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COMMENTARY

Plant-Derived Anticancer Agents

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PROGRAM FOR COLLABORATIVE RESEARCH IN THE PHARMACEUTICAL SCIENCES, DEPARTMENT OF MEDICINAL CHEMISTRY AND PHARMACOGNOSY, COLLEGE OF PHARMACY, AND DEPARTMENT OF SURGICAL ONCOLOGY, COLLEGE OF MEDICINE, UNIVERSITY OF ILLINOIS AT CHICAGO, CHICAGO, IL 60612, U.S.A.

ABSTRACT. Natural product drugs play a dominant role in pharmaceutical care. This is especially obvious in the case of antitumor drugs, as exemplified by paclitaxel (Taxol®), vincristine (Oncovin®), vinorelbine (Navelbine®), teniposide (Vumon®), and various water-soluble analogs of camptothecin (e.g., Hycamtin®). The most efficient method of discovering drugs such as these (i.e. novel chemical prototypes that may function through unique mechanisms of action) is bioactivity-guided fractionation, and it is certain that additional natural product drugs, some of which should be useful for the treatment of humans, remain to be discovered. For the commercial procurement of structurally complex natural product drugs, isolation from plant material may be most practical. With the advent of combinatorial chemistry and high throughput screening, however, even greater progress may now be expected with natural product leads. While systemic drug therapy, to an appreciable extent based on natural products, has been the mainstay of pharmaceutical care, the logic of disease prevention is overwhelming. Bearing in mind the pandemic nature of cancer, a proposal is put forth to create a cancer chemoprevention drug formulation for utilization on a widespread basis by the general population. Cancer chemopreventive agents, many of which are natural products, are capable of preventing or inhibiting the process of carcinogenesis. As with other pharmaceutical agents useful for disease prevention, a pharmacoeconomic analysis of a cancer chemopreventive formulation would need to be considered, and the composition of the formulation should improve over time. Nonetheless, implementation should commence immediately. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 53;2:121–133, 1997.

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For time immemorial, terrestrial plants have played a dominant therapeutic role in the treatment of human ailments. Although the actual rationale or process leading to incorporation of specific terrestrial plant materials into the therapeutic armamentarium utilized for various disease states is generally not known with certainty, some speculation can be made. For example, one practice that has led to the choice of plant materials is based on the so-called "doctrine of signatures." This entails utilizing plants for the treatment of specific diseases on the basis of visual analogy. Accordingly, as an example, a plant exuding a red-colored material could be utilized for amenorrhea. In other cases, it is likely that plant materials were identified originally due to serendipitous events. Along these lines, it is believed that Spaniards who originally occupied the Americas learned from Peruvian Indians to drink from defined bodies of water for the treatment of fever (malaria). It is now speculated that these bodies of water contained the bark of fallen *Cinchona* trees, a source of quinine. Irrespective of the validity of these methods of "drug discovery," it is a matter of fact that traditional medicines, largely based on terrestrial plants, currently embody something on the order of 85% of the treatment regimens utilized by the inhabitants of underdeveloped countries. Notably, since these individuals total approximately 4.5 billion, about 79% of the world's population is reliant on traditional medicines to some extent.

In "Western" civilization, terrestrial plants also continue to play an important role in health care. In fact, the intense influence plant-derived drugs exert on the practice of Western medicine may be viewed by many as rather surprising. As summarized recently, approximately 120 drugs are obtained from plants [1], a large number of therapeutic activities are mediated by these drugs, and a host of the drugs currently in use are still obtained from plants in which they are synthesized. Examples include steroids, cardiotonic glycosides (*Digitalis* glycosides), anticholinergics (belladonna-type tropane alkaloids), analgesics and antitussives (opium alkaloids), antihypertensives (reserpine), cholinergics (physostigmine, pilocarpine), antimalarials (*Cinchona* alkaloids), antigout (colchicine), anesthetic (cocaine), skeletal muscle relaxant (tubocurarine), and anticancer agents (see below) [2]. As an indication of the extent of utilization, it

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has been estimated that plant-drugs represent about 25% of the prescription drug market in the United States [3, 4], and, in 1990, this equated to a retail value of approximately $15.5 billion [5]. When scrutinized in a slightly different manner, it was noted that over one-half of the top 25 prescription products are plant-derived products [6]. Based on these data, clearly, terrestrial plants continue to play a striking role in all forms of modern-day pharmaceutical care.

PLANT-DERIVED ANTICANCER AGENTS: AN OVERVIEW

Very few drugs currently being used for the treatment of cancer were discovered on the basis of rational structural design. One notable exception is 5-fluorouracil, conceptualized and evaluated by Heidelberger and coworkers in the 1950s [7]. Most other agents have been discovered as a result of empiricism, serendipity, or large-scale evaluation (screening) programs. This should not be viewed as a criticism of the dedication, diligence, intellect, or intuition of the various investigators who have provided the agents currently in use, but the pathway from the discovery/concept phase to clinical utilization is generally ambiguous. Starting with the availability of nitrogen mustard in the late 1940s for systemic chemotherapy, there are currently over 50 anticancer drugs available. Many of these clinically useful entities are either natural products or derivatives of natural products. Four examples are illustrated in Fig. 1: *Taxus brevifolia* L., *Catharanthus roseus* G. Don, *Podophyllum peltatum* L., and *Camptotheca acuminata* Decne. The respective antitumor agents derived from these plants are paclitaxel (Taxol®), vincristine (Oncovin®), podophyllotoxin, and camptothecin (Fig. 1, insets). Interestingly, these substances embrace some of the most exciting new chemotherapeutic agents currently available for use in clinical settings. Use of paclitaxel has been expanded to include a greater variety of cancers and, more recently, Taxotere® has received approval by the F.D.A. Further, the semi-synthetic bisindole alkaloid, vinorelbine (Navelbine®), which is closely related to vincristine, as well as a podophyllotoxin analog, teniposide (Vumon®), and the water-soluble camptothecin analog topotecan hydrochloride (Hycamtin®) have been approved for human use during the last several months. Based on this impressive array of structures and activities, it is clear that plants and plant-derived drugs play a dominant role in contemporary cancer chemotherapy.

APPROACHES FOR THE DISCOVERY OF NOVEL PLANT-DERIVED ANTICANCER AGENTS

Various methods may be employed for the discovery of naturally occurring drugs. For example, phytochemical screening or exploitation of chemotaxonomic relationships may be of some practical benefit. In general terms, however, this approach is not directed toward the discovery of new, therapeutically useful substances. Of course serendipity may come into play, and straightforward isolation procedures could lead to the discovery of extremely valuable therapeutic agents. Nonetheless, for most drug discovery programs it is not acceptable to await the occurrence of a serendipitous series of events that lead to success.

