Seminars in HEMATOLOGY
VOL. XIX, NO. 4, OCTOBER 1982

New Approaches in Chronic Granulocytic Leukemia—Origin, Prognosis, and Treatment

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A SERIES of remarkable discoveries has-contributed in the last 20 yr to our understanding of the nature of chronic granulocytic leukemia (CGL)—notable landmarks have been the discovery of the Philadelphia chromosome, the recognition of the translocation t(9;22), the realization that CGL is a panmyelopathy involving all cell lines in the myeloid series. and the finding that a- minority of transformations have blast-cells with a lymphoid phenotype. For the patient with CGL, however. the progress has been less impressive. Drugs that controlled to some extent the myeloproliferation were available at the beginning of the century, and the use of radiotherapy when it became available in 1902 was an important further devclopmcnt. In 1952 the introduction of busulfan revolutionized treatment and it was indeed some years before it was fully realized that busulfan was in a sense a nixed blessing—the very ease of its administration directed attention away from the fact that urvival of patients treated with busulfan was rolonged only very modestly. Subsequent attempts to improve survival by the use of other cytotoxic drugs, alone or in combination. have ot in general &en valuable.

Many aspects of the etiology, epidemiology. cytogenetics. clinical features and hematology of 7L have been ably reviewed in the last few ars.[16,67,70,98,113,119] In this short paper we will limit our attention to three areas in which new i-formation has accumulated very recently: the dencc for the clonal origin of CGL. the attempts to predict the duration of the chronic phase and of survival, and the newer approaches t reatment of paticnts in chronic phase and in tr nsformation.

CLONAL ORIGIN OF CGL

Cytogenetic and Enzymologic Data for Unicellular Origin

In 1960 Nowell and Hungerford described an abnormai small chromosome in patients with CGL." This abnormality is now known to arise as a result of translocation of a variable quantity of genetic material from the long arm of one of the 22 chromosomes to another site. In perhaps 90% of patients the material is translocated on to the long arm of chromosome 9 and the translocation is then referred to as t(9q+; 22q-).[96] Although originally thought to be confined to cells of the granulocytic series. the Ph' chromosome has since &en identified in cells of erythroid, rnegakaryoblastic, monocytic, eosinophilic and probably also basophilic lineages.[25,44,66,94,123] It is regularly absent in dividing marrow fibroblasts. in somatic cells and in most peripheral blood lymphocytes stimulated to divide by phytohemagglutinin.[54,75,123] One report suggested that it was present in T-lymphocytes (or their precursors).' but we studied a 13-yr-old boy who had had CGL since the age of 7 without finding any Ph'-positive T-cells in the peripheral

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Supported in pari by the Leukaemia Research Fund.

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0037-1963/82/1904-0001$02.00/0

blood.[54] Conversely the simultaneous application of immunofluorescence techniques and cytogenetics showed that substantial proportions of B-lymphocytes may be Ph¹-positive[7] and B-lymphoid cell lines bearing the Ph¹ chromosome have been established from a patient with CGL.[74] Such evidence suggests that the Ph' abnormality may originate in a single pluripotential stem cell. In patients in whom the two 22 chromosomes can be distinguished one from the other the finding that the Ph' chromosome always involves the same member of the pair further supports the concept that the Ph¹ abnormality has developed originally in a single progenitor or stem cell.[70]

Further evidence in favor of the unicellular or clonal origin of CGL comes from the elegant studies of Fialkow and his co-workers. They made use of the observation that individual somatic cells of black female patients heterozygous for the two enzymes of glucose-6-phosphate dehydrogenase (G6PD) contain only either enzyme type A or enzyme type B. Studies in a small series of women have uniformly shown that leukemic cells and their progeny contain exclusively enzyme type A or enzyme type B but not both. This finding suggests that the CGL is derived from a single hemopoietic stem cell of one or other enzyme type.[34,35] One such patient treated with cytotoxic drugs has achieved a true remission. Her marrow was restored to Ph¹-negative status; at that time the marrow contained presumably normal myeloid cells that were of both enzyme types.'"

Other cytogenetic evidence also favors the clonal origin of CGL. In one patient whose somatic cells showed him to be a sex chromosome mosaic (XY/XXY) only the 46, XY clone showed the Ph' chromosome." A similar patient was reported by Moore and his colleagues": a boy with CGL proved to be a constitutional mosaic with 46. XY/47, XYY chromosomes in skin, blood and marrow. In ihc chronic phase a third cell line, 46. XY. Ph¹ prcdominatcd in the marrow and was identified also in transformation.

Finally. one must consider the evidence that some paticnts with CGL enter a phasc of transformation in which the blast cells havc predominantly lymphoid characteristics.[1] Such lymphoid blast cells contain terminal deoxynucleotidyl transferase[74,102] and sometimes cytoplasmic immunoglobulin"*"that suggests features in c mon with an early progenitor of B-cell lineage one reported case the blast cells had immunol cal feature of T-cells.[34] The finding that s lymphoid transformations involve Ph¹-posit progenitor cells adds further weight to the ar ment that CGL starts in a cell with both myel and lymphoid potential. in other words, a plu potential hemopoietic stem cell."

Significance of Ph¹-Negative Myeloid Cells in Patients with Ph¹-Positive CGL

In the majority of patients with CGL, 100% marrow metaphases show the Ph¹ chromosom and the anomaly persists throughout the cours of the disease. A small proportion, perhaps les than 8%, have a similar disease in which the Ph chromosome is never detected. Most suci patients with Ph¹-negative disease have a leuke mia that is hematologically distinct from Ph¹-positive CGL and could perhaps be better classified as "chronic myeloid leukemia."[41] It is generally agreed that the survival of patients with Ph¹-negative CGL is inferior to that of patients with Ph¹-positive disease.[15,30,104,122] In occasional cases. however, the leukemia is hematologically indistinguishable from classical CGL and differs only in lacking the Ph¹ chromosome;[100] the survival of such patients may be no different from survival of patients with Ph¹-positive disease.

