

Antitumor Principles Derived from *Vinca rosea* Linn

I. Vincalukoblastine and Leurosine

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SUMMARY

The experimental activity of a new clinically confirmed antitumor compound, Vincalukoblastine ($C_{46}H_{58}O_9N_4$), (VLB) as the sulfate has been described. Greatest activity was seen against the P-1534 acute lymphocytic leukemia in DBA/2 mice. Late as well as early stages of this leukemia were significantly affected by this compound. No synergistic or additive effects have been observed in combination therapy with other antitumor compounds.

A second indole-indoline alkaloid, leurosine, isomeric with VLB, has also been obtained from *Vinca rosea* Linn, with similar demonstrable experimental antitumor activity.

Two other alkaloids, vindoline ($C_{25}H_{32}O_2$) and catharanthine ($C_{21}H_{24}O_2N_2$), also obtained from *Vinca rosea*, were devoid of antitumor activity singly or in equimolar concentrations, but have been postulated as the biogenetic precursors of VLB and leurosine.

Preliminary studies *in vitro* demonstrated that certain compounds were capable of reversing the growth-inhibitory activity of VLB against human monocytic leukemia cells. These compounds were coenzyme A, aspartic acid, tryptophan, α -keto-glutaric acid, ornithine, citrulline, arginine, and glutamic acid.

VLB and leurosine are representatives of a new class of clinically active antitumor compounds which may interfere with the cellular metabolic pathways leading from glutamic acid to urea, and from glutamic acid to the citric acid cycle.

Concomitant with the preparation of clinically useful compounds from ancient plant remedies there has been an increased interest in studying the pharmacological action of plant alkaloids. In the course of investigating *Vinca rosea* Linn, certain crude fractions of the plant were submitted for evaluation in our cancer screening program. The fractions were discovered to give interesting and, in some cases, profound activity against the P-1534 leukemia, an acute lymphocytic leukemia transplanted in DBA/2 mice. These findings led to a prompt phytochemical investigation in these laboratories which resulted in the obtaining of three new alkaloids: leurosine, virosine, and perivine (24). Leurosine was found to have some activity against this experimental neo-

plasm. Shortly after these compounds were obtained Noble, Beer, and Cutts¹ reported obtaining another new alkaloid as a sulfate from *Vinca rosea*. They suggested the name Vincalukoblastine (VLB) for this compound. The most striking biological action of VLB was its leukopenic action in normal rats, which was used for its bioassay (4, 21). It was subsequently demonstrated in both the Collip (20) and Lilly Laboratories (13) that VLB also markedly inhibited the P-1534 leukemia. It is the purpose of this report to describe some of the experimental results obtained with these compounds and to suggest a possible hypothesis for the mechanism of action of this example of a new class of antineoplastic agents.

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MATERIALS AND METHODS

The P-1534 leukemia was implanted in DBA/2 mice with 0.5 ml. of a 10^{-4} dilution of spleen cells in medium 199 given intraperitoneally. This tumor is widely dispersed within 24 hours. Treatment started 24 hours after implantation, except where stated otherwise, and consisted of the daily administration of 0.5 ml. of the test compounds in physiological saline for 10 days, except when the test compound was not water-soluble. In such cases 0.125 per cent carboxymethylcellulose was added as a dispersant. Groups of five animals were used per compound, and treatment of all tumor-bearing animals was intraperitoneal unless otherwise indicated. Effects of a drug are in terms of increase in average survival times over that of saline-treated control groups. At the inoculum

Briefly, the method involved the mixing of defatted ground whole plant with an aqueous solution of 2 per cent tartaric acid, followed by extraction with benzene. After partial purification by acid-base treatment, the crude alkaloidal mixture was chromatographed on alumina. In a typical chromatographic column, a benzene solution of weak bases was chromatographed on Alcoa Grade F-20 activated alumina which had been previously treated with 10 per cent acetic acid.