In dealing with terrestrial plants, conservative estimates suggest the existence of 250,000 species. With the advent of high capacity screening (see below), it is quite feasible that samples derived from the entire flora covering the face of the earth could be evaluated for potential to mediate specific biologic responses, should they be available. However, the specimens are not available *in toto*, and a formidable task relates to the selection of starting materials. A number of approaches have been devised for the selection process. First, it is comforting when plant materials are reputed to mediate a certain therapeutic effect, in a traditional medicine sense, and scores of manuscripts are currently available describing such ethnomedical usage. Similarly, epidemiological data may come into play that are suggestive of therapeutic efficacy in human populations. We have implemented a literature-based correlative approach that interrelates datasets containing active compounds, plants with active compounds, plants reported to mediate activity, and plants that are reported to be in medical use [9, 10]. The keystone for this entire process is the NAPRALERT database, originally created and developed by Dr. Norman R. Farnsworth and coworkers [11], and this process represents the hallmark of rational plant selection. By and large, however, plant materials are procured based on geographical location and various logistical factors often come into play. One key requirement often mandated by our group is that the plant materials are endemic to the site of collection.

Once materials are procured, it becomes of the utmost importance to rapidly identify promising leads and to obtain the active chemical principles affiliated with these substances. In this endeavor, recently, a technique in which HPLC/MS is coupled to bioassay procedures was employed [12, 13]. The methodology is illustrated in Fig. 2. Using this approach, it is feasible that active principles contained in crude plant samples can be identified in a very short period of time. Identification is based on mass, and again, the process is dependent on the NAPRALERT database. Of course, this procedure does not permit identification of structurally novel compounds. Although this aspect is sometimes criticized, the identification of known compounds capable of mediating hitherto unknown biological responses is, in fact, very important. New uses of known compounds is as important as discovering novel compounds with the same activity as the known compound, both in terms of actual benefit to humanity and exclusivity (i.e. patent protection). Also, known compounds capable of mediating new activities can enhance our understanding of factors such as molecular recognition sites and structure–activity relationships. Further, such agents could play a role

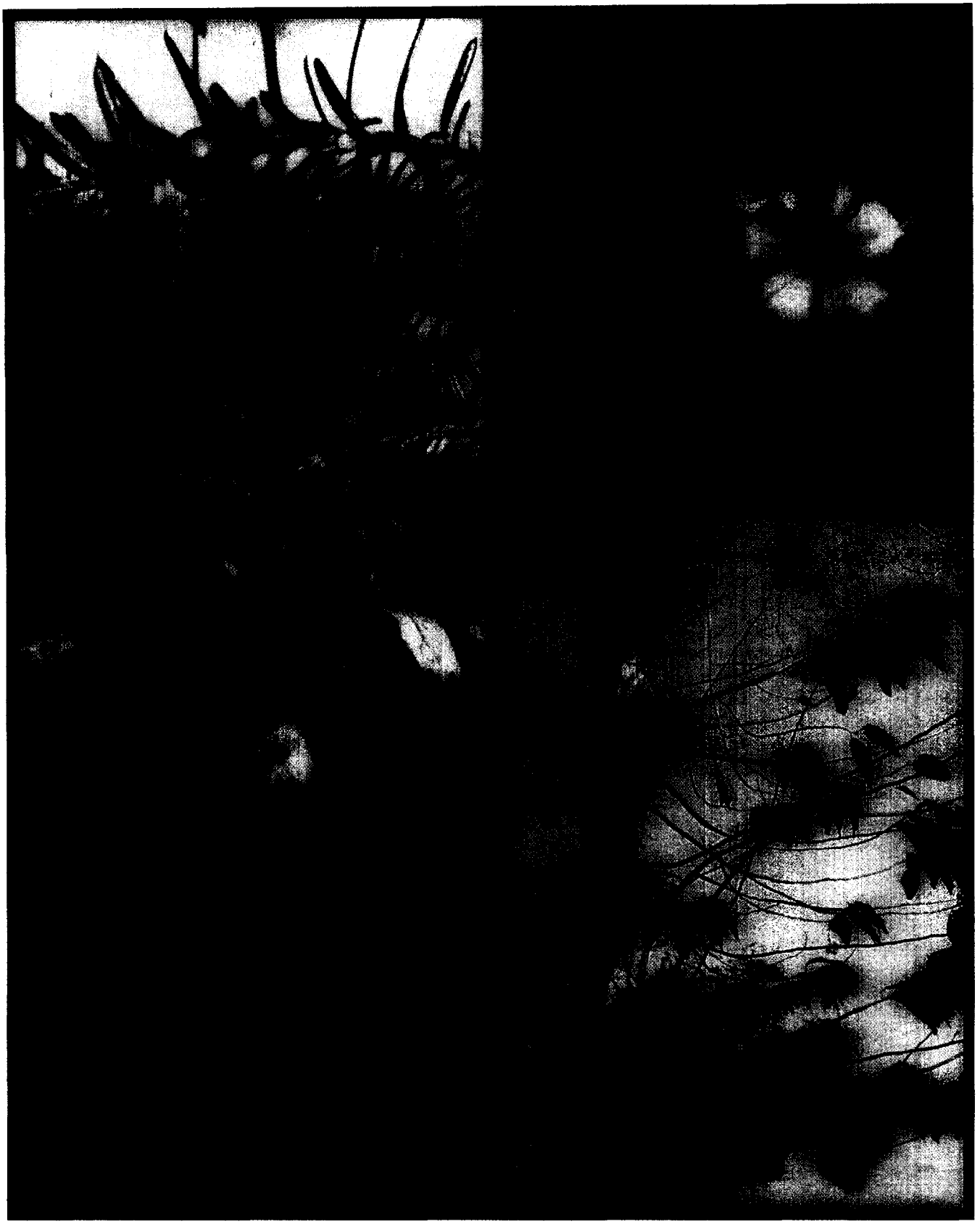

FIG. 1. Illustration of *Taxus brevifolia* L. (Taxaceae) (top left), *Catharanthus roseus* G. Don (Apocynaceae) (top right), *Podophyllum peltatum* L. (Berberidaceae) (bottom left), and *Camptotheca acuminata* Decne. (Nyssaceae) (bottom right). The respective antitumor agents derived from these plants are paclitaxel (Taxol®), vincristine (Oncovin®), podophyllotoxin (a natural product precursor for etoposide or teniposide), and camptothecin (a natural product precursor for water-soluble derivatives such as topotecan) (Fig. 1, insets). The original discoveries leading to the isolation and identification of these compounds are generally ascribed to Dr. Gordon H. Svoboda (vincristine), Dr. Morris S. Kupchan (podophyllotoxin), and Drs. Monroe E. Wall and Mansukh C. Wani (paclitaxel and camptothecin), irrefutably a group of preeminent scientists who epitomize the science of natural product drug discovery. This illustration is modified from the cover photograph of Ref. 8 with permission of the *International Journal of Pharmacognosy*, Swets & Zeitlinger, publishers.