There are other patients in whom Ph¹-positive and Ph¹-negative metaphases coexist when the disease is first diagnosed. Rarely. such mosaicism may be recognized even before the leukemia is clearly established or when the leukocyte count is still comparatively low." Mort often. the patient already has pronounced leukocytosis and perhaps symptomatic disease. The frequency with which some Ph¹-negative metaphases are identified in patients with Ph¹-positive disease must depend to somc extent on the number of metaphases analyzed: some Ph¹-negative cells have bccn found in 6% of patients in one series,[39] in 29% of cases in a second series[110] and in 51% of cases in a third.[1] In the majority of cases in which Ph¹-negative metaphases have bccn observed in Sokal's series. less than 2 yr have elapsed from diagnosis and the author concluded that the probability of finding Ph¹-negative mitoses was inversely related to the duration of

the CGL.[119] In another series of paticnts the finding of **Ph'-positive/Ph'-negative** mosaicism indicated a relatively prolonged duration of chronic phase disease[79] but in earlier and later studies **no** such survival advantage could **be** demonstratcd.[17,110,129]

In **1964** Speed and Lawler rcportcd brief details of a patient with CGL who was treated with busulfan and sustained a period of bone marrow hypoplasia; subsequently his marrow was found to **be Ph'-negative.**[112] Thereafter **a** number of similar patients was reported whose marrows, Ph'-positive at diagnosis. showed a majority or virtually all mitoses to **bc** Ph'-negative after treatment with busulfan that had produced variable **periods** of **hypoplasia.**[37,79,93] In some reports the patienu had been treated with amounts of busulfan that were excessive by conventional standards, but in other **cases** the hypo**plasia** had followed administration of quite small doses of the drug.[40] Such observations were interpreted as compatible with the hypothesis that Ph'-negative, and presumably normal, hemopoietic stem cell: were present in the marrow, albeit their proliferation suppressed. in the majority of ***cases*** of CGL at diagnosis. Appropriate therapy might then selectively suppress the Ph'-positive clone and permit the marrow to **be** repopulated by normal hemopoietic cells."

This possible approach **to** therapy appeared to have particular appeal because some of these patients who survived periods of marrow hypoplasia and showed Ph'-negative metaphases had **very long survivals.**[5,9,26,37,43,79,112,127] In one remarkablc example a 42-yr-old woman was treated with busulfan (185 mg) in 1956 (before the Ph' chromsome had been recognized). In 1964 her marrow was Ph'-negative but later in the year it became Ph'-positive; she then received further treatment with busulfan (110 mg) and her marrow was restored to Ph'-negativity. Thus, her first true remission may be presumed to have lasted 7 **yr**[26]; her second remission continued and had lasted 14 years at the time of the morc recent report.' More often, the treatment has Icd to a stable pattern where both Ph'-positive and Ph'-negative cells were present for long periods in the marrow **in** approximately constant proportions." Such "stable mosaicism" after treatment with busulfan might be explained **by** postulating that busulfan caused a mutation or other sublethal damage in the Ph'-positive clone whereby these cells lost their proliferative advantage." A murine model of chronic hypoplastic marrow failure following treatment with busulfan was developed by Morlcy and might parallel in the mouse the effects of busulfan in selected ***cases*** in man."

These observations has led Sandberg to propose a scheme **of** "staging" for patients with Ph'-positive **CGL based** on cytogenetic findings? *

Stage I—The presence of only cytogenetically normal marrow metaphases (only achieved **on** rare occasions following treatment)

Stage II—Cytogenetically normal **cells** coexist with Ph'-positive cells

Stage **111—All** marrow metaphases are Ph'-positive

Stage IV—All marrow metaphases are Ph'-positive but some **cells** have additional chromosomal anomalies

Stage V—All metaphases show the **Ph'** change **plus** additional chromosomal anomalies

Most patients with CGL are in Stage III when the disease is diagnosed. Clearly, **Stage** I is achieved only after "successful" therapy and might correlate with prolonged survival. Whether Stage II recognized before treatment or achieved after administration of cytotoxic drugs *is* of benefit to the patient remains an open question.

Is the Ph' Chromosomal **Anomaly** Primary or **Secondary** in CGL?

It was assumed for some years after its discovery that the acquisition by a hemopoietic stem cell ***of*** the Ph' chromosome anomaly represented the primary event in the pathogenesis of CGL, and such may indeed be the case. Certainly the Ph' chromosome is almost universally present, usually in 100% of marrow metaphases. very early in the clinical evolution of CGL.[41] Recent evidence, however, **casts** some doubt on the conclusion that the acquisition of a Ph' chromosome is always the seminal event. There are a number of individual reports in which the patient's marrow is apparently entirely Ph'-negative at diag**nosis.** but Ph'-positive cells are identified during subsequent follow-up (Table 1). Of course. if the number of metaphases analyzed originally is

Table 1. Reported Cases in Which Either (A) the Ph' Chromosome was Identified Only Some Time After the Original Diagnosis of CGL, or (B) in Which a Ph' Chromosome was Identified When the Leukemia was Diagnosed but was Subsequently "Lost"

Patient	Age/Sex	Diagnosis and Initial Cytogenetics	Subsequent Course	Reference
1	38/F	MPD (45,XX,−6)	BT (45,XX,−6,−22,+Ph')	Kohn et al., 1975[68]
2	NS/M	CGL-CP (46,XY)	CGL-CP (46,XY,Ph')	Tanzer, 1977[70]
3	5/M	CGL-CP (100% Ph')	CGL-BT (100% Ph'-neg)	Lawler, 1977[70] Chessells et al., 1979[20]
4	39/M	CGL-CP (Ph'-neg)	Subsequent CGL-CP (Ph'-pos)	Hayata et al., 1975[66] Sandberg et al., 1978[100]
5	42/M	AML (46,XY,Ph'[40%])	Relapse, 100% Ph'-neg	Wayne et al., 1979[70]
6	19/M	CGL-CP (100% 46,XY,Ph')	CGL-BT (49,XY,+9,+10,+12; no Ph')	Hagemeijer et al., 1979[57]
7	53/M	CGL-CP (46,XY)	CGL-CP (46,XY,Ph')	Lisker et al., 1980[73]
8	42/F	CGL-CP (46,XX)	CGL-CP (46,XX,Ph')	Lisker et al., 1980[73]

