2 Murine L1210 and P388 Leukemias

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CONTENTS

1. INTRODUCTION

Mouse leukemia models were a central component of the initial drug discovery programs employed by the Division of Cancer Treatment (DCT) of the National Cancer Institute (NCI) during the early 1960s and 1970s. The L1210 and P388 leukemias, developed in 1948 *(1)* and 1955 *(2),* respectively, played a major role in both screening and detailed evaluations of candidate anticancer agents. Today, 40 yr later, these models are still used to evaluate anticancer activity, although at a greatly reduced level, and to study mechanisms of drug resistance. This chapter reviews their past contributions, updates their present role in the evaluation of anticancer drugs, and summarizes data for the drug sensitivity of these two leukemias and various drug-resistant P388 sublines to clinically useful drugs.

2. ROLE IN DRUG SCREENING

Spontaneous tumors in animals were first used as models for screening potential anticancer agents. In fact, these types of studies occurred even prior to the beginning of the twentieth century *(3),* and provided the basis for modern drug-screening programs. However, large-scale screening and the ability to conduct detailed drug-evaluation studies with anticancer agents increased greatly in the 1920s through the development of inbred strains of mice that allowed investigators to propagate tumor lines by serial transplantation in vivo *(4).*

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The US Congress became interested in cancer research when it was recognized in the 1940s that systemic cancer could be influenced by drug treatment. This was demonstrated at Memorial Sloan-Kettering, one of the first of several institutions in the United States and Europe to begin drug-screening programs. In this program, the mouse sarcoma SA-180 was used as its screening model. However, as drugs exhibited anticancer activity and the supply of new candidate agents exceeded the screening capacity of that program, the need for additional drug development capabilities became apparent. With this impetus, Congress directed NCI to implement a national drug development program, which went into effect in 1955 as the Cancer Chemotherapy National Service Center (CCNSC).

Initially, the CCNSC primary screening program consisted of L1210 leukemia, SA-180, and mammary adenocarcinoma 755 *(5).* Over the years, the composition of the primary screen changed several times—i.e., from the original three tumors to L1210 and two arbitrarily selected tumors; to L1210 and Walker 256 carcinosarcoma; to L1210 and P388 leukemia; and finally, to L1210, P388, and either B16 melanoma or Lewis lung carcinoma (LLC) *(6).* Several other models were also used during this period for special, detailed drug evaluation.

The primary screening program underwent a major change in 1976, when DCT incorporated the use of three human tumor xenograft models. The new screen now consisted of a panel of colon, breast, and lung tumors, both murine and human. However, all drugs intended this screen were still initially evaluated for activity against the sensitive P388 leukemia model *(7).* During this period, the small number of drugs discovered with marked antitumor activity against human solid tumors led to a radical change in the screening program that had used murine leukemia models as the primary screen. In the mid-1980s, NCI developed a new primary screen based on the use of established human tumor-cell lines in vitro *(8).* The new and old screen programs were to be conducted in parallel to permit a comparison; however, in early 1987, budget cuts at NCI forced an end to large-scale P388 screening *(9).*

3. CHARACTERISTICS

Both L1210 and P388 leukemias were chemically induced in a DBA/2 mouse by painting the skin with methylcholanthrene *(1,2).* Propagation of the leukemia lines occurs in the host of origin by intraperitoneal (ip) implantation of diluted ascitic fluid containing either 10^5 (L1210) or 10^6 (P388) cells per animal. Testing is generally conducted in a hybrid of DBA/2 (e.g., $CD2F_1$ or $B6D2F_1$), because the hybrids are somewhat heartier. However, DBA/2 mice may be used for special studies, and should be used for serial in vivo propagation of the leukemias. Frequently used implant sites are ip, subcutaneous (sc), intravenous (iv), or intracerebral (ic). For L1210 leukemia with an implant of 10^5 cells, the median days of death and the tumor doubling times for these implant sites are 8.8, 9.9, 6.4, and 7.0 d and 0.34, 0.46, 0.45, and 0.37 d, respectively. For P388 leukemia with an implant of 10^6 cells, the median days of death and the tumor doubling times for these implant sites are 10.3, 13.0, 8.0, and 8.0 d and 0.44, 0.52, 0.68, and 0.63 d, respectively.

Skipper and colleagues at the Southern Research Institute determined the rate of distribution and proliferation of L1210 leukemia cells using bioassays of untreated mice after ip, iv, and ic inoculation *(10).* Following ip inoculation, most of the L1210 cells were found in the ascites fluid of the peritoneal cavity. Using the median day of death

as the evaluation time-point, the most commonly infiltrated tissues were the bone marrow, liver, and spleen. Following iv inoculation, the majority of L1210 cells appeared in the bone marrow. On the median day of death from the iv implant, the most infiltrated tissues were also the bone marrow, liver, and spleen. After ic inoculation, most of the L1210 cells remained in the brain (for 3–5 d). On the median day of death from the ic implant, the spleen was heavily infiltrated (the extent of the leukemia in other tissues was not reported).

Southern Research was one of the first institutions to become involved in the CCNSC screening program, and was heavily involved in designing protocols for the program. One aspect essential to the operation of a screening program is the development of appropriate parameters for measuring antitumor activity. At Southern Research, antitumor activity for leukemia studies is assessed on the basis of percent median increase in lifespan (% ILS), net $\log_{10}$ cell-kill, and long-term survivors. Calculations of net $\log_{10}$ cell-kill are made from the tumor-cell population doubling time that is determined from an internal tumor titration consisting of implants from serial 10-fold dilutions *(11)*. Long-term survivors are excluded from calculations of % ILS and tumor-cell-kill. To assess tumor-cell-kill at the end of treatment, the survival time difference between treated and control groups is adjusted to account for regrowth of tumor-cell populations that may occur between individual treatments *(12)*. The net $\log_{10}$ cell-kill is calculated as follows:

$$\text{Net } \log_{10} \text{ cell-kill} = \frac{\text{(T-C)} - \text{(duration of treatment in days)}}{3.32 \times T_d}$$

where (T-C) is the difference in the median day of death between the treated (T) and the control (C) groups, 3.32 is the number of doublings required for a population to increase 1-$\log_{10}$ unit, and T_d is the mean tumor doubling time (days) calculated from a log-linear least-squares fit of the implant sizes and the median days of death of the titration groups.

4. SENSITIVITY TO CLINICAL AGENTS

The majority of clinically useful compounds in current use was first detected in the murine leukemia models. The sensitivities of L1210 and P388 leukemias (ip implantation) to most of these agents (ip administration) are shown in Figs. 1 and 2 and Figs. 3 and 4, respectively. Overall, P388 leukemia is somewhat more sensitive than L1210 leukemia. For alkylating agents, the sensitivities are similar. Notable exceptions are chlorambucil, mitomycin C, and carboplatin, for which P388 is markedly more sensitive. For antimetabolites, the sensitivities are also similar. Exceptions are floxuridine (P388 being markedly more sensitive) and hydroxyurea (L1210 being markedly more sensitive). For DNA-binding agents, P388 leukemia is clearly more sensitive (e.g., actinomycin D, mithramycin, daunorubicin, teniposide, doxorubicin, and amsacrine). For tubulin-binding agents, P388 leukemia is again clearly more sensitive. The vinca alkaloids are active against P388 leukemia, but ineffective against L1210 leukemia.

