The Effects of Two Alkaloids Derived from *Vinca Rosea* on the Malignant Cells of Hodgkin’s Disease, Lymphosarcoma and Acute Leukemia in Vivo

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Several plant alkaloids endowed with the ability to poison karyokinesis by damaging the mitotic spindle have been used in the treatment of human tumors; however, the two key alkaloids derived from *Vinca rosea* Linn., vinblastine (VLB) and vincristine (VCR), have received in recent times much attention because of their encouraging and sometimes striking effects, especially in the field of hemopoietic malignancies. This paper’s purpose is to present some morphologic investigations on the mitosis-arresting effects of these alkaloids on the malignant cells of patients affected with Hodgkin’s disease, lymphosarcoma, and acute leukemia; these appear to be of interest regarding both the mechanism of action of the drugs and some properties of the diseases. Because of the preeminently biological character of this study, reference to clinical results was restricted to the essential. The authors’ clinical investigations have already been reported elsewhere.1-4

Biological Aspects

The mechanism by which the *Vinca rosea* alkaloids affect cellular division consists chiefly in a blockade of mitosis in metaphase, as a result of the inactivation of the mitotic spindle.5,8 This effect appears practically superimposable to the C-mitotic effect produced by colchicine. Since the entrance of cells into mitosis is not hindered (only very high doses would seem to exert some additional prophase inhibition according to Cardinali et al.5), the increased number of mitotic cells is due to an accumulation of cells in early metaphase, rather than to a stimulation of mitosis. These properties account not only for the universally adopted methods of colchicine blockade for...
chromosomal studies, but also for the introduction of the stathmokinetic method for evaluating the proliferating activity of blood cells.  

The well-known transformation of the achromatic spindle's polarized fibrous structure into a "pseudospindle," also called "hyaline globule," or "lakelike body," has been recently elucidated by George et al., who have shown by means of electronmicroscopic studies that VCR interferes with the normal assembly of preformed spindle fiber proteins into an oriented tubular structure. Without organization of structural units, functional spindle tubules will not be performed, and chromosome movements are prevented in spite of centriole duplication.

It has been demonstrated that there are two different systems of polarized, achromatic fibers operating during karyokinesis, the former consisting of fibers joining the centromeres to the cellular poles ("karyokinetic spindle") and the latter of continuous fibers extending from pole to opposite pole ("cytokinetic spindle"). As these indications suggest, the first system is especially concerned with chromosome traction, the second with the division of the cell. Treatment with colchicine causes a progressive destruction of both systems, which is reflected in an increasing loss of birefringence; the central achromatic fibers are even more sensitive and may be destroyed almost selectively by lesser concentrations of colchicine.

The ability of the C-mitotic poisons to damage one or both the fibrillar spindle systems or their interaction may account for the extreme variability of mitotic alterations induced by these substances, including VLB. In a recent, careful study on the effects of VLB in vitro cultures, Siebs has distinguished between unoriented damaged mitoses, induced by highly concentrated solutions with total loss of chromosomal orientation, and oriented damaged mitoses, induced by lesser concentrations, with premetaphasic, metaphasic, or even anaphasic-telophasic spindle blockade and equatorial or even bipolar orientation of the chromosomes. The first category includes so-called "exploded metaphases," with chromosomes irregularly floating in a spindleless cell ("chromosomes éparpillés," "scattered chromosomes"), and "clumped C-mitoses," sometimes progressing to so-called "ball metaphases," in which the tightly coiled chromosomes are irregularly condensed in the center of the cell, and may conglutinate toward the "mitotic pyknosis" described by Dustin. The oriented C-mitoses include the classical "star metaphases," also known as "oriented arrested metaphases" or "métaphases en étoiles," "distorted star metaphases" and the so-called "double-star anaphases" or "anaphases en double étoile."

The mechanism by which colchicine and other C-mitotic agents cause this damage is not clear, so that, as remarked by Bieselet in 1958, each new category of metaphase poison has tended to spawn a fresh theory. There certainly exist certain critical points in the structure and function of the mitotic apparatus which may be particularly subject to attack, resulting in metaphasic arrest. They include the synthesis of the apparatus itself, the disulfide bridge formation of long fibrous molecules, and the movement toward the poles depending on contraction of the chromosomal fibers of the spindle, which...
probably involves a splitting of ATP, providing the necessary energy from its high energy phosphate bonds (~P~).

