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Chemotherapy of the Transplantable Acute Leukemia L5222 in Rats

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SUMMARY

This study presents results of single-drug and combination chemotherapy of the transplantable acute leukemia L5222 in BD IX rats.

In leukemia L5222 there is a direct relationship between the number of transplanted cells and mean life expectancy.

After single-drug therapy with L-asparaginase, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), cyclophosphamide, cytosine arabinoside, daunomycin, 6-mercaptopurine, methylglyoxal bis(guanylhydrazone) dihydrochloride, prednisolone, or vincristine, the best therapeutic effect was observed with BCNU and cyclophosphamide. A massive-dose therapy with BCNU repeated twice or a combination of vincristine with cyclophosphamide or BCNU with cyclophosphamide yielded a high percentage of cures.

Moreover, leukemia L5222 seems to be suitable for studying the influence of drugs on the proliferation kinetics of leukemia cells.

INTRODUCTION

It has been pointed out on several occasions that results of chemotherapy should not be judged only in animal experiments with transplanted tumors, but that these therapy schemes should also be tested on autochthonous animal tumors, *i.e.*, on tumors that either have developed spontaneously or have been induced by chemical carcinogens (4, 5, 16). It is obvious that autochthonous animal tumors can be better compared with human tumors in their biological behavior and their susceptibility to chemotherapy than can transplanted tumors.

Since it was our intention to carry out these comparative studies on rat leukemias, we first had to gain experience with therapy of transplanted leukemias in rats. For this purpose we chose the transplantable rat leukemia L5222; the therapeutic results obtained led to a complete cure of the experimental leukemia.

There are thus far no publications on the influence of chemotherapy on rat leukemia L5222, which has been adopted during the past 2 years as a model at several international institutions, among them the European Organization for Research on Treatment of Cancer. Recently, Harriss and Hoelzer (6) and Hoelzer *et al.* (7-9) reported in detail on the growth behavior of this leukemia, its proliferation kinetics, and the influence on the normal hemopoiesis during its growth.

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There are few studies of rat leukemias, as compared to the great number of fundamental studies of experimental leukemias of the mouse, particularly leukemia L1210 (5, 14, 15, 18). Thus, in addition to investigating mouse leukemias, it appeared advantageous to carry out chemotherapy studies on rat leukemias since these can in part be classified on the basis of cytochemical characteristics (10).

Recently, Pearson *et al.* (11) reported on chemotherapeutic trials on Nova rat leukemia. They did not succeed in effecting cures with BCNU,[1] Cytoxan, or melphalan. However, we have found with rat leukemia L5222 that experience gained with mouse leukemias can also be applied in principle to rat leukemias. Cures can be effected by optimal scheduling. In this study it was possible to eliminate 10^9 leukemia cells by means of suitable therapy.

MATERIALS AND METHODS

Tumor. The leukemia L5222 was induced in 1967, 326 days after a single i.v. injection of ethylnitrosourea, 200 mg/kg body weight, in a 3-month-old female BD IX rat (10). Since then we have maintained the leukemia exclusively in BD IX rats, and it is at present in the 360th passage. A cell-free transfer of L5222 to newborn rats, using the technique of Gross, has remained negative on repeated occasions.

According to the investigations of Harriss and Hoelzer (6), the generation time of L5222 cells is 12 hr and the duration of DNA synthesis is 7 to 9 hr. This determination of the generation time was made in a late stage of the leukemia. Rajewsky (personal communication) found that the growth fraction was almost 100%.

Rats. The investigations were carried out on more than 1000 BD IX rats of both sexes (2). The animals had been bred in our own laboratory. The growth of L5222 does not depend on the sex of the rats. Once sexual maturity has been reached (3 months), the age of the animals no longer has any influence on the growth of leukemia L5222. All rats were 3 to 6 months old at the time of treatment.

Drugs. The drugs were kindly placed at our disposal by the following firms: cyclophosphamide (Endoxan) by Asta-Werke GmbH, Brackwede, Federal Republic of Germany; L-asparaginase (Crasnitin) by Bayer AG, Leverkusen, Federal Republic of Germany; VCR (Vincristin-Lilly) by Eli

[1] The abbreviations used are: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; VCR, vincristine sulfate; ara-C, cytosine arabinoside; 6-MP, 6-mercaptopurine; LD_{10}, lethal dose to 10% of animals; MST, median survival time; ILS, increased life-span.