NS = Not stated in original report.
CGL-CP = Chronic granulocytic leukemia—chronic phase.
CGL-BT = Chronic granulocytic leukemia—blast-cell transformation.
MPD = Myeloproliferative disease.
AML = Acute myeloid leukemia.

small (as was the case in the report by Lisker et al.),[73] it remains possible that a Ph'-positive line was present ab initio but was simply not identified. In other cases a Ph'-positive line was identified originally at diagnosis but disappeared subsequently in the patient's clinical course (Table I). One such patient was studied with particular care by Hagemeijer and his colleagues.[57] A 19-yr-old boy presented with typical Ph'-positive CCL. He was treated intermittently over four years with busulfan; his blood and marrow stilt showed a picture consistent with CGL but cytogenetic analysis of blood. marrow. and spleen cells was normal. During two separate episodes of blast-cell transformation, he had a hyperdipioid karyotype without evidence of the Ph' chromsome. Another paticnt described by Chessells and her colleagues is of interest.[20] A 5-yr-old boy presented with classic Ph'-positive CGL in chronic phase. The disease was controlled initially with busulfan. but some months later blast-cell transformation supervened; at this time the blast cells were Ph'-negative. These various clinical reports are difficult to reconcile with the concept of CGL as a monoclonal disease in which the neoplastic clone, marked by rhc Ph' chromosome. coexists with 3 population of strictly normal cells. the proliferation of which is usually effectively supprcsscd.

Further study of the patient with CGL and constitutional mosaicism originally described by Moore et al.[33,34] has yielded intriguing results. Though initially blastic transformation was recognized in the 46. XX. Ph' cell line, later in the evolution of the disease a major aneuploid 46. XYY, -C, Ph' line was identified. One possible explanation for this curious sequence of events is that both lines of the mosaic were involved early by the leukemic process, the Ph' anomaly being acquired or expressed in the XYY line only late in the disease.

Fialkow and his colleagues have recently reported the combined use of cytogenetic analysis and assay of cellular G6PD with valuable results.[71] They were able to infect with EB virus B-lymphocytes collected from a patient with CCL who was heterozygous for the two enzymes of G6PD and to establish from different lymphoid progenitor cells a series of short-term cell lincs of undoubted B-cell lineage. Some of these lincs were Ph'-positive but the majority were Ph'-negative. The Ph'-positive lincs were all of the same G6PD phenotype as the patient's myeloid leukemia (type B); amongst the Ph'-negative lines some were of type A and others of type E. Of the Ph'-negative lines only those of G6PD type B had karyotypic abnormalities; the G6PD type A lincs wcrc all essentially normal. The authors concluded that their studies were compatible with a multi-step origin of CGL—a neoplastic cell line occurring first as a result of a hcritablc change in a single pluripotential stem cell and the Ph' chromosome appearing thereafter. If this is indeed the universal sequence of

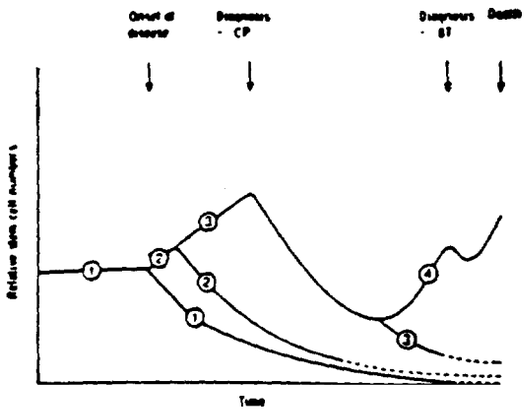

Fig. 1. Schematic representation of the evolution of hemopoietic stem cells in CGL. The progress of the disease in a hypothetical patient is represented by progression from left to right: (1) Ph¹-negative normal stem cells; (2) Ph¹-negative stem cells with a heritable defect characteristic of CGL; (3) Ph¹-positive stem cells with a proliferative advantage characteristic of chronic phase CGL; (4) Ph¹-positive stem cells (usually with additional cytogenetic changes) characteristic of blast-cell transformation of CGL. CP = Chronic phase. BT = Blastic transformation.

events, it seems probable that the acquisition of the Ph¹ anomaly confers on that clone of cells (in most cases) a proliferative advantage over both normal and Ph¹-negative neoplastic hemopoietic stem cells. This hypothesis is shown schematically in Fig. 1.

PROGNOSTIC FEATURES IN CGL

For some years. writers have studied personal series of paticnts with *CGL* and have sought like a holy grail a feature or a constellation of features that would help *to* predict prognosis — the duration of the chronic phase or the duration of survival—in an individual patient. Results of such analysts have in general been confusing, and features that appeared in one study *to* have definite prognostic significance have frequently failed to gain confirmation in other studies. This result can only mean either that the originai positive findings reached conventional Icvcls of statistical significance only by chance or that different series of paticnts are genuinely different. In either case, the value of the original findings is reduced.

In more general terms, the attempt *to* identify a feature or features characterizing a paticnt at diagnosis that correlates well with survival may be conceptually wrong. The features that determine the duration of the chronic phase or of survival in a given paticnt are not understood, but it may reasonably be postulated that they include at least somc measure of the rate of progression or "tempo" of the disease, some measure of the point in its progression at which the disease is diagnosed and other unspecified factors.[1,4] An assessment that takes account in individual patients of "static" features at diagnosis may reflect the stage to which the disease has progressed but may provide no measure of the disease tempo. Conversely, measurement of *"dynamic"* features such as change in leukocyte numbers following treatment or cytokinetic data may give some evidence of the disease tempo but may not accurately reflect the point in its progression at which the disease is diagnosed. The ideal procedure for assessing prognosis should reflect both these aspects of a patient's disease. A possible solution to this problem will be discussed later.