RESULTS

The activities observed with the initial total extracts of the plant and with the first crude fractions, including some total alkaloidal fractions, are seen in Table 1. In all these early experiments there were a certain number of animals which sur-

TABLE 1
ACTIVITY OF ORIGINAL EXTRACT OF WHOLE PLANT AND CRUDE
FRACTIONS AGAINST P-1534 LEUKEMIA

Material	Dosage (mg/kg/day)	Av. wt. chg. (gm.) T/C	Av. surv. time (days)	Per cent increase in surv. time	Per cent indefinite surv.
Defatted whole plant*	120.0	+0.1/+2.7	25.6/14.8	73	0
Fraction A*	5	-0.6/+1.2	26.5/19.2	38	20
Fraction B*	30.0	-1.2/+1.2	24.0/19.2	25	40
Total alkaloids†	6.0	+0.8/+2.9	27.7/17.2	61	20
"	7.5	+0.2/+0.6	29.8/13.4	122	0
"	15.0	-2.3/+0.6	20.3/13.4	51	60
"	15.0	+0.3/+0.5	30.0/13.0	130	0
"	75(Oral)	-1.5/+0.6	20.6/13.4	53	0

* For chemical description, see reference (25).

† Total ethanol extracts, defatted and extracted with benzene and 2 per cent tartaric acid.

of malignant cells used, the average survival time of the controls usually varied from 13 to 16 days after implantation. An increase in survival time of 20 per cent, or more, over the controls was considered significant. All solid tumors were implanted subcutaneously by trocar, and groups of ten animals were used per compound. Inhibitions were in terms of mean tumor diameters derived from two-dimensional caliper measurements and compared with the diameters of control groups treated with physiological saline. An inhibition of 30 per cent, or more, was considered significant.

Tissue culture studies involved the planting of replicate cultures in Medium 199 for routine drug evaluation. Growth was determined by measuring the original inoculum and the volume of cells produced after a 1-week growth period in a Van Allen hematocrit as described by Waymouth (11). All tests were done in duplicate.

The procedures used to obtain the alkaloids of *Vinca rosea* have been described elsewhere (25).

vived "indefinitely." These experiments were usually terminated after 90–120 days. All such animals were still susceptible to the P-1534 leukemia when re-implanted.

A number of alkaloids have been obtained from *Vinca rosea* Linn in these and other laboratories (Table 2). It can be seen that one, leurosine, obtained in our laboratories, has the same empirical formula as VLB. It has been shown that the two alkaloids are isomeric and represent a new class of dimeric alkaloids containing both indole and dihydroindole moieties. The chemical and physical properties have been reported elsewhere (7, 19). Biologically, leurosine has also been shown to have demonstrable activity against the P-1534 leukemia (Table 3). In our experience leurosine as a free base has not been as active or as consistent an inhibitor of the P-1534 leukemia as has VLB. Consequently, the major portion of this study was devoted to work with VLB.

A dose-response effect could be demonstrated

in the P-1534 leukemia (Table 3) with VLB. "Indefinite" survivors observed with the crude preparations were never observed following administration of the pure alkaloids reported here. A study was made of VLB in combination with other antitumor agents at levels which have given antitumor effects in this laboratory in tumor systems sensitive to the other drugs studied. This was done with the hope that some synergistic effect might be noted which would yield a clue to possible mechanism of action (Table 4). None

TABLE 2
ALKALOIDS PREVIOUSLY REPORTED FROM
Vinca rosea

Name	Empirical formula	M.P. (° C.)
Ajmalacine	C ₂₁ H ₃₄ O ₃ N ₂	253-254
Tetrahydroalstonine	C ₂₁ H ₂₄ O ₃ N ₂	230-231
Serpentine	C ₂₁ H ₂₂ O ₃ N ₂	156-157
Lochnerine	C ₂₀ H ₂₄ O ₂ N ₂	202-203
Reserpine*	C ₃₃ H ₄₀ O ₆ N ₂	264-265
Akuammine*	C ₂₂ H ₂₆ O ₄ N ₂	258-260

NEW ALKALOIDS REPORTED FROM LILLY
AND OTHER LABORATORIES

Vindoline	C ₂₅ H ₃₂ O ₆ H ₂	154-155
Vindolinine-2 HCl	C ₂₁ H ₂₄₋₆ O ₂ N ₂ -2 HCl	210-212
Lochnericine	C ₂₁ H ₂₄ O ₂ N ₂	190-193
Vincalukoblastine	C ₄₆ H ₅₈ O ₉ N ₄	211-216

NEW ALKALOIDS REPORTED FROM
LILLY LABORATORIES

Leurosine	C ₄₆ H ₅₈ O ₉ N ₄	202-205
Catharanthine	C ₂₁ H ₂₄ O ₂ N ₂	126-128
Perivine	C ₂₀ H ₂₄ O ₃ N ₂	180-181
Virosine	C ₂₂ H ₂₆ O ₄ N ₂	258-264

* Not encountered in the studies reported here.

was observed, which indicated that the mechanism of action of VLB was probably different from that of any of the other compounds studied.