Although most of the sensitivity data are for ip-implanted leukemia and ip-administered drugs, valuable information can be obtained from separating the implant site and the route of administration. Table 1 shows the activity of melphalan (ip administration) against both L1210 and P388 leukemias implanted through ip, iv, and ic meth-

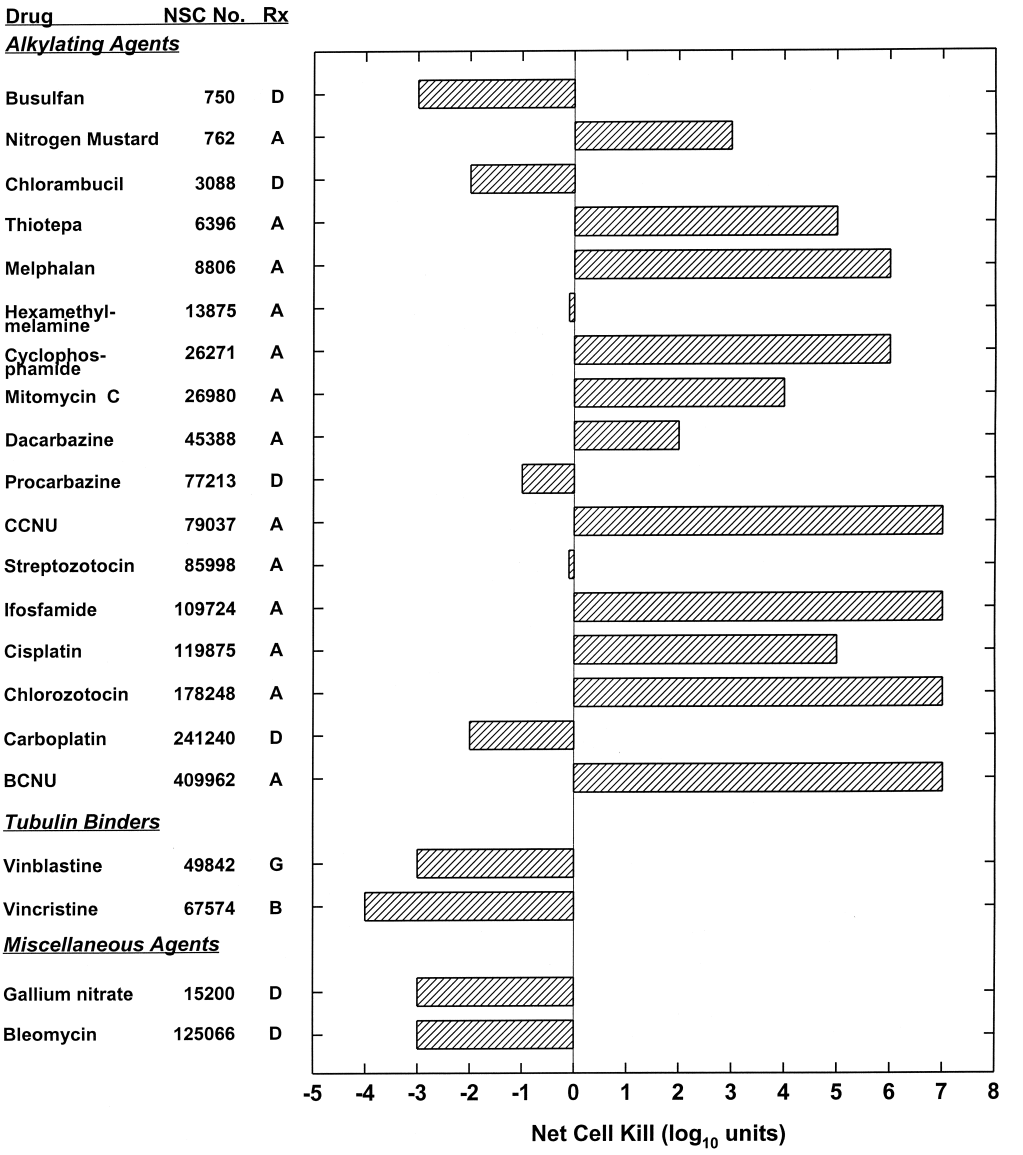

Fig. 1. Sensitivity of ip-implanted L1210 leukemia to clinically useful alkylating agents, tubulin binders, and other miscellaneous agents. L1210 leukemia (10^5 cells except for hexamethylmelamine, which used 10^6 cells) was ip-implanted on d 0. Beginning on d 1, the agents were ip-administered using the indicated schedules. Treatment schedule (R_x): A = d 1; B = d 1, 5, 9; C = d 1–5; D = d 1–9; E = d 1, 4, 7, 10; F = q3h × 8, d 1, 5, 9; G = d 1–15.

ods. The use of ip melphalan is very effective against both ip-implanted leukemias. The activity is reduced to less than one-half when changed to an iv implant site. The activity is further reduced with change to an ic implant site; however, melphalan can cross the blood-brain barrier to some extent. This principle is illustrated more fully with the data in Figs. 5 (L1210) and 6 (P388) for the leukemias with ic implantation, and various clinically useful agents with ip administration. Thiotepa, CCNU, BCNU, and ara-