Lilly GmbH, Giessen, Federal Republic of Germany; daunomycin (Daunoblastin) by Deutsche Farmitalia GmbH, Freiburg, Federal Republic of Germany; ara-C (Alexan) by Heinrich Mack Nachf., Illertissen, Federal Republic of Germany; prednisolone (Solu-Decortin-H) by Merck AG, Darmstadt, Federal Republic of Germany; and 6-MP (puri-Nethol) by Deutsche Wellcome GmbH, Grossburgwedel, Federal Republic of Germany. We obtained BCNU and methylglyoxal bis(guanylhydrazone) dihydrochloride from Dr. T. Schuchhardt GmbH, Hohenbrunn, Federal Republic of Germany.

Cyclophosphamide, 2% in 0.9% sodium chloride solution; L-asparaginase, 1000 units/ml 0.9% sodium chloride solution; VCR, 0.02% solution in water for injection; daunomycin, 0.2% in 0.9% sodium chloride solution; ara-C, 2% solution; prednisolone, 5% solution in water for injection; 6-MP, 1% suspension in water for injection; BCNU, 6% solution in dimethyl sulfoxide, then diluted with water to make up a 0.3% suspension for injection; methylglyoxal bis(guanylhydrazone) dihydrochloride, 0.5% solution in water for injection. All drugs were given i.p. Equitoxic doses ($\leq LD_{10}$) were chosen as a basis for single-drug chemotherapy as well as for combination chemotherapy.

Those rats that survived 60 days after the last day of treatment were considered cured. In over 1000 leukemic rats treated (including the results of other experiments), occurrence of leukemia after that time was not observed in a single case.

RESULTS

Pathology of Untreated L5222 Leukemia. As soon as 3 days after i.v. and 4 days after i.p. injection of 1×10^6 L5222 cells, pathological cells could be found in the peripheral blood. After the 4th or 5th day, respectively, a rapid increase of leukemia cells in the peripheral blood could be observed. In the terminal stage, values up to 0.5×10^6 leukemic cells/cu mm were counted in the peripheral blood. At the same time, we found a decrease of erythrocytes to $3.5 \times 10^6 \pm 0.5 \times 10^6$/cu mm, of thrombocytes to $0.1 \times 10^6 \pm 20{,}000$/cu mm, and of hemoglobin to 6.5 ± 0.3 g/100 ml. Normal values in BD rats (peripheral blood) are: leukocytes, $8{,}000 \pm 3{,}000$/cu mm; erythrocytes, $8.0 \times 10^6 \pm 1.2 \times 10^6$/cu mm; thrombocytes, $0.8 \times 10^6 \pm 0.15 \times 10^6$/cu mm; and hemoglobin, 15.1 ± 0.8 g/100 ml. At the autopsy of the deceased leukemic rats a marked hepatosplenomegaly and only moderately enlarged lymphatic nodes were found in each case.

With rat leukemia L5222, there is evidence of a direct relation between the number of implanted cells and the MST of the animals (Chart 1). The i.p. transplantation of 10^9 leukemia cells caused the recipients to die of leukemia after 5 ± 0.5 days (10 animals examined); with the i.p. transplantation of 2.5×10^6 cells death occurred after 10 ± 1.5 days (>500 animals examined). After i.p. inoculation of 10^2 cells 11 of 11 rats died after a median time of 14.5 ± 1 days; after i.p. inoculation of 10^1 cells 17 of 18 rats died of leukemia after a median time of 16.5 ± 1 days. After i.p. inoculation of 1 L5222 cell (calculated by dilution of hemocytometer-counted leukemic blood) 8 of 40 rats (20%) died of leukemia after 17.5 ± 1.5 days. This shows that with this transplantable rat leukemia successful transmission by 1 cell can be assumed.

