Hematopoietic Growth Factors as Adjuncts to the Treatment of Acute Myeloid Leukemia

By Charles A. Schiffer

A number of randomized trials have recently been completed evaluating the effect of hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor or granulocyte colony-stimulating factor) as adjuncts to the treatment of patients with acute myeloid leukemia. Most studies used the growth factors to decrease the duration of neutropenia with the hope of reducing infectious morbidity and mortality. The results of these trials are generally quite consistent. Virtually all trials showed a modest reduction in the duration of severe neutropenia with a variable effect on the incidence of severe infections, antibiotic usage, and the duration of hospitalization. There was no consistent benefit in terms of improvements in complete response rate, complete response duration, or overall survival. However, it is important that there does not appear to be an increase in the incidence of drug-resistant leukemia in trials in which the growth factor was begun after completion of the chemotherapy. Other trials administered growth factors either before or simultaneously with the chemotherapy in an attempt to enhance chemosensitivity and decrease drug resistance.

None of these trials, whether conducted as part of initial induction therapy or in relapse, showed improvements in response rate or survival. Lastly, some anecdotal reports have suggested that occasional patients who receive growth factors as the only therapy for overt leukemia can achieve remission, possibly through a differentiating effect of the growth factor. However, there are very few such reports, and growth factor use in this situation is potentially dangerous and should be performed only in the context of a clinical trial. In summary, there appears to be no role at this time for priming of leukemia cells by growth factors to enhance the effect of chemotherapy, and more in vitro studies should be performed before further clinical trials of this approach. It is clear that growth factors administered after induction and possibly consolidation chemotherapy can shorten the duration of neutropenia, without a significant effect on treatment outcome. It is as yet unclear whether the use of growth factors in this fashion is cost effective.

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Japan conducted in a heterogenous group of patients with three following general clinical situations.

The myeloid CSFs (granulocyte-CSF [G-CSF] and granulocyte-macrophage-CSF [GM-CSF]) have been used in the three following general clinical situations.

ATTENUATION OF NEUTROPENIA

Most of the completed clinical trials have used G-CSF or GM-CSF to shorten the duration of neutropenia after either induction or consolidation chemotherapy in an attempt to decrease the incidence and severity of neutropenia-associated infections. Bacterial and fungal infections represent the major source of morbidity and mortality, particularly in older patients with AML. Recent studies show approximately a 50% to 60% complete remission (CR) rate in patients greater than 60 years of age, with the causes of treatment failure divided equally between patient survival with drug-resistant leukemia and death during marrow aplasia, almost always due to infectious causes. In contrast, in younger patients, although infectious morbidity is appreciable, treatment failure is much more frequently due to drug resistance to chemotherapy.

Consequently, most clinical trials focused on patients greater than 60 years of age beginning G-CSF or GM-CSF shortly after completion of chemotherapy in a manner analogous to the licensing trials initially performed in patients with solid tumors. By beginning the growth factor after the completion of chemotherapy, it was hypothesized that the leukemic burden was sufficiently reduced so as to diminish potential problems with stimulation of leukemia cell growth. The primary goal of these trials was to decrease the mortality rate due to infection with the hope that some patients who would otherwise have succumbed during aplasia would survive long enough to have marrow regeneration and CR.

Similarly, it was hoped that the use of growth factors after intensive consolidation therapy would allow such therapy to be administered more safely, perhaps permitting either higher doses or more repeated courses. Higher dose postremission therapy is now used very commonly, with a recent clinical trial from the Cancer and Leukemia Group B (CALGB) showing a significant increase in disease-free survival for younger patients receiving multiple courses of a high-dose cytarabine based regimen compared with lower dose regimens. It has also been the hope, albeit with little supporting data, that the CSF-stimulated granulocytes might have improved functional activity.

