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AN INTENSIVE TREATMENT REGIMEN FOR CHRONIC MYELOGENOUS LEUKEMIA

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CHRONIC MYELOGENOUS LEUKEMIA

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INTRODUCTION

In 1960 Nowell and Hungerford[1] discovered that dividing cells in the bone marrow of patients with chronic myelogenous leukemia (CML) are characterized by a deletion in the long arm of one of the group G chromosomes, and this marker became known as the Philadelphia (Ph') chromosome. Until the advent of the banding techniques, the identity of this chromosome was unknown, but Rowley has since established that the Ph' chromosome is not a deletion but a balanced reciprocal translocation between the long arms of chromosomes No. 9 and 22.[2,3] Translocations involving the No. 22 chromosome and chromosomes other than No. 9 have also been observed in a small number of patients with Ph'positive CML[3]. Because of its presence in erythrocyte, megakaryocyte, granulocyte, and monocyte precursors, leukemic transformation is considered to take place in an ancestral cell common to these myeloid cell types.[4,5]

There is now strong evidence pointing to a uniclonal origin of the Ph' positive leukemic clone. Patients with CML who are heterozygous for the enzyme glucose 6-phosphate dehydrogenase[6,7], and two patients with sex chromosome mosaicism,[8,9] were found to have the Ph' chromosome restricted to just one of their dual cell lines, strongly suggesting that the Ph'+ stemline arises from a single cell. Although many investigators have found only Ph'+ cells in colonies growing in semi-solid media derived from marrow and peripheral blood of patients with CML,[10,11] Chervenick et al.[12] found that some patients had both Ph'+ and Ph'- colonies, but no mixed colonies, indicating that populations of both normal and leukemic cells exist in the marrow of at least some patients with CML.

The defect is an acquired one, since the Ph' chromosome is not found in the lymphocytes[5] nor in fibroblasts of skin[13] or marrow,[7,14] and

monozygous twins of patients with CML do not have Ph'+ cells in their marrows[15-19]. Only rare instances of familial occurrence have been reported[20].

The Ph' chromosome is found in the marrow of about 85% of patients with CML,[21] but not all patients with the Ph' marker have it in 100% of marrow metaphases at diagnosis. Sakurai et al. have suggested that patients with some normal cells at diagnosis may survive longer.[22] Two interesting examples are patients reported by Gatti[23] and Canellos[24], both of whom had some normal metaphases at diagnosis of Ph'+ CML. Both were followed for five years before chemotherapy was instituted.

The literature contains isolated reports of patients with reduced populations of Ph'+ cells after therapy of chronic phase CML, and many of these patients are among the long survivors (7 to 17 years) in Busulfan - treated series.[25-35] Seven patients were reported with reduced Ph'+ cells on examination 3 to 16 years after diagnosis and there are two reports of complete absence of the marker at 9[29] and 13[35] years. All of these patients were treated with Busulfan, some to marrow hypoplasia[29,32,35] and others not so intensively.[33,34] There have also been a few reports of reduction in Ph'+ cells with treatment other than busulfan (i.e., P^{32}, 6-mercaptopurine (6-MP), methotrexate (MTX), vincristine (VCR), and prednisone)[36-39]. One of these latter patients[38] survived 24 months and the other, who was seen at Memorial Hospital, survived 13 months, with transient complete loss of the Ph'+ marker from the marrow.[39]

In 1924, the median survival of untreated CML patients was reported by Minot et al.[40] to be 31 months from onset of symptoms. Since then, therapeutic attempts to improve survival have been generally disappointing; median survivals from diagnosis of from 1¼ to 3½ years have been reported in several large series[26,30,41,42,43], and there has been little improvement in survival after onset of blastic transformation.

The retrospective study by Monfardini et al.[44] of all CML patients seen at Memorial Sloan-Kettering Cancer Center from 1948 through 1967 revealed an overall median survival from diagnosis of 31 months, with a longer median (47 months) for the 19 patients who received P^{32}, splenic irradiation and chemotherapy. Because conventional treatment had not appreciably extended survival in almost 50 years, it was decided that a more aggressive therapeutic approach to treating early CML might be beneficial.

In recent years attention has focused on the role of the spleen in CML. Both the Ph' chromosome and additional stemlines, which characterize progression of the disease, have been noted in the spleen[45,46], and a few reports have suggested that both these markers may be seen in the spleen before the marrow[47-50]. Armenta et al.[48] reported a patient with a long history of polycythemia vera whose disease changed to CML in blastic transformation; the Ph' chromosome was found in the spleen and lymph nodes but not in the marrow or peripheral blood when examined up to 5 months later. There are also several reports of more typical cases of CML in which blastic transformation first appears to have occurred in the spleen[49-51] or other extra-medullary sites[52,53]. Loss of aneuploidy as well as decrease in the percentage of Ph'+ cells have been noted with remission of blastic crisis induced by chemotherapy[37-39,54-56]. There has been one report of response to splenectomy which resulted in loss of hyperdiploidy and reduction of the Ph'+ metaphases from 100% to 23%, with survival of 3 years after transformation.[51,57]

The therapeutic program, the L-5 Protocol, was designed to include early splenectomy (Fig. 1). It was reasoned that splenectomy would remove a large mass of leukemic cells and eliminate a potential site for blastic transformation, and, in addition, obviate the late complications of massive splenic enlargement and hypersplenism. Irradiation was administered prior

to splenectomy to reduce the size of the spleen and to control the leukocyte and platelet counts, because elevated levels may be associated with hemorrhagic and/or thromboembolic complications during or following surgical procedures.

It was further reasoned that drugs which are only effective against proliferating cells should be selectively lethal to the Ph'+ cells which have an (undefined) proliferative advantage[58]. Arabinosylcytosine (Ara-C) and 6-thioguanine (TG) were chosen as the primary chemotherapy because this combination had been found to have at least some selectivity of action in acute myelogenous leukemia (AML)[59]; three courses were given, because some patients with AML required three courses before achieving remission[60]. It had previously been shown that L-Asparaginase destroyed leukemic cells in CML with fair regularity and that it had relatively little effect on normal hematopoiesis[61], and thus this drug was added to the regimen to further reduce the leukemic population. The decision regarding further treatment depended on the results of chromosome analysis at the end of the intensive phase of treatment.

MATERIALS AND METHODS

Patient Population

Between January 1970 and July 1976, 37 adults with Ph'+ CML were entered on the L-5 Protocol. Patients were excluded if they were more than 60 years old, if they were considered to be poor operative risks or too unreliable to follow the portocol, or if they elected to receive conventional treatment after being told that the L-5 Protocol was experimental. Initially, patients were also excluded if they were thought to be undergoing blastic transformation, but later two (No.31 and 37) were

entered to determine whether there would be any beneficial effect on advanced disease. Previously untreated patients were preferred, but some patients with prior therapy were accepted if there were none of the contraindications listed above. There were 26 males and 11 females ranging in age from 16 to 52 years; the median age was 33.

Tables 1 and 2 show the hematologic status of each patient at the time of beginning the protocol. Sixteen had received no prior treamtment, except for short-term leukepheresis in three. Fifteen had had prior Busulfan; the duration of treatment was 4 months or less, except in two patients who had received Busulfan for 12 and 24 months. One patient had received 6-MP, MTX, and splenic irradiation (300 rads). Six patients were given Ara-C, either by continuous 24-hour infusion, or every twelve hours with TG (one patient), just prior to starting the protocol, because of very high leukocyte counts (160 to 548 x 10^9/1) and the clinical necessity of rapidly reducing these counts. Three of the latter patients presented with priapism which resolved after the Ara-C infusions[62].

The number of Ph'+ cells present in the marrow of each patient before beginning the L-5 Protocol is shown in Tables 1 and 2. All patients initially had normal 46 stemlines except Patient 26, who had both 46 and 47 stemlines. Hyperdiploidy has been reported to herald blastic transformation,[21,22,49,56] but this did not occur in her case until 29 months after this was first noted. All but three patients initially showed 75-100% Ph'+ metaphases; the others were Patient 19, who had 52% and 50% Ph'+ cells on two determinations 10 days apart prior to initiation of therapy, Patient 12, who had 86% initially and 38% after Busulfan, and Patient 21 who had two pretreatment studies - one showed 100% and the other 26% (see below).

Treatment Program

The protocol began with irradiation to the spleen (Fig.1). Radiation was delivered through anterior and posterior portals[63], using Cobalt 60, in doses of 50-100 rads three times weekly for a total of 150 to 1950 rads. The median dose was 825 rads given over 24 days. Three patients (No.24,26, and 12) were not given irradiation as their peripheral blood counts were already near normal after 4,8,and 12 weeks of Busulfan.