A further problem related to the question of predicting the duration of the chronic phase or of survival requires mention. It has been suggested that the chronic phase *of* CGL might better be described as a "pre-leukemic" condition that predisposes to but does not inevitably terminate in blastic transformation." The observation that transition to a more aggressive phase of the disease is usually accompanied by evidence of cytogenetic evolution has been interpreted as support for this concept. Alternatively, it is plausible that even in chronic phase the disease is already truly leukemic and is progressing inexorably towards a terminal blastic transformation.'" Evidence for kinetic changer occurring progressively during the course of chronic phase disease. referred to below. is compatible with this interpretation. Whichever view more correctly describes the natural history of CGL. one can recognize two different categories of prognostic features. On the one hand, there is a reasonably well defined series of features which, occurring during the course of the chronic phase, appear to predict poor subsequent survival but are in fact early signs of transformation.'.".'" 'These features include fever. refractory splenomegaly. nodular skin lesions. significant lymphadenopathy, lyric lesions of bone, excess of blasts or blasts plus promyelocytes in the blood or the marrow, high neutrophil alkaline phosphatase and per-

haps marrow myelofibrosis. Cytogenetic evidence of clonal evolution or changing patterns of colony and cluster formation in agar culture of marrow are probably also in this category.[70,85,98] Any of these features may in reality be better regarded as evidence that the initial events in transformation have already occurred and the relevant question relates only to the speed at which transformation will proceed thereafter. On the other hand. there are other features. some identifiable at diagnosis (static features) and others based on changes occurring during the first months or years after diagnosis (dynamic features). that do not definitely represent early transformation but may yet have prognostic significance. The possible prognostic features in this second category are reviewed briefly bclow.

Possible Prognostic Features Definable at Diagnosis

Age

In one large series. younger patients (less than 20 yr) survived significantly longer than older patients (more than 70).[61] The same trend was noted in other series.[30,104,130] Young patients survived longer also in the multicenter study organized in Italy' but age carried no prognostic weight in a recent report of patients treated in Bologna.[124] In the majority of other reports the age of the patient did not appear to influence prognosis.[18,50,65,82,87,106]

Sex

There was no difference in survival between males and females in the series of patients reported by Minot ct al,[61] and there was no influence of sex in series rcportcd recently from Italy. Spain and the United States.[15,50,124] In almost every other series in which sex was considered in relation to survival. howcver. males survived distinctly or significantly worse than females.[33,61,65,104,106,130] It seems fair to conclude that in general fcmalcs with CGL can expect to live lonncr than males.

Symptoms at Diagnosis

With respect to symptoms it is particularly necessary to differentiate bctwcen clinical features that actually denote early transformation and those that correlate merely with cxtcnsivc chronic phase disease. With this proviso. Jacquillat has rcportcd that patients with symptoms (including asthenia, weight loss. bone pin. fever, night sweats and gastrointestinal disturbance) at diagnosis have significantly shorter survivals than those without symptoms." Based on a series of 130 patients. Haanen and Oehlen have confirmed that patients with weight loss or night sweats have poorer prognoses than those without such symptoms." Leavell has reported that patients with a bleeding tendency have poor prognoses.[71] Others. however, have found that asthenia and weight loss lack prognostic significance.[104]

Spleen Size at Diagnosis

Measurement of spleen size is not particularly objective and writers have used various criteria to dowment splenomegaly. In the two Italian series and in the Chinese series. patients with spleens descending more than IS cm below the costal margin have survived for shorter periods than those with smaller spleens.[124,130] In the Spanish series. an impalpable spleen has k e n a good prognostic feature"; in five other series patients with minor degrees of splenomegaly have survived longer than those with larger spleens.[2,22,23,50,61] Other reports have failed to show any effect of spleen size at diagnosis on survival.[59,60,82,104]

Liver Sire at Diagnosis

If the measurement of spleen size at diagnosis is sonicwhat inexact. this applies even more to measurement of liver size. Frequently the organ is enlarged but the consistency of the edge is such that it is difficult to feel per abdomen. Nevertheless, patients with an impalpable or small (less than 6 cm bclow the costal margin) liver have survived significantly longer than patients with a larger livcr in the scrics rcportcd from Italy and Spain."[124] Conversely, large liver size has carricd a poor prognosis in some series[50,82] but the association has not bccn confirmed by others."[104]

Leukocyte Count at Diagnosis

In the two Italian series patients with presenting leukocyte counts bclow 100 x 10^9/l survived significantly longer than those with higher leukocyte counts.[124] Using a cut-off point at 25 x 10^9/l, the same superior survival for

patients with low leukocyte counts was noted in the series reported by Jacquillat.[31] Leavell also noted a favorable influence of relatively low leukocyte counts,[11] although in a German series patients with presenting leukocyte counts below 20 x 10^9/l had short survivals.[11] In line with the possible benefit of a low leukocyte count at diagnosis. a high leukocyte count (>300 x 10^9/l) was found to predict short survival in one series.[130] No effect of leukocyte count on survival was noted in five other series.[18,50,59,60,82]

Proportion of Blasts and Promyelocytes in Blood and Marrow at Diagnosis

There seems to be no general agreement as to whether the proportion of blast-cells or blasts plus promyelocytes has significance in determining prognosis in patients who are not in incipient transformation. In five series a relatively high percentage of blast cells in the blood or the blood and marrow has correlated with poor prognosis.[18,50,60,61,108] In the Italian series patients with less than 1% of blasts in their blood, who surprisingly constituted 33% of patients. have had significantly longer survivals[124]; the patients with fewer than 20% of granulated precursors (promyelocytes and myelocytes) have also survived longer than those with more than 20%. On the other hand, no influence of blast cell numbers at presentation has been noted in two other studies.[82,104]

Hemoglobin a: Presentation

In some series patients with relatively lower hemoglobin values had poorer survivals than those with higher values.[18,50,60,61,71,130] In other series no effect of hemoglobin level on survival was discerned.[59,82,104,124]

Platelet Count at Diagnosis

The level of the platelet count at diagnosis has had prognostic significance in most reports. Though the level defining "high" platelet counts was quite variable, it was a general finding that patients with high counts had short survivals.[59,61,77,90,124] Conversely, patients with abnormally low platelet counts also had a poor prognosis.[50,61,90,104,124,130] In two reports no dctinite effect of platelet count on survival could be detected.[18,82] There seems general agreement that a persistent rise of platelet numbers refractory to therapy and falling numbers both herald transformation.

