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Perspectives in Cancer Research

Current Results of the Screening Program at the Division of Cancer Treatment, National Cancer Institute*

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Abstract—*The prospective screening program at the Division of Cancer Treatment, National Cancer Institute, has now been in operation for several years and is making steady progress in the identification of new synthetic compounds and natural products of potential interest for the clinic. Data are presented on four categories of drugs that have been tested in the new screening panel: (a) clinically established antitumor agents; (b) new drugs and drugs for which there is renewed clinical interest based on activity in the new screen and previously inadequate clinical trial; (c) drugs in the initial phases of clinical trial; (d) compounds in development. An analysis of the data is presented, taking into account a series of important questions that are being addressed prospectively to the new screen. Although the ability to provide definitive answers must await feedback from clinical testing of compounds recommended by the screen, some generalizations appear to be emerging, and these are discussed. A comparison is made of the activity of drugs in the treatment of human tumors growing in two sites, subcutaneously and under the renal capsule. The subrenal capsule model appears to be somewhat more sensitive to drugs than the subcutaneous model and may provide certain advantages for initial panel testing. Attention is drawn to the potential usefulness in a screening program of the newly developed clonogenic techniques for growing human tumors. The screening program at the Division of Cancer Treatment is viewed as a dynamic entity, subject to modification in accordance with acquired experience.*

INTRODUCTION

FOR CERTAIN types of cancer, chemotherapy has been capable of rendering patients free of disease, with achievement of a normal life span (Table 1) [1, 2]. However, this responsive category does not include the most frequently encountered forms of malignant tumor and although with the availability of new drugs and the use of combinations of drugs and combined modalites significant responses are being obtained for the common solid tumors [1, 2], there remains a great need for new and more effective antitumor agents. It was this need which in 1975 prompted a reexamination of the screening systems at the Division of Cancer Treatment, National Cancer Institute, and led to the institution of

Table 1. *Cancers in which drugs have been responsible for a fraction of patients achieving a normal life span*

Acute lymphocytic leukemia—pediatric
Acute myelogenous leukemia—adult
Hodgkin's disease
Diffuse histiocytic lymphoma
Nodular mixed lymphomas
Burkitt's lymphoma
Ewing's sarcoma
Rhabdomyosarcoma
Wilms' tumor
Choriocarcinoma
Testicular cancer
Ovarian cancer

See [2].

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a new prospective screening program [3]. A serious possible lesion in the extant screening program appeared to be the preferential selection of drugs active against rapidly growing tumors. Attention was therefore focused on the utilization of slow growing tumors for drug selection and evaluation. The availability of athymic (nude) mice capable of supporting the growth of slow growing human tumors facilitated the institution of a balanced screening program incorporating both murine and corresponding human tumors.

The new screening program has been making steady progress since 1975 in the testing of synthetic compounds and natural products and in the identification of new drugs of potential interest for further development, characterization and clinical evaluation. It is the purpose of this report to summarize the results of the program, to assess the status of its acomplishment and to indicate new directions under consideration, as part of an evolving dynamic approach to the screening for new and more effective antitumor agents for the clinic. A number of important questions, such as those listed below, have been addressed to the new screen.

(1) Does the new screen increase the yield of true positive compounds (active in the screen and active in the clinic)?

(2) Does extensive and/or broad spectrum activity in the screening panel result in increased probability of clinical antitumor effectiveness?

(3) Do human tumor xenografts and animal tumor screens select the same or different drugs as active?

(4) Are the xenograft positives more active in the clinic than those selected by animal screens?

(5) Does the screen reduce the number of false positives (active in the screen but inactive in the clinic)?

(6) Does it reduce the number of false negatives (inactive in the screen, but active in the clinic)?

(7) Is there a correspondence of activity against animal tumors and/or human tumor xenografts with activity against clinical tumors for specific histologic types or specific organ systems?

(8) Are compounds that bypass the P388 prescreen because of activity in other screening programs or in selected biochemical or biological assays more effective in the screening panel and in the clinic than compounds initially selected for further testing by the prescreen?

(9) What contribution will the utilization of the new screening panel make to prediction of clinical effectiveness of new drugs with respect to structure–activity analysis, analogs of known antitumor agents, and mathematical approaches to activity prediction?

METHODOLOGY

A schema of the new prospective screen is shown in Fig. 1 [2–5]. Prior to initiation of the new prospective screen, the testing level in the Division of Cancer Treatment program had been approximately forty thousand new materials per year, but because of the more extensive effort of testing involved in the new screen the number was reduced to fifteen thousand materials per year. The compounds to be subjected to screening are no longer selected entirely at random but rather on the basis of review of the world's literature and through voluntary submissions of compounds of potential interest. These compounds are tested in a prescreen *in vivo* against leukemia P388. All of the compounds demonstrating activity against leukemia P388 are then tested in a panel of tumor screens including mouse colon, human colon xenograft, mouse breast, human breast xenograft, mouse lung, human lung xenograft, B16 melanoma in the mouse and leukemia L1210 in the mouse. Compounds of interest because of reported activity in other antitumor screening programs and compounds selected on the basis of biochemical or biological assays may bypass the P388 prescreen and go directly to testing in the screening panel. Although they are incidentally also tested in the P388 system, activity in that system is not requisite for testing in the panel. Natural product isolates are tested *in vivo* against leukemia P388 and also *in vitro* in the KB tissue culture system, and those which demonstrate activity are then tested in the entire screening panel. Approximetaly 500 or more compounds per year are becoming eligible for testing against the Division of Cancer Treatment screening panel.

The tumor systems currently being employed are shown in Table 2. They include leukemia P388, L1210 leukemia, B16 melanoma, Lewis lung tumor, colon 26 (employed for special comparisons), colon 38 and $CD8F_1$ mammary tumor in mice, and the human tumor xenografts mammary MX-1, lung LX-1 and colon CX-1. Included also are

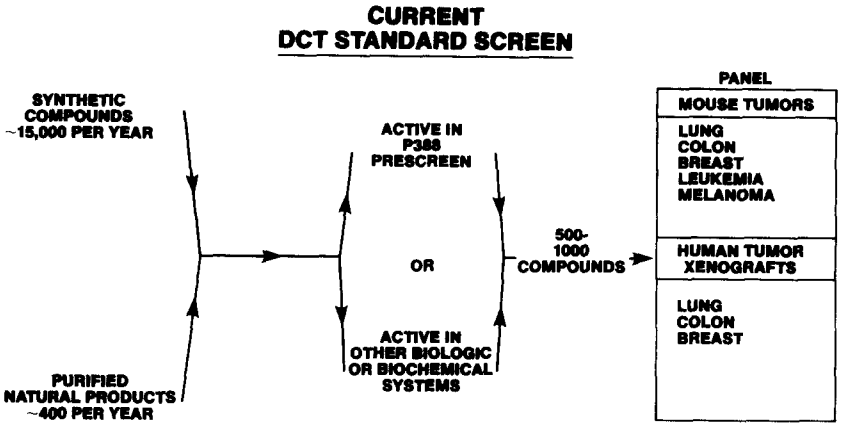

Fig. 1. Flow of drugs through the Division of Cancer Treatment screens.