An important consideration in the experimental evaluation of a potentially useful therapeutic agent for the treatment of neoplastic disease is the ability of the agent to retard terminal phases of the experimental malignancy. VLB did significantly prolong the life span of animals given implants of the P-1534 leukemia (Table 5).

Experimentally, VLB has not been a "broad spectrum" antitumor agent, although limited effects against some other transplantable neoplasms have been obtained. They include activity against the Walker carcinosarcoma 256 in rats and a mammary adenocarcinoma of DBA/1 mice, which were inhibited 64 and 65 per cent after 10 days' treatment at 0.3 and 0.6 mg/kg/day, respectively. Three ascites tumors—the Ehrlich adenocarcinoma, an ascitic form of Sarcoma 180, and

the undifferentiated, chemically induced Freund tumor—were completely inhibited with respect to growth of ascitic cells following 10 days of treatment, but did develop occasional solid tumors at the site of inoculation. The L1210 leukemia in DBA/2 mice; the C-1498 leukemia, the E 0771 and 755 mammary adenocarcinomas in C57BL/6; the Mecca lymphosarcoma and Ridgway osteogenic sarcoma in AKR mice; and solid Sarcoma 180 in Swiss mice were unaffected. Dosage levels in these studies were usually in the range of 0.3-0.45 mg/kg, but perhaps if these were increased there might have been some effect. Noble, Beer, and Cutts have also reported some carcinostatic effects with VLB against a transplanted mammary

TABLE 3
RESULTS OBTAINED WITH LEUROSINE AND VLB TREATMENT OF THE P-1534 LEUKEMIA

Material	Dosage (mg/kg/ day)	Av. wt. chg. (gm.) T/C	Av. surv. time (days) T/C	Per cent increase in surv. time
Leurosine	7.5	+0.2/+1.9	22.0/14.6	50
"	3.0	+1.4/0.0	27.3/13.0	110
"	6.0	+1.4/+1.3	26.4/17.2	53
"	150(Oral)	-1.2/+0.6	19.6/13.4	46
VLB	.05	+2.7/+0.7	23.0/16.2	41
"	.1	+1.7/+0.7	24.8/16.2	53
"	.3	+0.6/+0.9	22.8/13.4	70
"	.45	+0.3/+0.3	27.0/13.6	98
"	.45	+0.8/+0.6	30.6/13.2	131
"	.6*	-1.7/-1.6	28.6/11.4	150
"	.6*	-2.0/-1.6	26.0/11.4	128
"	.6*	+0.3/-1.6	24.6/11.4	115
"	1.5 (Oral)	-1.2/+0.7	27.6/16.2	70

* Three different lots of VLB-SO₄.

tumor in DBA mice, a transplantable rat sarcoma, and more recently the L1210 and P-1534 leukemias in BDF¹ hybrid mice, IRC 741/139B leukemia in rats, and Sarcoma 180 in Swiss (20, 21). The ineffectiveness of VLB in our studies in L1210 and Sarcoma 180 may have been due to differences in dosage and strains of host animals used. Increased "curability" of transplantable tumors where some host histo-incompatibility exists is well known (14).

Mechanism studies.—The mechanism of action of VLB is not yet known, although some preliminary observations may indicate the direction which such studies should pursue. Tissue culture studies have indicated a selective inhibition of growth of certain cells of malignant origin. An example is the J96 cell derived from peripheral blood of human monocytic leukemia, and an em-

