

## HUMAN LEUKEMIA – AN OVERVIEW

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Prior to twenty-five years ago, there was no specific therapy for acute leukemia and survival of individuals with these diseases was usually no more than 3 or 4 months. There was no useful specific therapy, treatment consisting largely of blood transfusions and other supportive measures. X-irradiation, radioactive phosphorous, benzene, potassium arsenite, and nitrogen mustard, although of some use in chronic leukemia, were of little value in acute leukemia. Then, in 1948, Farber and his colleagues (1) reported that folic acid antagonists could induce complete remission in acute lymphocytic leukemia of children.

Subsequent work demonstrated that these agents, particularly aminopterin, would induce remissions in approximately 30 % of children with acute leukemia

### EFFECTIVE DRUGS IN CHILDHOOD ACUTE LYMPHOCYTIC LEUKEMIA

	NO. PATIENTS	CR (%)
6 MP	43	27
MTX	48	22
VCR	103	57
CYTOXAN	45	40
ASPARAGINASE	32	44
DAUNOMYCIN	82	15
ADRIAMYCIN	144	25
PREDNISON	337	63
ARA-C	122	7
BCNU		
TG		
HYDROXYUREA		

CR= COMPLETE REMISSION

MODIFIED, HENDERSON, 1969

Fig. 1: Useful drugs in the treatment of childhood acute lymphocytic leukemia. Modified from Henderson, E. S.: Treatment of Acute Leukemia. Seminars in Hematology 6: 271–319, 1969.

but in far fewer adults with acute leukemia. Unfortunately, remissions were temporary, the patients soon became refractory to the agents and survival was affected little, if at all. Important as these observations were, there followed little systematic fundamental work aimed at the control of cancer and specifically, leukemia. However, the observations with aminopterin and amethopterin gave rise to a good deal of optimism that curative treatment could soon be achieved and there followed a gradually intensifying effort to discover other drugs which could induce remissions. During the last two decades approximately one dozen agents (Figs. 1 & 2) have been found which are effective in acute leukemia. Some of these were discovered empirically and others were developed as an outgrowth of biochemical or other rationale. As a consequence, the incidence and duration of remissions have increased greatly and survival has gradually been extended so that median survival is now 36 months or more for childhood acute lymphocytic leukemia (Fig. 3). In some studies, this is now at approximately 5 years. Unfortunately, in adult acute leukemia, progress has been far slower. Remission rates of 50 % are not unusual but survival has been lengthened only relatively little. These results in childhood and adult leukemia have not been achieved with any single agent but are due to the use of combinations of drugs, a better understanding of the importance of drug scheduling, supportive care, and patient protection.

#### EFFECTIVE DRUGS IN AML (ADULT)

	CR (%)
<b>PREDNISONE</b>	15
<b>6 MP</b>	10
<b>MTX</b>	3, 16
<b>ARA-C</b>	21, 37, 44
<b>DAUNORUBICIN</b>	38, 50
<b>ADRIAMYCIN</b>	27
<b>METHYL GAG</b>	45
<b>VINCRISTINE</b>	20

**BCNU**

**TG**

**HYDROXYUREA**

CR= COMPLETE REMISSION

MODIFIED, HENDERSON, 1969

Fig. 2 Drugs for the treatment of adult acute myelocytic leukemia. For many of these, the number of patients treated with the individual drugs, is too few to make complete remission rates meaningful.

**CHANGING PROGNOSIS IN ACUTE LYMPHOCYTIC LEUKEMIA**

YEARS	GROUP OR INVESTIGATOR	TYPE OF THERAPY	MEDIAN SURVIVAL (MO. FROM DIAGNOSIS)
1937-53	Tivey	Supportive	3.5
1946-47	Burchenal	Supportive	3.5
1944-60	Boggs	Supportive → Pred., MTX, 6-MP	7.0
1956-57	Hendersen	Pred., MTX, 6-MP	5.0
1961	Boggs	Pred., MTX, 6-MP	12.0
1954-62	Cutler	Pred., 6-MP, MTX	12.0
1958-62	ALGB	Pred., MTX, 6-MP, Cytosan	12.0
1959-63	Burchenal	Pred., MTX, 6-MP, Cytosan	13.0
1955-63	Zuelzer	Pred., MTX, 6-MP	16.0
1963-64	NCI	VAMP	24.0
1963-65	ALGB	Combination of Pred., 6-MP, VCR, MTX, Cytosan	24.0
1965-66	NCI	POMP	33.0
1966-68	ALGB	Combination of Pred., VCR, MTX, 6-MP, daunomycin	> 36.0
1966-71	St. Jude's	Combination of VCR, Pred., 6-MP, MTX, Cytosan	> 36.0

**Fig. 3:** Progressive improvement in survival in patients with acute lymphocytic leukemia. Carter, S. K.: *The Chemotherapeutic Approach to Cancer Therapy: A Quick Overview*. In *Year Book of Cancer*, 1972. Clark, R. L. & Cumley, R. W. (eds). Year Book Medical Publishers, Chicago. pgs. 475-498.

