Chapter 20. Anticancer drug discovery and development at NCI 220818cg

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 20

Anticancer drug discovery and development at the National Cancer Institute (NCI).

Attempts to find anticancer medicines date back to before the beginning of the 20th century and were undertaken by many researchers and institutions in many countries. In this chapter, I review the anticancer drug discovery program at the National Cancer Institute (NCI), particularly the parts of it that I have some direct knowledge of between the mid-1950's to the second decade of the 21st century. I came to the NCI as a Clinical Associate in the childhood leukemia and adult cancer wards and joined research in a clinical pharmacology unit of the medicine branch (more in the Introduction). As research expanded and diversified, one of several new Laboratories established was a Laboratory of Molecular Pharmacology, which I was appointed to lead. Since the mid-1960's, I served on various committees of the Developmental Therapeutics Program (DTP), although I had direct responsibility only for my own Laboratory. My role in the broader part of the DTP was to contribute basic science input, and I was free to make suggestions or point out problems. Here, I relate some of the successes and disappointments of the Program based on articles I was able to obtain through the NIH library and on my possibly imperfect memory, as well as some old items that remained in my possession. I leave to a future historian or investigative writer to review archival material for a proper history of this complex and instructive story.

"Chemotherapy" dates back to 1909, when German Nobel Prize winner physician and chemist Paul Ehrlich (Figure 20.1) developed the first effective medicine for treatment of syphilis: the arsenic-containing drug, arsphenamine. Ehrlich coined the term "chemotherapy" to denote the treatment of disease using chemical drugs. He was also the first to use an animal screen to test chemicals for their effectiveness against a disease: in 1908 he used rabbits to test chemicals for their effectiveness against syphilis.

A screen for potential anticancer activity required a suitable test system, which was first provided in the early 1910s by George Clowes at Roswell Park Memorial Institute in Buffalo, New York, who developed in mice and rats the first transplantable tumors. The first anticancer screen was set up in 1935 by Murray Shear at the newly established National Cancer Institute, which however was dropped in 1953 because of unacceptable toxicity (DeVita and Chu, 2008). Screening for potential anticancer chemical agents however received new emphasis from the effectiveness of nitrogen mustards against lymphomas (Chapter 1). The recurrence of the tumors, which then no longer responded to the drugs, caused many physicians to feel that trying to cure cancers by means of chemotherapy was hopeless. It was with this pessimistic view that the research physicians newly recruited to NCI in the 1950s to conduct cancer chemotherapy research were faced.

So, where did these intrepid research physicians come from? Perhaps surprisingly, it traces back to the anti-malaria research during World War II. Soldiers fighting in malaria-infested areas of the Pacific and Asia often came down with the disease within a few weeks, and the available drugs to supplement quinine, such as quinacrine (then commonly called Atabrine), were inadequate. A program was therefore established at several hospitals and universities to find more effective drugs (Condon-Rall, 1994).

One of the hospitals that was prominent in the anti-malaria drug discovery research was Goldwater Memorial Hospital located on Roosevelt Island (then called Welfare Island) in the East River between Manhattan and Queens, New York (Figure 20.2). Screening chemicals for potential drugs required an animal test system. Early in the research, they found that a suitable test species were certain birds. Using malaria-infected birds to test several thousand chemicals, they discovered what they were looking for: the suitably effective new drug was chloroquine. (Both quinacrine and chloroquine, by the way, are DNA intercalating agents, see Chapter 4.)

From the success of the anti-malaria drug screen, it was thought that something akin to that success might be accomplished in the cancer field. Accordingly, several of the clinician scientists who were prominent in the anti-malaria program were recruited to head a new anticancer research program at the National Institutes of Health that was expanded in1955 to include a research hospital, the NIH Clinical Center. Chosen to head the new cancer chemotherapy research program was Gordon Zubrod (Figure 20.1), who had a major role in the anti-malaria research at Goldwater. Much of the credit for the eventual cure of leukemias and lymphomas is attributed to Zubrod's directorship, accomplished despite strong headwinds.

Zubrod's research career began in 1943 at Goldwater Hospital in New York, where he worked on the search for treatments for malaria — the first nationally organized drug discovery program – after which he had a fellowship in pharmacology and medicine at Johns Hopkins University. The NIH recruited Zubrod in 1954 to provide leadership in clinical research and chemotherapy programs at the brand new 500-bed Clinical Center.

Zubrod became clinical director of the National Cancer Institute in 1954 and became head of its Division of Cancer Treatment in 1956 and scientific director in 1961.

It was with considerable apprehension that Zubrod reported to NCI on October 1, 1954. "Could I adapt to government service after 20 years of university life?" he wrote. "How would I, without experience in cancer research, provide leadership to scientists who had spent a lifetime studying cancer?" He is reported to have said, "my friends at Hopkins teased me about joining a non-clinical group to which they mockingly attached the sobriquet 'The National Mouse Cancer Institute.'" (The Cancer Letter Archives, January 29, 1999).

Zubrod received a medical degree from Columbia University's College of Physicians & Surgeons in 1940 and had house staff training at its Presbyterian Hospital in New York. I graduated from the same medical school in 1956 and had a 3-month elective at Columbia's medical research unit at Goldwater. That was how I found out about the newly expanded NIH and the possibility of starting on a research career there. Indeed, while at Goldwater, I heard the NIH called "the Goldwater on the Potomac." In view of my background and interest in chemistry and physics, Zubrod hired me as a Clinical Associate assigned to work with David Rall in a clinical pharmacology group in the Division of Cancer Treatment.

Zubrod targeted leukemia as the initial disease for intensive study, and his proposed trials of methotrexate became the first prospective cancer chemotherapy trial in the U.S. My first assignment when I arrived at NIH in July 1957, was to help in the care of children who had acute leukemia and were in the first trials of methotrexate and 6-mercaptopurine as single agents. Although the drugs did not cure, we could at least bring them out of the acute phase of blast crisis and sometimes prolong their lives for a few weeks. Fortunately, the childhood leukemia and adult solid tumor units were expertly directed by Emil "Jay" Freireich and Emil "Tom" Frei. I have recorded in the Introduction chapter my impressions of them and their clinical units of the time.

Freireich gives Zubrod credit for launching him on the work that led to a first step in the control acute leukemia: the use of platelets to stop hemorrhage. According to Freireich, Zubrod would come on rounds, and in those days there would be blood splattered over over the linens and the staff. Freireich said, "Zubrod said to me, 'You're a hematologist, why don't you do something about this bleeding.' I took that as an order." Freireich found that fresh platelets could be effective, but the NIH blood bank would not give him the fresh blood needed, because at the time everyone thought that platelets wouldn't work and might even be harmful. A grand rounds meeting on blood transfusion was called. "We presented our data, but during the discussion, the director of the blood bank said platelet transfusions were not effective and the bank would not issue fresh blood," Freireich said. Recalled Frei: "Zubrod got up and said something like, 'Speaking for NCI and patients with cancer currently and in the future, we truly don't know whether we can cure cancer in the near future or if ever, but we are here to try. Progress is going to come incrementally and not all at once, and one hurdle is to control bleeding, and platelets offer the best chance to do that. I plan to support platelet research to get it done.'" "It took a lot of courage to say

that" Frei said. "Within five years, we had eliminated hemorrhage as a cause of death in 90 percent of the patients," Freireich said. "I always give Zubrod credit for that." "He kept Frei and Freireich out of trouble," said DeVita, who arrived at NCI in 1963. "They were doing what was considered very wild stuff. They needed a distinguished guy like Zubrod over them." Zubrod is sometimes remembered as the organizer, enabler and pacifier who managed to shepherd an unruly bunch of NCI scientists, particularly Tom Frei and Jay Freireich, through a wild ride that demonstrated the efficacy of chemotherapy in the treatment of childhood acute leukemia, resulting in the first cures of this disease.

However, the malaria program that led to the leukemia research program also had a history. Zubrod had participated in the treatment of syphilis of the central nervous system, in which the patients were given malaria to induce fever that was the only successful treatment. It was based on that experience, that Zubrod was recruited in 1943 to military service at Goldwater Hospital to work on the malaria program. As director of NCI's cancer treatment program, Zubrod then hired Frei, age 31, who had been a resident at St. Louis University, to manage the studies. A year later, he hired Freireich, a 28-year-old hematology trainee at Boston University. "Zubrod said, 'I see you have training in hematology. Do you know anything about leukemia?'" Freireich said. "I said, 'Of course,' even though I didn't know much. He said, 'I've decided we need to make progress in leukemia, and therefore, you're hired'."

The headwinds Zubrod's program encountered are further shown by the following recollections. "At a conference, a pathologist once said finding a drug for cancer was like finding a drug that could dissolve off the left ear and leave the right ear intact," Frei said. "General medicine thought we were members of the Poison-of-the-Month Club," Holland said. "There was little confidence in chemotherapy." DeVita recalled attending a seminar Zubrod gave at Mt. Desert Island Biological Laboratory in Bar Harbor, Maine, in the summer of 1959. Zubrod spoke about the NCI drug development program. "I remember being stunned at how hostile the crowd was that there would be any success at random screening," he said. "He deserves a lot of credit for that program, which I would submit has been a great success."
(Sources: https://www.library-archives.cumc.columbia.edu/obit/c-gordon-zubrod; The Cancer Letter Archives, January 29, 1999)

In 1966, Zubrod reviewed the history of anticancer drug discovery attempts from the beginning of the 20th century and the development by mid-century of the cancer chemotherapy program at the National Cancer Institute (Zubrod, 1966). The general view at the time was that effective cancer chemotherapy would require molecules that could reach and act on every cancer cell in the body. An important early development was the production by the R. B. Jackson Laboratories around 1946 of inbred strains of mice and transplantable tumors. These were among the first things needed for consistent and reproducible animal tests of potential anticancer drugs and chemicals. In another important early development, Lloyd Law at NCI isolated from x-rayed mice a transplantable leukemia whose characteristics were highly suited for quantitative studies of chemicals that inhibited the growth of these cells in the mice. This leukemia, which was

called L1210, was to have an important role in anticancer drug discovery for many years. It was the first of a panel of transplantable mouse tumors that came to be used to evaluate a great many chemicals as potential anticancer drugs.

After extensive reviews and head-scratching, NCI officials proposed to the Congressional Appropriations Committee to build on the wartime success in producing new antimalaria drugs and to support a similarly designed research program to discover drugs against leukemia. Impressed by this proposal, Congress appropriated one million dollars for fiscal year 1954 to support the research. From that modest investment, funding in the following years grew exponentially.