Table 2. Tumor panel systems

	L1210	P388	B16 Melanoma	Lewis lung	Colon 26	Colon 38	$CD8F_1$ Mammary	Mammary xenograft MX-1	Lung xenograft LX-1	Colon xenograft CX-1
Host	CDF_1 or BDF_1	CDF_1 or BDF_1	BDF_1 or B6C3	BDF_1	CDF_1	BDF_1	$CD8F_1$	Nu/Nu Swiss	Nu/Nu Swiss	Nu/Nu Swiss
Inoculum	10^5 Ascites	10^6 Ascites	1:10 Homogenate	1×10^5 Viable cells	1% Brei	Fragment	5×10^5 cells	Fragment	Fragment	Fragment
Site	IP	IP	IP	IV	IP	SC	SC	SC; SRC	SC; SRC	SC; SRC
Parameter	Mean survival time	Median Survival time	→	→	→	Tumor weight inhibition	→	→	→	→
Activity criteria	T/C ≧125%	T/C ≧120%	T/C ≧125%	T/C ≧140%	T/C ≧130%	T/C ≦42%	T/C ≦42%	T/C ≦42% ≦20%	T/C ≦42%; ≦20%	T/C ≦42%; ≦20%

the site of inoculation, the parameter of effect and criteria of activity.

The protocols for screening against leukemias L1210 and P388, B16 melanoma and Lewis lung carcinoma have been described previously [6]. The origins and the experimental methods employed in the screening against the carcinogen-induced transplantable tumors colon 26 and 38 were reported by Corbett *et al.* [7] and the spontaneous mammary carcinoma in $CD8F_1$ mice was described by Martin *et al.* [8]. In the screening with the $CD8F_1$ mammary carcinoma, the first generation transplant is employed. The human tumor xenografts CX-1, MX-1 and LX-1 are carried in serial transplantation in athymic mice. The CX-1 tumor model was initiated by A. Bogden at the Mason Research Institute. The MX-1 and LX-1 xenografts were developed by B. Giovanella at the Stehlin Foundation for Cancer Research. The biological characteristics of the tumors that are included in the Division of Cancer Treatment tumor panel are shown in Table 3 [3].

With the human tumor xenografts, the primary parameter of response is extent of inhibition of tumor growth as compared with controls, with treatment initiated when the tumors are well established and palpable at the site of implantation. Because of the relatively slow growth of the human tumor xenografts at the subcutaneous site of inoculation each test requires approximately 60–90 days for accomplishment. This demand in time of observation necessitated a reduction of the number of models for chemotherapy trials for established tumors.

In order to minimize the time required for testing, and to permit a broadening of the base of drug evaluation and more detailed study of the matching of therapy to individual patients, further investigations are ongoing in the program, employing human tumors growing in various sites in the athymic animal. Attention is focused on optimization of tumor

Table 3. Biological characterization of tumors included in the DCT tumor panel

Tumor and code	Host of origin	Origin of tumor	Histological description	Miscellaneous
Human				
Colon CX-1	Isolated in tissue culture, subsequently maintained in nude mice	Human colon	Adenocarcinoma of the colon	—
Breast-MX-1	Isolated in nude mice	Human breast	Infiltrating duct cell carcinoma	—
Lung-LX-1	Isolated in nude mice	Human lung	Oat cell carcinoma	—
Mouse				
Colon-C 26	BALB/c mouse	Induced by chemical carcinogen *N*-methyl-*N*-nitrosourethane	Undifferentiated colon mucosal carcinoma	Very high rate of metastases
Colon-C 38	$C_{57}BL/6$ mouse	Induced by chemical carcinogen, 1,2-dimethylhydrazine	Colon adenocarcinoma	Very low rate of metastases
Melanoma B16	$C_{57}BL/6$ mouse	Spontaneous at base of ear	Melanoma	—
Lung (Lewis lung)	$C_{57}BL/6$ mouse	Spontaneous in the lung	Anaplastic carcinoma	Metastases
Breast	$CD8F_1$ mouse	Spontaneous	Mammary adenocarcinoma	—
Leukemia L1210	DBA/2 mouse	Chemically induced with methylcholanthrene	Lymphocytic leukemia	—
Leukemia P388	DBA/2 mouse	Chemically induced with methylcholanthrene	Lymphocytic leukemia	—

take, rate of growth, precision of measurement, extent of metastasis, uniformity of survival time and other parameters that may lend themselves to precise quantitation of the inhibitory effect of antitumor agents. One of these systems, the subrenal capsule model, is under intensive investigation. The technique employed and preliminary data for the subrenal capsule system have been reported by Bogden *et al.* [9]. The technique [9] involves insertion of small fragments (approximately $1.0\,mm^3$) of human tumor xenografts under the renal capsule, where there is a rich vascular bed, ensuring adequate nutrient for tumor growth and ready drug delivery. Employing a stereoscopic microscope in which a micrometer disc is inserted into one eyepiece, it is possible to measure, *in situ*, the size of the initial graft and the ultimate size achieved at the termination of the experiment. An assay time frame of eleven days was selected since it was long enough to permit measurement of extent of growth and of drug-induced inhibition of the human tumor xenografts.

The screening data for the xenograft models in which the tumors are inoculated subcutaneously were obtained from D. Houchens and T. Ovejera at the Battelle Columbus Laboratories. The screening data for the xenografts inoculated under the renal capsule were obtained from A. Bogden at the Mason Research Institute.