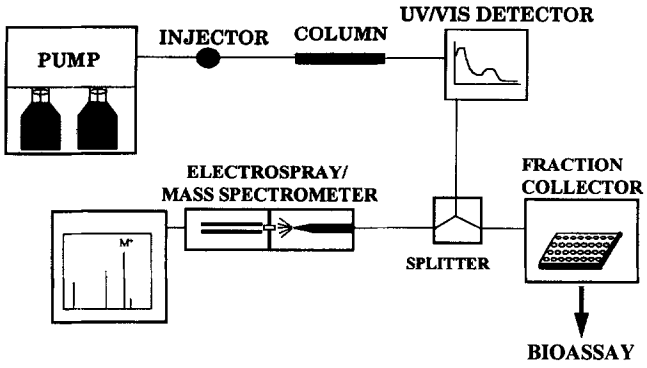

FIG. 2. Illustration of the coupling of HPLC/MS analyses with biological evaluation for the rapid identification of active principles from plant extracts. Active plant extracts are subjected to HPLC, and the post-column stream is split as indicated. The 96-well plates are then evaluated, utilizing the same bioassay procedures originally employed to classify the extract as active, and profiles of data (mass spectra, UV/vis spectra, and biological response) are then compared with the intent of identifying the active principles. This figure is reproduced from Ref. 13 with permission.

in rationally designed combination chemotherapy regimens, once mechanisms are elucidated. In essence, therefore, novel drug discovery is a biology-driven process.

For situations wherein the biologically active constituent of a plant extract is structurally unique, the approach generally regarded as most practical for drug discovery is referred to as bioassay-directed fractionation. As illustrated in Fig. 3, this entails evaluating plant extracts in a bioassay system, and substances demonstrating a positive response are considered as active leads. After a number of active leads are identified, decisions are made to fractionate the

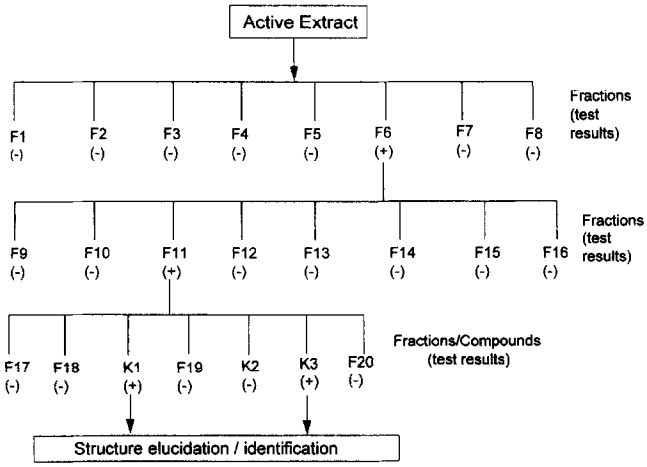

FIG. 3. Bioassay-directed fractionation. This diagram illustrates chromatographic separation of biologically active plant extracts (Active Extract). Each resulting fraction (designated F) is tested in a bioassay system, with the intent of establishing a positive (+) or negative (−) response. Only active fractions are subfractionated, with the objective of ultimately obtaining pure, biologically active compounds (designated K). The active principles are subjected to procedures of structure elucidation/identification.

most promising materials [cf. Ref. 10]. Each fraction is monitored for potential to mediate a positive response in the bioassay test system, and this process continues until a pure active substance is obtained. The resulting substance is then subjected to procedures of structure elucidation. Once an active isolate is obtained, more thorough biological evaluation procedures are often performed and, based on the accumulated data, the material is considered as a candidate for more advanced testing and development.

A question of paramount importance relates to the bioassay test system. In the area of antitumor drug discovery, a large number of *in vitro* test systems, and the issues that need to be considered when attempting to interrelate *in vitro* test results with *in vivo* efficacy studies, have been described [cf. Ref. 14]. In general, recent advances in molecular biology have led to a greater understanding of the molecular basis of human disease states. As a correlate, *in vitro* systems that monitor a response that is either closely related to or identical with the molecular event yielding the disease condition can be devised.

An axiom in the field of drug discovery worth bearing in mind follows: "It's not what you miss that is important, it's what you find." In dealing with plant extracts, it may be generalized that the activity of approximately 50% of the samples will reconfirm on recollection, and the activity of about 50% of the samples will be lost during the process of bioassay-directed fractionation. Since neither botanists, nor chemists, nor biologists generally acknowledge the cause of these losses in activity, the notion of lack of chemical stability or synergistic activity often arises. With a few exceptions, such as the amazing work of Dr. Koji Nakanishi, compounds lacking chemical stability are rarely isolated. In the synergistic activity scenario, it is hypothesized that activity results from the presence of multiple active principles, which must be admixed to observe the desirable response. As a correlate, through the process of fractionation, the principles are separated and activity is lost. This is certainly a legitimate query from a theoretical viewpoint. In the area of bioassay-directed fractionation, however, synergism remains a largely unstudied phenomenon. It is not necessarily difficult to establish the cause of such a result, but it is very likely unproductive. For example, if synergism is suggested by demonstrating that biological activity can be reconstituted after remixing aliquots derived from 2–3 otherwise inactive fractions, the next step of purification may involve the generation of 15 fractions. In this case, 37,767 (i.e. 2^{15}-1) possible combinations of fractions would need to be tested. This assumes certain parameters can remain fixed (such as equal volumes of each fraction); otherwise the situation is actually much more complex. Next, should the correct fractions be identified, subsequent fractionation leads to a larger number of possible combinations, and clearly this is an unmanageable situation. Therefore, it is unreasonable to contemplate the isolation of two or more synergistic unknown active principles using a bioassay-directed fractionation approach; this may be considered a

short-coming but, as noted above, "It's not what you miss that is important, it's what you find."

RECURRING THEMES: THE "THIS IS ONLY A FISHING EXPEDITION" COMPLAINT

As highlighted in the preceding section, estimates suggest that approximately 250,000 species of flowering plants exist on the face of the earth, and there are some rational approaches by which certain plants can be selected with the hope that they may have a higher probability of mediating some type of positive antitumor response. From a realistic viewpoint, however, too little is known about the constituents of the flora of the earth to eliminate plants that are not targeted by any known "rational" selection strategy. In turn, this leads to the notion of screening, and the identification of active leads on the basis of empiricism. Is this awful, unscientific, anti-intellectual, and an utter waste of time and money, as has been implied by various reviewers of grant applications over the past 15 years? Resounding comments such as: "This 'screening' exercise can be regarded as a 'fishing expedition' that will lead nowhere," echo through the halls of grant review bodies and haunt natural product scientists. Is the criticism valid? Should the "fishing expedition" theme be propagated and tossed in the faces of natural product scientists for the rest of eternity?