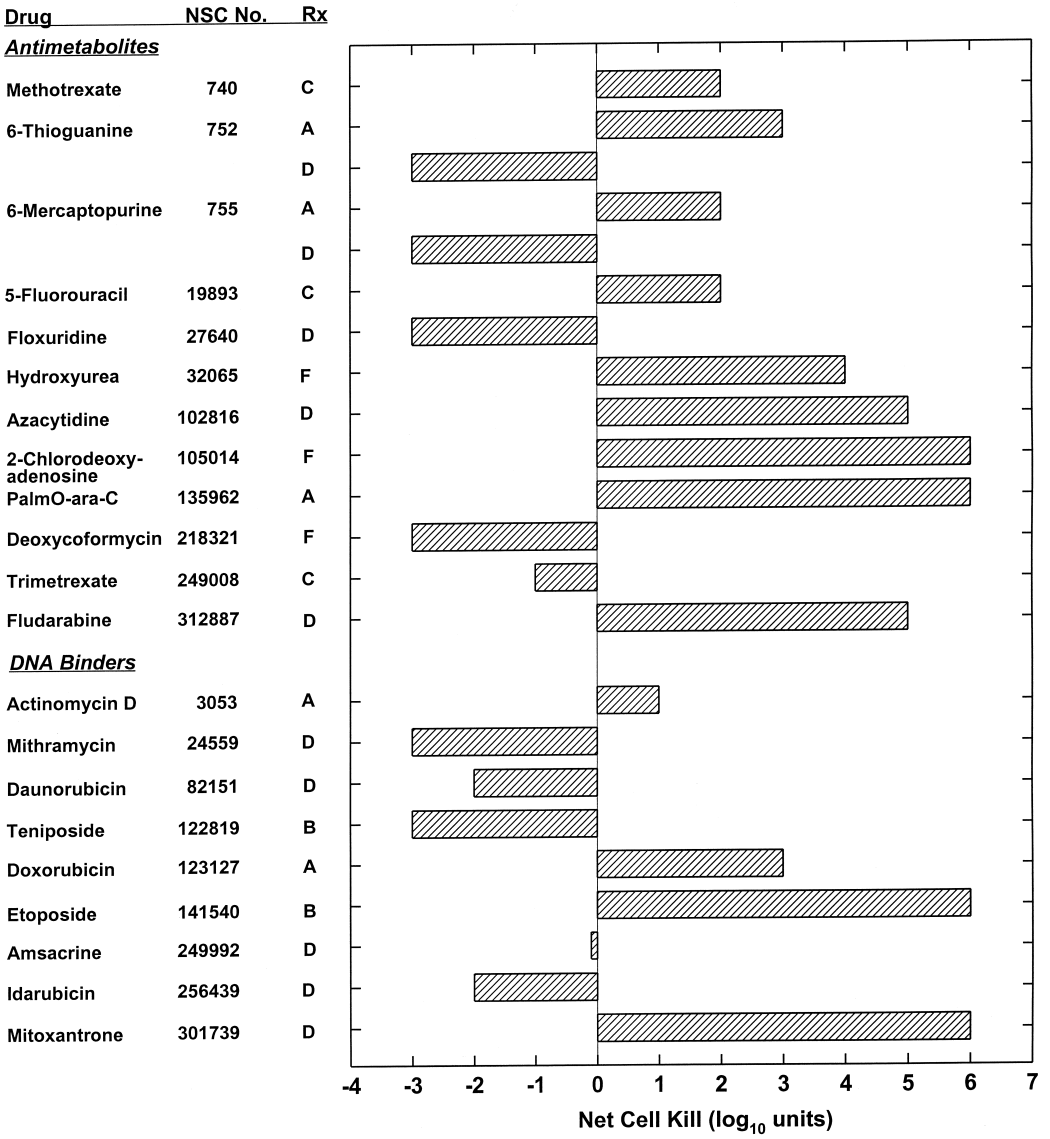

Fig. 2. Sensitivity of ip-implanted L1210 leukemia to clinically useful antimetabolites and DNA binders. L1210 leukemia (10^5 cells, except for hydroxyurea, which used 10^4 cells and 6-thioguanine (d 1-only treatment) and daunorubicin, which used 10^6 cells) was ip-implanted on d 0. Beginning on d 1 (d 2 for daunorubicin), the agents were ip-administered using the indicated schedules. Treatment schedule (R_x): see legend for Fig. 1.

C/palmO-ara-C, with ip administration, exhibit comparable activity against either ip- or ic-implanted leukemias. Cisplatin, cyclophosphamide, ifosfamide, and 6-mercaptopurine (L1210), in addition to melphalan, have reduced activity with an ic implant site. Several agents become inactive with an ic implant site—methotrexate (P388), 5-fluorouracil (5-Fu), floxuridine, actinomycin D, vincristine, doxorubicin, and etoposide. Comparisons among different treatment schedules can be misleading. Although all values have been expressed as net cell-kill (i.e., corrected for the treatment schedule),

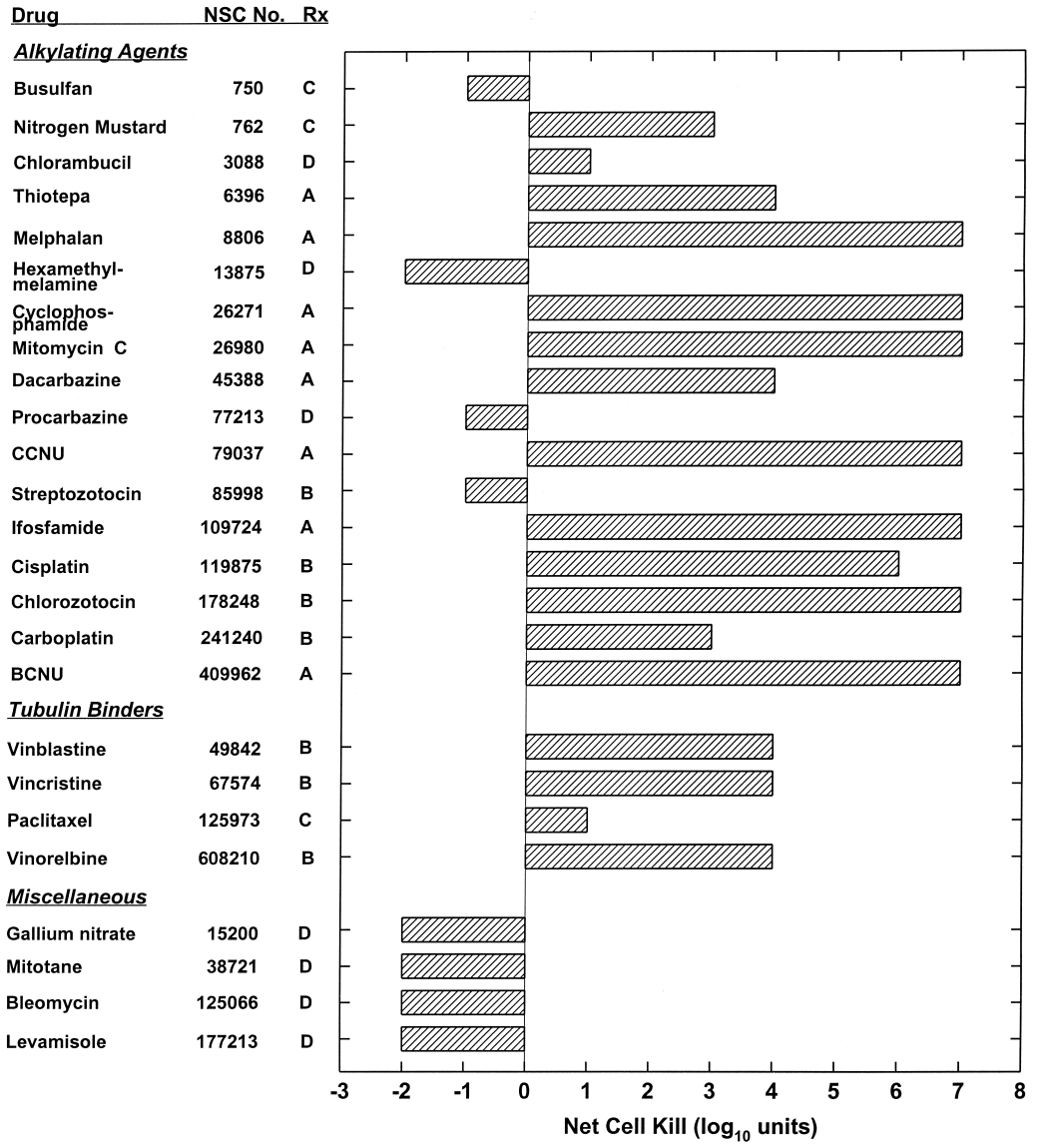

Fig. 3. Sensitivity of ip-implanted P388 leukemia to clinically useful alkylating agents, tubulin binders, and other miscellaneous agents. P388 leukemia (10^6 cells except for CCNU, which used 10^7 cells) was ip-implanted on d 0. Beginning on d 1 (d 2 for CCNU, streptozotocin, and chlorozotocin), the agents were ip-administered using the indicated schedules. Treatment schedule (R_x): see legend for Fig. 1.

one schedule can be optimal, whereas another schedule is suboptimal. For nitrogen mustard, no conclusion can be drawn from the data about its ability to cross the blood-brain barrier. The agent is active against the ip-implanted leukemia using a single ip injection (optimal), and is inactive against the ic-implanted leukemia using 15 daily ip injections (suboptimal). This is further illustrated by chlorambucil, which is active against ic-implanted L1210 (using a single ip injection), and inactive against ip-implanted L1210 (using nine daily ip injections).