SENSITIZATION OF LEUKEMIC CELLS TO CYTOTOXIC THERAPY

There is a considerable body of data using both cell lines and clinical samples showing that there are receptors for CSFs on leukemic cells and that G-CSF and GM-CSF can stimulate the proliferation of leukemic cells in vitro. Importantly, a number of investigators have also shown that coculturing the growth factors with relatively S-phase-specific agents, such as cytarabine, can increase cytotoxicity to leukemic cells and that normal hematopoietic precursors can be relatively spared from this enhanced cytotoxic activity. These in vitro studies vary considerably in methodology, the criteria used to assess cytotoxicity, and whether evaluations were made on the bulk population or on clonogenic subpopulations. In addition, differing doses of growth factors and chemotherapy were used. Some studies suggested schedule dependence for the growth factors, whereas others showed further benefit from combinations of cytokines. These issues have recently been reviewed and will not be considered in detail here.

However, it should be noted that the doses and schedules subsequently used in clinical trials were generally empirically derived. Nonetheless, early clinical trials administering growth factors before and during chemotherapy to patients with active leukemia showed increases in the number of leukemia cells in S phase, thereby providing further impetus for more expanded clinical trials of this so-called priming approach.

INDUCTION OF TERMINAL DIFFERENTIATION

In addition to stimulating proliferation in vitro, both G-CSF and GM-CSF can promote differentiation of leukemia cells to more mature myeloid elements. The therapeutic expectation here is that leukemia cells will undergo apoptosis or differentiate into a postmitotic pool incapable of self-renewal. This has been the least studied of the three approaches and only anecdotal reports are available.

POTENTIAL SIDE EFFECTS AND CONCERNS

The most obvious concern about the use of CSFs in AML was the possibility of stimulation of leukemia growth that could not be controlled by chemotherapy, particularly if there were residual leukemia cells after the completion of chemotherapy. Certainly, the in vitro evidence that provided the basis for clinical trials of priming leukemia cells to enhance cytotoxicity served to amplify these concerns. It was therefore critical in randomized clinical trials of induction chemotherapy to monitor for the incidence of obvious and rapid leukemia regrowth, differences in rates of treatment failure due to persistence of drug-resistant leukemia cells, and duration of complete response. The latter depends on relative standardization of postremission approaches.

Other potential concerns were somewhat less obvious. The mechanisms by which normal hematopoiesis is suppressed in leukemia and by which residual normal hematopoietic...
cells remain relatively impervious to the effects of the high doses of chemotherapy capable of reducing or eliminating the leukemia clone are poorly understood. One protective mechanism that is frequently invoked is that the normal progenitors are cytokinetically quiescent. Thus, there is the potential in trials designed to attenuate neutropenia and, particularly in priming trials, of enhanced cytotoxicity against normal cells with more prolonged durations of marrow aplasia. It was therefore important in clinical trials to carefully monitor the kinetics of neutrophil and platelet recovery.

In addition to their appreciable costs, some of the cytokines are associated with a variety of side effects including skin rash, fevers, gastrointestinal symptoms, and, less frequently, pulmonary symptomatology. Such side effects are significant in any patient population, but in patients with leukemia who are severely neutropenic, they can necessitate changes in antibiotic therapy, or sometimes the implementation of therapy with amphotericin B because of the development of fever in patients already covered by antibacterial antibiotics. Amphotericin B itself actually may be somewhat marrow suppressive, thereby prolonging the duration of neutropenia. In addition, renal toxicity from amphotericin could influence the ability to safely give postremission chemotherapy to some patients. Thus, the incidence and types of ancillary therapy required by patients in clinical trials of CSFs should be monitored carefully.