A patient with acute leukemia dies because leukemic cells have compromised the function of an organ or normal tissue to the extent that some vital function is no longer possible. Suppression of marrow function is frequent either as a consequence of the disease itself or due to the use of myelotoxic drugs. Bleeding due to thrombocytopenia was until relatively recently the most common cause of death but at present, infections, particularly gram negative infections, are the most serious problem (2). The generous use of platelet transfusions has been responsible for the diminution of fatal thrombocytopenic hemorrhage but granulocyte transfusions have not been widely accepted probably due to the fact that until the last few years the procurement of normal granulocytes in large quantities has not been possible. In addition, there were problems in designing controlled studies to evaluate their effectiveness. However, it has recently been shown that histocompatible granulocyte transfusions are useful in the management of serious infections when given repeatedly to granulocytopenic patients (3). Another approach to the control of infection has been the use of protected environments and although their ultimate role in cancer therapy is yet to be defined, there is strong evidence that the incidence of infection is greatly reduced (4).

The strategy in the management of patients with acute leukemia has been to attempt to achieve rapid reduction of the leukemic cell population and restoration of normal bone marrow function followed by therapy designed to eradicate the neoplastic cells. Subsequently, maintenance therapy is instituted to keep the patient in remission and prevent overt appearance of the disease.

Although with the years more agents with activity in leukemia have been discovered, the most important factor in the improved prognosis in acute leukemia has

been the employment of drug combinations based on the underlying principle of using agents with different dose-limiting toxicities and with different mechanisms of action in order to minimize the development of drug resistance. There is abundant evidence that combinations of drugs can achieve remission rates as great or greater than predicted for additive effects of the single drugs employed (Fig. 4).

The role of immunotherapy in the management of patients with acute leukemia remains to be determined. There is evidence for tumor associated or tumor specific antigens on the surface of acute leukemia blast cells and prognosis appears to be related to immune reactivity. There have been many attempts to manipulate the immune mechanism to therapeutic advantage using immunization with syngeneic, allogeneic or isogenic cells, BCG and other immune enhancers, transfusion of immune sera, and syngeneic or allogeneic bone marrow transplants. Unfortunately, in spite of all these efforts, the role of immunotherapy in acute leukemia remains uncertain.

The success of chemotherapy in acute leukemia is undoubtedly dependent on exploitation of differences in cell uptake, biochemical control mechanisms, and cell kinetics and other factors which are not completely understood. Most of the advances in the treatment of leukemia have been achieved through the empirical search for anti-tumor drugs. Contributing factors include: 1. Synthesis or isolation of drugs from natural products and their evaluation for anti-tumor activity in animal

#### SINGLE VS. DRUG COMBINATIONS IN A.L.L.

	MARROW REMISSION (%)
PREDNISONE	67
VINCRIStINE	57
6 MP	27
MTX	22
PREDNISONE + MTX	80
PREDNISONE + VINCRIStINE	87
PREDNISONE + 6 MP	82
VAMP	88
POMP	94

Fig. 4: Examples of superiority of drug combinations compared to individual drugs.

systems. 2. Elucidation of their effects at the biochemical level. 3. Pharmacological and toxicological studies in animals in order to anticipate better pharmacologic disposition and toxicity in man and to provide guidance as to the route, dose, and schedule to be employed in man. 4. Pharmacologic studies in man. 5. Experimental trials in cancer patients to determine optimal dosage and schedules.

Undoubtedly, one of the major factors contributing to the success of chemotherapy, particularly against the rapidly growing tumors has been an understanding of the importance of drug scheduling concentrations at the target site and duration of effect. There are now numerous examples, both experimental and clinical, where a drug may be relatively ineffective on one schedule of administration yet result in a total remission with prolongation of survival on another schedule. The toxicity of an agent against both normal and neoplastic cells is directly related to its concentration (C) at the target and the duration of time (T) that this level is maintained. This so-called C x T concept is markedly affected by dose and schedules and optimally, the maximum number of tumor cells will be destroyed with minimal effect on the normal cells. It follows, of course, that different drugs are metabolized at different rates and their distribution in the body may vary.

