CURRENT STATUS OF THE NCI PLANT AND ANIMAL PRODUCT PROGRAM

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Background

The National Cancer Institute began an organized program to screen for new anticancer agents in 1955 with the founding of the Cancer Chemotherapy National Service Center (CCNSC). The original objective of the CCNSC was the acquisition and screening of chemical substances for anticancer activity together with conducting the necessary preclinical development studies needed to bring active drugs to clinical trials. The program was intended to be a service to researchers in academia, research institutes and the pharmaceutical industry who were working in the cancer field but lacked resources to follow up their investigations. The existence of an integrated program to develop antitumor agents was quickly recognized by Congress as a valuable resource in the fight against cancer. The CCNSC was rapidly developed into a large, highly targeted drug development program encompassing all areas of drug development from acquisition of substances for screening through advanced clinical trials. By 1958, the CCNSC had evolved from a modest service program to an entire drug development effort and one of the first decisions which had to be made was how the program would attempt to discover useful anticancer agents. Targeted programs to synthesize and screen analogs of existing anticancer agents were an obvious place to start, but it was recognized that the number of known effective drugs was very small and that there was also a major need to discover new types of agents. NCI decided that since there was not enough information available for extensive rational drug design based on the biology and chemistry of tumor cells, an empirical approach should be used based on screening large numbers of materials. 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 chemicals with a wide variety of biological activities. By 1957, large numbers of fermentation broths were being tested, and in 1960 a major effort was begun in screening plant extracts. A small number of animal extracts, mainly of marine origin, were also tested beginning in 1960 but by the end of 1968 only 1,000 animal extracts had been screened whereas 39,000 plant extracts had been screened at that time. Table 1 shows the statistics on the plant and animal extracts screened as of the end of 1980.

TABLE 1. Plant and animal extracts screened by NCI to January, 1981.

Plant extracts	
Total screened	114,045
Confirmed actives	4,897 (4.3%)
number of genera	1,551
number of species	3,394
Animal extracts	
Total screened	16,196
Confirmed actives	660 (4.1%)
number of genera	413
number of species	561

[1]Presented as a plenary lecture of the joint meeting of the American Society of Pharmacognosy and the Society for Economic Botany, Boston, Massachusetts, July 13–17, 1981.

The decision by CCNSC in 1956 to include natural products extracts as an important component of the NCI screening program has proven to be very wise. Many active compounds which have unusual structures and are worthy of development to clinical trials have been discovered. This has opened up new areas for investigation of related compounds as antitumor agents and has also provided new biochemical tools for cell biology. The inclusion of natural products of plant, animal, and microbial origin in the screening program has resulted in the discovery of different substances from each type of natural product; there has been almost no overlap among the compounds isolated. Although many compounds of both natural and synthetic origin have been discovered which have good activity in the experimental models used by the NCI, only a small number have proven useful in the clinic and there is a continuing need for active compounds with novel structures and mechanisms of action. It is, therefore, clear that screening of new materials must be an ongoing process. Today this is complicated by many factors including the large number of materials previously screened, the increased expense of newer, more sophisticated screens, inflation, and, in the case of natural products, difficulties in obtaining collection and export permits from the countries of origin.

Selection of Materials for Screening

During the early years of the NCI drug development program, most materials screened represented new structural classes in the case of synthetic agents and pure natural products or represented novel botanical or zoological groups in the case of crude extracts. In the synthetic compound acquisition program, all of the 340,000 different structures tested have been computerized, and programs have been developed to relate new structures to the master file in order to identify novel combinations of structural features and to reject compounds which are in classes which have a low probability of showing activity. The fermentation program eliminates repetitive isolation of the same compounds by an extensive program of early identification of active compounds in crude broths which includes antimicrobial spectrum, bioautography, thin layer and paper chromatography, uv absorption and spectrum of activity against biochemical screens. The plant program relies heavily on taxonomy in both the selection of plants for collection and screening and in the identification of known compounds in active extracts. The first approximation in 1975 was to evaluate the percentage of confirmed active plant extracts by family; several families were designated Families of No Interest (FONI) due to the low percentage of activity found. Families with a high percentage of active compounds were designated Families of Special Interest (FOSI) and were emphasized in future collections. The FOSI families clearly gave high percentages of active extracts; because there was also a high incidence of repetitive isolation of the same compounds or closely related analogs, this was subsequently abandoned. The FONI concept was likewise discontinued since the data on distribution of the anticancer compounds isolated in the NCI program or reported in the literature showed that active compounds were commonly restricted to a genus or a few related genera or a tribe or sub-tribe; therefore, it was apparent that elimination of whole plant families could easily result in missing good leads. Although active compounds may sometimes be found in only one species in a genus, the more general case is that several or many species in a genus and, indeed, several related genera will contain the same active compound. It was decided to use the generic level to eliminate plants from the collection program, and a compilation was made of genera that had been screened extensively without activity being shown or for which the active components were known from NCI data or from the literature. This listing was added to a list of particular species which had been screened sufficiently even though their genus might not have been. This combined listing was called the Species Low on Priority (SLOP) list which is currently in use for rejecting plants for the NCI screening program. While this works well as a