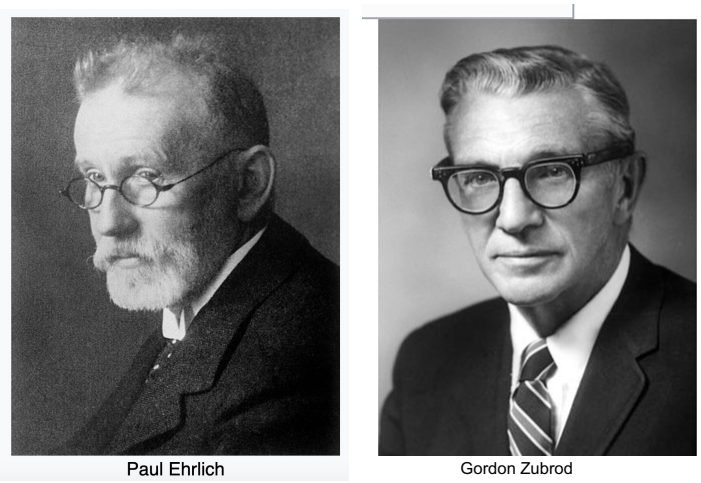


Figure 20.1. *Left*, Paul Ehrlich (1854-1915), the founder of chemotherapy.
Right, C. Gordon Zubrod (1914-1999) was clinical and research director of the National Cancer Institute (NCI) from 1956 until 1974, when he moved to direct the oncology program at the University of Miami Medical School and the Florida Comprehensive Cancer Center. He received an MD degree at Columbia College of Physicians and Surgeons in 1940. During World War II, he worked at Goldwater Memorial Hospital to find more effective drugs for prevention and treatment of malaria. That work, using birds as a test system, led to a better drug: chloroquine. Based on the success of that program, Zubrod was recruited to organize a program to discover anticancer drugs at NCI, NIH.

Figure 20.2. Goldwater Memorial Hospital in 1938, as seen from the Queensborough Bridge. This vast chronic disease hospital was located on Welfare Island (later called Roosevelt Island), a two-mile sliver of land in the East River nestled between the Upper East Side and Astoria, Queens, New York. In addition to caring for a large number of chronic disease patients, Goldwater included clinical research departments associated with the Columbia, Cornell, and NYU medical schools. The hospital, opened in 1939, was an immense facility designed to be a new model of medical care for patients with chronic illnesses. Researchers in the Columbia unit solved the anti-malaria drug problem during World War II. Many of those researchers, including Gordon Zubrod, were recruited to lead the clinical and research programs of the newly expanded NCI at NIH. The hospital closed in December 2013, but before its destruction, a detailed photographic record was made: (http://urbanomnibus.net/2014/04/autopsy-of-a-hospital-a-photographic-record-of-coler-goldwater-on-roosevelt-island/).

NCI's cancer chemotherapy program in 1970.

A milestone in the early development of the cancer chemotherapy program was reviewed in the First Joint Working Conference on the NCI Chemotherapy Program, which convened on December 16-18, 1970, in the Hilton Inn in Annapolis, Maryland. The report summarized a milestone in the development of the program. I found a copy of this 50-year-old report among my admittedly disorderly records and will summarize it here, because the report may or may not exist in the NCI archives. A thorough historical investigation is more than I could undertake and leave it for future historians or investigative writers. In

the course of the following summary of the 1970 report, I will take the liberty of inserting a few personal impressions that may perhaps add a little to the story.

The overall program was led by NCI's Scientific Director, Gordon Zubrod and was carried out in NCI's laboratories and clinics in conjunction with contracts with industry and grants to universities. Components of the program ranged from acquisition of large numbers of new compounds, to screening those compounds for anticancer action, to selection of compounds for toxicology, pharmacology, and biochemical studies, to clinical trials. Major segments of the program carried out pharmacologic and toxicologic studies in animals and patients under the Associate Scientific Director for Experimental Therapeutics, David P. Rall, MD, PhD.

Gordon Zubrod was highly admired and respected for his skillful and thoughtful leadership, which was dignified yet flexible. He made courageous decisions that supported uncertain efforts, which ultimately led to the cures of childhood leukemia and Hodgkin's lymphoma.

Dave Rall had a very relaxed and pleasant leadership style that encouraged everybody in our phrmacology group to do our best to make significant progress. He was chief of the laboratory to which I was assigned during my early years at NCI (1959-1965). The papers on his desk seemed highly disorganized, yet he would immediately find whatever he needed. Our meetings in his office were enjoyable and provoking of new ideas to investigate. One day, a wall of his office was covered with an enormous diagram of the new Linear Array, about which there will be more to say. It was already well established in 1970 for guiding the progress of new compounds through the development sequence.

Clinical investigations, as well as studies of the natural history of cancer and cell population kinetics in relation to chemotherapy, were led by the Associate Scientific Director for Clinical Trials, Seymour M. Perry, MD. This component of the program had in it a childhood leukemia service, an adult solid tumor service, and a branch that investigated human tumor cell biology and cell control mechanisms.

A Cancer Therapy Evaluation Branch, headed by Stephen K. Carter, MD, monitored and coordinated the clinical trials of investigational new drugs undertaken by the chemotherapy program. The Branch also connected with the Food and Drug Administration (FDA) for the preparation of new drug applications.

In addition, NCI had a Medical Oncology Program, headed by Jerome B. Block, MD, at the VA hospital in Baltimore. This program had its own clinical and laboratory branches. We regularly went up to Baltimore for joint conferences between the Bethesda and Baltimore NCI scientists. I looked forward to these conferences, in part because they were occasions for detailed and enjoyable conversations with David Ludlum about our related research interest -- as well as other matters where our views diverged during protracted dinner conversations, since his leanings were Republican while mine were Democrat. However, that difference did not impair, and may actually have enhanced, our friendship, and we collaborated in studies, some of which we coauthored.

Another major part of the overall program was the Cancer Chemotherapy National Service Center (CCNSC), which was led by Saul A. Shepartz, PhD. A particularly consequential part of the CCNSC was an experimental chemotherapy unit led by Abraham Goldin, PhD, Associate Chief of Laboratory Research. Abe Goldin was highly respected and admired for his insightful innovations and personal qualities that set the tone and scientific discipline. It was carried forth, after his death, ironically of cancer, by those he had supervised, including John M. Venditti, PhD and John S. Driscoll, PhD, as well as others whom I did not know as well.

Abe established the methods and protocols to evaluate the anticancer potential of new compounds, which led to a large body of reliable information about a large number of compounds that had anticancer potential. The protocol used a well-characterized strain of mouse leukemia cells (L1210) that allowed precise estimations of prolongation of life span after implantation of the L1210 cells. Three days after implantation, mice were injected with the test compound. Groups of 10 mice received a wide range of doses (according to a Fibonacci sequence). With increasing dose, an active compound would first give increased survival time relative to controls, but further increase in dose, would decrease survival due to toxicity. This gave good estimates of both the degree of activity (maximum lifetime extension at the optimal dose) and the range of doses over which survival increased*, i.e.,* the therapeutic ratio (Figure 20.3). Abe's successors, however, tended to be locked in with the concepts and methods used during their early research and lacked the flexibility of thinking that the originator (Goldin) had.

Here I would like to insert another personal and perhaps instructive recollection. Anthony (Tony) Schrecker, PhD, whom the 1970 report listed as Associate Chief for Laboratory Research of the CCNSC had an unfortunate dislocation of his research career. Earlier, during my first few years at NCI (late 1950s and early 1960s), Tony was admired as the best organic chemist in our part of the program. I frequently visited him for advice and to borrow chemicals. He had a precise Germanic no-nonsense style that I found admirable, although sometimes overbearing. In later years, Tony responded to the perception of a demand for "relevance," which seemed like a politically inspired pressure. Trying to respond positively to this new policy, Tony changed the focus of his work from organic chemistry to biochemistry. However, the area of anticancer biochemistry in which he was engaged was not amenable to his accustomed precise discipline of methods. It was a case, I think, where an expert in one field became mediocre when redirected into another.

As experience with various mouse tumors accrued, three were added to the standard screen: P388, a leukemia that was generally more sensitive than L1210; B16 melanoma, a relatively slow growing solid tumor, less sensitive than the leukemias, but nevertheless responsive to the large majority of the known clinically effective drugs, drugs that at least temporarily shrank a cancer or increased the survival time of some patients; and Lewis Lung carcinoma, a slow growing solid tumor that responded to few of the drugs, initially only cyclophosphamide, nitrosoureas, and bleomycin. A newly tested compound that was active against Lewis Lung was considered particularly noteworthy.

Table 20.1. shows an example of a significant finding in the four-mouse-tumors test system. It showed that doxorubicin (also known as Adriamycin) was better than its close chemical relative, daunorubicin (see Chapter 8). Most telling was the result in B16 melanoma in which an optimal dosage of Adriamycin cured 8 of 10 animals (Johnson and Goldin, 1975).

Another example of an important finding (Table 20.2) was that drugs, such as methotrexate, that mainly killed cells that were undergoing DNA replication, were more effective when given intermittently, such as every four days, rather than daily. This was later found to be true also for leukemia and lymphoma patients (Goldin et al., 1971). It seemed that during the few days without drug, the normal cells of the bone marrow recovered more rapidly than the leukemia or lymphoma cells.

That is where the anticancer drug screen stood for many years, until an objective analysis after a few successes, such as cisplatin (which was actually not initially discovered in the screen; see Chapter 3), showed that the screen was picking up only drugs effective solely against rapidly growing cancers, such as leukemias and lymphomas. There was almost no success in finding drugs against the common cancers of lung, breast, and colon. It was therefore decided to change the drug screen to include slow growing human cancers implanted in immune-deficient mice that did not reject the foreign tissue.

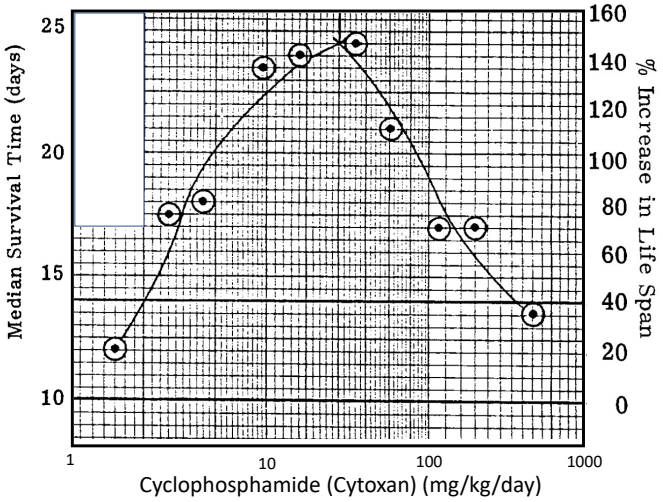


Figure 20.3. An example of a dose-response test as designed by Abraham Goldin for screening of chemical compounds in search of potential new anticancer drugs. Each point represents the median survival time of a group of 10 mice injected with the test compound (in this case cyclophosphamide) with the dose indicated in the horizontal axis. Eight days before the start of treatment, the mice were implanted with L1210 leukemia. Untreated mice had a median survival time of 10 days. With increasing dose, the survival time increased to a peak of 29 days, corresponding to 145% increase in survival time. Beyond that optimum survival, further increase in dose, reduced the survival time, as mice were dying due to toxicity of the test compound. A positive test in the screen was considered to require that, at the optimal dose, the test compound

increased the survival time by more than 40%. An increase beyond 100%, as in this test of cyclophosphamide, was considered to indicate a powerful drug action. (From (Goldin and Venditti, 1962).)