After the spleen was reduced in size, and the peripheral blood counts were nearing normal levels, irradiation was stopped and the patient was allowed to rest until the blood counts stabilized at satisfactory levels. The amount of time between completing irradiation and surgery depended on the time required for the blood counts to recover, and ranged from a few days to two months, with an average of 30 days. At the time of surgery the white blood cell counts (WBC) and platelet counts were within the normal range in the majority of patients. In three patients there was difficulty in controlling the WBC with splenic irradiation. Patient 37, whose disease was accelerating at entry (16% blasts) had progression of disease while receiving 400 rads, with rapid increase in WBC, marrow blasts, and spleen size. He was subsequently treated with chemotherapy and splenectomy without control of his disease. The other two patients (No.20 and 22) initially had good responses to 790 and 590 rads, but within four weeks of stopping irradiation the WBC rose rapidly and it was necessary to reinstitute irradiation. Blastic transformation occurred in one of these patients (No.20) during his second course of irradiation, and in the other (No.22) a few weeks after splenectomy.

Splenectomy was performed on 36 patients; the one patient who was not operated on was No. 20. There were no complications during surgery and no operative deaths. One patient (No.15) was found to have extensive para-aortic node involvement at surgery and shortly thereafter developed

extramedullary blastic transformation in his peripheral lymph nodes[52]. He
was withdrawn from the protocol and given other treatment, but his marrow
became blastic six months later. Another patient (No.35) was found to have
a blastic marrow after recovery from surgery and she was similarly given
other therapy. Complications during the postoperative period included
a pulmonary embolism eight days after surgery in one patient (No.13) when
his platelet count was over 1000×10^9/l. One patient developed a wound
infection, and two had episodes of gastrointestinal bleeding postoperatively;
the sites of bleeding were not determined. All recovered completely.

Chemotherapy was begun after recovery from surgery, usually about one
month later, in the 32 patients who remained in the "chronic" phase. The
protocol was discontinued in the 5 patients who had already undergone blastic
transformation and they received other chemotherapy. In most cases, the
WBC remained stable postoperatively, but three patients (No.13,18,25) required
Hydroxyurea in the postoperative period to control rapidly rising WBC until
they had recovered from the operation and could tolerate more intensive
chemotherapy. Postoperative thrombocytosis occurred to $>500 \times 10^9$/l in
13 patients and to $>1000 \times 10^9$/l in 9 others. Ara-C was given (3.0 mg/kg i.v.)
with TG (2.5 mg/kg p.o.) every 24 hours to the first few patients, and later
every 12 hours for the majority of patients. The patients were treated
similarly to those with AML on the L-6 Protocol[60], but not so intensively
as to cause severe marrow hypoplasia. Patients usually received three
courses of Ara-C and TG, each time in sufficient dosage to cause moderate
marrow hypocellularity, with rest periods of 3-4 weeks between courses.
Immediately following the last course of Ara-C and TG most of the
patients (24/32) were given L-Asparaginase (E.coli) at 200 IU/kg i.v.
three times a week for six doses.

The choice of treatment at the conclusion of the intensive part of
the protocol depended on the chromosome response. Several patients who

initially showed a reduction of Ph'+ cells in the marrow with subsequent

return to Ph'+ predominance were given additional courses of intensive

chemotherapy in an attempt to re-induce a response, either at the end

of the protocol or during maintenance. Three of these patients (No.6,11,12)

received Daunorubicin and Ara-C, and two of them (No.6,11) were treated

to severe marrow hypoplasia. In an attempt to continue suppression of the

Ph'+ population in the responders, therapy was continued with the

maintenance part of the L-6 Protocol for AML, which consists of rotating

courses of multiple drugs as previously described[60] and as outlined in

Figure 2. In cases in which there had been no significant decrease in the

percentage of Ph'+ cells or in whom attempts to re-induce a response had

failed, Hydroxyurea was given and the dosage was adjusted according to

the results of weekly blood counts (usually 20 to 40 mg/kg per day as a

single dose). Maintenance therapy was continued indefinitely until blastic

transformation or acceleration of the disease dictated a change to more

aggressive treatment. Several patients required additional treatment with

Thiotepa and/or Melphalan to reduce excessively high platelet counts which

could not be controlled with Hydroxyurea.

Evaluation of Chromosome Response

Before and after each phase of the protocol, bone marrow was aspirated

for evaluation of cellularity and the differential count and analyzed for the

presence of the Ph' chromosome, in the Bone Marrow and Cytogenetics

laboratories at Memorial Hospital. Whenever possible, 25 to 50 marrow

metaphases were counted. Responses were arbitrarily considered significant

only if the percentage of Ph'+ metaphases decreased to less than 1/3 of

the value obtained just prior to starting the L-5 Protocol.

Leukocyte alkaline phosphatase (LAP) scores were not followed serially

in most patients, and were not used as criteria for response. Only a few

patients were studied for terminal deoxynucleotidyl transferase activity at blastic transformation, as this test was not available during the major part of the study.

RESULTS

Survival

The median survival of the 37 patients from diagnosis is 50 months (Figure 3). Fourteen patients are alive, all in the chronic state at 17 to 107 months after diagnosis, as of January 1, 1978. Twenty-three have died - 19 after blastic transformation at 5 to 61 months after diagnosis. Three of the patients who underwent transformation (No.31,26,32) also developed myelofibrosis; their survivals were 17,34,and 50 months. None of the 19 patients with blastic transformation responded satisfactorily to subsequent chemotherapy, and their median survival from transformation was $3\frac{1}{2}$ months. Two patients (No. 18 and 13) died in an accelerated phase of the disease at 12 and 40 months; they were thus termed because the disease was clearly out of control and poorly responsive to therapy, although they did not fully meet our criteria for blastic transformation (30% blasts in the blood and 50% in the marrow). The cause of death of Patient 34 is unknown, as he died in another country. One patient (No. 11) died of lung cancer while his CML was in complete remission, 52 months after diagnosis.

Ph' Chromosome Response

Of the 37 patients who began the protocol, five became blastic before chemotherapy was begun, one failed to follow the protocol and had protracted intervals between phases of treatment, and two others were inevaluable for

chromosome response because most of their marrow aspirations were inadequate for study due to development of myelofibrosis. Of the remaining 29 cases evaluable for chromosome response, 12 were considered to have a significant reduction in the percentage of Ph'+ cells in their marrows ($<$1/3 of baseline value) and 17 did not respond. Transient reduction occurred in some of the non-responders, but in no case was the minimum value less than 50% of baseline, except in Patient 21 who will be described below. The lowest percentages of Ph'+ metaphases achieved in each patient are noted in Tables 1 and 2.

The median survival of those patients with reduced populations of Ph'+ cells has not been reached as only four have died, but it appears that it will be appreciably longer than the 34 month median of non-responders (Fig.3). Three of the responders (No.8,10,9) died between 3 and 7 months after undergoing blastic transformation, which occurred at 36,45, and 59 months after diagnosis, respectively. One responder (No.11) died in remission as noted above. The other 8 responders continue in the chronic phase at 17 to 107 months.

There was considerable variation in the duration of the Ph' chromosome reduction. Reduction lasted $<$ 2 months in 3 patients (No.7,8,12), 2 to 4 months in 5 others (No. 2,3,4,5,9), and was inevaluable in Patient 10 whose follow-up study was not done for 9 months. One patient (No.1) had two periods of reduction of 8 months each (Fig.5), and only two patients had a sustained reduction for more than 3 years (No.6,11).

Reduction was first noted at different points in the protocol: after irradiation in two patients (No.7,11), after splenectomy in two others (No.4,6),and after the first or second courses of Ara-C and TG in seven (Table 3). One other patient (No.5) showed absence of Ph'+ cells after treatment with Ara-C and TG, but since no examinations were made after irradiation or splenectomy it is not possible to determine at which

point the reduction began.

Two of the above mentioned patients who showed a reduction in Ph'+ cells before receiving chemotherapy had the longest duration of response. Patient 11 showed 2% Ph'+ cells after completion of the intensive chemo-therapy and his marrow remained 0-8% for the remaining 42 months of his life. Patient 6 maintained 0-2% Ph'+ cells in her marrow for 43 months while she received first maintenance L-6 therapy and later Hydroxyurea. At her request, all therapy was then discontinued; her marrow metaphases were 16% Ph'+ at that time. Subsequently, the percentage of Ph'+ cells gradually increased to 82% over a period of 10 months, and later to 100%. However, she remained clinically well and required no treatment for 31 months after stopping chemotherapy, at which time her WBC rose from around $30 \times 10^9/1$ to nearly $100 \times 10^9/1$. Hydroxyurea was again initiated with a satisfactory response of the WBC, but her marrow remains 100% Ph'+ at 107 months after diagnosis.

