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EFFECTS OF VITAMIN A ON SURVIVAL IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA: A SWOG RANDOMIZED TRIAL

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Abstract—A national cooperative group trial was conducted in 153 patients with chronic myelogenous leukemia (CML) in chronic phase treated with oral pulse busulfan to determine if oral vitamin A can increase the time to blast crisis and enhance survival of patients. Patients diagnosed within 1 year and in the chronic phase of CML were randomized to receive oral pulse busulfan or the alkylator plus continuous oral vitamin A. Distributions of clinical progression and overall survival were estimated using the method of Kaplan and Meier. Associations of these endpoints with treatment and other patient characteristics were analyzed using the proportional hazards regression method of Cox. Both regimes were well tolerated. Patients in the busulfan plus vitamin A arm had somewhat longer durations of clinical progression-free survival (median 46 months) and overall survival (51 months) compared to those in the busulfan arm (medians 38 and 44 months). However, the differences were not statistically significant (one-tailed $P=0.11$ for clinical progression-free survival, 0.081 for survival). After adjustment for significant factors identified in an additional exploratory multivariate analysis, risk of clinical progression or death was 53% ($P=0.022$) greater and risk of death 60% ($P=0.014$) greater among busulfan patients. Given the relatively large though non-significant difference between treatment arms, the limited statistical power of the study, and the likelihood that oral vitamin A may not be the most effective means of delivering retinoid therapy, we conclude that further investigation of retinoids in chronic phase CML is warranted.

Key words: Chronic myelogenous leukemia, busulfan, vitamin A, retinoids, differentiation.

Introduction

The use of alkylator chemotherapy in the chronic stage of chronic myelogenous leukemia (CML) led to a definite, but small, increase in overall survival from 2.6 to 3.3 years [1]. However, at the time the current trial was initiated, no further survival benefit of other agents or modalities had been shown. The main cause of death in approximately 70% of patients with CML is blastic transformation which occurs secondary to unknown intrinsic or extrinsic factors. Since standard chemotherapeutic approaches do not appear to significantly retard blastic transformation, further advances in therapy are

most likely to arise from different approaches toward the management of the chronic phase of this disease. Alternative treatment modalities include bone marrow transplantation, immunotherapy, cytokines, lymphokines, differentiation compounds, and chemopreventive agents.

Recently, there has been a great deal of interest in the use of retinoids in the treatment of malignancies [2]. Data supporting the use of these compounds as potential therapeutic agents has existed for over 20 years in both leukemic and solid tumor model systems [3, 4]. In the 1970s, a substantial amount of evidence demonstrated that vitamin A and its natural and synthetic derivatives (retinoids) could suppress chemically-induced carcinogenesis in epithelial cell systems [5]. Topical retinoic acid was shown to cause regression of premalignant epithelial lesions, including actinic keratosis, oral leukoplakia, and urinary papillomas [4, 6]. We postu-

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lated that the transition of CML cells in chronic phase to blast crisis was analogous to the progression of epithelial cells from preneoplasia to frank malignancy and might be sensitive to retinoid therapy.

We report here the results of a randomized trial of intermittent pulse busulfan with or without a modest dose of oral vitamin A in patients in the chronic phase of CML. Both time to clinical progression and overall survival were somewhat lengthened in the group receiving the oral retinoid.

Patients and Methods

Patient eligibility

Patients with the diagnosis of chronic phase CML for less than 1 year were eligible. No prior treatment was allowed except with hydroxyurea and/or leukopheresis for less than 7 days. Patients were eligible who had a splenectomy.