It has been reported by Creasey and Markiw\textsuperscript{28} that VCR, VLB, and colchicine inhibit the synthesis of sRNA in Ehrlich ascites cells; these investigators then speculated that this interference could prevent the formation of specific proteins required for the mitotic process. The observation, however, that cells with normal metaphase plates—therefore containing a well-formed and functional spindle mechanism—when exposed to VCR were unable to complete division indicates that the drug does not act by such a mechanism alone.\textsuperscript{12} The uncoupling of oxidative phosphorylation has been also suggested by some workers,\textsuperscript{24} while a decrease in vivo and in vitro of anaerobic glycolysis could also be demonstrated.\textsuperscript{25} An inhibiting effect of ATP on the embryotoxic activity of VLB in in vitro cultures of chick embryos was observed by Taglioretti et al.\textsuperscript{26} In addition, the demonstration that glutamic acid and/or tryptophane may partially reverse the effect of VLB has lent support to the conception\textsuperscript{*} that VLB possesses an antimetabolitic effect not shared by colchicine (which is in turn antagonized by other tropolones and, to some extent, by mesoinositol and ATP) nor, surprisingly enough, by VCR. Also glucose and d-ribose, according to Di Marco et al.,\textsuperscript{27} are capable of temporarily inhibiting VLB.

It has been speculated that mitotic arrest is not the only operative mechanism of these alkaloids, and this hypothesis appeared supported by the demonstration that metaphase blockade occurred at more than 100 times the concentration necessary to cause 50 per cent inhibition of growth as estimated by protein determination or cell count. However, recent extensive studies have failed to reveal any detectable effect on cellular respiration, glycolysis, protein, or nucleic acid synthesis,\textsuperscript{8} with the exception of soluble RNA in Ehrlich ascites cells.\textsuperscript{23} It would appear, in addition, that not only mitotic rates, but also intrinsic aspects of cellular metabolism, may influence the sensitivity of these substances.

**Previous Stathmokinetic Studies in Man**

Both VLB and VCR have been utilized for direct chromosome analyses of bone marrow cells after systemic administration.\textsuperscript{29} In addition, Vaiktevicius et al.\textsuperscript{30} found mitotic arrest in malignant serous exudate cells in solid tumor biopsy specimens at 18 hours after the administration of VLB. They also gave an excellent description of the mitotic alterations occurring in the normal bone marrow cells of their patients.

**Material and Methods**

Over 70 cases of generalized Hodgkin's disease (stages III and IV, subgroups A and B according to Kaplan\textsuperscript{31}), besides a group of miscellaneous diffuse malignancies, have been treated with VLB in our Medical Department. Successful lymph node aspirations before and 24 hours after standard (0.15–0.25 mg./Kg.) doses of VLB were obtained in 15 cases; actual surgical biopsies before and after VLB were performed in 4 patients. Smear and touch preparations and/or histologic sections were examined by phase contrast and after staining according to the May-Grünwald-Giemsa and fluorescent Feulgen\textsuperscript{92,35} technics. No specific chromosome studies were performed.
Twelve cases of lymphoblastic lymphosarcoma, 2 of reticulum cell sarcoma, 2 of plasmacytoma, and 31 of acute leukemia were treated with standard (0.05-0.10 mg./Kg.) doses of vincristine. Mitotic studies on lymph node and/or bone marrow aspirates could be performed in all the lymphosarcoma, reticulum cell sarcoma, and multiple myeloma patients, and in 12 leukemic patients. The mitotic indexes (number of mitoses per 1000) of the malignant cells were determined before and 24 hours after the administration of VCR. Since the number of prophases and telophases was practically negligible in the post-VCR specimens, only metaphases were tabulated. The reported values are the means of 3 determinations for each preparation.

The differences between the mitotic (metaphasic) indexes before and after administration of VCR were construed as differential stathmokinetic indexes (D.S.I.). These indexes were subsequently correlated with (a) the magnitude of oncolytic effect, and (b) medullar remission—if any—in leukemia.

I. VLB and Hodgkin’s Disease

Although a few arrested metaphases in what appeared to be lymphoid-like cells could be occasionally seen in the post-VLB aspirates, in the great majority of cases the lymphatic tissue proper appeared to be in the interphasic, resting stage.

The bulk of the arrested mitoses was clearly derived from the large mononuclear cells, also known as pre-Sternberg, Hodgkin, or mononuclear Sternberg cells. On sections the chromosomes appeared tightly clumped at the center of the cells (Fig. 2), thus distinctly differing from the pattern of mitoses in the untreated specimens; the cytoplasm was peculiarly clear, contrasting with the dark central mass of already conglutinated chromosomes. On the pre-VLB aspirates some few atypical mitoses could be seen, but their number was at least tenfold higher, and the clumping and/or scattering of chromosomes distinctly greater, in the post-VLB specimens (Figs. 3 and 4).

On occasion, a restricted number of larger metaphasic cells could also be seen, sometimes suggesting a double-star anaphase (Fig. 5), but multiple or multipolar mitoses were never observed. The genuine polynuclear giant Reed-Sternberg cells were always seen in the resting stage (Fig. 6).

II. VCR, Lymphosarcomas and Acute Leukemias

Cytomorphologic observations on lymph node and bone marrow aspirates (in 2 cases of acute blastic crisis of chronic myelogeneous leukemia with secondary myelofibrosis also in peripheral blood concentrates) showed typical arrested metaphases of the so-called oriented type in the malignant cells. Except for the degree of metaphasic blockade, which appeared dependent from dosage and—up to a certain point—cellular type of leukemia, the morphology of the stathmokinetic phenomenon was similar. Some of these findings are shown on Figures 7–18.

It could be observed that the immobile chromosomes of the arrested star metaphases, generally tightly spiralized and hyperchromatic, not infrequently coalesced, gradually forming a characteristically homogeneous, hyaline, spherico nuclear mass, readily identifiable with the stathmokinetic phenomenon described by Dustin29 (Fig. 10). These late effects of mitotic poisoning were often phagocytosed by macrophages in the lymph node aspirates, but not in the bone marrow.
Fig. 1.—Histologic sections of a lymph node in a patient with Hodgkin's disease. One multinuclear giant Reed-Sternberg cell and a few Hodgkin cells (see text) are visible. All cells are in a resting stage, and no mitoses are visible.

Fig. 2.—Histologic section of a lymph node from the same case, 24 hours after a standard dose of VLB. Many arrested mitoses of Hodgkin cells are visible. Chromosomes appear tightly clumped at center of cells, displaying a "ball-néphase" pattern.

The quantitative results of these studies have been tabulated in Tables 1 (acute leukemias), 2 (lymphosarcomas), and 3 (miscellaneous malignancies), respectively.

In all these malignant blood diseases, and especially so in lymphoblastic lymphosarcoma, the oncolytic effects correlated markedly with D.S.I.
Fig. 3.—Same section at higher magnification. Metaphasic “Hodgkin” cells display tightly clumped chromosomes and clear cytoplasm, sharply contrasting with surrounding lymphocytes.

Fig. 4.—Lymph node aspirate from a patient with Hodgkin's disease 24 hours after VLB. One arrested metaphase in a Hodgkin cell is visible. The chromosomes appear tightly clumped at the center of the cells. Two other similar cells and one giant Reed-Sternberg cell are in the resting stage.

which in some cases reached 150. In the acute leukemias a good correlation was also found between oncolytic effect and the magnitude of the D.S.I., and one case with very high D.S.I. went into complete remission; however, in other cases, the correlation between oncolytic effect and medullary remission was poor. Finally, in other hemopoietic malignancies, such as multiple myeloma
Fig. 5.—Two arrested mitoses in Hodgkin cells. In a larger cell irregular, hyaline, pyknotic clumps of chromosome material are visible, besides one nucleolus and a superimposed lymphocyte.

Fig. 6.—Double-star anaphase in Hodgkin cell, 24 hours after VLB.

and reticulum cell sarcoma, the low D.S.I. correlated rather well with their clinical refractoriness.

COMMENT

1. Hodgkin’s Disease

The cytology of Hodgkin’s disease by the aspiration, smear, and imprint method has been exhaustively studied in a classical series of monographs dealing with lymph node puncture.36 The thesis of the reticular origin of Sternberg cells has been most conclusively demonstrated by means of phase40 and electron41 microscopy.
Fig. 7.—Giant multinuclear Sternberg cell, in the resting stage even after VLB.

Fig. 8.—A bone marrow preparation from a case of acute myeloid leukemia with some (pseudo) monocytoid aspects. One mitotic figure is visible.

Although, in this first group of investigations, the focal character of the lesions and of the mitotic alterations did not warrant, in our opinion, a direct quantitation of the results, there is no doubt that there was an accumulation of arrested mitoses in the post-VLB specimens, and that these interested the mononuclear Sternberg cells. These two points will be discussed separately.

It has been speculated that mitotic blockade is not the only operative mechanism in the antitumor activity of VLB; in addition, Vaiktevicius et al.
apparently found no correlation between histologic evidence of metaphasic arrest and solid tumor regression. However, our present small series consistently suggested that, in Hodgkin’s disease, oncolytic effects were indeed correlated with an interference with mitosis. A totally superimposable effect could be seen in a few miscellaneous malignancies treated with VLB, and notably so in a case of metastasizing melanoma.

Although recent clinical observations have illustrated four cases in which apparently healthy infants were delivered from Hodgkin’s-affected mothers treated with VLB, three of whom had received the drug also during the first,
critical trimester of pregnancy, it has been demonstrated that VLB and VCR are markedly embryocidal and/or teratogenic when administered to pregnant golden hamsters and rats. Cohlan and Kitay have shown that transplacentally acquired VLB will inhibit cell mitosis in fetal tissues, as evidenced by a sixfold increase in mitotic figures after a single injection, again strongly suggesting that mitosis inhibition probably plays an important role in VLB
Fig. 13.—Bone marrow in acute lymphoblastic leukemia in relapse after steroid-induced complete remission of over one year.
Fig. 14.—Same case 16 hours after .075 mg./Kg. of VCR. Metaphasic arrest is prominent. Thromboeytosis in the peripheral blood could be ascertained on third day after administration, and leukocytes fell to 200 × ml. after 5 days, after which a neutrophile response took place. This is an additional, nontabulated case, in which the D.S.I. reached 120.

embriopathy. These findings are in good agreement with the demonstration that the growth-inhibiting effects of VLB on Earle’s L-strain mouse leukemia cells were closely linked to the effects on cell proliferative capacity.48 Finally, in recent studies on the suppression of immune response by means of these
alkaloids, Aisenberg and Wilkes\textsuperscript{19} have considered the immunosuppressive effect as a manifestation of the inhibition of cell division of a number of drug-sensitive immunocytes.

In a recent discussion on how certain drugs act in order to relieve the signs and symptoms of Hodgkin's disease, the question has been put "whether they act directly on the primary derangement responsible for the disease, or indirectly to inhibit host reactions to an underlying process.\textsuperscript{36} Because of the established ability of VLB to affect dividing cells, the observation that the
greater number of arrested mitoses is apparently derived from the large mononuclear Hodgkin cells is an additional, functional criterion for regarding them as the fundamentally proliferating tissue in Hodgkin's disease, as already proposed by an impressive array of previous and more recent investigations.

It must still be pointed out, however, that our stathmokinetic observations...
are in favor of a functional distinction between the giant, polynuclear, genuine Reed-Sternberg cells and the mononucleated Sternberg cells. The progression from the latter to the former type would seem to coincide with an appreciable reduction of their proliferating ability, as already remarked on the basis of simple morphologic criteria.\textsuperscript{56}

II. Leukemias and Lymphosarcomas

The kinetics of cellular proliferation in logarithmically growing populations, such as tumor cells, have been intensively investigated, both in vivo and in vitro. At any given moment it may be postulated that a small portion of cell population is in mitosis, while the interphase cells are in the G\textsubscript{1}-period, the S-period (DNA-synthetizing), and the G\textsubscript{2}-period. It has been demonstrated that the S-period has a duration which, under given environment conditions, is species-specific and cell line-specific.\textsuperscript{57}

Although the percentage of labeled nuclei—also known as thymidine labeling index—measured autoradiographically after a pulse exposure to \(^{3}\text{H}\)- or \(^{14}\text{C}\)-thymidine is generally considered\textsuperscript{58} a valuable indication of the rate of cellular proliferation, the so-called stathmokinetic or colchicine method, by demonstrating regions of high mitotic activity, is also capable of indicating cells with shorter lifespan, since it has been shown that lifespan and mitotic index are inversely related.\textsuperscript{59} Although the use of some proposed formulae\textsuperscript{60} has been invalidated to some extent by the complexity of factors which regulate cell growth and differentiation in mammals, the stathmokinetic method holds much of its significance if some biological requirements are satisfied.\textsuperscript{61} Thus, the kinetics of neutrophile generation in dogs have been recently investigated with the VLB method by Boggs et al.\textsuperscript{62}

Returning to our clinical experiments, at least two criticisms must be made concerning the time after which cells were examined and their origin. A 24-hour-period for the acquisition of the second sample (marrow lymph node aspirate) would seem too long a period for determining the mitotic arrest; however, practical reasons made it difficult to operate again on the patient after a shorter period, while, on the other hand, the inclusion of the stathmokinetic phenomena made it possible to include late effects of mitotic damage.

Another debatable point is that, in some cases where marrow aspirations could not be performed (dry taps or other reasons), peripheral blood metaphases were counted. It is well known, however, that although the blood picture may be morphologically closely similar to the bone marrow's when intensive leukoblastosis is present, the number of actively proliferating cells may be quite different, for, in addition to cells which may be regarded as proliferative, there may be many which have lost this capacity. Mauer\textsuperscript{63} has shown that the number of nonproliferative leukemic cells, whether they are at the end of their lifespan or simply in prolonged interphase, is notably higher in the circulating blood than in the bone marrow. The term "growth fraction" has also been employed to indicate the size of the proliferative compartment.\textsuperscript{64}

Notwithstanding such limitations, we do not feel that they invalidate the fundamental significance of our experiments. In the first place, the mitosis-arresting effect in human leukemic cells could be clearly demonstrated in the
bone marrow and, to a lesser extent, in the blood. The same was true for the lymphosarcoma cells. Some cytomorphologic observations suggested that the majority of arrested mitoses degenerate and are ultimately destroyed, although a less severely damaged fraction may still be capable of progressing into anaphase and telophase, as in Ehrlich’s ascites, cancer cells in mice,4 and other instances.30 VLB would seem to possess, in addition, an antimetabolic activity because of the folic acid-dependency of the meta-anaphase transition,65 but, by and large, cells with high proliferative rates appear to be better targets for these fundamentally aspecific spindle poisons than slowly reproducing cells.

Some perplexity might arise in view of the demonstration of the reduced proliferative capacity of most acute leukemia cells, according to the stathmokinetic66 and tritiated thymidine methods.67,68 It has been shown subsequently, however, that the reduction in proliferative capacity in an acute leukemia cell population is not a homogeneous phenomenon,70,71 since “the values obtained from the population as a whole are simply the mean values between the younger blast cells still in possession of a high proliferative capacity and the
older blast cells which have gradually lost their proliferating capacity. Conceivably, these "younger" and actively reproducing leukoblasts are the real targets of VCR. It has been calculated theoretically that the acute lymphatic leukemia cell population may be reduced by about 3 logarithms (from more than $10^{12}$ to a median of $10^8$) in patients achieving complete remission on VCR, although combination therapy ("VAMP") would appear to be able to drive the leukemic cell population to much lower levels than those achieved by prednisone or VCR alone. The problems of obliterating "the last few logs" of leukemic cells surviving maximum tolerated therapy have been recently outlined by Skipper.

From the quantitative point of view, a good correlation could be established in the leukemic patients between oncolytic effect and the magnitude of D.S.I. (Table 1). That remission did not always follow in cases with high oncolysis
Table 3.—Differential Stathmokinetic Indexes in Four Miscellaneous Patients Treated with VCR. Mitotic Observations Performed on Malignant Cells

<table>
<thead>
<tr>
<th>Cases</th>
<th>Diagnoses</th>
<th>VCR mg/Kg</th>
<th>Mitotic studies</th>
<th>Oncolytic effects</th>
<th>Clinical effectiveness</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multiple myeloma</td>
<td>0.05</td>
<td>0 1 2</td>
<td>?</td>
<td>none</td>
<td>moderate neurotoxicity</td>
</tr>
<tr>
<td>2</td>
<td>Id.</td>
<td>0.05</td>
<td>?</td>
<td>none</td>
<td></td>
<td>thrombocytopenia</td>
</tr>
<tr>
<td>3</td>
<td>Reticulum cell sarcoma</td>
<td>0.05</td>
<td>scarce</td>
<td>none</td>
<td></td>
<td>slight neurotoxicity</td>
</tr>
<tr>
<td>4</td>
<td>Id.</td>
<td>0.05</td>
<td>scarce</td>
<td>none</td>
<td></td>
<td>slight neurotoxicity</td>
</tr>
</tbody>
</table>

Met%0 before VCR, Met%0 after VCR, Differential stathmokinetic index

would seem to suggest that the restoration of a normal marrow is dependent not only upon the destruction of leukemic cells, but also upon the preservation of a sufficient reserve of normal hematopoietic cells.