In the present analysis the criteria for drug activity against human tumor xenografts implanted subcutaneously and under the renal capsule are those in current use by the Division of Cancer Treatment. These are 58% inhibition from controls ($T/C\% \leqq 42$) for the subcutaneous model and 80% inhibition ($T/C\% \leqq 20$) for the subrenal capsule model. The investigators who have used these models most extensively—Ovejera *et al.* [10] in the case of the subcutaneous model and Bogden *et al.* [9] in the case of the subrenal capsule model—have employed various cutoff points to distinguish 'activity' from 'inactivity'. Also, the activities listed herein (Tables 4, 5–8, 10 and 11 and Figs. 2–4) as reported by the investigators were, derived using different methods of computation. For the subcutaneously implanted tumor model, Ovejera *et al.* [10] estimated tumor weight (W) in mg from caliper measurements according to the formula $W = (a^2 \times b)/2$, where a is the width and b is the length in mm. In addition, in an effort to standardize variability in tumor size among test groups at the initiation of treatment, these authors calculated relative weights (RW) using the formula $RW = Wi/Wo$, where Wo is the mean tumor weight of a group at the beginning of treatment and Wi is the mean tumor weight at any subsequent time. A significant response to treatment is indicated when a test group shows an $RW \leqq 42\%$ of that of the control at any time during a specified range of days after the last treatment.

In contrast, Bogden *et al.* [9] using the subrenal capsule model measured tumor length (b) and width (a) in ocular micrometer units (OMU), the micrometer disc of the

microscope eye-piece having been calibrated so that 10 OMU = 1.0 mm. Treatment activity was based on the change in average tumor diameter over the prescribed course of treatment compared with the change in average control diameter. Thus, $T/C\% = DT/DC \times 100$, where DT is the mean tumor diameter $(a+b)/2$ of the treated group at the end of treatment less the mean tumor diameter at the beginning of treatment, and where DC is the change in mean tumor diameter of controls over the same period.

RESULTS AND DISCUSSION

The data in the screening panel for a series of the more established antitumor agents are summarized in Table 4. In a previous retrospective analysis it had been suggested that compounds that are active in a number of screening systems in rodents could have more likelihood of demonstrating activity against hematologic malignancies and solid tumors in the clinic [11, 12]. Also, the more extensive the response in the tumor systems, the greater the possibility appeared to be that the compounds would exert clinical antitumor activity. That such high and broad spectrum activity in the screening panel could be indicative of greater probability of antitumor effectiveness in the clinic is reflected also in the data of the new screening panel where for the more established clinically active antitumor agents high and broad spectrum activities were obtained (Table 4). Taking into account the total number of animal tumors plus human tumor xenografts, including the subcutaneous and subrenal capsule sites, all but one of the drugs (L-asparaginase) were active in greater than 45% of the tumor systems, ranging from 46% of the tumor systems for methotrexate, 6-mercaptopurine, adriamycin and bleomycin to over 80% for nitrogen mustard, melphalan, cyclophosphamide, mitomycin C, CCNU and cisplatinum II (Table 4). Overall, the animal tumor systems rated a higher percentage of drugs as active than did the human tumor xenografts in either the subcutaneous or subrenal capsule sites (Table 4). The reduced sensitivity of the human tumor xenografts could provide an important advantage in drug selection if it is also accompanied by the identification of new types of antitumor drugs.

*Table 4. Activity in the new screening panel for clinically active antitumor agents**

NSC number	Drug	L1210	P388	B16 Melanoma	Lewis lung	Colon 26	Colon 38	$CD8F_1$	Subcutaneous MX-1	Subcutaneous LX-1	Subcutaneous CX-1	Subrenal capsule MX-1	Subrenal capsule LX-1	Subrenal capsule CX-1	Active systems Number	Active systems Per cent
740	Methotrexate	272	296	120	148	106	76	20	34	41	66	93	37	54	6/13	46
752	6-Thioguanine	228	145	128	192	>200	10	6	71	76	81	70	NT	121	7/13	54
755	6-Mercaptopurine	263	150	134	121	246	10	21	75	77	99	90	61	103	6/13	46
762	Nitrogen mustard	304	251	235	125	262	30	11	NT†	NT	NT	NT	NT	NT	6/7	86
3053	Actinomycin D	173	618	191	124	154	15	0	70	46	73	5	NT	10	9/12	75
3088	Chlorambucil	149	171	140	125	190	59	16	24	46	60	−55	16	NT	8/12	67
8806	Melphalan	237	281	257	154	>309	8	1	2	35	101	−25	47	−13	11/13	85
13875	Hexamethylmelamine	132	117	126	202	150	12	16	10	81	85	−17	72	12	9/13	69
19893	5-Fluorouracil	180	220	140	150	200	0	0	73	70	88	56	36	60	7/13	54
26271	Cyclophosphamide	236	>300	176	222	165	9	0	1	37	113	−37	41	0	11/13	85
26980	Mitomycin C	178	242	181	142	187	9	16	1	41	62	NT	NT	NT	9/10	90
45388	DTIC	160	130	145	267	126	13	20	55	40	92	37	NT	NT	7/11	64
49842	Vinblastine	154	252	280	111	188	0	3	NT	117	119	NT	NT	NT	6/9	67
63878	Cytosine arabinoside	285	255	159	143	164	34	15	71	102	73	NT	NT	NT	7/10	70
67574	Vincristine	147	300	189	116	130	23	7	8	69	89	NT	NT	NT	7/10	70
77213	Procarbazine	188	180	168	154	115	38	102	46	8	60	NT	−17	NT	7/11	64
79037	CCNU	243	278	287	253	>363	0	15	43	15	77	19	−11	NT	10/12	83
82151	Daunomycin	161	>266	>350	122	155	88	55	NT	NT	NT	NT	NT	NT	4/7	57
95441	Methyl CCNU	>310	>275	>279	>242	345	4	7	30	48	83	NT	NT	NT	8/10	80
109229	L-Asparaginase	117	154	104	NT	113	109	55	NT	NT	NT	NT	NT	NT	1/7	14
119875	*Cis*-Platinum	207	264	288	261	245	27	0	20	86	66	−17	6	NT	10/12	83
122819	VM 26	239	>350	>285	113	220	48	16	NT	NT	NT	NT	NT	NT	5/7	71
123127	Adriamycin	>300	>300	>300	>252	310	68	1	68	73	72	59	43	37	6/13	46
125066	Bleomycin	120	193	144	142	116	10	9	27	83	51	66	26	51	6/13	46
178248	Chlorozotocin	>439	>251	>356	164	>322	15	9	51	68	75	NT	NT	NT	7/10	70
409962	BCNU	>563	>298	267	>305	>340	36	6	43	85	68	17	−28	73	9/13	69
Number of drugs active		24/26	25/26	24/26	17/25	21/26	20/26	23/26	10/21	7/22	0/22	8/15	5/13	4/11		
Percentage		92	96	92	68	81	77	88	44	32	0	53	38	36		

*Underlining means drugs are active.
†NT = Not tested.