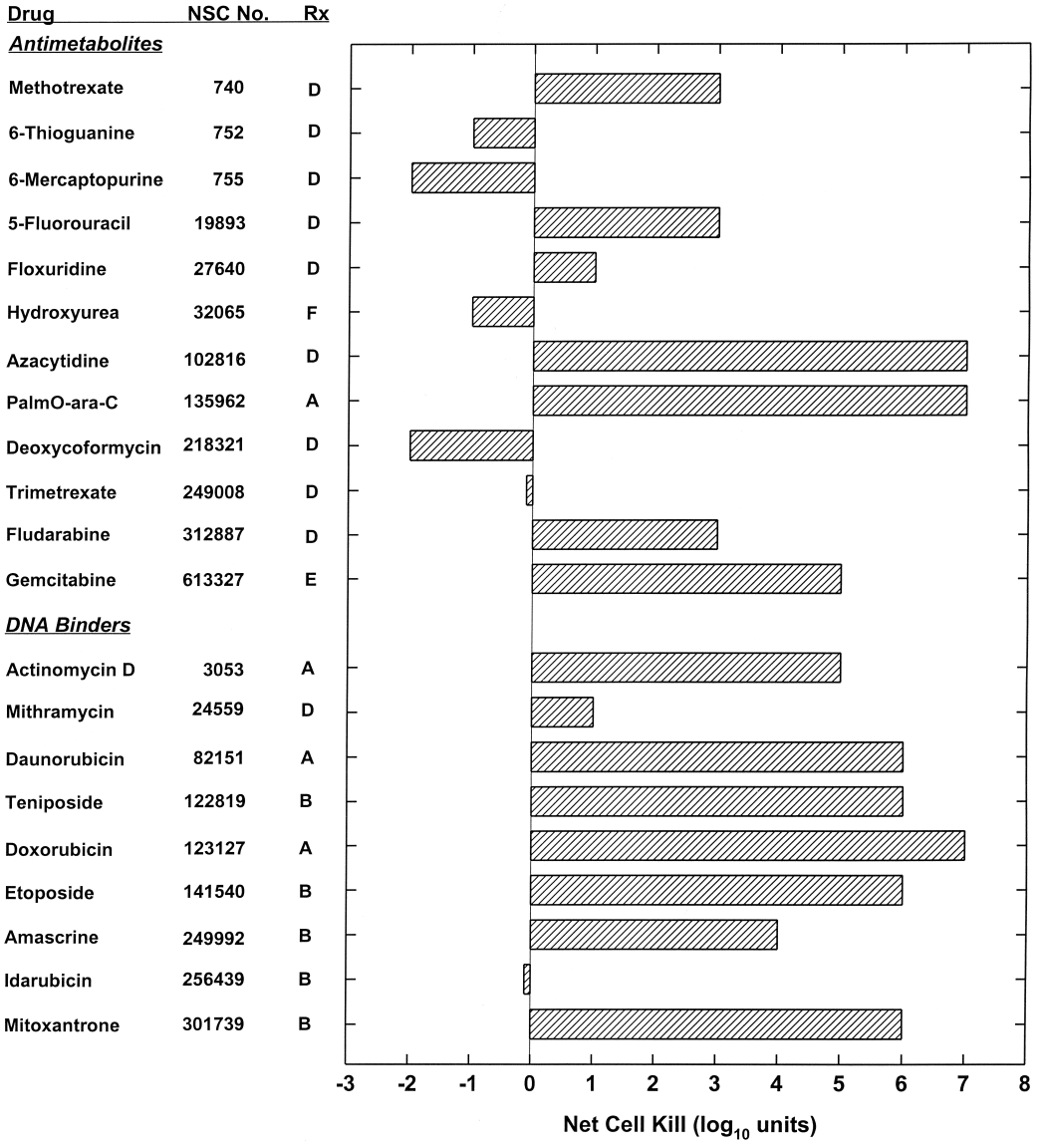

Fig. 4. Sensitivity of ip-implanted P388 leukemia to clinically useful antimetabolites and DNA binders. P388 leukemia (10^6 cells) was ip-implanted on d 0. Beginning on d 1, the agents were ip-administered using the indicated schedules. Treatment schedule (R_x): see legend for Fig. 1.

Studies with these screening models revealed that drug sensitivity was, in some cases, heavily dependent on drug concentration and exposure time, which in turn was impacted by the in vivo treatment schedule. As an example, studies conducted with 1-β-D-arabinofuranosylcytosine (ara-C) pointed out the need for concentration and time of exposure studies. Using L1210 leukemia in mice, it was shown that the optimal dosage and schedule for ara-C was 15–20 mg/kg/dose, given every 3 h for eight doses, then repeated three times at 4-d intervals *(13)*. This regimen was "curative." The single-dose LD_{10} for mice was between 2500 and 3000 mg/kg, and using a single dose within

Table 1
Activity of Melphalan Administered as a Single IP Injection Against L1210 and P388 Leukemias Implanted ip, iv, and ic

		Net cell-kill (log_{10} units)	
Site	*Inoculum size*	*L1210*	*P388*
IP	10^6	4.7	>6.5
IV	10^6	2.0	2.9
IC	10^4	1.2	2.4

that range would effect a 3-log_{10}-unit reduction in L1210 cells but was not "curative." Although these in vivo results might give the appearance of a concentration-dependent effect, in vitro studies have clearly shown that cell-kill of L1210 in culture was time-dependent at the higher concentration levels employed. The apparent concentration dependence observed in vivo over a range of single doses resulted from the extended time of exposure of those extremely high dosage levels.

5. PREDICTIVE VALUE

Many investigators have questioned the use of experimental leukemias as primary screening models over the years. Some have argued that since L1210 or P388 leukemia was used for many years as the initial screening model, continued evaluation of compounds emerging from this screening configuration—even using solid-tumor models for secondary evaluation—would only produce antileukemic drugs *(14)*. If compounds active against solid tumors were being missed by the primary screen composed of leukemias, it would appear reasonable that in order to obtain agents that are active against specific tumor types or solid tumors in general, then the primary screen should consist of specific tumor types or solid tumors. Although this would appear to be a reasonable approach, it will depend on whether or not there are existing agents or whether agents can be developed that will selectively kill specific cancer histotypes.

