Eur J Cancer Clin Oncol, Vol. 22, No. 7, pp. 773–780, 1986.
Printed in Great Britain.

0277–5379/86$3.00+0.00
Pergamon Journals Ltd.

Development and Some Characteristics of a P388 Leukemia Strain Resistant to 1, 2 : 5, 6-Dianhydrogalactitol

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Abstract—*Repeated intraperitoneal treatment of standard P388 mouse leukemia with dianhydrogalactitol (DAG) resulted in the development of a P388/DAG experimental mouse tumor which was resistant to the drug. Resistance was stable without DAG treatment throughout 80 passages.*

P388/DAG shows cross-resistance to alkylating agents such as nitrogen-mustard, cyclophosphamide and diacetyl-DAG but not to selected antimetabolites and tubulin binders and exhibits reduced sensitivity to nitrosoureas.

Resistance to DAG could not be overcome by the administration of maximally tolerated dose of DAG to tumor bearing mice. The resistant tumor is one chromosome short and shows a 13-fold increase of cells possessing a submetacentric marker chromosome.

INTRODUCTION

RESISTANCE to antitumor agents is one factor influencing the effectiveness of tumor therapy. The use of experimental tumor systems is important in selecting combinations of agents which are effective against drug resistant tumors and provides valuable information utilizable for determining the proper sequence of drug administration in order to preserve tumor sensitivity even during resistance developed to one of the drugs.

Drug-resistant tumor lines induced by alkylating agents are generally cross-resistant to most other alkylating agents [1–2]. Some chemically related agents, however, did not develop cross-resistance [1–7]. Schabel *et al.* [7,8,11] also found that L1210 leukemia lines resistant to BCNU or cyclophosphamide retained sensitivity to DAG.

DAG is one of the conversion products of dibromodulcitol (DBD) [12] and exhibits antitumor activity in a wide spectrum of experimental rodent tumors [13]. There is a direct evidence for the DNA alkylating property of DAG [14]. Clinical trials have also been performed [15–18].

Since resistance to DAG has not as yet been described, we attempted to render a P388 leukemia resistant to DAG and to determine some characteristics of the new tumor line including its sensitivity to other anticancer agents.

Accepted 6 November 1985.

MATERIALS AND METHODS

Dianhydrogalactitol, NSC 132313, was obtained from the Chinoin Pharmaceutical and Chemical Works, Ltd., Budapest, Hungary. Diacetyldianhydrogalactitol (diac-DAG), an analogue of DAG used in the cross resistance studies, was also obtained from Chinoin. The other agents were: dibromodulcitol (Elobromol) (DBD), NSC 104800, Chinoin, Budapest; CCNU, NSC 79037, Division of Cancer Treatment, National Cancer Institute, USA; BCNU (Carmustine), NSC 409962, Bristol; 1-(2-hydroxyethyl)-3-(2-chloroethyl)-3-nitrosourea (HeCNU), NSC 294895 new nitrosourea derivative was kindly provided by Dr. Eisenbrand DKFZ, Heidelberg, F.R.G., in the frames of the Screening and Pharmacology Group of EORTC; methotrexate (MTX), NSC 740, Lederle Ltd., U.S.A.; 6-mercaptopurine (6-MP), NSC 755, Wellcome; 5-fluorouracil (5-FU), NSC 19893, Hoffmann–La Roche, Basel; adriamycin, NSC 123127, Farmitalia, Milan; cyclophosphamide, NSC 26271, Asta, Bielefeld; nitrogen mustard (HN_2), NSC 762, Sigma, St.Louis; vincristine (VCR), NSC 67574, Gedeon Richter Ltd., Budapest, Hungary; and vinblastine (VBL), NSC 49842, Gedeon Richter Ltd., Budapest, Hungary, commercially available.

The P388 mouse leukemia strain generally used for chemotherapeutic screenings was kindly provided by Dr. Wodinsky, Arthur D. Little, Inc., Cambridge, MA. Up to the beginning of the

present studies, the tumor had undergone 27 transplantations, and this generation was considered to be passage 0.

Experimental animals were the F_1 hybrids of our own inbred H-Riop DBA_2 and H-Riop $C_{57}Bl/_6$ mouse strains: $C_{57}Bl/_6$ ♀ × DBA_2 ♂. Males and females comprised separate groups.

Tumor transplantations were carried out with i.p. inoculation of 10^6 cells per animal.