RESULTS OF CLINICAL TRIALS

There have been a large number of publications describing the use of myeloid growth factors in patients with AML, including more than 20 published in the 1995 Proceedings of the Annual Meeting of the American Society of Hematology. Until recently, most have represented relatively small series of patients, sometimes with attempts made to compare results with historical controls. Because of subjectivity in decisions regarding initiation and cessation of antibiotics and discharge from hospital and potential difficulties in rigorous classification of the severity of infections and side effects of the cytokines, it was critical that the clinical benefit be evaluated in placebo-controlled, preferably double-blinded clinical trials. A number of these trials have now been fully published or are available in reasonably complete abstract form and will represent the focus of this review. There are substantial differences in the design of these trials as well as in the dosage and schedule of growth factors so that it is sometimes difficult to precisely compare results between trials. In addition, one cannot directly compare the reported durations of neutropenia because trials began counting at different times, with some beginning at the initiation of therapy, others at the time that the neutrophil count fell below a specified level, and yet others at the time of completion of chemotherapy. In retrospect, it might have been relevant to standardize these criteria beforehand. In addition, white blood cell (WBC) counts and differentials are sometimes not available on a daily basis on all patients, necessitating the use of different formulae to extrapolate results surrounding missing data points. However, it is likely that these methodologic issues wash out in the larger trials and affect both arms equally.

Attenuation of neutropenia. The results of randomized trials using GM-CSF and G-CSF administered after (and sometimes during) initial induction therapy for AML are summarized in Tables 1 and 2, respectively. Escherichia coli- and yeast-derived (glycosolated) preparations of GM-CSF were used in different trials. In most trials, the growth factor or placebo were begun the day after completion of therapy, although the Eastern Cooperative Oncology Group (ECOG) and Southwest Oncology Group (SWOG) trials did not begin growth factor until a bone marrow biopsy performed on day 10 (2 to 3 days after the completion of chemotherapy) was shown to be hypocellular or aplastic. It is not known how many patients in these two trials did not receive the growth factor because of persistence of leukemia. However, because the overall results of the studies in Table 1 are reasonably reproducible, it is unlikely that routine monitoring of bone marrow samples before starting growth factors in patients rendered leukopenic by induction therapy is mandatory. Some studies randomized patients at the start of chemotherapy, whereas others performed the randomization when the growth factors were scheduled to begin. However, it is unlikely that this small methodologic variable would have substantially influenced the comparisons among the trials. Most studies evaluated patients greater than 55 to 60 years of age for the reasons enumerated above.

However, there were potentially important differences among the trials in the approach to patients who required a second course of induction therapy. In most published trials, approximately 25% to 40% of older patients who enter remission receive two courses of chemotherapy during induction. Although details in some trials are sparse, some groups continued the study drug while the second course was being administered, whereas others stopped administering the study drug and restarted it after the completion of the second course. The latter approach perhaps naively assumed no hangover effect in terms of priming from the previously administered growth factor. Of interest is the observation made in the CALGB study with regard to second courses of chemotherapy. Because there was little information about the morphology of early regenerative bone marrow samples in patients receiving growth factors, there was concern that one might see populations of immature cells in patients having growth factor-stimulated normal marrow regeneration and that an inappropriate course of chemotherapy could be administered based on the appearance of such marrows. Therefore, a second course of chemotherapy could not be administered until day 22 after induction, as compared with the more traditional model of administering a second course beginning on day 14. Of interest is that greater than 90% of the patients who achieved remission on this protocol did so with a single course of chemotherapy, suggesting perhaps that, in the past, many patients may have received second courses of treatment for what in retrospect represented marrow regeneration rather than persistent leukemia. Nonetheless, no firm guidelines can be provided at this time about whether to discontinue growth factors should patients require a second course of induction therapy.