Unfortunately, for many drugs, there appears to be little correlation between schedule dependency studies in L1210 or other experimental systems and clinical results. One of the difficulties lies in the fact that the cellular growth characteristics of L1210 leukemia and normal mouse marrow and the relationship between the two does not resemble any of the cancers in man including acute leukemia. More data are needed, not only of pharmacologic characteristics of drugs but also of the cell kinetics of both normal and tumor tissues at any given moment.

6. Supportive care, as already indicated, has allowed the clinician to treat more aggressively resulting in a greater cell kill. 7. Appropriate therapy to eradicate sequestered leukemic cells (as in the central nervous system). 8. The appreciation of the fact that acute leukemia is not a single entity and that the response to a given treatment varies according to the type of leukemia. The traditional classification of leukemia is based on morphologic description and clinical course and recently, cytogenetic analysis has been added to help in identifying certain subclasses and as a guide in prognosis. Many characteristics of leukemic cell populations – biochemical, kinetic, colony forming, cytochemical and ultrastructural – have been studied but most new classification proposals have been based on the use of finer cytological characteristics than those presently employed. Unfortunately, these are generally too difficult and controversial for general adoption. Nevertheless, it is obvious that the current classification is inadequate and a better scheme is needed in order to predict the course of leukemia and response to therapy.

In a broad sense, it can be stated that the vast amount of knowledge of leukemia including cell kinetics, biochemistry, molecular biology, cytogenetics, virology, and immunology has had relatively little impact on the management of patients with these diseases. This is true in spite of many optimistic opinions often expressed by investigators involved in these studies. The literature abounds with presumably logical concepts of leukemia cell growth and with sequences of macromolecular synthesis but who can say with real conviction that these reports have as yet had any impact in changing the prognosis of even a single patient with leukemia?

It is true that within the last decade, the relevance of cell kinetics of leukemic and normal leukocytes to successful chemotherapy of cancer has come to be recognized. An integral part of the anti-tumor development effort has been the constant search for drugs with "selective toxicity", i. e., drugs which could selectively destroy cancer cells without undue damage to normal cells. Unfortunately, this goal has never really been achieved and most clinically useful agents have significant and usually serious effects on normal tissue, particularly those with relatively rapid turnover times, the bone marrow and the gastrointestinal tract.

As is well known, under normal circumstances granulocytopoiesis is a cell renewal system so that cell production equals cell death. In patients with leukemia in relapse, granulocytopoiesis usually exceeds cell loss and an expanding cell population is the result (5). Granulocytes in the adult are produced in the bone marrow where there is an orderly division and maturation from the earliest cell, the stem cell, successively through the various cell types to the mature polymorphonuclear leukocytes so that fairly distinct morphologic compartments are identifiable (Fig. 5). In leukemia, in contrast to the normal situation, there is evidence from cell kinetic studies and from histological examination that leukemic cells may be produced in a variety of sites in addition to the bone marrow, i. e., lymph nodes, liver, spleen, testes, etc. The process of maturation and differentiation is disturbed and morphologic classification based on maturation is usually not possible.

Available evidence suggests that a cell perhaps similar to a small lymphocyte may be the common stem cell and that the erythroid, myeloid and megakaryocytic cell lines are probably derived from this pluri-potential cell. The stem cell compartment must be able to maintain itself against continued removal of cells for differentiation, reconstitute itself if depletion occurs, and be capable of increasing its rate of cell production upon demand. There is now good evidence in man that there is a single compartment which gives rise to these various cell lines. Support for this concept is provided from the observations by Whang *et al.* that the Ph<sup>1</sup> chromosome is present not only in granulocyte precursors but also in erythrocytic and megakaryocytic precursors (6). This suggests that the chromosomal defect arises in a cell