Cytogenetic Analysis to Predict Prognosis in CGL

This subject has been extensively reviewed in recent years[7,77] and results will be summarized here only briefly:

(1) In almost all reports. patients with Ph^1-negative CGL survive less well than those with Ph^1-positive disease, in fact the presence or absence of the Ph^1 chromosome is probably the single most important prognostic feature.

(2) Patients with non-standard translocations (translocations other than t(9;22)) do not differ in survival from those with standard translocations.[111]

(3) The survival of patients with abnormalities in addition to Ph^1 at diagnosis is *not* definitely shorter than when the Ph^1 chromosome is the only abnormality. Those who acquire such additional abnormalities after diagnosis do. however. have shorter survivals.

(4) In most reports, loss of the Y-chromosome leading to the Ph^1-positive, XO myeloid line carries a relatively favorable prognosis[7,77]; not all, however, agree.[70]

Prognostic Features Defined After Initial Diagnosis

Response *of rhr Leukocyte Count* io *Chemotherapy*

In early studies Galton noted that the rate at which the leukocyte count fell after beginning treatment was related to the daily dose of busulfan.[18] He also noted that the rate of fall differed in different patients and that the doubling time for the leukocyte count after each successive course of busulfan tended to shorten in an individual paticnt. These observations were extended by Bergsagel who plotted the leukocyte count doubling time after the first course of busulfan against the patients' survival and observed a significant correlation': a similar relationship between circulating immature myeloid cell numbcrs and survival has also been demonstrated.[116]

Cytokinetic Studies

Cytokinetic studies carried out on the blood and marrow of patients with CGL have recently been reviewed.[77] Using specific cytokinetic fea-

tures (mitotic index, stathmokinetic index following vincristine in vivo and flash-labeling index with ^{3}H-TdR). Baccarani and Killmann were able to show that promyelocytes and myelocytes in CGL resembled normal progenitor cells but blast cells had a distinctly lower labeling index and resembled in this regard the blasts of acute myelogenous leukemia rather than their normal counterpart.' These studies were interpreted as evidence for the existence early in the disease of a minority of cells that might characterize subsequent transformation. Others have suggested that the labeling index in vitro was related rather to the level of the leukocyte count and was unlikely in itself to have prognostic significance."' There was. however, a correlation between high proportions of blasts plus promyelocytes with low mitotic indices and survival.[108] Though the authors suggested it, they did not show in their study a separate influence of low mitotic index and high proportion of blasts plus promyelocytes.

Cytogenetic Studies

The majority of patients with Ph'-positive CGL have no other cytogenetic abnormalities when first diagnosed. Perhaps 80% of patients in blast-cell lransformation have evidence of additional cytogenetic change. and the occurrence of such change in patients still in apparently uncomplicated chronic phase disease has bccn held to indicate impending transformation. The subject has been reviewed by a number of writers in recent years[70,98,100] and will not be referred to here in further detail. For the sake of this discussion, the central question is really whether the occurrence of further cytogenetic change in chronic phase represents the earliest evidence of transformation or merely reflects a disease that is genetically unstable and thus more likely than others to enter transformation in the near future.

Myelofibrosis

Marrow fibrosis is present at diagnosis in some paticnts with CGL and dcvclops during the course of the chronic phase in oihcrs. As to whcthcr the idcntiticntion of mild or moderate degrees of fibrosis indicates impending transformation. opinions differ. In rhc scrics rcportcd by Gralnick and his colleagues, 8 of 30 paticnts had some increase in marrow content of reticulin fibers at diagnosis and 39 of 181 patients developed fibrosis after diagnosis: the mean duration of survival after recognition of fibrosis in these paticnts was only 4.9 mo.[32] In another more recent but smaller series of patients the finding of myelofibrosis "warned of imminent death."[13] The amount of fibrosis was graded in serial biopsies performed in 45 patients by Clough and her colleagues." They established no definite correlation between the amount of fibrosis and survival and noted that patients with major marrow fibrosis did not always have a rapidly fatal course. Others also failed to find a correlation between myelofibrosis and survival in the first three years of the chronic phase but fibrosis was a poor prognostic feature in patients more than 45 mo aftcr diagnosis." Myelofibrosis is seen with qual frequency in patients with lymphoid and myeloid transformations."