The results of these studies are generally quite similar. All show a modest reduction in the median duration of neutropenia that is generally statistically significant because of
the large numbers of patients in the trials. In some studies, this is associated with a reduction in the duration of hospitalization and, therefore, presumably antibiotic use. Most studies do not show any difference in the incidence of severe infections or deaths during induction; hence, there appears to be no important effect on CR rate, CR duration, or overall survival. The one exception to the latter statement is the relatively small trial reported by the ECOG in which there was a survival advantage albeit with relatively short follow-up, due to what appeared to be a reduction in early mortality in the patients receiving GM-CSF ($P = .048$). The reasons for this are not entirely clear, because there was no significant difference in what was termed therapy-related mortality during induction. Some of the differences in the ECOG survival curves also seemed to occur after consolidation therapy was administered and there was no apparent effect of the GM-CSF on neutrophil recovery in patients receiving consolidation. Lastly, approximately 15% of patients randomized to receive either growth factor or placebo did not receive their treatment due to either early death or other unspecified clinical events. When reviewed as an isolated clinical trial, the study served as the basis for approval in the United States of the use of GM-CSF in patients with AML. The results from other larger studies are less promising with regard to endpoints other than shortening of the duration of neutropenia, and the ECOG study should certainly not be regarded as definitive.

Sensitization of leukemic cells to cytotoxic therapy. Fewer randomized trials have been performed evaluating the priming approach. The EORTC-HOVON Groups recently

| Table 1. Randomized Trials Using GM-CSF During and/or After Initial Induction Therapy for Patients With AML |
|-------------------------------------------------|-----------------|------------------|-----------------|------------------|-----------------|-----------------|
| Source                                          | Age (yr)        | Days After Start of | Arms             | Median Days      | Induction       | CR (%)          |
|                                                 | (median)        | Chemotherapy       |                  | <500/μL PMN      | Deaths (%)      |                 |
| CALGB[8] (386 patients)                         | ≥60 (69)        | 8                 | GM (E coli)      | 15*             | 27             | 52              |
|                                                 |                 |                   | Placebo          | 17              | 23             | 54              |
| ECOG[31] (118 patients)                         | 55-70 (64)      | 11 (if marrow aplastic) | GM (yeast)      | 11*             | 13             | 61              |
|                                                 |                 |                   | Placebo          | 14              | 21             | 46              |
| GOELAM[32] (244 patients)                       | 55-75 (67)      | 1                 | GM (E coli)      | 22*             | 18             | 62              |
|                                                 |                 |                   | Placebo          | 27              | 15             | 61              |
| EORTC-GIMEMA[33] (102 patients)                 | 15-60           | (not supplied)    | GM               | ND              | 6              | 47              |
|                                                 |                 |                   | Placebo          | 8               | 8              | 87              |
| Buchner[34] (63 patients)                        | 16-75 (51)      | 1                 | GM               | Reduced by 2 days | —              | 77              |
|                                                 |                 |                   | Control          | —               | 76              |
| HOVON/SAKK[35] (253 patients)                   | <60 (42)        | 8-9               | GM (E coli)      | Reduced by 2 days | —              | 84              |
|                                                 |                 |                   | GM               | Reduced by 2 days | —              | 75              |
| EORTC-HOVON[36] (326 patients)                  | >60             | —1→8 (during)     | GM (E coli)      | Reduced by 3-4 d* | 17             | 42              |
|                                                 |                 | —1→PMN (during + after) | GM              |                 | 14             | 49              |
|                                                 |                 |                   | Placebo          |                 | (infectious death) |
| **Abbreviation:** ND, not determined.           |                 |                   | Control          |                 | 56              | 57              |
| * P < .05.                                       |                 |                   | Reduced**        |                 |                 |                 |

| Table 2. Randomized Trials Using G-CSF After Initial Induction Therapy for Patients With AML |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Source                                          | Age (yr) (median) | Days After Start of Chemotherapy | Arms | Median Days <500/μL PMN | Induction Deaths (%) | CR (%) |
| Amgen[37] multi-institutional (521 patients)     | 16-89 (54)       | 8                | G | 20* | —             | 69              |
|                                                 |                 |                   | Placebo | 25 | —             | 68              |
| (521 patients)                                  |                 |                   |                               | (<1,000/μL) |
| SWOG[38] (243 patients, only 193 "eligible," 23% had secondary AML) | ≥55 (66) | 11 (if marrow hypocellular) | G | Reduced by 3-4 d* | 17             | 42              |
|                                                 |                 |                   | Placebo | 14 | (infectious death) |
| (173 patients)                                  | ≥65 (71)        | 8                | G | 21* | —             | 70*             |
|                                                 |                 |                   | Placebo | 27 | —             | 47              |