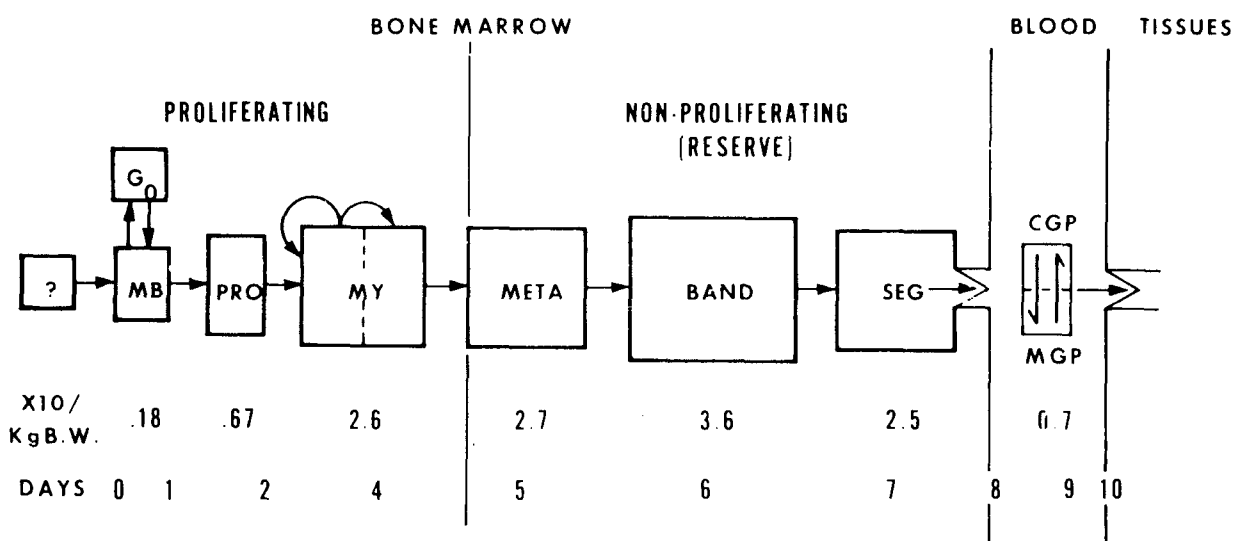


Fig. 5: Model for normal leukocyte kinetics.

which is a common stem cell for the three cell lines. Similarly, studies of the hematopoietic system of the mouse utilizing the spleen colony technique have also provided data suggesting that there is a single pluripotential stem cell.

In addition to the stem cell compartment there is also a large differential proliferating pool consisting of myeloblasts and promyelocytes. The next compartment in the sequence is the myelocyte pool composed of large and small myelocytes; the large cells representing a dividing pool supplying cells to the small cell maturation pool.

The proportion of proliferative cells in the bone marrow of patients with acute leukemia is relatively low compared to normal marrow (7–10). In normal bone marrow, approximately one-third of the myeloid cells are in proliferation with an average labeling index of about 30 % (11). Generation times for the myeloblasts, promyelocyte, and myelocyte have been estimated at 24, 60, and 54 hours respectively (12) with a maximum DNA synthesis time of 24 hours. It is now known that there may be a wide distribution of intervals for each of the phases. The variability in length of the  $G_1$  phase has the most relevance to the chemotherapy of patients with leukemia since most of the presently available anti-leukemic agents do not affect cells in the long  $G_1$  or so-called  $G_0$  phase. This will be considered at greater length below.

With the completion of maturation, the granulocyte enters the so-called “mature granulocyte reserve” of the bone marrow. Estimates vary, but there are approximately  $2-3 \times 10^{11}$  granulocytes in this compartment (13), and there are thus 10–20 times as many bands and segmented granulocytes in reserve as there are circulating in the blood.

The release of granulocytes into the blood is an interesting phenomenon which unfortunately is not well understood. Recent work suggests that changes in the biophysical properties of the cytoplasm as differentiation and maturation occur may be important factors (14).

It is important at this point to mention, if only briefly, some of the observations which have been made in recent years concerning granulocyte production *in vitro* (15). With both mouse and human bone marrow cells, colonies grown *in vitro* and arising from the colony-forming cell (CFC) require the continuous presence of a stimulatory substance, colony stimulating factor (CSF), which is found in sera and urine from normal and leukemic individuals and from mice. In the absence of this material, colony growth is not sustained and the cells rapidly die. It has been suggested that CSF is specific for neutrophils and that its major source are mature granulocytes. If this were the case, there would be no stimulus if an individual were rendered neutropenic and increasing myelopoiesis would result in the presence of granulocytosis. To confuse the issue further, there is good evidence that mature granulocytes are inhibitory (16) and that monocytes may be the source of material controlling granulocytosis (17).

CSF is a glycoprotein with a molecular weight of approximately 190,000 and is considered by many to be a growth regulator or granulopoietin for the granulocytic series analogous to erythropoietin for the red cell series. The function of CSF *in vivo* has not yet been elucidated; however, patients with acute lymphocytic or stem cell leukemia generally have elevated levels while those with acute myelocytic

leukemia have depressed levels (18). During remission, the levels in patients with acute myelocytic leukemia rise to normal or high values.

Diffusible granulocytopoietic stimulator (DGS) has been reported to be present *in vivo* in mice following the injection of endotoxin or after irradiation and has been shown to stimulate granulocyte production in Millipore filters implanted intraperitoneally (19). Preliminary data suggest that this material is different from CSF.