Requirement for Treatment as a Prognostic Feature

The finding that the leukocyte count doubling time after a single course of busulfan can be correlated with survival' could be due to the fact that this measure reflects to some degree both the point in its evolution at which the disease is diagnosed and the tempo of disease in a given patient. Along similar lines, Haanen and his colleagues note that splenomegaly or leukocytesis that persists aftcr three months' treatment with busulfan defines patients in a "poor-risk" category.[56] Wc identified 25 patients whose chronic phase disease has lasted from 13 to 72 mo and who have received treatment with busulfan only within the first year of diagnosis. We found that ihc total dose of busulfan administercd during this time was inversely related to the duration of chronic phase in individual patients, and a mathematical derivation of the relationship gave a highly significant correlation.':' When busulfan-treated patients are included in thc original comparison of busulfan and radiotherapy undertaken by the Medical Rcscarch Council in the United Kingdom[30] wcrc analyzed in the same way, a similar correlation was observed (Fig. 2). We havc also identified in our scrics 11 patients with rclativcly long durations of chronic phase, continuing 47 to 136 mo aftcr diagnosis. These patients either had very low

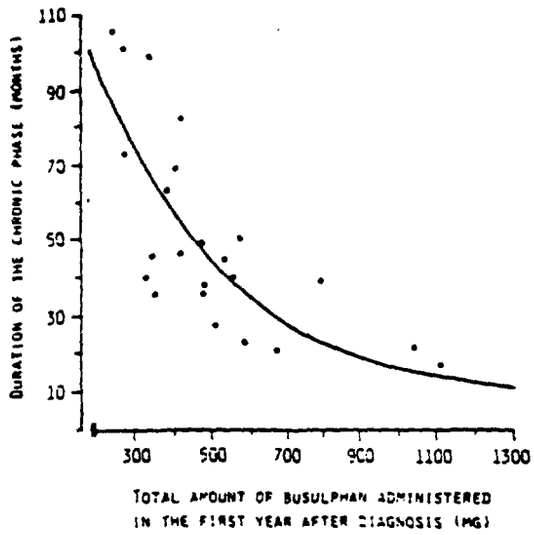

Fig. 2. Relationship of duration of chronic phase to total dose of busulfan administered to patients within the first year of diagnosis. Of 48 patients randomized to receive busulfan in the original MRC comparison of busulfan and radiotherapy[10] we could accurately assess the duration of chronic phase in 25; 23 patients could not be assessed for a variety of reasons (not treated only with busulfan, insidious transformation, myelosclerosis, Ph¹-negative, etc.) The line of best fit (solid line) is very similar to that obtained by analysis of 25 other patients treated at the Hammersmith Hospital since 1972 (Wareham et al.).[126]

rquirements for busulfan or were untreated within the first year of diagnosis."' Thus the requirement for treatment reflected in the use of cytotoxic drugs can be an important prognostic feature; the idea must be tested in other series of patients.

TREATMENT OF CGL

Many aspects of the treatment of CGL have been comprehensively reviewed in recent years.[16,67,113,115,119] We will discuss here only the various attempts that have been made to eliminate the Ph¹-positive myeloid population by use of cytotoxic drugs during the chronic phase and the place of autografting and allogeneic marrow transplantation in the management of patients in chronic phase and in transformation.

Attempts to Eliminate the Ph¹-Positive Myeloid Populatton During the Chrontc Phase

Because it now seems likely that residual Ph¹-negative (and perhaps therefore normal) hemopoietic stem cells survive in the marrow of most or all patients when CGL is diagnosed and bccausc occasional patients whose marrows are Ph¹-positive at diagnosis but become largely Ph¹-negative after treatment with busulfan[9,37,79,112] have had unexpectedly long survivals. deliberate attempts have made to suppress the Ph¹-positive population of cells in the hope of permitting re-emergence of Ph¹-negative hemopoiesis.[10,23,51,105,109]

The interim results of the L-5 protocol *used* at the Sloan-Kettering Institute in New York were published in 1979.[23] Thirty-seven patients with newly diagnosed CGL were treated first by splenic irradiation, then subjected to splenectomy and thereafter received a variety of *cyto*toxic drugs more commonly employed for patients with acute myeloid leukemia. The median survival for all 37 patients was 50 mo, slightly longer than that reponed in previous series. In 12 patienu there was a temporary reduction in the proportion of Ph¹-positive marrow metaphases to one-third or less of their initial value. These reductions lasted from I to 43 mo. and the median survival for these I2 patients. designated "responders" was significantly longer than the survival of the remaining 25 patients. Similar results were obtained in a subsquent protocol. designated L-15. in which additional cytotoxic drugs were introduced. Of *28* patients treated. 12 had marrow studies that showed Ph¹-negative hemopoiesis but such cytogenetic "remissions" tended to *be* short-lived."

Broadly similar results have been reported for patients treated with cycle-active cytotoxic drugs at other centers. In Philadelphia 23 patients were treated with arabinosyl cytosine and 6-thioguanine followed by splenectomy and *6* (24%) showed some reduction in the percentage of Phi-positive metaphases in the marrow.[10] In another study also reported from Philadelphia patients were treated with the same drugs but without splenectomy. Two of 16 patients achieved Ph¹-negative hemopoiesis.[109] Twelve patients were treated each with six courses of combination chemotherapy at the King's College Hospital in London."' In 6 the proportion of Ph¹-positive marrow metaphases fell to 50% or lower and 4 patients had values less than 10%. The splenectomy included in most of these protocols may be an important component of this approach since the spleen may play a role in

facilitating the proliferation of Ph¹-positive cells.[78]

Taken together, the results of these studies provide strong support for the concept that some Ph¹-negative stem cells 8re present when CGL is diagnosed and that suppression of the Ph¹-positive clone with cytotoxic drugs can restore to tbe progeny of Ph¹-negative stem cells. albeit temporarily, a proliferative advantage. Unfortunately, one cannot conclude that such "responders" those patients whose marrows show a temporary reduction in proportion of Ph¹-positive metaphases, have in fact been benefited by the treatment. It is possible that aggressive treatment merely helps to identify a subpopulation of. patients whose prognosis would in my case have been favorable. Further studies along these lines can only be justified after due consideration has been given to the toxicity of the drugs[10,109] and to the interference with the patients' life-style.