TABLE 4—RESULTS OBTAINED BY COMBINING VLB THERAPY OF P-1534
LEUKEMIA WITH OTHER ANTITUMOR DRUGS

DRUG	DOSAGE (MG/KG/DAY)		AV. WT. CHG. (GM.)	FREQ.	AV. SURV. (DAYS)	PER CENT INCREASE IN SURV. TIME
	Other drug	VLB				
6-Mercaptopurine		0.9	+0.3	×10	27.0	98
		0.9	+0.2	×5*	26.0	91
		0.45	+0.7	×10	21.6	58
	60		-1.6	×10	15.0	10
	30	0.45	-1.3	×10	22.4	64
	60	0.9	+1.1	×5*	23.2	70
0-Diazoacetylserine	6		+0.6	×10	17.6	29
	3	0.45	+1.7	×10	17.0	25
	6	0.9	-0.3	×5*	25.6	88
6-Diazo-5-oxo-L-norleucine	0.6		+0.3	×10	15.2	10
	0.3	0.45	+0.3	×10	20.8	52
	0.6	0.9	×0.7	×5*	20.2	48
Saline			+0.3	×10	13.6	
HN ₂		.45	+0.5	×10	30.6	131
	0.6		-2.0	×10	15.0	
	0.3	.225	-1.3	×10	19.8	50
Triethylene melamine	0.6	.45	-2.1	×5*	19.8	50
	0.3		-1.4	×10	18.8	42
	0.15	.225	0.0	×10	19.0	43
8-Azaguanine	0.3	.45	-0.8	×5*	21.8	64
	60.0		+1.8	×10	18.0	36
	30.0	.225	+2.4	×10	18.0	36
Cortisone	60.0	.45	+0.5	×5*	23.6	78
	60.0		+0.1	×10	16.0	21
	30.0	.225	+0.3	×10	23.6	78
Colchicine	60.0	.45	+0.1	×5*	21.2	60
	0.6		+2.1	×10	20.8	57
	0.3	.225	+0.2	×10	25.4	92
2,6-Diaminopurine	0.6	.45	+0.1	×5*	22.2	68
	60.0		+0.3	×10	13.6	
	30.0	.225	+1.6	×10	21.2	60
6-Mercaptopurine	60.0	.45	-0.9	×5*	21.0	59
	3.0		+4.6	×10	14.8	
	1.5	.225	+1.7	×10	22.0	66
TESPA†	3.0	.45	+0.2	×5*	22.2	68
	1.2		+0.6	×10	14.6	
	0.6	.225	+1.5	×10	22.2	68
Myleran‡	1.2	.45	-0.2	×5*	27.8	110
	15.0		+0.2	×10	14.2	
	7.5	.225	+0.2	×10	24.2	83
Epoxypropidine§	15.0	.45	+0.9	×5*	22.0	66
	4.5		+0.2	×10	21.4	62
	2.25	.225	0.0	×10	22.2	68
Methotrexate#	4.5	.45	+0.8	×5*	25.2	90
	1.8		+1.9	×10	16.0	21
	0.9	.225	+0.8	×10	18.6	40
6-Diazo-5-oxo-L-norleucine	1.8	.45	+0.8	×5*	20.4	54
	0.6		+3.0	×10	14.2	
	0.3	.225	+2.7	×10	19.8	50
Saline	0.6	.45	+1.3	×5*	20.0	51
			+0.6	×10	13.2	

* Each compound given once daily on alternate days for 5 days each, as compared with simultaneous administration daily of the lower levels of the drugs for 10 days. Total dosage for each group is the same.

† TESPA = N-N'-N''-triethylenethiophosphoramidate. ‡ Myleran = 1,4-dimethanesulfonybutane.

§ Epoxypropidine = 1,1-di(2,3-epoxypropyl)-4,4-bipiperidine.

Methotrexate = 4-amino-N¹⁰methyl folic acid.

brionic connective tissue cell (LLC-He₁) isolated in our laboratories. A TCD/50 for the J96 cell has been 0.00083 $\mu\text{g}/\text{ml}$ and, for the LLC-He₁, 0.002380 $\mu\text{g}/\text{ml}$ in the same experiment. A TCD/50 is interpreted as the dose/ml of tissue culture medium which causes a 50 per cent inhibition of growth compared with that of untreated replicate control cultures during a 1-week period of growth. This figure will vary with and is dependent upon the size of the cell inoculum. This type of study has been used to study reversal of the cell inhibitory activity of VLB. The results obtained in a typical experiment are seen in Table 6. Glutamic acid completely reversed the growth inhibition of VLB in the concentrations used. Tryptophan

and coenzyme A gave partial reversal of this effect. Of the materials tested in these preliminary studies, glutamic acid has been the most consistent. Since this is a delicate system, its reliability may be open to question. However, we have pursued this approach, since VLB has no easily demonstrable microbiological activity. Reversal studies *in vivo* with VLB and the P-1534 leukemia are in progress, based upon these preliminary tissue culture studies with VLB as well as with leucosine. Partial reversal of the antileukemic activity of VLB has been confirmed *in vivo* with tryptophan and glutamic acid. A summary of the substances studied by the *in vitro* system is seen in Table 7. All these compounds have been tested