The relationship of CSF, DGS, chalone and other inhibitors, antichalone and leukocyte inducing factor is at present unclear and certainly somewhat bewildering. If there is a defect in this system in leukemia, its precise location is difficult to ascertain from reports in the literature. Finally, the significance, if any, of these observations for the treatment of patients with leukemia remains to be determined.

In contrast to the orderly unidirectional progression of division, maturation and release from the bone marrow of leukocytes in the hematologically normal individual, the picture in leukemia is largely one of confusion with marked deviation from the steady state (Fig. 6). In acute leukemia, normal leukocytes are replaced by large numbers of blasts both in the bone marrow and in the peripheral blood where the count may or may not be elevated. The spleen, liver and lymph nodes may be infiltrated with these cells and enlarged.

Years ago, it was assumed that in leukemia the orderly process of normal myelopoiesis was greatly disturbed owing to some unidentified influence and the myeloid precursors were rapidly and excessively proliferating. This hypothesis was never substantiated and was replaced by the current concept, first suggested by Astaldi and Mauri (7) that leukemic cells do not proliferate wildly, but that there is

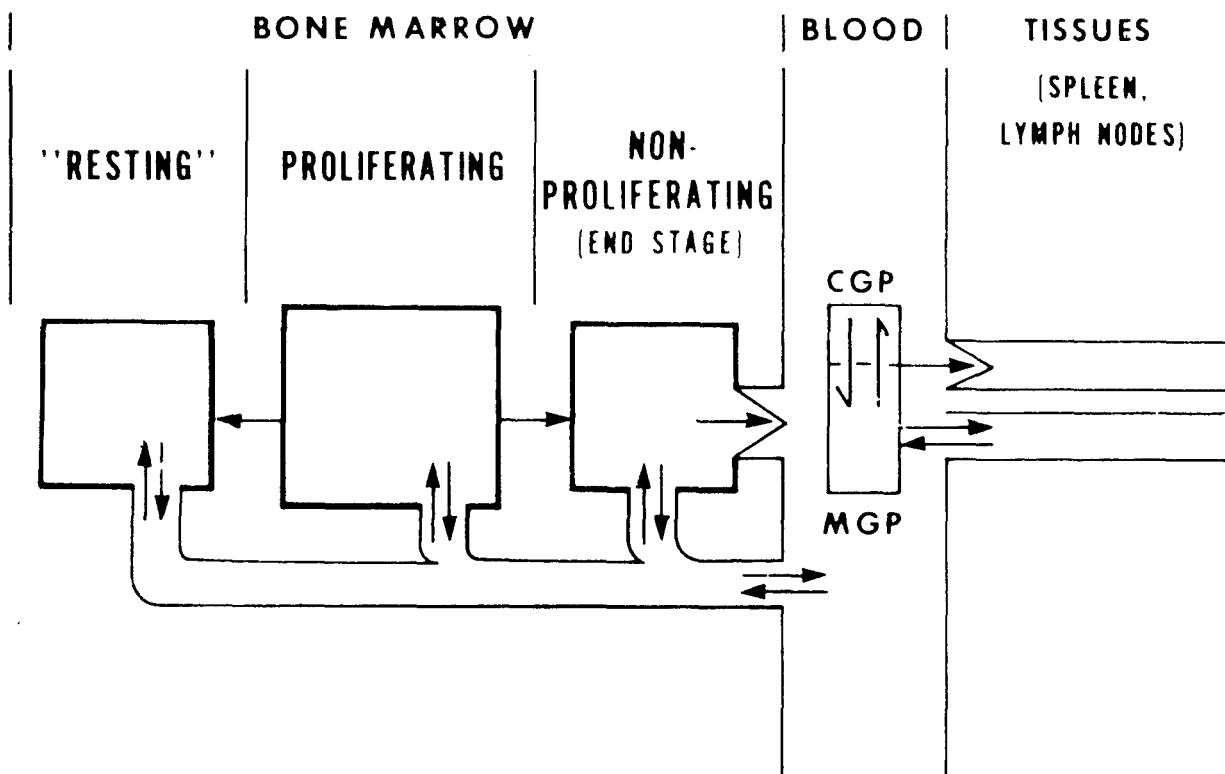


Fig. 6: Model for leukocyte kinetics in leukemia.



some maturation defect accompanied by the accumulation of large numbers of immature myeloid cells. Based on stathmokinetic and *in vitro* labeling studies with  $^3\text{HTdR}$ , Gavosto et al (8, 9), suggested that the proliferative capacity in acute leukemia was very low compared to normal bone marrow and that the labeling index of blast cells in acute leukemia was in proportion to the size of the cells, the larger cells being considered the younger ones. These cells, in both AML and ALL, comprised a relatively small percentage of leukemia cells in the bone marrow and had a high labeling index (range 24–52) both after *in vitro* labeling with  $^3\text{HTdR}$  and after a pulse label *in vivo* (20). In contrast, the labeling index of the small cells was quite low. It is now generally accepted that the large cells are the dividing or cycling population and that the small cells are the “resting” ( $G_0$ ) or non-proliferating population. However, this population is obviously not “resting” in the strict sense and most likely is comprised of cells in a very prolonged  $G_1$  phase. It is hypothesized, based on the interpretation of data obtained in patients with acute leukemia using  $^3\text{HTdR}$  labeling (21), that the small “non-dividing” leukemia cells are capable of re-entering the proliferative cycle.