CML in chronic phase was defined as: (1) persistent leukocytosis of at least $30,000/\text{mm}^3$ demonstrated on two occasions (no less than 24 h or more than 60 days apart) and not ascribed to other causes; (2) differential counting of blood leukocytes with myeloblasts less than 10%, lymphocytes less than 15%, the sum of the percentage of segmented neutrophils plus metamyelocytes greater than the percentage of promyelocytes, and metamyelocytes and myelocytes present in the circulation and comprising more than 10% of cells; (3) bone marrow aspiration and biopsy demonstrating hypercellularity, no significant fibrosis, a myeloid/erythroid ratio of at least 3:1, and a differential count satisfying the requirements in (2) above. Blood or bone marrow aspiration was obtained for chromosomal analysis. Initially, a positive Ph^1 chromosome analysis was required before randomization; however, this delayed care and on 4 December 1981 the protocol was amended to delete this requirement for eligibility. Patients with myelofibrosis and agnogenic myeloid metaplasia were allowed only if a Ph^1 chromosome test was done before study entry and was positive.

Renal and liver function tests, leukocyte alkaline phosphatase, protein, quantitative immunoglobulin, and serum vitamin A level were also performed.

All patients gave informed consent according to institutional and federal guidelines before entry into the study.

Therapeutic regimen

Patients were randomly assigned to receive intermittent pulse busulfan or intermittent pulse busulfan plus continuous oral vitamin A. This randomization was stratified by age (≤ 20 vs. >20 years) and prior splenectomy (yes vs. no), which were parameters thought to affect outcome.

All patients received busulfan orally at a dose of $8 \text{ mg}/\text{m}^2/\text{day}$ for 4 days every 4 weeks until a chronic stable phase was reached (total leukocyte count $<50,000/\text{mm}^3$ but $>6,000/\text{mm}^3$) at which time busulfan was discontinued. Busulfan was restarted only when the total leukocyte count exceeded $50,000/\text{mm}^3$. Treatment was reinstated until the total leukocyte count was $<50,000$ but $>6,000$. Busulfan was discontinued and reinstituted as necessary under the above conditions. Patients who were resistant to busulfan or who developed long-term toxicity (pulmonary fibrosis, skin pigmentation, hypogonadism, or features of Addison's disease) were allowed to switch to another alkylating agent. Patients in the second arm of the trial were also given $50,000 \text{ IU}/\text{day}$ of oral vitamin A (Aquasol

A, USV Pharmaceuticals [currently Armour Pharmaceuticals, Fort Washington, Pennsylvania] or equivalent). Until mid-1982 the active compound in Aquasol A was retinol, and thereafter changed to retinol palmitate by the manufacturer. The vitamin A was given continuously as long as the patient was on the study and was not interrupted when busulfan therapy was discontinued.

Toxicity criteria

Toxicities were rated according to SWOG guidelines. In addition, specialized guidelines were developed for the unique side effects of vitamin A and included central nervous system (emotional lability, personality changes, mental processing, headaches, papilledema), dermatology (skin and mucosa), endocrine/metabolism (appetite, weight loss, menstrual changes, libido, and impotence) and systemic (fatigue, joint pain). Grades of toxicity were based on what was known about the side effects of vitamin A [7]. Since that time the range and extent of side effects of the retinoids has become more deeply appreciated [8]. Adjustments of vitamin A dosage were made in relation to these side effects and to serum vitamin A levels.

Statistical considerations

The major objective of this study was to determine the efficacy of standard pulse, intermittent busulfan therapy plus oral vitamin A in prolonging the chronic phase of CML, and hence, in prolonging patient survival. All eligible patients were included in the primary analysis.

The analysis of time to progression was based on an endpoint defined as 'clinical progression'. This approach was considered appropriate since a review of the records indicated that a large number of patients were considered by their physicians to have progressed, even though the criteria for progression defined in the protocol were not (or were not shown to be) satisfied. According to the protocol, progression was defined as: (1) an increasing leukocyte count with or without progressive anemia; plus (2) more than 25% blasts plus promyelocytes in the bone marrow and/or peripheral blood; plus (3) progressive splenic enlargement in a non-splenectomized patient. Where information was available, the basis for the conclusion that progression had occurred was also recorded. For the purposes of this analysis, 'clinical progression' was defined as any of the following: progression as defined in the protocol, the start of treatment for progression, or documentation of the physician's diagnosis of progression.