Development of P388 mouse leukemia strain resistant to DAG (P388/DAG)

The P388 tumor of BDF_1 mice was passaged on every 7th day and treated i.p. with rising doses of DAG. During the first 10 passages single, subeffective doses of DAG (the initial being 1.25 mg/kg) were given on the day after transplantation. After the 10th passage, the total dose of DAG per passage was gradually raised every one to five passages either by repeated treatments or by raising the dosage levels. Total dose was elevated only when the previous one had not caused any prolongation in the lifespan of animals. The maximum amount of DAG administered within one passage was four daily treatments at 3 mg/kg. The tumor that had been rendered resistant to DAG was maintained, from the 56th passage, in two lines: (a) P388/DAG was treated in every passage with 1 × 7.5 mg/kg DAG i.p. and (b) P388/DAG-U (untreated), which did not receive further treatmcnt with DAG. For sensitivity, cross resistance and chromosome analyses and cytological examinations untreated animals of the P388/DAG strain served as donors.

Sensitivity studies

Every 2 months 30–36 BDF_1 mice were inoculated with tumor cells derived from the P388/DAG strain, and of P388 sensitive and of P388/DAG-U. On the following day groups of six mice were injectcd intraperitoneally with single doses of DAG: 1.25; 2.5; 5.0; 7.5; 9.0; 10.0 mg/kg. Drug sensitivity was evaluated on the basis of survival time in days (T/C × 100%).

Cytological examination

On the fourth day after transplantation, eight groups of three mice bearing the sensitive tumor and eight groups of three mice bearing the resistant tumor were injected i.p. with single 7.5 mg/kg DAG, then, after 12, 24, 48, 72, 96, 120, 144 and 288 hr one group of each tumor bearing mice was examined. Untreated animals were killed daily, simultaneously until death. The ascites fluid was weighed and the per cent of the ascitocrit value (tumor cell volume related to total tumor volume) determined by centrifugation in a hematocrit centrifuge. Tumor cells suspended in Tyrode solution containing 0.5% eosin were counted in Buerker's chamber. Total cell number was expressed as the product of ascites weight and cell number per ml. In the smears stained according to the May–Grünwald–Giemsa technique, the proportions of dividing cells were determined. Five hundred cells per smear were counted.

Determination of the grade of resistance

Sensitive and resistant P388 cells were drained off under aseptic conditions and diluted with 3 ml Fischer medium to attain the 4×10^6 cells per ml ascites concentration. Then the cells were incubated for 1 hr at 37°C with the following concentration series of DAG:

P388 sensitive: –, 1.25, 2.5, 5.0, 10 μg/ml
P388/DAG: –, 1.25, 2.5, 5.0, 10, 20, 40, 80 μg/ml.

After incubation DAG was removed from the culture medium by triple washing in 3-3 ml Fischer medium devoid of the drug. The drug-free cell suspension was inoculated i.p., in 0.5 ml volume in BDF_1 mice. Resistance is expressed by the ratio of the smallest dose of the drug effective in the resistant and sensitive tumors.

Chromosome analysis

Chromosome analyses were performed with the ascites cells of P388/DAG (67th passage) and P388/DAG-U (100th passage) tumor lines and simultaneously with the ascites cells of the parent P388 sensitive tumor maintained under identical conditions and passaged with the same frequency. On the fourth day after transplantation cells were treated with 5 mg/kg colchicine. After 4 hr the ascites cells were pooled and centrifuged for 5 min at 500 ***g***, swollen in distilled water for 10 min and fixed 4 times in methanol: acetic acid, 3 : 1. The cells were placed on iced slides, dried and stained with Giemsa. Chromosome sets of 50 cells in metaphase per tumor line were counted.

Cross resistance studies

Groups of BDF_1 mice, each comprising six animals were inoculated with tumor cells of untreated donors of P388/DAG strain in 60–130th passage or with cells of the sensitive tumor. Doses were chosen to reach optimal effect in the sensitive tumor. If the selected dose was ineffective or toxic new groups treated with lower and/or higher doses respectively were also introduced in the resistant tumor line. The two tumors were compared for the increase in lifespan attainable with single doses or by repeated treatment with agents of various mechanism of action.

RESULTS

Development of P388/DAG and sensitivity studies

The results of drug sensitivity tests performed during development of resistance are presented in Table 1.

Sensitivity to DAG in the initial phase of maintaining the strain, i.e. when the strain was treated with single subeffective doses, did not change. Repeated treatment initiated a decrease in sensitivity, first indicated by an examination done after the 23rd passage, 6 months after the studies were initiated. The strain appeared to be solidly resistant to 5 mg/kg after the 31st passage. Resistance to DAG continued to increase until even the maximal tolerable doses did not increase the lifespan of animals. Treatment with high doses of DAG (9–10 mg/kg) resulted in occasional deaths due to toxicity. Consequently, the maximum tolerable dose of DAG was 7.5 mg/kg.