Studies in the spontaneous AKR mouse leukemia employing a cell separation technique conclusively demonstrate that the small cells have a normal component of DNA and even after labeling with  $^3\text{HTdR}$  for a period equivalent to 5 cell cycle times, unlabeled cells are still present. These small cells are heterogeneous consisting of both non-clonogenic cells and clonogenic cells residing in either a  $G_0$  or a long  $G_1$  phase of the cell cycle (22–24). Upon transplantation to young normal AKR mice, the small cells are capable of proliferating and causing death due to leukemia.

There have been many cell kinetic studies in acute leukemia and although some of the data on cell cycle characteristics of leukemic leukocytes may be suspect it appears that (1) the majority of leukemic cells are capable of DNA synthesis but that most of the blasts are not in active proliferation (2) cell cycle times vary greatly (25–28), ranging from 60 to 200 hours and are generally somewhat longer than those for the early normal myeloid precursors and (3) the intravascular life of leukemic leukocytes is prolonged.

In contrast to the simple exponential intravascular disappearance pattern of normal granulocytes, leukocyte disappearance curves in patients with acute leukemia are often complex and prolonged (29–30). This may be present even when the patients are in remission and suggests that morphologically normal appearing granulocytes in these patients are still defective. On the other hand, extra-corporeal factors cannot be ruled out since prolonged intravascular curves have been reported in patients with non-leukemic malignancies (31).

In hematologically normal individuals, granulocytes once having left the vascular tree, do not return but in AML (32, 33) as in CML (34) leukemic cells may enter the spleen and then recycle to the blood and the bone marrow. Leukemic cells are rarely seen dividing in the peripheral blood and the proportion able to incorporate  $^3\text{HTdR}$  is less than that in the bone marrow.

The foregoing is a brief review of the current status of information concerning leukocyte kinetics in acute leukemia. The precise defect in acute leukemia specifically acute myelocytic leukemia, is not known but as has been postulated by Gallo (35) and others, the findings are consistent with a block in the normal process of

maturation of myeloid elements. Until the *in vitro* colony work discussed above this was considered irreversible but it now appears that leukemic cells can be made to mature under appropriate circumstances in the presence of a certain protein factor(s).

The cause of this disturbance in maturation is also not clear at the present time but in the last two or three years, a great deal of evidence has been accumulated strongly suggesting that RNA tumor viruses are involved. It is beyond the scope of this paper to review this evidence but regardless of whether one accepts the oncogene theory or the provirus theory, the finding of the enzyme, reverse transcriptase, may be a most important development as far as the potential for controlling or curing acute leukemia. This enzyme appears to be distinct from RNA dependent DNA polymerase activities which have been reported in normal cells (36, 37). If reverse transcriptase is unique to leukemic cells it represents a prime target for therapeutic attack providing its presence is required for maintenance of the neoplastic state. Other DNA polymerases in leukemic cells, if qualitatively different from their counterparts may also be important targets. In any case, the reports of selective toxicity of rifamycin derivatives for leukemic cells are exciting (38) even though the precise mechanism for this toxicity is still unclear (37). Undoubtedly, other compounds will be found with similar or better selectivity.

The accumulating evidence suggesting that a virus may be the etiologic agent in leukemia and that reverse transcriptase plays an important role in the initiation of the disease and perhaps, in its maintenance raise important questions particularly in relation to relapses in patients after long apparently disease free intervals. Such relapses have been postulated to be due to 1) persistence of resting cells and their re-entry into cycle 2) a failure of the immune mechanism in preventing the appearance of clinically detectable leukemic cells arising from a small cluster of cycling cells 3) re-induction by the agent responsible for the initial event. The latter possibility gains some support from the experience with normal marrow transplants into leukemic patients in which leukemic transformation of donor cells were observed. However, other explanations for this phenomenon are possible. In addition, specific cytogenetic abnormalities when present in acute leukemia tend to disappear when the patient is in remission but the same abnormalities recur in late relapses. It would be most unlikely that a virus would cause precisely the same abnormality upon re-infection. However, it is conceivable that a sub-virus moiety might bind at the same site and produce the same karyotypic defect.