The responses first noted after Ara-C and TG were all of short duration; in five of the seven patients, the majority of marrow cells were again Ph'+ by the end of the intensive part of the protocol. In four of these patients (No. 1,8,9,12) an attempt was made to re-induce a response with additional intensive treatment. This was successful transiently in three (No. 1,9,12). The best response was in Patient 1 whose marrow remained consistently negative for over 8 months after the first course of Ara-C and TG, after which Ph'+ cells reappeared in the marrow while he continued on Hydroxyurea. The Ph'+ cells did not diminish on retreatment with Ara-C and TG, but largely disappeared for another 8 months after institution of the L-6 maintenance regimen.

Response was never noted later than the Ara-C and TG phase of the protocol, unless re-induction was attempted. Eleven of the twelve patients

who showed reduction received L-Asparaginase, but in all cases it was given after the initial decrease had been demonstrated, and in four of these the Ph' marker had already reappeared (Table 3). L-Asparaginase did not effect reduction in any of the patients. The maintenance therapy did not result in reduction except in the case of Patient 1 who showed a second reduction after starting the L-6 maintenance as noted above.

In addition to the 12 patients who responded, there were two "non-responders" with significant numbers of Ph' negative cells, but in neither was this attributable to the L-5 Protocol. Patient 21 had two studies done prior to receiving splenic irradiation - one showed 100% Ph'+ metaphases of 11 counted, and the other 26% of 19. He had received no chemotherapy and was receiving only an (unidentified) anticoagulant after a myocardial infarction. After receiving a total of 475 rads to the spleen his marrow showed 10% Ph'+ cells of 19 scored, but after splenectomy the marker was found in 88% and there was no change thereafter. Another patient (No.19) who had about 50% Ph'+ cells on repeated studies prior to any treatment, continued to show similar proportions of Ph'+ and Ph'- cells in the marrow throughout the L-5 Protocol, and at its completion there were still 36% Ph'+ cells in the marrow. His blood counts remained stable with very little treatment except intermittent Hydroxyurea for the next 2½ years, but the percentage of Ph'+ cells in the marrow gradually increased to nearly 100%. He has remained clinically well with his disease under control on Hydroxyurea 43 months after diagnosis, with nearly all marrow metaphases demonstrating the Ph' marker.

Factors Which May Influence Response

Among possible variables which might predict a favorable prognosis

for survival or loss of the Ph' marker, it appears that the size of the

spleen at diagnosis may be important. Measurements of the spleen size on

palpation at diagnosis were available for 34 patients. Sixteen had

relatively small spleens (<7 cm.below the left costal margin) at

diagnosis, whereas the other 18 had larger spleens (>10 cm.). The

median survivals of the two groups are similar at the present time:

44+ months in the former group and 37 months in the latter group.

However, 63% of the patients with small spleens are still alive, and

the eventual median survival of this group will probably be appreciably

longer than than that of the larger-spleen group in which only 22% of

patients are alive.

It is noteworthy that 8 of the 12 patients with Ph' chromosome

reduction had small spleens at diagnosis (Table 1), and their diseases

appeared to be slowly progressive. Additionally, in three of these

patients (No.1,2,6) the WBC and platelet counts remained stable at moderate

levels or rose only very gradually for many months before the institution

of any treatment. This group with small spleens and indolent disease

included the 3 patients with the longest chromosome response. Seven

of the eight responders with small spleens are still alive; the only

death resulted from an unrelated carcinoma (No.11). Four of the

responders had large spleens at diagnosis (Table 1). The duration of

chromosome response was very brief in all of these patients and in

three of them the disease underwent blastic transformation and they died.

At the time of beginning the protocol there was a total of 25

patients with small spleens because some patients who had large spleens

when diagnosed had responded to chemotherapy before coming to Memorial

Hospital. This group with small spleens had an appreciably better

response; their median survival is 45+ months with 48% still alive, compared to 29 months with only 16% still alive for the 12 patients with larger spleens, half of whom had received no prior treatment.

Of the 25 patients with small spleens, 22 were given splenic irradiation (the other three were considered to have had adequate control of spleen size and WBC following Busulfan). In reviewing this study it became evident that these 22 patients had received a higher dose of irradiation per gram of splenic tissue than the group with larger spleens. The median dose in the former group was 875 rads (range 200-1550 rads), compared to a median dose of 600 rads (150-1950 rads) for the 12 with larger spleens. The median weight of the 24 spleens in the small-spleen group was 318 grams and that of the larger spleens was 795 grams (one of the patients in the former group did not undergo splenectomy). It is interesting to note that the four patients with larger spleens who received higher amounts of irradiation (875 rads) had smaller spleen weight (525g v. 930g) and longer survival than those who received less irradiation (46 v. 25 months). There was no correlation between pretreatment WBC level and amount of irradiation. The amount of irradiation received depended on the rate at which the WBC and spleen size decreased and the appraisal of the examining physician.

These findings suggest a correlation of small spleen size at diagnosis with longer survival and greater probability of Ph' chromosome reduction; however, as is evident from examination of Table 2, some patients with small spleens failed to show a reduction in Ph'+ cells. The dose of splenic irradiation may also be important, but this study was not designed to answer that question and no conclusions can be drawn concerning the significance of irradiation dosage.

Another difference between responders and non-responders was that

marked thrombocytosis ($>$1000 x 10^9/1) occurred at some time in the protocol in only four patients in the group with Ph' reduction, whereas it was frequently observed in the non-responders (16 of 20). The four responders were Patients 4,7,8, and 10, and all of these patients had only very transient reduction in Ph'+ cells. Platelet counts between 500 and 1000 x 10^9/1 were observed at some time in most patients in both groups and no correlation of this moderate thrombocytosis with response can be made.

In only two cases was thrombocytosis a life-threatening problem. The one postoperative pulmonary embolism has already been noted. Another patient (No.26) experienced an abrupt rise in platelets to 7000 x 10^9/1 while recovering from the last course of Ara-C and TG, and she developed multiple pulmonary emboli and cardiac arrest. She was successfully resuscitated, and with chemotherapy her platelet count was reduced to 357 x 10^9/1. She lived another 2 years before her death due to myelofibrosis and megakaryocytic leukemia with massive liver involvement.

One other patient, No.6, who was a responder, had a thrombotic complication while on the protocol, but it was not related to thrombocytosis. About two months after completing the intensive phase of chemotherapy, she developed thrombophlebitis of an iliac vein and pulmonary emboli while on the L-6 maintenance arm of the protocol. Her platelet count remained between 180 and 350 x 10^9/1. She eventually underwent plication of the inferior vena cava and had no further complications until 6 years later when she developed thrombophlebitis of the other iliac vein; on this occasion the platelet count was around 500 x 10^9/1. The thrombophlebitis responded to conservative therapy.

DISCUSSION

Analysis of the L-5 Protocol has shown that: 1) A significant
reduction in the Ph'+ population is possible in a certain number or cases
(32% in this study); 2) This reduction occasionally can be achieved by
splenic irradiation followed by splenectomy as well as by chemotherapeutic
treatment; 3) Severe marrow hypoplasia is not required; 4) The reduction
is usually transient and difficult to maintain for more than a few
months; 5) The reduction may sometimes be re-induced by further
intensive treatment with Ara-C and TG or Daunorubicin and Ara-C, but
not by L-Asparaginase or Hydroxyurea in conventional dosages; 6) The
reduction of the Ph'+ population most often occurs in patients with
small spleens and slowly progressive disease at the time of diagnosis,

and it is in this group that duration of response is longest; 7) Patients
showing a reduction may survive longer than those who do not; it is not
known whether or not this longer survival is related to treatment with
the L-5 Protocol, because it is quite possible that such patients with
slowly progressive or highly responsive disease might also live longer
with less intensive treatment; 8) Although the 50 month median survival
for all patients treated with the L-5 Protocol is longer than that of the
Memorial Hospital historical control series (31 months)[44], it cannot be
concluded that the L-5 Protocol is responsible for prolonging survival
because the patients entered on this protocol were a selected group in
that some patients with early blastic transformation were excluded.
Moreover, unlike the historical series, only Ph'+ patients were included
in the L-5 study and they are known to have a longer survival than
patients with Ph' negative CML.

