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Development and characterization of a new murine renal tumor model

Chemotherapeutic results

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Summary. *A murine renal cell carcinoma model, the RC tumor, was pharmacologically and histologically characterized and was used for the evaluation of 20 chemotherapeutic agents. This model, when implanted IP or SC showed reproducible behavior and was found to be very sensitive to many drugs with different mechanisms of action, and particularly to alkylating agents. The IP-implanted tumor was sensitive to some clinically active drugs which were reported inactive against L1210 or P388 murine leukemias.*

The higher sensitivity of this model than of L1210 and P388 leukemias makes the RC tumor a good prescreening system for testing potential chemotherapeutic agents.

Introduction

Renal cell carcinoma accounts for approximately 3% of adult malignancies [3]. Most patients bearing this tumor (75%–80%) die within 10 years with distant metastases [4]. Surgical excision remains the only effective method of curing renal cell carcinomas. Pre- or postoperative radiotherapy has not been shown to affect the survival of patients [3]. Progestational therapy was previously advocated [1] but no recent report has substantiated the role of progestational agents [3]. Immunotherapy did not markedly influence survival [8].

Chemotherapeutic agents have a very limited effectiveness in the treatment of renal cell carcinoma. CCNU, BCNU, and vinblastine showed a moderate objective response rate and may be considered the most active agents [7, 9, 10, 14]. Since most patients develop metastases there is an obvious need for more effective drugs. Some agents have not been given adequate trials and it could be that agents effective against this tumor are already in our possession [10, 14].

Few murine renal tumor models have been described [2, 6, 11–13]. A hormone-dependent model was developed in the Syrian hamster [2]. However, the poor clinical results obtained with progestational agents suggest that this tumor model may not accurately represent the human disease [13]. The object of our study is to develop a new murine transplantable model of renal carcinoma which may be more clearly analogous to the human renal cancer and be used as a possible screen for new potentially active drugs. For this purpose, we used the RC murine renal adenocarcinoma from the Tumor Bank of the National Cancer Institute (Division of Cancer Treatment, Bethesda, Maryland).

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Materials and methods

Tumor. The RC renal adenocarcinoma, obtained from Dr Bogden (Mason Research Institute, Worcester, Massachusetts), was maintained in CDF1 mice by serial transfers every 14 days. The ascitic fluid was removed from tumor-bearing animals under aseptic conditions between day 12 and day 15 after transplantation and diluted in saline. Cell viability, determined by trypan blue staining, ranged from 90% to 100%. The CDF1 hosts received a total dose of 10^6 live tumor cells IP or SC in 0.1 ml. The tumor could also be propagated from SC-implanted solid nodules. In this case, tumor fragments were homogenized and suspended in saline before cell counting and transplantation. The tumor was syngeneic in CDF1 mice. DBA/2 and Balb/c mice that received 10^6 RC cells IP or SC showed no evidence of tumor growth.

Animals. CDF1 mice weighing between 18 and 22 g were used in this study. In each experiment, untreated tumor-bearing animals served as controls and their survival was recorded until death. The animals were examined daily. They were weighed before implantation and on day 5 to detect a possible toxicity. Seven to 10 animals were used per test and control group.

Drugs. Drug treatment started on day 1 after implantation. In most experiments drugs were administered IP every 4 days until day 17 (i.e., on days 1, 5, 9, 13, and 17). In others, they were given daily on days 1–5, days 1–9, or days 1–15. For each drug evaluation three dose levels were selected, based on the effective dose range for our treatment schedule.

Assessment of drug activity. The evaluation criteria established by the National Cancer Institute (Bethesda, Maryland) were used in this work [5]. The T/C ratio (median survival time of treated animals divided by that of the control group) and the number of long-term survivors on day 60 were used to evaluate the effectiveness of the drugs. Tumor size was estimated every 2 days from caliper measurements converted to weight, using the following formula: A chemotherapy activity index was established as follows: weight (mg) = length (mm) × width $(\text{mm})^2 \cdot 2$ + = T/C > 120%; ++ = T/C > 150%; +++ = T/C > 200%; ++++ = T/C > 200% and presence of survivors in the treated group.