Let us return to the examples presented in Fig. 1 (insets): paclitaxel (Taxol®), vincristine (Oncovin®), podophyllotoxin, and camptothecin. These agents and close structural analogs derived from these agents epitomize natural product drug discovery. What human being could possibly conceive of such elegant structures? And, moreover, once the structures were conceptualized, who could dream of the unique mechanisms of action facilitated by the agents, i.e. tubulin stabilization (paclitaxel), tubulin depolymerization (vincristine), or inhibition of topoisomerases (camptothecin, teniposide)? To take this one step further, once these breakthrough concepts were made, who could produce sufficient quantities of the agents, with proper stereochemistry, to enable appropriate antitumor testing and eventual use in humans? Clearly, if the lead compound had not been obtained from nature, and if nature was not capable of providing adequate amounts of material for clinical use, these discoveries would be nonexistent or irrelevant. Instead, natural product drugs such as these have altered the practice of clinical oncology as profoundly as any other discovery in the history of the world. By the sentiments of some, all as the result of a "fishing expedition." One thing is clear, if we don't go fishing, we will catch none. It is imperative that people in positions of authority permit the search to continue.

ARE ALTERNATIVE APPROACHES FOR THE DISCOVERY OF ANTICANCER AGENTS MORE SENSIBLE?

Over the last few years, there have been very rapid and exciting developments in the areas of combinatorial chemistry, high throughput screening, and molecular recognition. This is illustrated by the creation of an entirely new journal completely dedicated to the subject: *Combinatorial Chemistry and High Throughput Screening*. Peptidomimetic inhibitors of Ras farnesyltransferase and HIV-1 protease inhibitors are fine examples of computational chemistry-assisted drug discovery. Through various methods of combinatorial chemistry, heretofore unimaginable strides in molecular diversity are being realized, and this will undoubtedly have a profound effect on the drug discovery process. High throughput screening, an integral component of the overall process, will certainly lead to breakthroughs that will enhance our understanding of drug–receptor interaction/recognition sites, and this information will likely be brought to bear to enhance drug development. The pharmaceutical industry has responded to these technological breakthroughs in a rapid and vigorous manner. Multimillion dollar investments are numerous, and anticipation of important discoveries runs high. There is great promise, and optimism is warranted.

Unfortunately, however, some of these programs have been initiated at the expense of existing natural product drug discovery programs. Obviously, in the private sector, this is a management decision that is based on a myriad of factors unbeknownst to all but a few insiders. Accordingly, the rationale of such decisions is not subject to dispute. In generic terms, however, the question may legitimately be posed, "On an exclusive basis, are alternative approaches for the discovery of anticancer agents more sensible than natural product-based approaches?" To explore this question, one could first ask, "Would some of our most useful anticancer drugs, such as taxol, have been found by the use of high throughput or combinatorial approaches?" The most probable answer is "No." It may still be the case that lead optimization could and should be accomplished through utilization of this technology, and new receptor sites may be identified for known antitumor agents through the process of high throughput screening, but it is doubtful that molecular diversity as profound as that illustrated by the molecular entities illustrated in Fig. 1 will result from methodology other than natural product drug discovery. I believe it is fair to state, unequivocally, that there is no more effective means of uncovering new and useful antitumor agents than the approach of natural product drug discovery.

HUMAN DISEASE AND THE ECONOMIC LOGIC OF DISEASE PREVENTION STRATEGIES

The ten leading causes of death in the U.S. are listed in Table 1. Topping the list are heart disease and cancer. In 1995, these diseases manifested annual economic burdens of $137.7 billion [16] and $104 billion [17], respectively (Table 2). Roughly estimating the population of the United States at 250 million, this equates to annual economic burdens of $550 and $416, due to heart disease and cancer, respectively, for each man, woman, and child, or a total of

TABLE 1. Leading causes of death in the United States [15]

Cause of death	Deaths per 100,000 (1990)
Heart disease	189.8
Cancer	135
Accidents	32.5
Chronic pulmonary disease (e.g., asthma)	19.7
Pneumonia	14.0
Infectious and parasitic diseases	12.0
Diabetes mellitus	11.7
Suicide	11.5
Homocide	10.7
Chronic liver disease	8.6

$3,864 per year for a hypothetical family of four. As an economic burden affiliated with only two diseases, this amount is staggering. One might guess, *a priori*, that it would be worth an economic outlay of, let's say $2,500 per family of four per year, to prevent these diseases. Not only would over $1,000 be saved, but think of the myriad of ancillary benefits. It is certain that money could be (and is) spent for disease control, and positive results are observed. Diseases are delayed or prevented, and quality and length of life are enhanced, but at what expense? One may rationally ask, "What *is* the monetary value of a human life?" Certainly, if one considers the monetary value of their own life, or the lives of their loved ones, the question is moot. In these highly personalized situations, invariably, the value is effectively equivalent to the maximal ability to pay. Thus, rather than a specific personal case, it is reasonable to query the monetary value of a "statistical" or "theoretical" human life. Perhaps the value could be related to income: a human capital approach. In the United States, per capita income ranges from a low of $15,793 (Mississippi), to a high of $29,044 (Connecticut) [20], which corresponds to roughly $750,000 or $1,500,000, respectively, if the number of years of earning power (say 50) is multiplied times these amounts. Obviously, this is a crude estimation, but it gives us at least some notion of the economic value of human life.

Perhaps a more rational concept in the arena of disease prevention is the willingness-to-pay approach [21]. As a simple example, consider that a population of 100,000 is affected by a particular program, and the program is anticipated to reduce the overall probability of death from 0.009 to 0.008. The question becomes, how much will *you* pay for the program? If the amount is $100, for example, and the program reduces the death rate from 90 per 100,000 to 80 per 100,000, the economic value of each of the 10 "statistical" lives saved is $1,000,000 (100,000 people × $100/person = $10,000,000 invested; $10,000,000/10 lives saved = $1,000,000/life). Is the program worth the investment? The question would typically not be a matter of life or death, but rather the potential of extending life. In this context, Goldman *et al.* [22] estimated the economic value of life extension in terms of quality-adjusted years of life (full quality = 1 full year for each year of life extension; lower quality years are equivalent to <1 year for each year of life extension) as follows (cost in dollars for each additional full quality year of life): <$20,000, very attractive; $20,000–40,000, equivalent to currently accepted programs (e.g. hemodialysis, treatment of mild hypertension); $40,000–100,000, higher than currently accepted programs; >$100,000, unattractive.

TABLE 2. Annual economic burdens affiliated with major diseases in the United States

Disease state	Cost	Ref.
Heart disease	$137.7 billion (1995)	[16]
Cancer	$104 billion (1995)	[17]
Diabetes mellitus	$91.8 billion (1990)	[18]
Chronic pulmonary disease (e.g. asthma)	$6.2 billion (1990)	[19]

In the context of pharmacoeconomics, certain programs such as vaccination against polio and smallpox are so obvious and well-accepted that consideration is not even necessary. This applies in any situation wherein the entire society is at severe risk. How about newer vaccines, however, such as those useful for the prevention of hepatitis B or chicken pox? Each has been documented as economically favorable under certain specific conditions [23, 24]. How about drug-based strategies for the reduction of heart disease? As concluded by T. R. Pedersen and the Scandinavian Simvastatin Survival Study Group [25] in a randomized trial of cholesterol lowering in 4,444 patients with coronary heart disease, this approach, for example, does serve to extend life. But is it worth it in economic terms? The cost of the drug plays a dominant role in the analysis, but the answer is probably "Yes" with certain high-risk patient populations, and probably "No" with patients at lower risk [cf. Ref. 22].