The correlation between drugs active against L1210 or P388 leukemia and solid experimental tumor models has not been good. For example, only 1.7% of 1493 agents that were active against P388 leukemia were also active against murine LLC. Further, only 2% of 1507 agents active against P388 leukemia were also active against murine colon 38 adenocarcinoma. Finally, only 2% of 1133 agents that were active against P388 leukemia were also active against human CX-1 (HT29) colon tumor. However, when comparing leukemias, a correlation less than expected was obtained—only 15% of 1564 active agents against P388 leukemia were also active against L1210 leukemia *(15)*.

One common observation is that some drugs that are active against experimental solid tumors are inactive against P388 leukemia. For example, 15% of 84 agents that were inactive against P388 leukemia were active against at least one of eight solid tumors tested *(15)*. Flavone acetic acid has been cited as an example *(14)*. This compound was inactive in the initial P388 screen, although it was later shown to exhibit

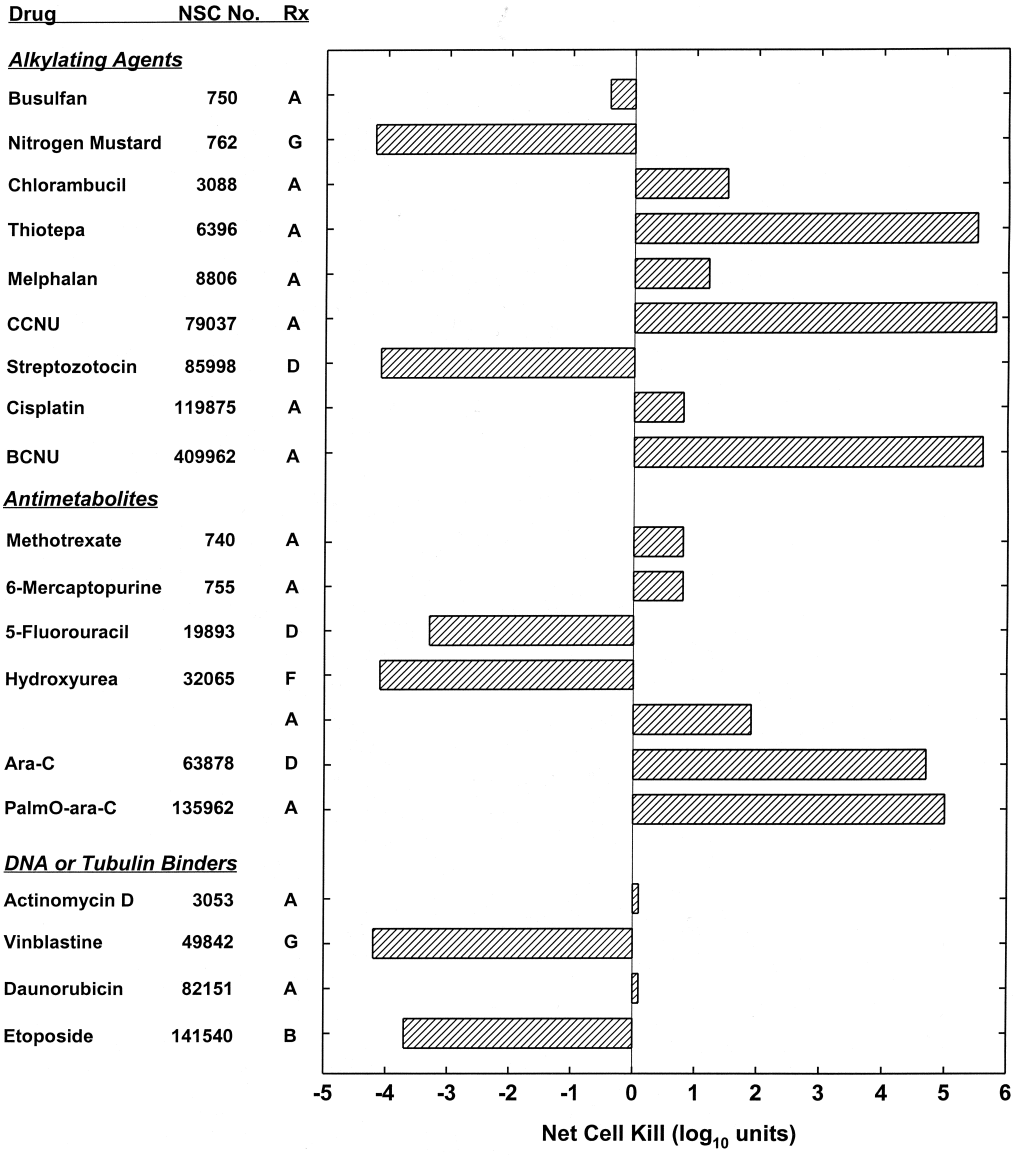

Fig. 5. Sensitivity of ic-implanted L1210 leukemia to clinically useful agents. L1210 leukemia (10^4 cells except for CCNU, which used 10^5 cells) was ic-implanted on d 0. Beginning on d 1 (d 2 for busulfan, chlorambucil, thiotepa, melphalan, hydroxyurea (single injection), cisplatin, BCNU, and daunorubicin), the agents were ip-administered using the indicated schedules. Treatment schedule (R_x): see legend for Fig. 1.

activity against the leukemia when the appropriate treatment schedule was used *(16)*. This example reveals a problem with large-scale screening programs—it is not logistically feasible to conduct preliminary schedule-dependency trials.

Another observation is that there are experimental solid tumors (e.g., murine pancreatic 02 ductal adenocarcinoma) that are not responsive in vivo to any clinically used agents, including many P388-active agents *(14)*. It may be noted, however, that this

