selective toxicity to the normal adrenal cortex. There is also a concern that the selection of cell lines for long-term growth in tissue culture has altered phenotype sufficiently to mask histotype-selective properties. Conversely, variance in drug sensitivity among cell lines with the same histotype may preclude detection of histispecific toxicity.

The most important question is whether there are targets that will allow us to selectively and effectively treat tumors such as colon adenocarcinoma and bronchogenic carcinoma. It is critical that compounds showing promise in selective cytoxicity screens be subject to rigorous and timely study at the biochemical level to identify molecular targets for therapeutic intervention.

Ideally, discovery of drugs to treat the major cancers will evolve to mechanism-based approaches. The discovery of a number of appropriately expressed or mutated genes that are conclusively linked to malignant transformation provides the means for establishment of screens designed to detect specific inhibitors of the enzymes or receptors that are products of these genes. Unfortunately, the subtlety of the biochemical changes in malignancy makes this a formidable task. An agent capable of blocking the tyrosine kinase activity of an oncogene product might also interfere with other tyrosine kinases in normal cell physiology. On the other hand, inhibition of the ras gene product could be associated with severe toxic effects related to inhibition of signal transduction mediated by G proteins in normal cells. Despite these caveats, it is important to devise screens for identifying agents that will interact with targets being defined in tumor biology, as selectivity can only be addressed when inhibitors are available.

Advances in cancer treatment will continue to be made by the discovery and/or design of new agents that act by traditional mechanisms such as binding to DNA, producing topoisomerase-mediated DNA damage, inhibiting nucleotide biosynthesis, and interfering with tubulin function. The use of such agents will probably be limited by the same factor that limits the use of established anticancer drugs—toxicity to normal proliferating tissues. Hopefully, there are targets in tumor cells that will allow more selective chemotherapeutic attack. Histispecific cytotoxicity assays may help to identify such targets, and mechanism-based screening and drug design will identify compounds that selectively interact with these targets.

References


In Defense of Cell-Line Screening

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In 1984, the Division of Cancer Treatment of the National Cancer Institute took a long and hard look at the accomplishments of its then 30-year-old program for the discovery and development of new anticancer drugs. The heart of this effort was a large-scale drug screening operation that tested large numbers of compounds (up to 40,000 per year at its zenith in the late-1970s) in a variety of murine leukemia models, most recently P388 lymphocytic leukemia (1). The product of this screening effort was a series of modestly useful compounds (1). The most important of these drugs are the nitrosoureas, mitoxantrone, hydroxyurea and deoxycoformycin, all of which are currently used for the treatment of specific malignancies and most of which have limited roles in the effort to treat and cure advanced malignancies.

The NCI screen, in its later years, attempted to introduce more rational criteria into the selection of candidates for initial screening, emphasizing novelty of structure and known biological activity; yet the majority of compounds tested were random chemical entities submitted by chemical and pharmaceutical companies, academic laboratories, and NCI procurement contractors.

The cost of the drug discovery operation, including the screening and preclinical-development phases, was approximately $29 million in 1984, and for that sum approximately 3-5 new chemical entities reached clinical trial each year.

The need to reassess the drug-discovery phase of the discovery-development program was obvious to NCI staff, the Division of Cancer Treatment's Board of Scientific Counselors, and the academic community. Although the development activities of the previous 30 years were crucial to bringing a number of known active compounds—such as cisplatin, deoxycoformycin, fludarabine, carboplatin, and others—to clinical trial, we had not discovered any significant new agents for treatment of solid tumors.

Received May 29, 1990; accepted May 30, 1990.
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tumors. A number of alternatives were considered by Division of Cancer Treatment staff and by its Board of Scientific Counselors, including:

1. Abandonment of all discovery activities supported by contract. Contract funds would be redirected to support of drug-discovery grants and clinical development of compounds submitted from outside NCI. This alternative was rejected because of the limited confidence that the private sector would be willing and able to commit major resources to cancer drug discovery; indeed, an exit by NCI from drug screening and early drug development would be perceived as a strong negative signal by those few companies supporting active cancer drug discovery programs.

2. Development of new molecular screening targets. The foremost of those considered were growth-factor inhibitors, oncogene products, protein kinases, and other proteins implicated in malignant transformation and maintenance of the transformed phenotype. This alternative, which has subsequently been adopted by a number of private-sector firms, had the apparent disadvantage of using a cell-free target. In addition, there was considerable uncertainty as to the importance of any specific target in human neoplasia. Nonetheless, this approach has considerable appeal because it incorporates our rapidly expanding knowledge of tumor biology.