Current Treatment Protocols for Early CML

There have been seven other series reported in which splenectomy was performed in early CML[64-71], including more than 150 comparable patients. All were prospective studies except that of Didolkar et al.[68] All or most of the patients in each series were Ph'+, and most of the operations were performed within a year of diagnosis. Median survivals have been reached in three studies and they were 40 months[68], 43 months[65], and 44 months[67] from diagnosis. These median survivals are comparable to the 50 month median in the L-5 series. The median survival times for the other series have not been established, but two were reported to be on-going at 30+ months[71] and 36+ months[69]. It is thus likely that all studies will show similar median survival times of between 3 and 4 years, and we can conclude that early splenectomy in CML does not appreciably extend survival. The incidence of blastic transformation was reported for only two of these series[65,67] and it was 100% and 76%. Median survival from transformation was 2 and 4 months, respectively, which is similar to the 3½ months found in the L-5 series.

In almost all cases, early splenectomy was relatively free of serious complications and probably beneficial in preventing late complications associated with massive splenomegaly. Operative mortality was reported by three groups, from infection in 17%[65], and 2%[66] of patients, and from bleeding in 9%[68]. As in this study, there were no operative deaths in three other series[64,67,69]. Operative morbidity varied, from 12% to 34% in the 3 studies in which it was reported[64,66,67], as did the seriousness of the morbidity observed. Postoperative thrombocytosis to levels over 1000 x 10^9/l was reported in 16%[67], 33%[65], 54%[64] and a "majority"[66] of patients in the series in which platelet counts were recorded. The median spleen weight at operation varied as

did the choice of pre-operative chemotherapy. Didolkar et al.[68] reported

mean spleen weights in chronic (1408 g) and blastic (1830 g) cases.

The chronic cases had received Busulfan, 6-Mercaptopurine, or

Hydroxyurea, and the operation was carried out when the disease was

out of control. Tura et al.[66] reported a median spleen weight of 906 grams

in patients who had received Hydroxyurea and were not in remission.

Ihde et al.[67] found the median spleen weight to be less than 500 grams

in patients who were in "remission" from Busulfan.

Chemotherapeutic regimens varied in the series of splenectomized

patients. Most groups relied on "conventional" treatment with Busulfan,

Dibromomannitol, or Hydroxyurea prior to surgery[64-69], and the study by

Didolkar et al. included some who had also received splenic irradiation.[68]

Three groups employed intensive antimetabolite therapy at various points

in their protocols: 6-Thioguanine[64-72], Ara-C[66], and Ara-C and TG[69].

The final results of these studies have not been reported. The

preliminary report by Fuscaldo et al.[69] suggests that this approach may

improve survival, as the only three deaths in the group of 16 were at

5, 5½, and 7½ years.

Only three of these reports[64,65,70] included evaluation of

chromomsome response to therapy. Schwarzenberg et al.[65] concluded that

the Ph'marker "persisted" and Spiers noted that in a group of 17 patients

treated intensively with Thioguanine prior to splenectomy all maintained

100% Ph'+ marrows[72]. However, in the group reported by Fuscaldo et al.[69]

and Brodsky et al.[70] at least 2 of 16 patients have shown a reduction in

Ph'+ cells to date. One had a decrease from 100% to 13% Ph'+ metaphases

after splenectomy and another showed a reduction from 67% to 16% after

cell cycle-specific chemotherapy.[70]

The seven prospective early splenectomy protocols are similar with

regard to basic treatment approach and probable ultimate median survival.

Of the 7 early splenectomy series, the L-5 study is one of only two in which Ph'+ population reduction was apparent. The reasons for this are not clear. However, as has been noted, the duration of repopulation of the marrow with Ph' negative cells was short in some of the patients, and might have been missed if frequent serial cytogenetic examinations had not been performed. It seems possible that similar instances of transient repopulations with Ph' negative cells might have occurred in some of the other series but were not observed because of infrequent examinations after treatment. In the L-5 series there was, in addition, a relatively low operative morbidity (11%), a low incidence of post - operative thrombocytosis to levels above 1000 x 10^9/l (23%), and the median spleen weight (358 g) was smaller than in the other series noting it. The principal difference between the L-5 and other protocols was that splenic irradiation rather than chemotherapy preceded splenectomy. It is possible that this treatment achieved better control of the disease and played a role in the decrease in the Ph' cell populations which we observed. The Medical Research Council study[42] concluded that splenic irradiation was not as effective as Busulfan in terms of extending survival, but the timing and doses of irradiation were not consistent in the irradiated group and the patients received various chemotherapeutic agents in addition. The role of irradiation as primary treatment is currently being investigated by Jacobs et al.[73]

Smalley et al.[74] treated a series of selected patients intensively with Ara-C and TG after their diseases had been brought under good control with Busulfan; they had nearly normal blood counts and no splenomegaly at the time of beginning Ara-C and TG. Splenectomy was not done. Two of the 10 evaluable patients showed a significant reduction in Ph'+ cells. One showed complete absence of the Ph' marker on one determination after 3 months of treatment, with 11% after 6 courses.

She was alive at 6 years after diagnosis. The other patient showed only 7% Ph'+ cells after the 6th course, which is particularly interesting because his marrow had been consistently 100% Ph'+ through five courses, as was the study following the 7% determination.

Although overall median survival may not have been significantly prolonged by the aggressive approach that all these studies demonstrate, the possibility remains that survival may be longer in those patients who show evidence of decrease in the percentage of the Ph'+ metaphases in their marrows, as suggested by the occasional reports prior to these current studies[25,32-35], the preliminary reports of Fuscaldo et al.[69] and Smalley et al.[74] and the results of the L-5 Protocol.

TABLE 1

HEMATOLOGIC STATUS AT START OF L-5 PROTOCOL:

PATIENTS SHOWING REDUCTION IN Ph'+ CELLS

PATIENT	AGE	SEX	PRIOR THERAPY	DURATION	WBC* (×10⁹/1)	HGB* (g/dl)	PLT* (×10⁹/1)	SPLEEN* (cm)	PERCENT BLASTS+ BLOOD	MARROW	Ph'+ METAPHASES AT START (%)	LOWEST (%)
1	48	M	None		27	13.8	318	0	0	3	100	0
2	32	M	None		148	14.2	456	3	3	2	100	23
3	46	F	None		155	10.3	536	0	1	3.5	100	0
4	28	F	None		43	12.5	750	0	0	2	100	6
5	41	F	None		48	13.0	535	0	0	2	100	0
6	17	F	BUS	1 m	53	?	"↑"	?				
					110	12.2	811	0	1	1.5	82	0
7	25	M	BUS,ARA	4 d	327	?	"N"	?				
					200	11.0	250	12	4	0.5	97	27
8	29	M	BUS	1 m	177	16.3	439	"↑↑"				
					67	14.1	380	2	0	1.5	87	0
9	36	M	ARA,TG	10 d	475	14.6	278	15				
					55	13.4	246	0	0	0	100	0
10	47	M	HU,BUS	3/4 m	587	5.0	?	"↑↑"				
					98	13.5	523	6	0	5.5	96	20
11	43	M	ARA	5 d	225	13.7	342	6				
					109	12.2	276	3	0	0	96	0
12	19	M	BUS	2 m	57	"N"	295	4				
					10	15.6	244	4	0	0.5	38	11

*   values at beginning of L-5 Protocol

+   for previously treated patients, values at diagnosis and at beginning of L-5 Protocol are given

spleen = cm. below left costal margin

abbreviations: BUS=busulfan, ARA=arabinosylcytosine. TG=thioguanine, MTX=methotrexate, 6-MP=6-mercaptopurine, HU=hydroxyurea

TABLE 2

HEMATOLOGIC STATUS AT START OF L-5 PROTOCOL: PATIENTS SHOWING NO REDUCTION IN Ph'+ CELLS

PATIENT	AGE	SEX	PRIOR THERAPY	DURATION	WBC* (x10^9/1)	HGB* (g/dl)	PLT* (x10^9/1)	SPLEEN* (cm)	PERCENT BLOOD	BLASTS[+] MARROW	Ph'+ METAPHASES AT START (%)	LOWEST (%)
13	27	M	None		156	8.3	276	17	1	4	93	90
14	46	F	None		150	8.4	520	10	7	1.5	92	91
15	31	M	None		98	9.2	310	"↑↑"	8	3.5	30(p.b.)	48
16	25	F	None		257	12.0	310	16	2	1	88	66
17	52	F	None		141	12.8	1060	6	8	6	100	90
18	40	M	None		230	14.0	113	15	5	1	95	56
19	45	M	None		126	13.3	283	2	0	1	52	36
20	36	M	None		75	13.6	674	2	2	7	75	56
21	38	M	None		210	9.9	393	2	0	1.5	100,26	10
22	46	M	Leukepheresis		525	8.9	372	16	3	1.5	100	73
23	46	M	Leukepheresis		239	10.2	305	5	1	0.5	100	100
24	32	M	BUS	1½ m	120 6	10.0 13.2	245 79	10 1	0	0	96	80
25	31	M	BUS	1 m	94 31	12.3 13.6	1018 1101	? 0	4	9	100	83
26	22	F	BUS	2 m	180 22	"↓" 14.3	465 220	"↑↑" 4	0	0.5	100	98
27	16	M	ARA	5 d	548 150	12.4 13.5	570 450	13 18	2	0	100	96
28	30	M	BUS	½ m	173 214	11.4 10.0	430 668	"↑↑" 9	0	4	96	84

