Aids in the Management of Leukemia (Cellremoval by Continuous Flow Leukapheresis and Impulsecytophotometry)

P. Höcker, E. Pittermann, D. Lutz and A. Stacher

1st Med. Dept and the Ludwig-Boltzmann-Institute for Leukemia Research and Hematologie
Hanusch-Krankenhaus, Vienna

During the last years the mechanical withdrawal of white blood cells (WBC) by a continuous flow cellseparator (CFC) was shown to be an effective method to treat chronic leukemias, especially in certain situations (1, 2, 3, 6, 7, 8). We want

Table I: Clinical datas of patients with CML and CLL submitted to leukapheresis

	NUMBER	FEMALE	MALE	AVERAGE AGE	LEUKOCYTES x 10^3 /CMM	UNTREATED	PRETREATED
CML	20	9	11	51.05 (23-79)	228 (63-700)	17	3
CLL	12	8	4	67.3 (49-81)	267 (110-660)	6	6

Table II: Indication for leukapheresis in patients with CML and CLL

	CML	CLL
HIGH PERIPHERAL COUNT	11	2
INCIPIENT BLAST CRISIS	2	0
RESISTANCE AGAINST CYTOSTATICS	2	0
INCOMPATIBILITY OF CYTOSTATICS	2	4
INITIALTHERAPY	3	6
TOTAL	20	12

Table III: Results of leukapheresis therapy

	NUMBER OF LPH	MEAN DURATION OF A SINGLE PROCEDURE/HOURS	BLOOD VOLUME POSESSED PER PROCEDURE	LEUKOCYTE REDUCTION %	LEUKOCYTES REMOVED X 10^{11} PER PROCEDURE
CML	108	4,03 1,5 - 5,2	7,95 2,7 - 11,8	56 21,6-88,4	3,02 0,9 - 11,2
CLL	142	4,16 0,9 - 6,7	7,44 2,1 - 10,2	77,06 48,89-96,2	4,00 0,4 - 16,07

to demonstrate our results obtained in 20 patients with CML and in 12 patients with CLL who underwent repeated leukapheresis using an AMINCO cellseparator.

The clinical datas were shown in tab. 1. The indication for leukapheresis was mainly high peripheral cell count with signs of hyperviscosity in some of the patients, resistance to cytostatic treatment or incompatibility of cytostatic drugs. Also untreated patients entered this study (tab. 2).

The results obtained by leukapheresis are shown in tab. 3. In all cases a quick decrease in the peripheral cell count could be obtained by a single serie of 3–4 subsequent procedures. The number of removed cells varied from 0,9 – 11,2 x 10^{11} in cases of CML and from 0,4 – 16,7 x 10^{11} in cases of CLL. A close relationship

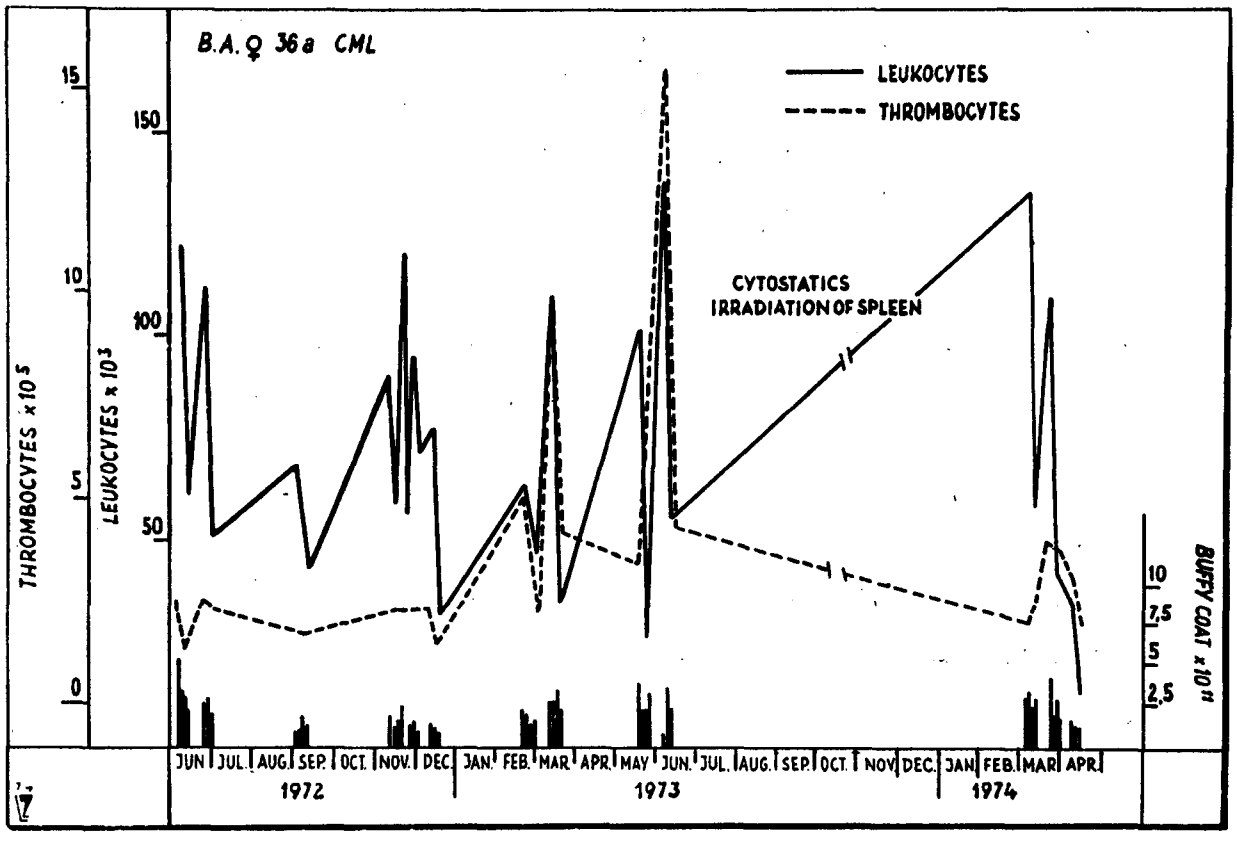

Fig. 1: Clinical course of a long time leukapheresis therapy in a patient with CML.

Table IV: Mean values of removed leukocytes in patients with CML and CLL with different pretreatment values

peripheral leukocyte count before Leucapheresis ($10^3/mm^3$)	CML			CLL		
	n	$\bar{x}$	s	n	$\bar{x}$	s
40 - 60	11	1,43	0,64	14	2.59	0.86
60 - 80	22	1.49	0.62	16	3.36	1.02
80 - 100	17	2.04	0.79	13	4.06	1.07
100 - 150	24	3.13	1.91	26	5.31	1.64
150 - 200	5	4.70	1.49	7	6.85	1.93

was seen between the initial cell count and the number of removed cells (tab. 4). The procedure itself was well tolerated and many of the patients were treated as out patients. One patient with CML was treated with 55 leukaphereses over a period of more than 2 years until her disease underwent malignant transforma-

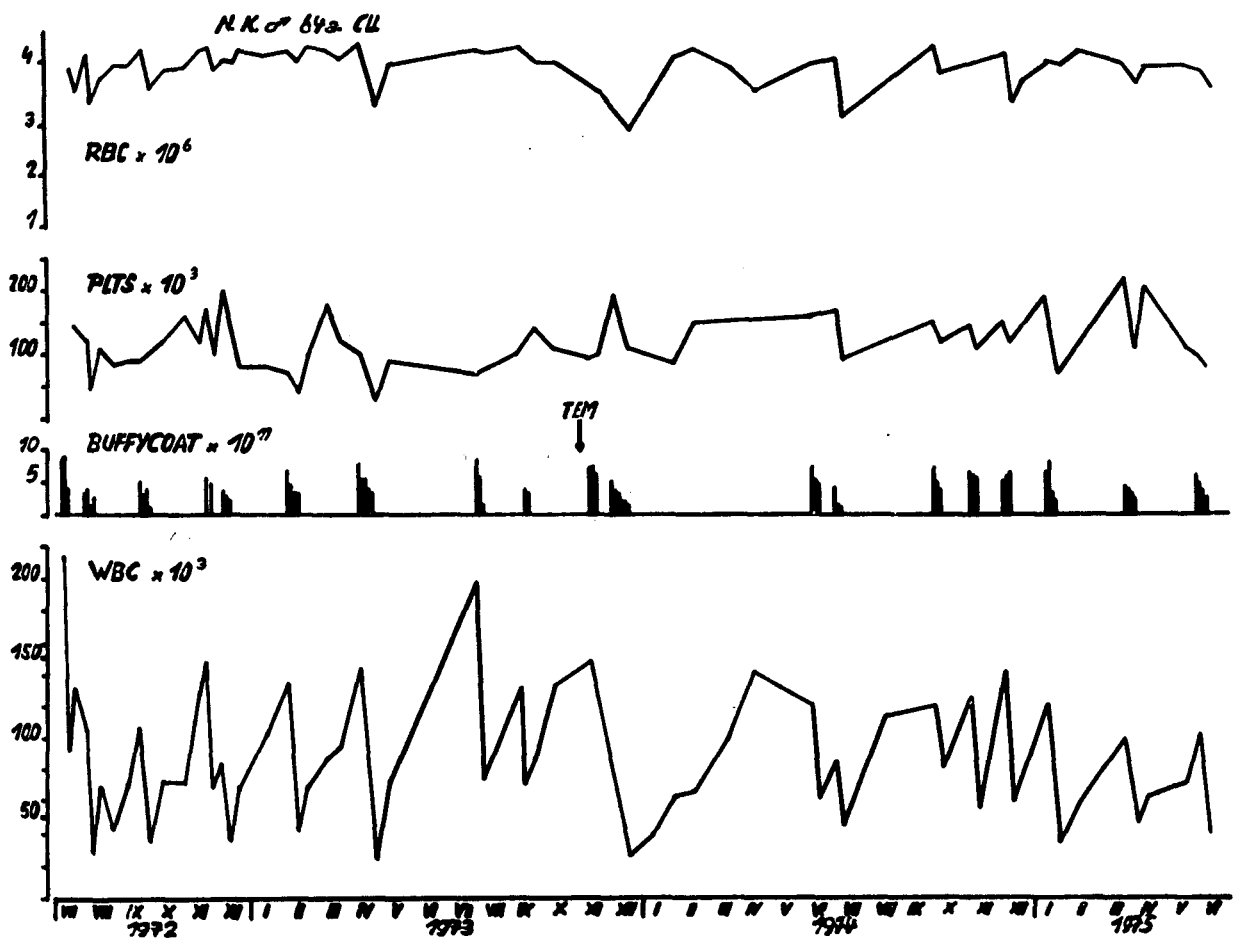

Fig. 2: Clinical course of a long time leukapheresis therapy in a patient with CLL.

Table V: Increase of the S and G_2+M fraction in the bone marrow and peripheral blood in a patient with CML after 4 subsequent leukaphereses

K.R. ♂ CML	LI	DNA-histogram G_1	S	G_2/M	Leuko	Enlarged Spleen	Liver
BLOOD	11.8%	60%	18%	22%	320000	+++	+
B.M.	4.5%	65%	17%	18%			
	after 4 Leukaphereses (Cell separator)						
BLOOD	23.5%	51%	26%	23%	275000	++(+)	+
B.M.	13.6%	59%	21%	20%			

tion (fig. 1). Another patient with CLL was also treated by 69 leukaphereses alone over a period of 3 years (fig. 2). This way a possibility is demonstrated to treat patients with chronic leukemia over a longer period of time without using any cytostatic drugs.

Kinetic studies were done by autoradiography and impulsecytophotometry in 6 cases of CML during a serie of 4 leukaphereses. Only in one case a slight increase of the S and G_2/M fraction was observed after the serie (tab. 5). This

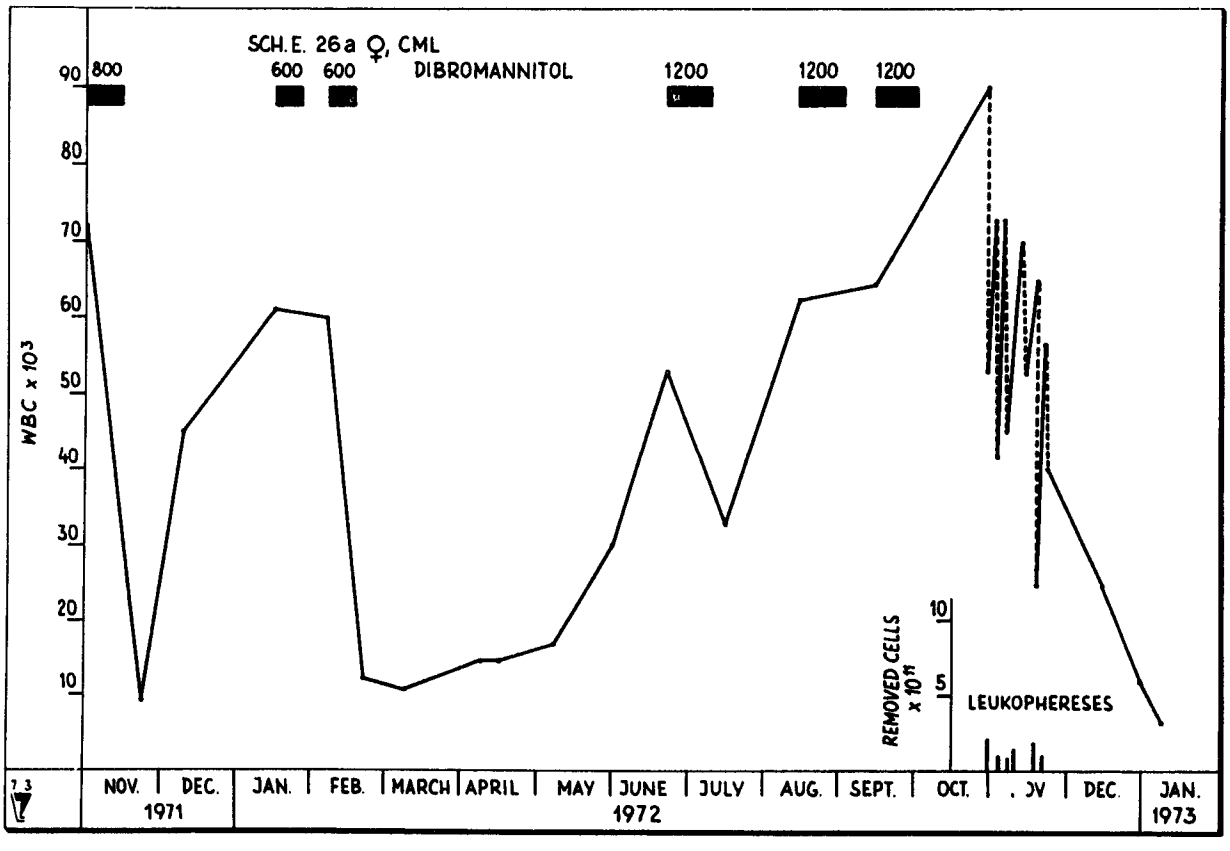

Fig. 3: Repeated leukapheresis in a patient with CML who developed resistance to dibromannitol.

Table VI: Loss of blood cells during leukapheresis

NUMBER OF LEUKAPHERESIS	LOSS OF PLATELETS %	LOSS OF ERYTHROCYTES %
67	28,57	4,98

Table VII: Side effects of leukapheresis

LOSS OF ERYTHROCYTES
LOSS OF THROMBOCYTES
HYPOVOLUMINIA
ANAPHYLACTOID REACTION CAUSED BY PLASMAEXPANDER
INCOMPATIBILITY AGAINST PROTAMIN
LOCAL THROMBOPHLEBITIS

proves that proliferation of leukemic cells is probably not enhanced by mechanical cellremoval. However, in one case of resistance to dibromannitol a good response to the same drug was seen after 6 leukaphereses had been performed (fig. 3).

In patients with CLL leukapheresis led to an increased blastic transformation rate and in some cases changes in the cytochemical findings were seen (4). Side effects were mainly the loss of platelets and the loss of erythrocytes which in some cases required a transfusion of packed red cells (tab. 6). Therefore patients with platelet counts below 20.000/cmm were not treated by leukapheresis. Sometimes anaphylactoid reactions to dextran and protamin were seen as well as local thombophlebitis (5) (tab. 7). According to our experience with leukaphereses by CFC in the treatment of chronic leukemias the following points might be taken into consideration (tab. 8). Leukapheresis is an expensive, time and personal consuming procedure which is accompanied by a loss of erythrocytes and platelets and which does not influence the basic disease. The benefits of the procedure are: it is well tolerated and side effects such as hyperuricemia and bone marrow aplasia

Table VIII:

DISADVANTAGES	ADVANTAGES OF LEUKAPHERESIS
EXPENSIVE PROCEDURE	WELL TOLERATED
LOSS OF ERYTHROCYTES	MINIMAL SIDE EFFECTS
BASIC DISEASE IS NOT INFLUENCED	NO BONE MARROW TOXICITY

Table IX: Indication for leukapheresis

A) HIGH PERIPHERAL CELL COUNTS WITH CLINICAL SYMPTOMS, WHICH REQUIRES BRISK CELL REDUKTION.
(INCREASED BLOOD VISCOSITY)
B) RESISTANCE AGAINST CYTOSTATICS
C) INCOMPATIBILITY OF CYTOSTATICS
D) PREGNANCY

are absent. It has also to be mentioned that large amounts of granulocytes can be obtained of patients with CML, wich permits granulocyte transfusions in leukopenic patients. Leukapheresis should therefore be used in patients with a high peripheral cell count which requires a brisk cell reduction as well as in cases of resistance to cytostatic drugs and in cases of inability to use irradiation or cytostatic treatment, f. i. in pregnancy (tab. 9).

Since most cytostatic agents currently used in the treatment of acute leukemia are strongly cell cycle dependent, their cytotoxic effect correlates with the proliferation rate of a certain cell population. The proliferation kinetics of leukemic cells vary from one case to another, therefore the effect of the same kind of leukemia can be different. Also the proliferation pattern of a leukemic population is often changed after the application of cytostatic drugs. Therefore the knowledge of the proliferative parameters of a leukemic cell population might help to predict the response to a certain drug. The possibility presented by the flow system analysis of impulsecytophotometry to obtain within a short time useful informations about the proliferative state of a given cell suspension is a considerable contribution to the cytostatic treatment of leukemic patients.

Method:

Blood and bone marrow samples are fixed with ethanol and stained with ethidium bromide. Ethidium bromide is a fluorescent dye which binds quantitatively the double stranded nucleic acids. The amount of ehtidium bromide bound per cell corresponds with the DNA content of each cell. Since the DNA content of a cell is a characteristic parameter in each phase of the cell cycle, it represents a good marker for determining the cellular proliferation kinetics. By this flow

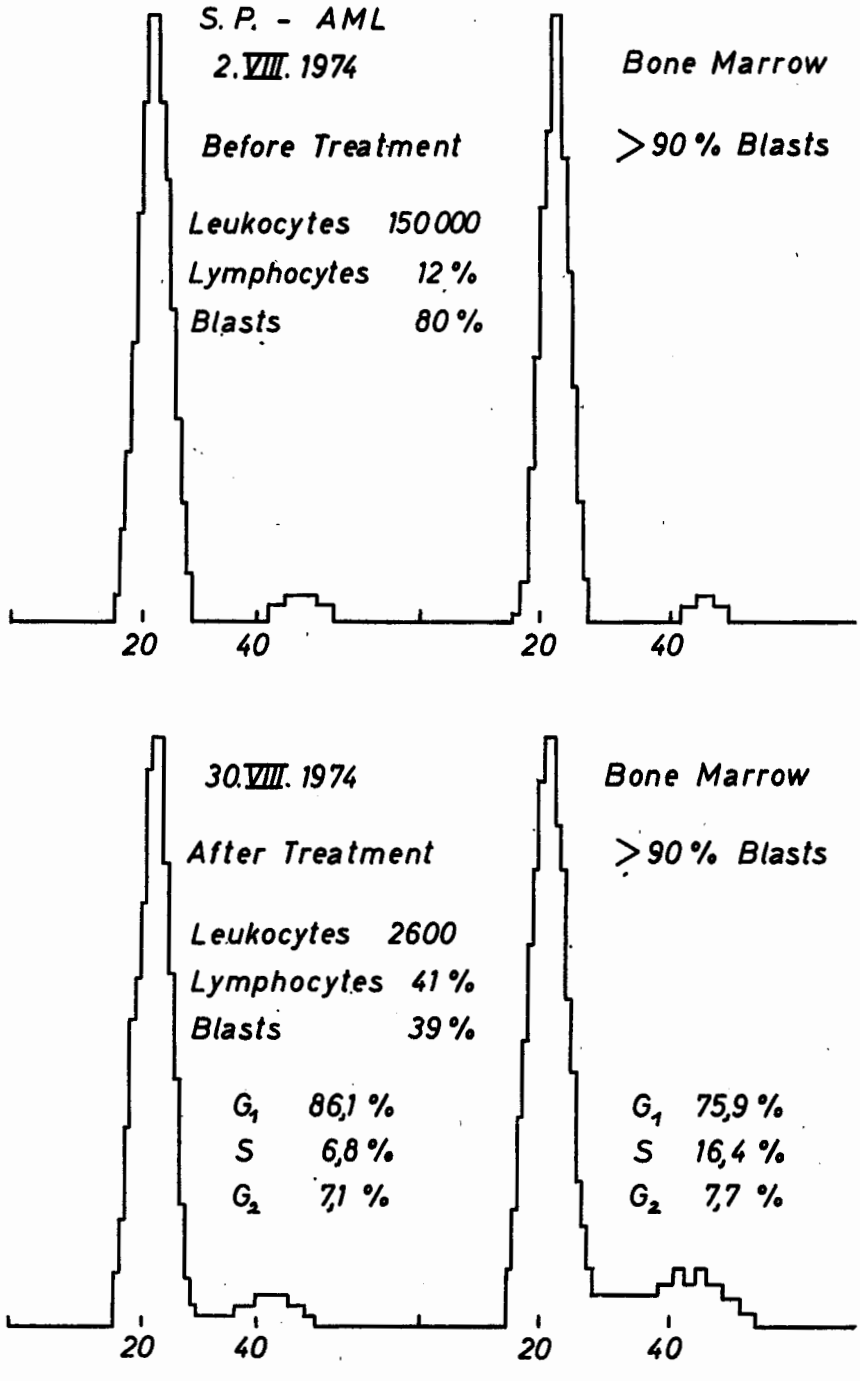

Fig. 4: Low proliferation activity of leukemic cells in a patient with AL, unchanged despite of the application of several cytotoxic drugs.

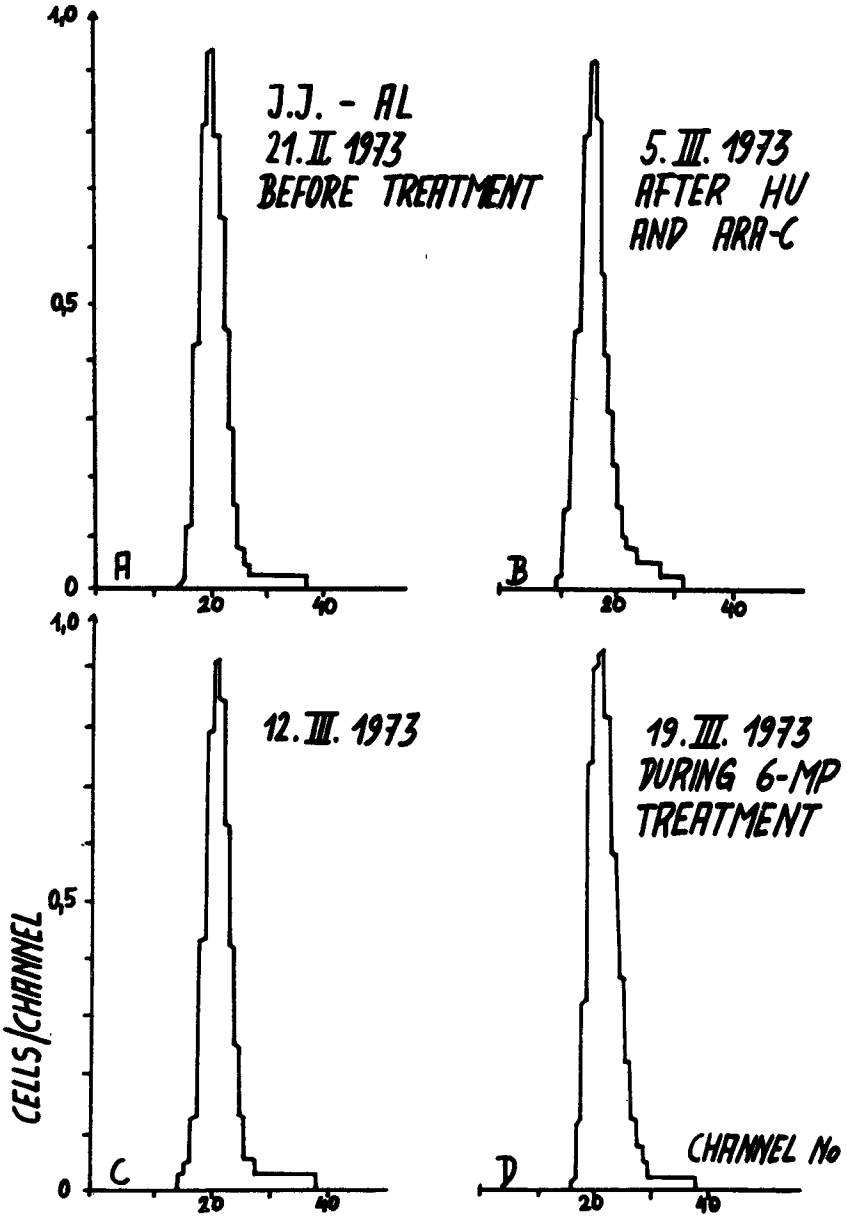

Fig. 5: Increase of proliferation activity in a patient with AL after 3 courses with Adriamycine and ARA-C, followed by a complete remission after two further courses.

system 50,000 to 100,000 cells in suspension are measured and each cell is recorded in a multichannel analyzer according to its ethidium bromide impulse intensity which corresponds to the DNA content of each cell. Such a measurement results in a DNA histogram representing the distribution pattern of measured cells according to their DNA content. The planimetric evaluation of the DNA histograms indicates the percentage of cells in the different phases of the cell cycle.

Using this method for measuring the proliferation pattern of peripheral blood and bone marrow cells in leukemic patients, the following observations were done:

1.) Patients suffering from acute leukemia with no changes in the blood and bone marrow histogram before and during therapy did not respond to various cytostatic drugs and had a bad outcome (fig. 4). This is in accordance with other informations in which the prognosis was better when the labelling index of the

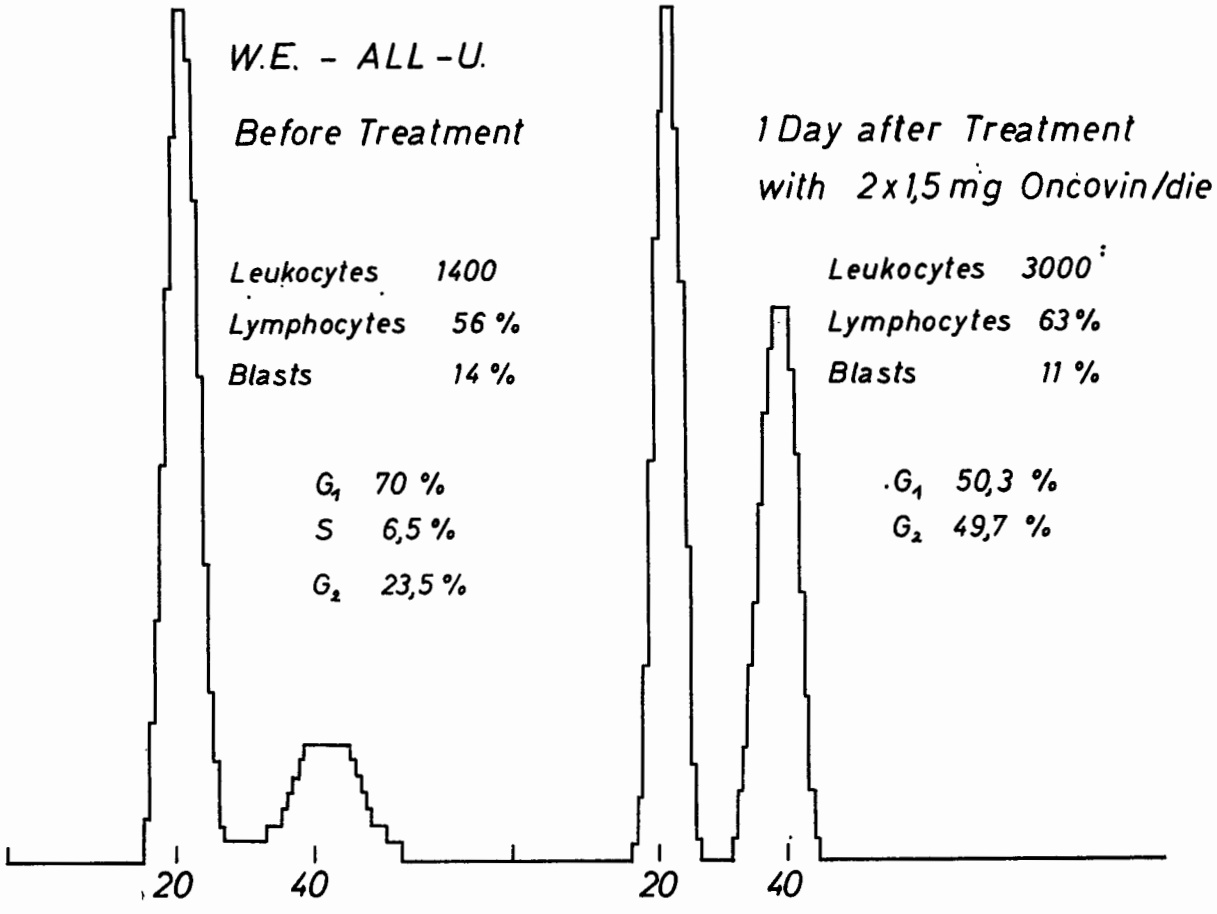

Fig. 6: Increase of the G_2 + M fraction after the application of vincristine.

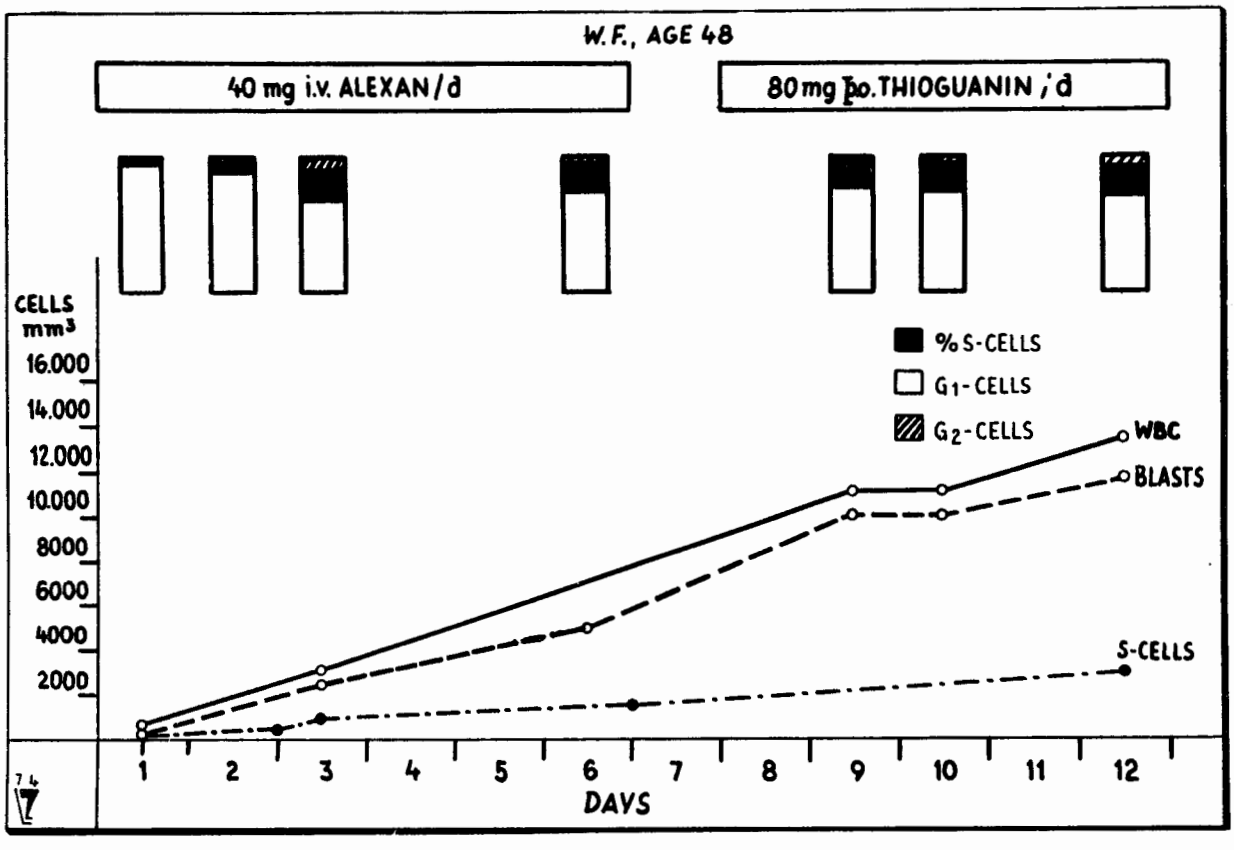

Fig. 7: Effect of a low dosage of thioguanine following to ARA-C Application leading to an increase of blastcells in the peripheral count and unchanged proliferation activity.

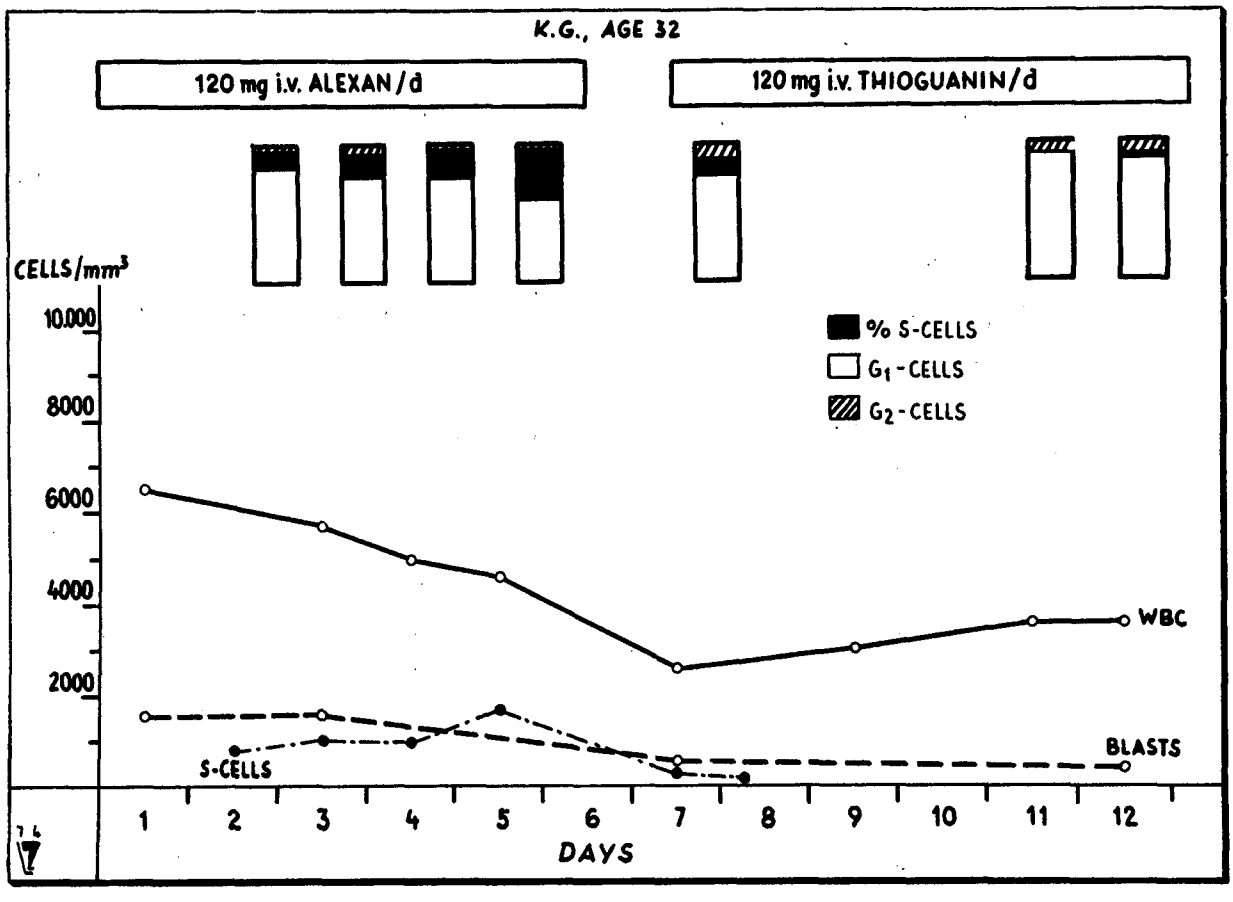

Fig. 8: Effect of high dosage of thioguanine following to ARA-C application leading to a decrease of blastcells in the peripheral count.

blood was increased. Therefore the success of any cell dependent drug should be related to the extent of the proliferative reactivity of a malignant cell population.

2.) A good response to chemotherapy was obtained in leukemic patients who either showed a high proliferative activity of their blast cells prior to treatment or who exhibited an increasing amount of proliferating leukemic cells during cytostatic therapy. In most cases such changes of the proliferation pattern were observed in the bone marrow histograms as well as in the peripheral blood. As soon as a high proliferative activity of leukemic blast cells was reached during chemotherapy, a good response was obtained by the cytotoxic treatment with cycle specific drugs (fig. 5).

Therefore the follow up of the proliferative activity of a leukemic cell population might be of prognostic importance. 4.) Cytokinetic changes which are specific for the action of an applied drug can be measured easily and rapidly by impulse-cytophotometry. This way the response of leukemic cells to a certain drug can be observed in each leukemic patient, so that individual changes of the chemotherapeutic treatment are possible (fig. 6). It is our opinion that the follow up of the proliferative activity of a leukemic cell population during chemotherapy might facilitate the individual treatment of leukemic patients on the base of cell proliferation and drug interaction (fig. 7, 8).

Acknowledgement: We are indepted to the late Federal President of Austria Dr. h. c. Franz Jonas for support of our work by means of his "Leukämieforschungsspende".

This research has been made by order of the Austrian Bundesministerium für Wissenschaft und Forschung.

Address of author: Dr. Paul Höcker, Ludwig Boltzmann-Institut für Leukämieforschung und Hämatologie, Hanusch Krankenhaus, Heinrich Collinstraße 30, 1140 Wien/Austria.

References:

1. Buckner, D., Graw, R. G., Robert, Jr., Eisel, J., Henderson, E. S. and Perry, S.: Leukapheresis by Continuous Flow Centrifugation (CFC) in Patients with Chronic Myelocytic Leukemia (CML). Blood *33*, 353 (1969).
2. Curtis, J. E., Hersh, E. M. and Freireich, E. J.: Leukapheresis Therapy of Chronic Lymphocytic Leukemia. Blood *39*, 163 (1972).
3. Hadlock, D. C., Mac Cullough, J. J., Deinard, A., Kennedy, B. J. and Fortuny, I. E.: Role of Continuous – Flow – Centrifugation (CFC) Leukapheresis in the Menagement of Chronic Myelogenous Leukemia (CML). Int. Congress about Cancer, Florenz 1974, p. 514 (Abstract).
4. Höcker, P., B. Haist, M. Goberts und A. Stacher: Die PHA-Stimulierung bei chronisch lymphatischen Leukämien vor und nach Leukapherese mit dem Zellseparator. 4. Arbeitstagung über Leukozytenkulturen "Lymphozytenfunktion in vitro", Innsbruck 1973 (Abstract).
5. P. Höcker, E. Pittermann, M. Goebets, A. Stacher: Treatment of patients with Chronic Myeloid Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL) by Leukapheresis with a Continuous Flow Cell Separator. Int. Symp. on Leukocyte Separation and Transfusion, London, September 1974, in press.
6. Lowenthal, R. M. and Graubner, M.: Leukapheresis as Initial Therapy of chronic myeloid leukemia, In: 3. Int. Arbeitstagung "Proliferative Erkrankungen des myeloischen Systems" Wien, März 1975, in press.
7. Schwarzenberg, L., Mathe, G., Pouillant, P., Weiner, R., Locour, J., Genin, J., Schneider, M., de Vassal, F., Hayat, M., Amiel, J. L., Schlumberger J. R., Jasmin C. and Rosenfeld, C.: Hydroxyurea, Leukapheresis and Splenectomy in Chronic Myeloid Leukemia at the problastic phase. Brit. Med. J., *1*, 700 (1973).
8. Vallejos, C. S., Mac. Credie, K. B., Brittin, G. M. and Freireich, E. J.: Biological Effects of Repeated Leukapheresis of Patients with Chronic Myelogenous Leukemia. Blood, *42*, 925 (1973).