Chemotherapy of L5222. Leukemia L5222 was chemotherapeutically treated in its advanced stage. As stated above, a generalization of leukemia with an increase of the pathological cells in the peripheral blood exists at the end of the 5th day after i.p. implantation of $\geq 1 \times 10^6$ leukemic cells. In our studies we usually injected between 2.0 and 3.0 $\times 10^6$ leukemic cells i.p. Treatment was started between the end of the 5th and the 7th days after inoculation.

Single Agents. L-Asparaginase proved to be only weakly effective in a total dose of 2000 units/kg body weight given in a single dose as well as after fractionated or daily therapy. ara-C and prednisolone also had only borderline activity. A single dose of methylglyoxal bis(guanylhydrazone) dihydrochloride on the 6th day after implant showed no therapeutic effect. All the same, scheduling will be necessary with this substance before it can be finally declared ineffective. 6-MP, daunomycin, and VCR proved to be of medium effectiveness.

When 6-MP was administered in a daily dose of 10 mg/kg on Days 5 to 11 after implant of 2.2×10^6 leukemia L5222 cells, there was an ILS of 208%. A single dose of 6-MP, 70 mg/kg, on Day 5 led to an ILS of 164%. The median time between the day of last treatment and death from leukemia was 18.9 and 20.6 days, respectively. When therapy with 6-MP was started 1 day later (Day 6), the MST of the treated rats was significantly reduced (Table 1).

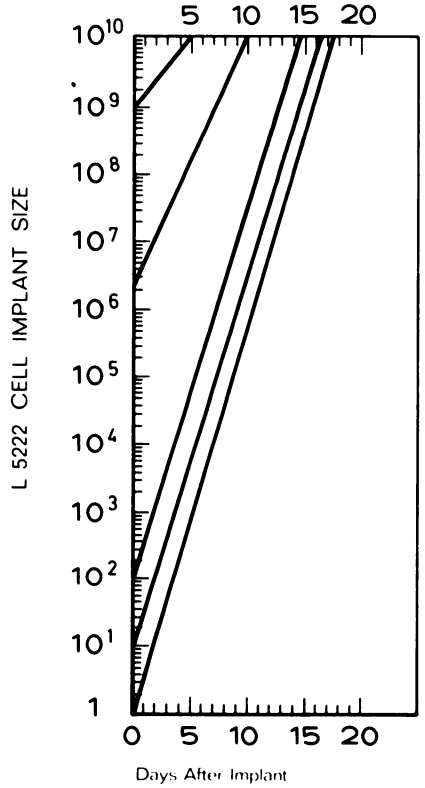

Chart 1. Growth of the transplantable acute leukemia L5222 in rats. L5222 cells were implanted i.p. into BD IX rats.

A single dose of daunomycin, 10 mg/kg, on Day 5 gave 135% ILS with a median time of 17.8 days between the day of treatment and death from leukemia, whereas a daily dose of 1 mg/kg on Days 5 to 14 (total dose, 10 mg/kg) gave 201% ILS with a median time of 15.2 days. It has not been possible to achieve a cure of leukemic rats with daunomycin by any of the therapeutic schemes applied (Table 1). A fractionated therapy with VCR, 0.25 mg/kg, on Days 5 and 8 resulted in an MST of 21.6 days (123% ILS). This schedule was superior to a single dose or daily therapy. However, no cures were achieved with VCR either (Table 1). BCNU showed a high activity against advanced L5222 (Table 2). This activity also existed in a very late stage of leukemia (Day 7 after i.p. transplantation of 2.5×10^6 cells). A single dose of BCNU, 20 mg/kg, on Day 6 led to an MST of 30.9 days (219% ILS). A fractionated dose of BCNU, 20 mg/kg, (10 mg/kg on Days 7 and 9 after i.p. inoculation of 2.5×10^6 cells) resulted in a cure of 9 of 10 treated rats.

Cyclophosphamide also showed itself very active against L5222 cells, although a delay in beginning therapy reduced the number of cured rats. A single dose of 80 mg/kg on Day 5 resulted in a cure for 4 of 10 treated rats. Later administration of the same dose did not result in any cures. Raising the dose of cyclophosphamide to 100 mg/kg on Day 6 gave cures in 2 of 10 rats. A total cyclophosphamide dose of 120 mg/kg in 2 single doses of 60 mg/kg on Day 6 and Day 8 cured 4 of 10 rats. When this therapy schedule started on Day 7, 1 of 10 rats was cured (Table 2).