* P < .05.
described results in 326 newly diagnosed elderly patients who received GM-CSF (5 μg/kg) by continuous infusion both during (beginning on the day of chemotherapy) and after induction therapy with cytosine arabinoside and daunorubicin. Although the rate of neutrophil recovery was more rapid, there was no difference in CR rate (57% v 56%), transfusion requirements, incidence of infection, days of antibiotics, and overall or disease-free survival. The number of febrile days and the incidence of fluid retention were greater in the patients receiving GM-CSF.

Other studies conducted during initial induction also failed to show improved CR rates, including two studies that also included younger patients. Although there are some differences in the duration of growth factor use, the interval between starting growth factor and chemotherapy, and the doses of GM-CSF, the conclusions from these studies are remarkably consistent. Heil et al randomized 80 patients (median age, 50 years) to GM-CSF or placebo beginning 48 hours before the second course of a so-called double induction regimen and continuing until neutrophil recovery. This is not precisely analogous to the previous studies because patients have already been substantially cytoreduced by one course of induction therapy. Nonetheless, there was no difference in response rates or other important clinical endpoints and, in this circumstance, the duration of neutropenia was not shortened and there was some prolongation of the duration of thrombocytopenia. Lastly, the CALGB has just completed a randomized trial using E coli-derived GM-CSF or placebo begun 5 days before the initiation of a high-dose Ara-C regimen and continued 24 hours after completion of chemotherapy in patients with either refractory AML or in first relapse. The CR rate was 33% in the patients primed with GM-CSF and 38% in the control group.

There is only one small randomized study using G-CSF as priming. Ohno et al randomized 58 patients with relapsed or refractory AML to G-CSF or placebo beginning 2 days before reinduction therapy. There was no statistically significant difference in CR rates, event-free survival, or disease-free survival, although granulocyte recovery was faster in the patients receiving G-CSF. Estey et al. from the MD Anderson Cancer Center, treated 112 patients with either newly diagnosed AML or myelodysplasia with the so-called FLAG regimen (fludarabine, high-dose Ara-C, and G-CSF), comparing these results with a previous group of patients treated with the same chemotherapy without the G-CSF. There was a reduction in the duration of neutropenia in the patients receiving G-CSF, but no difference in CR rate or survival, in this somewhat heterogeneous group of patients. Of note, in 1992, the same group of investigators reported potentially adverse effects of concurrent administration of GM-CSF and chemotherapy compared with a historical control group with similar risk characteristics treated with a variety of regimens. After statistical adjustment for what was felt to be prognostically significant variables, the patients treated with GM-CSF had a shorter overall survival and, with what appears to have been appropriate prescience, the investigators suggested "caution in the use of GM-CSF to sensitize myeloid leukemia cells to daunorubicin and Ara-C chemotherapy." Given the negative results from the clinical trials reported to date, it would appear that this warning was appropriate and that growth factors should only be used for priming purposes in the context of well-designed clinical trials with in vitro correlates.

Induction of terminal differentiation. There are no systematic clinical trials evaluating this approach, although some provocative case studies have been published. Giralt et al administered G-CSF to seven patients with different types of myeloid malignancies who had relapsed after allogeneic bone marrow transplantation. Three patients achieved a complete hematologic and cytogenetic remission with restoration of donor hematopoiesis, whereas the other four patients had increases in their peripheral blast counts. The mechanism for these responses is not defined. There was no evidence of increased graft-versus-host disease (GVHD), although it is possible that the G-CSF induced an independent enhancement of graft-versus-leukemia effect, particularly because GVHD prophylaxis had been discontinued in some patients. Fluorescence in situ hybridization studies did not show progressive differentiation of the leukemic clone but certainly could have been missed in this small number of patients.