(continued)

TABLE 2
(continued)

PATIENT	AGE	SEX	PRIOR THERAPY	DURATION	WBC* (x10⁹/l)	HGB* (g/dl)	PLT* (x10⁹/l)	SPLEEN (cm)	PERCENT BLASTS+ BLOOD	MARROW	Ph'+ AT START (%)	METAPHASES LOWEST (%)
29	34	M	ARA	3 d	160	7.3	252	5	0	3	100	93
					98	13.7	550	2				
30	45	M	BUS	1 m	370	15.9	"N"	"→"	0	2.5	84	80
					320	9.9	589	"→"				
31	33	M	BUS	4 m	186	9.0	720	7	33	6	100	ineval
					21	8.6	560	25				
32	19	F	BUS	12 m	88	12.7	"→"	?	0	6.5	100	ineval
					13	12.0	1080	2				
33	22	M	ARA	5 d	>300	"N"	550	"→"	3	0	100	55
					184	11.3	"→"	6				
34	31	M	BUS	24 m	259	6.4	744	6	0	0.5	92	72
					78	11.7	134	0				
35	36	F	6-MP; MTX;RT	2 m	365	12.9	82	15	2	1.5	92	92
					151	11.6	280	6				
36	26	M	BUS	1 m	292	8.0	?	"→"	1	2.5	100	77
					80	9.9	727	17				
37	33	M	BUS	1 m	42	15.1	566	?	3	16	91	72
					71	10.1	104	3				

TABLE 3

REDUCTION IN PERCENTAGE OF Ph'+ METAPHASES

ACCORDING TO PHASE OF L-5 PROTOCOL

PATIENT	PRIOR THERAPY	BEFORE L-5	AFTER SPLENIC R.T.	AFTER SPLENEC-TOMY	AFTER ARA-C + TG COURSES #1	#2	#3	(additional)	AFTER L-ASPARAGINASE
				percentage	of	Ph'+	marrow	metaphases	
1	0	3/3	89	81	0	0	0	ND (1)	ND
2	0	100	100	93	23	ND	96,88	95 (1)	100
3	0	100	98	71,86	0	5	ND	x	ND
4	0	100	80	40,50	ND	ND	ND	x	13,6
5	0	100	ND	ND	3/8	0	ND	x	68
6	+	82	89	92,32	13	2	x	x	0
7	+	97	92,65	27,90	76	68	88	x	83
8	+	87	ND	5/6	4	50	10	0-62 (2)	x
9	+	4/4	ND	71	2	0	2	ND (1)	15
10	+	96	ND	98	8/10	2/2	65	ND (1)	20
11	+	96	94,46	14	ND	1/2	20	ND (1)	10,2
12	+	86,38	no RT	100	87	43	35,11,16	x	61,80

x = course not given
ND = karyotype not done

Figure 1

L-5 PROTOCOL

SPLENIC IRRADIATION

↓

SPLENECTOMY

↓

ARABINOSYLCYTOSINE + 6-THIOGUANINE

x 3 courses

↓

L-ASPARAGINASE

↓

RE-INDUCTION ATTEMPT :   HYDROXYUREA   L-6 MAINTENANCE

ARABINOSYLCYTOSINE
6-THIOGUANINE
or
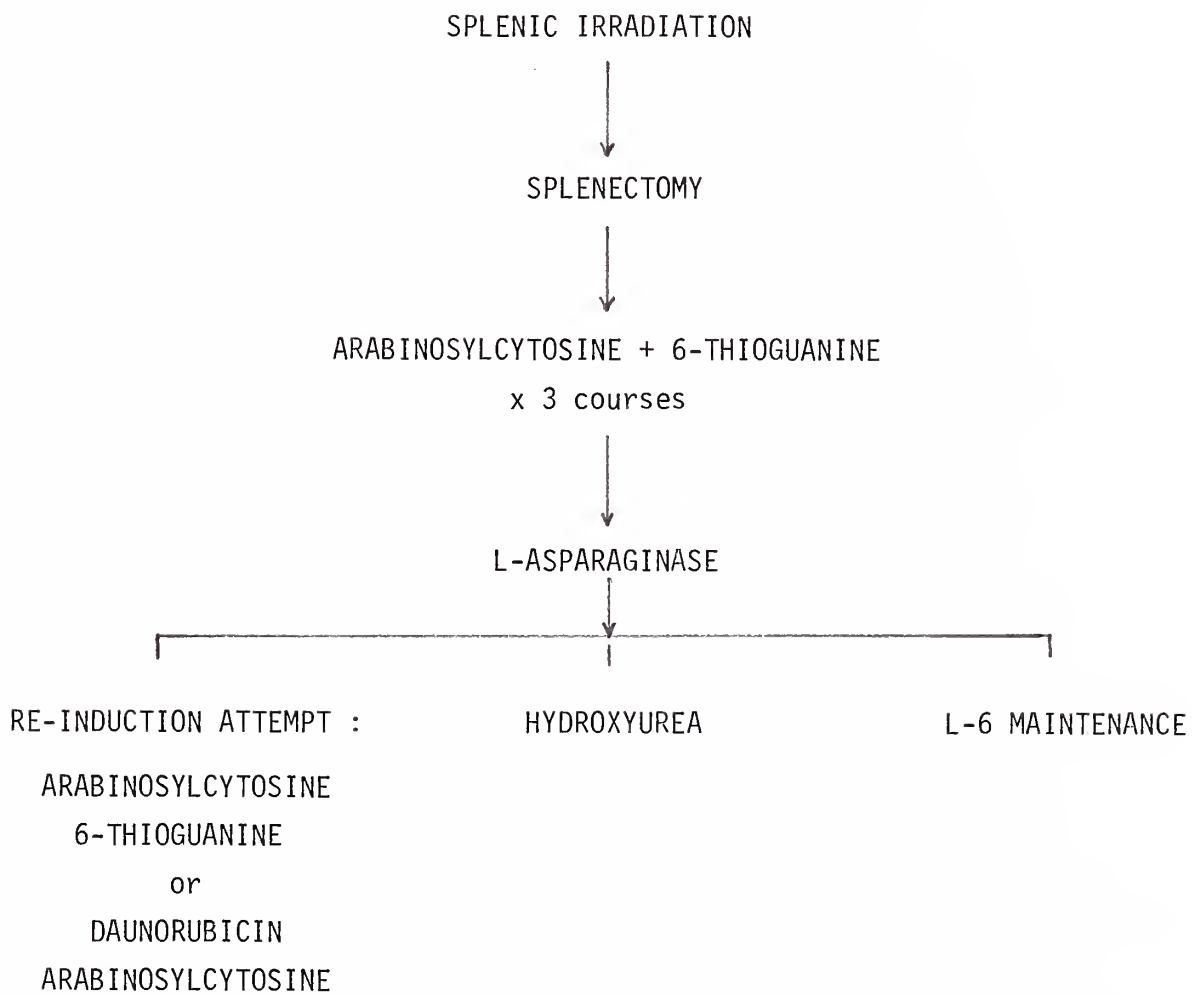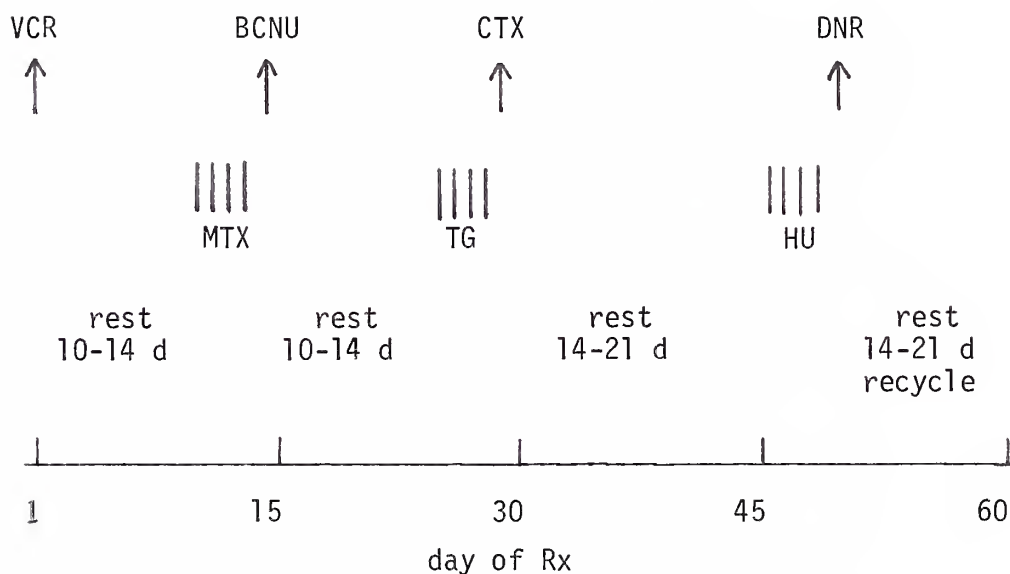DAUNORUBICIN
ARABINOSYLCYTOSINE