The P388 system was the most sensitive, indicating activity for 96% of the drugs. The L1210 and B16 melanoma systems were next in responsiveness, each indicating activity for 92% of the drugs, whereas the Lewis lung system rated 68% of the drugs as active.

Of the three types of xenografts employed the mammary tumor xenograft MX-1 was the most responsive, yielding 44% active with the subcutaneous tumor and 53% with the subrenal capsule tumor. The LX-1 lung xenograft was second in responsiveness, giving 32% active with the subcutaneous site and 38% active, with the subrenal capsule site. The CX-1 tumor was the least responsive, resulting in zero% selection as active with the subcutaneous site, but 36% as active with the subrenal capsule site. The latter result is suggestive of overprediction for this human colon tumor with the criterion of effectiveness employed.

In order to examine the question of whether there is any correspondence of activity in animal tumors and human tumors for specific types of tumor, the screening panel data are listed for the breast tumor, lung tumor and colon tumor models for drugs that have been reported to have activity against breast, lung and colon carcinoma in the clinic (Tables 5, 6, 7).

For the drugs reported as active against breast tumor in the clinic the $CD8F_1$ mammary tumor model in the mouse identified activity for 3/3 (Table 5). The MX-1 subcutaneous model identified 2/3 drugs, failing to identify adriamycin as active. The subrenal capsule model classified only 1/3 drugs as active, missing methotrexate and adriamycin. The active and marginally active drugs were identified in essentially the same proportion.

For drugs reported to be active or marginally active against small cell lung cancer, the Lewis lung (IV) tumor system identified all as active whereas the lung tumor xenograft LX-1 both subcutaneously and in the subrenal capsule indicated activity for a reduced number (Table 6). In the active drug category, LX-1 SC and LX-1 SRC systems failed to identify adriamycin as active. The LX-1 SRC system also rated cyclophosphamide and methotrexate as negative. For the drugs listed as marginally active, CCNU was identified as active by the LX-1 SC and LX-1 SRC systems, but hexamethylmelamine was rated as inactive.

No drugs are listed as definitely active against epidermoid lung cancer. Of the 5 drugs listed as marginally active, only nit-

Table 5. Activity in breast tumor models of the screening panel. Drugs with reported activity in the treatment of clinical breast cancer

	Breast tumor models		
	$CD8F_1$	MX-1 SC	MX-1 SRC
Active in clinic*			
Cyclophosphamide	0+‡	1+	−37+
Methotrexate	20+	34+	93−
Adriamycin	1+	68−	59−
Actives in tumor models	3/3	2/3	1/3
Marginal activity in clinic†			
5-Fluorouracil	0+	73−	56−
Melphalan	1+	2+	−35+
Mitomycin C	1+	41+	NT
Actives in tumor models	3/3	2/3	1/2

*Active in clinic: Drug consistently produces partial regressions and at least occasionally produces complete regressions. It may improve survival in some patients.

†Marginal activity in clinic: Drug has been observed to produce only partial regressions of tumor and has no definite effect on patient survival.

‡Activity followed by rating of + (active) or − (inactive).

Table 6. Activity in lung tumor models of the screening panel. Drugs with reported activity in the treatment of clinical lung cancer

	Lung tumor models		
	Lewis lung	LX-1 SC	LX-1 SRC
SMALL CELL			
Active in clinic*			
Cyclophosphamide	222+	37+	41−
Methotrexate	148+	41+	37−
Adriamycin	>252+	73−	43−
Procarbazine	154+	8+	−17+
VP 16	No data		
Actives in tumor models	4/4	3/4	1/4
Marginal activity in the clinic*			
CCNU	253+	15+	−11+
Hexamethylmelamine	202+	81−	72−
Actives in tumor models	2/2	1/2	1/2
EPIDERMOID			
Marginal activity in clinic*			
Cyclophosphamide	222+	37+	41−
Methotrexate	148+	41+	37−
Ariamycin	>252+	73−	43−
Nitrogen mustard	125−	NT	NT
cis-Platinum II	261+	86−	6+
Actives in tumor models	4/5	2/4	1/4

*See footnotes to Table 5.

Table 7. Activity in colon tumor models of the screening panel. Drugs with reported activity in the treatment of clinical colon cancer

	Colon tumor models			
Marginal activity in clinic*	Colon 26	Colon 38	CX-1 SC	CX-1 SRC
5-Fluorouracil	200+	0+	88−	60−
Mitomycin C	187+	9+	62−	NT
Methyl CCNU	345+	4+	83−	NT
Actives in tumor models	3/3	3/3	0/3	0/1

*See footnotes to Table 5.

rogen mustard was inactive in the Lewis lung model, but this drug was not tested in the LX-1 models. The LX-1 SC model failed to identify adriamycin and cisplatinum II as active and the LX-1 SRC system failed to identify cyclophosphamide, methotrexate and adriamycin as active.

For the drugs 5-fluorouracil, mitomycin C and Methyl CCNU, reported as having marginal activity against colon tumor in the clinic, the colon 26 and colon 38 tumor models identified 3/3 as active and the CX-1 SC model failed to identify any as active (Table 7). Only 5-fluorouracil was tested in the CX-1 SRC system and it was inactive. The lack of activity of these antitumor agents against the CX-1 human colon tumor model suggests that this system may have important discriminatory value in drug selection for treatment of this highly refractory clinical tumor category.

Thus, the data (Tables 5, 6, 7) indicate only a partial correspondence between the activity in animal tumor models and that which might be expected in the clinic for specific histologic types. Since the xenograft models, particularly the colon tumor, are resistant to therapy, they may prove to be important in drug selection. The human tumor xenograft models do not appear to yield as many false positives as the animal tumor models (Table 4). Overprediction by the animal models was especially indicated for the $CD8F_1$ breast tumor model for clinical breast cancer, and by the colon 26 and colon 38 animal models for colon cancer. The Lewis lung carcinoma did not appear to yield as many false positives.