Results of sensitivity studies performed with the tumor line untreated since the 56th passage are shown in Table 2. The untreated tumor line has undergone 76 passages since the discontinuation of treatment. No reduction in resistance to DAG has been observed.

The sensitive tumor line did not exhibit altered sensitivity to DAG during this period (Table 3).

The mean survival time of untreated mice inoculated with 10^6 cells deriving from P388/DAG and P388/DAG-U tumors, respectively, was nearly identical with that of the parent P388 sensitive tumor bearing animals:

Subline	Average survival time Days	± S.D.	No. of mice
P388 sensitive	10.3	1.3	50
P388/DAG	10.1	0.9	42
P388/DAG-U	9.5	0.8	49

Cytological examinations

Total cell number in the parent (wild) P388 tumor gradually decreased after the administration of 1 × 7.5 mg/kg DAG and by the 120th hour it was merely 5% of the initial value. Regrowth started within 4–6 days. Total cell number in the P388/DAG tumor also decreased reaching its lowest value (50% of the initial one) by 48 hr and remained at this level up to the 120th hour, i.e. until the beginning of rapid regrowth (Fig. 1).

The dramatic cell death in the sensitive P388 tumor following drug administration was accompanied by continuously rising mitotic rate in the surviving cell population (6% at 120th hr), while in the P388/DAG the highest mitotic rate of the surviving population elevated up to 4–4.5% (at

Table 1. Sensitivity of P388/DAG during the development of resistance (T/C × 100%)†

Passage No.	Sex	DAG doses (mg/kg i.p. 1 × on day 1) 1.25	2.5	5.0	7.5	9.0	10.0
4	male	110	133	158			269
9	female	121	135	169			267
16	female	110	129	155			77*†
23	male	124	155	130			155
31	female	106	114	119			68*†
39	male		119	120	127	140	120
52	female		111	122	141	98*†	109*†
64	male		104	102	122	127†	
70	female		107		120		
86	male		116	102	104	108	
99	female		104	117	122	125	
105	male		91		105		
117	male		100		106		
123	male			99	102		
132	female			105		111*†	

* † toxic death.
† T/C × 100%: [lifespan of the treated group (days)/ lifespan of the control group (days)] × 100.

Table 2. Sensitivity of P388/DAG-U to DAG (T/C × 100%)†

Passage No.	Sex	DAG doses (mg/kg i.p. 1 × on day 1) 2.5	5.0	7.5	9.0
64	male	107	121	103	102
70	female	105		118	
86	male	117	128	130	120
89	male	102	112	137	121
99	female	105	117	121	120
105	male	108		119	
117	male	102		116	
123	male		109	111	
132	female		102		105*†

* † toxic death
† T/C × 100%: [lifespan of the treated group (days)/ lifespan of the control group (days)] × 100.

Table 3. Sensitivity of P388 sensitive tumor line to DAG (T/C × 100%)

Passage No.	Sex	DAG doses (mg/kg i.p. 1 × on day 1) 1.25	2.5	5.0	7.5	9.0	10.0
0	male	122	144	180			220
17	male		134	174	186		
35	female		182	238	232		118*†
64	male		162	198	200	168*†	
86	male		140	168	217	182	
105	female		121		163		
117	male		190		192		
123	male			167	181		
132	female			215		226	

* † toxic death.
† T/C × 100%: [lifespan of the treated group (days)/ lifespan of the control group (days)] × 100.

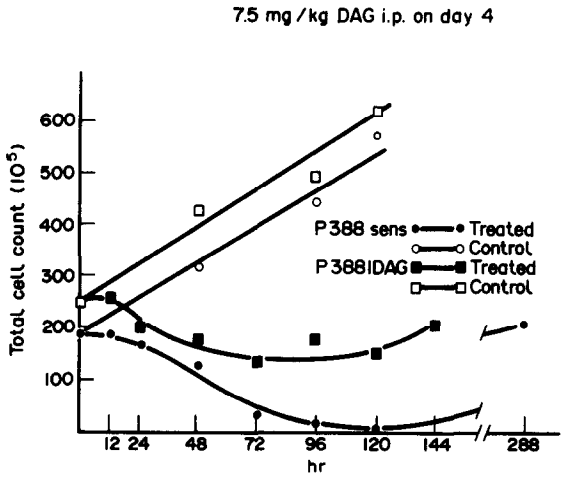

Fig. 1. Changes in total cell count after 1 × 7.5 mg/kg DAG in P388 sensitive and P388/DAG tumors.