negative list enabling NCI to screen fewer plants more efficiently, we also wanted to make a positive list of plants which had not been screened and, again, the genus level was chosen. The biggest problem was in getting a complete listing of the genera of higher plants and resolving the nomenclatural confusions and synonymy. Fortunately, the International Association of Plant Taxonomy has recently completed the Index Nomenum Genericorum (ING) which contains and cross-references all accepted and non-accepted generic names and we have been able to obtain selected information from that index through the courtesy of the Smithsonian Institution. We are currently doing the computer programming work necessary to make the ING data compatable with the NCI master file of plant accessions. We will be able to develop a comprehensive list of unscreened genera in a few months. The Economic Botany Laboratory of the United States Department of Agriculture will then investigate these genera as to the species they contain, where they occur, whether the plants are large enough to be collectable, etc. and will use this information to develop a priority list for NCI collections.

The use of taxonomy in identifying known active compounds can be illustrated by the case of *Merrilliodendron megacarpum*, a monotypic tree in the family Icacinaceae, native to Western Pacific Islands. A collection of this plant made in Guam showed high activity against the P388 leukemia and a literature search revealed no prior chemical work on this genus. The only highly active compounds previously isolated from the Icacinaceae were camptothecin and analogs isolated from *Nothapodytes foedita* (syn. *Mappia foetida*). Comparison of the *Merrilliodendron* crude extract with reference samples of camptothecin and derivatives immediately led to tentative identification of the active components which was quickly confirmed by isolation saving considerable time and expense over bioassay-directed isolation.

The NCI plant screening program has been based since its inception on "random" screening of plants, i.e., plants that have no reason for being screened other than novelty to the program. There is a fairly extensive literature on folklore and traditional medicine in treatment of cancer, most of which has been compiled by Jonathan Hartwell, formerly head of the Natural Products Section at NCI (1). Spjut and Perdue (2), in 1976, did a retrospective correlation of several groups of medicinal and poisonous plants with NCI screening data and found substantially increased activity for plants used as anthelmintics, arrow poisons and fish poisons when compared with plants selected at random. It must be noted, however, that these authors considered activity in all NCI systems including KB cell culture. Further examination of this data indicates that the highest correlation is between poisonous or toxic plants and *in vitro* activity (cell cytotoxicity) and that the correlation between these plants and activity *in vivo* is not strong. It is certainly not surprising that poisonous plants are toxic to cells in culture.

Terminology and Data Interpretation

When one reads the literature on natural products with biological activity in the cancer area and when one talks to fellow chemists about their interpretation of the significance of the work, it is striking to note how loosely terminology is used and how often the information presented is misinterpreted by the authors themselves or by the readers.

There is a great deal of loose usage of terminology with reference to *in vitro* and *in vivo* antitumor activity. Many compounds are cited as anticancer or antitumor agents which are, in fact, only cytotoxic to tumor cells *in vitro*. This group of compounds contains a wide variety of toxic substances which display no particular selectivity toward tumor cells as opposed to normal cells and have no hope of being useful anticancer agents. Some examples of such compounds are found in table 2. Put simply, most *in vitro* active agents are poisons of one type or another.

TABLE 2. KB active compounds with no useful *in vivo* activity.

Plant derived

- cardenolides and bufadienolides
- saponins
- cucurbitacins
- various toxic alkaloids
  - — aconitine type
  - — many indoles
  - — aporphines
  - — curare type
  - — lycorine type
- most lignans
- sesquiterpene lactones
- flavonoids

Animal derived

- toad poisons
- snake toxins
- starfish saponins
- palytoxins
- cembranolides
- dinoflagellate toxins

We wish to put forward definitions of terminology which we hope will be broadly adopted to eliminate the confusion caused in the literature which is often misleading to the uninitiated reader. We define **CYTOTOXICITY** as toxicity to tumor cells in culture and do not use such terms as antitumor, anticancer or antineoplastic to relate to *in vitro* results. For *in vivo* activity in experimental systems we use the terms **ANTITUMOR** or **ANTINEOPLASTIC** in reporting results. We reserve the term **ANTICANCER** for reporting clinical trials data in man in order to avoid the sensationalism which often results from the use of this word and to avoid the false hopes that cancer patients have when they hear of new "anticancer" agents which are only in early experimental stages of development.

Screening Methods

The single most important factor in drug discovery for any type of biological activity is the quality of the screening methodology employed. The predictability of the screens for clinical activity is absolutely critical since if the screens identify compounds which are clinically inactive all of the effort which goes into the development of such compounds is wasted. The correlation of screening "actives" with clinical activity is an extremely difficult process since it takes a number of years from when active compounds are detected until results of clinical trials are available, and the data base is very small since few compounds ultimately reach clinical trials.

New screens are usually validated by testing a wide variety of agents which have been in clinical trials and comparing the detection of known clinically active compounds (true positives) with the detection of compounds which show activity in experimental screens but not in man (false positives) and compounds which are clinically active but undetected in experimental screens (false negatives). The data thus obtained can be used to judge the value of a new screening system. The background and history of the *in vivo* screening systems currently in use at NCI have been reviewed by Goldin *et al.* (3).