3. Development of a cell-line-based screen that would represent the major classes of solid tumors. While lacking the obvious pharmacological advantages of an in vivo assay, this approach had a number of positive features. It would allow relatively inexpensive and rapid testing of a candidate compound against broad panels of human tumors, and thus might have greater chances of recognizing drugs active against human solid tumors. In addition, it would be particularly adaptable to the needs of a natural-product screening effort. The latter was considered an essential component of any new screen (vide infra).

After months of debate (2), in January 1985 the Board of Scientific Counselors approved initiation of a pilot effort to establish an in vitro, cell-line-based screen (3). The major reservations of those dissenting from this change of course were:

a) a reluctance to give up the advantages of a primary in vivo screen,
b) the strong positive correlation of P388 activity and clinical efficacy for existing commercial agents, although few of them had been discovered in primary screening against P388,
c) the absence of a plan to compare in vivo P388 and the in vitro cell-line assay head-to-head, an expensive undertaking rejected by NCI staff, and
d) the intrinsic disadvantages with respect to the inability to recognize compounds that require systemic metabolic activation.

Over the next few years, the machinery for a large-scale (10,000 compounds yearly) cell-line-based screen was set in place at the NCI-Frederick Cancer Research and Development Center, largely through the tenacity of Michael Boyd and staff of the NCI Developmental Therapeutics Program. Reviews of the rationale, development, and current status of this program have been published elsewhere (4–7). Details of the methodology have been described in a series of papers in this and other journals (8–18), the most recent being the sulforhodamine-B dye for protein as a quantitative end point for measurement of cell proliferation (14,16), discussed in two papers in this issue of the Journal.

All of the developmental work was reviewed periodically, and most recently in November 1989 (19–24), by an ad hoc advisory committee chaired by Prof. Ken Harrap of the Institute for Cancer Research, Surrey, United Kingdom; based on their favorable assessment and further reviews by the National Cancer Advisory Board and the Division of Cancer Treatment’s Board of Scientific Counselors, full-scale screening began in early 1990 (24–27). As presently constituted, the screen incorporates approximately 60 cell lines representing lung cancers, colon cancers, acute myeloid leukemias, ovarian cancers, melanomas, brain tumors, and renal cancers. Active efforts are now directed at developing appropriate breast and prostate cancer cell lines.

It is important to emphasize that each of the three earlier-mentioned alternatives considered in 1984–1985 has been implemented at least partially. A total of $13 million in grant funding, formerly associated with the Developmental Therapeutics Program’s contract effort, now supports National Cancer Drug Discovery Groups through a cooperative agreement mechanism that encourages collaboration between industry, academia, and government. These groups have targeted molecular mechanisms such as growth-factor receptors, protein kinases, and polyamine biosynthetic pathways and clearly complement the NCI’s cell-based approach. In addition, grant-supported molecular modeling has increased dramatically in recent years.

In considering the status of drug discovery in 1984, NCI staff, the Division of Cancer Treatment’s Board, and many investigators in the extramural community felt that significant changes should be made not only in the basic screening systems, but also in the source of compounds screened. In particular, natural products represented an untapped source of unique structures, particularly the tropical plants and marine animals. The yield of cytotoxic entities from these sources is, in general, several orders of magnitude greater than that from randomly selected synthetic chemicals, although the natural products pose a series of unique problems: isolation and characterization of active fractions and synthesis or procurement of active principles (7). Each of these steps represents a formidable obstacle to the discovery and development of a new natural-product entity. Despite these obstacles, a comprehensive new natural products program was launched in 1985 with the enthusiastic endorsement of the Board of Scientific Counselors (5) and has since become a major source of new chemical entities entering the NCI-National Institute of Allergy and Infectious Diseases preclinical AIDS drug discovery effort (28).

The NCI screening effort is still under evolution. In vivo confirmation of in vitro activity, a necessary step in the progression towards clinical trial, will require reproducible growth of the cell lines in athymic, nude mice or other suitable animal models. Consideration must also be given to the inclusion of non-neoplastic cells such as myeloid colony-forming cells to provide a routine therapeutic index.

While the essential elements for testing the usefulness of the in vitro screen are now in place, possibilities for more complete understanding of its output will require more complete characterization of its cell lines in terms of growth-factor dependence, oncogene expression, protein kinase activities, and other factors relevant to growth regulation and differentiation. This back-
ground information might allow rational interpretation of the patterns produced by active agents, and might, in fact, allow the cell line to be used as a device for mechanism-based screening.

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