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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

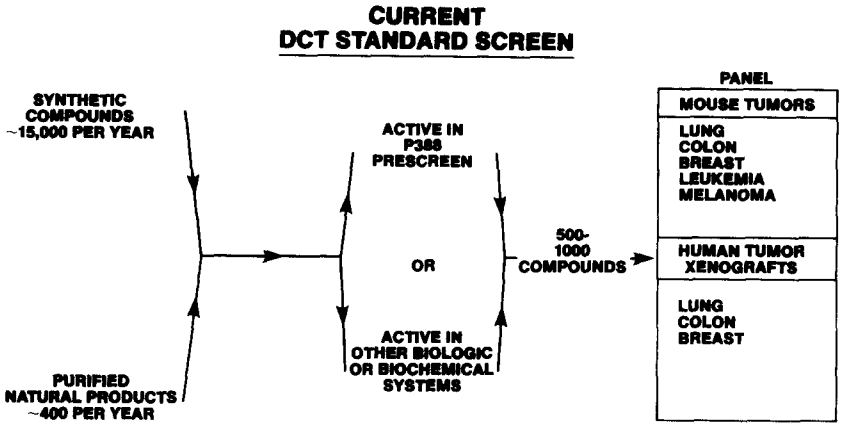

Fig. 1. Flow of drugs through the Division of Cancer Treatment screens.

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 cis-platinum 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.

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