When compared with the single dose, the effectiveness of 2 consecutive doses of cyclophosphamide showed that most of the MST was achieved with the 1st dose. A single cyclophosphamide dose of 10 mg/kg (Day 6 after implant) resulted in a MST of 20.1 days (105% ILS). By an additional dose of 10 mg/kg on Day 7, a MST of 22.0 days (124% ILS) was achieved. A comparison of the time between the last day of treatment and death from leukemia in these 2 groups (14.1 and 15.0 days, respectively) makes it clear that the effect of the 2nd dose of cyclophosphamide was considerably smaller than that of the 1st dose. This result was also obtained after raising the single doses to 33 mg/kg.

Combination Therapy. A sequential combination of cyclophosphamide with 6-MP, daunomycin with 6-MP, cyclophosphamide with prednisolone, as well as the combination of daunomycin with 6-MP and cyclophosphamide with

Table 1

Single-drug therapy of L5222 given in different schedules

L5222 i.p. inoculum size ($\times 10^6$)	Schedule[a]	Dose (mg/kg)	Total dose (mg/kg)	Median survival time T/C (days)[c]	Median survival time % ILS[d]	Median time between last treatment and death (days)[b]	60-day post-therapy survivors/total
			6-MP				
2.2	Day 5	70	70	25.6/9.7	164	20.6	0/8
2.2	Days 5, 8	35	70	24.9/9.7	157	16.9	0/8
2.2	Daily, days 5-11	10	70	29.9/9.7	208	18.9	0/8
3.0	Days 6-8	20	60	17.4/9.8	78	9.4	0/12
3.0	Days 6, 11	30	60	12.8/9.8	31	1.8	0/12
			Daunomycin				
2.2	Day 5	10	10	22.8/9.7	135	17.8	0/7
				21.9/9.7[e]	*126 (1)*[f]		
2.2	Days 5, 8	5	10	26.2/9.7	170	18.2	0/7
2.2	Daily, Days 5-14	1	10	29.2/9.7	201	15.2	0/8
				29.3/9.7	*202 (1)*		
			VCR				
2.2	Day 5	0.5	0.5	16.9/9.7	74	11.9	0/8
2.2	Days 5, 8	0.25	0.5	21.6/9.7	123	13.6	0/8
2.2	Daily, Days 5-9	0.1	0.5	17.0/9.7	75	8.0	0/8
2.5	Day 5	0.3	0.3	12.1/9.8	22	7.1	0/10
2.5	Day 6	0.3	0.3	11.5/9.8	17	5.5	0/10
2.5	Days 5, 7[g]	0.3	0.6	14.2/9.8	45	7.2	0/10
2.5	Days 6, 8[g]	0.3	0.6	13.0/9.8	33	5.0	0/10
3.0	Daily, Days 6-8	0.15	0.45	14.2/9.8	45	6.2	0/10

[a] Therapy was given at the end of the day, except where noted.
[b] Leukemic rats only.
[c] T, treated rats; C, control (untreated leukemic) rats.
[d] Percentage of ILS of treated rats over untreated controls.
[e] Numbers in *italics* include also the animals that died of late toxicity.
[f] Numbers in parentheses, number of animals dying of late toxicity.
[g] Second treatment after 36 hr.

daunomycin given simultaneously did not yield any cures in the schedules tested despite high dosage ($\leq LD_{10}$) (Table 3). On the other hand, the application of VCR, 0.3 mg/kg, at the end of the 5th day after implant, followed by cyclophosphamide, 80 mg/kg, at an interval of 36 hr, led to cures in 9 of 10 treated rats. A delay of the start of the therapy with this combination by 1 day reduced the number of cured animals to 5 of 10, and a delay of 2 days reduced it to 1 of 10 rats (Table 3).

The application of BCNU, 10 mg/kg, at the end of the 5th day after implant followed by cyclophosphamide, 80 mg/kg, at an interval of 36 hr, led to the cure of all rats. The effectiveness of this combination is such that, in spite of the 2-day delay in starting the therapy (start of therapy was on Day 7), 8 of 10 rats were cured. This corresponds to a log 9 tumor cell kill (Table 4).