Results

1. Tumor characterization

Structure of the tumor. Grossly, following SC implantation, the tumor was pink to grey, of a firm consistency, moderately,

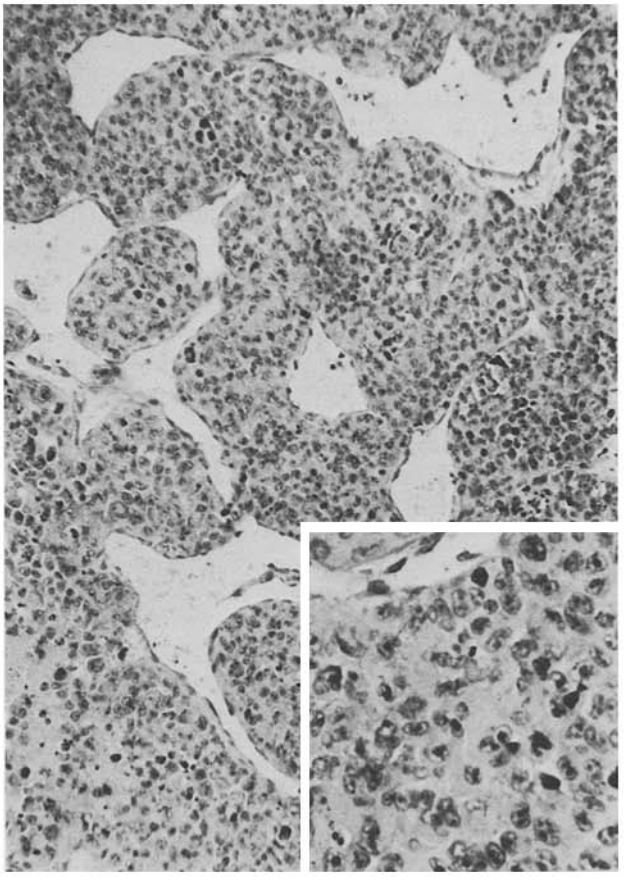

Fig. 1. Magnification showing the compact trabecular pattern of this tumor. Trabeculae are separated by wide vascular channels. (H & E, × 160) *Inset* High-power magnification revealing the anaplastic and monotonus pattern of the neoplastic cells. Numerous mitotic figures can be observed. H & E, × 420

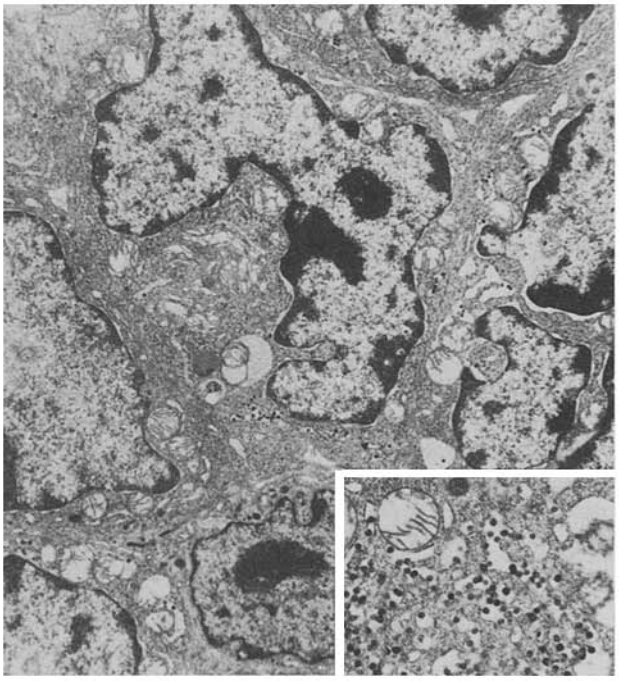

Fig. 2. Electron micrograph of malignant cells, showing indented nuclei with large nucleolae. Cytoplasm contains very few organelles except for numerous mitochondria with irregular cristae. × 7,500. *Inset* Numerous retrovirus particles appearing as small black dots in the cytoplasm. Electron micrograph, × 15,000

vascularized, with no distinct encapsulation and occasionally with necrotic areas. Following IP implantation bloody ascites was observed. In both SC- and IP-inoculated mice, macroscopic involvement of the spleen and para-aortic adenopathies were observed.

Numerous metastases are microscopically detected in the spleen and the liver. The tumor is characterized by trabeculae of large anaplastic cells separated by wide vascular sinuses (Fig. 1). The cellular pattern is monotonous: the nuclei are large, polygonal, and indented, with a thick nuclear membrane and large nucleoli. Some cells are multinucleated, and atypical mitotic figures are frequent. The cytoplasm is rather large and amphophilic with standard Hematoxylin and Eosin staining, and has well-defined borders. The compact trabeculae are well underlined by reticulin fibers, and press against the endothelium of the vascular sinuses. No glandular differentiation is observed.