Data in the screening panel for new drugs and drugs for which there is renewed clinical interest based in large measure on activity in the new screen are summarized in Table 8. Clinical data for these compounds are summarized in Table 9.

4′-(9-acridinylamino) methanesulfon-*m*-anisidide (AMSA), an acridine derivative [13], showed broad spectrum activity in the new screening panel, including activity against leukemias P388 and L1210, B16 melanoma, colon tumors 26 and 38 and the $CD8F_1$ mammary tumor. It was inactive, however,

Table 8. New drugs and drugs of renewed clinical interest. Activity in tumor models*

NSC		L1210	P388	B16 Mela-noma	Lewis lung	Colon 26	Colon 38	$CD8F_1$	SC MX-1	SC LX-1	SC CX-1	SRC MX-1	SRC LX-1	SRC CX-1	Active systems Number	Active systems Percent
249992	AMSA	185	216	243	125	241	25	3	74	75	67	63	96	NT†	6/12	50
224131	PALA	120	135	192	215	197	11	1	35	41	78	32	80	76	8/13	62
7365	D-O-Norleucine	174	166	112	121	162	14	3	11	2	56	-25	51	52	8/13	62
32946	Methyl G	176	176	96	103	106	75	88	116	83	92	57	85	39	2/13	15
157365	Neocarzinostatin	175	190	175	109	200	37	43	75	89	NT	51	41	69	5/12	42
Number of drugs																
	Active	4/5	5/5	3/5	1/5	4/5	4/5	3/5	2/5	2/5	0/4	1/5	0/5	0/4		
	Per cent	80	100	60	20	80	80	60	40	40	0	20	0	0		

*Underlining means drugs are active.
†NT = Not tested.

Table 9. New drugs and drugs of renewed clinical interest. Clinical activity

			No. Patients	CR	PR	MR
249992	AMSA	Acute leukemia in adults	22	3		
		Acute leukemia in adults	13	2		
		Breast cancer	22		5	
224131	PALA	Non small cell lung cancer	21			3
		Bladder cancer	10			2
7365	D-O-Norleucine	Breast	14		2	
		Lung carcinoma	9		2	
		Hodgkin's disease	11		2	
32946	Methyl G	Transitional cell carcinoma	2	1		
		Colon	9		2	
		Esophageal	2		1	
		Renal cell	4		1	
		Adenocarcinoma unknown primary	3	1		
157365	Neocarzinostatin	Pancreatic	88	3	8	
		Gastric	217	2	4	
		Acute leukemia	76	11	4	

against Lewis lung carcinoma and the human tumor xenografts. AMSA is being subjected to broad spectrum Phase II testing in the clinic and has been reported to be active in acute leukemia in adults [14, 15] and in breast cancer [15]. In initial studies it has also shown activity against lymphoma, ovarian and renal cell tumors. AMSA is a likely candidate for clinical investigation employing drug combinations.

Phosphonacetyl-L-aspartic acid (PALA) is an inhibitor of aspartate transcarbamylase [16]. It showed only borderline activity in the treatment of leukemia P388 and little to no activity against leukemia L1210. It did, however, show broad spectrum activity in the new screen, including activity against Lewis lung carcinoma [17, 18], B16 melanoma, colon tumors 26 and 38 and the mammary tumor $CD8F_1$. It also showed moderate activity against the mammary xenograft MX-1 and the lung xenograft LX-1. In addition, PALA has been reported to show broad spectrum activity against a variety of experimental solid tumors [19]. PALA has entered clinical trial, and to date minimal responses have been seen with non small cell lung tumor and bladder cancer [20].

Clinical interest was also renewed in the glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) [21] as a result of its activity in the new screen. In addition to activity in leukemias P388 and L1210 it was active in colon 26, colon 38 and $CD8F_1$ mammary tumor, and the human tumor xenografts MX-1 and LX-1. DON had been reported initially to produce partial remissions against breast tumor and lung carcinoma [22] and Hodgkin's disease [23] in the clinic. The drug has also evidenced activity against choriocarcinoma. A new Phase I study has been activated with DON, employing intermittent high doses.

Renewal of interest in the drug methyl glyoxal bis guanyl hydrazone (Methyl G) is attributable to the influence of scheduling characteristics. In the new screen Methyl G was observed to be active against leukemias L1210 and P388, with possible activity against the CX-1 xenograft in the subrenal capsule site. A complete remission has been reported recently against transitional cell carcinoma and also against adenocarcinoma, and partial remissions against colon, esophageal and renal cell tumors [24].

Neocarzinostatin was active against leukemias P388 and L1210, B16 melanoma and colon tumors 26 and 38 of the new screen. It was inactive against Lewis lung carcinoma and the human tumor xenografts. In the clinic it has evidenced activity against pancreatic [25] and gastric tumors [26], acute leukemia [26], and hepatoma.

As for the well established drugs, so too with the new drugs and drugs of renewed clinical interest, high and broad spectrum activity were observed in the experimental tumor systems (Table 8). Methyl G provided the only marked exception, being active in only 2/13 of the test systems. Again, in general, a higher incidence of actives was observed for the animal test systems as compared with the human tumor xenografts.

A listing of the screening data for drugs about to enter or primarily in initial clinical trials is presented in Table 10. Many of these drugs elicited high and broad spectrum activity in the new screen.

'Bypass' compounds include Baker's antifol (NSC 139105) and 2'deoxycoformycin (NSC 218321), which were not active in any of the systems of the new screening panel, and dichloroallyl lawsone (NSC 126771), which had only marginal activity in the P388 system. The remaining compounds listed in Table 10 were active in three or more test systems of the new screen.

Data in the new screening panel for compounds in development are listed in Table 11. High and broad spectrum activity were observed for many of these materials. With some exceptions such as the 'bypass' compounds, ADI (EHNA) (NSC 263164), an adenosine deaminase inhibitor, and trimethyl melamine (NSC 57552), an older drug of interest, the compounds were active in at least one and usually more systems of the new screen.

Since in general there is greater activity against the murine tumors than against the human tumor xenografts growing in athymic mice it may be of interest to focus attention on drugs that have demonstrated activity against the human tumor xenografts. Among the drugs about to enter or in initial clinical testing (Table 10) PCNU and AT-125 were active in two of the three subcutaneous human tumor xenografts. Others such as indicine *N*-oxide and piperazinedione showed activity in one of the systems. Some compounds, although not active in the subcutaneous xenograft systems, were nevertheless active in at least one of the subrenal systems. These include maytansine and aclacinomycin A.