For cancer prevention, the situation is not well-developed and is fairly ambiguous. Most current emphasis is on early diagnosis, with the hope that the disease may be definitively treated by surgery or other means prior to metastatic spread. In accord with this premise, a favorable economic analysis is borne out by certain screening procedures. For women, for example, it has been estimated that an annual physical exam with biannual mammography costs $30,000/life year saved [26], whereas the corresponding amount for Pap tests ranges from $1,595 (one test at age 65) to $97,000/life year saved (annual test) [27]. For males, application of PSA screening for prostate cancer, alone or in conjunction with ultrasound, is not economically favorable, ranging in cost from $113,000 to $729,000/life year saved, depending on patient age [28]. In other cancer cases wherein prognosis is not favorable at the time the screen is capable of yielding a true positive response, the economic analysis is also not favorable.

Due in part to significant decreases in annual death rate due to heart disease [15, 16] concomitant with annual in-

creases in annual death rate due to certain types of cancer [17], it is generally accepted that cancer will be the leading cause of death by the year 2000 in the United States. As cogently espoused by Sporn [29], at the present time, widespread strategies for cancer prevention and control significantly lag behind approaches that have been successfully implemented for averting the consequences of heart disease. Certainly, cancer does not begin at the time it can be detected, but rather invasion, spread, and metastasis characterize the end stage of the disease. The disease begins through the process of carcinogenesis and, clearly, at this time, no perceivable alterations are present. Of key importance, there is a myriad of opportunities to alter the course of the disease prior to reaching the end stage, and currently, relatively little is being done along the lines of intervention.

To a certain extent, diseases, including cancer, may be viewed as "avoidable predicaments." These are circumstances where it is obviously much easier to avoid the predicament, rather than finding oneself in the situation and having then to attempt corrections with varying degrees of success. As related to the cancer problem, the National Cancer Institute developed comprehension strategies to reduce the 1985 cancer mortality rate by 50% by the year 2000 [cf. Refs. 30 and 31]. Currently, it appears that this specific goal will not be achieved in the proposed timeframe, but the premise is well-founded and reflective and clear, definitive, forward thinking. In the context of this Commentary, only drug-based approaches of cancer prevention will be discussed in any detail, but it should be borne in mind that only comprehensive approaches of cancer prevention are realistic.

THE CONCEPT OF CANCER CHEMOPREVENTION

During the 1960s and 1970s, pacesetting studies were performed by Dr. Lee W. Wattenberg and his associates at the University of Minnesota, in which it was demonstrated that various compounds, especially those associated with fruits and vegetables such as indoles and isothiocyanates, could inhibit chemically induced tumors in laboratory animals. This was the advent of the "chemoprophylaxis of carcinogenesis" [32], and the implications of these observations in terms of human health maintenance were immediately apparent. Subsequently, during the course of a series of hallmark studies performed with a myriad of retinoids, Dr. Michael B. Sporn coined the term "cancer chemoprevention" [33] to further define the phenomenon. In general terms, cancer chemoprevention may be considered as the prevention of cancer in human populations by ingestion of chemical agents that prevent carcinogenesis. It is important to differentiate the concept of cancer chemoprevention from primary cancer prevention, such as the cessation of cigarette smoking, and cancer chemotherapy, the therapy used after the diagnosis of cancer.

Since the pioneering work of Wattenberg, a number of world-renowned scientists have made fundamental contributions that have given credence to the concept of cancer chemoprevention as a viable intervention strategy for the reduction of human cancers. In addition to Dr. Michael B. Sporn mentioned above, other prominent names in chemoprevention are the officers and affiliates of the newly formed *International Society of Cancer Chemoprevention*, viz. Drs. Umberto Veronesi, Trevor J. Powles, Takashi Sugimura, Michael P. Osborne, Nico de Vries, Reuben Lotan, Richard C. Moon, Jack Cuzick, R. Sankaranarayanan, W. Ki Hong, Martin Lipkin, Gary J. Kelloff, Hoyoki Nishina, Charles W. Boone, Alberto Costa, Peter Boyle, Jack Fishman, V. Craig Jordan, Hiroshi Kobayashi, Frank Meyskens, Virgilio Sacchini, and Hans-Jörg Senn. As demonstrated by this organization and the number of monographs, symposia, and plenary sessions at national meetings, cancer chemoprevention has developed as a well-defined and distinct discipline of science. Some ramifications in terms of human health and lifestyle are beginning to be appreciated. As examples, certain epidemiological studies have demonstrated that dietary factors may reduce the incidence of cancers [34–36], presumably through chemopreventive mechanisms, and various prospective studies are currently underway [37]. In essence, the notion of eating five or more servings of fruits and vegetables per day [38], as put forth by the National Cancer Institute, the American Cancer Society, and others, encourages a rather crude form of cancer chemoprevention. Also, the extraordinary work of Dr. Paul Talalay and coworkers [cf. Ref. 39] and a recent cover story in Newsweek [40] further emphasize the potential chemopreventive role of phytochemicals in our diet.

THE ARMAMENTARIUM OF CANCER CHEMOPREVENTIVE AGENTS

There is a diverse structural array of compounds that may be considered "chemopreventive," and over 600 agents may be considered in this category. Examples include inhibitors of initiation such as phenols, flavones, aromatic isothiocyanates, diallyldisulfide, ellagic acid, antioxidants, glutathione, and S_2O_3, and inhibitors of post-initiation events such as β-carotene, retinoids, tamoxifen, dehydroepiandrosterone, terpenes, protease inhibitors, prostaglandin inhibitors, Ca^{2+}, and nerolidol. Agents can be categorized as micronutrients, intentional food additives, non-nutritive food molecules, industrial reagents, pharmaceutical agents, hormones, and anti-hormones [41]. Agents are often categorized as inhibitors of specific stages of carcinogenesis [42], but pleiotropic mechanisms of chemopreventive agents are well-known.

RECURRING THEMES: THE "IS THE BIOASSAY PHYSIOLOGICALLY SIGNIFICANT?" COMPLAINT

Drug discovery is an applied science wherein the objective is to find agents suitable for use in humans. Since we do not

experiment with humans in the early stages of the drug discovery process, under all circumstances, even those in which the loss of human life is near certain, model test systems come into play. As a result, we rely on model systems to provide an indication of potential human efficacy. Accordingly, it is certainly legitimate to scrutinize the validity of model systems, and a great deal of work is required to devise suitable model systems. Aside from the ultimate test system, the human, animal models have traditionally been regarded as being capable of providing the most salient indication of human efficacy. So, for example, a chemical agent capable of inhibiting carcinogen-induced mammary tumors in rats could be considered a good cancer chemopreventive candidate. In the context of natural product drug discovery, however, it is not feasible to conduct bioassay-directed isolation of active chemicals utilizing animal models. It is simply too costly and too time-consuming, and ethical considerations also preclude such an approach [43].