The Linear Array and a Decision Network Committee.

In 1966, the NCI chemotherapy program undertook a concerted effort to diagram the logic of the many decision steps through which a chemical compound or natural (biological) product would pass from acquisition through screening, toxicology, pharmacology, etc. to approval for clinical trial. The steps in the decision network logic were diagrammed in the form of a Linear Array based on a Convergence Technique developed by Louis M. Carrese and Carl G. Baker of the NCI staff. The Linear Array of the decision network logic was then developed over a period of several weeks by a team of NCI working scientists and planning specialists (Rothenberg and Terselic, 1970). The result was a huge logic diagram, far too large to show here.

To implement the operation of the Linear Array, a Decision Network Committee of about 30 NCI scientists was appointed, whose job was to decide, based on evidence provided by relevant parts of the NCI program, whether a compound passes from one step to another through the Linear Array's logic network. The Linear Array served to focus the attention of the Committee on what decisions had to be made for each compound, thereby greatly increasing the number of compounds that could be managed at each meeting.

As a member of the Decision Network Committee, however, I sometimes felt called upon to mark a decision ballot yes or no based on meager relevant data. Over perhaps about 10 years on the Committee in the late 1960s – 1970's, there were no more than one or two truly new types of clinically effective compounds detected for the first time by the screen.

There were nevertheless some useful findings already mentioned. One was that doxorubicin (Adriamycin) was better than daunorubicin (daunomycin) in the test systems (Table 20.1). Another was that that intermittent treatment of leukemia in an intermittent schedule (every 4 days) was better than daily treatment (Table 20.2).

Table 20.1. Comparison of two chemically closely related drugs, daunorubicin and Adriamycin (doxorubicin), for their activity in the four-mouse-tumors test systems, showing the superiority of the latter (Johnson and Goldin, 1975).

Experimental tumor system	Optimal % increase in lifespan (cures)	
	Daunorubicin	Adriamycin
Leukemia L1210	40 (0/10)	68 (0/10)
P388 leukemia	125 (0/10)	>200 (5/10)
B16 melanoma	145 (2/10)	>200 (8/10)
Lewis lung carcinoma	0 (0/8)	20 (0/8)

Table 20.2. Intermittent treatment with methotrexate (drug given every 4 days) was better than daily treatment for mouse leukemia L1210 and for patients with acute lymphatic leukemia ((Goldin et al., 1971).

Leukemia L1210		Acute lymphocytic leukemia		
Schedule	Median survival time (days)	Schedule	Median duration of remission (weeks)	Median lifespan (weeks)
Daily, days 1-death	34	Daily	9	64
Every 4 days, day 1-death	53	Twice weekly	50	91+
Control	14			

Mini- and econo-screens.

In 1970, or thereabouts, two types of smaller screens were evaluated. The first, called mini-screen, addressed the problem that many new compounds could not be tested because the amount of compound produced by synthesis was insufficient for testing in the full screen. The second, called econo-screen, aimed to reduce the cost per compound tested. Both screens reduced the number of animals used and reduced the number of injections from daily to two or three at specified times after the tumor was implanted. Eventually, a standard first screening of a new compound used three animals per dose. The cost per compound tested was thereby approximately halved (not to mention the reduced animal suffering – an ethical issue that could be debated). It was concluded that this reduced screen was almost as effective as the previous full screen (Goldin, 1973).

A new screen: human cancer xenografts in immune-deficient mice.

The leaders of the NCI mouse cancer screen felt increasingly frustrated that after many years and testing of huge numbers of chemical compounds the screen had picked up hardly any truely new drugs clinically active against the major solid tumors, such as cancers of lung or colon. The previous mouse screen was therefor replaced in 1975 by a screen that included human cancers implanted in mice that were immune-deficient, so that they did not reject the human cancer tissues (DeVita and Chu, 2008). The screen included tumors derived from a human lung cancer, a human colon cancer, and a human breast cancer. These "xenograft" tumors were slow growing, and a complete test could require 60-90 days making it necessary to reduce the number of compounds that could be tested per year. What was looked for was a reduction in the growth rate of the tumors, as measured either by reduced tumor size or weight.

This drastic overhaul of the anticancer drug screening process entailed major reorientation of staff, procedures, and of the laboratories and companies that were contracted to do the work. The Associate Director of the drug screening and development program, Vincent DeVita, discussing the objectives of the new screen in a staff meeting, said that it was to be viewed as a 5-year experiment to see whether the human tumor xenograft testing would pick up new, previously unknown, drugs that would be active against some of the major human solid tumor cancers in the clinic. Because of the complexity and expense of this new screening panel, however, the number of drugs screened was reduced from about 40,000 per year to about 10,000 (DeVita and Chu, 2008).

The flow through the screen was designed to start with 15,000 compounds per year selected from literature reports or voluntary submissions. The compounds would first be tested in a prescreen for activity in mouse leukemia P388, the most sensitive of the mouse tumors of the previous screens. An estimated 500-1000 compounds that passed the prescreen would then be tested in the new screen's xenograft tumors derived from human breast, colon, and lung cancers. Natural products would flow through the same screen sequence, except that the prescreen could be in a KB-cell culture when quantity of test material was limiting. Compounds could bypass the prescreen based on data from other anticancer systems or from biochemical or biological assays. The xenograft screen however had the downside that a complete test would take much longer than was the case for the previous mouse tumor screen: an estimated 60-90 days, which severely limited the number of compounds screened per year (Goldin et al., 1979). Another problem was that the immune-deficient mice used in the xenograft screen may become infected, which on at least one occasion decimated the mouse colony.

Figure 20.4 shows an example of a response of a human breast cancer xenograft to a drug, hexamethylmelamine (HMM) that had failed to produce positive responses in the L1210, P388, B16, or Lewis lung tumor mouse cancers of the previous screen. This was perhaps

one of the few significant findings of the xenograft testing program. However, the drug had already passed a phase I clinical trial in 1965 (Wilson and De la Garza, 1965). It seems that the new screen failed to discover any truly new clinically active drugs and that the 5-year experiment of screening in human tumor xenografts announced by then Associate Director DeVita in 1975 was not a success. The failure could be attributed in part to the long time required per test and by the difficulty managing the infection-prone immune-deficient animals.

HMM, by the way, is an interesting drug; it is activated by enzymes in the liver and intestines to produce an alkylating agent that can attack DNA by binding covalently to it and producing DNA inter-strand crosslinks. The drug produced tumor responses in patients with cancers of ovary, breast, lymphoma, and small cell lung cancer (Ames, 1991; Ross et al., 1981).

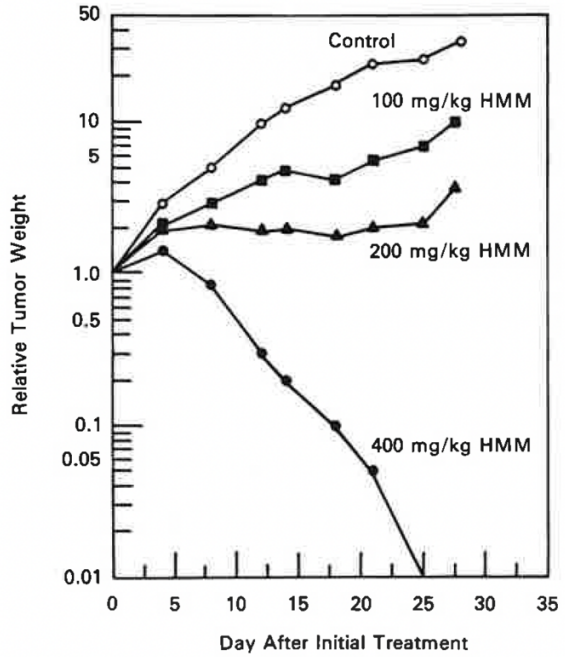


Figure 20.4. Response to hexamethylmelamine (HMM) by MX1 human breast cancer xenografts in immune-deficient mice (Goldin et al., 1979). HMM inhibited the net growth of the tumor and, at the highest dose (400 mg/kg), destroyed the tumor almost completely.

A new screen: human tumor "stem cell" or colony-forming assays.

I must now tell the story, as I remember it, of how a so-called human tumor stem cell assay temporarily became a new screen for cancer drug discovery. It was in 1980, I think, that then Associate Director Vincent DeVita, having decided that the human tumor xenograft screen was not fruitful, called a Technical Review Committee to review a proposal for a

new screen submitted by Sidney E. Salmon of the University of Arizona College of Medicine, Tucson, Arizona. In my first reading of the proposal as a member of the committee, I felt that at last we might replace the mouse tumor screens by one focused on human cancer cells in culture. On more careful reading, however, I was distressed to find what I thought to be a fatal flaw. The proposal was to screen using a "human tumor stem cell" assay developed by Sidney Salmon and Anne Hamburger (Salmon et al., 1978). The assay started with human tumor tissues derived from surgical specimens, which were then minced by a procedure that yielded a suspension of mostly single cells. A given number of cells were then deposited onto a layer of soft agar on a plate. After being incubated for 2 weeks, the number of colonies that had grown were counted. A drug, chemical compound, or natural product that reduced significantly the number of colonies grown was considered to have given a positive result.

A major problem, as I saw it, was that only a limited number of assays could be done from any one tissue samples. Therefore, tissues had to come from a series of patients. Although they came from the same cancer type, say breast cancer, the drug sensitivities of the tissues likely would vary from patient to patient, making interpretation difficult. I argued against approval, but the Committee voted by a narrow margin to approve the proposal. DeVita may have felt that the existing large apparatus for moving drug candidates through the Decision Network could not continue to sit idle, and no viable alternative screen proposal was at hand. A massive screening program, however, would have an inertia that would resist attempts to change its direction.

As the human "stem cell" assay proceeded, some of us felt that significant problems were becoming increasingly apparent. To begin with, the ratio of colonies grown to number of cells put on the plate (the so-called plating efficiency) was extremely low (Figure 20.5). Typically, only one colony grew for every 10,000 cells put on the plate. That meant that the colonies that grew in the presence or absence of test drug might have come from different kinds of cells, clouding interpretation.

Also, unusual colony survival patterns suggested another problem – that the tissue mincing often left cell aggregates or clumps, which might be what was being counted rather than colonies growing from single cells. I had wondered why I had not seen microscopic confirmation that the tumor mincing was generally effective in producing dispersions of single cells on the agar plates. The researchers, even though highly respected, seem to have been blind to checking this out carefully, since Agrez and colleagues at the Mayo Clinic in Madison, Wisconsin soon reported that the various tumor disruption methods generally produced cell aggregates that could be seen within a day after plating and that grew into colonies over the next 14 days (Agrez et al., 1982) (Figure 20.6.). The presence of cell aggregates was found to affect the dose dependence of a drug effect in a way that would complicate the apparent cell kill fraction and thus whether a test was positive or negative (Rockwell, 1985), which may be one reason why the researchers judged a large fraction the drug tests to be uninterpretable. Moreover, the NCI researchers who reported those difficulties with the screening results had by 1985 stopped using the term "stem cell assay" and instead referred it as "colony-forming assay" (Shoemaker et al., 1985).