This study was closed in April 1987 with the expectation that about 150 patients would be eligible, as called for by the study design. Subsequently, a careful review of the pre-study data, particularly for patients accrued during the first several years, indicated that more patients failed to meet the hematologic eligibility criteria than originally thought: 24 (29%) of the 84 patients registered before 1986 were ineligible, as were five (7%) of the 69 subsequent patients. This improvement in the ineligibility rate resulted largely from the imposition of stricter registration and evaluation procedures following the establishment of a new Statistical Center for the Group in October 1985.

Overall survival was measured from randomization until death from any cause, with censorship at the date of last contact for patients last known to be alive. Dates of last contact were within 6 months of the present analysis for 15 of the 22 censored eligible patients; for only two of these patients was the last contact more than 1 year before the analysis. The duration of clinical progression-free survival was measured

from randomization until the earlier of clinical progression or death from any cause; observation was censored at last contact for patients known to be alive without clinical progression. Distributions of overall and clinical progression-free survival were estimated by the method of Kaplan and Meier [9].

The effects of treatment and patient and disease characteristics and treatment on these endpoints were analyzed using the proportional hazards regression model of Cox [10]. These included analyses for the specific hypotheses of the study (i.e. treatment comparisons stratified by age group and prior splenectomy, with no adjustment for other factors), as well as additional analyses allowing adjustment for possible effects of other factors which, while not regarded as established prognostic factors in CML, might enhance or reduce an effect of vitamin A. The magnitude of treatment differences was expressed as the relative risk, for busulfan alone relative to the busulfan plus vitamin A, of the endpoint in question (death, or clinical progression or death), as estimated from proportional hazards regression analysis. For example, a relative risk of 1.5 for death would indicate that on average the risk of death at any point in time was 50% greater with busulfan alone, compared to busulfan plus vitamin A.

Comparisons of toxicity rates between the treatment arms were based on small sample methods [11], since large sample approximations might misrepresent the statistical significance of differences. All eligible patients were included in analyses of toxicity, except one in the busulfan plus vitamin A group who refused protocol treatment.

Treatment comparisons were based on one-sided tests, since the hypotheses of superior overall or clinical progression-free survival and of greater toxicity in the busulfan plus vitamin A arm were of interest. The significance of other factors in multivariate analyses was expressed using two-sided *P*-values, since the concern was to identify factors with prognostic influence, regardless of the directions of their effects [12].

Results

Patient characteristics

From 12 July 1980 to 1 April 1987 a total of 153 patients from 58 SWOG institutions were randomized between busulfan only (79 patients) and busulfan plus vitamin A (74 patients). Twenty-nine patients (19%; 12 busulfan, 17 busulfan plus vitamin A) were subsequently found to be ineligible. Twenty-two did not satisfy one or more of the protocol requirements regarding peripheral blood and bone marrow differential counts, five did not have required pre-study data, one had myelofibrosis without a documented pre-study Ph¹ chromosome, and one received protocol treatment before registration. There were 67 and 57 fully eligible patients on the busulfan alone and busulfan plus vitamin A arms, respectively. Initially patients were required to be positive for the Philadelphia chromosome (Ph¹). However, the requirement for pretreatment cytogenetics was removed in December 1981. Of the 124 fully eligible patients, 111 were Ph¹-positive and six were Ph¹-negative, although four of these six were based on less than 10 karyotypes. For the other seven patients, pretreatment cytogenetics were either not done, un-

Fig. 1. Clinical progression-free survival by treatment. Symbols: —, busulfan plus vitamin A arm; - - - busulfan arm.

usable, or not reported. Exclusion from analysis of patients who had Ph¹-negative disease or in whom cytogenetic results were unavailable did not affect the clinical outcome of the two arms. The treatment arms were fairly well balanced. Patients in the two arms were similar for age, although patients on the busulfan plus vitamin A arm had a somewhat higher median age (51 vs. 46 years), higher proportion of females (53 vs. 47), and percentage of whites (84 vs. 73). Disease characteristics of the two groups were similar for prior splenectomy, presence of splenomegaly, distribution of marrow and peripheral blood cells, number of platelets, and hemoglobin level. The median number of peripheral white cells in the busulfan plus vitamin A group was higher (132 vs. 114 $\times 1000/\mu\text{l}$). Additional analyses of age were carried out since the age stratification was ineffective (only two eligible patients less than or equal to 20 years of age).