There are special problems encountered in screening crude natural products. It is essential to discuss these problems in some detail since failure to deal with them will result in an ineffective program. These factors are summarized in table

3. High sensitivity is required because most natural products which are biologically active are generally present in the crude extract or broth at dilutions of 1:1,000 or more even up to 1:1,000,000. Therefore, the screen is being required to pick the needle out of the haystack. If one calculates the amount of an individual compound present in a 400 mg/kg dose as used by NCI, it quickly becomes clear that in order to be detected an active compound would have to be either quite abundant or extremely potent and that it is highly likely that *in vivo* screening alone is going to miss compounds which may be quite active but are not potent enough or not concentrated enough in a crude extract for detection. There is a solution to this problem which is the use of *in vitro* methods which are often much more sensitive than *in vivo* screens.

TABLE 3. Special considerations in screening crude extracts.

Selectivity	—must be high enough to limit the number of leads for follow-up evaluation
Sensitivity	—must be very high in order to detect low concentration of active compounds
Specificity	—assay must be insensitive to a wide variety of inactive compounds
Methodology	—must be adaptable to materials which are 1) highly colored 2) tarry 3) poorly soluble in water 4) chemically complex

Before proceeding further it is necessary to define key terminology (table 4) to differentiate between prescreens, screens and bioassays since the terms are all too frequently used incorrectly and a clear understanding of these terms is required for the discussion which follows.

TABLE 4. Definitions of screening terminology.

Screen	—Assay used to select compounds or extracts for further development
Pre-screen	—Assay used to select compounds or extracts for screening
Bioassay	—Assay used to guide isolation of pure compounds from complex mixtures

The second problem in screening crude natural products is that the prescreen, screen or bioassay used must be insensitive to the thousands of inactive compounds which are potential interfering substances. Direct spectrophotometric assays, for example, are extremely difficult to carry out reproducibly. A related problem is that the assay system must be insensitive to ubiquitous compounds which will give false actives. NCI dropped the Walker 256 screen from use in the plant program because it was highly sensitive to tannins. The last probem to be mentioned is that the assay must be highly selective in order to ignore all of the many negative materials and to detect a relatively small number of actives. These requirements are special needs for screening natural products, but the assay chosen must still meet the other requirements of *any* good assay including validity, predictability, correlation, reproducibility, and reasonableness of cost.

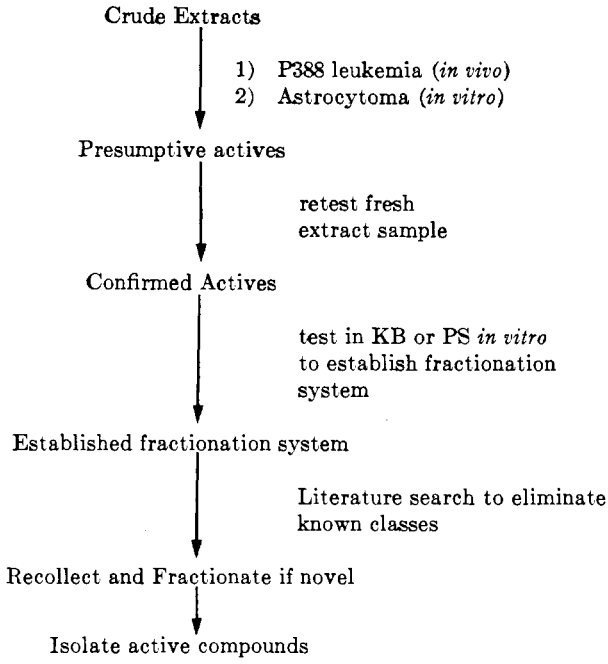


CHART 1. Current program flow for plant extracts.

The current approach at NCI for crude plant and animal extracts is to use a primary *in vivo* screen, the P388 leukemia, and to use *in vitro* systems as bioassay tools to guide isolation of *in vivo* active compounds (chart 1). This has been successful over the years, but data from the NCI fermentation program indicate that the number of *in vivo* active leads can be increased by the use of *in vitro* prescreens first (table 5). The proposed scheme for screening crude plant and animal extracts is shown in chart 2. The plan is to use several prescreens which detect different types of biological activities and mechanisms of action in order to be sure of detecting a variety of different types of structures. While it is possible to prescreen using only one general type of system such as cytotoxicity to tumor cells in culture, this type of screen will miss compounds which are not cytotoxic and selects only for toxic compounds. The advantages of a battery of prescreens are that 1) there is a greater chance to detect novel compounds, 2) compounds with

TABLE 5. Effect of using prescreens in assay of fermentation broths.

Current data

For one NCI contractor from 1978–1980

Cultures isolated and prescreened *in vitro*	2,793
Cultures active *in vitro*	460 (17%)
Cultures active *in vivo* (only in vitro actives screened)	182 (39%)
Total cultures active *in vivo*	182/2,793 (6.5%)

Historical data

Using no prescreens all cultures tested *in vivo* showed an activity rate of 5 to 7%.

Conclusions

1) Using selected *in vitro* prescreens few if any *in vivo* active leads are missed.
2) *In vivo* screening was reduced by 83%.
3) Using *in vitro* prescreens reduces costs and allows more rapid identification of active leads.