Figure 2

L-6 MAINTENANCE PROTOCOL

VCR   = Vincristine 0.03-0.04 mg/kg i.v. x 1
MTX   = Methotrexate 10 mg p.o. x 4 d
BCNU = 1,3-bis (2-chloroethyl)-1-nitrosurea 1-2 mg/kg i.v. x 1
TG   = 6-Thioguanine 10mg/kg p.o. x 4 d
CTX   = Cyclophosphamide 10-20 mg/kg i.v. x 1
HU   = Hydroxyurea 60-80 mg/kg p.o. x 4 d
DNR   = Daunorubicin 0.5-1.0 mg/kg i.v. x 1

Figure 3

L-5 PROTOCOL : SURVIVAL

Symbols indicate last follow-up of living patients (1/1/78)

□ Whole group (37 pts, 14 alive)

○ Non-responders (25 pts, 6 alive)

△ Responders (12 pts, 8 alive)

months from diagnosis
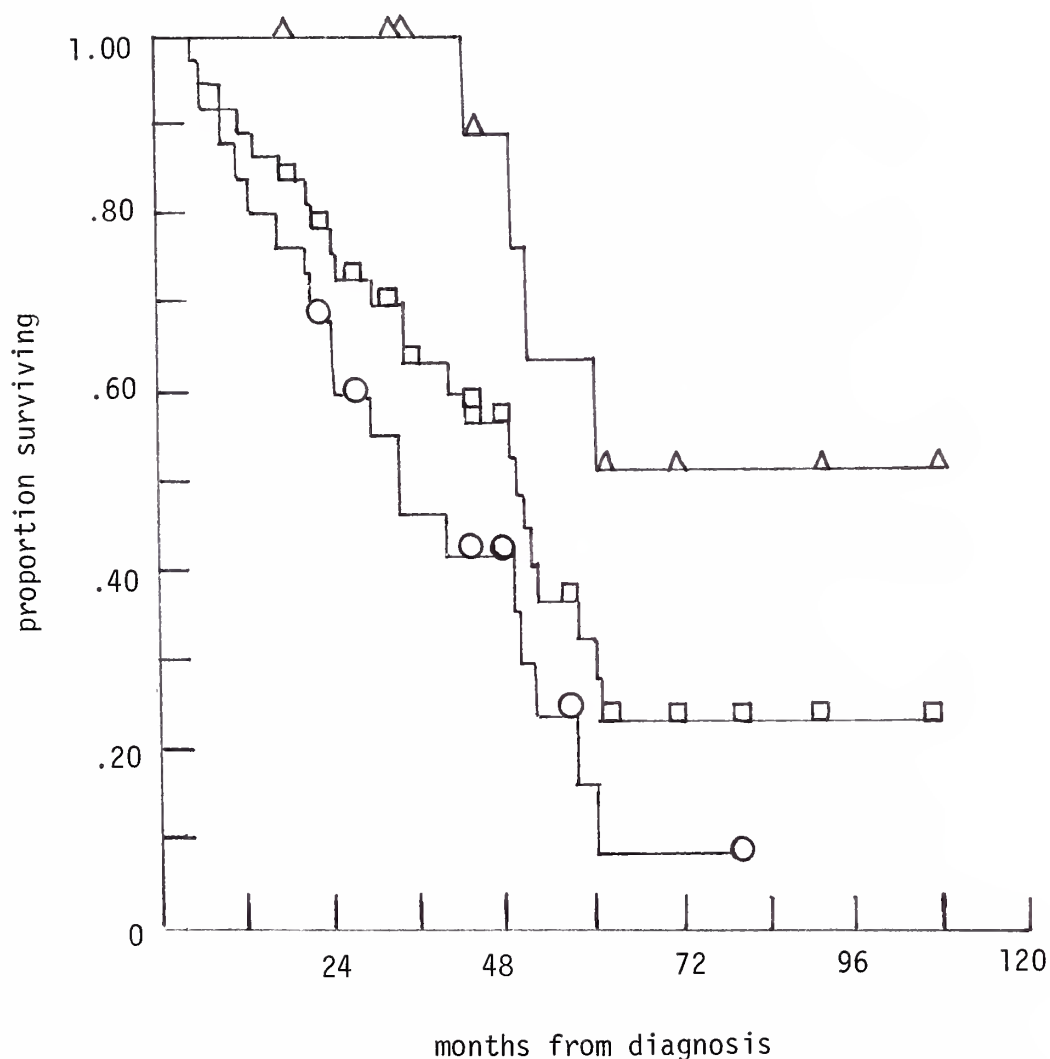
Survival curves calculated by the Kaplan-Meier product limit method(75). Application of the logrank test(76) comparing survival patterns of responders and non-responders yielded p=0.005. Median survival times are 50 months for the whole group, 34 months for non-responders, and has not been reached for responders.

REFERENCES

1.   Nowell, P.C., Hungerford, D.A.: Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst 25:85,1960

2.   Rowley, J.D.: A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and giemsa staining. Nature 243:299, 1973

3.   Rowley, J.D.: Chromosomes in leukemia and lymphoma. Sem Hem 15:301, 1978

4.   Tough, I.M., Jacobs, P.A., Court-Brown, W.M., Baikie, A.G., Williamson, E.R.D.: Cytogenetic studies on bone marrow in chronic myeloid leukaemia. Lancet i:844, 1963

5.   Whang, J., Frei E.III, Tjio, J.H., Carbone, P.P., Brecher, G.: The distribution of the Philadelphia chromosome in patients with chronic myelogenous leukemia. Blood 22: 664, 1963

6.   Fialkow, P.J., Gartler,S.M., Yoshida, A.: Clonal origin of chronic myelocytic leukemia in man. Proc Nat Acad Sci 58: 1468, 1967

7.   Fialkow, P.J., Jacobson, R.J., Papayannopoulou, T.: Chronic myelocytic leukemia: Clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med 63: 125,1977

8.   Fitzgerald, P.H., Pickering, A.F., Elby, J.R.: Clonal origin of the Philadelphia chromosome and chronic myeloid leukaemia: evidence from a sex chromosome mosaic. Br J Haem 21:473, 1971

9.   Moore, M.A.S., Ekert, H., Fitzgerald, M.G., Carmichael, A.: Evidence for the clonal origin of chronic myeloid leukemia from a sex chromosome mosaic: clinical, cytogenetic, and marrow culture studies. Blood 43: 15, 1974

10. Moore, M.A.S., Metcalf,D.: Cytogenetic analysis of human acute and chronic myeloid leukemic cells cloned in agar culture. Int J Cancer 11:143, 1973

11. Shadduck, R.K., Nankin, H.R.: Cellular origin of granulocyte colonies in chronic myeloid leukaemia. Lancet ii:1097, 1971

12. Chervenick, P.A., Ellis, L.D., Pan, S.F., Lawson, A.L.: Human leukemic cells: in vitro growth of colonies containing the Philadelphia (Ph') chromosome. Science 174:1134, 1971

13. Baikie, A.G., Court-Brown, W.M., Buckton, K.E., Harnden, D.G., Jacobs, P.A., Tough, I.M.: A possible specific chromosome abnormality in human chronic myeloid leukaemia. Nature 188:1165, 1960