Becoming concerned about the presence of cell clumps in the disrupted tumor samples, the screen researchers tried to subtract the initially present cell clumps from the final "colony" count (Shoemaker et al., 1984). But, as can be seen in Figure 20.6. in the report by Agrez et al., colonies can grow from small cell aggregates that may not appear as obvious clumps (Agrez et al., 1982). The researchers were also concerned about the likely large and variable component of inherently non-dividing cells in the tumor tissue samples (Shoemaker et al., 1984). Another difficulty may have been that the tissue samples of a given human cancer type, derived from different patients, and then subjected to a cell dispersion process could have led to variable cell clumping and different cell types with different sensitivities to the test drug.

As these problems became apparent in practice, it was eventually decided that they could be evaded by carrying out the assays instead on cultures of well-characterized cell lines that were originally derived from a single tumor of one of several cancer types. The new screening assay was to be on 60 cell lines (the "NCI-60") of several cancer types (Shoemaker et al., 1988). That story will be told shortly.

The human "stem cell" or colony-forming assay, however, was dropped on or a little after 1985, a few years after a new Associate Director, Michael R. Boyd, had come to lead the program. Although the screening staff was still trying to improve the assay, Boyd had doubts. My recollection is that he called the entire staff together and began by saying that he had a problem and needed their help. There was about to be a Board meeting where he was called upon to report the progress of the screening program. But, after reviewing the data, it seemed to him that the current screen was failing. Several of the staff then agreed that the screen wasn't working adequately. It seemed to me as if it could now be admitted that the Emperor Had No Clothes. That is my recollection of how Boyd cleared the way that then led to the development by some of the same researchers of the NCI-60 assays and made possible the highly successful gene expression analysis program (Shoemaker et al., 1988), which is the subject of the next section of this chapter.

TABLE III. Plating Efficiencies in the Human Tumor Stem Cell System*

Tumor type	Median number of colonies/dish (range)	Median % plating efficiency (range)
Neuroblastoma	81 (12–20,000)	0.01 (0.002–4.0)
Ovarian	54 (6–650)	0.01 (0.001–0.1)
Breast	50 (4–60)	0.01 (0.0008–0.01)
Melanoma	32 (10–210)	0.006 (0.002–0.04)
Colorectal	26 (10–118)	0.005 (0.002–0.02)

*Based on 500,000 nucleated cells plated per dish.

Figure 20.5. Colonies formed from 500,000 cells, derived from dispersed human tumor tissue, that were deposited on a plate of soft agar. The number of colonies that grew from a given tumor type varied greatly ("range") and was tiny compared to the number of cells put on the plate (plating efficiency). A colony might have grown from a single "stem" cell or from a cluster of viable cells on the plate. Moreover, the cells plated would have included normal fibroblasts and lymphoid cells that are normally present in tumor tissues and could help neighboring tumor cells to grow. (Copied from (Von Hoff et al., 1980).)

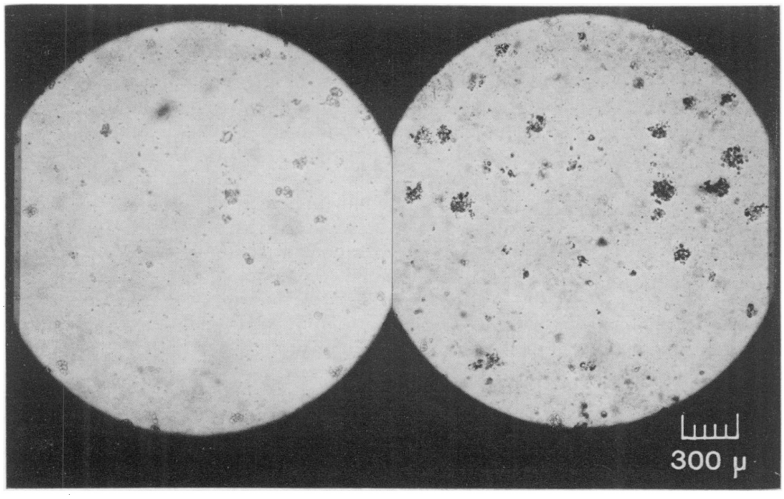


Figure 20.6. Aggregates of cells from disrupted ovarian carcinoma tissues after 1 day (*left*) or 10 days (*right*) of incubation on an agar plate (Agrez et al., 1982). The cell clumps on the plate after 10 days could have grown larger and been mistaken for colonies grown from single cells. Most of the clumps could be seen to have grown from small cell aggregates already visible after 1 day.

The NCI-60 and CellMiner stories.

A new screen: human NCI-60 cell lines.

Having deemed the previous screen using human tumor colony-forming assays a failure, the anticancer drug screening staff directed their attention to human cell lines derived from various human tumors and designed survival assays to gauge the responses of the cells to many anticancer drugs and candidate compounds (Alley et al., 1988; Shoemaker et al., 1988). The new screen avoided the major pitfalls of the previous screen. First, cells of each line used in the survival assays came from the same passaged cultures, so the cells of a line were always of the same kind -- which is what it means to be a cell line. That helped to get reproducible results. Second, the cultures grew largely as single cells, free of clumps or aggregates. This would be less problematic also because the assay measured the growth of viable cells on a plate, rather than survival of colony-formation number. The staff, particularly Robert H. Shoemaker and his colleagues, devoted much effort developing a colorimetric viability assay that was sufficiently sensitive, reproducible, and that could be automated to measure the large numbers of compounds required by the screen. The protocol for how the original assay was conducted is summarized in Figure 20.7. Later modifications further improved the reproducibility of the assay.

The new primary screen included a large number of lines derived from some of the major human tumors, including leukemia, melanoma, and cancers of breast, ovary, lung, colon, kidney, and brain (Shoemaker et al., 1988). It was hoped thereby to find new drugs that may target one or another of those tumor types. In all, a panel of 60 cell lines was eventually selected that came to be known as the NCI-60.

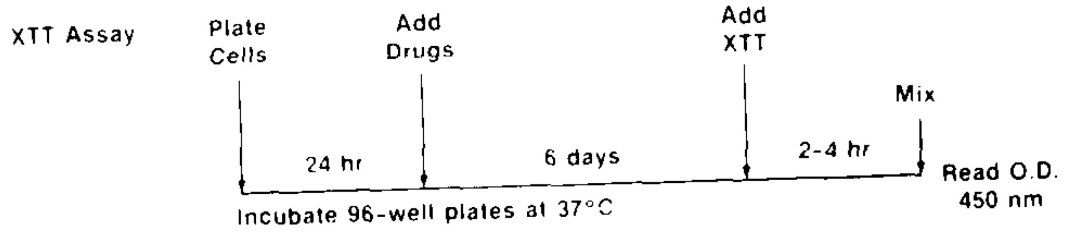


Figure 20.7. The protocol for the colorimetric assay using a tetrazolium reagent (XTT) to measure inhibition of the growth of viable cells (Schoemaker et al., 1988). Only viable cells were able to produce the color that the assay measured. The test drug or compound was added 24 hours after seeding the cells of a given line on the plate. After allowing the cells to grow for 6 days, the XTT reagent was added and 2-4 hours later the intensity of the resulting blue-violet color (450 nm) was measured.

A drug-sensitivity analysis called COMPARE applied to the NCI-60 cell lines.

In 1988, Kenneth D. Paull of the NCI staff devised a bar-graph display of the survival pattern of the cell lines in response to a drug or compound. He then developed an algorithm to quantify the difference between the survival patterns of individual compounds or group averages. This drug sensitivity display method became standard and extensively used. Paull gauged the sensitivity of a cell line to a given drug or compound as the logarithm of the ID50 (dose producing 50% inhibition of the growth of viable cells). He then calculated a "mean graph" display centered on the mean sensitivity for the entire cell line panel (mean log(ID50) for all the cell lines) (Paull et al., 1989). Figure 20.8 shows the first published mean graph displays using this procedure, which he dubbed COMPARE analysis, because it facilitated comparing the sensitivity patterns of different drugs or compounds (Shoemaker et al., 1988). This early example had 50 cell lines; later a standard set of 60 human cancer cell lines was used: the NCI-60.

In 1998, Glenda Kohlhagen, Ken Paull, Yves Pommier, and others in our Laboratory noted that a compound of distinctive chemical structure, NSC 314622, an indenoisoquinoline, produced a mean graph for growth inhibition of the NCI-60 cell lines that was highly correlated with the topoisomerase-I blocking drugs camptothecin and topotecan (Chapter 11) (Figure 20.9) (Kohlhagen et al., 1998). They showed, using our DNA filter elution methodology (Chapter 9), that the novel compound indeed produced the protein-associated DNA strand breaks we had found to be characteristic of topoisomerase inhibitors (Ross et al., 1979). Pommier and his colleagues went on to prepare several indenoisoquinoline derivatives (Figure 20.10) and found that the compounds had properties differing from camptothecin that merited further investigation (Kohlhagen et al., 1998; Marzi et al., 2018; Marzi et al., 2019).