48th hr) and quickly dropped to the control level (1.5–2%) (not presented in figure).

Mean cell volume increased in the parent tumor due to the treatment indicating a high ratio of giant cells. In the P388/DAG, however, the rise in mean cell volume was minimal and reversible (Fig. 2).

Grade of resistance

Inoculation with sensitive cells previously incubated with 10 μg/ml DAG evoked a 52% prolongation in lifespan. Similar effect (32%) in the resistant line could be achieved, however, by 80 μg/ml DAG dose. Consequently the grade of resistance of P388/DAG to DAG was equal to 8.

Karyotypes of tumor lines

The P388 sensitive mouse leukemia had a hyperdiploid stemline with 41 chromosomes consisting of acro- and telocentric ones. The chromosome number of the diploid stemline of P388/DAG and of P388/DAG-U tumors was 40. In the resistant

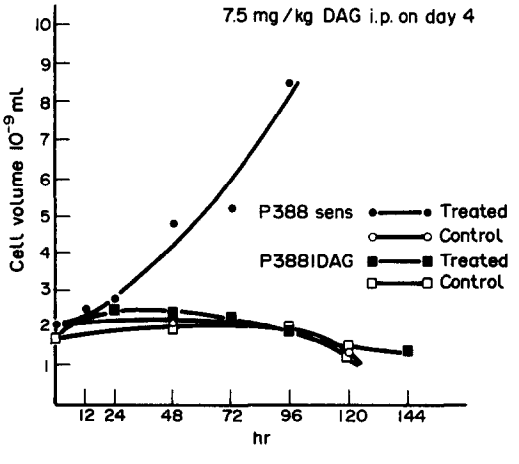

Fig. 2. Changes in mean cell volume under the effect of 1 × 7.5 mg/kg DAG in P388 sensitive and P388/DAG tumors.

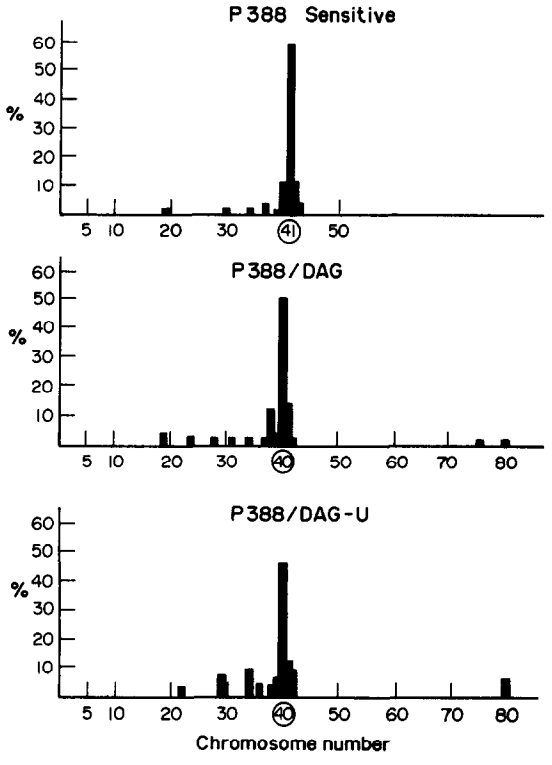

Fig. 3. Chromosome patterns of the P388 sensitive, P388/DAG and P388/DAG-U tumors.

tumors tetraploid cells were also observed (Fig. 3).

In 80% of the P388/DAG and of P388/DAG-U tumor cells a large submetacentric marker chromosome was present, that occurred in the P388 sensitive tumor only in 6%.

Cross resistance studies

Among the alkylating agents examined complete cross resistance to DAG was found in the case of nitrogen mustard, cyclophosphamide and diacetyl-DAG. Dibromodulcitol, however, did not affect either the wild or the P388/DAG leukemia. The P388/DAG line showed only decreased sensitivity to nitrosoureas compared to the sensitive P388 line (Table 4).

Agents with other mechanisms of action like vincristine, adriamycin, methotrexate and 5-fluorouracil exhibited no differential antitumor activity against either tumor. 6-mercaptopurine was ineffective in both tumor lines.