Among the compounds in development (Table 11), trimethyl-trimethylol melamine

Table 10. Activity in the new screening panel for additional drugs about to enter or primarily in early clinical trials*

NSC number	Drug	L1210	P388	B16 Melanoma	Lewis lung	Colon 26	Colon 38	$CD8F_1$	Subcutaneous MX-1	Subcutaneous LX-1	Subcutaneous CX-1	Subrenal capsule MX-1	Subrenal capsule LX-1	Subrenal capsule CX-1
4728	Aminothiadiazole	155	200	111	121	145	22	24	58	60	57	NT†	NT	NT
51143	Dihydroimidazo pyrazole	144	120	106	169	120	50	46	68	64	53	55	104	128
95466	PCNU	>300	118	179	>260	346	6	4	39	26	88	−36	−9	133
102816	5-Azacytidine	>300	>300	160	222	141	16	21	68	71	86	61	62	88
126771	Dichloroallyl lawsone	113	120	120	117	107	45	52	87	78	83	71	102	73
132319	Indicine-*N*-oxide	147	262	182	115	176	5	21	14	98	85	−21	65	38
135758	Piperazinedione	>470	223	141	126	171	20	0	3	69	80	9	43	19
139105	Baker's antifol	120	115	112	NT	98	55	58	87	58	88	68	77	96
139490	Methyl tetrahydro homofolate	147	139	117	129	147	54	33	72	57	68	40	37	68
153353	L-Alanosine	161	178	117	125	141	53	30	57	84	116	80	77	60
153858	Maytansine	126	186	207	104	126	62	27	77	71	69	−25	2	−42
163501	AT-125	191	250	131	147	144	69	6	9	31	55	−40	78	33
169780	Soluble ICRF	172	229	142	213	177	33	10	80	58	78	56	NT	35
182986	AZQ	269	238	170	121	325	17	11	26	58	93	0	63	103
208734	Aclacinomycin A	139	236	148	NT	123	60	11	53	62	101	20	39	−14
218321	2'Deoxycoformycin	105	118	NT	127	NT	NT	51	74	70	65	44	31	118
246131	AD-32	>521	560	288	118	175	6	3	59	74	52	NT	37	NT
301739	Anthracenedione dihydrochloride	274	450	506	276	303	23	29	64	92	69	63	70	43

*Underlining means drugs are active.
†NT = Not tested.

*Table 11. Data in the new screening panel for compounds in development**

		L1210	P388	B16 Melanoma	Lewis lung	Colon 26	Colon 38	$CD8F_1$	Subcutaneous MX-1	Subcutaneous LX-1	Subcutaneous CX-1	Subrenal capsule MX-1	Subrenal capsule LX-1	Subrenal capsule CX-1
2014	Nitrophenyl benzene	<125	140	111	102	117	68	69	87	93	73	66	52	88
3087	Oxophenarsine	110	145	110	105	118	55	67	81	73	NT	27	102	NT
3136	Benzohydroxamic acid	150	150	101	114	NT†	87	40	NT	18	84	48	NT	NT
5356	Dimethyl formamide	110	115	<125	121	137	75	57	64	70	73	−13	84	50
57198	Diazoniabicyclo-heptane deriv.	190	213	157	126	272	48	6	35	66	67	21	34	53
57552	Trimethyl melamine	103	NT	113	123	110	74	69	88	111	NT	96	102	90
71795	Ellipticine	>495	204	147	147	263	0	9	67	96	93	72	75	64
74437	Epoxypropylpiperazine	160	179	135	124	180	40	3	58	74	87	60	44	NT
122023	Valinomycin	131	183	183	<140	200	40	49	72	89	81	67	99	8
125973	Taxol	139	190	226	98	161	69	57	91	93	70	−23	24	−22
127755	Dihydrotriazene deriv.	141	230	191	139	175	13	3	63	59	88	77	86	47
137679	Selenoguanosine	229	247	136	145	179	28	12	50	104	101	113	115	85
141633	Homoharringtonine	150	338	134	116	113	8	7	82	131	87	103	74	38
154020	Tricyclic nucleoside	215	156	123	113	125	75	0	89	93	75	−34	65	−24
156315	Tantalum deriv.	110	135	118	130	170	51	0	59	52	70	78	58	75
166100	Prospidine	114	159	183	145	185	37	5	72	71	55	93	NT	43
172112	Spirohydantoin Mustard	142	>290	263	149	286	25	4	50	85	85	−14	77	32
196473	Anthraquinone	>428	>467	>260	297	180	8	2	55	NT	NT	NT	NT	NT
208642	Lymphosarcin	127	169	170	NT	132	96	133	68	78	17	NT	NT	NT
219733	Diacridine deriv.	143	180	206	125	NT	70	47	75	68	118	68	106	18
234714	Aphidicolin	122	155	176	126	220	65	26	77	84	92	51	15	67
237020	Largomycin	118	225	200	107	184	32	18	67	55	88	NT	NT	NT
241240	Cyclobutanedicarbo-xylato platinum deriv.	140	150	170	135	250	18	8	48	106	60	−29	65	NT
249008	Quinazoline deriv. (JB-11)	158	NT	157	132	215	10	2	180	57	74	59	66	26
250427	Sulfato platinum deriv.	300	200	160	117	178	25	8	58	116	46	NT	82	NT
256927	Di-isopropylamine	170	175	166	129	170	46	5	57	96	47	NT	66	NT
259272	Ara-A-5'phosphate	134	NT	131	126	130	83	59	69	103	87	13	57	87
259968	Bouvardin	129	188	152	119	120	44	80	78	81	85	55	72	7
263164	ADI (EHNA)	100	105	106	130	106	57	43	94	71	91	67	57	NT
264880	Dihydro-5-azacytine	228	227	125	120	133	40	35	36	82	62	12	89	38
265328	Hematoporphyrin deriv.	100	NT	123	116	127	66	38	61	100	100	NT	NT	NT
283162	Trimethyltrimethylo melamine	147	NT	140	128	170	4	15	3	40	NT	3	80	12
287513	9,10 Anthracenedione	>513	>463	197	131	180	0	0	63	79	87	17	82	−9
288411	Indequinoline carboxylic acid	141	172	169	103	125	62	>42	71	70	88	49	75	83
289642	Macromycin	182	218	265	116	227	38	45	89	44	68	19	52	49
296961	WR-2721	106	123	125	115	NT	72	43	60	94	69	NT	NT	NT
301463	Poly IC:Poly L-lysine	116	149	153	108	NT	40	39	NT	49	73	NT	NT	NT
526417	Quinomycin A	<125	175	180	<140	<130	128	>42	71	85	78	55	52	22

*Underlining means drugs are active.
†NT = Not tested.