TABLE 5
RESULTS OBTAINED WITH TREATMENT DELAYED FOR VARIOUS TIMES
AFTER IMPLANTATION OF THE P-1534 LEUKEMIA

DRUG	DOSAGE (MG/KG/DAY) FREQ.	AV. SURV. TIME (DAYS) WITH TREATMENT STARTING ON DAY				
		1	3	6	8	10
VLB	0.6 × 3	>52	24.7	25.2	19.4	
"	0.45 × 7					
"	0.6 × 2					
"	0.45 × 8					
"	0.45 × 10					
Saline	0.45 × 10	18.4				
6-Mercaptopurine	3.0 × 10	17.6	19.0	18.4	18.2	18.4
Methotrexate	0.9 × 10	20.0	21.6	21.4	19.0	18.6
Epoxypropidine	4.5 × 10	>38.5	24.8	23.2	30.4	19.6
VLB	0.45 × 10	>38.0	37.0	31.8	28.8	25.2
Saline		16.0				

TABLE 6
EXAMPLE OF EFFECTS OBTAINED WITH VARIOUS AGENTS
UPON THE INHIBITION OF J96 CELLS BY
VLB TISSUE CULTURE

Agents	Hematocrit readings (H.R.)	Average H.R.	Per cent inhibition
Controls	1.6, 1.8	1.70	
VLB	0.9, 0.9	0.90	47
Glutamine+VLB	0.7, 0.8	0.75	56
Coenzyme A+VLB	1.3, 1.6	1.45	15*
Folic acid+VLB	0.7, 1.0	0.85	50
Ca pantothenate+VLB	0.9, 1.0	0.95	45
Phenylalanine+VLB	0.9, 1.0	0.95	45
Glutamic acid+VLB	1.9, 1.6	1.75	0†
Tryptophan+VLB	1.6, 1.4	1.50	12*

All compounds including VLB were in T. C. medium 199 at concentrations of .005 $\mu\text{g}/\text{ml}$. Hematocrit reading of original cell inoculum was 0.3.

* Partial reversal.

† Complete reversal.

against a single TCD/50 level of VLB while four levels of the test compound were utilized, usually at .05, 0.25, .005, and .0025 $\mu\text{g}/\text{ml}$ of tissue culture medium. These preliminary observations suggest an interference with the metabolic pathways leading from glutamic acid to urea via ornithine, arginine, etc., and from glutamic acid to the citric acid cycle via α -ketoglutaric acid. A more complete report of these studies *in vivo* and *in vitro* will be presented elsewhere.

The J96 cell has also been used to study some cytological aspects of the mechanism of action of VLB. In one series of experiments it was found that VLB caused an arrested metaphase.² This has been similarly observed in tissue cultures inoculated with urine from VLB-treated patients,

² C. G. Palmer, D. Livengood, A. Warren, P. J. Simpson, and I. S. Johnson. The Action of Vincalukoblastine on Mitosis *In Vitro* (unpublished manuscript).

indicating excretion of VLB in the urine.³ The type of arrest caused was that which is termed C-mitosis, which indicates an effect upon the mitotic spindle. At the same concentration no postmetaphase stages were observed with VLB-treated J96 cells, but were observed in VLB-treated LLC-He₁ cells. In similar studies with Ehrlich ascites cells *in vivo* Cutts has seen cells in anaphase showing bridging and multipolar divisions.⁴

DISCUSSION

The plant *Vinca rosea* Linn (periwinkle) is an apocynaceous, ever-blooming, pubescent herb or sub-shrub which has been shown to be a source of many alkaloids. It has enjoyed a popular reputation in indigenous medicine in various parts of the world. For example, Peckolt, in 1910, described the use in Brazil of an infusion of the leaves to control hemorrhage and scurvy, as a mouthwash for toothache, and for the healing and cleaning of chronic wounds (22).