In certain ways, therefore, the discovery of cancer chemopreventive agents is more difficult than the discovery of cancer chemotherapeutic agents. Short-term model systems must be used due to logistical considerations, but efforts must be focused toward limiting the number of "false positive" compounds discovered, i.e. compounds that mediate a positive response in the short-term model system but not the more relevant *in vivo* model or humans. In this endeavor, various *in vitro* test systems have been proposed and utilized. For example, since some correlation exists between the mutagenic and carcinogenic potential of chemicals, the discovery of anti- or desmutagens has been widely reported [44]. Other examples of short-term test systems include studies conducted with cultured Chinese hamster ovary cells to assess effects on carcinogen metabolism and inhibition of carcinogen binding to DNA [45], studies conducted to assess inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced early antigen of Epstein-Barr virus in cell culture [46], and assessment of potential to inhibit C3H/10T½ cell transformation by enhancing gap junction communication [47]. These and a variety of other test systems [48] have proven of use in the discovery and characterization of cancer chemopreventive agents. Nonetheless, it is a fact that *all* short-term test systems suffer from drawbacks since the physiological complexity cannot even begin to approach the physiological complexity of humans or other mammals. Hence, the haunting question faced continuously by biologists: "Is the bioassay physiologically significant?" The answer is proven by performing the work, the work is dependent on funding, and hence, the archetypal example of the concept put forth in Joseph Heller's *Catch-22* [49].

The approach we have developed for the discovery of natural product cancer chemopreventive agents is distinctive [43, 50]. It was devised, in part, by tenacious interaction with reviewers assigned by the National Cancer Institute as part of the grant review process. Although the names of these individuals never appear on publications or acknowledgements, they do, in fact, deserve accolades for their persistent and constructive input. The resultant experimental design is illustrated in Fig. 4. By means of a program project mechanism, we are capable of bringing discoveries from the field to the level of establishing *in vivo* efficacy with pure (structurally and mechanistically characterized) chemical entities. Plant extracts are first evaluated with a panel of *in vitro* bioassays (designed to monitor inhibition of the initiation, promotion, or progression stages of carcinogenesis), and active leads are then evaluated in a more complicated and time-consuming assay that employs carcinogen-treated mouse mammary glands. Active leads in this latter test system are fractionated, using an *in vitro* bioassay as a monitor, and resulting active principles are subjected to more advanced test systems (Fig. 4).

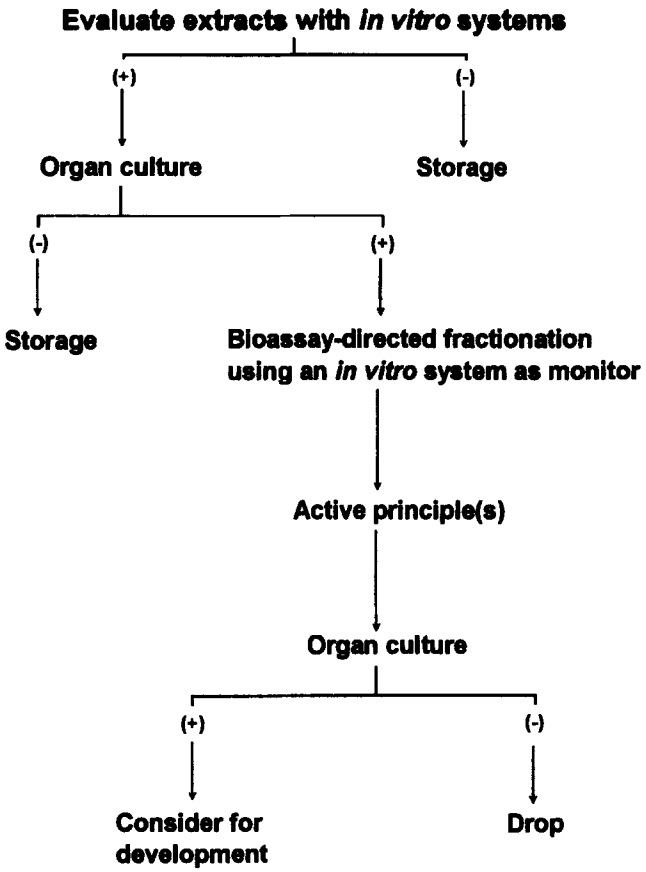

FIG. 4. Experimental approach for the bioassay-directed isolation of cancer chemopreventive agents. Extracts are first tested through a battery of *in vitro* bioassay test systems to monitor potential inhibition of the initiation, promotion, or progression stages of carcinogenesis. Active extracts are then tested for potential to inhibit preneoplastic lesion formation with mouse mammary organ culture. Materials found to be active in this system are then subjected to bioassay-directed fractionation utilizing the *in vitro* monitor in which activity was originally observed, and resulting active principles are then tested in the organ culture model. These active principles are structurally defined and considered for more advanced development.

Following this experimental design, approximately 600 plant materials have been evaluated, and this has resulted in over 5,000 bioassay results. Several compounds have been discovered that are active in preventing the formation of preneoplastic lesions in mammary organ culture, and thus far three lead compounds have been shown to mediate considerable cancer chemopreventive activity in full-term tumorigenesis models [51–53]. We remain hopeful that one or more discoveries resulting from this project will be deemed worthy of human intervention trials.

FUTURE OF CANCER CONTROL, PREVENTION, AND CHEMOPREVENTION

Cancer claims over 6 million lives each year. On a worldwide basis, cancer represents the single largest cause of death in both men and women. Bearing in mind the level of morbidity that is often affiliated with this disease, comprehension of such a high incidence is horrifying. Obviously, the treatment of cancer and most other systemic diseases involves administration of drugs. At the current time, it is apparent that drug-based therapeutic strategies will predominate into the 21st century. The paramount role of therapeutic efficacy, originally described on an intellectual level by Paul Ehrlich and since consuming the dedicated effort of innumerable scientists throughout the world, remains a keystone in the hierarchy of disease control. As new molecular targets are defined, novel anticancer drugs may be developed, and modern methods of rational drug design may come into play with greater frequency. At the present time, semi-empirical methods are still required for the provision of useful drugs, and it is certain that additional natural product antitumor agents remain to be discovered. As demonstrated in the past, these distinctive agents may be of direct use for the treatment of cancer patients. Alternatively, they may serve useful functions in defining novel molecular targets, atypical aspects of cancer cells versus normal cells, or unique mechanisms of action. They may also serve as prototype chemical skeletons useful for semi-synthetic procedures, and certain chemotherapeutic agents, which mediate sufficiently low levels of toxicity, may be of value in the chemopreventive arena [cf. Refs. 54 and 55].