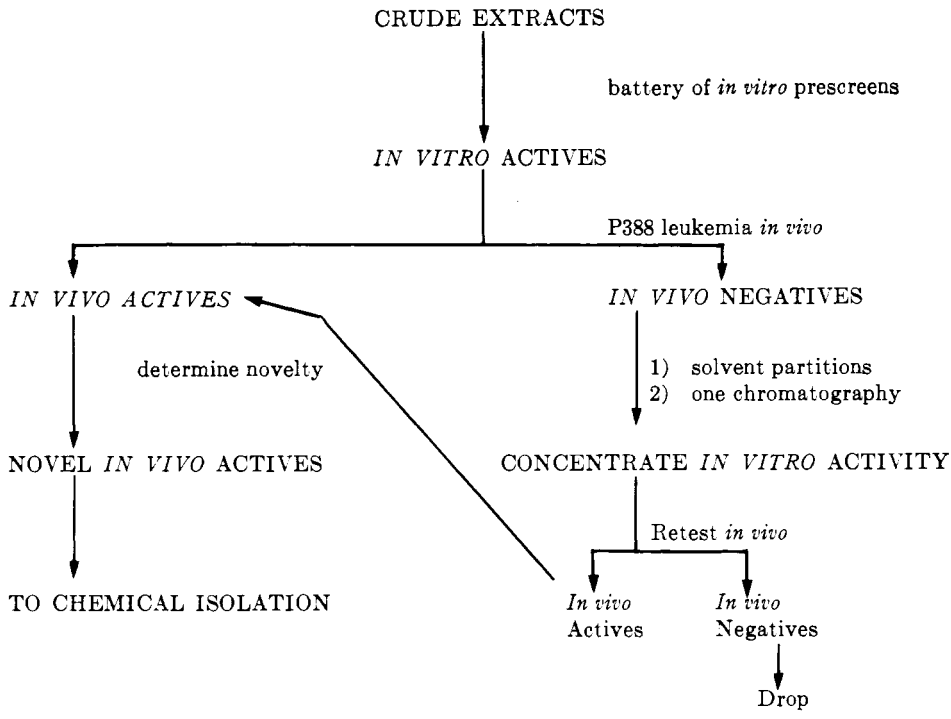


CHART 2. Proposed program flow for plant extracts.

differing mechanisms of action can be selected, 3) identification of known compounds or known classes of compounds is simplified since only certain classes of compounds will show activity in particular prescreens, and 4) classes of compounds of little interest can be selected against by judicious choice of prescreens. The prescreens now in development for future use are shown in table 6. Validation of these prescreens and development of detailed protocols should be complete in about two years.

TABLE 6. Prescreens in development at NCI.

Assay	*Biological Activities Selected*
Astrocytoma	mitotic inhibition
Phage induction	DNA damage
Aminopeptidase B	cell surface changes
Candida	cell membrane actives, antifungals
Xanthomonas	glycosylation inhibitors
Agrobacterium	plasmid transfer

The first of these systems, the astrocytoma (9ASK) bioassay, is shown schematically in figure 1. Dibutyryl cyclic AMP is used to convert rat glioma cells from a fibroepithelial morphology to a neuroglial or astrocyte form. This conversion is reversed in the presence of mitotic inhibitors in a concentration-dependent fashion. The assay readily detects such known antimitotic agents as vincristine, vinblastine, colchicine, maytansine, and podophyllotoxin and has detected activity in a number of plants which are not reported to contain antimitotic principles. Several of these plants are currently undergoing fractionation studies using the 9ASK system as a bioassay, but no pure active compounds have been isolated as yet. Antimitotic agents are of particular interest because they are generally not mutagenic as many alkylating agents or DNA interactive agents are.

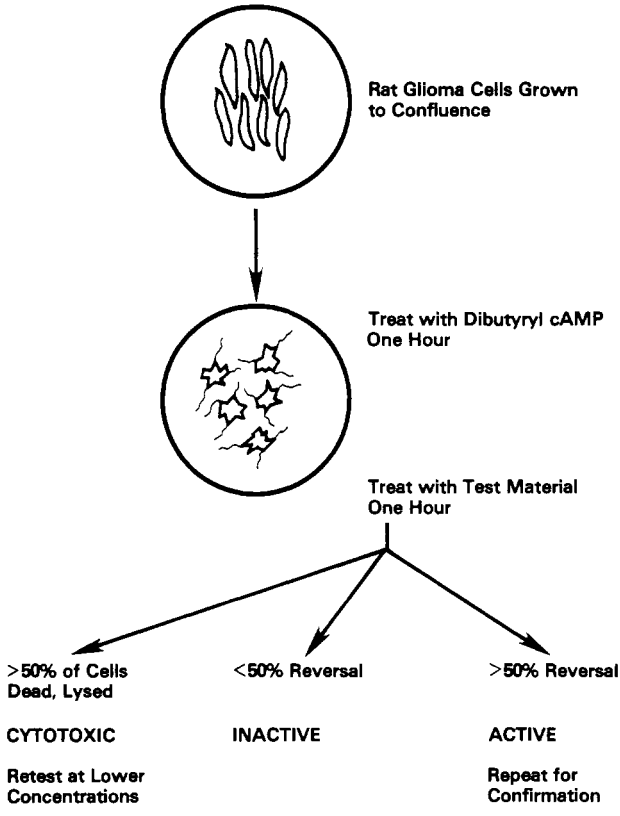


FIGURE 1

The NCI program has always used bioassay-directed isolation to purify active compounds from crude mixtures. It is our experience that the biologically active compounds of interest are nearly always present in low concentrations (generally from 1 to 100 mg per kilogram of dried plant), and the chances of finding these compounds by the standard phytochemical method of purifying and characterizing the most abundant compounds present in an active extract are quite small. Bio-