DISCUSSION

Administration of dianhydrogalactitol in single, sub-effective doses and later in gradually increasing repeated ones to mice bearing the P388 mouse leukemia resulted in the development of a P388 mouse leukemia resistant to the drug. Transplantable mouse tumors resistant to agents with different mechanisms of action have been induced in a similar way, i.e. by drug administration per pas-

Table 4. Effects of antitumor agents on P388 sensitive and P388/DAG tumors. Cross resistance studies

Agents	Doses (mg/kg i.p.)	P388 sensitive (T/C %)	P388/DAG (T/C %)	Pass.
DAG	9 × 2.5	197	116	60
	5 × 2.5	200	112	63
Diac-DAG	1 × 14	193	105	64
	7 × 6.3	183	91	77
DBD	1 × 200	115	95	60
	1 × 380	119	95	60
Nitrogen	8 × 0.25	257	93	77
mustard	8 × 0.5	289	106	77
Cyclophos-	8 × 5.0	227	102	130
phamide	8 × 10.0	239	97	130
	1 × 60.0	203	109	131
Cis-DDP	8 × 0.5	107	94	128
	8 × 1.0	151	97	128
	8 × 2.0	225	85	128
Adriamycin	8 × 0.75	213	209	73
	8 × 1.5	235	217	73
BCNU	1 × 15.0	248	137	98
	1 × 20.0	400	176	98
	1 × 25.0	350	203	98
	1 × 30.0	367	209	98
CCNU	7 × 2.0	137	97	60
	7 × 5.0	216	146	60
HeCNU	1 × 15.0	174	132	110
	1 × 20.0	296	180	99
VCR	8 × 0.1	208	181	71
	8 × 0.2	198	187	71
MTX	8 × 0.5	159	163	65
	8 × 0.75	176	168	65
5-FU	8 × 5.0	136	122	65
	8 × 10.0	202	200	77
	8 × 20.0	248	246	73
6-MP	8 × 20.0	143	124	65
	8 × 40.0	112	118	65

sages followed by inoculations [3–5, 7, 9, 19–23]. Some experiments of development of resistance to alkylating agents [24–26] showed the necessity of high doses when trying various dose-schedules. In agreement with their observations, DAG-sensitivity of our P388 tumor also did not change during the first 10 passages when suboptimal total dose of less than 2.5 mg/kg had been administered. Higher total doses (5–7.5 mg/kg), however, resulted in the gradual development of resistance to DAG. After passage 53 the maximal tolerable dose of the drug (1 × 9 mg/kg, or 4 × 3 mg/kg) did not cause any significant prolongation in lifespan. The drug tolerability of the tumor-bearing animals did not change during the development of resistance.

Grade of resistance was calculated on the basis of the cell killing effect of DAG measured after short term incubation. There was an 8-fold degree of resistance cca. the double of the therapeutic index (MTD/MED) of DAG on the P388 sensitive tumor. Resistance of the P388/DAG tumor can thus be considered to be high grade and, taking into consideration the *in vivo* data, complete.

Tumors resistant to alkylating agents generally need maintenance therapy for preserving their resistance [7, 19, 22], although the L1210/CPA [7, 25], L1210/BCNU, B16/meCCNU [27] and Yoshida/MDMS [28] have preserved their drug resistance over a long period of time.

Our studies demonstrate that the sensitivity of the P388/DAG-U remained identical with that of P388/DAG after the cessation of the treatment.

Experimental tumor lines resistant to alkylating agents are often accompanied by quantitative or qualitative chromosome changes detectable by light microscopy [19, 29–33] but the causal relationship between resistance and chromosome changes has not been proven. Ujházy succeeded in proving the drugspecific causal relationship of resistance and altered karyotype with the aid of HN_3 resistant tumor lines developed in three different ways but having identical karyotype [32]. The karyotype characteristics of a resistant tumor can be attributed to the non-specific, mutagenic effect of the inducer alkylating agent or to the selection of a resistant tumor cell population with different karyotype existing in the tumor from the very first.

Our karyogram of the original *in vivo* P388 sensitive tumor corresponds to Kuznyecova's analysis [34]. During the development of DAG-resistance the chromosome number of the stemline decreased by one and 80% of the resistant cells contained the submetacentric marker chromosome present in 6% of the cells in the wild tumor. P388/DAG probably was derived from selection since the chromosome set of the stemline of the DAG resistant line was also present in the parent tumor cell population. There is little likelihood that resistance developed by mutation since DAG showed only moderate mutagenicity by the Ames test [35].

In case of complete selection, successive generations preserve the characteristics of the tumor cell population. When selection is incomplete, the stability of resistance is influenced by the growth rate of the selected cells. Resistance remains stable even in case of incomplete selection provided that the growth rate of the resistant subpopulation is not lower than that of the sensitive line [26]. Since the cell number of P388/DAG is not lower than that of its sensitive counterpart of the same age and its doubling time is not longer either, there is a chance for survival (maintenance) of the P388/DAG over many generations. The presence of a small sensitive subpopulation within the resistant tumor is also reflected by the fact that in 20% of the cells the karyotype corresponds to that of the sensitive tumor and that high doses of DAG are capable of producing a limited cell death in the resistant tumor.