Clinical progression-free survival

An analysis of clinical progression-free survival in the 124 eligible patients was performed (Fig. 1). One hundred and seven patients had clinical progression and/or died. The remaining 17 were last known to be

Fig. 2. Overall survival by treatment. Symbols as for Fig. 1.

Table 1. Multivariate analyses and treatment effect

	Regression coefficient	Standard error	P-value
Clinical progression-free survival			
Age	0.019	0.007	0.010
PMNs (1000s)	0.004	0.002	0.044
Marrow lymphocytes (%)	0.089	0.044	0.046
Treatment arm	0.425	0.210	0.022 [0.023]*
Overall survival			
Age	0.023	0.007	0.002
PMNs (1000s)	0.004	0.002	0.038
Marrow lymphocytes (%)	0.085	0.046	0.064
Treatment arm	0.470	0.214	0.014 [0.010]*

* Number in parentheses is the *P*-value for secondary analysis that included an additional 20 ineligible patients who were Ph¹-positive, as defined in Patients and Methods.

alive and progression-free between 37 and 111 months after entering the study (median 76 months). Six of these 17 patients were still on protocol treatment at last contact. The median duration of clinical progression-free survival with busulfan plus vitamin A was 46 months, compared to 38 months for busulfan alone. Proportional hazards regression analysis, with adjustment for stratification by age and prior splenectomy, indicated that the risk of clinical progression or death was 26% greater among busulfan patients, compared to busulfan plus vitamin A patients (relative risk=1.26, 95% confidence interval 0.86–1.86). This treatment effect was not statistically significant (one-tailed log rank, adjusted for stratification, *P*=0.11).

Among the patient and disease characteristics, only age (*P*=0.041) and marrow lymphocyte percentage (*P*=0.070) were even marginally related to clinical progression-free survival in univariate analyses. However, in the multivariate analysis (Table 1), risk increased with increasing age (two-tailed *P*=0.010), marrow lymphocyte percentage (*P*=0.046), and with increasing absolute polymorphonuclear leukocyte (PMN) count (*P*=0.044). Adjusting for these three factors in the multivariate analyses increased the apparent treatment effect: risk of clinical progression or death was 53% greater among busulfan patients compared to busulfan plus vitamin A patients (i.e. relative risk=1.53, 95% confidence interval 1.01–2.31). This treatment effect was statistically significant (one-tailed *P*=0.022).

Analyses of the possible effects of total white blood cell count (WBC), duration of symptoms, and duration from diagnosis to study entry found no significant association with clinical progression-free survival. This was true in univariate analyses [WBC (*P*=0.58), duration

of symptoms (*P*=0.67), diagnosis to entry (*P*=0.77)], as well as after adjusting for treatment and the significant variables listed above [WBC (*P*=0.99), duration of symptoms (*P*=0.55), diagnosis to study entry (*P*=0.96)].

Survival

The effect of treatment on overall survival was also analyzed (Fig. 2). Of the 124 eligible patients, 102 have died. The remaining 22 patients were last known to be alive between 37 and 111 months after entering the study (median 76 months). Patients in the busulfan plus vitamin A arm had longer survival (median 51 months) compared to those in the busulfan arm (median 44 months). Proportional hazards regression analysis, with adjustment for stratification by age and prior splenectomy, indicated that the risk of death was 33% greater among busulfan patients compared to busulfan plus vitamin A patients (i.e. relative risk=1.33, 95% confidence interval 0.89–1.97). This treatment difference was not statistically significant (one-tailed log rank adjusted for stratification *P*=0.081).