TABLE 7. Comparison of *in vivo* and *in vitro* bioassays.

IN VIVO	*IN VITRO*
ADVANTAGES	ADVANTAGES
• Activity data	• Speed • Cost • Sensitivity • Small sample size
DISADVANTAGES	DISADVANTAGES
• Long turn around • Expensive • Often less sensitive • Large sample needed	• In vitro data only • Activity may not correspond to *in vivo* activity

assays can be performed either *in vivo* or *in vitro* and a comparison of these methods is found in table 7. Due to the more rapid turn around time and lower cost of *in vitro* bioassays, they are normally to be preferred but it is also important to periodically check the *in vitro* active fractions *in vivo* since it is not uncommon for there to be two or more classes of active compounds present in a crude extract

and the *in vivo* active compounds can be missed if only cytotoxicity tests are used for bioassay. Examples are found in the family Celastraceae where cytotoxic, but *in vivo* inactive, quinone methides co-occur with maytansine in the genus *Maytenus*, and with tripdiolide in the genus *Tripterygium*. The major bioassays currently in use in the NCI program are the KB and P388 cell lines. While these *in vitro* systems are excellent bioassays, they are poor screens because of their sensitivity to cytotoxic substances which are devoid of *in vivo* activity.

The most dramatic development in screening in the last several years is the stem cell assay. It is now believed by many investigators that only a small percentage of cells in a tumor can reproduce (stem cells) and that all other tumor cells are derived from these stem cells. Stem cells are therefore responsible for tumor growth, metastasis and regrowth after treatment and inhibition or eradication of stem cells is the key to successful cancer chemotherapy.

Methods have been developed to clone stem cells directly from biopsy specimens of individual patient tumors (4) and drug sensitivities of these stem cells *in vitro* have been determined. Retrospective studies have been conducted in which physicians' selections of drugs to treat individual patients were subsequently compared with the results of drug inhibition of stem cells from the same tumor. In $>90\%$ of the cases where the therapy was ineffective, the drug chosen was inactive in the stem cell assay on that tumor. In over 70% of the cases where therapy was effective the stem cell assay showed strong inhibition of growth of the patient's tumor. The correlations are incomplete, however, because not all tumors could be cloned successfully and some assay results were equivocal. The results of this retrospective study were successful enough to warrant a prospective study in which tumors were cloned and stem cell inhibition was used to select drugs for therapy. Not all patients were treated with the drugs selected because some drugs were contraindicated in certain patients and other patients were not evaluable because the drugs had to be withdrawn due to side effects but for those patients who were able to complete at least one course of therapy there was a greater than 70% correlation between activity of drugs in the stem cell assay and clinical activity. This study is ongoing. It appears that it is now possible to tailor drug therapy to the individual patient and this should have a major impact on the success of cancer chemotherapy in coming years.

NCI is now attempting to develop this assay as a screen for new compounds and a validation study using drugs known to be effective in animals and man, in animals only, and in cell culture only is underway. When this is completed, screening of new compounds in the stem cell system will begin. This assay is closer to the human situation than any assay previously used in cancer drug screening and the current feeling is that it has a tremendous potential for discovering clinically effective agents.

Conceptually, this screening will be very different from anything which has been previously undertaken since each new compound will be tested against a variety of human tumors to determine its activity. The tumors used will be different for nearly every compound tested because of the limited number of cells which can be isolated as first generation clones from biopsies.

There are a number of problems with the assay at present, including the availability of biopsy specimens, variations in cloning efficiencies, high cost per assay, and solubility requirements for the test compounds. These problems are all being worked out. Since this is a cytotoxicity assay, it may be sensitive to toxic substances which don't have *in vivo* activity so a companion *in vivo* system possibly using athymic mice will also be developed.