Some data demonstrated cross resistance among alkylating agents [2–5, 9, 10, 36–39], but much

experimental evidence suggests that the resistant tumors preserved their sensitivity to other alkylating agents in a measure observed with the parent (sensitive) tumor or showed decreased sensitivity, the value of which, however, did not reach the level considered 'resistant' [8, 28, 40, 41]. This phenomenon refers to the fine differences in the mechanism of action of the alkylating agents. The several intermediate positions in the relation of drug sensitivity and resistance have been pointed out by Schabel [7, 11] and Skipper [42] by quantifying the order of magnitude of the drug induced changes in cell number.

The P388/DAG showed complete cross resistance to all the alkylating agents examined except to the nitrosoureas. DBD, structurally related to DAG, was ineffective in both DAG resistant and sensitive tumors. The resistant line preserved its sensitivity to some antimetabolites, tubulin binders, and intercalating agents. So, this is not the case of pleiotrop resistance.

In agreement with others [1, 7, 8, 11 ,27, 43–46], and particularly with Schabel *et al.* [7, 8, 11] who found the L1210/BCNU to be sensitive to DAG, we also observed the lack of cross resistance between nitrosoureas and other alkylating agents.

The cause of resistance developing to the alkylating agents is generally sought in altered transport processes, defective drug metabolism, enhanced inactivation or increased DNA-repair capacity [47].

DAG produced, under identical conditions, identical numbers of cross links in the DNA of P388 sensitive and P388/DAG tumors [48] thus altered transport process or metabolic changes leading to inactivation are not likely to play a role in the mechanism of resistance.

In addition to alkylating the DNA, the nitrosoureas are capable of carbamoylating the proteins and nucleic acids. There are experimental results suggesting that DNA repair may be influenced by this process [49, 50].

It is presumable that the incomplete cross resistance of P388/DAG to nitrosoureas is to be attributed to the effect of nitrosoureas delaying DNA repair.

As it can be expected from the lack of cross resistance between nitrosoureas and other alkylating agents the combined administration of hexitols and nitrosoureas may be reasonable both under experimental conditions [51] and in clinical practice [18].

REFERENCES

1. Hill BT, Collateral sensitivity and cross-resistance. In: Fox BW, Fox M, eds. *Antitumor Drug Resistance. Handbook of Experimental Pharmacology*. New York, Springer, 1984, Vol. 72, 673–697.
2. Skipper HE, Hutchinson DJ, Schabel FM Jr. *et al.* A quick reference chart on cross resistance between anticancer agents. *Cancer Chemother Rep* 1972, **56**, Pt. 1, 493–498.
3. Csányi E, Halász M. Cross-resistance studies on 1.6-dibromo-dideoxy-D-Mannitol (DBM)-resistant Yoshida s.c. sarcoma. *Br J Cancer* 1967, **21**, 353–357.
4. Gáti É. Keresztrezisztencia-vizsgálatok alkiláló szerekkel és alkaloidokkal szemben rezisztens átoltható daganatokon. Orvostudomány 1970, **21**, 231–248.
5. Gáti É. Studies on cross-resistance to degranol and dibromodulcitol-resistant Yoshida tumours. *Int J Cancer* 1968, **3**, 260–264.
6. Wheeler PG. Studies related to mechanism of resistance to biological alkylating agents. *Cancer Res* 1963, **23**, 1334–1349.
7. Schabel FM Jr, Trader MW, Laster WR, Wheeler PG, Witt MH. Patterns of resistance and therapeutic synergism among alkylating agents. In: Schönfeld H *et al.*, eds. *Antibiotics Chemother.* Basel, Karger, 1978, Vol. 23, 200–215.
8. Schabel FM Jr, Skipper HE, Trader MW, Laster WR Jr, Corbett TH, Griswold DP Jr. Concepts for controlling drug-resistant tumor cell. In: Mouridsen HT, Pafshof T, eds. *Breast Cancer Experimental and Clinical Aspects.* Oxford, Pergamon Press, 1980, 199–211.
9. Ujházy V, Winkler A. Nitrogen mustard-resistant Yoshida sarcoma and cross-resistance studies. *Neoplasma* 1965, **12**, 11–14.
10. Ujházy V. Chromosomal studies with the nitrogen mustard-sensitive and resistant Yoshida tumour. *Neoplasma* 1968, **15**, 657–661.
11. Schabel FM Jr, Skipper HE, Trader MW, Laster WR Jr, Griswold DP Jr, Corbett TH. Establishment of cross-resistance profiles for new agents. *Cancer Treat Rep* 1983, **67**, 905–922.
12. Institoris L, Németh L, Somfai S, Gál F, Hercsei I, Zaka S, Kellner B. Investigation into the correlation of cytostatic activity with the *in vitro* diepoxide formation on some terminally substituted hexitols. *Neoplasma* 1970, **17**, 15–22.
13. Németh L, Institoris L, Somfai S *et al.* Pharmacologic and antitumor effects of 1,2:5,6-dianhydrogalactitol (NSC 132313). *Cancer Chemother Rep* 1972, **56**, Part 1, 593–602.
14. Institoris E, Tamás J. Alkylation by 1,2:5,6-Dianhydrogalactitol of deoxyribonucleic acid and guanosine. *Biochem J* 1980, **185**, 659–666.
15. Ahmann DL, O'Comell MJ, Bisel HF, Edmondson JH, Halm RG, Frytak S. Phase II.

