbach, M.D.
Director, National Cancer Institute

Date

mailing and contact address:

Technology Transfer Branch
National Cancer Institute at Frederick
Fairview Center, Suite 502
1003 – W. 7th Street
Frederick, Maryland 21701-8512
U.S.A.
Telephone: 301-846-5465
Facsimile: 301-846-6820

For [SCI] or [SCO]:

Name (typed):
Title:

Date

mailing and contact address: