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Proliferation Kinetics of Acute Leukemia Cells in Relation to the Chemotherapy[1]

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The ultimate task of the chemotherapy of leukaemias is to inhibit leukaemia cell proliferation selectively, but there is an important obstacle in the way of such therapy, namely that some actively proliferating normal tissues, including the normal blood cells themselves, are particularly vulnerable to the various cytostatic substances. Thus it will never be possible to treat leukaemias rationally until more is known about the proliferative characteristics of both normal and leukaemia blood cells.

Great advances have been made in the last few years in this field by using some specific precursors of DNA (such as thymidine and desoxycytidilic acid) and employing high resolution autoradiographic techniques (Bond *et al.*, 1959; Gavosto *et al.*, 1959, 1960; Milton and Cooper, 1964; Pileri *et al.*, 1965). These studies have been made both in vitro and particularly in vivo where pulse labelling with thymidine has made it possible to evaluate both the percentage variations of labelled cells and the mean number of grains in the labelled population. Useful kinetic information regarding normal and leukaemic blood cells has thus been obtained (Gavosto *et al.*, 1964; Killman *et al.*, 1963).

It has been observed that in the bone marrow on progressive myeloid and erythroid maturation, proliferative capacity decreases progressively until it stops altogether at the myelocytic and polychromatic erythroblastic stage. For chronic myeloid leukaemia the labelling index of each proliferating cell type is similar to the values of the corresponding normal cells (Gavosto *et al.*, 1959).

In 1958 we used tritiated thymidine to study many cases of acute leukaemia, and we found a clear fall in proliferative capacity of these blast cell (Gavosto *et al.*, 1959, 1960) (Fig. 1) in accordance with stathmokinetic investigation of Astaldi *et al.* (1950, 1953). Furthermore, in the case of particular total remission of the acute leukaemia, we observed, at the same time as a fall in the blast content, a clear recovery in the proliferative capacity (Pileri *et al.*, 1964). These results have been successfully confirmed in other laboratories and now, quite the contrary to what was thought a few years ago that the leukemic cell grew faster than the normal, we know that the

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rate of growth of these cells is often much slower that the corresponding normal cells (Gavosto, 1962; Mauer and Fisher, 1962, 1963; Killman, 1965).

Furthermore, some recent studies on leukaemic cell populations have pointed to another fact: leukaemic transformation depresses cell differentiation when it does not totally interrupt it. In some cases, such as the chronic leukaemias, this event

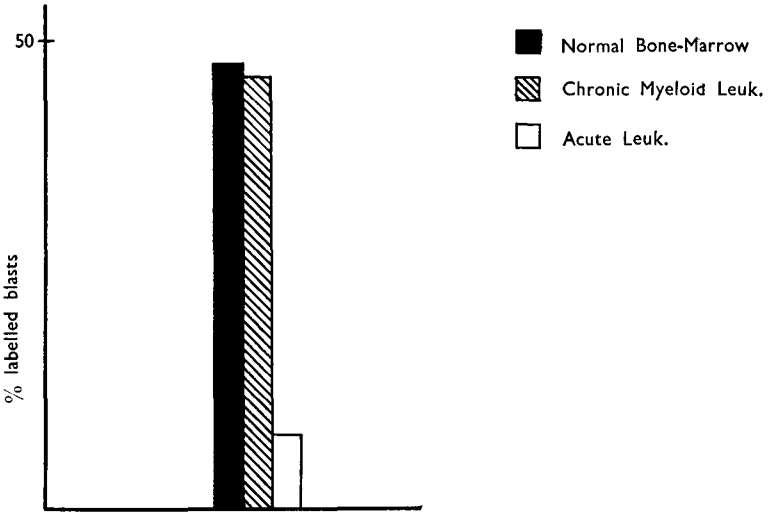

Fig. 1. Labelling index of normal and leukaemic blast cells

cannot readily be observed and only occurs in an appreciable way in advanced stages of the disease. In other cases, such as the acute leukaemias, this arrested differentiation is absolute and constitutes one of the most typical features of the disease.

Some investigations have been undertaken in our laboratory to search for a possible relationships between proliferative defect and the differentiation block in leukemias as well as to examine the significance of the low proliferative rate of acute leukaemic cells.

First, the proliferative activity has been studied in the blasts of acute leukemias, of chronic myeloid leukemia and then in various intermediate conditions, such as terminal blast crises of chronic myeloid leukaemia and acute leukaemia in remission phase. It was concluded that the progressive accentuation of the differentiation defect is accompanied by a progressive fall in the total blast population proliferative capacity (Gavosto *et al.*, 1964) (Fig. 2).

Secondly, within these same blast cell populations the proliferative activity has been analyzed in different cell classes distinguished by size (cell diameter), number of nucleoli, and cytoplasmatic basophilia. The analysis of the proliferative capacity

in these various classes showed that the fall in this function is not a characteristic of the whole blast leukemic population but is typical to only part of it (Pileri *et al.*, 1964).

It has been also shown that these differences of proliferative activity among the various classes of blast cells are not adequately explained by the increase in size which takes place during the cell cycle. We must therefore be up against a real cellular

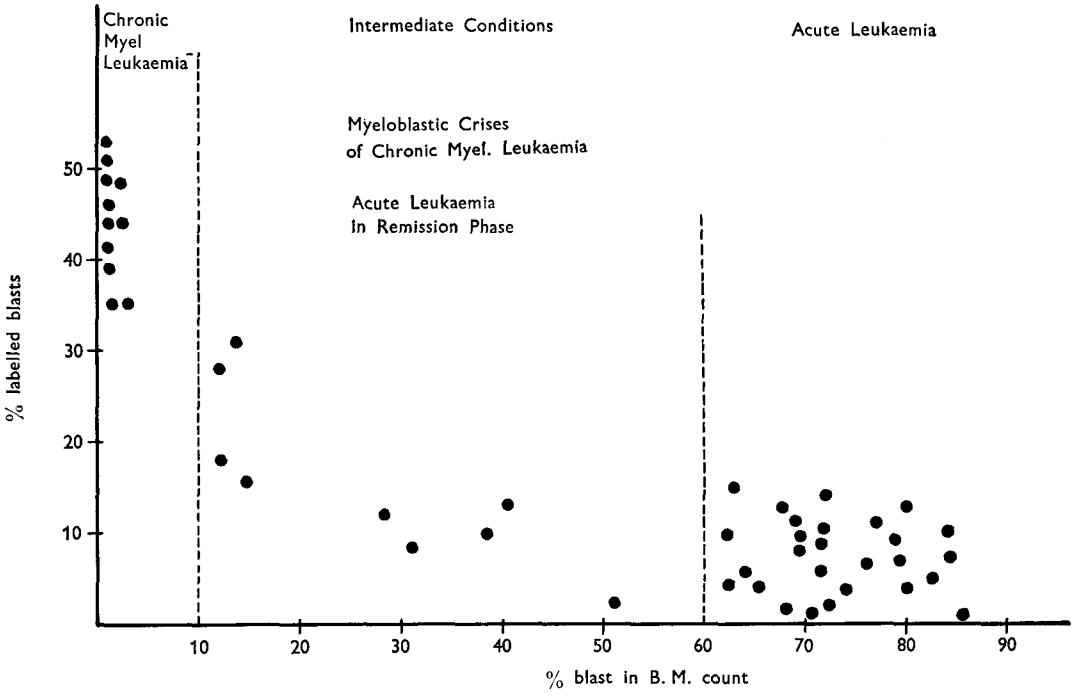

Fig. 2. Correlation between percentage of blast cells and labelling index in the different leukaemia conditions

heterogeneity and not just cells at various moments of the mitotic cycle. This fact has been demonstrated by comparing the size of the labelled and not labelled normal blasts, by observing that the difference between the mean diameters of the two groups is only of 10%, and by considering that the difference between the largest and smallest blasts of an acute leukaemia population is more than 100% (Gavosto *et al.*, 1964).

Finally, the fate of various classes of thymidine pulse-labelled cells in an investigation in vivo clearly showed that in a single population of acute leukaemia, those cells larger are generally the less aged and that with subsequent divisions (altogether ineffective as regards maturation) these blasts become smaller in diameter thereby increasing the groups of the smaller cell-diameter classes (Gavosto *et al.*, 1964). In all probability this transformation of some blast cells from large to small is imme-

diately post-mitotic. This suggests that the acute leukemic blast cells grow old as they are because of their incapacity to differentiate and thus progressively lose their proliferative potential. Clearly then the reduction in proliferative capacity observed in the blasts population as a whole is simply the mean value between the younger blast cells still in possession of a high proliferative capacity and the older blast cells which have gradually lost their proliferative capacity.

In normal bone marrow cells, therefore, the progressive falling off in proliferative capacity is associated with contemporaneous maturative development whereas in acute leukaemia cells this falling off in proliferative capacity is not accompanied by any real specific qualitative maturative phenomenon but only by quantitative alterations in some cell parameters (cell dimensions, number of nucleoles, basophilia, intensity of nucleic acid and protein metabolism) (Gavosto, 1965). These concepts are illustrated in Fig. 3: in normal and chronic myeloid leukaemia cells, a progressive fall in the proliferative capacity is observed during the progressive differentiation

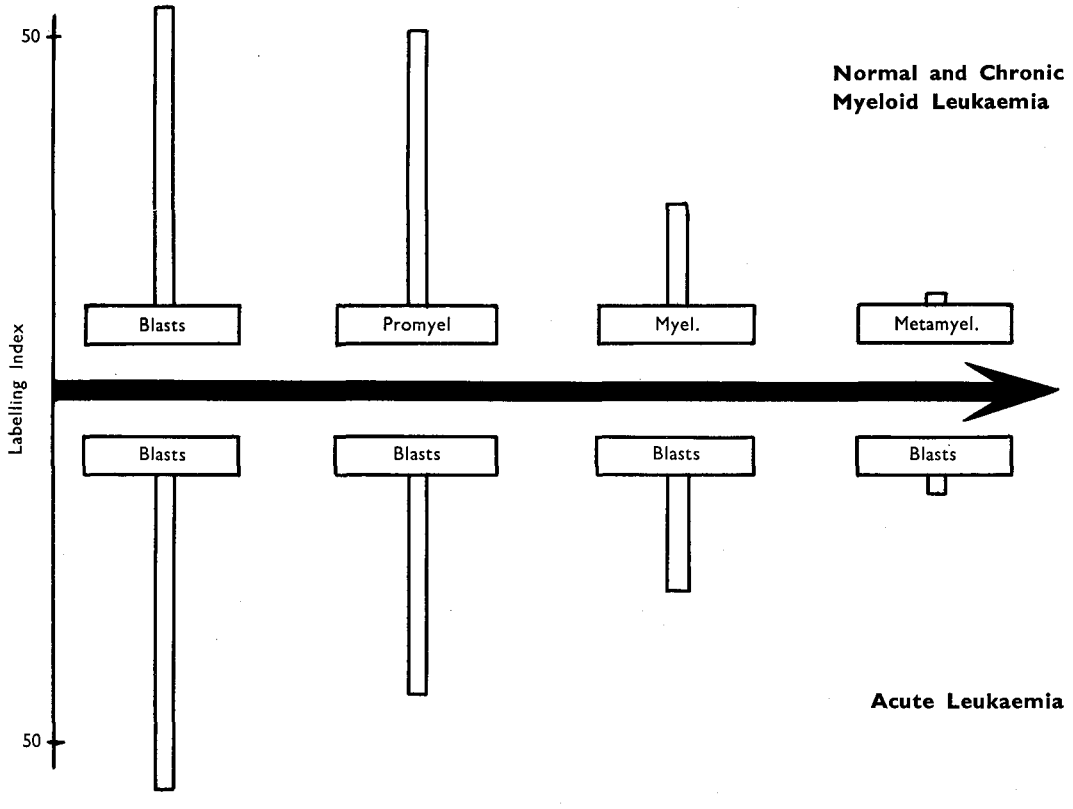

Fig. 3. Evolution of bone-marrow cells with and without differentiation in relation to the proliferative capacity

(maturation) of the cells: *evolution with differentiation*; in acute leukaemia cells this drop in proliferative capacity is not accompanied by any real qualitative differentiation phenomenon, but only by quantitative variations in some cell parameters: *evolution without differentiation.*

As regard chemotherapy, the existence of a real proliferative defect most certainly represents a serious difficulty for the radical cytostatic therapy of acute leukaemias because sensitivity to the various cytostatic agents is principally based on the degree of proliferative activity of the tissues themselves. In this connection we should immediately point out that, as we have seen, the acute leukaemia population is not uniform and the younger blasts gradually lose their proliferative potential in the course of cell division. It is evident that the influence of the various cytostatics will be felt quite differently within the same acute leukaemia population. The antimitotic effect will be more direct and intense in the first blast generations which have greater proliferative potential, while it will made itself felt indirectly and with less intensity in later blast generations. Thus cytostatic therapy reduces the proliferating blast compartments and only indirectly affects the non-proliferating compartments (Dogliotti and Pileri, 1966).

The above also applies for cytostatics acting directly on RNA metabolism instead of on that of DNA. As in the case of DNA, in fact, the highest incorporation values of RNA precursors are observed in the youngest acute leukaemia blasts (Gavosto *et al.*, 1965).

These observations stimulate some remarks about a more general problem, which can be expressed as follows: should the largest and most actively proliferating blasts be considered as precursor cells or do these instead consist of another pool of cells which generally escape direct observation, for example reticular cells?

An approach to this important problem of histogenesis of leukaemia cells can be made in those cases of acute leukaemia where a considerable number of very early reticular cells can be picked out. Some of these cells can be recognized as being intermediate between reticular cells and blasts. In two cases of acute leukaemia with a high number of reticular cells in the bone marrow we observed a very high proliferative capacity. In these cases we can probably admit that the evolution is: reticular cells — reticular cells with blast modulation — proliferating blasts — non-proliferating blasts. This evolution is often accompanied by a gradual falling off in proliferative activity.

The blast population of acute leukaemias is probably not selfperpetuating but continually primed by a stem cell compartment. Some information on the kinetics of this progenitor compartment could be obtained by following the labelling index of the largest blasts after a pulse thymidine in vivo injection. The guiding idea of this experiment could be as follows: if the proliferative activity of the stem cell compartment is less than that of the largest blast compartment, there will be a steady fall in the latter's labelling index. If, on the contrary, the proliferative rate of the stem cell compartment proves to be greater than that of the largest blast compartment there will be a steady rise in the labelling index (Killman, 1965). It is evident

that in the first case the cytostatic effect would be felt more in the blast compartment while in the second case it would be more marked at stem cell level.

Finally additional information can be drawn from the study of proliferative capacity in the course of the various cytostatic therapies. In chronic myeloid leukaemias, during treatment, the proliferative capacity of the surviving cells is not different from their capacity before treatment (Dogliotti and Pileri, 1966; Bond *et al.*, 1962). Furthermore, extreme variability in degree of response to the same cytostatic is also encountered in different cases, like those reported, for example, by Sandberg between Ph^+ and Ph^- forms of chronic myeloid leukaemia (1965). Proliferative activity being equal, the pharmacological response can also vary with the different clone characteristics of the leukaemic tissue.

During the treatment of acute leukaemia, the proliferative activity of the blasts can rise or fall. We have seen that, when it falls, only the largest blasts are involved (Fig. 4).

Increases in proliferative activity can occur spontaneously in the terminal blast

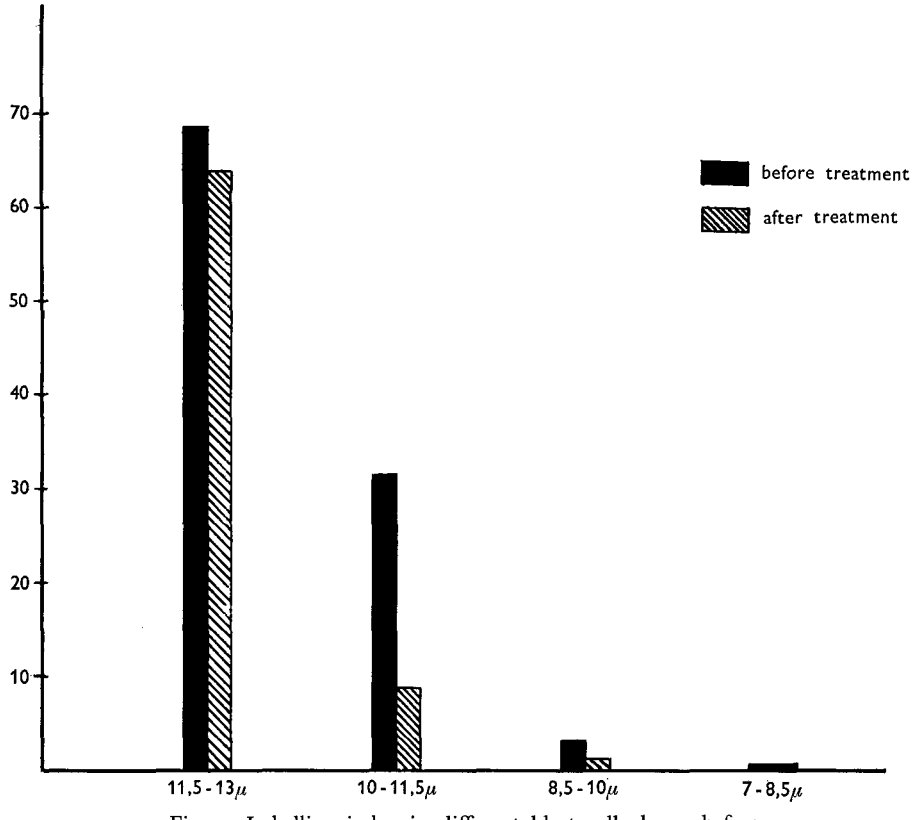

Fig. 4. Labelling index in different blast cell classes before and after a 4 days treatment with 6-Mercaptopurine

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crises with dramatic formation of new blast cells (Fig. 5). However, the highest values of proliferative activity in the blast compartment are observed in cases of total and partial remission and are due to the destruction of the leukaemia cells and to the consequent recovery in normal bone marrow development. There are reports

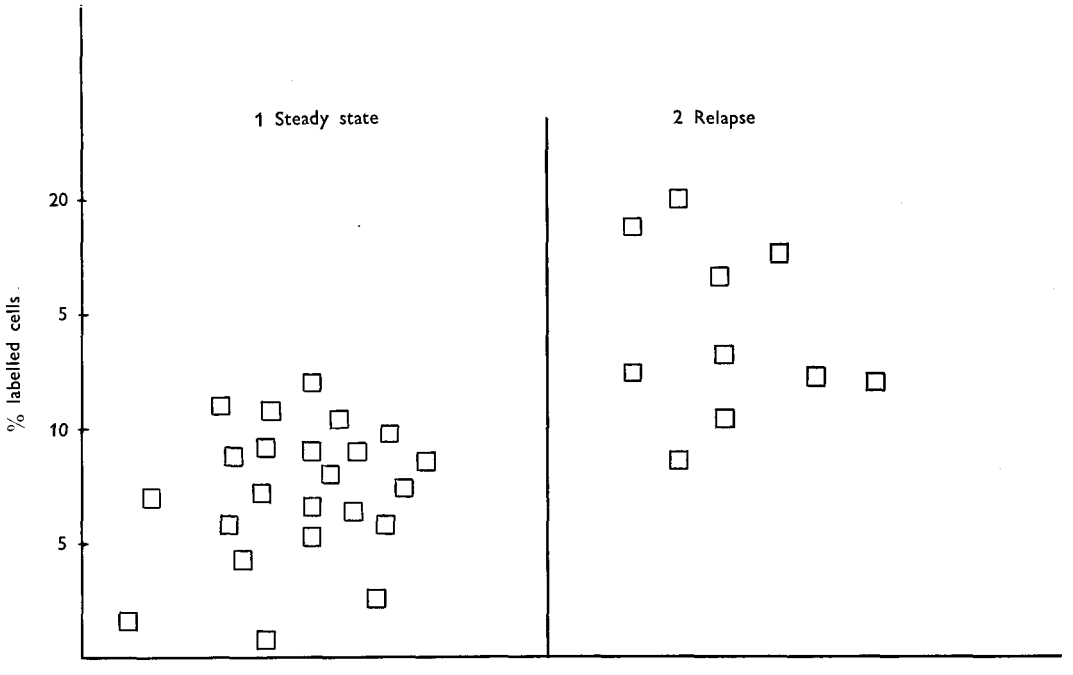

Fig. 5. Labelling index of acute leukaemia blast cells, during (1) steady state, and (2) relapse

of temporary cure with complete sterilization of the leukaemic content (Zuelzer, 1964; Keidan, 1964).

Up to now we have considered chemotherapy as an antagonist of cell proliferation. It should not be forgotten, however, that new chemotherapeutic agents are needed to damage leukaemic cells selectively in the various phases of their mitotic cyle, including cells which no longer proliferate.

The metabolic study of DNA as an index of proliferative activity has up to now only been considered at cell level. These same studies, however, must also be extended to chromosome level and the synthesis pattern of DNA evaluated in both normal and leukaemic cells (Gavosto *et al.*, 1965). This investigation is now possible always by using a high resolution autoradiographic tecnique. These studies at chromosome level should therefore enable us to evaluate the synthesis pattern of DNA in relation to the chemotherapic agents even more analytically. In fact we can consider that

most of the cytostatic agents normally employed in therapy act prevalently on DNA metabolism and might therefore induce various chromosome abnormalities in cases of leukaemia which are treated with various types of intercurrent alterations.

Summary

The proliferative characteristics of human acute leukaemia cells are reported and the relationships between the proliferative alterations and differentiation defect of these cells discussed. The proliferative activity of acute leukaemia cells was also studied in relation to cytostatic treatment.

Emphasis is laid on the fact that in all cases of acute leukaemia the characteristic blast cells of the disease do not constitute a homogenous cell population but can be divided into various sub-classes with different kinetic and proliferative characteristics. It is also pointed out that all cytostatic treatment acts on the most actively proliferating classes and only indirectly on the non-proliferating classes.

Finally, the need for more detailed study of DNA synthesis at chromosome and sub-chromosome level for the purpose of more fully understanding the response of leukaemic cells to the various chemiotherapies is underlined.

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RIASSUNTO

Sono riportate le caratteristiche proliferative delle cellule di leucemia acuta umana e sono discussi i rapporti tra alterazioni proliferative e difetto differenziativo di queste cellule. L'attività proliferativa delle cellule di leucemia acuta è stata studiata anche in rapporto al trattamento citostatico.

È stato sottolineato il fatto che in ogni caso di leucemia acuta gli elementi blastici caratteristici della malattia non costituiscono una popolazione cellulare omogenea, ma possono essere divisi in diverse sottoclassi con caratteristiche cinetiche e proliferative differenti ed è stato rilevato il fatto che ogni trattamento citostatico agisce sulle classi più attivamente proliferanti e soltanto indirettamente sulle classi non proliferanti.

È stata infine sottolineata la necessità di una più dettagliata indagine della sintesi dell'ADN a livello cromosomico e subcromosomico anche ai fini di una migliore comprensione della risposta degli elementi leucemici ai vari trattamenti chemioterapici.

RÉSUMÉ

Les auteurs rapportent les caractéristiques prolifératives des cellules de la leucémie aiguë humaine et discutent les rapports entre altération proliférative et défaut de différentiation de ces cellules. Ils ont étudié aussi l'activité proliférative des cellules de la leucémie aiguë en relation avec le traitement cytostatique.

Ils soulignent le fait que, dans chaque cas de leucémie aiguë, les éléments blastiques, qui sont caractéristiques de la maladie, ne constituent pas une population cellulaire homogène, mais peuvent être divisés en diverses sous-classes aux caractéristiques cinétiques et prolifératives différentes; ils relèvent aussi le fait que tout traitement cytostatique agit sur les classes les plus activement proliférantes et seulement indirectement sur les classes non proliférantes.

Ils soulignent enfin la nécessité d'une investigation plus détaillée de la synthèse de l'ADN au niveau chromosomique et sous-chromosomique, et cela aussi en vue d'une meilleure compréhension de la réponse des éléments leucémiques aux différents traitements chimiothérapeutiques.

ZUSAMMENFASSUNG

Die Verfasser beschreiben die proliferativen Merkmale der Zelle der akuten Leukämie beim Menschen und besprechen die Beziehungen zwischen proliferativer Aenderung und Differenzierungs-Defekt dieser Zellen. Die Wucherungstaetigkeit der Zellen bei akuter Leukaemie wurde auch in Bezug auf die cytostatische Behandlung untersucht.

Die Verfasser unterstreichen die Tatsache, dass die für diese Krankheit typischen Blastzellen keine homogene Zellenbevoelkerung bilden, sondern in mehrere Untergruppen eingeteilt werden koennen, welche verschiedene kinetische und proliferative Kennzeichen aufweisen; sie bemerken, dass jede cytostatische Behandlung auf die am meisten proliferierenden Klassen und nur indirekt auf die nicht proliferierenden Klassen wirkt.

Sie unterstreichen zum Schluss die Notwendigkeit einer mehr in die Einzelheiten gehenden Untersuchung der DNS-Synthese auf Chromosom-und Subchromosomebene, und dies vor allem, um das Ansprechen der leukämischen Elemente auf die Chemotherapien besser zu verstehen.

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