Table 4 shows the results of chemotherapy trials on L5222 with 3 and 4 drugs. It becomes evident that, despite the increase in the number of drugs applied, no therapeutic results can be attained that are equivalent to an optimal dosage of BCNU or an optimal 2-drug combination with VCR and cyclophosphamide or BCNU and cyclophosphamide.

DISCUSSION

The results presented in this study are being tested in further experiments (which are not at present complete) with autochthonous rat leukemias. Special attention will then have to be paid to the optimal number of drugs to be applied. The leukemia L5222, which represents a rapidly proliferating tumor cell population, can be more favorably influenced by high-dose ($\leq LD_{10}$) therapy with BCNU in 2 fractions, by a sequential combination therapy with single doses of VCR and cyclophosphamide, or by combination therapy with single doses of BCNU and cyclophosphamide, than it can by multiple-drug therapy. It becomes clear that an increase in the number of drugs applied cannot replace favorable timing and optimal dosage with fewer drugs.

The use of an antimetabolite such as 6-MP in attempting to maintain remission did not lead to an appreciable increase in survival time or cures. These findings are in accord with the observations made by Frei *et al.* (4) on the AKR system of the mouse.

The clinical effectiveness of a combination therapy with VCR and cyclophosphamide has already been shown, particularly in treatment of advanced childhood leukemia

Table 2

Single-drug therapy of L5222 given in different schedules

L5222 i.p. inoculum size ($\times 10^6$)	Schedule[a]	Dose (mg/kg)	Total dose (mg/kg)	Median survival time[b]		Median time between last treatment and death (days)[c]	60-day post-therapy survivors/total
				T/C (days)[d]	% ILS[e]		
				BCNU			
2.5	Day 5	10	10	>27.5/9.8	>181	18.3	1/10
2.5	Day 6	10	10	24.1/9.8	146	18.1	0/10
2.5	Days 7, 9[f]	10	20	>65.4/9.8	>567	24.0	9/10
2.5	Days 6, 11	10	20	>66.6/9.8	>580	20.0	8/10
				>64.9/9.8[g]	*>562 (1)*[h]		
2.0	Day 6	20	20	30.9/9.7	219	24.9	0/7
				Cyclophosphamide			
2.5	Day 6	100	100	>35.9/9.8	>266	22.4	2/10
2.5	Daily, Days 6–8	33	99	>32.7/9.8	>234	20.8	1/10
2.5	Daily, Days 6–15	10	100	>36.4/9.8	>271	17.1	1/10
2.5	Day 6	33	33	22.1/9.8	126	16.1	0/10
2.5	Days 6, 7	33	66	27.6/9.8	182	20.6	0/10
2.5	Day 6	10	10	20.1/9.8	105	14.1	0/10
2.5	Days 6, 7	10	20	22.0/9.8	124	15.0	0/10
2.5	Day 5	80	80	>42.3/9.8	>332	22.2	4/10
3.0	Day 6	80	80	22.2/9.8	127	16.2	0/12
2.5	Day 7[i]	80	80	25.6/9.8	161	18.6	0/10
1.0	Days 6, 8[f]	60	120	>49.1/10.0	>391	22.3	4/10
				>46.7/10.0	*>367 (1)*		
2.5	Days 7, 9[f]	60	120	>34.6/9.8	*>253*	21.8	1/10

[a] Therapy was given at the end of the day, except where noted.
[b] The results include cured rats counted as 60-day posttherapy survivors.
[c] Leukemic rats only.
[d] T, treated rats; C, control (untreated leukemic) rats.
[e] Percentage of ILS of treated rats over untreated controls.
[f] Second treatment after 36 hr.
[g] Numbers in *italics* include also the animals that died of late toxicity.
[h] Numbers in parentheses, number of animals dying of late toxicity.
[i] Treatment in middle of 7th day after implant.