14. Maniatis, A.K., Amsel, S., Mitus, W.J., Coleman,N.: Chromosome pattern of bone marrow fibroblasts in patients with chronic granulocytic leukemia. Nature 222: 1278, 1969

15. Jacobs, E.M., Luce, J.K., Cailleau, R.: Chromosome abnormalities in human cancer: report of a patient with chronic myelocytic leukemia and his non-leukemic twin. Cancer 19:869, 1966

16. Dougan, L., Scott, I.D., Woodliff, H.J.: A pair of twins, one of whom has chronic granulocytic leukemia. J Med Genet 3:217, 1966

17. Goh,K., Swisher, S.N.: Identical twins and chronic myelocytic leukemia. Arch Intern Med 115:475, 1965

18. Goh, K., Swisher, S.N., Herman, E.C.: Chronic myelocytic leukemia and identical twins. Arch Intern Med 120: 214, 1967

19. Woodliff, H.J., Dougan, L., Onesti, P.: Cytogenetic studies in twins, one with chronic granulocytic leukemia. Nature 211:533, 1966

20. Tokuhata, G.K., Neely, C.L., Williams, D.L.: Chronic myelocytic leukemia in identical twins and a sibling. Blood 31:216, 1968

21. Whang-Peng, J., Canellos, G.P., Carbone, P.P., Tjio, J.H.: Clinical implications of cytogenetic variants in chronic myelocytic leukemia. Blood 32:755, 1968

22. Sakurai, M., Hayata,I., Sandberg, A.A.: Prognostic value of chromosomal findings in Ph' postivie chronic myelocytic leukemia. Cancer Research 36:313, 1976

23. Gatti, R.A., Robinson, W.A., Deinard, A.S., Nesbit, M., McCullough,J.J., Ballow, M., Good, R.A.: Cyclic leukocytosis in chronic myelogenous leukemia: new perspectives on pathogenesis and therapy. Blood 41:771, 1973

24. Canellos, G.P., Whang-Peng, J.: Philadelphia-chromosome positive preleukaemic state. Lancet ii:1227, 1972

25. Kenis,Y., Koulischer, L.: Etude clinique et cytogénétique de 21 patients atteints de leucémie myéloïde chronique. Europ J Cnacer 3:83, 1967

26. Conrad, R.F.: Survival in chronic granulocytic leukemia. Arch Intern Med 131:684, 1973

27. Djaldetti, M., Padeh, B., Pinkhas,J., DeVries,A.: Prolonged remission in chronic myeloid leukemia after one course of busulfan. Blood 27:103,1966

28. German, H.J., Smith, J.A., Lindenbaum, J.: Chronic intravascular coagulation associated with chronic myelocytic leukemia. Am J Med 61:547, 1976

29. Maurice,P.A., Alberto, P., Ferrier, S., Freund, M.: Leucémie myélocytaire chronique:"Guerison" apparente depuis plus de 9 ans, consécutive à une hypoplasie medullaire thérapeutique. Schweiz Med Wochenschr 101:1781, 1971

30. Haut, A., Abbott, W.S., Wintrobe, M.M., Cartwright, G.E.: Busulfan in the treatment of chronic myelocytic leukemia. The effect of long term intermittent therapy. Blood 17:1, 1961

31. Perreau, P., Gardais, J.: Survival in chronic myeloid leukaemia following aplasia caused by busulphan. Seminaires Hôpital 45: 964,1969

32. Galton, D.A.G.: Radical therapy for chronic granulocytic leukaemia. In: Proceedings of the II Padua Seminar on Clincal Oncology, Padua, Oct. 1972, p.95

33. Brandt,L., Mitelman, F., Panani, A., Lenner,H.C.: Extremely long duration of chronic myeloid leukaemia with Ph' negative and Ph' positive bone marrow cells. Scand J Haematol 16: 321, 1976
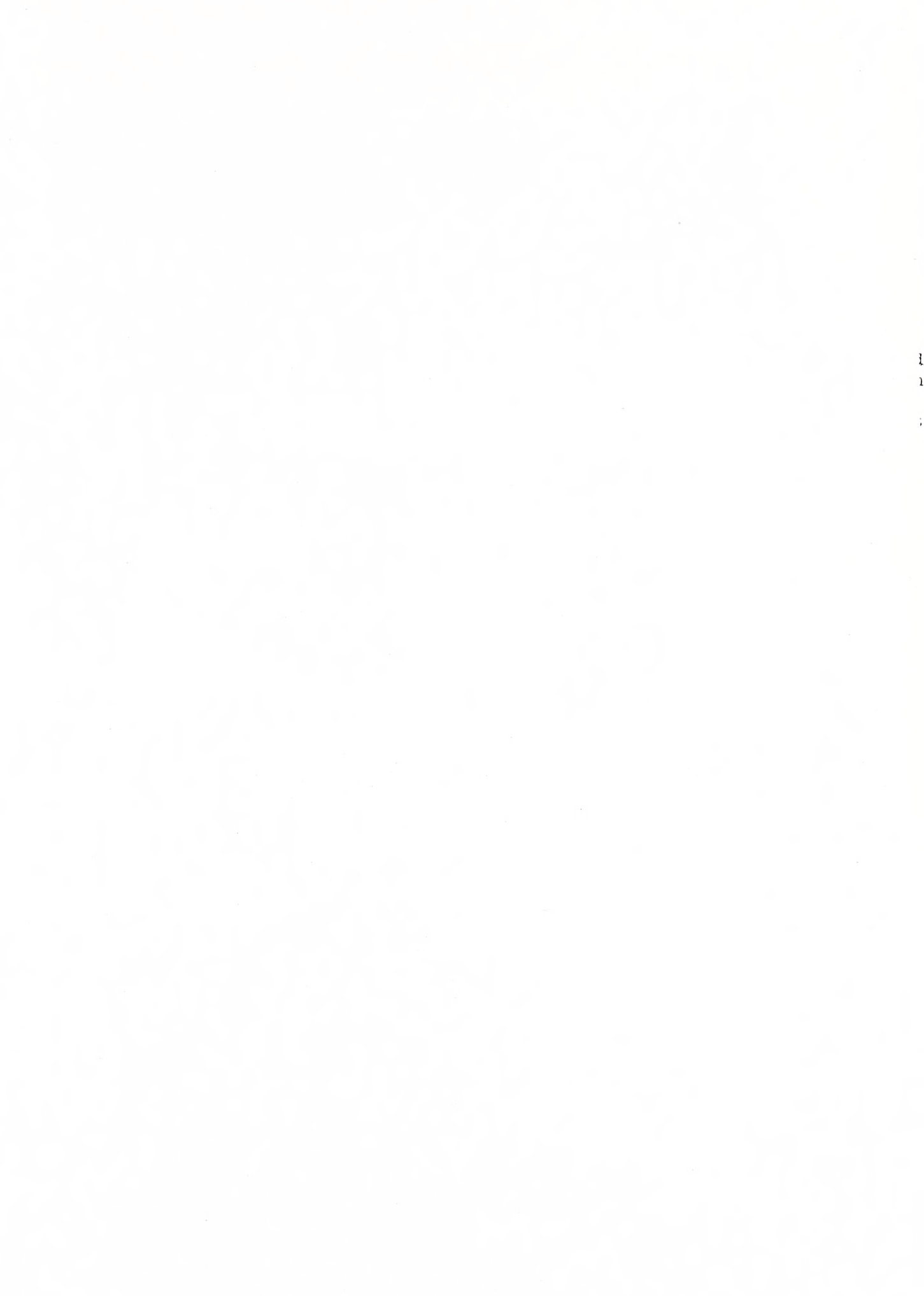
34. Golde,D.W., Bersch, N.L., Sparkes, R.S.: Chromosomal mosaicism associated with prolonged remission in chronic myelogenous leukemia. Cancer 37: 1849, 1976

35. Finney, R., McDonald, G.A., Baikie, A.G., Douglas, A.S.: Chronic granulocytic leukemia with Ph' negative cells in the bone marrow and a ten year remission after busulfan therapy. Br J Haem 23: 283,1972

36. Fitzgerald, P.H., Adams, A., Gunz, F.W.: Chronic granulocytic leukemia and the Philadelphia chromosome. Blood 21: 183, 1963

37. Canellos, G.P., Whang-Peng, J., Schnipper,L., Brown, C.H.III: Prolonged cytogenetic and hematologic remission of blastic transformation in chronic granulocytic leukemia. Cancer 30: 288, 1972

38. Mastrangelo, R., Zuelzer, W.W., Thompson,R.I.: The significance of the Ph'chromosome in acute myeloblastic leukemia: serial cytogenetic studies in a critical case. Pediatrics 40:834, 1967