Drug Development Flow

When pure compounds are isolated as a result of bioactivity-directed fractionation, they enter the screening flow for pure compounds shown in chart 3. All pure compounds entering the NCI program, whether of synthetic or natural pro-

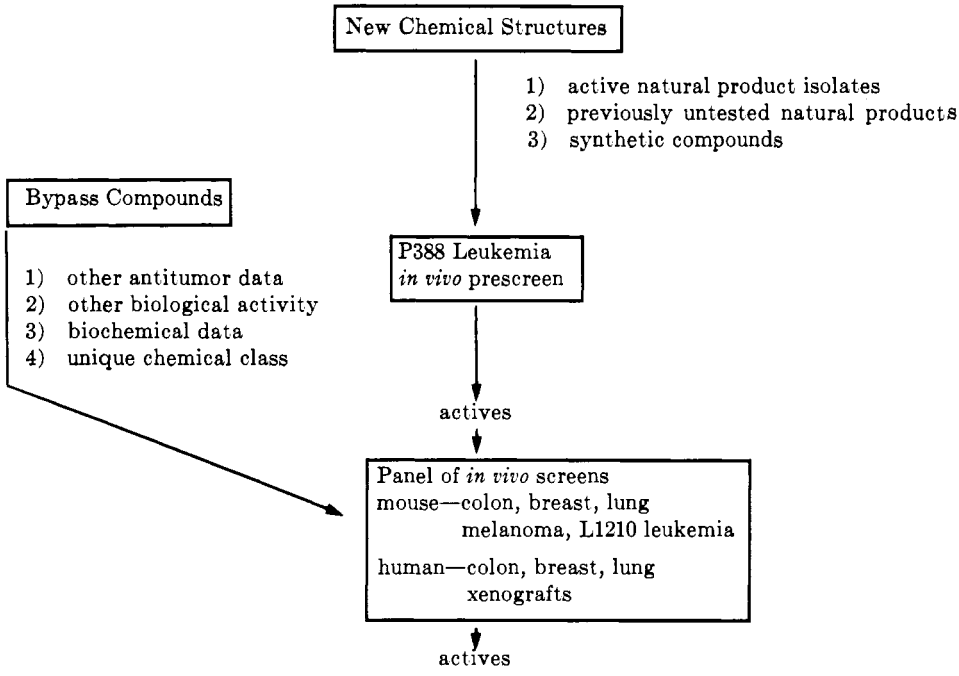


CHART 3. Flow through NCI screens.

duct origin, undergo initial screening in the P388 leukemia system with two classes of exceptions, certain analogs and bypass compounds. Some classes of compounds are extremely active in P388 and many analogs give the same high activity, so other tumor systems are much more advantageous for screening. NCI has established individual testing protocols for about 20 classes of antitumor agents which all involve direct comparison with a parent compound in one or more *in vivo* screening models which may or may not include P388 leukemia testing depending on the class of compound. It is also recognized that although the P388 leukemia detects greater than 90% of known clinically effective agents, it is not perfect. Consequently an alternate mechanism has been established to get other compounds into more detailed screening. This is the bypass mechanism by which compounds which have never been tested in P388 or which are negative in P388 can go on to further testing. Such bypasses are made on the basis of antitumor or cytotoxic activity from outside sources, interesting biochemical data, biological activity which may be related to anticancer activity, or highly unusual chemical structures.

All novel compounds which are reproducibly active in the P388 leukemia prescreen or which have been selected as bypass compounds then go into tumor panel testing. The panel as presently constituted consists of eight systems of which five are mouse tumor lines and three are human tumor lines carried in athymic mice. This panel was begun in 1976, and the data thus far evaluated show that there are significant differences in the active compounds detected by the mouse tumors and the human xenografts. We are not yet able to evaluate the predictability of these models for clinical activity since it takes several years to develop active compounds to clinical trials and another two years of clinical testing to get sufficient clinical data to make a judgement on clinical efficacy.

There are two activity levels for each tumor in the panel, a level of statistically significant activity (minimal activity) and a level of biologically important activity

TABLE 8. NCI tumor panel systems and activity criteria.

Tumor	Tumor code	Evaluation parameter	Confirmed T/C%	
			Minimal	DN2
B16 melanocarcinoma	B1	ILS[a]	125	150
$CD8F_1$ mammary	CD	TWI[b]	20	0
Colon 38	C8	TWI	≤ 42	≤ 10
L1210 lymphoid leukemia	LE	ILS	125	150
Lewis lung carcinoma	LL	ILS	140	150
CX-1 colon xenograft	C2	TWI	≤ 20	≤ 10
LX-1 lung xenograft	LK	TWI	≤ 20	≤ 10
MX-1 breast xenograft	MB	TWI	≤ 20	≤ 10

[a]ILS = increase in life span.
[b]TWI = tumor weight inhibition.

(Decision Network 2 level activity) as shown in table 8. Compounds meeting Decision Network 2 level activity in the panel become candidates for development to clinical trials as shown in chart 4 which outlines the criteria for acceptance and the flow to clinical trials. To put the program in perspective, NCI screens about 10,000 new synthetic compounds and 400 pure natural products per year plus about 14,000 crude natural products extracts (8,000 fermentation, 5,000 plant and 1,000 marine animal). From 8–12 compounds pass Decision Network 2A as preclinical candidates. About 6–8 compounds enter clinical trials each year of which slightly less than half are natural products.