Autografting for CGL in Transformation

Autologous hemopoietic stem cells can be used in the management of CGL in transformation. This approach was pioneered by Buckner and his colleagues in Seattle.[11] Nucleated cells were harvested from the patient's marrow at diagnosis or soon after. stored in liquid nitrogen and used when the patient entered blastic transformation to reconstitute the bone marrow after treatment with high-dose cyclophosphamide and whole body irradiation (TBI). Two of the 7 patients treated in this way were rapidly restored to a hematological picture consistent with chronic phase disease. Engraftment was apparently facilitated in patients who received substantial quantities of cryopreserved autologous blood cells as well as marrow cells.[12]

Because we encountered technical difficulties in harvesting adequate numbers of marrow cells and because we speculated that pluripotential stem cells might. uniquely in CGL, be present in the circulation in vast excess, we began at the Hammersmith Hospital to store nucleated cells collected from the blood of newly diagnosed patients: we abandoned collection of marrow cells. Subsequently. we showed that reconstituted blood cells alone would re-establish chronic phase hemopoiesis in patients in blastic transformation after treatment with chemotherapy or chemotherapy plus TBI.[13] Between July 1977 and June 1981 we treated 29 patients by this approach: I7 patients received only cytotoxic drugs before autografting and 12 received cytotoxic drugs plus TBI. Definite and in most cases rapid re-establishment of chronic phase hemopoiesis occurred in 28 patients. The median survival from date of transformation was 20 wk, which was significantly longer than the 10-wk median survival in a contemporary group of patients treated by chemotherapy alone.[14] We noted that patients treated by chemotherapy only before autografting fared better than those who received chemotherapy plus TBI (Fig. 3).

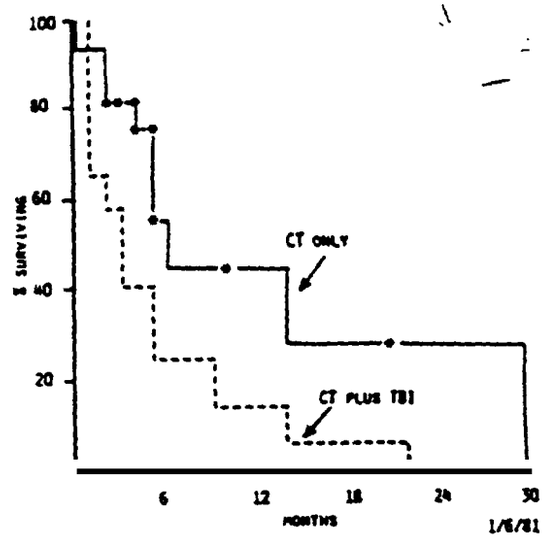

Fig. 3. Actuarial survival for 29 patients with CGL in transformation from date of autografting analyzed by treatment received before the autograft. The median survival for patients treated by chemotherapy only (CT only, $n = 17$) was 24 wk; the corresponding value for patients who received chemotherapy and TBI (CT plus TBI, $n = 12$) was 11 wk ($p < 0.05$). Asterisks indicate surviving patients (1st June 1981).

It is possible to draw some general conclusions about the place of autografting in the management of patients in transformation. Hemopoietic reconstitution is highly predictable and seems not to be a problem. Unfortunately, the value of the technique is strictly limited by the relative resistance to therapy of the transformed population of cells and their propensity to reappear in the marrow and blood in most cases within 3–6 mo of autografting. It is worth noting that occasional patients have. however. had second chronic phases lasting more than a year.[47,89] For patients whose transformation has recurred,

autografting has been attempted on a second or a third occasion with some success. At present it seems reasonable to recommend that a quantity of blood leukocytes should be collected and stored from every new patient with CGL before treatment is started. The questions of how or when. or indeed whether, to use the stored cells can be tackled at a later date.

A major conceptual objection to autografting is the fact that hemopoieticstem cells collected in the chronic phase. be it from the blood or from the marrow, are all presumably Ph¹-positive. However, this may not necessarily be so if stem cells are harvested after the patient has been treated with cytotoxic drugs.[10,23,30a,31,104,109] A recent report described a patient whose marrow, initially Ph¹-positive, was restored to Ph¹-negativity by treatment with high-dose cyclophosphamide.[77] Blood stem cells were then collected and cryopreserved. When Ph¹-positive transformation occurred, the patient was treated with high-dose busulfan and cyclophosphamide, followed by transfusion of autologous stem cells. Hemopoiesis was reestablished with Ph¹-negative myeloid cells. If one assumes for the sake of argumcnt that the cytogenetically normal cells in this patient were not leukemic, the sequence of events suggests a new wry in which autografting with circulating stem cells might be exploited in the future.

Bone Marrow Transplantation

The successful transplantation of bone marrow collected from a normal donor could in theory cure a patient with CGL. Cure would, however. be more difficult if constitutional or marrow microenvironmental factors play a role in the pathogenesis of the leukemia—hitherto unresolved questions. Such considerations apart, for the rare patient fortunate enough to have a hematologically normal genetically identical twin the major problem may be simply the design of a regimen of chemoradiotherapy preceding the syngeneic transplantation that will permanently eradicate the Ph¹-positive myeloid cell line. A larger number of patients is likeiy to have HLA-identical sibs, and allogeneic marrow transplantation may then be undertaken. in such cases the problem of eradicating the leukemia is compounded by the dangers inherent in transplantation of HLA-matched non-identical marrow, principally those of graft-versus-host disuse (GVHD) and interstitial pneumonitis.

Syngeneic Marrow Transplantation

Results of treating patienu with CGL in transformation by chemoradiotherapy followed by transplantation of normal marrow from identical twins are not encouraging. Only 2 of 10 patients so treated in Seattle remain alive and the single long-term survivor (>71 mo) may have been transplanted in accelerated phase rather than frank transformation of CGL.[32] As with autografting in transformation, the main problem appears to be the extreme resistance of the transformed clone of cells to high-dose chemoradiotherapy; moreover the patient is often ia poor clinical condition when the procedure is undertaken.