(NSC 283162) was active in two of the subcutaneous and two of the subrenal capsule xenograft systems. Taxol (NSC 125973), the tricyclic nucleoside (NSC 154020) and 9,10-anthracenedione (NSC 287513) were not active in the subcutaneous xenograft systems but were active in two of the subrenal capsule systems. Dihydro-5-azacytidine (NSC 264880) was active against the MX-1 mammary tumor in both the subcutaneous and subrenal systems. Additional drugs were active in only one of the xenograft systems employing either the subcutaneous or subrenal sites of tumor inoculation. Lymphosarcin (NSC 208642) was the only drug active against the SC colon tumor CX-1.

It is of interest to examine the relationship between the subcutaneous and subrenal capsule systems for the human tumor xenografts (Figs. 2–4). For the MX-1 breast tumor a generally good correspondence of results was obtained. Out of sixty-five compounds for which there was data for both systems there were thirteen (20.0%) compounds active in both systems and thirty-five (53.8%) inactive in both systems. There were four compounds (6.2%) active only in the SC system, but a higher incidence of thirteen compounds (20.0%) active only in the SRC system.

Similarly, there was a reasonable correspondence of ratings for the LX-1 lung tumor xenograft. For sixty-four compounds with ratings in both systems, three (4.7%) of the drugs were active in both systems and forty-nine (76.6%) of the drugs were inactive in both systems. There were seven (10.9%) com-

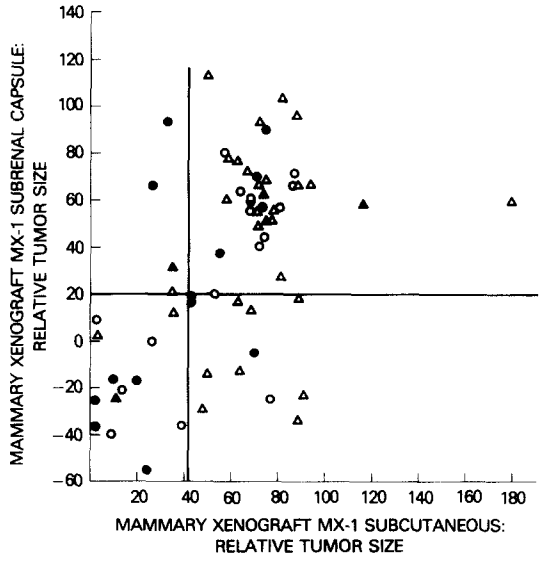

Fig. 2. Comparison of drug activity against the human mammary tumor xenograft MX-1 in the subcutaneous and subrenal capsule systems. ●, *Clinically active antitumor agents (see Table 4);* ▲, *new drugs and drugs of renewed clinical interest (see Table 8);* ○, *drugs about to enter or primarily in early clinical trials (see Table 10);* △, *compounds in development (see Table 11). Activity requirement: subcutaneous system* ≦42; *subrenal capsule system* ≦20.

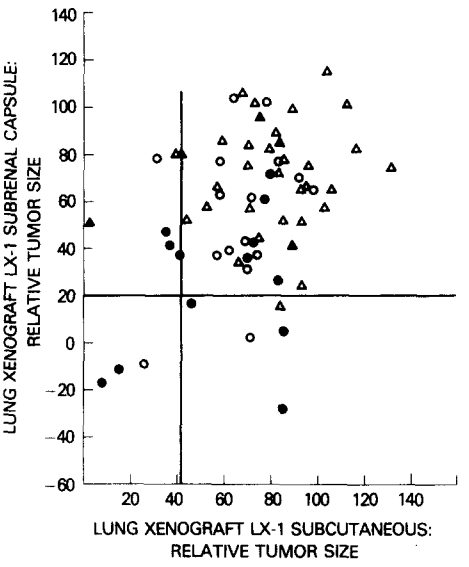

Fig. 3. Comparison of drug activity against the human lung tumor xenograft LX-1 in the subcutaneous and subrenal capsule systems. ●, *Clinically active antitumor agents (see Table 4);* ▲, *new drugs and drugs of renewed clinical interest (see Table 8);* ○, *drugs about to enter or primarily in each clinical trials (see Table 10);* △, *compounds in development (see Table 11). Activity requirement: subcutaneous system* ≦42; *subrenal capsule system* ≦20.

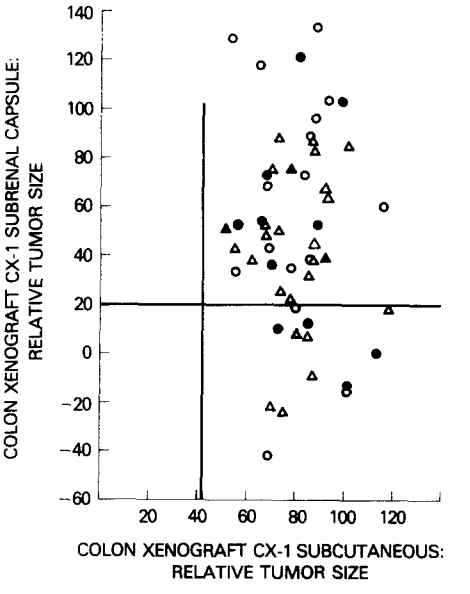

Fig. 4. Comparison of drug activity against the human colon tumor xenograft CX-1 in the subcutaneous and subrenal capsule systems. ●, *Clinically active antitumor agents (see Table 4);* ▲, *new drugs and drugs of renewed clinical interest (see Table 8);* ○, *drugs about to enter or primarily in each clinical trials (see Table 10);* △, *compounds in development (see Table 11). Activity requirement: subcutaneous system* ≦42; *subrenal capsule system* ≦20.

pounds active in only the SC system and five (7.8%) active only in the SRC system.