Study of dianhydrogalactitol and ICRF-159 in patients with advanced breast cancer previously exposed to cytotoxic chemotherapy. *Cancer Treat Rep* 1977, **61**, 81–82.
16. Haas CD, Baker L, Thigpen T. Phase II. Evaluation of dianhydrogalactitol in lung cancer: a southwest oncology group study. *Cancer Treat Rep* 1981, **65**, 115–117.
17. Eagan RT, Ingle JN, Frytak S *et al.* Platinum based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. *Cancer Treat Rep* 1977, **61**, 1339–1345.
18. Eagan RT, Dinapoli RP, Hermann RC Jr, Groover RV, Layton DD Jr, Scott M. Combination carmustine (BCNU) and dianhydrogalactitol in the treatment of primary brain tumors recurring after irradiation. *Cancer Treat Rep* 1982, **66**, 1647–1649.
19. Gáti É. Chromosomal analysis on Yoshida tumor cells sensitive and resistant to dibromodulcitol. *Neoplasma* 1979, **26**, 79–83.
20. Johnson RK, Chitnis MP, Embrey WM, Gregory EG. *In vivo* characteristics of resistance and cross resistance of an adriamycin-resistant subline of P388 leukemia. *Cancer Treat Rep* 1978, **62**, 1535–1547.
21. Skovsgaard T. Development of resistance to rubidazone (NSC 164011) in Ehrlich ascites tumor *in vivo*. *Cancer Chemother Rep* 1975, **59**, 301–308.
22. Ujházy V, Winkler A. The degree and stability of resistance of a nitrogen mustard-resistant subline of Yoshida tumor. *Folia Biologica* 1965, **11**, 434–437.
23. Danø K. Development of resistance to adriamycin (NSC 123127) in Ehrlich ascites tumor in vivo. *Cancer Chemother Rep* 1972, **56**, 321–326.
24. Schmid FA, Hutchinson DJ. Effect of different doses of methotrexate (NSC 740), cytosine arabinoside (NSC 63878) and cyclophosphamide (NSC 26271) on drug resistance in mice with L1210 leukemia. *Cancer Chemother Rep* 1972, **56**, Part 1, 473–481.
25. de Wys WD. A dose-response study of resistance of leukemia L1210 to cyclophosphamide. *J Nat Cancer Inst* 1973, **50**, 783–789.
26. Inaba M, Fujikura R, Sakurai Y. Comparative study on *in vivo* development of resistance to various classes of antitumor agents in P388 leukemia. *Gann* 1979, **70**, 607–613.
27. Schabel FM Jr. Nitrosoureas: a review of experimental antitumor activity. *Cancer Treat Rep* 1976, **60**, 665–698.
28. Fox M, Fox BW. The establishment of cloned cell lines from Yoshida sarcoma having differential sensitivities to methylene dimethane sulphonate *in vivo* and their cross-sensitivity to X-rays, UV and other alkylating agents. *Chem-Biol Interactions* 1971, **4**, 363–375.
29. Schmid FA, Hutchinson DJ. Collateral sensitivity of resistant lines of mouse leukemia L1210 and L5178Y. *Cancer Res* 1972, **32**, 808–812.
30. Voitovitskii VK, Edygenova Ak, Kabiev OK. Comparative cytogenetic study of the resistance of tumor cells to sarcolysine. *Genetika* 1971, **7**, 89–93.
31. Schuette BP, Rabinovitz M, Vistica DT. A comparative cytogenetic study of melphalan-sensitive and -resistant murine L1210 leukemia cells. *Cancer Lett* 1980, **8**, 335–341.
32. Ujházy V. Identical karyotypes in acquired drug-resistant subline of Yoshida sarcoma. *Neoplasma* 1974, **21**, 665–669.
33. Parsons PG, Morrison L. Melphalan-induced chromosome damage in sensitive and resistant human melanoma cell lines. *Int J Cancer* 1978, **21**, 438–443.
34. Kuznyecova LE, Samsonova LI. Citogeneticeskaja characteristica nekotorih perevivaemih opuholej musej. *Vopr Onkol* 1979, **11**, 54–57.
35. Tóth K, Sugár J, Somfai-Relle S, Hegedüs L. Mutagenicity of the dibromodulcitol (DBD, an alkylating anticancer drug) and its mono- and bifunctional conversion products studied by the Salmonella/microsome assay. *Carcinogenesis* 1982, **3**, 333–336.
36. Gáti É, Horváth IP. The role of the carrying molecule in the cross-resistance against sugar alcohol derivatives. *Eur J Cancer* 1969, **5**, 553–557.
37. Goldenberg GJ. The role of drug transport in resistance to nitrogen mustard and other alkylating agents in L5178Y lymphoblasts. *Cancer Res* 1975, **35**, 1687–1692.
38. Somfai-Relle S, Gáti É, Bence J, Gál F. New derivatives of Dianhydrogalactitol (NSC 132313) with significant antitumor activity. In: Siegenthaler W, Lüthy, eds. *Current Chemotherapy*. Washington, DC, American Society for Microbiology, 1978, Vol. II. 1302–1304.
39. Jeney A, Szabó J, Vályi-Nagy T. Investigation on the mechanism of action of cytotoxic hexitols. I. Cross resistance investigations on Yoshida sarcoma tumours. *Neoplasma* 1968, **15**, 231–240.
40. Wampler GL, Regelson W, Bardos TJ. Absence of cross-resistance to alkylating agents in cyclophosphamide-resistant L1210 leukemia. *Eur J Cancer* 1978, **14**, 977–982.
41. Tyrer DD, Kline I, Gang M, Goldin A, Venditti JM. Effectiveness of antileukemic agents in mice inoculated with a leukemia L1210 variant resistant to 5-[3,5-bis(2-chloroethyl)-1-triazeno] imidazole-4-carboxamide (NSC 82196). *Cancer Chemother Rep* 1969, **53**, Part 1, 229–241.
42. Skipper HE, Schabel FM Jr, Lloyd HH. Experimental therapeutics and kinetics: selection and overgrowth of specifically and permanently drug-resistant tumor cells. *Semin Hematol* 1978, **15**, 207–219.
43. Griswold DP, Dykes DJ, Kelley CA, Roberts BJ, Dominick CA. Approaches to combina-