In Europe related species have been used for the proprietary suppression of the flow of milk (23). In the British West Indies it has been used to treat diabetic ulcer (21) and in the Philippines has been reported as being an effective oral hypoglycemic agent (5). More recently, Chopra *et al.* (3) have reported that the total alkaloids possess a limited antibacterial activity as well as a significant and sustained hypotensive action. The hypoglycemic and antibacterial activities have not been confirmed, although one of the alkaloids isolated from this plant, ajmalicine, has been reported to possess transient depressor action on arterial blood pressure (16).

The detection of activity against the P-1534 leukemia was considered particularly significant, owing to the fact that this tumor system has detected other clinically useful antitumor agents in our laboratory (12) and has been sensitive enough to study structure-activity relationships of active compounds which correlated with the clinical activity (6, 15, 17, 18). VLB has also been clinically confirmed (9). The lack of a so-called "broad spectrum" of activity against a battery of transplantable murine neoplasms seems of little importance in the face of clinical activity and suggests that broader use and evaluation of less commonly used tumor systems might offer a potential advantage in the search for clinically useful compounds.

³ Personal communication from Drs. Thomas H. Weller and James B. Hanshaw, Harvard University School of Public Health, Boston, Massachusetts.

⁴ Personal communication.

Of the alkaloids studied, one isomeric with VLB—leurosine—has also shown a demonstrable retardation of the P-1534 leukemia. It has generally been of a lower order of activity than VLB, and less consistent. Of theoretical interest are the indole alkaloid, catharanthine, and the dihydroindole alkaloid, vindoline (8). Vindoline-like and catharanthine-like molecules each approximate one-half of the leurosine and VLB molecules. A solution containing equimolar proportions of these two alkaloids has an infrared absorption spectrum which approximates those of VLB and leurosine (5). These compounds either singly or in an equimolar solution have been devoid of any anti-P-1534 activity, as have all other pure alkaloids from this plant which have been tested.

It would seem possible on biogenetic, chemical, and physical grounds to speculate that vindoline

TABLE 7
COMPOUNDS TESTED FOR VLB REVERSAL
IN TISSUE CULTURE

No reversal		Partial	Complete
Serotonin	Nicotinic acid	Tryptophan Coenzyme A	Glutamic acid Aspartic acid α -Ketoglutaric acid
Serine	Glutathione		
TPN	DPN		
Cysteine	Folic acid	Ornithine Citrulline Arginine	
Glutamine	Phenylalanine		
Kynurenine	Ca pantothenate		
	Indole acetic acid		

and catharanthine may represent precursors of the biologically active VLB and leurosine molecules. The interesting chemical and biological relationships prompt a continuing study of these and other compounds on a structure-activity basis.

The mechanism of action of VLB is of considerable practical importance. Metaphase arrest of the type referred to as C-mitosis has been observed *in vitro* in human cells of malignant origin, but not all cells examined were equally susceptible. The classical example of an agent with C-mitotic activity is the alkaloid colchicine. It is the authors' opinion that this may be only coincidental insofar as usefulness of VLB is concerned. Alkaloids are substances of widely divergent structures whose chief similarity are their natural origin and basic nature. Biogenetically, the origin of colchicine remains obscure, and no relationship with any other groups of alkaloids has been detected (1). The biologically active antitumor compounds described here represent a new and previously unknown class of indole-indoline alkaloids. Indole alkaloids are believed to be derived from tryptophan (1). Previously described indole alkaloids

include the ergot alkaloids, the harmala alkaloids of the yohimbine and physostigmine groups, *Strychnos* alkaloids which include strychnine, and the *Rauwolfia* alkaloids. In our experience, colchicine has not had the selective effects seen with VLB. Arrested metaphases of the C-mitotic type have also been demonstrated by such diverse agents as other alkaloids, sulfhydryl reagents, antifolics, purines, certain amino acid analogs, quinones, phenols, and polyanions (2). The preliminary tissue culture studies seem to indicate an antimetabolite action, perhaps connected with glutamic acid. An additional observation which is suggestive of antimetabolite action is the clinical remissions obtained by Hertz, Lipsett, and May in women with Methotrexate-resistant choriocarcinoma.⁵ The clinical results of Hodes, Rohn, and Bond indicate that VLB not only affects a different clinical spectrum of malignancies than is affected by colchicine, but also has fewer toxic or side-effects (9, 10). VLB and related active compounds, regardless of mechanism of action, are examples of clinically active substances of new chemical compositions which provide a new and heretofore unknown lead in cancer chemotherapy.

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