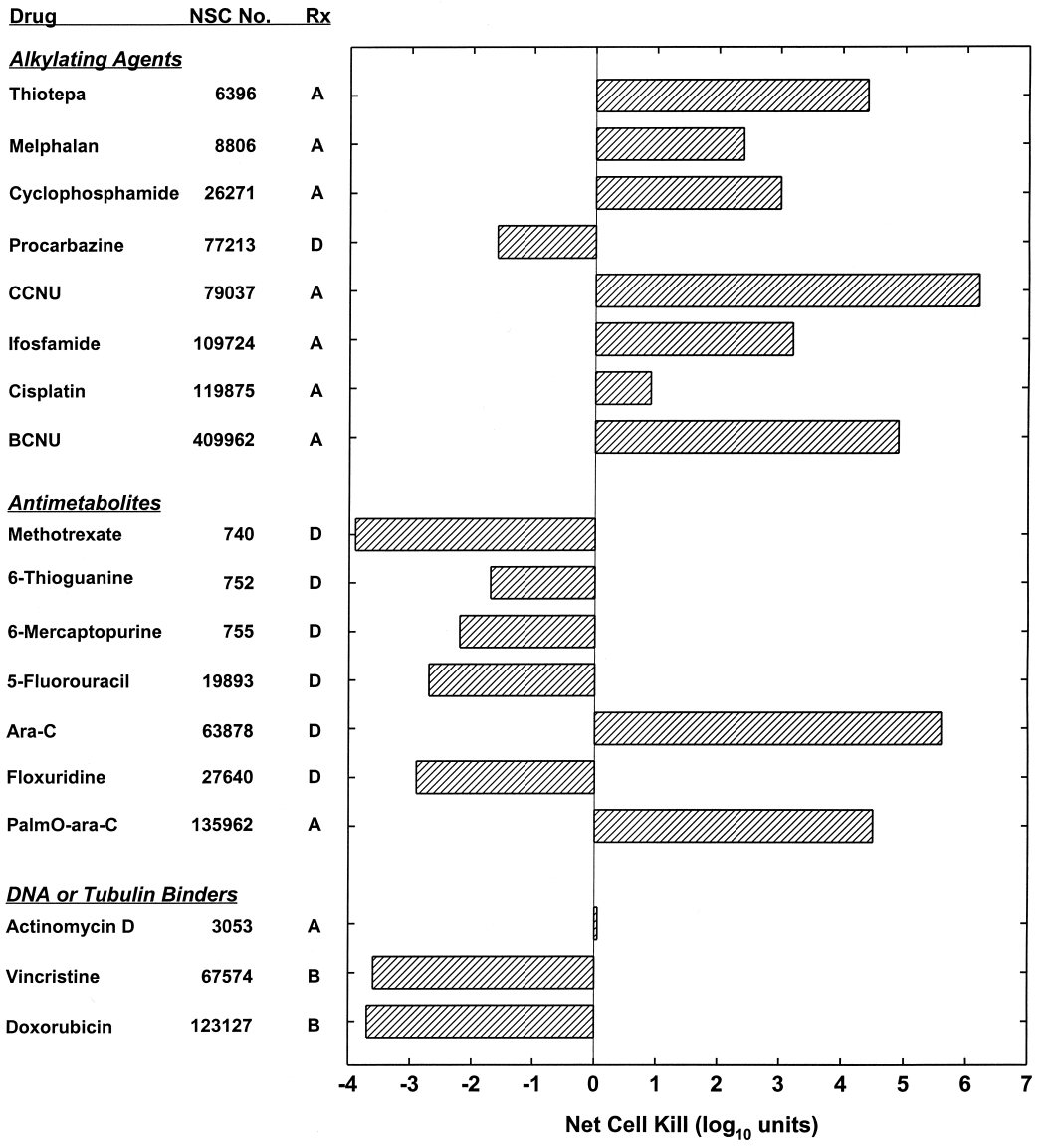

Fig. 6. Sensitivity of ic-implanted P388 leukemia to clinically useful agents. P388 leukemia (10^4 cells except for ifosfamide, methotrexate, 6-thioguanine, 6-mercaptopurine, 5-FU, and floxuridine, which used 10^3 cells and CCNU and ara-C, which used 10^5 cells) was ic-implanted on d 0. Beginning on d 1 (d 2 for ifosfamide), the agents were ip-administered using the indicated schedules. Treatment schedule (R_x): see legend for Fig. 1.

tumor is sensitive to numerous clinical agents in vitro after a 24-h exposure *(17),* suggesting that the in vivo insensitivity of this tumor may not be caused by cellular characteristics, but may be a result of physiological or architectural constraints of the animal.

Southern Research has evaluated a spectrum of compounds in the ip-implanted P388 model in order to evaluate this model as a predictor for the response of human tumor xenografts to new candidate antitumor agents (unpublished data). The P388 data col-

lected were compared to the data for various sc-implanted human tumor xenografts, which were selected on the basis of the results of the NCI in vitro screen. In general, compounds that were active against P388 leukemia were active to a lesser degree in one or more of the xenografts in the in vivo tumor panel. However, there were isolated examples of a P388-active agent being inactive in the human tumor xenograft models tested, and vice versa. There was no indication that the P388 model could predict compound efficacy for specific tumor xenografts.

Whether or not the murine leukemias are poor predictors of activity in solid tumors is still somewhat questionable, and will only be determined with the availability of drugs without antileukemic model activity but with proven value in the treatment of human solid tumors.

6. DRUG-RESISTANT LEUKEMIAS

Panels of in vivo drug-resistant murine L1210 and P388 leukemia models have been developed at Southern Research for use in the evaluation of crossresistance and collateral sensitivity. These models have been used for the evaluation of new drugs of potential clinical interest. An extensive summary of in vivo drug resistance and crossresistance data has been published by Schabel and colleagues *(18).* Their initial manuscript included results of in vivo crossresistance studies on 79 antitumor drugs in seven drug-resistant L1210 leukemias and 74 antitumor drugs in 12 drug-resistant P388 leukemias. Previously, we expanded this crossresistance data base for the drug-resistant P388 leukemias to include two new drug-resistant lines and more clinically useful drugs. Also, we updated the database to include new candidate antitumor agents entering clinical trials *(19).* Recently, another drug-resistant P388 leukemia (P388/VP-16) was added to this database *(20).* This section examines the crossresistance database for 16 drug-resistant P388 leukemias and many of the clinically useful agents.

6.1. Resistance to Alkylating Agents

The crossresistance profile of cyclophosphamide-resistant P388 leukemia (P388/CPA) to 14 different clinical agents is shown in Table 2. The P388/CPA line was crossresistant[1] to one of the five alkylating agents, no antimetabolites, no DNA-binding agents, and no tubulin-binding agents. Crossresistance of P388/CPA has also been observed for two other alkylating agents (chlorambucil and ifosfamide) *(20).* Interestingly, there are differences among these three agents. Chlorambucil and ifosfamide, like cyclophosphamide, each have two chloroethylating moieties, whereas mitomycin C is from a different chemical class. Whereas ifosfamide, cyclophosphamide, and mitomycin C require metabolic activation, chlorambucil does not. Although P388/CPA is crossresistant to two chloroethylating agents, the line is not crossresistant to other chloroethylating agents (melphalan and BCNU). Therefore, P388/CPA appears to be crossresistant only to a select group of alkylating agents with differing characteristics. P388/CPA appeared to be collaterally sensitive to fludarabine.

[1] Crossresistance is defined as decreased sensitivity (by >2-$\log_{10}$ units of cell-kill) of a drug-resistant P388 leukemia to a drug compared to that observed concurrently in P388/0 leukemia. Similarly, marginal crossresistance is defined as a decrease in sensitivity of approx 2-$\log_{10}$ units. Collateral sensitivity is defined as increased sensitivity (by >2-$\log_{10}$ units of cell kill) of a drug-resistant P388 leukemia to a drug over that observed concurrently in P388/0 leukemia.