Electron microscope examination reveals marked irregularity and indentations of the nuclei. The heterochromatin is dispersed as heavy blocks against the nuclear membrane (Fig. 2). The cytoplasm only contains very few organelles, except for numerous swollen mitochondria with irregular cristae. Countless retrovirus particles are observed and also a few elementary junctional complexes.

IP implants and titration curve. In five experiments in which the IP route was was used there were no survivors, and 100% tumor takes were observed in 113 untreated tumor-bearing mice (inoculated with 10^6 live tumor cells). The median survival time (MST) was 16.6 days, with a range of 9–24 days in individual mice. Similarly, in another experiment mice received IP implants of various amounts of live tumor cells (10^6, 10^5, 10^4, 10^3, and 10^2 cells). A relationship between the number of cells implanted and the host lifespan was observed (Fig. 3). The correlation between the two variables was excellent ($r = -0.78$).

SC implants and tumor growth. Mice were inoculated SC in the right flank with 10^6 tumor cells. There were no survivors and 100% tumor takes were recorded in 98 control mice. The tumor was palpable in 100% of them on day 10. The median survival time of mice following SC implants mice was 21.4 days, with a range of 12–26 days. The growth of the SC-implanted tumors is described by a Gompertz function (Fig. 4). A tumor weight of 1 g is reached on day 13. The doubling time of the tumor increases from 1.5 days for a 0.1 g tumor to 3.0 days for a 2.0 g tumor.

Metastases. An important increase in the volume of the spleen was observed. Microscopic examination gave evidence of the liver and spleen involvement. To assess the metastatic involvement of other organs bioassays were performed, starting with mice killed on day 18 after receiving SC implants. Metastases were similarly detected in the liver, the spleen, the

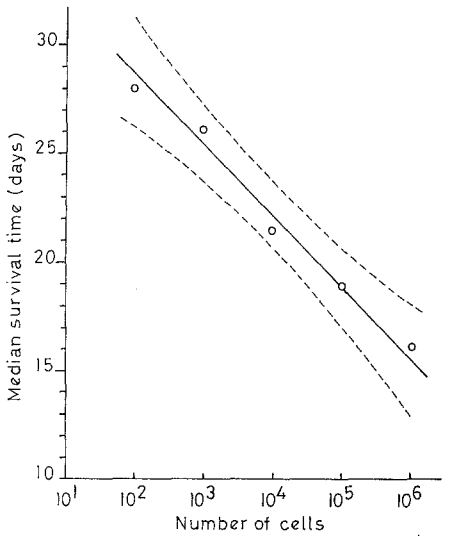

Fig. 3. Linear regression line analysis between mouse survival and the number of RC cells implanted IP. *Dottes lines* indicate 95% confidence limits

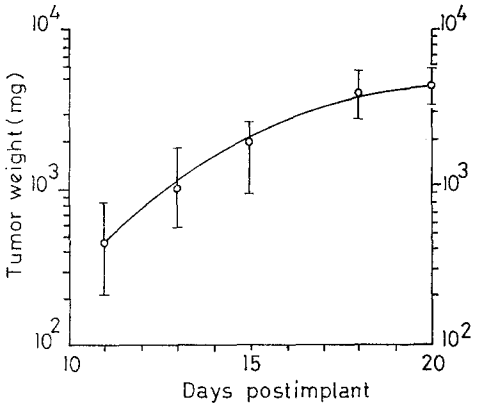

Fig. 4. Gompertz-fitted growth curve for SC-implanted RC tumor. Size range of the tumor at each measurement is indicated

Table 1. Results of the IP bioassays of various tissues of mice following SC tumor implantation

Organ	Number of mice	MST (days)	Number of survivors on day 60
Liver	8	14,0	0
Spleen	6	18.5	0
Kidneys	6	19.7	0
Lungs	8	19.8	0
Brain	9	26.0	5/9

kidneys, and the lungs, and 100% of mice to which homogenized fragments of these organs were implanted died (Table 1). On the other hand, several survivors were observed in the group of mice that received implants of brain fragments.