How has this knowledge I have reviewed been utilized in the management of patients and has it been useful? Based on data from animal studies and certain kinetic considerations it is possible to conceptualize (Fig. 7) neoplastic cell populations, including leukemia (20). Populations with a high proportion of cells in active proliferation and with a high clonogenic potential are classified into compartment A; cells temporarily non-dividing but capable of re-entering the growth cycle (cells in  $G_0$  or in with a prolonged  $G_1$  phase) are in compartment B; cells which are incapable of reverting to proliferation and are end-stage or mature are in compartment C; and finally, dying cells and cells undergoing lysis and resorption belong to compartment D.

In leukemia and in other neoplastic populations, growth occurs when the input

from compartment A exceeds the loss in compartment D (or with A constant, the loss in D decreases, a very unusual situation). At an early stage, the proportion of cells in active cycle (i. e. in A) is high and the proportion in a resting phase (i. e. in B) is low. As the disease progresses, the proportion in B increases and the doubling time of the whole population lengthens. This change in proliferative characteristics from early exponential growth is best described by a Gompertzian function (39). Obviously, the deviation from exponential growth may also occur from an increase in cell loss, a lengthening of  $T_c$ , or a combination of these factors. It is important to note, however, that growth fraction and cell loss are probably the prime determinants governing the rate of tumor growth although growth characteristics may be changed as a consequence of therapy. It has been shown, in fact, that regrowth of L1210 following treatment with BCNU is accompanied by cells dividing with a longer  $T_c$  (40) and similar observations have also been reported in acute leukemia (41).

Remissions occur when the loss in compartment D exceeds the input from A. Most clinical by useful anti-tumor drugs affect compartment A cells. These are the so-called cycle active drugs and include the anti-metabolites and the mitotic inhibitors. Alkylating agents and functionally related compounds probably have their major effect on compartment A cells but do also exert an effect on cells in compartment B. Unfortunately, the effects on these cells are not well understood and as will be seen, the persistence of these cells after treatment represents one of the serious problems in the management of patients with leukemia.

Kinetic data on normal and leukemic animal and human leukocyte populations have been examined with relation to response to chemotherapy (20). A number of observations emerge including 1) there is a direct relationship between the labeling index and the response to chemotherapy; 2) there is an inverse relationship between doubling time and response and 3) alkylating agents are more effective against

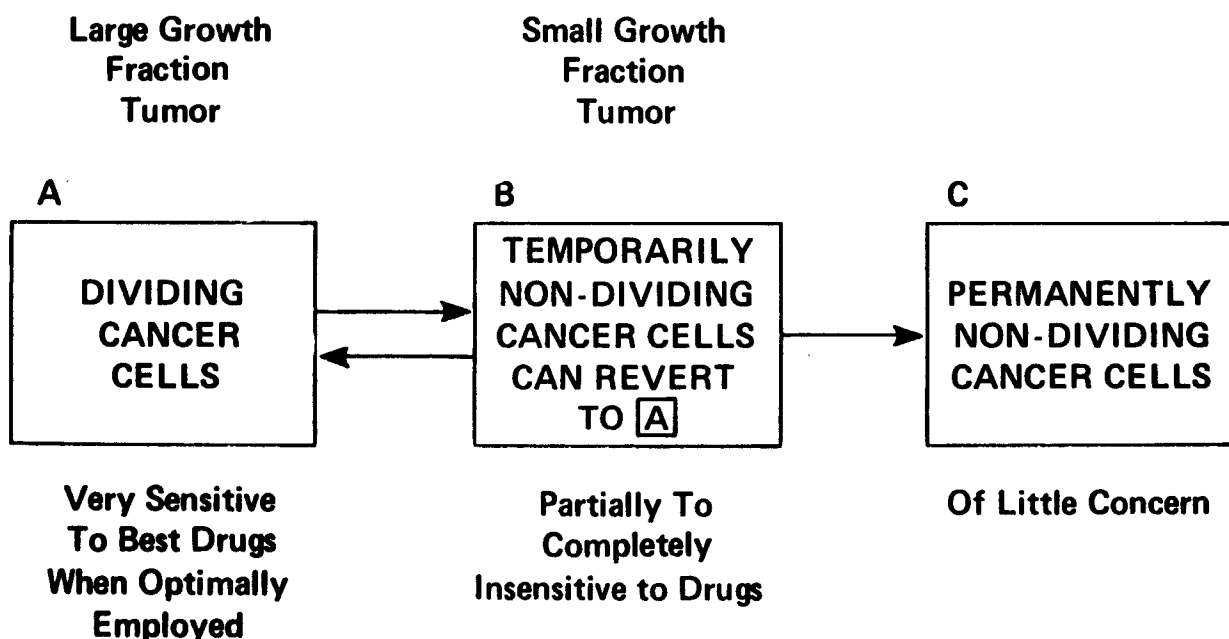


Fig. 7: Relationship between tumor growth characteristics and response to therapy (modified from ref. 20).

tumors with long doubling times and low growth fractions (compartment B) compared to anti-metabolites. Responsiveness appears to be related to the size of the growth fraction since as the growth fraction decreases with advancing disease, the likelihood of obtaining a tumor regression or cure declines.