The use of syngeneic marrow transplantation for patients still in the chronic phase of CGL u much more promising. In *1979* the Seattle group reported preliminary results of treating 4 patients with Ph¹-positive CGL with dimethyl busulfan, cyclophosphamide. a d TBI followed by transfusion of twin marrow.[33] On follow-up the 4 patients were hematologically normal without Ph¹-positive marrow metaphases. Subsequently. a further 8 patients wen treated by transplantation of syngeneic bone marrow. Of all 12 patients transplanted, the most recent report noted that one subsequently died of interstitial pneumonitis and another entered blastic transformation and died; two other patients relapsed with cytogenetic but not clinical evidence of CGL. Eight patients remained apparently free of all evidence of leukemia at follow-up timer ranging from 21 to 65 mo post-transplant.[32]

Similar experience with a smaller number of patients has been gained at other centers. At the Hammersmith Hospital we treated two patients, one in uncomplicated chronic phase and the other in early accelerated phase, with chemoradiotherapy followed by syngeneic marrow transplantation.[77] Both patients are alive and well 30 and 33 mo. respectively. after transplantation: both have only cytogenetically normal cells identifiable in the bone marrow. Two other patients with CGL in chronic phase wen truted with twin marrow transplantation in Paris.[77] In one case the transplant was complicated by a clinical picture closely resembling GVHD but

the patient recovered and both patients were well at the time of the report.[27]

It thus appears that for the patient in chronic phase, syngeneic marrow transplantation has the capacity either to cure or at least to prolong the duration of life. Conversely, for the patient already in transformation the procedure offers little benefit.

Allogeneic Morrow Transplantation

For the patient with CGL in transformation who has an HLA-identical sib. the results of allogeneic transplantation are generally poor. At the time of their most recent report. 28 patients in aplastic. accelerated or frank blastic phases of CGL had been treated in Seattle.[28,29] Three patients were alive at times ranging from 18 to 37 mo after transplantation. It is of interest that one of these survivors is a woman who was transplanted at the age of *50.*

The success achieved with allogeneic transplantation of patients with acute leukemia in remission and the observation that syngeneic transplantation for patients in the chronic phase of CGL an suppress the Ph¹-positive clone of cells at least for some years has led a number of groups to explore the possibility of treating patients by allogeneic transplantation before the onset of transformation. Again. the Seattle group has pioneered this approach. A 4-yr-old boy with Ph¹-negative chronic myeloid leukemia was successfully treated by transplantation of marrow from his histocompatible brother.[19] Of 10 other patients with Ph¹-positive CGL transplanted in the chronic phase, 4 died of transplant-related complications but 6 survive at follow-up times ranging from 13 to 37 mo: these patients are all clinically well.[20a,29] At the Hammersmith Hospital 12 patients in the chronic phase of CGL were treated with cyclophosphamide at high dosage and fractioned TBI (total 1000 or 1200 cGy) followed by transplantation of marrow from HLA-identical sibs; 2 patients died of infection and GVHD but 10 survive at follow-up times ranging from 1 to 14 mo.[19] Similar results with smaller numbers of patients have ken reported from Los Angeles, Toronto, Minneapolis and Basel.[19,24] Though the duration of follow-up is still short in most casu and some patients may ultimately relapse initially with cytogenetic and tubsqucntly with hematological evidence of CGL, no recurrence of Phi-positive marrow metaphases after transplantation has yet & reported in any of these series. No recurrence of Ph¹-positive marrow metaphases after transplantation has yet been reported in any of these series.

At present, allogeneic transplantation is available only for the relatively young patient (usually under the age of 40) who has an HLA-identical brother or sister. Even then the risks remain considerable. The especial hazards are GVHD and interstitial pneumonitis. One hopes, however, that increased understanding of the pathogenesis of these two major complications will lead to measures that prevent them. At some time in the future it may be possible to offer marrow transplantation to older patients with CGL and to those who. lacking HLA-identical sibs. can only be transplanted with marrow from mismatched relations or matched unrelated donors.

CONCLUSIONS

Though there is persuasive *evidence* to support the concept that Ph¹-positive CGL is a clonal disease derived originally from a single ancestral stem cell, the assumption that all Ph¹-negative marrow cells in a patient with Phi-positive disease are necessarily normal is no longer tenable. It is probable, however. that some Ph¹-negative and truly normal hemopoietic stem cells do survive in the marrow of the newly diagnosed patient with CGL; possibly such residual normal stem cells are gradually destroyed by chemotherapy.

In assessing the prognostic value of clinical and hematological features defined at diagnosis (or soon after), one must first exclude features that indicate that the disease is already in early or established transformation. The remaining 'prognostic features' can then be divided into static features that reflect the extent of disease and dynamic features which reflect its tempo; some of the latter may only be measurable after a period of observation or after initial treatment. These two categories of prognostic features must to some extent be interdependent. but this approach may heip to discriminate between *advanced* disease that is not necessarily aggressive and *aggressive* disease that may or may not be advanced. Though there is no general agree-

ment about the prognostic valuc of individual features, possibly significant static features include the patient's sex, spleen size, liver size, hemoglobin, leukocyte count, platelet count, the proportion of immature granulocytes in the blood or marrow, and the presence or absence of the Ph' chromosome. Possibly significant dynamic features include the doubling time of the leukocyte count, the response of the leukocyte count to chemotherapy and its ratc of rise thereafter, the mitotic index of the blast cells and the acquisition of cytogenetic changes in addition to the Ph' chromosome.

Occasional patients treated with busulfan have had true "cylogenetic" remissions and some of these patients have been long-term survivors. Attempts, however, to reproduce such clinical benefit routinely with combinations of cytotoxic drugs havc in general been disappointing. Results of syngcncic marrow transplantation suggest that most patients in the chronic phase benefit from such treatment and some m y perhaps be cured. Preliminary results of allogeneic marrow transplantation for patients still in the chronic phase of CGL suggest one possible avenue for progress in the current decade.

ACKNOWLEDGMENT

We are very grateful to Professor David Galton who read the manuscript and nude valuable comments; he also made available hematologic data on patients treated in the Medical Research Council study" comparing busulfan with radiotherapy. We must thank also Professor Joseph Sokal whose constructive criticism led, we hope, to an improved manuscript.

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