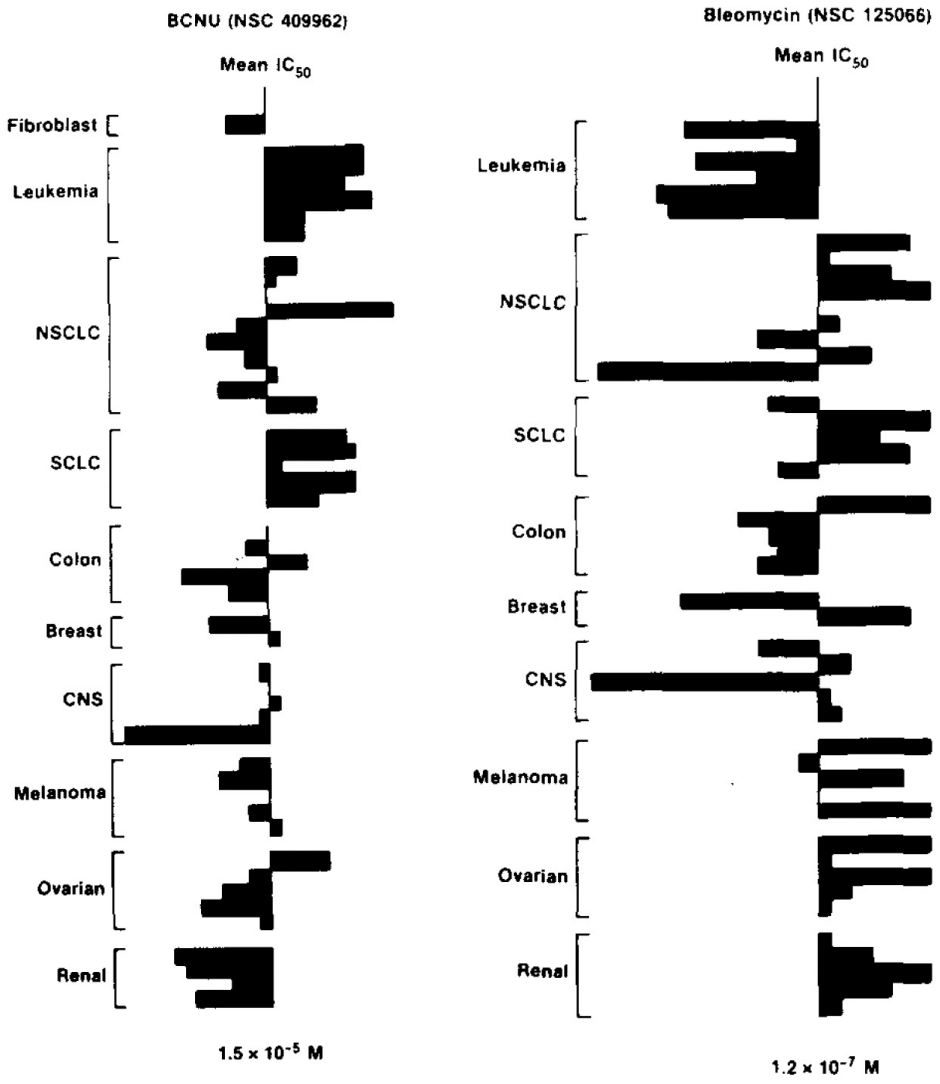


Figure 20.8. The first published example of Ken Paull's mean graph method (COMPARE analysis) for comparing the sensitivity patterns of different drugs (Schoemaker et al., 1988). This early example included 50 human cancer cell lines derived from several tissues of origin. It showed that the two chemotherapy drugs, BCNU (bis(chloroethyl) nitrosourea) and bleomycin, had very different sensitivity patterns. In this display, bars to the right of the center line, indicated high sensitivity relative to the mean IC50 (drug concentration producing 50% inhibition of viable cell growth). Thus, leukemia cell lines were sensitive to BCNU but relatively insensitive to bleomycin, whereas the converse was true particularly for the renal cell lines. Drugs of the same type, such as chloroethylnitrosoureas, on the other hand gave similar mean graphs.

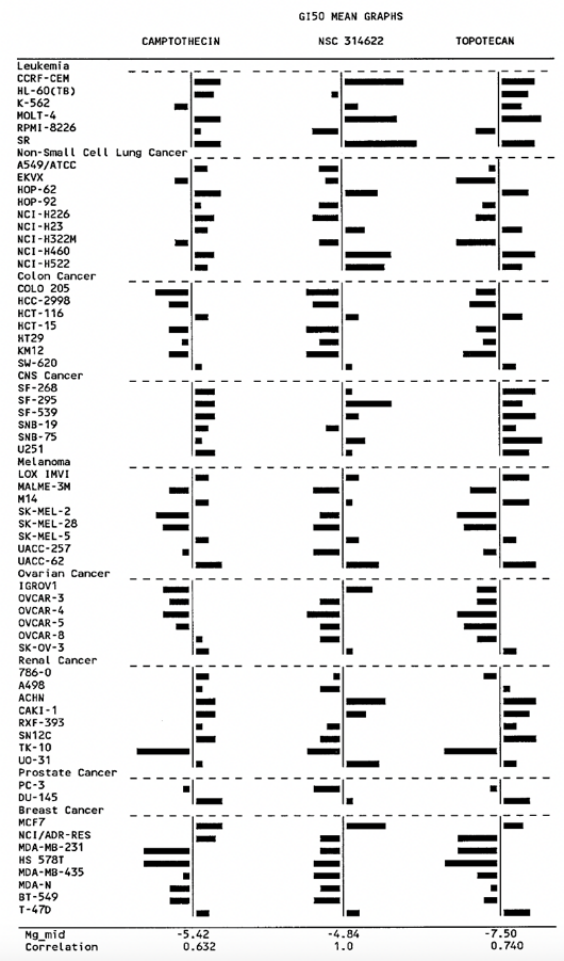
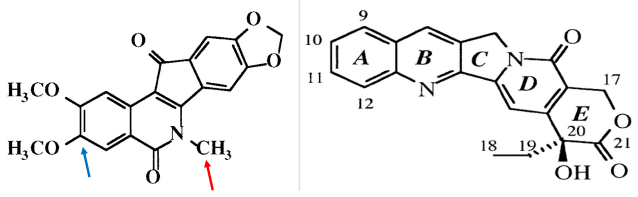


Figure 20.9. The novel compound, NSC 314622 had a mean graph for growth inhibition of the NCI-60 cell lines (*middle*) that correlated well with the mean graphs for the topoisomerase-I inhibitors camptothecin (*left*) and topotecan (*right*) (Kohlhagen et al., 1998).



NSC 314622 Camptothecin

Figure 20.10. The novel topoisomerase I inhibitor, NSC 314622, an indenoisoquinoline (*left*), had a multi-ring structure resembling in shape that of camptothecin (*right*). Several variant compounds were prepared in which the methyl group at the red arrow was replaced by other substituents. In some compounds, the two methoxy groups on the ring on the left were removed and a fluorine was added at the blue arrow (Kohlhagen et al., 1998; Marzi et al., 2018; Marzi et al., 2019).

Gene expression data for the NCI-60 human cancer cell lines.

At staff meetings, I had often urged we think about acquiring molecular data for the NCI-60 cell lines and relating it to drug sensitivity data. It was some years, however, before the technology and data analysis tools, as well as staff members with the necessary skills and determination were available to us. This effort came to a head when John N. Weinstein joined our Laboratory. John had the necessary skills and determination in spades. He also had the organizational skills to take on and lead a group within the Laboratory, as well as outside collaborators to push ahead on this complex task. Their success in this effort with data analysis methods they developed were shown in two sentinel papers published in 2000 in Nature Genetics (Ross et al., 2000; Scherf et al., 2000).

In the first of those papers (Ross et al., 2000), they explained how they measured gene expression by first robotically spotting 9,703 human cDNAs as microarrays on glass microscope slides, and then subjecting those cDNA microarrays to hybridization with fluorescence-labeled cDNA obtained by reverse-transcription of mRNA from each cell line. In that way, they got a measure of the complementary mRNA (reverse-transcribed cDNA) of each gene in each cell line. There were many quality-control issues to overcome, and they explained the details of how they did that; one of the consequences was that the number of well-defined human genes that they could measure reliably with the technology of the time was only 3,700.

In the first analysis reported by Ross, Scherf, Weinstein, and their many coworkers and collaborators (Ross et al., 2000) (Figures 36.11 and 36.12), they applied a clustering algorithm to the average expression difference of a panel of genes between every pair of cell lines. The genes selected for the panel, 1,161 in number, were those that showed relatively large expression differences over the cell lines, so as to be able to contribute significantly to the expression differences to be analyzed. It was satisfying to see that the expression differences of the genes sorted the cell lines, to a large degree, according to the tissue type each cell line came from. This was particularly striking for cell lines that came from leukemias, colon cancers, kidney cancers, melanomas, and cancers of the ovary (Figure 20.11).

They also displayed the data as a two-dimensional cluster diagram. The vertical axis showed the genes, clustered according to their expression in the cell lines. The horizontal axis showed the cell lines, clustered according to expression of the genes. Figure 20.12 shows the section of the cluster diagram where the melanoma cell lines clustered together. Several genes known to be expressed mainly in melanoma were present in the section shown in the figure (Ross et al., 2000). However, several other genes known to be expressed particularly in melanoma were absent, probably because of the number of melanoma cell lines in the NCI-60 was too small. When data for a larger number of cell lines

became available, the missing melanoma genes showed up clearly, for example, the melanoma driver gene MITF (see Figure 20.19).

In the second paper (Scherf et al., 2000), they selected 1,376 genes for analysis in the NCI-60 cell lines, based on showing large differenced in expression in the cell lines, and the cell lines again tended to cluster according to tissue of origin (Figure 20.13A). The cell lines also tended to cluster according to tissue of origin when clustered based in sensitivities of the cell lines to 1,400 compounds (Figure 20.13B). So, tissue of origin was reflected in both gene expression pattern and drug sensitivity pattern.

Moreover, the sensitivities of the cell lines to a set of 118 drugs of known likely mechanisms of action tended to cluster according to those mechanisms of action (Figure 20.13C). Clustering according to mechanism was also seen when based on the correlations between drug sensitivity and gene expression patterns (Figure 20.13D). (Those correlation values were derived by multiplying the cell line-drug sensitivity matrix by the transpose of the cell line-gene expression matrix.) The central conclusion here was that the drug sensitivity and gene expression data for the NCI-60 human cancer cell lines both contained information about tissues of origin of the cell lines and the mechanisms of action of the drugs. It encouraged the development of similar data sets for larger numbers of cell lines that could lead to more and firmer conclusions.

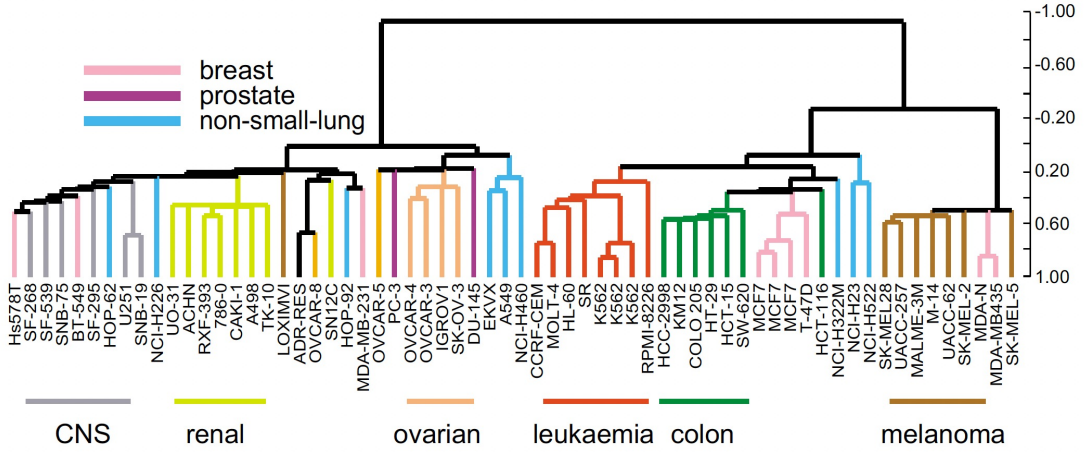


Figure 20.11. Cell-line dendrogram with colored branches to reflect tissue of origin, based on hierarchical clustering of the expression of 1,161 cDNAs in the NCI-60 cell lines (Ross et al., 2000). The 1,161 cDNAs were chosen (out of a total of 9,703) whose expression levels varied at least 7-fold in at least 4 of the 60 cell lines; that was done in order to select genes with the greatest differences in expression over the NCI-60 cell lines. The dendrogram shows that the cell lines tended to cluster according to tissue of origin.