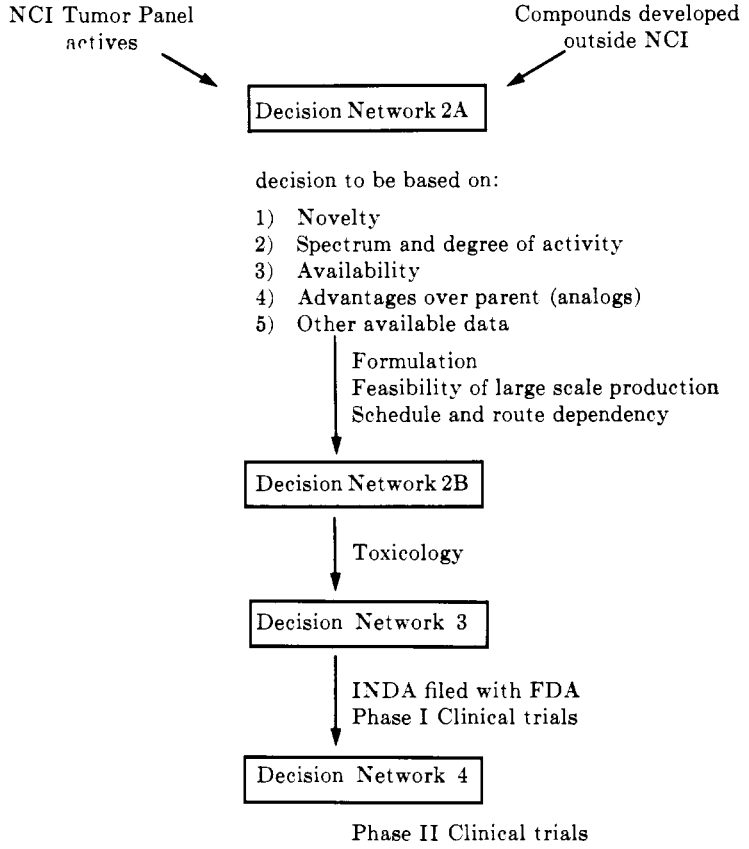


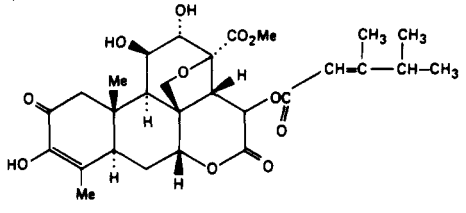
CHART 4. Drug development flow.

Results

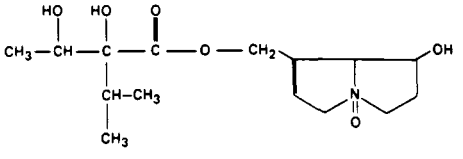
Table 9 shows the current status of plant- and animal-derived compounds which are in clinical trials or which have passed Decision Network 2A or are candidates for advanced preclinical development. Structures of these compounds are shown

TABLE 9. Status of drugs in advanced development

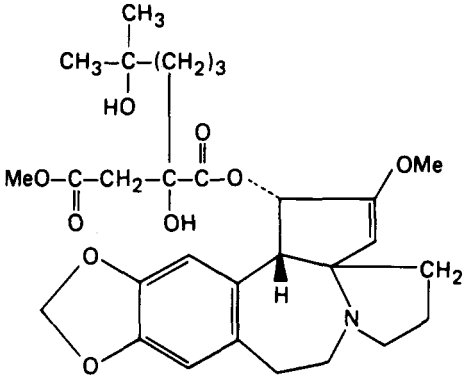
Maytansine	— Phase II trials have shown little activity
Bruceantin	— Phase II trials show minimal activity thus far
Indicine-*N*-oxide	— Phase II trials ongoing. Good responses in leukemia
Homoharringtonine	— Phase I trials to start September, 1981
Ellipticine	— Oral toxicology studies ongoing
Taxol	— Toxicology studies in progress
4-Beta-hydroxywithanolide E	— Formulation development
Didemnin B	— Formulation and analytical studies