The current therapeutic situation, while not humorous in the least, can be graphically depicted as illustrated in Fig. 5. Once the tumor is visualized through diagnosis, various types of therapeutic assaults are brought to bear, and this entire process results in a great economic burden. As indicated in the lower portion of the cartoon, and as discussed in greater detail above, it is the process of carcinogenesis that should be aggressively attacked, well before the advent of tumorigenesis. The approach can and should be comprehensive, involving social as well as therapeutic aspects. As purported by the World Health Organization, smoking is the world's largest single preventable cause of illness and death, claiming the lives of six people per minute, 3 million people per year, and expected to kill 10 million per year by 2020.

In the context of broad-based cancer prevention, cancer chemoprevention has a role to play. Recently, this subject received the cogent attention of the Committee on Comparative Toxicity of Naturally Occurring Carcinogens that was convened by the National Research Council. Their report [57] lucidly describes the complexity of diet and cancer, and provides a vision for the future. Under normal circumstances, dietary chemicals do not pose an appreciable cancer risk. Conversely, cancer chemopreventive agents are found in the diet of humans, and it is feasible that their presence already affects the incidence of human cancer. Recommendations for dietary modification, including reduction of meat and fat consumption as well as increasing consumption of fruits and vegetables, is clearly sound advice that should be followed by many inhabitants of the developed world. The vast importance of a simpler concept, that of reduced caloric intake, should also be emphasized. But is this enough? It is clear that chemopreventive agents, many of which are natural products, can reduce the cancer risk in highly challenged animal models. All scientific logic dictates that cancer chemopreventive agents could also reduce the risk of cancer in humans. Dietary suggestions are fine, but it is not sufficient to challenge a disease with the severity of cancer with a banana. It is not sufficient to rely on "An apple a day keeping the doctor away." It is necessary to fight cancer with the full armamentarium of drugs that are available, and this already includes cancer chemopreventive agents that are several orders of magnitude more potent than compounds found in the human diet. Of course, risk versus benefit, pharmacoeconomics, and a host of other relevant factors need to be taken into account, but the consequences are much too severe not to take action. The fragility of humans, as illustrated by susceptibility to cancer, presents an ongoing challenge for individuals who are involved in the discipline of therapeutic intervention. It is time for full recognition of the benefits of disease prevention through therapeutic intervention, and it is time for aggressive implementation.

CONCLUSIONS

On January 22, 1971, the "war on cancer" was proclaimed by President Richard M. Nixon. Clearly, a large number of Americans found this proclamation to be heartening. The hopelessness of cancer was well-known among the masses, and, on the other hand, the perceived omnipotence of American ingenuity was broadly entrenched. After all, in May 1961 President John F. Kennedy went before Congress and proclaimed that America would lead all of humanity by placing a man on the moon and having him return safely to earth, and this "incredible" feat was accomplished with amazing grace just eight years later, with the touchdown of Neil A. Armstrong on July 20, 1969. Tacitly, by some diffuse analogy, now that "war on cancer" was declared, could

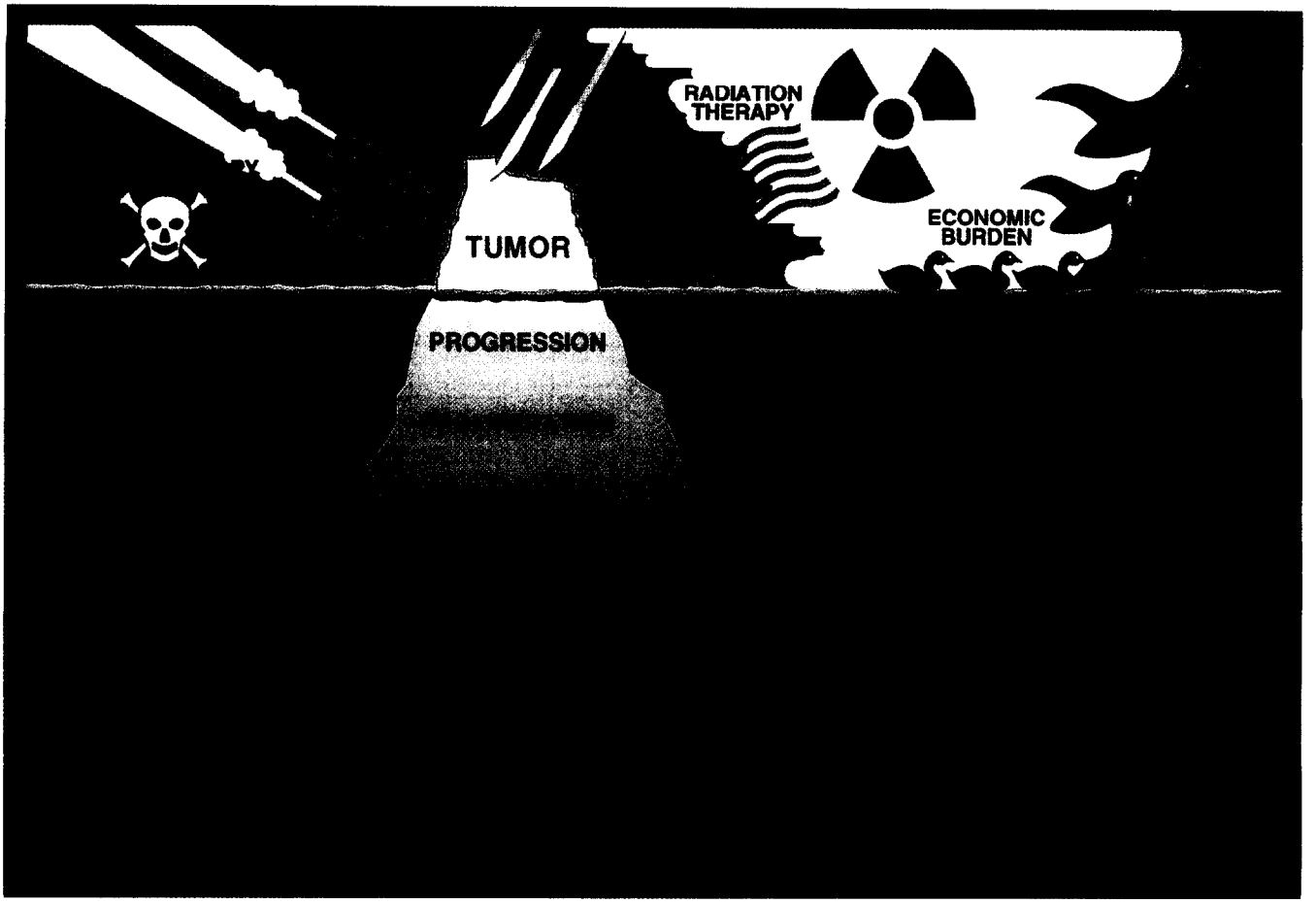

FIG. 5. Cancer chemotherapy versus cancer prevention. This illustration, loosely based on the paper of De Flora *et al.* [56], graphically depicts the treatment of cancer subsequent to presentation with metastatic disease, and the economic burden affiliated therewith, versus the host of preventive opportunities, including chemoprevention, that are available during or prior to the process of carcinogenesis.

a defeat (a cure) be far off? Certainly not, in the mind of the lay public, especially since the work of eminent scientists was pointing toward a viral origin of the disease, and it was well-known that viral-based diseases (such as polio) could be prevented through vaccination.