Among the patient and disease characteristics, only three were significantly related to survival in univariate analyses: the risk of death increased with increasing age (two-tailed *P*=0.014), absolute PMN count (*P*=0.099) and decreasing hemoglobin (*P*=0.086). However, in the multivariate analysis, the following three factors were identified as significant: increasing age (*P*=0.002), absolute PMN count (*P*=0.038), and marrow lymphocyte percentage (*P*=0.064) increased risk. After adjusting for the joint effect of these three factors, the risk of death was estimated to be 60% greater among busulfan patients, compared to busulfan plus vitamin A patients (relative risk=1.60, 95% confidence interval 1.05–2.43).

This represents a statistically significant treatment effect (one-tailed $P=0.014$).

Total WBC might be expected to have a prognostic significance. However, this was not seen to be the case, in either univariate analysis ($P=0.37$) or after adjusting for the effects of age, absolute PMN count, marrow lymphocyte percentage, and treatment in the multivariate analysis ($P=0.82$).

Duration from diagnosis of CML until entry into the study was not significantly related to overall survival in either, univariate analysis ($P=0.89$), or after adjusting for treatment and the significant variables in the multivariate analysis ($P=0.86$).

Duration from onset of symptoms until entry into the study was not significantly related to overall survival in the univariate analysis ($P=0.19$). However, after adjusting for the variables listed above in the multivariate analysis, there was a marginally significant tendency for survival to decrease with increasing duration of symptoms ($P=0.086$). Including this variable in the multivariate model had virtually no effect on the apparent treatment effect (relative risk=1.62, 95% confidence interval 1.05–2.51, $P=0.015$).

In evaluating the results of this study, it is important to assess the statistical power that was actually achieved, since the number of eligible patients, 124, fell well below the planned accrual of 150. Using the formula of Schoenfeld [12], the analysis of overall survival based on 100 deaths has adequate statistical power (i.e. >80%) to detect relative risks of 1.64 or greater (one-sided test at 0.05 critical level). The analysis of clinical progression-free survival, based on 107 events, has only slightly greater power. The statistical power in the present analysis can perhaps best be characterized as barely adequate.

Secondary analysis

Owing to the large proportion of ineligible patients in the study, a secondary analysis was performed, which included the 124 fully eligible patients, plus all ineligible patients who were positive for the Philadelphia chromosome. The latter group included 20 patients (eight busulfan, 12 busulfan plus vitamin A). Their reasons for ineligibility included failure to meet the protocol requirements regarding peripheral blood and bone marrow differential counts (17 patients), failure to obtain pre-study leukocyte alkaline phosphatase (two), and initiation of treatment prior to registration (one). The secondary analysis of time to clinical progression and overall survival gave results that were generally similar to those found in the primary analysis. In the secondary analyses of clinical progression-free and overall survival, the relative risks were 1.19 ($P=0.17$) and 1.26 ($P=0.08$), respectively. Multivariate analyses produced similar results to those of the primary analyses

suggesting a possible beneficial effect of vitamin A: the relative risks for clinical progression-free and overall survival were 1.48 ($P=0.023$) and 1.59 ($P=0.010$), respectively.

Toxicities

Toxicities seen in the eligible patients included allergy, alopecia, anemia, bone pain, dizziness/hot flashes, general neurologic, headache, nausea/vomiting/diarrhea, other GI, weight loss, and changes in hepatic enzymes, prothrombin time, platelet count, and white count.

Sixty-seven eligible patients in the busulfan only arm were evaluated for toxicity. One patient had mucositis, gastric and esophageal ulceration, vomiting and anorexia, all Grade 3. Two patients had Grade 2 nausea and vomiting, one had Grade 1 abdominal cramps, and one had Grade 1 thrombocytopenia. One patient experienced dizziness and faint feeling of unknown grade.