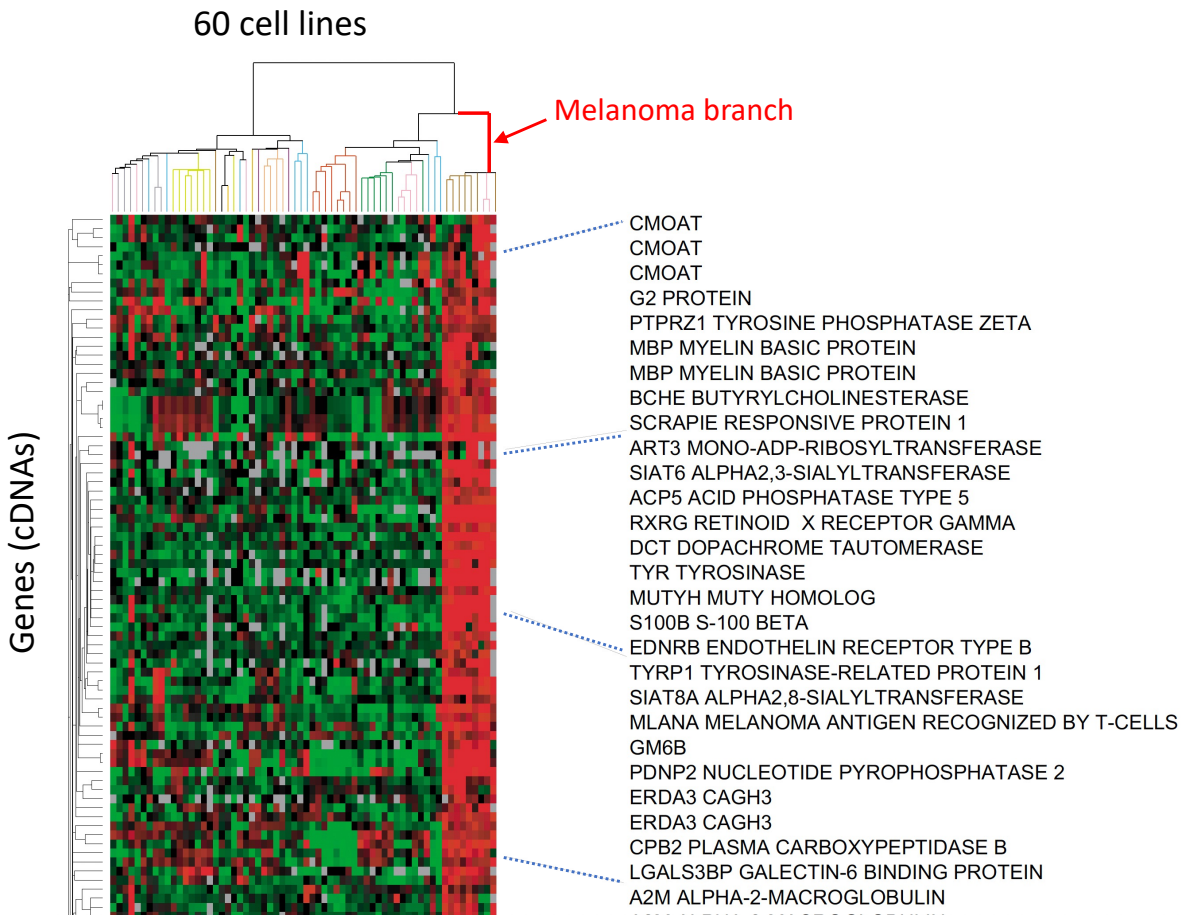


Figure 20.12. Part of a two-dimensional hierarchical cluster diagram of genes (vertical axis) versus the NCI-60 cell lines (horizontal axis). The part of the full cluster diagram shown is the section where the melanoma cell lines clustered together, as shown by the vertical strip of red pixels on the right. A red pixel indicated high expression of a gene (cDNA) in a cell line. (Additional genes known to be highly and specifically expressed in melanoma were later revealed when a larger number of melanoma cell line became available for analysis.)

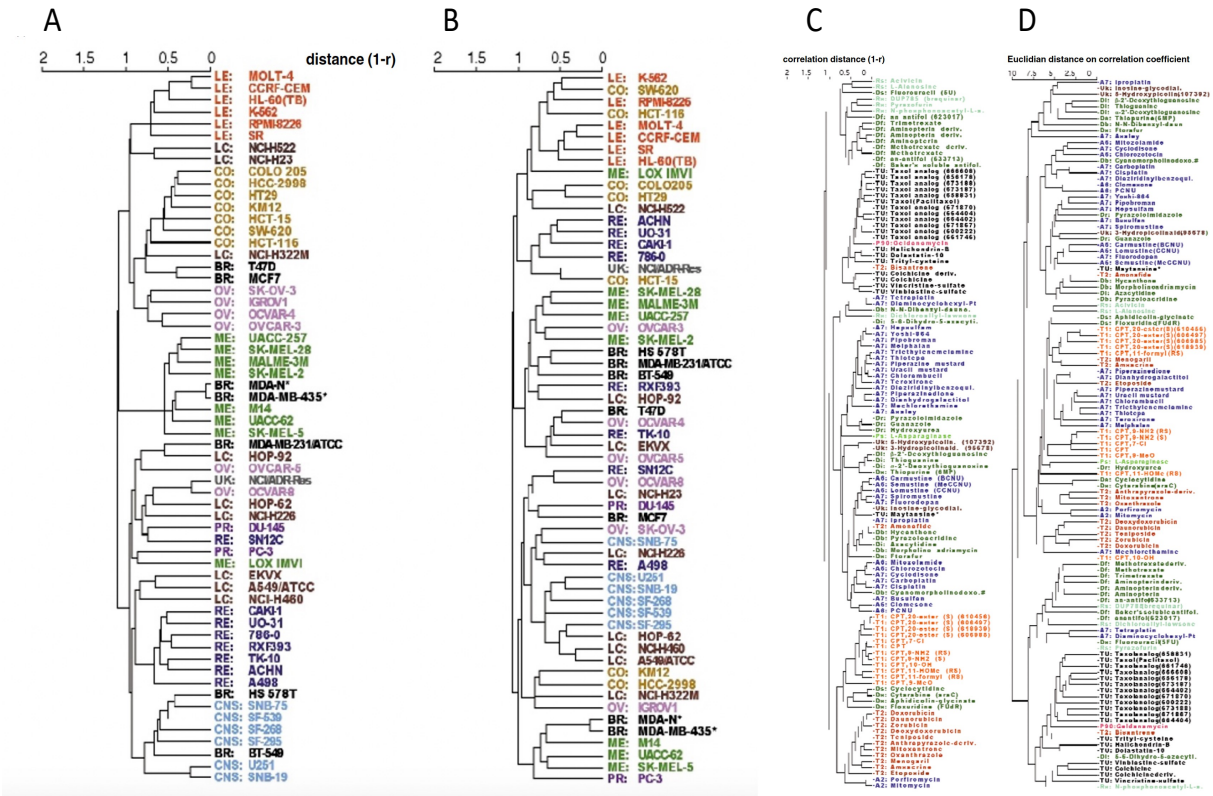


Figure 20.13. Dendrograms for average-linkage hierarchical clustering of the NCI-60 human cancer cell lines. For the analysis, 1376 genes were selected, based on showing large differences in expression in the cell lines (Scherf et al., 2000).
A. Linkage hierarchy cluster tree of the NCI-60 cell lines based on their gene expression patterns, showing clustering according to tissue of origin.
B. Linkage hierarchy cluster tree of the NCI-60 cell lines based on their sensitivities to 1,400 compounds, again showing clustering according to tissue of origin.
Abbreviations in **A** and **B** of the tissues of origin of the cell lines: LE, leukemia; LC, lung cancer; CO, colon cancer; BR, breast cancer; OV, ovarian cancer; ME, melanoma; RE, renal cancer; CNS, brain cancer; PR; prostate cancer.
C. Linkage hierarchy cluster tree of 118 drugs of likely mechanism of action based on the sensitivities of the NCI-60 cell lines to the drugs, showing clustering according to mechanism of action.
D. Linkage hierarchy cluster tree of the 118 drugs of likely mechanism of action based on correlation of their drug activity patterns with their gene expression patterns in the NCI-60 cell lines, again showing clustering according to mechanism of action.
Abbreviations in **C** and **D** of likely mechanisms of the drugs: A7, alkylation at guanine N7; A6, alkylation at guanine N6; A2, alkylation at guanine N2; Db, DNA binder (non-covalent); Df, antifol DNA synthesis inhibitor; Di, incorporation in DNA; Dr, ribonucleotide reductase inhibitor; Ds, DNA synthesis inhibitor; Pi, protein synthesis inhibitor; P90, binds HSP90; Rs,

RNA synthesis inhibitor; T1, topoisomerase-I blocker; T2, topoisomerase-II blocker; TU, tubulin binder; Uk, unknown.

COMPARE analysis for gene expression patterns of human cancer cell lines.

With the success of COMPARE analysis for growth inhibition by drugs and compounds (Figures 36.8 and 36.9), an attractive idea was to apply the method to gene expression patterns in the cell lines. That meant acquiring a large amount of gene expression data on each of the NCI-60 cell lines, as described in the previous section and Figures 36.11-36.13.

The power of the method was shown by our use of COMPARE analysis to examine gene expression expected in cells having an epithelial phenotype (Kohn et al., 2014). Among the NCI-60 human tumor cell lines there was indeed a very consistent pattern for expression of several genes characteristic for epithelial cell types (Figure 20.14B). Moreover, there was a remarkably consistent inverse of expression patterns of epithelial versus mesenchymal cell types, as expected from the epithelial-mesenchymal-transition that many cancer cells undergo. The inverse patterns were, for example, clearly seen between the epithelial marker gene, E-cadherin, and the mesenchymal marker gene, vimentin (Figure 20.14A). COMPARE also revealed genes expressed in particular cell types. For example, the cadherin-17 gene tended to be expressed particularly in the colon cell lines (Figure 20.14C). The functional relationships of the epithelial genes were diagrammed in a molecular interaction map using the notation we had developed (Kohn, 1999) (Figure 20.15). The map shows the molecular interaction of the genes that hold epithelial genes together via tight junctions and adherence junctions.