2. Chemotherapeutic experiments

Twenty drugs were evaluated in the study, being 16 drugs with known clinical activity and four compounds still in development programs. The clinically active drugs included alkylating agents (cyclophosphamide, DTIC, *cis*-platinum, Me-CCNU, BCNU, and busulfan), antimetabolites (methotrexate, hydroxyurea, and 5-fluorouracil), antimitotic agents (vinblastine and vindesine), compounds reacting with nucleic acids (Adriamycin and bleomycin), and miscellaneous drugs (hexamethylmelamine, op'-DDD). The compounds in development included two epoxide derivatives: α-triglycidyl triazinetrione or α-TGT (NSC-269934) and triglycidyl urazol or TGU (NSC-332844), and also 2 tin derivatives: 3,4,7,8 tetramethyl-1,10-phenanthroline (NSC-328378) and 2-2' pyridyl-4-benzimidazol-diethyl-tin dichloride (NSC-314263).

RC implanted IP. Eighteen chemotherapeutic agents were evaluated in the group of mice with IP-implanted tumor (Tables 2 and 3). Fourteen of these drugs produced a significant increase in the T/C ratio in at least one experiment ($P < 0.001$, two-tailed t-test for unrelated samples). The most active drugs were methyl-CCNU, BCNU, cyclophosphamide, α-TGT, and hexamethylmelamine. These drugs produced a considerable increase in the T/C values and induced more than 50% cures in at least one experiment. No ascites was found in the peritoneal cavity of cured animals at autopsy on day 60. Cyclophosphamide and α-TGT cured most of the mice in all experiments in which they were used (except for a toxic dose of cyclophosphamide). BCNU, methyl-CCNU, and hexamethylmelamine displayed a marked activity, producing T/C values of almost 300% or more with a very satisfactory cure rate. Other drugs produced a T/C value of 200% or more: 5-fluorouracil, Adriamycin, DTIC, cisplatin, hydroxyurea, and the tin derivative NSC-314263. Variable results were found when methotrexate, vinblastine, vindesine, and the tin derivative NSC-328378 were evaluated.

Four drugs, hexamethylmelamine (HMM), busulfan, bleomycin, and op'-DDD, were tested against the RC tumor because they are clinically active but remain ineffective against L1210 leukemia [17]. Since this tumor system is commonly used to screen new compounds, we were interested to check the responsiveness of our tumor model to these four drugs to evaluate its potential usefulness for screening new drugs. The RC tumor showed a good response to HMM, busulfan, and bleomycin. Only op'-DDD was totally ineffective (Table 3). The most active compound was HMM, which resulted in 50% survival on day 60. This drug is even inactive against P388 leukemia, a model currently used as prescreen in many programs [17].

RC implanted SC. Fourteen drugs were evaluated in SC-implanted tumor (Table 4). Nine drugs were judged effective against this tumor. None of the survivors at day 60 showed any residual tumor, except for the *cis*-platinum-treated group. As observed in the IP experiments, α-TGT and cyclophosphamide were the most effective, producing 90% of cures except with doses in the toxic range. TGU, not evaluated in the IP experiments, also produced a very marked effect and a high cure rate. BCNU, methyl-CCNU, and cisplatin showed good activity against the tumor and were curative. Other agents were less active: methotrexate, bleomycin, and DTIC. Vindesine and vinblastine were ineffective, as were Adriamycin and the tin derivatives NSC-314263 and NSC-328378.

Table 2. Responses to chemotherapy[a] of mice following IP implantation of RC

Substance	mg/kg per dose	B.W.C.[b] (g)	T/C (%)	Survivors on day 60	Activity index
Methotrexate	30	− 1.9	195	1/10	++
	20	− 1.0	179	−	++
	10	+ 0.5	190	−	++
5-FU	120	− 0.5	103	−	
	60	0	255	−	+++
	30	+ 0.9	154	−	++
Adriamycin	6	− 0.1	195	1/10	++
	3	− 0.3	201	−	+++
	1.5	+ 0.2	175	1/10	++
Me-CCNU	15	0	> 309	5/10	++++
	10	+ 0.2	237	3/10	++++
	5	+ 0.6	175	2/10	++
BCNU	12	+ 0.5	> 309	8/10	++++
	6	+ 1.5	247	3/10	++++
	3	+ 1.2	191	−	++
Vinblastine	0.8	− 0.1	159	−	++
	0.4	+ 0.4	171	−	++
	0.2	+ 0.7	153	−	++
Vindesine	0.8	− 0.8	149	−	+
	0.4	− 0.4	130	−	+
	0.2	− 0.2	122	−	+
Cyclophosphamide	80	− 2.0	317	−	+++
	60	+ 0.3	> 487	6/6	++++
	40	+ 0.7	> 487	4/6	++++
DTIC	300	− 1.0	221	−	+++
	150	− 0.3	162	−	++
	75	− 0.3	146	−	+
cis-Platinum	4	− 0.3	380	−	+++
	2	+ 0.9	243	−	+++
	1	+ 1.1	219	−	+++
α-TGT	60	− 1.3	> 435	9/10	++++
	40	+ 0.2	> 435	9/10	++++
	20	+ 0.6	> 435	7/10	++++
Hydroxyurea	300	+ 0.1	220	−	+++
	150	+ 0.6	150	−	++
NSC-314263	40	+ 0.5	218	−	+++
	20	+ 1.5	99	−	−
	10	+ 1.2	82	−	−
NSC-328378	12.5	+ 0.7	173	−	++
	6.25	+ 0.7	100	−	−
	3.12	+ 1.2	136	−	+