tion chemotherapy in rat, mouse, and hamster tumors. *Cancer Chemother Rep* 1974, **4**, Part 2, 99–108.

44. Sandberg J, Goldin A. Antileukemic action of a new sugar derivative dimethansulphonate (NSC 122402) and related compounds in mice. *Cancer Chemother Rep* 1969, **53**, Part 1, 367–376.
45. Connors TA, Hare JR. The binding of ^{14}C labelled 1-(2-chloroethyl))-3-cyclohexyl-1-nitrosourea (CCNU) to macromolecules of sensitive and resistant tumours. *Br J Cancer* 1974, **30**, 477–480.
46. Kline I, Woodman RJ, Gang M, Venditti JM. Effectiveness of antileukemic agents in mice inoculated with leukemia L1210 variants resistant to 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (NSC 45388) or 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC 82196). *Cancer Chemother Rep* 1971, **55**, Part 1, 9–28.
47. Curt GA, Clendeninn NJ, Chabner BA. Drug resistance in cancer. *Cancer Treat Rep* 1984, **68**, 87–99.
48. Institoris E. Personal communication.
49. Kann HE Jr, Kohn KW, Lyles JM. Inhibition of DNA repair by the 1,3-bis(2-chloroethyl)-1-nitrosourea breakdown product 2-chloroethyl isocyanate. *Cancer Res* 1974, **34**, 398–402.
50. Erickson LC, Laurent G, Sharkey NA, Kohn KW. DNA crosslinking and monoadduct repair in nitrosourea-treated human tumour cells. *Nature London* 1980, **288**, 727–729.
51. Levin VA, Wheeler KT. Chemotherapeutic approaches to brain tumors: experimental observations with dianhydrogalactitol and dibromodulcitol. *Cancer Chemother Pharmacol* 1982, **8**, 125–131.