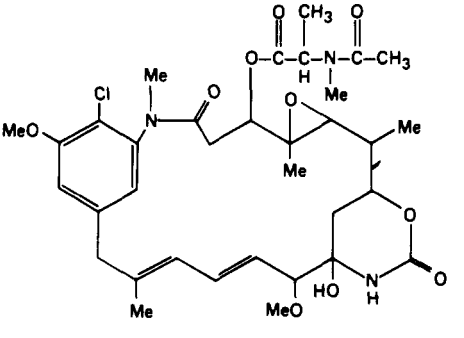
Bruceantin



Indicine, *N*-oxide

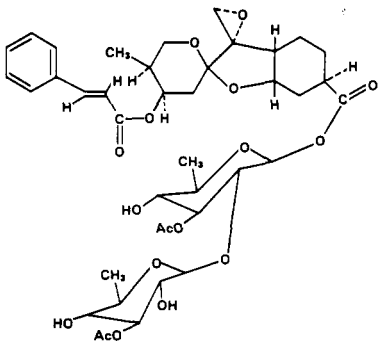


Homoharringtonine

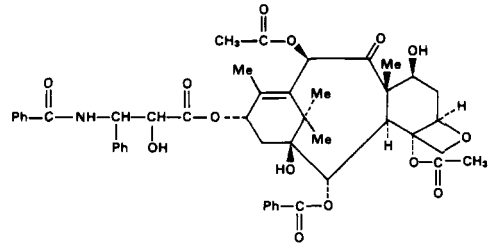


Maytansine

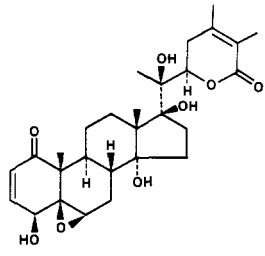
figures 2–4. In clinical trials both maytansine and bruceantin have shown a small amount of activity in which is insufficient to develop them further. Indicine-*N*-oxide showed good activity in leukemia and hints of possible activity in colon cancer and melanomas in early trials. A Phase II study in leukemia was encouraging but was complicated by delayed hepatotoxicity. This study will be continued at lower doses. A recent Phase II study in colon cancer resulted in stable disease in some patients but no significant remissions were noted. Additional supplies of drug are currently being produced for additional clinical studies. Homoharringtonine, which was originally discovered as an antileukemic agent by Powell *et al.* (5) in the United States, could not be developed to clinical trials for a number of years due to a lack of drug supply, and the first clinical trials were done in the People's Republic of China. These trials showed very good results in leukemia and NCI was subsequently able to buy supplies of the drug from China.



Phyllanthoside
NSC 328426



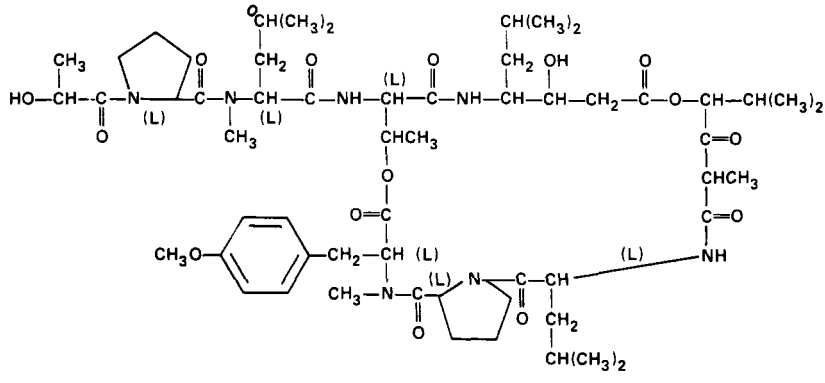
Taxol
NSC 125973



4-β-Hydroxywithanolide E
NSC 212509

Toxicology studies have been completed within the last month and an Investigational New Drug Application is now being filed with FDA. Clinical trials in the United States will be starting in about one month at several institutions. Clinical studies of other tumor types have not yet been reported from China, but the NCI tumor panel data show that homoharringtonine is effective against colon tumors and melanoma in mice in addition to its antileukemic activity. This is the most promising plant-derived drug to go to clinical trials in several years.

Taxol is another plant-derived agent which will probably be in clinical trials within the next year. It is a mitotic inhibitor which has a very unusual mechanism of action in that it stabilizes microtubules and prevents disaggregation in contrast to other agents like maytansine, *Vinca* alkaloids, and colchicine which inhibit



Didemnin B
NSC 325319

microtubule formation (6). Taxol has shown activity against a number of tumor panel systems including: B16 melanoma, L1210 leukemia, and all three human tumor xenografts.

4-Beta-hydroxywithanolide E is one of the few steroidal lactones which are active in current NCI screens in contrast to the bufadienolides, cardenolides, and other withanolides which are generally cytotoxic only. The interesting feature of this molecule is that the orientation of the side chain at C–17 is alpha instead of the more usual beta, which confers a markedly different overall shape to the molecule. This drug is now undergoing formulation studies in preparation for toxicology.

Phyllanthoside was originally isolated by the late S. M. Kupchan's group who established the structure of the aglycone (7). Recent results (8) from G. R. Pettit's group at Arizona State University have established the structure of the glycosidic linkages and have provided additional material for further testing. It is interesting that phyllanthoside shows only modest activity in the P388 leukemia screen and is considerably more active against the B16 melanoma. A decision on development of phyllanthoside towards clinical trials will be made in the next few months when antitumor testing is completed.

In the animal area there is one compound which has passed Decision Network 2A and is in preclinical development. This is didemnin B (fig. 4), which was isolated by two groups, Rinehart's at the University of Illinois (9) and Weinheimer's at the University of Houston. Didemnin B is an extremely potent agent which shows activity against the B16 melanoma system in μg/kg doses and has also shown a broad spectrum of activity against stem cells in culture. A closely related compound, didemnin A, is of interest because of its antiviral activity. Several other peptides have been isolated from marine organisms recently including work by Ireland and Scheuer (10) and Pettit (11) and these also have shown antitumor activity in preliminary screening. There are several non-peptidyl compounds isolated recently from marine organisms which show excellent activity in the P388 leukemia and which appear to be likely prospects for further development, but structural studies are incomplete at present.

It has not been possible to discuss many of the agents in earlier stages of development in this brief review, but these have been summarized in other recent publications (12, 13).

In conclusion, it is expected that the NCI program will continue to discover novel compounds of natural origin with antitumor activity. It is hoped that these new agents will ultimately become clinically useful in cancer treatment as well as being novel biochemical probes for the investigation of tumor cell biology.

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