Fifty-six patients in the busulfan plus vitamin A arm were evaluated for toxicity. Two patients experienced Grade 4 thrombocytopenia; one of these patients also had Grade 3 leukopenia and Grade 1 hyper-pigmentation; the other patient additionally had Grade 2 weight loss, anorexia and fatigue with muscle aches. A third patient had Grade 2 thrombocytopenia. One patient underwent personality changes of Grade 3, and another of Grade 2. Four patients had rashes or dry skin, including one of Grade 3. One patient had stomach cramps of Grade 1. Five patients had hepatic toxicities. 'Miscellaneous other' toxicities included dry mouth of Grade 3 (one patient), Grade 2 fatigue with joint pain (one patient), Grade 2 anxiety (one patient), Grade 1 restrictive lung disease (one patient), and pancreatitis and hyperlipidemia of unknown severity (one patient).

Grade 2+ toxicities were reported for more patients in the busulfan plus vitamin A arm (13/56) than in the busulfan only arm (3/67); one-tailed $P=0.002$). However, both regimens were well tolerated.

Discussion

This study suggests that a modest dose of vitamin A may favorably alter the natural history of alkylator-treated CML in chronic phase. This observation is strengthened by the almost identical time to clinical progression seen in the busulfan alone arms in the current trial (38 months) and that of Hehlmann *et al.* (37 months) [41]. In patients treated with vitamin A there was a prolongation of 8 months in the median duration of clinical progression-free survival, which translated into an increase in median survival of 7 months. After 5 years (Fig. 2), 48% of patients in the busulfan plus vitamin A arm were alive, while only 30% were alive in the busulfan alone arm. In the early 1980s the first

prognostic factor studies in CML were published and the results have been used for stratification purposes in subsequent trials [43]. A detailed analysis of the results by stage showed an overall advantage in clinical progression-free survival and survival that was most pronounced in the lower stages (data not shown).

The toxicities of the two regimens were comparable and busulfan plus vitamin A was well tolerated. Although these effects are modest, they contribute to the growing body of literature that the natural history of CML can be altered, whether with interferon or bone marrow transplantation [13, 14]. Given the relatively large, though nonsignificant, difference between treatment arms, the limited statistical power of the study, and the likelihood that oral vitamin A may not be the most effective means of delivering retinoid therapy, we conclude that further investigation of retinoids in chronic phase CML is warranted. What might be the biologic basis for such an effect?

In the 1970s, several studies documented that vitamin A could serve as an adjuvant in murine tumor models, including Cloudman S-91 melanoma and Lewis lung carcinoma [15, 16]. Several studies also demonstrated that various retinoids, including vitamin A, enhanced serum or cell-mediated immunity in tumor bearing mice [17, 18]. These observations were particularly relevant at the time of the design of this trial, as a pilot study in CML patients had suggested that patients vaccinated with BCG and leukemic cells had increased survival [19].

Since this trial began, we have learned a great deal about the basic mechanisms of action and clinical effects of retinoids in epithelial and non-epithelial systems, as well as their effect on cells of the hematopoietic and immune system [2, 20–28]. Different retinoids produce transient and modest effects on cells in myelodysplasia, most notably a decrease in transfusion requirement. However, at least one retinoid, 4-hydroxyphenylretinamide, may stimulate leukemic clones in this disease [23]. Retinoids also alter T-cell response *in vitro* [24] and a variety of vitamin A derivatives have been shown to produce substantial clinical responses in the cutaneous helper T-cell lymphoma, mycosis fungoides [25]. Arlin *et al.* concluded that the addition of 13-*cis*-retinoic acid to chemotherapy did not increase the incidence and duration of remission in chronic phase CML but the trial involved only 17 patients, was not randomized, and was asking a different question than was addressed in the current trial [26]. Interestingly, patients with juvenile CML appear to respond with durable clinical and laboratory responses to oral 13-*cis*-retinoic acid [29].