39. Arlin,Z., Chaganti, R.S.K., Gee, T., Clarkson, B.: Complete remission of the blastic phase of chronic myelocytic leukemia. Proc AACR 18:196 (abstract 782), 1977

40. Minot, J.B., Buckman, T.E., Isaacs, R.: Chronic myelogenous leukemia: age, incidence, duration and benefit derived from irradiations. JAMA 82, 1489, 1924

41. End Results in Cancer. Report No. 4 DHEW Pub No. (NIH) 73-272, 1972

42. Medical Research Council: Chronic granulocytic leukemia: comparison of radiotherapy and busulphan therapy. Br Med J 1:201, 1968

43. Dibromomannitol Cooperative Study Group: Survival of chronic myeloid leukemia patients treated by dibromomannitol. Europ J Cancer 9:583, 1973

44. Monfardini, S., Gee, T., Fried, J., Clarkson, B.: Survival in chronic myelogenous leukemia: influence of treatment and extent of disease at diagnosis. Cancer 31:492, 1973

45. Spiers, A.S.D., Baikie, A.G.: Chronic granulocytic leukaemia:

demonstration of the Philadelphia chromosome in cultures of spleen cells. Nature 208:497, 1965

46. Mitelman, F.: Comparative cytogenetic studies of bone marrow and extramedullary tissues in chronic myeloid leukemia. Ser Haemat 8:113, 1975

47. Baccarini, M., Zaccaria, A., Santucci, A.M., Bagnara, G.P., Ricci, P., Gobbi, M., Ruggero, D., Brunelli, M.A., Tura, S.: A simultaneous study of bone marrow, spleen, and liver in chronic myeloid leukemia: Evidence for differences in cell composition and karyotypes. Ser Haemat 8:81, 1975

48. Armenta, D., Cadotte, M., Beaulieu, R., Neemeh, J., Long, L., Pretty, H., Gosselin, G.: Evidence cytogénétique de l'origine splénique de la leucémie myéloïde chronique. Union Med Can 105:922, 1976

49. Mitelman, F., Brandt, L., Nilsson, P.G.: Cytogenetic evidence for splenic origin of blastic transformation in chronic myeloid leukaemia. Scand J Haemat 13:87, 1974

50. Mitelman, F., Nilsson, P.G., Brandt, L.: Abnormal clones resembling those seen in blast crisis arising in the spleen in chronic myelocytic leukemia. J Natl Cancer Inst 54:1319, 1975

51. Hossfeld, D.K., Schmidt, C.G.: Chromosomal data suggesting a primary role of the spleen in the pathogenesis of chronic myelocytic leukemia and blastic phase of CML. In: Garatini, S. and Franchi, G. (eds), Chemotherapy of Cancer Dissemination and Metastasis. New York, Raven Press, 1973, p.223

52. Gee, T.S.: Extramedullary blast crisis. Proc Amer Assoc Cancer Res 3:55 (abstract 217), 1973

53. Rosenthal, S., Canellos, G.P., DeVita, V.T. Jr., Gralnick, H.R.: Characteristics of blast crisis in chronic granulocytic leukemia. Blood 49: 705, 1977

54. Whang-Peng, J., Henderson, E.S., Knutsen, T., Freireich, E.J.,

Gart, J.J.: Cytogenetic studies in acute myelocytic leukemia with special emphasis on the occurrence of Ph' chromosome. Blood 36:448, 1970

55. Garson, O.M., Burgess, M.A., Stanley, L.G.: Cytogenetic remission in acute transformation of chronic granulocytic leukaemia. Brit Med J 2:556, 1969

56. Canellos, G.P., DeVita, V.T., Whang-Peng, J., Carbone, P.P.: Hematologic and cytogenetic remission of blastic transformation in chronic granulocytic leukemia. Blood 38:671, 1971

57. Gomez, G., Hossfeld, D.K., Sokal, J.E.: Removal of abnormal clone of leukemic cells by splenectomy. Br Med J 2:421, 1975

58. Clarkson, B., Rubinow, S.I.: Growth kinetics in human leukemia. In: Drewinko, B. and Humphrey, R.M. (eds), Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells. Baltimore, Williams and Wilkins Co., 1977, p.591

59. Gee, T.S., Yu, K-P, Clarkson, B.D.: Treatment of adult acute leukemia with arabinosylcytosine and thioguanine. Cancer 23:1019, 1969

60. Clarkson, B.D., Dowling, M.D., Gee, T.S., Cunningham, I.B., Burchenal, J.H.: Treatment of acute leukemia in adults. Cancer 36:775, 1975

61. Clarkson, B., Krakoff, I., Burchenal, J., Karnofsky, D., Golbey, R., Oettgen, H., Dowling, M., Lipton, A.: Clinical results of treatment with E. coli L-Asparaginase in adults with leukemia, lymphoma, and solid tumors. Cancer 25:279, 1970

62. Schreibman, S.M., Gee, T.S., Grabstald, H.: Management of priapism in patients with chronic granulocytic leukemia. J Urol 111:786, 1974

63. Hopfan, S., Watson, R., Benua, R.: Splenic irradiation portals. Radiology 112:417, 1974

64. Spiers, A.S.D., Baikie, A.G., Galton, D.A.G., Richards, H.G.H.,
    Wiltshaw, E., Goldman, J.M., Catovsky, D., Spencer, J., Peto, R.:
    Chronic granulocytic leukaemia: effect of elective splenectomy on
    the course of disease. Brit Med J 1:175, 1975

65. Schwarzenberg, L., Mathe,G., Pouillart, P., Weiner, R., Locour, J.,
    Genin, J., Schneider, M., deVassal, F., Hayat, M., Amiel, J.L.,
    Schlumberger, J.R., Jasmin, C., Rosenfeld, C.: Hydroxyurea,
    leucopheresis, and splenectomy in chronic myeloid leukaemia at
    the pro-blastic phase. Brit Med J 1:700, 1973

66. Tura, S., Baccarini, M., Gugliotta, L., Lauria, F., Fiacchini, M.,
    Tomasini, I.: A clinical trial of early splenectomy, hydroxyurea,
    and cyclic arabinosylcytosine, vincristine, and prednisone in
    chronic myeloid leukemia. Ser Haemat 8:121, 1975

67. Ihde, D.C., Canellos, G.P., Schwartz, J.H., DeVita, V.T.:
    Splenectomy in the chronic phase of chronic granulocytic leukemia.
    Ann Int Med 84:17, 1976

68. Didolkar, M.S., Mittelman, A., Gomez, G., Elias, E.G.: Evaluation
    of splenectomy in chronic myelogenous leukemia. Surg Gyn Obstet
    142:689, 1976

69. Fuscaldo, K.E., Brodsky, I., Kahn, S.B., Conroy, J.F.: CGL: effect of
    sequential chromosomal analysis, splenectomy, and intensive chemotherapy
    on survival. Proc Amer Soc Clin Onc 18:294 (abstract 111), 1977

70. Brodsky, I., Fuscaldo, K.E., Kahn, S.B., Conroy, J.F., Lamping, C.G.:
    Chronic myelogenous leukemia: a clinical and experimental evaluation
    of splenectomy and intensive chemotherapy. Sem Haemat 8:143, 1975

71. Hester, J.P., Kwaan, H.C., Moake, J., Trujillo, J., Hart, J.,
    McBride, C., McCredie, K.B., Freireich, E.J.: Cytokinetic, cytogenetic,
    coagulation profiles of chronic myelogenous leukemia patients undergoing
    splenectomy. Proc Amer Soc Clin Onc 18:280 (abstract C-55), 1977

72. Spiers, A.S.D., Galton, D.A.G., Kaur, J., Goldman, J.M.: Thioguanine as primary treatment for chronic granulocytic leukaemia. Lancet i:829,1975

73. Jacobs, P., Dubovsky, D., King, H., Sealey, R.: Splenic irradiation in the management of chronic granulocytic leukaemia. Cent Afr J Med 21:207, 1975

74. Smalley, R.V., Vogel, J., Huguley, C.M., Miller, D.: Chronic granulocytic leukemia: cytogenetic conversion of the bone marrow with cycle-specific chemotherapy. Blood 50:107, 1977

75. Kaplan, E.L., Meier, P.: Nonparametric estimation from incomplete observations. J Amer Statis Assoc 53: 457, 1958

76. Peto,R, Pike,M.C.: Conservatism of the approximation $\sum(O-E)^2/E$ in the logrank test for survival data or tumor incidence data. Biometrics 29: 579, 1973

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