[a] All drugs were administered IP on days 1, 5, 9, 13, and 17, except for hydroxyurea which was administered daily IP from days 1 to 15 post implant

[b] Body weight change

Table 3. Chemotherapy[a] of mice with drugs inactive against L1210 leukemia, following IP tumor implantation

Substance	mg/kg per dose	B.W.C. (g)	Treatment schedule	T/C (%)	Survivors on day 60	Activity index
Busulfan	60	+ 0.3	Day 1–9	150	−	++
	30	+ 1.0		96	−	−
	15	+ 0.7		103	−	−
op'-DDD	100	+ 0.3	Day 1–9	95	−	−
	50	+ 0.6		103	−	−
	25	+ 1.0		103	−	−
Hexamethylmelamine	120	+ 1.7	Day 1–9	> 292	5/10	++++
	100	+ 0.7		> 292	4/10	++++
	80	+ 0.5		268	1/10	+++
Bleomycin	40	− 0.7	Days 1, 5, 9,	165	−	++
	30	− 0.3	13, 17	178	−	++
	20	− 0.9		163	−	++

[a] All drugs were administered IP

Table 4. Responses of mice to chemotherapy[a] following SC implantation of RC

Substance	mg/kg per dose	B.W.C. (g)	T/C (%)	Survivors on day 60	Activity index
Methotrexate	30	+ 0.4	62	–	–
	20	– 0.4	182	–	++
	10	– 0.4	134	–	+
Adriamycin	6	+ 0.8	92	–	–
	3	+ 0.3	111	–	–
	1.5	+ 0.4	107	–	–
Bleomycin	40	– 1.2	131	–	+
	30	+ 0.8	126	–	+
	20	+ 1.2	110	–	–
Me-CCNU	15	+ 0.8	225	3/10	++++
	10	+ 0.6	117	–	–
	5	+ 1.6	109	–	–
BCNU	12	+ 0.3	> 257	7/10	++++
	6	+ 1.0	134	–	+
	3	+ 0.6	120	–	–
Vindesine	0.8	– 0.5	108	–	–
	0.4	+ 0.4	104	–	–
	0.2	+ 0.5	103	–	–
Vinblastine	0.8	+ 0.1	113	–	–
	0.4	+ 0.1	116	–	–
	0.2	+ 1.1	103	–	–
Cyclophosphamide	80	+ 0.3	130	2/10	+
	60	+ 0.9	> 300	9/10	++++
	40	+ 0.8	> 300	9/10	++++
DTIC	300	– 0.4	158	1/10	++
	150	+ 0.5	121	–	–
	75	+ 1.0	110	–	–
cis-Platinum	4	– 0.3	86	–	–
	2	+ 0.7	> 300	6/10	++++
	1	+ 1.3	236	–	++
α-TGT	60	– 2.1	62	–	–
	40	+ 0.6	> 258	9/10	++++
	20	+ 0.7	> 258	10/10	++++
TGU	50	– 0.9	130	4/9	+
	25	– 0.9	> 285	8/9	++++
NSC-314263	60	+ 1.5	106	–	–
	40	+ 1.3	103	–	–
	20	+ 1.0	89	–	–
NSC-328378	12.5	+ 1.5	128	–	+
	6.25	+ 1.0	104	–	–
	3.12	+ 1.5	96	–	–

[a] All drugs were administered IP on days 1, 5, 9, 13, and 17 post implant

Discussion

Murine tumors are valuable assets for screening new drugs and helping to develop clinically useful chemotherapeutic agents. They are essential to the programs of the National Cancer Institute and of the European Organization for Research on Treatment of Cancer (EORTC). Our RC tumor has many of the characteristics desirable for an experimental tumor model. The RC tumor is transplantable, it develops metastases, and it shows reproducible behavior. The survival times recorded after IP or SC implantation of 10^6 cells and the size of the primary subcutaneous tumor are constant and reproducible. The extremely low percentage of no-takes observed with inoculum levels as low as 10^2 cells means the long survivals in drug-treated mice are indubitable evidence of the drugs' effectiveness. The titrating ability of the IP-implanted tumor allows assays with the surviving viable cells and chemotherapy trials against a small number of cells.