In the lymphoblastosarcomas the D.S.I. were consistently high (Table 2), even to a peak of 150, and the clinical results were proportional. In other hemopoietic malignancies the lower D.S.I. correlated rather well with their clinical refractoriness (Table 3). The well-known shift of maximal chemotherapeutic effectiveness from the malignant histiocyte of Hodgkin's disease to the lymphoblast, in relation to the substitution of formylc (in VCR) to a methylc (in VLB) group in the dimeric alkaloidal molecule, would seem to implicate additional, possibly metabolic or enzymatic, factors. Similar factors might also be at the origin of the different sensitivity between lymphoblastic and granulocytoblastic leukemias.

The need of reliable methods for selecting cancer chemotherapy to replace the present system of “blind” chemotherapy selection has been stressed recently.74 Several in vitro methods have been devised,74,75 but they are generally outside the scope of the average hospital laboratory. It is conceivable that the in vivo determination, by needle aspiration or biopsy, or arrested mitoses in malignant tissues after one course of VLB or VCR, may prove itself helpful in selected cases of neoplastic diseases.

**Summary**

Hodgkin's disease, lymphosarcoma, and acute leukemia have been studied after treatment with the Vinca rosea alkaloids (vinblastine and vincristine), in order to demonstrate and evaluate the mitosis-arresting effects of the two drugs on the malignant cells. Histologic sections, lymph node and bone marrow aspirations were performed immediately before and 24 hours after the
intravenous administration of the drugs in 15 cases of Hodgkin's disease, 12 of lymphoblastic lymphosarcoma, and 12 of acute leukemia, besides other miscellaneous cases.

In Hodgkin's disease aspirates and sections showed a clear-cut metaphase arrest in the post-MLB specimens, chiefly affecting the pre-Sternberg or Hodgkin cells. This effect, besides corroborating the fundamentally stathmokinetic mechanism of VLB in Hodgkin's disease, was considered an additional factor in confirming the widely proposed conception that these cells represent the fundamentally proliferating and malignant tissue of this disease.

In the acute leukemias and lymphoblastosarcomas, cytomorphologic and quantitative studies demonstrated that the oncolytic effects correlated well with the magnitude of metaphasic blockade. It is postulated that only actively proliferating cells—the so-called "growth fraction"—are the target for these alkaloids.

**SUMMARIO IN INTERLINGUA**

Morbo de Hodgkin, lymphosarcoma, e leucemia acute esseva studiate post therapia con le alcaloides de *Vinca rosea* (vinblastina e vincristina), con le objectivo de demonstrar e evalutar le effectos antimitotic del duo pharmacos in le cellula maligne. Sectiones histologic e aspirationes de nodo lymphatic e de medulla ossee esseva effectuate immediamente ante e 24 horas post le administration intravenose del pharmacos in 15 casos de morbo de Hodgkin, 12 casos de lymphosarcoma lymphoblastic, e 12 casos de leucemia acute, e etiam de altere casos de conditiones miscellanee.

In morbo de Hodgkin, le aspiratos e le sectiones monstrava un nette arresto del metaphase in specimens post-vinblastinic, afficiente primarimente le cellulas pre-Sternberg o Hodgkin. Iste effecto—a parte le facto que illo corroborava le mechanismo fundamentalmente stathmokinetic de vinblastina in morbo de Hodgkin—esseva considerate como capace a confirmar additionalmente le popularissime conception que iste cellulas representa le fundamentalmente proliferante e maligne tissu del morbo.

In casos de leucemia acute e de lymphoblastosarcoma, studios cytomorphologic e quantitative demonstrava que le effectos oncolytic esseva ben correlacionate con le magnitude del blocage metaphasic. Es postulate que solo active-mente proliferante cellulas—le si-appellate "fraction crescential"—es le appropriate centro de attacco pro iste alcaloides.

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