The recent description of dramatic, although short-lived, responses of patients with acute promyelocytic leukemia to oral *trans*-retinoic acid (RA) provides further documentation of the potent effect of retinoids

on hematopoietic cells [27, 28]. These observations led to studies of the underlying mechanisms. The effect of RA on APL may well be mediated through a fusion protein that is produced during the translocation of genetic material from chromosome 15 to chromosome 17 and involves the retinoic acid receptor- α gene [30, 31]. It is unlikely that a similar phenomenon accounts for the effect of vitamin A in CML, as the disease predominantly involves chromosomes 9 and 22, and no molecules related to retinoids have been identified on these chromosomes. However, at the time of blastic transformation, clones of cells with abnormalities in chromosomal 17q and 3p changes may appear [32–34]. This is noteworthy in that RAR- α is located at the 17q21.1 locus while RAR- β and retinol binding protein I and II are located at 3p24, 3q21 and 3p11, respectively [35]. Exploration for gene rearrangement of these retinoid-related genes in CML, particularly during the accelerated/early blast phase, may prove informative.

At what stage of CML might vitamin A be working? Three major possibilities exist. In one model the duration of both the chronic and blast phases are increased by vitamin A. A second possibility is that only the cells in the blast phase are affected. A third possibility is that the vitamin A selectively increases the time in chronic phase or delays transition to blast crisis. Based on the analyses of the trial results, it seems very likely that the effect we are describing corresponds more closely to the third model rather than to either of the other two; i.e. that adding vitamin A to busulfan is associated with longer durations of clinical progression-free survival. This is the question directly addressed in the analyses. The second alternative model implies no effect on clinical progression-free survival, but prolonged survival after progression with vitamin A. This possibility seems to be excluded since the period after entry into blast crisis was not lengthened. There is, of course, the possibility that both clinical progression-free survival and survival after progression are prolonged by the addition of vitamin A. This also seems unlikely, since the improvement in clinical progression-free survival with vitamin A was 8 months (measured as the difference in medians), while the improvement in overall survival was 7 months. However, conclusions regarding the stage at which vitamin A may be working can be made more formal using proportional hazards regression models in which the effect of treatment on overall survival is allowed to differ according to whether a patient has or has not suffered clinical progression. Following this strategy we found no significant treatment effect on survival following progression (using multivariate models; one-tailed $P=0.53$ in the primary analysis, $P=0.46$ in the secondary analysis).

Can pharmacological observations provide us with an

insight into the possible effect of vitamin A in CML? Since busulfan is metabolized by the liver and high doses of vitamin A can effect hepatic metabolism, perhaps the increased response was secondary to a higher effective plasma level of busulfan. We have no direct data to address this question but the absence of change in liver-related chemistries in the trial and the comparable hematopoietic effects in the two arms argues against this explanation.

Can biologic studies provide us with an insight into the effect of vitamin A in CML? Retinoids inhibit colony-forming units of CML cells in chronic phase as well as accelerated phase and blast crisis [36–40]. The recent data generated by Gallagher *et al.* may offer, in part, an explanation for the effect seen in the current trial [37]. He has measured the effect of RA on clonogenic cells (CFU-GM) obtained from patients in chronic and accelerated/blast phases. Accelerated/blast phase cells were 1000-fold more sensitive to RA (ID_{50} 10^{-9} vs. 10^{-6} M) than cells obtained from the chronic phase. These data suggest that retinoids may act at the critical transition point from chronic to blast phase. Designing appropriate experiments *in vitro* to understand this phenomenon will clearly be very important.

What is the next step? A phase II trial of RA in accelerated phase/blast crisis is underway [37]. Hydroxyurea has been shown to be more effective than busulfan in controlling the chronic phase of CML and with considerably less toxicity [41]. Recently, therapy with IFN- α has been shown to induce hematologic remission with a percentage of patients demonstrating cytogenetic remissions, some of which show complete suppression of malignant Ph+ clones [13, 42]. Results of a recent randomized trial do suggest, however, that interferon alpha-2a is significantly better than chemotherapy alone and that time to progression to blast crisis and overall survival can be markedly lengthened [13]. Combining hydroxyurea or IFN- α with vitamin A or retinoic acid would seem a logical next step, and studying the effects of these agents in combinations *in vitro* should provide useful information. Evidence already exists *in vitro* that RA and interferon are synergistic in their action against acute and chronic myeloid leukemic cells [37, 39].

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