Comparison of the tests performed in mice after IP and SC implantation showed that drug sensitivity of the tumor was much higher in the group of mice with IP-implanted tumor. A number of factors may affect tumor responsiveness to chemotherapy: direct contact between tumor cells and the drug, which would favor antitumor activity; drug pharmacokinetics; drug distribution into the various organs; and tumor dependence on the localization and degree of vascularization. However, good drug activity correlations were seen in both IP and SC groups, and the outcome of the experiments remained

unaffected. The RC tumor exhibited high sensitivity to alkylating agents. With both sites of implantation the tumor was highly responsive to cyclophosphamide, α-TGT, BCNU, methyl-CCNU, and cisplatin. In addition, HMM, in the IP group, and TGU, in the SC group, had similarly high levels of effectiveness. Methotrexate, bleomycin, DTIC, and NSC-328378 were moderately active against RC in both groups. In contrast, mitotic inhibitors showed a lower activity against RC.

Vinblastine and nitrosoureas have been found to have limited usefulness in clinical chemotherapy of advanced renal cell carcinomas [7, 9, 10]. These drugs were effective against our animal model, extending the survival time substantially. However, it would be premature to consider RC a well-established model for selecting drugs with potential clinical activity against renal cell carcinoma.

Murphy developed a tumor model analogous to ours in which some chemotherapeutic experiments were performed [11]. Microscopically, Murphy's model was quite different from our tumor. This model was also sensitive to nitrosoureas and vinblastine, but did not respond to HMM [15, 16].

In our experiments, the RC model was as responsive to alkylating agents and antimetabolites as P388 leukemia, and almost as responsive to antimitotics as L1210 leukemia. The IP-implanted RC tumor showed a good responsiveness to drugs that are clinically active but inactive against L1210 (HMM, busulfan, and bleomycin). Similarly, good activity against RC was produced by the two tin derivatives (NSC-314263 and NSC-328378), whereas they showed only marginal activity in routine testing in IP P388 leukemia. These results suggest that the RC tumor is more sensitive to chemotherapeutic agents than P388 and L1210 leukemias. Indeed, the RC tumor offers a high yield and a high sensitivity, which are important requirements for a primary screen in experimental chemotherapy. For that reason, the RC model could provide a good prescreen for further testing of potential chemotherapeutic agents.

Acknowledgements. This work was performed under the anspices of the EORTC Screening and Pharmacology Group and was partially supported by the Banque Nationale de Belgique and by the National Cancer Institute (USA).

List of compounds

Adriamycin, hydrochloride: NSC-123127
BCNU: NSC-409962; 1,3-bis(2-chloroethyl)-1-nitrosourea
Bleomycin A2: NSC-125066; (2,4′-bithiarole)-4-carboxylic, 2′(2-aminoethyl)-monohydrate
Busulfan: NSC-750; 1,4(tetramethylene) dimethylsulfate
cis-Diamminedichloroplatinum: NSC-119875
Cyclophosphamide: NSC-26271; 2 *H*-1,3,2-oxazophosphorine, 2-(bis(2 chloroethyl)amino)tetrahydro-2 oxide, monohydrate
DTIC: NSC 45388; 5-(3,3-dimethyl-1-triazene) Imidazole-4-carboxamide
5-Fluorouracil: NSC-19893
Hexamethylmelamine: NSC-13875
Hydroxyurea: NSC-32065
Methyl-CCNU: NSC-95441; 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
Methotrexate: NSC-740; 4-amino-4 deoxy-N^{10}-methylpteroyglutamic acid
op′-DDD: NSC-38721; 1-(O-chlorophenyl)1-(*p*-chlorophenyl)2,2 dichloroethane
α-TGT: NSC-296934; α-triglycidyl triazinetrione
TGU: NSC-332844; triglycidyl urazol
Vinblastine: NSC-49842; vincoleukoblastine sulfate (1 : 1), monohydrate
Vindesine: desacetyl vinblastine amide sulfate
2-2′-Pyridyl-4-benzimidazol-diethyl-tin-dichloride: NSC-314263
3,4,7,8 Tetramethyl-1,10-phenanthroline: NSC-328378

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