Table 3
Combination chemotherapy of L5222 (2-drug combinations)

Drug combination	L5222 i.p. inoculum size ($\times 10^6$)	Schedule[a]	Dose (mg/kg)	Total dose (mg/kg)	Median survival time[b] T/C (days)[d]	% ILS[e]	Median time between last treatment and death (days)[c]	60-day post-therapy survivors/total
Cyclophosphamide		Day 6	60	60				
+	3.0				24.6/9.8	151	4.6	0/15
6-MP		Days 14, 20	25	50				
Cyclophosphamide		Day 6	80	80				
+	3.0				22.9/9.8	134	14.9	0/12
6-MP		Daily, Days 6-8	20	60	*20.8/9.8*	*112 (2)*		
Daunomycin		Day 6	7	7				
+	3.0				21.2/9.8	116	13.2	0/12
6-MP		Daily, Days 6-8	20	60	*20.3/9.8*[f]	*107 (1)*[g]		
Daunomycin		Days 6, 11, 16	6	18				
+	3.0				26.3/9.8	168	10.3	0/12
6-MP		Days 6, 11, 16	30	90	*26.8/9.8*	*173 (1)*		
Cyclophosphamide		Days 8, 15	20	40				
+	2.0				31.0/9.7	220	16.0	0/11
Daunomycin		Days 8, 15	5	10	*29.9/9.7*	*208 (1)*		
Cyclophosphamide		Day 8	20	20				
+	2.0				22.9/9.7	136	12.9	0/10
Prednisolone		Daily, Days 8-10	50	150				
VCR		Day 5	0.3	0.3				
+	2.5				>62.8/9.8	>541	18.0	9/10
Cyclophosphamide		Day 7[h]	80	80				
VCR		Day 6	0.3	0.3				
+	2.5				>50.2/9.8	>412	20.0	5/10
Cyclophosphamide		Day 8[h]	80	80				
VCR		Day 7	0.3	0.3				
+	2.5				>37.2/9.8	>280	21.8	1/10
Cyclophosphamide		Day 9[h]	80	80	*>34.5/9.8*	*>252 (2)*		
Cyclophosphamide		Day 6	80	80				
+	3.0				23.9/9.8	144	15.9	0/12
VCR		Daily, Days 6-8	0.15	0.45				

[a] Therapy was given at the end of the day, except where noted.
[b] The results include cured rats counted as 60-day posttherapy survivors.
[c] Leukemic rats only.
[d] T, treated rats; C, control (untreated leukemic) rats.
[e] Percentage of ILS of treated rats over untreated controls.
[f] Numbers in *italics* include also animals that died of late toxicity.
[g] Numbers in parentheses, number of animals dying of late toxicity.
[h] Treatment in middle of 7th (8th, 9th) day after implant.

and generalized neuroblastoma (3, 13). Razek *et al.* (12) very recently reported a therapeutic synergism between VCR and cyclophosphamide on AKR lymphoma in the mouse and also against L1210 leukemia in the mouse.

Combination of BCNU with cyclophosphamide, which also acted synergistically against AKR lymphoma and L1210 leukemia (15, 19), has been applied successfully in patients (D. E. Bergsagel. Timed Drug Combinations and Chemotherapy: Do Clinical Results Justify Our Expectations? Workshop on Clinical Usefulness of Cell Kinetic Information for Tumor Chemotherapy. Rijswijk Z. H., October 14 to 15, 1974, in preparation.)

Thus, it is clear that rat leukemia L5222 is a useful model for developing treatment schedules that might be feasible for clinical trials. Furthermore, its high sensitivity to nitrosoureas affords a means of testing the chemotherapeutic activity of new nitrosoureas synthesized in our own institute.

Finally, the rat leukemia L5222 appears to be a suitable model for studies of proliferation kinetics. Depending on the weight of the rat, the lethal number of leukemia cells was 1.0 to 1.5 $\times 10^{10}$ leukemia blast cells per rat. At the time of death there were 4.0 to 5.0 $\times 10^9$ leukemic cells in the liver and 1.0 to 1.5 $\times 10^9$ leukemic cells in the spleen. These figures are approximations determined by the increase in weight of these organs in a total of 20 leukemic BD IX rats, about 250 g in weight, compared with controls. On the basis of a blood volume of 6.7 ml/100 g body weight (1), the number of leukemic blast cells in the peripheral blood is about 3 $\times 10^9$ cells/100 g body weight (taking into account 0.3 to 0.5 $\times 10^6$ blast cells/cu mm at the time of death). The approximate number of cells calculated by D. Hoelzer and E. B. Harriss (personal communication) for the bone marrow, lymph nodes, thymus, and other organs, such as lungs and gut, was about 1.0 $\times 10^9$ leukemic cells per rat at the time of death.