Table 2
Crossresistance of P388 Sublines Resistant to Various Alkylating Agents and Antimetabolites to Clinically Useful Agents

Drug	*NSC No.*	R_x[a]	*CPA*	*L-PAM*	*DPt*	*BCNU*	*MMC*[b]	*MTX*	*5-FU*	*ARA-C*
Alkylating Agents										
Melphalan	8806	A	–	+	–	–				±
Cyclophosphamide	26271	A	+	–	–	–	–			+
Mitomycin C	26980	A	±	+	–	–	+	–		+
Procarbazine	77213	D	–							
Cisplatin	119875	B	–	+	+	–	±[c]	–		+
BCNU	409962	A	–	–	–	+	–			–
Antimetabolites										
Methotrexate	740	D			–		–[d]	+	+	+
6-Thioguanine	752	A					–[d]	–		
6-Mercaptopurine	755	D						–		
5-Fluorouracil	19893	D	–		–		–[d]		+	=
PalmO-ara-C	135962	A	–	–	–		–[d]	–	–	+
Trimetrexate	249008	D		±	–			–		–
Fludarabine	312887	D	=	=	=			–	=	+
Gemcitabine	613327	E	–	–	–			–		+
DNA Binders										
Actinomycin D	3053	A	–	±	–		±			
Doxorubicin	123127	A	–	–	–		+	–		–
Etoposide	141540	B	–	–	–		+[c]	–		–
Amsacrine	249992	B	–	+	=		–[c]	–		–
Mitoxantrone	301739	B		+	=			–		–
Tubulin Binders										
Vinblastine	49842	A					+			
Vincristine	67574	B	–	+	–		+[c]	–		+
Paclitaxel	125973	C		–	–			–		–

$CD2F_1$ mice were ip-implanted with 10^6 P388/0 or drug-resistant P388 cells on d 0. Data presented are for ip drug treatment at an optimal ($\leq LD_{10}$) dosage. Symbols: resistance/crossresistance, +; marginal crossresistance, ±; no crossresistance, –; and collateral sensitivity, =.

[a] Treatment schedule (R_x): A = d 1; B = d 1, 5, 9; C = d 1–5; D = d 1–9; E = d 1, 4, 7, 10.

[b] Data from *In Vivo* 1987; 1:47–52.

[c] Treatment schedule was d 1.

[d] Treatment schedule was d 1 and 5.

The effect of 15 different clinical agents on melphalan-resistant P388 leukemia (P388/L-PAM) is shown in Table 2. The P388/L-PAM line was crossresistant to approximately one-half of the agents—2 of 4 alkylating agents, 1 of 4 antimetabolites, 3 of 5 DNA-binding agents, and 1 of 2 tubulin-binding agents. The alkylating agents involved in crossresistance represent different chemical classes. Similarly, the DNA-interacting agents involved in crossresistance include agents with different mechanisms of action—inhibitors of DNA topoisomerase II (amsacrine and mitoxantrone) and a DNA-binding agent (actinomycin D). However, the melphalan-resistant line did not exhibit crossresistance to other inhibitors of DNA topoisomerase II (e.g., doxorubicin and etoposide) or another DNA-binding agent (e.g., doxorubicin).

The sensitivity of cisplatin-resistant P388 leukemia (P388/DDPt) to 17 different clinical agents is shown in Table 2. The P388/DDPt line was not crossresistant to any of these agents. Interestingly, the cisplatin-resistant line was collaterally sensitive to three agents (fludarabine, amsacrine, and mitoxantrone). Of these three agents, the latter two have been reported to interact with DNA topoisomerase II *(21,22).*

The crossresistance data for N,N′-*bis*(2-chloroethy1)-N-nitrosourea-resistant P388 leukemia (P388/BCNU) have been limited to the evaluation of alkylating agents. The crossresistance profile of P388/BCNU to four different clinical agents is shown in Table 2. The BCNU-resistant line was not crossresistant to melphalan, cyclophosphamide, mitomycin C, or cisplatin.

The crossresistance profile of mitomycin C-resistant P388 leukemia (P388/MMC) to 13 different clinical agents is shown in Table 2 *(23).* The P388/MMC line was crossresistant to approximately one-half of the agents—1 of 3 alkylating agents, 0 of 4 antimetabolites, 3 of 4 DNA-binding agents, and two of two tubulin-binding agents. The pattern was similar to that observed for P388/L-PAM.

6.2. Resistance to Antimetabolites

The effect of 14 different clinical agents on methotrexate-resistant P388 leukemia (P388/MTX) is shown in Table 2. The P388/MTX line was not crossresistant to any of these agents.

The crossresistance data for 5-fluorouracil-resistant P388 leukemia (P388/5-FU) have been limited to antimetabolites. The sensitivity of the P388/5-FU to three different agents is shown in Table 2. The P388/5-FU line was not crossresistant to palmO-ara-C (a slow-releasing form of ara-C) or fludarabine (possible collateral sensitivity). Crossresistance was observed for methotrexate.

The crossresistance profile of 1-β-D-arabinofuranosylcytosine-resistant P388 leukemia (P388/ARA-C) to 16 different clinical agents is shown in Table 2. The P388/ARA-C line was crossresistant to members of several functionally different classes of antitumor agents—four of five alkylating agents, three of five antimetabolites, none of four DNA-binding agents, and one of two tubulin-binding agents. Interestingly, the line was collaterally sensitive to 5-FU.

6.3. Resistance to DNA- and Tubulin-Binding Agents

The effect of 17 different clinical agents on actinomycin D-resistant P388 leukemia (P388/ACT-D) is shown in Table 3. P388/ACT-D was not crossresistant to any alkylating agents or antimetabolites. However, it was crossresistant to all of the drugs tested that are involved in multidrug resistance, except for amsacrine.

Table 3
Crossresistance of P388 Sublines Resistant to Various DNA and Tubulin Binders to Clinically Useful Agents

Drug	*NSC No.*	*R_x*[a]	*ACT-D*	*ADR*	*AMSA*	*DIOHA*	*VP-16*	*CPT*[b]	*VCR*	*PTX*
Alkylating Agents										
Melphalan	8806	A	–	–	–		–		–	
Cyclophosphamide	26271	A	–	–	–	–	–		–	
Mitomycin C	26980	A		±	–			–[e]	+	
Procarbazine	77213	D	–	–					–	
Cisplatin	119875	C	–	–	–[c]		–[c]	–[e]	±	
BCNU	409962	A	–	–			–		–	
Antimetabolites										
Methotrexate	740	D	–	–	±		–		–	
6-Thioguanine	752	D	–	–					–	
6-Mercaptopurine	755	D	–	–					–	
5-Fluorouracil	19893	D	–	–[d]	–[d]		–		–	
PalmO-ara-C	135962	A	–	–	–				–	
Trimetrexate	249008	D		–					–	
Fludarabine	312887	D		=					–	
Gemcitabine	613327	E		–			–		–	
DNA Binders										
Actinomycin D	3053	A	+	+	+	–	+	–[e]	–	
Doxorubicin	123127	A	±	+	+	–	+	–[e]	–	+
Etoposide	141540	B	+	+	+	–	+		–	+
Amsacrine	249992	B	–	+	+	+	+	–[e]	–	
Mitoxantrone	301739	B	+	+	+	+	+	–[e]	–	
Tubulin Binders										
Vinblastine	49842	B	+	+	+		+		+	
Vincristine	67574	B	+	+	+	+	+		+	+
Paclitaxel	125973	C	±	±	+	–		–[e]	–	+

$CD2F_1$ mice were ip-implanted with 10^6 P388/0 or drug-resistant P388 cells on d 0. Data presented are for ip drug treatment at an optimal ($\leq LD_{10}$) dosage. Symbols: resistance/crossresistance, +; marginal crossresistance, ±; no crossresistance, –; and collateral sensitivity, =.