Has the "war on cancer" been won? Over the past 25 years, the United States government, through the National Cancer Institute, has expended a total of approximately $30 billion, and undeniable progress has been made. Certain cancers can be effectively controlled by treatment regimens, such as childhood leukemia, Hodgkin's disease, and testicular cancer, and, relative to 1971, the consequences of chemotherapy are managed with greater efficacy through the use of antiemetics and immunostimulants, for example. Also, notable improvements have been made in the areas of pain management and diagnosis. On the other hand, more people will die as a result of cancer in 1996 relative to 1971, and our current ability to manage metastatic diseases such as those arising from lung or pancreatic cancers is dismal. I think it is fair to state that the "war on cancer" has not been won.

Has the "war on cancer" been fully waged? In travelling to the earth's moon, the greatest obstacle faced was financing: technology was within reach and implementation was required. While not a trivial matter, sufficient funds were invested and the challenge was met. As summarized in Table 2, in the United States alone, the annual economic burden associated with cancer is approximately $104 billion. The total budget of the National Cancer Institute for the current fiscal year is approximately $2.25 billion. In my opinion, this ratio is ludicrous. An annual economic burden of over $100 billion is indicative of an inordinate amount of human sacrifice, and an annual disbursement equivalent to about 2% of the burden is not sensible. Virtually anyone who has had the opportunity to participate in the grant peer review process would agree that meritorious research is presently restricted due to insufficient funding, and it is certain that a larger number of high caliber scientists would focus on the cancer problem if additional funds were made available.

Michael Milken, former head of junk bonds at Drexel Burnham Lambert Inc., who pleaded guilty to securities fraud in 1990, has been particularly outspoken on this issue. Mr. Milken's proposals, which include an annual budget of $20 billion to fight the "war on cancer" [58], are certainly more rational than the current state of affairs. Progress is

related to knowledge, knowledge is related to research, and research is dependent on fiscal support. At present, fiscal support is the limiting factor. Given additional and (hopefully) ample fiscal support, it is incontrovertible that new discoveries would be made and these discoveries would be directly applicable to the improved treatment of human cancer. Natural product drug discovery should remain one of the focal points of discovery. As described in this Commentary, natural products continue to represent a rich and largely untapped resource for the discovery of drugs with potential application for the treatment of contemporary diseases that inflict humans. It is solely dependent on our ingenuity to devise and implement cost-effective methods of natural product drug discovery that do not adversely affect the environment.

Irrespective of how drugs are discovered, however, one of the great mysteries of our time is why the discovery of drugs useful for the treatment or prevention of human cancer should be limited by fiscal restraints. Another great mystery is the lack of full implementation of cancer prevention strategies, particularly in the area of therapeutic intervention, i.e. chemoprevention. As inscribed by Benjamin Franklin in *Poor Richard for 1735*, "An ounce of prevention is worth a pound of cure." This is well-stated and particularly apropos to the situation of cancer. Perhaps argumentatively, the following may be presented as facts: (a) To an appreciable extent, cancer is a preventable disease, (b) efficacious drugs can be given to humans to augment the prevention of cancer, and (c) this drug-based approach of cancer chemoprevention is not being utilized on a widespread basis. To implement a straightforward concept of "Healthy people through disease prevention," a proposal follows: (a) Assemble "Expert Panels" to design the "best" cancer chemoprevention formulation that is currently available, (b) study the cost-effectiveness aspect of the proposed cancer chemoprevention formulation and make adjustments as required, and (c) provide the final formulation for wide-scale human consumption. It may be noted that managed health care is a perfect forum for implementing this plan. It is fair to suggest that health care plans should take active and leading roles in the promotion of human disease prevention, including the supply, distribution, or payment of drugs that prevent disease.

It is realized that this plan is subject to scrutiny. We can see already the controversy caused by certain clinical trials conducted with cancer chemopreventive agents. As one example, a trial involving β-carotene administration to smokers was terminated recently because no effect was being observed, and there was a "trend" toward a greater incidence of cancer among the group taking the drug [59]. Assuming this information were not available, would the "Expert Panel" proposed above recommend inclusion of β-carotene? Obviously, since the clinical trial with β-carotene actually was initiated, some experts would believe effectiveness was possible. Due to lack of efficacy in animal models, however, other experts would undoubtedly argue against inclusion of β-carotene. Thus, the answer is unknown.

How about tamoxifen? This agent is currently being evaluated in breast cancer intervention trials involving tens of thousands of women [60], but if this were not the case, would the "Expert Panel" recommend inclusion? Tamoxifen, too, has generated controversy, at least in part due to the potential of promoting endometrial cancer [61]. Again, since the agent is actually being evaluated in a large-scale trial, it is rather certain that some experts would tout the use of tamoxifen. On the other hand, since clear indications of potential side-effects have been reported [62–64], it is nearly certain that other experts would recommend against the use of tamoxifen. Thus, as is the case with β-carotene, the answer is unknown.

It may be speculated, however, that due to the conflicting nature of existing scientific data, neither of these agents (β-carotene or tamoxifen) would have been recommended for use in the final formulation. The question may therefore be asked, "Is it then possible to agree on *any* formulation that could be recommended for widespread use by the general population?" Again, without actually going through the exercise, the answer is unknown, but I believe this task could be accomplished in a thorough and conscientious manner. The issue could be approached wherein the "Expert Panel" is viewed as a jury in a United States court of law. The jury (panel) could be charged from the outset to reach a verdict (cancer chemopreventive formulation), and this verdict (formulation) could require unanimous consent. Unlike the court of law, however, the notion of double jeopardy does not apply. As clinical trials are completed with experimental agents, for example, the panel should reconvene, re-evaluate the verdict (formulation), and make adjustments as necessary. In essence, we can elect to do nothing, or we can do our very best, and we can do it now. It is not necessary to wait for some undefined time in the future. Action can be taken now for the benefit of the general population. The initial formulation may be somewhat "lame," unfortunately, due to gaps in our knowledge and the necessity of remaining conservative. In due course, however, the formulation will undoubtedly improve, in part, simply due to its existence. This may be viewed as a general characteristic of all product development and, more broadly, as a function of human nature. Without a start, there will be no development, and without any development, humanity will not benefit from important knowledge and technology that are currently available. Cancer, estimated to affect one in three during the course of their life, may be considered as an epidemic. It seems quite obvious that now is the best time for large-scale implementation of cancer chemoprevention as a therapeutic strategy.

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