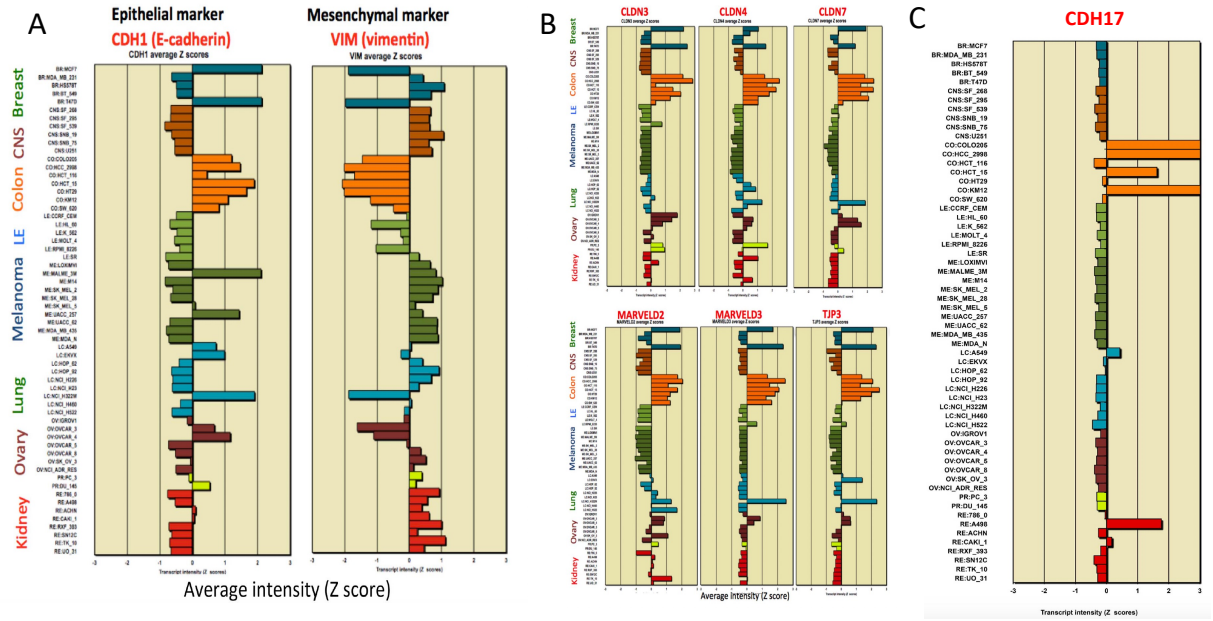


Figure 20.14. COMPARE analysis of expression of epithelial genes in the NCI-60 cell lines. (A) Inverse mean-graph gene expression patterns for an epithelial (E-cadherin) versus a mesenchymal (vimentin) gene. (B) Coherent of expression of several epithelial genes. (C) Gene expression pattern for a gene (cadherin-17) that was selectively expressed in the colon cancer cell lines (Kohn et al., 2014).

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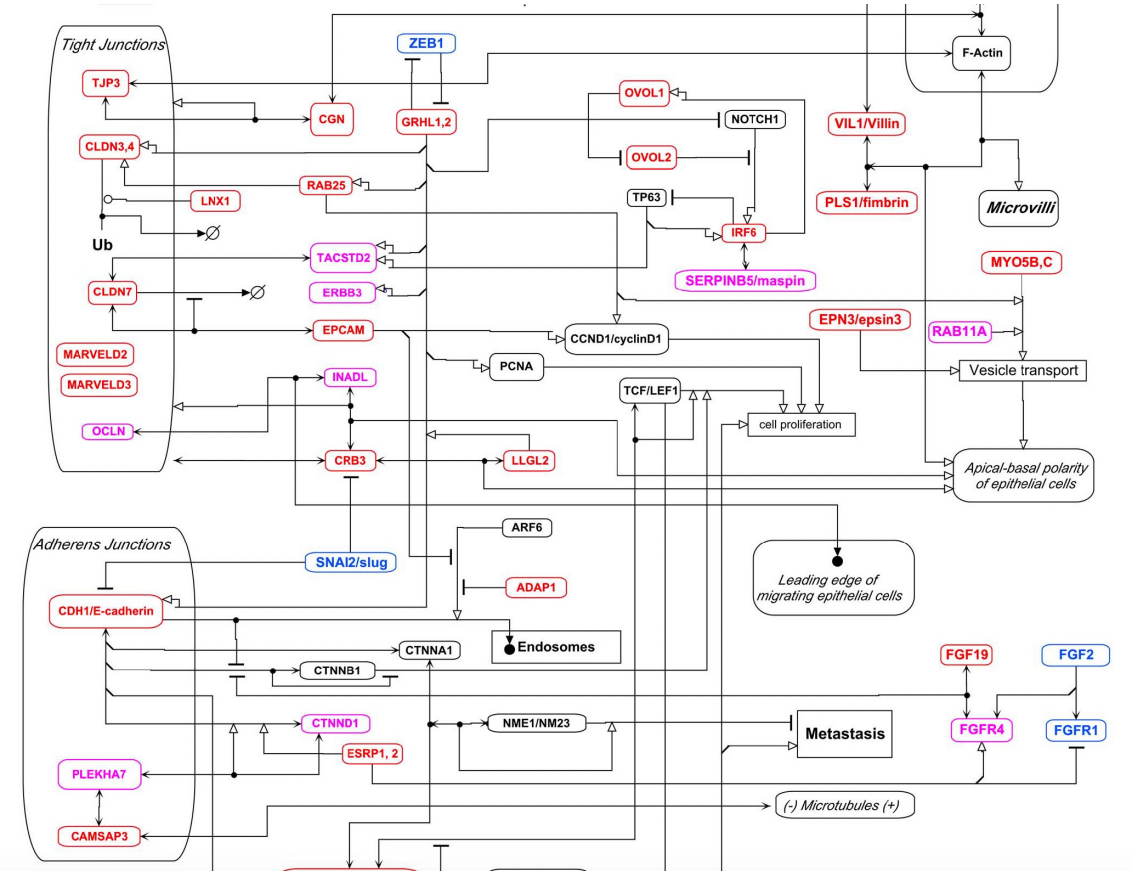

Figure 20.15. Part of a molecular interaction map showing functions at tight junctions and adherence junctions of epithelial cells (Kohn et al., 2014).

Finding new drugs using COMPARE analysis as a screen.

The pattern recognition feature of Ken Paull's COMPARE algorithm made it possible to search for compound that exhibited a unique growth inhibition pattern in the NCI-60 cell lines, which would suggest that the compound had a unique mechanism of action. As of March 1997, 74,196 compounds had been screened for unique growth inhibition pattern, and, after further studies, 5 went on to Phase I clinical trial as potential new anticancer drugs (Figure 20.16) (Monks et al., 1997).

One of the most notable findings of the new screen was flavopiridol (also known as alvocidib), a plant product modified by organic chemists. Flavopiridol was found to block the cell's progress through the cell cycle by inhibiting cyclin-dependent kinases (CDK's), particularly CDK9, and became the first CDK inhibitor to enter clinical trial. Also contributing to the drug's anticancer action may have been its ability to suppress the expression of several proteins in the programmed cell death (apoptosis) pathway and of

the vascular endothelium growth factor (VEGF) that stimulates the growth of tumor-nourishing blood vessels (Wang and Ren, 2010). Flavopiridol was found to be effective in about half of patients with advanced acute myeloid leukemia when used as initial treatment to reduce the number of malignant cells and to accumulate the cells in a phase of the cell cycle where they would be sensitive to other cytotoxic drugs (Zeidner and Karp, 2015).

Flavopiridol eventually failed in the clinic, however, because it inhibited a variety of cdk's with different functions. When inhibitors specific to cdk4 and cdk6 in the Rb pathways were developed, they became effective for treatment of common types of breast cancer (Chapter 33).

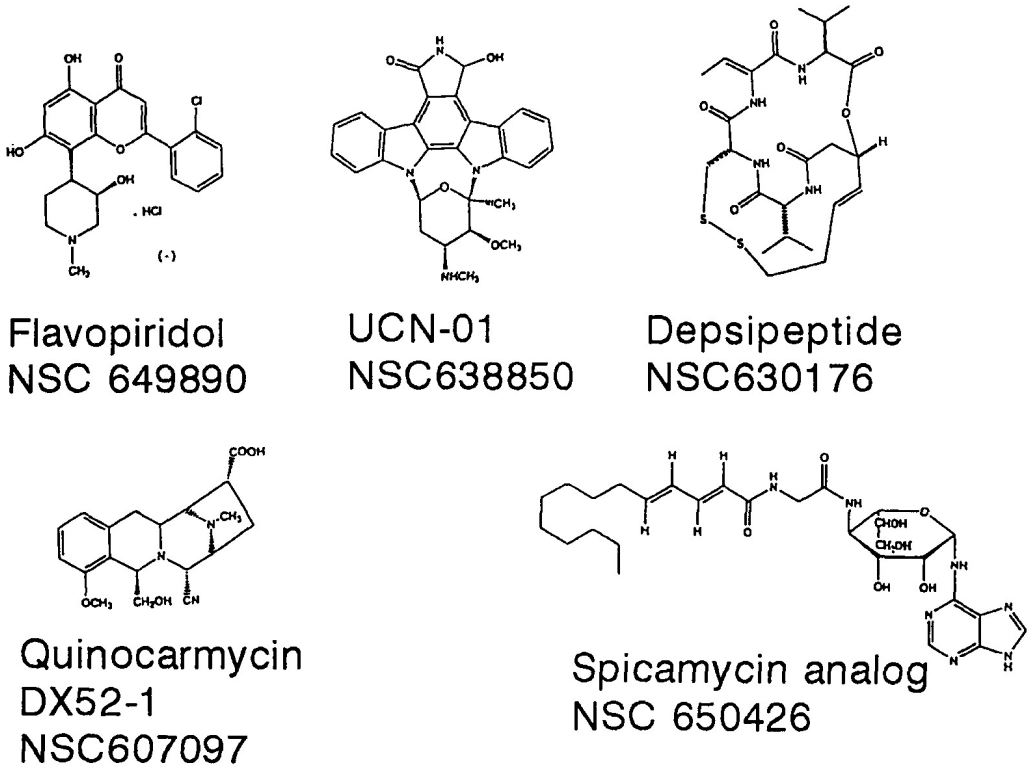

Figure 20.16. Five compounds that passed a screen for unique growth-inhibition in the NCI-60 cell lines, and, after passing additional tests, went on to Phase I clinical trial (Monks et al., 1997).

The CellMiner story: mining molecular and pharmacological data.

The NCI-60 data were limited by the few cell lines of each tissue type in the data set. The success of the NCI-60 analyses despite this limitation motivated the development of datasets for larger numbers of human cancer cell lines of various tissue types (Reinhold

et al., 2015). In addition to drug sensitivity and gene expression, data were assembled on gene mutations, gene copy number, DNA methylation, and protein expression. This presented a daunting complexity of analyses of various types that could be of interest. Our Laboratory met this problem by developing a set of software tools under the name *CellMiner* that in essence facilitated the mining of the data (Reinhold et al., 2012). This effort was conducted William C. Reinhold, head of our Genomics and Pharmacology group, together with John Weinstein, Augustin Luna, Ken Paul, and Yves Pommier (Figure 20.17), their coworkers and collaborators.

A recently enhanced version, *CellMinerCDB* (Luna et al., 2021; Reinhold et al., 2019), combined several databases covering larger numbers of cell lines and molecular as well as drug sensitivity data types (Figure 20.18). The new version allowed analyses between different databases. An example of a data plot for two genes expressed specifically in melanoma cell lines is shown in Figure 20.19. In addition, a database of drug sensitivity and gene expression was developed for cell lines derived from a particularly troublesome disease: small-cell lung cancers (Tlemsani et al., 2020).