After transplantation of a high number of cells (10^9), the increase in the number of cells to this lethal number appears less rapid than when fewer cells are inoculated (Chart 1). The exponential growth of L5222 becomes evidently asymp-

Table 4
Combination chemotherapy of L5222 (2-, 3-, and 4-drug combinations)

Drug combination	L5222 i.p. inoculum size ($\times 10^6$)	Schedule[a]	Dose (mg/kg)	Total dose (mg/kg)	Median survival time[b] T/C (days)[d]	Median survival time[b] % ILS[e]	Median time between last treatment and death (days)[c]	60-day post-therapy survivors/total
BCNU		Day 5	10	10				
+	2.5				>67.0/9.8	>584		10/10
Cyclophosphamide		Day 7[f]	80	80				
BCNU		Day 6	10	10				
+	2.5				*>64.2/9.8*	*>555(1)*		9/10
Cyclophosphamide		Day 8[f]	80	80				
BCNU		Day 7	10	10				
+	2.5				>64.7/9.8	>560	21	8/10
Cyclophosphamide		Day 9[f]	80	80	*>62.0/9.8*[g]	*>533(1)*[h]		
Cyclophosphamide		Days 8, 15	20	40				
+								
Daunomycin	2.0	Days 8, 15	5	10	>38.6/9.7	>298	20.0	1/12
+					*>37.8/9.7*	*>290(1)*		
6-MP		Day 13	30	30				
Cyclophosphamide		Days 8, 15	20	40				
+								
VCR	2.0	Days 8, 15	0.2	0.4	>40.2/9.7	>314	16.5	2/11
+					*>37.6/9.7*	*>288(1)*		
6-MP		Day 13	30	30				
Daunomycin		Days 6, 7	5	10				
+								
Cyclophosphamide		Days 6, 7	10	20				
+	3.0				24.8/9.8	153	17.8	0/7
VCR		Days 6, 7	0.1	0.2				
+					*26.7/9.8*	*172(1)*		
6-MP		Days 6, 7	10	20				
Daunomycin		Day 6	5	5				
+								
Cyclophosphamide		Days 6, 7, 8	10	30				
+	3.0				>38.0/9.8	>288	18.0	2/8
VCR		Days 6, 7	0.1	0.2	*>41.1/9.8*	*>319(1)*		
+								
6-MP		Days 6, 7, 8	10	30				

[a] Therapy was given at the end of the day, except where noted.
[b] The results include cured rats counted as 60-day posttherapy survivors.
[c] Leukemic rats only.
[d] T, treated rats; C, control (untreated leukemic) rats.
[e] Percentage of ILS of treated rats over untreated controls.
[f] Treatment in middle of 7th (8th, 9th) day after implant.
[g] Numbers in *italics* include also animals that died of late toxicity.
[h] Numbers in parentheses, numbers of animals dying of late toxicity.

totic in the final stage, a finding that is known for solid tumors and that may be caused by crowding, with competition for nutrients or humoral factors resulting either in increased cytolysis or in a slowing of proliferation (17).

The effect of chemotherapy on the growth characteristics of leukemia L5222 is also worthy of attention. Comparison of the interval between the last day of treatment and the day on which the noncured rats died because of regrowth of leukemia (Tables 1 to 4), with the maximum of 18 days until death after transplantation of a single L5222 cell, makes it clear that the doubling time must have increased after chemotherapy. This increase of the survival time with leukemia is particularly striking after a single dose of BCNU, 20 mg/kg ($\alpha \leq 0.01$; Wilcoxon test). Whether an increase in the generation time or an alteration in the size of the proliferating pool is responsible for this [as shown by Young and De Vita (20) on L1210 with BCNU] should be clarified in further studies.

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