For the colon CX-1 xenograft system there were no compounds rated as active in both systems, but 40/53 (75.5%) were rated as inactive in both systems. There were no compounds rated as active in the SC system, but thirteen (24.5%) showed activity in the SRC system. The higher incidence of positives in the SRC system was attributable primarily to the inclusion in this category of newer compounds undergoing development. A more stringent criterion of activity may be desirable for the colon tumor employing the SRC system in order to avoid the identification of too many false positive compounds.*

The apparent somewhat greater sensitivity of the SRC systems as compared with the SC systems, particularly for the mammary MX-1 and colon CX-1 xenografts, suggests that the SRC systems could constitute the initial test systems for the xenografts. However, it is also indicated that activity in the SRC xenograft test systems be followed by further testing in the SC systems in order to help narrow down the choice of drugs for further development. An experiment currently in progress in which a direct comparison is being made of one hundred and twelve compounds in both the SC and SRC systems should provide additional definitive information concerning this approach.

In addition to the *in vivo* test systems for drug screening there has been a strong interest in the program since its inception in the possibility of employing *in vitro* test systems [3, 11]. *In vitro* model systems have involved human tumor cells growing in tissue culture [3, 11, 27, 28] and as tumor explants [29, 30]. Currently, exploration is being conducted on the use of recently developed *in vitro* clonogenic assays [31, 32] from the point of view of eventually employing them in the screening flow. The intent is to develop and test the feasibility of *in vitro* assays as both pre-screens and screens and to serve as a guide for clinical emphasis of developed materials. Also, the possibility is attractive that both tumor xenograft models and *in vitro* assays employing human tumors in culture, such as with the clonogenic procedures, could be employed as indicators of selection and optimal utilization of drugs in the treatment of individual patients.

*The difference between SC and SRC results with the colon tumor does not appear to be attributable to differences in the methods of calculation of activity. Early initiation of treatment against a reduced tumor challenge may account at least in part for the increased responsiveness of the colon tumor in the SRC system.

SUMMARY

Although the prospective predictability value of the new screen must await further clinical testing of new drugs that emerge from the screen, important implications have already become available of pertinence to transfer of preclinical information to the clinic. In this regard it is of interest to examine the status of information on questions addressed to the new screen.

(1) Since the institution of the new screening panel, there are a substantial number of compounds in various stages of development. However, the question of whether the new screen increases the yield of true positive compounds must await determination of the effect of these compounds in the clinic.

(2) High and broad spectrum activity in a variety of tumor appears to prospectively increase the possibility for prediction for at least minimal clinical activity for one or more human tumors. This high and broad spectrum activity in the screening panel was observed not only for the more established clinically active antitumor agents, but also for the various other categories of drugs including new drugs and drugs of renewed clinical interest, drugs about to enter or primarily in early clinical trials, and compounds in development.

(3) In regard to the question of the extent to which tumor xenografts and animal tumor screens select the same or different drugs as active, the data indicate that compounds that have demonstrated activity in one or more of the animal test models have a lower incidence of activity in the human tumor xenografts systems. Conversely, to date compounds that have demonstrated activity in the human tumor xenograft systems have in general also demonstrated activity in one or more of the animal tumor test models. However, it is considered that eventually there will emerge a representative number of drugs active exclusively against at least one of the human tumor xenografts.

(4) The question of whether the xenograft positives are more active in the clinic than those selected by animal screens cannot be answered at this time, without the availability of additional clinical data. However, it is important to note that in comparability with the clinic, human tumors in xenograft appear

to be relatively resistant to therapy. In this regard it may be important to stress the clinical testing of drugs that have definitive activity in the xenograft systems.

(5) It is suggested that utilization of the new screening panel may provide a means for reducing the number of false positives, compounds that are active in the screen but inactive in the clinic. Not only do the animal tumor systems tend to rate a higher percentage of drugs as active as compared with the human tumor xenografts, but also, retrospectively, they yielded a relatively high incidence of false positives. In addition, the subrenal capsule systems, particularly the mammary MX-1 and colon CX-1 xenografts, had somewhat greater sensitivity as compared with the SC xenograft systems. In regard to initial screening, the greater sensitivity of the animal screens and the subrenal capsule systems may provide an advantage for the initial selection of potential drugs of interest. Although this selection undoubtedly would provide an increase in the number of false positives, this could be pared down by placing emphasis on the requirement of activity in the human tumor xenograft systems in order to create interest for further testing. If there is indeed greater comparability of activity in human tumor xenografts and in the clinic, this approach would result in a reduction of false positive compounds.

(6) False negatives, compounds that are inactive in the screen but which would have been active if tested in the clinic, are of serious concern for any antitumor screening program. Conceptually the question of whether the new screening panel reduces the number of false negatives cannot be answered adequately without the testing in the clinic of a prescribed number of compounds that are inactive in the screening panel. However, the question may ultimately be answered at least partially by comparison of the yield for the clinic of compounds that are introduced into the clinic despite inactivity in the screening panel, as the result of special biochemical, pharmacologic or other related considerations, or as the result of demonstrated activity in screening systems other than those in the screening panel. Also, it is considered that if compounds that are active exclusively in one or more of the human tumor xenograft systems are also active in the clinic, this could remove them from the false negative classification that would have resulted if the tumor screening had been restricted to the animal tumor systems.

(7) Although there was evidence of a partial correspondence of activity against animal tumors and/or human tumor xenografts with activity against clinical tumors for specific histologic types or specific organ systems, the question cannot be answered in a definitive manner without further feedback from the clinic. Testing of drugs in a battery of colon, lung, breast and other types of animal and human tumor xenografts as well as in animal and human tumor clonogenic assays may provide important information on the extent of predictability for the clinic of histologic and organ drug specificity.

(8) To date there have been too few compounds that have bypassed the P388 prescreen because of activity in other screening systems or in selected biochemical or biological assays to determine whether they will be more effective than P388 actives in the screening panel and in the clinic. Compounds such as Baker's antifol and 2′deoxycoformycin were inactive against leukemia P388 and also inactive in the screening panel and it will be of interest to determine whether such drugs have definitive activity in the clinic.

(9) Considerable additional experience is required in order to determine what contribution the new screening panel may make to prediction of the clinical activity of new drugs with respect to analogs of known antitumor agents and structure–activity analyses, as well as in mathematical approaches to activity prediction.

Nevertheless, it is clear that the new prospective screening program of the Division of Cancer Treatment, National Cancer Institute, is making steady progress in answering questions directed at it, of great pertinence to the discovery and development of new and more effective antitumor agents for clinical application.

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