Figure 20.17. Leaders who contributed to our Laboratory's information technology and CellMiner projects.
William C. Reinhold, leader of our Genomics and Pharmacology group.
Kenneth D. Paull, formerly chief of DTP's Information Technology Branch. Courtesy of *The NIH Record*, 7 April 1998.
https://dtp.cancer.gov/timeline/flash/milestones/M7_COMPARE.htm.
John N. Weinstein, formerly leader of our omics development group.
Yves Pommier, Director of the Developmental Therapeutics Branch.

CellMinerCDB drug, gene, and phenotype data

		NCI60	Almanac	CCLE	CTRP	GDSC	SCLC NCI/DTP	MD Anderson	Achilles
	Cell line	60	60	1,089	823	1,080	77	651	769
Drugs	Single	24,047		24	481	297	526		
	Combo		5,355						
DNA	Mutation	9,307		1,667	1,667	18,099			
	Copy #	23,232		23,316	23,316	24,502	25,568		
	Methylation	17,553		19,880		19,864	23,202		
RNA Expression	Microarray (zs)	25,040							
	Microarray (log2)	23,059		19,851	19,851	19,562	17,804		
	RNASeq	23,826		52,604					
	miRNA	417		734			800		
Protein Expression	RPPA	162		214				452	
	SWATH	3,167							
Metabolite Expression	LC-MS			225					
Gene knockout	CRISPR								18,119
Parameters	Phenotypic	70		7	7	6	3		2

Figure 20.18. Summary of data in CellMinerCDB version 1.4 (Luna et al., 2021; Reinhold et al., 2019). Drugs included activity levels, for example, for 24,047 compounds in the NCI-60 cell lines. There were 823 cell lines in the CTRP database and over 1,000 in the CCLE and GDSC databases. (RPPA, reverse-phase protein assay. SWATH, sequential window acquisition of theoretical spectra, mass spectroscopy.)

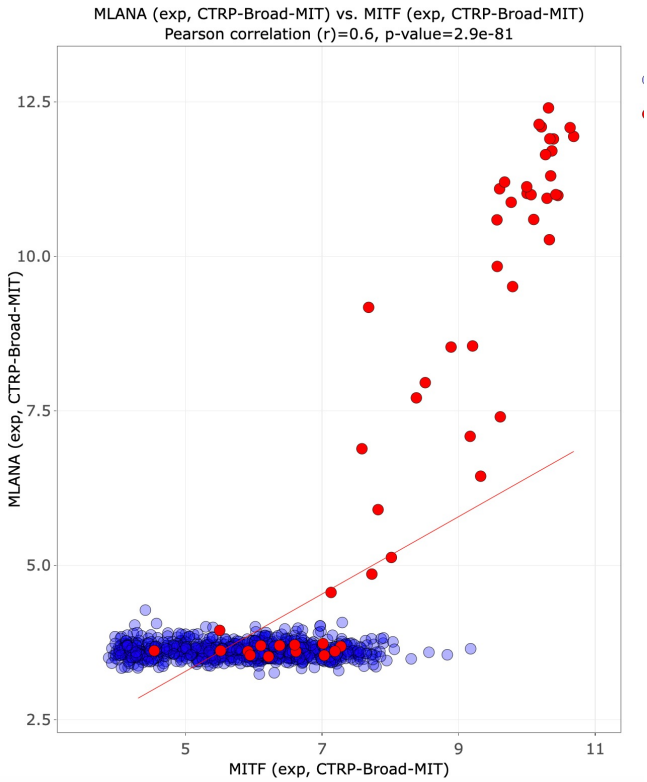

Figure 20.19. A CellMinerCDB data plot showing the specific expression of two genes known to function specifically in melanoma cells. *Red*, melanoma cell lines; *blue*, approximately 800 human cancer cell types of other tissue types. The plot shows that MLANA was expressed only when MITF was expressed beyond a certain level (about 7 on the horizontal scale), beyond which the expression of both genes rose. That was consistent with MITF being a factor that stimulates the transcription of MLANA. The plot also shows that MLANA was expressed exclusively in melanoma cell lines. (The axes are scaled in powers of 2.)

Using CellMinerCDB to explore the action of flavopiridol in acute myeloid leukemia (AML).

A previous section of this chapter discussed flavopiridol (also known as alvocidib) as one of the most notable discoveries in the NCI-60 cell line screen and the COMPARE algorithm. The drug went on to clinical trial and was found useful in the treatment of acute myeloid leukemia. In order to illustrate some of the capabilities of CellMinerCDB (Luna et al., 2021; Reinhold et al., 2019), I show some preliminary analyses of flavopiridol (alvocidib) in acute myeloid leukemia (AML) cell lines in the CTRP-Broad-MIT database (Figure 20.18) (Table 20.3; Figures 36.20 and 36.21).

Table 20.3 illustrates how genes can be found whose expression is highly correlated with sensitivity to a given drug in cell lines of a given tissue type. By modifying the options,

you can also find genes whose expression correlates with the expression a given gene or drugs whose sensitivity correlates with the expression of a given gene or with the sensitivity to a given drug. Also, the datasets used can be selected and even mixed. The facility is completely flexible in these regards.

Figure 20.20 is a plot of expression of S100G (the top gene in Table 20.3) versus sensitivity to flavopiridol (alvocidib) in AML cell lines. The plot suggests that sensitivity the drug would be high when expression of the gene is low, a relationship that has not previously been reported. This finding, however, is exclusive for AML cells.

Figure 20.21 is a multivariate plot showing how the expression of the 8 genes at the top of Table 20.3 correlated inversely with sensitivity to flavopiridol (alvocidib) in AML cells.

Explorations of these kinds could, and have indeed, translated to clinical application.

Table 20.3. A set of genes showing high negative correlation of expression relative to sensitivity to flavopiridol (alvocidib) in AML cell lines. The data in the Table came from CellMinerCDB version 1.4 using the *Compare Patterns* option (https://discover.nci.nih.gov/rsconnect/cellminercdb/).

GENE	LOCATION	Correlation	P-value
S100G	Xp22.2	-0.907	1.88E-05
TBC1D23	3q12.2	-0.898	3.01E-05
ABHD4	14q11.2	-0.872	1.02E-04
PARD3B	2q33.3	-0.869	1.15E-04
REXO2	11q23.2	-0.868	1.19E-04
MICAL2	11p15.3	-0.862	1.50E-04
UCA1	19p13.12	-0.859	1.69E-04
TXK	4p12	-0.857	1.79E-04
LEF1	4q23-q25	-0.849	2.40E-04
RABGEF1	7q11.21	-0.845	2.81E-04
TJP2	9q13-q21	-0.844	2.88E-04
STXBP6	14q12	-0.842	3.01E-04
AUH	9q22.31	-0.838	3.42E-04
PIP4K2C	12q13.3	-0.837	3.57E-04
DZIP3	3q13.13	-0.835	3.82E-04
PPFIA1	11q13.3	-0.835	3.83E-04
GOLPH3L	1q21.3	-0.835	3.86E-04
MTTP	4q24	-0.833	4.07E-04
LENG1	19q13.4	-0.832	4.19E-04
CTSL	9q21.33	-0.831	4.31E-04

HPSE2	10q23-q24	-0.830	4.49E-04
AVPI1	10q24.2	-0.830	4.52E-04
DNASE2B	1p22.3	-0.827	4.92E-04
TBK1	12q14.1	-0.827	4.93E-04
DSTYK	1q32.1	-0.826	4.96E-04

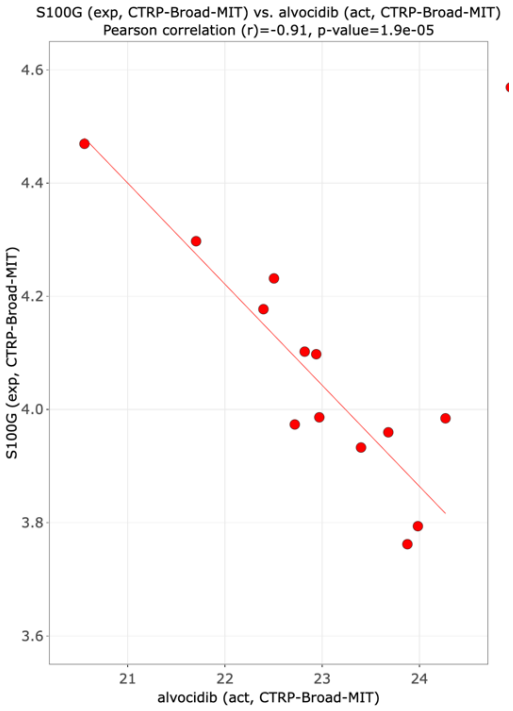

Figure 20.20. This plot shows how sensitivity to flavopiridol (alvocidib; horizontal axis) correlates with the expression of S100G (the top gene in Table 20.3; vertical axis) in acute myeloid leukemia (AML) cell lines. The plot came from the *Plot Data* option in CellMinerCDB version 1.4 (https://discover.nci.nih.gov/rsconnect/cellminercdb/). The facility is completely flexible in what data you chose for each axis of the plot. The result suggests that sensitivity of AML to flavopiridol (alvocidib) may be enhanced by reducing the expression of S100G.

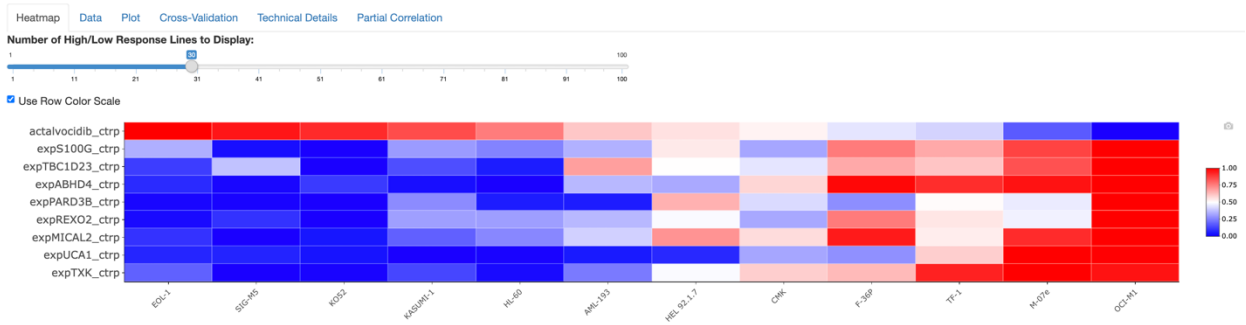

Figure 20.21. Multivariate display of a set of 8 genes whose expression is negatively correlated with sensitivity to flavopiridol (alvocidib) in acute myeloid leukemia (AML) cell lines (top 8 genes in Table 20.3). There were data for 12 AML cell lines (labeled at bottom) in the CTRP database. Sensitivity to alvocidib is in the top row. Expression of the 8 genes in the AML cell lines is in the lower 8 rows. Red to blue: high to low sensitivity or expression. The display suggests that low expression of the 8 genes would correlate with high sensitivity to alvocidib in AML cells. This display was obtained using the *Multivariate Analysis* option in CellMinerCDB version 1.4 (https://discover.nci.nih.gov/rsconnect/cellminercdb/).

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