At diagnosis, in an adult with acute leukemia, there are approximately  $10^{12}$  leukemic cells and the labeling index is quite low. A "remission" by current criteria is achieved when a 3 log reduction in cells is obtained with chemotherapy and although there may be  $10^9$  leukemic cells in the body, they are not detectable by the presently available techniques. Experience has shown, however, that continued aggressive therapy is necessary or the patient will quickly relapse. In any case, even though normal myeloid precursors are also affected by the agents employed, the normal elements regenerate more rapidly (shorter cell cycle time and higher growth fraction) and the leukemic cells are no longer detectable on blood or bone marrow examination.

If the body burden in acute leukemia at diagnosis or relapse totals approximately  $10^{12}$ , theoretically a 12 or 13 log reduction should affect a cure. The word "theoretically" needs to be emphasized since it may not be necessary to achieve at 12 or 13 log reduction for a cure if the immune mechanism is invoked to eradicate the last 2 or 3 logs of tumor cells.

In most cases of acute lymphocytic leukemia, the most responsive of the acute leukemias, the body burden of leukemic cells is reduced to  $10^3$  or  $10^4$  cells following vigorous combination chemotherapy. With prolonged therapy there is evidence that residual leukemic cells may number 100 or fewer and there are data in both man (25) and animals (23) that these remaining cells may be predominantly resting cells. Following continuous infusion of  $^3\text{HTdR}$  in patients with acute leukemia for as long as 20 days, a small but significant proportion of leukemic cells remain unlabeled (25). In spontaneous AKR leukemia, as discussed above, these small cells upon transplantation to young normal AKR mice, are capable of proliferating and causing death due to leukemia (22). There is good reason to believe that the kinetic behavior of leukemic cells in the advanced disease in man is similar to that of the leukemic population in spontaneous AKR leukemia and it appears quite likely that resting small cells in the human disease are also capable of resuming proliferation. Since resting cells are relatively insensitive to current chemotherapeutic agents, it would appear to be appropriate to use some form of immunotherapy in an attempt to eradicate them completely. However, thus far, as discussed above, this has not been achieved.

Both advanced L1210 leukemia and spontaneous AKR leukemia are relatively insensitive to cycle active agents, presumably due to the low growth fraction in both situations. However, if the total leukemic cell population is reduced by treating with a non-cycle active drug, the residual cells are stimulated to resume proliferation and are then susceptible to a cycle active agent such as arabinosylcytosine (42, 43). This concept underlies some of the attempts to gain a therapeutic advantage in human leukemia. For example, extracorporeal irradiation (44), intensive leukapheresis (45) and attempts at cell synchronization (46) have been employed in an effort to recruit resting cells to enter proliferation in AML. Unfortunately, these procedures have not led to a higher remission rate or to a prolongation of survival

following treatment. It is obvious that elucidation of the control mechanisms governing both the entry of cells into prolonged  $G_1$  or  $G_0$  is urgently needed.

A great deal of consideration in this paper has been given to attempts to achieve selective toxicity for tumor cells by trying to take advantage of a variety of differences between normal and neoplastic cells such as growth characteristics. Although these have not been totally successful, important progress has been achieved in controlling cancer in man. However, there are other avenues which deserve important emphasis and some of these, particularly following on the recent developments in molecular biology, have already been mentioned. Another approach which deserves attention lies in studies of the cell membrane. There is growing evidence that neoplastic cell surfaces may have therapeutically exploitable differences. The work with concanavalin A and wheat germ agglutinin has helped to elucidate cell membrane structure (47, 48). The agglutination of viral and chemically transformed cells is of great interest although some normal cells are also affected (49). These observations appear to deserve further work for potential application to treatment of patients with leukemia and other neoplastic disorders.

In summary, in this paper, I have attempted to review some of the concepts in acute leukemia and the status of treatment of patients with these diseases. Recent developments in several areas directly and indirectly related to leukemia add greatly to our knowledge of these disturbances and appear to have important implications for their control or cure.

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