[a] Treatment schedule (R_x): A = d 1; B = d 1, 5, 9; C = d 1–5; D = d 1–9; E = d 1, 4, 7, 10.

[b] Data from *Mol Pharmacol* 1990; 38:471–480.

[c] Treatment schedule was d 1, 5, 9.

[d] Treatment schedule was d 1–5.

[e] Treatment schedule was d 1 and 5.

The crossresistance profile of doxorubicin-resistant P388 leukemia (P388/ADR) to 21 different clinical agents is shown in Table 3. The P388/ADR line was not crossresistant to any of the antimetabolites, and was marginally crossresistant to only one alkylating agent (mitomycin C). Resistance was observed for all the drugs tested that are reported to be involved in multidrug resistance (actinomycin D, doxorubicin, etoposide, amsacrine, mitoxantrone, vinblastine, vincristine, and paclitaxel). P388/ADR was collaterally sensitive to fludarabine.

The sensitivity of amsacrine-resistant P388 leukemia (P388/AMSA) to 14 different clinical agents is shown in Table 3. P388/AMSA was not crossresistant to any of the alkylating agents, and was marginally crossresistant to only one antimetabolite. Crossresistance was observed for all the drugs tested that are involved in multidrug resistance.

The crossresistance data for mitoxantrone-resistant P388 leukemia (P388/DIOHA) have been limited mainly to agents involved in multidrug resistance. The sensitivity of P388/DIOHA to seven different clinical agents is shown in Table 3. The P388/DIOHA line exhibited mixed multidrug resistance—crossresistance to amsacrine and vincristine, but no crossresistance to actinomycin D, doxorubicin, etoposide, or paclitaxel.

The crossresistance profile of etoposide-resistant P388 leukemia (P388/VP-16) to 13 different clinical agents is shown in Table 3. The P388/VP-16 line was not crossresistant to any of the alkylating agents or antimetabolites. However, it was crossresistant to all of the drugs tested that are reported to be involved in multidrug resistance.

The sensitivity of camptothecin-resistant P388 leukemia (P388/CPT) to seven different clinical agents is shown in Table 3 *(24)*. P388/CPT was not crossresistant to any of these agents.

The effect of 21 different clinical agents on vincristine-resistant P388 leukemia (P388/VCR) is shown in Table 3. The P388/VCR line was crossresistant to three of the agents—mitomycin C, cisplatin (marginal), and vinblastine. Unexpectedly, P388/VCR was not crossresistant to many of the drugs tested that are involved in multidrug resistance (e.g., actinomycin D, doxorubicin, etoposide, amsacrine, mitoxantrone, and paclitaxel).

The crossresistance data for paclitaxel-resistant P388 leukemia (P388/PTX) have been limited to agents involved in multidrug resistance. The sensitivity of P388/PTX to three different clinical agents is shown in Table 3. The P388/PTX line was crossresistant to drugs that are involved in multidrug resistance (doxorubicin, etoposide, and vincristine).

CONCLUSION

Currently, biotechnology appears to be advancing in an almost exponential fashion. Today, advanced techniques and tools allow us to conduct research that could not even be imagined 40 yr ago, when the L1210 and P388 leukemia models were first used extensively (e.g., sequencing the human genome). Utilizing molecular biology techniques, the emphasis is now on the development of compounds designed for a specific target. Current NCI strategy suggests that models for evaluating these compounds contain the specific target, either naturally or by gene transfection. Successful treatment of such models will theoretically provide the necessary proof-of-concept required for continued development. This is a radical departure from the empirical approach to mass screening of compounds against murine leukemias.

The L1210 and P388 leukemia models do have some advantages—they are rapid, reproducible, and relatively inexpensive (in comparison to human tumor xenograft models). However, as with any experimental animal tumor model, there are limitations. Neither leukemia is a satisfactory drug discovery model for either human cancer in general or human leukemia in particular. Of course, this could be said of any animal tumor model. Of the two leukemias, P388 is the more sensitive, but overpredicts drug activity for both preclinical human tumor xenograft models and the clinic. However, the question of whether P388 leukemia (or L1210) is a poor predictor for solid tumor-active drugs has not yet been sufficiently answered.

Although the murine leukemia models have serious limitations, these models have been very useful in anticancer drug development, in the development of a number of therapeutic principles, and in understanding the biologic behavior of tumor and host. These models are still useful today in conducting detailed evaluations of new candidate anticancer drugs (e.g., schedule dependency, route-of-administration dependency, formulation comparison, analog comparison, and combination chemotherapy).

Perhaps the greatest utility of the murine leukemias today is derived from the evaluations of the drug-resistant sublines for crossresistance and collateral sensitivity. Analysis of the crossresistance data generated at Southern Research for clinical agents has revealed possible noncrossresistant drug combinations. The P388 leukemia lines selected for resistance to alkylating agents (e.g., P388/CPA, P388/L-PAM, P388/DDPt, P388/BCNU, and P388/MMC) differed in crossresistance profiles, both with respect to alkylating agents and other functional classes. Similarly, P388 leukemia lines selected for resistance to antimetabolites (e.g., P388/MTX, P388/5-FU, and P388/ARA-C) differed in crossresistance profiles, both with respect to antimetabolites and other functional classes. Clearly, the spectrum of crossresistance of an alkylating agent or an antimetabolite will depend on the individual agent. P388 leukemia lines selected for resistance to large polycyclic anticancer drugs (e.g., P388/ACT-D, P388/ADR, P388/AMSA, P388/DIOHA, P388/VP-16, P388/CPT, P388/VCR, and P388/PTX) were not generally crossresistant to alkylating agents or antimetabolites. However, the crossresistance profiles to DNA- and tubulin-binding agents were variable.

Five of the 16 drug-resistant leukemias exhibited collateral sensitivity to one or more drugs. These observations of collateral sensitivity suggest that a combination of one of the five drugs plus one of the corresponding agents for which collateral sensitivity was observed may exhibit therapeutic synergism.

Crossresistance data, coupled with knowledge of the mechanisms of resistance operative in the drug-resistant leukemias, may yield insights into the mechanisms of action of the agents being tested. Similarly, crossresistance data, coupled with the mechanisms of action of various agents, may yield insights into the mechanisms of resistance operative in the drug-resistant leukemias *(19).* Furthermore, crossresistance data may identify potentially useful guides for patient selection for clinical trials of new antitumor drugs *(19).*

In conclusion, the role of L1210 and P388 leukemias in the evaluation of anticancer agents has diminished considerably. Nevertheless, the majority of clinical agents now in use was first detected by the murine leukemias. These models are clearly still appropriate for answering certain questions, and the drug-resistant sublines can provide valuable information concerning crossresistance and collateral sensitivity.

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