Improvement in a patient with morphologically undifferentiated leukemia with minimal positivity for myeloid markers was seen after concurrent administration of G-CSF and high-dose methylprednisolone. The contribution of the steroid is difficult to determine. Another patient with minimally differentiated AML (French-American-British [FAB] M0) received GM-CSF therapy alone at the time of presentation of leukemia with extensive pneumonia. Her physicians were concerned about initiating cytotoxic chemotherapy because of the severe infection and attempted to transiently stimulate neutrophil production. Remarkably, a complete remission was obtained with only 7 days of GM-CSF therapy. A relapse occurred 9 months later and GM-CSF therapy produced a partial remission. No correlative in vitro studies were performed. Lastly, Dombret et al treated three newly diagnosed patients with AML and t(8;21) with G-CSF therapy alone. Two patients had moderate reductions in the percentage of marrow blasts, although none achieved a complete response. One patient was said to have improvement in erythropoiesis and thrombopoiesis, but further details are not given.

These case studies are provocative but do not provide real clues as to the mechanisms or frequency by which improvement in overt leukemia might occur in some patients with AML. It is also likely that there may be a publication bias in terms of a tendency to report more favorable outcomes. Although there is some controversy, it would appear that, in at least some patients, CR is either a consequence of or accompanied by attenuation of the block in differentiation of the leukemic clone. Further, careful evaluation of this approach would therefore be of interest. Patients must be observed carefully for evidence of rapid leukemic growth, and in vitro studies attempting to serially monitor leukemic clones with a definable marker should be performed whenever possible.

Discussion

Essentially all of the trials summarized in Tables 1 and 2 detected a shortening of neutropenia in the group of patients
receiving G-CSF or GM-CSF after induction therapy. However, in only one of the smaller studies did this translate into a decrease in the number of serious infections, and in only one study using G-CSF was there a statistically significant difference in CR rate. However, surprisingly, despite an apparent substantial difference in CR rates (70% v 47%), this did not translate into prolongation of event-free or overall survival; also, there was not a difference in the death rates during induction therapy, which would have been the expected benefit of the G-CSF. These discrepancies are difficult to explain and might suggest either a differentiating effect of the G-CSF (although not apparent in other trials) or concealment of residual blasts by the increased number of neutrophils produced by G-CSF stimulation in some patients who might otherwise have been in only partial response.

Indeed, most of the studies reported essentially identical CR rates. In retrospect, this is perhaps not particularly surprising. Because of the prolonged neutropenia, virtually all patients undergoing induction therapy for AML require antibiotic therapy for either presumed or documented infections. In many, if not most patients, antibiotics are initiated either at the time of presentation or within the first week to 10 days of treatment. Because growth factors are not begun until 7 to 10 days after chemotherapy is started, they could obviously have no effect on the frequency of early antibiotic use. Although practices vary, most physicians continue antibiotic therapy for the duration of neutropenia, again making it difficult to detect an effect on total antibiotic usage. A significant fraction of patients also receive amphotericin B, either empirically because of fevers of unknown origin while receiving antibacterial antibiotics or because of documented fungal infections. Although it is theoretically possible that a shortening of neutropenia by even a few days could decrease both the need for and the duration of amphotericin B therapy, these data are not available in either the published reports or those available in abstract form. Given these constraints, the therapeutic benefit of growth factors would be clinically apparent only as a reduction in the frequency and severity of complications occurring during the last few days of neutropenia. If this results in a shortening of antibiotic and amphotericin use and decreases in complications such as nephrotoxicity, this could be a highly clinically relevant benefit. Again, it is difficult to assess this directly from the available data. One might infer such a benefit by comparing the fraction of patients felt by their physicians to be medically fit to go on and receive subsequent postremission consolidation. This was not apparent in either the ECOG or CALGB trials, however; in both of these studies, an equal number of complete responders in each arm went on to receive postremission therapy. The accelerated recovery to either greater than 500 or greater than 1,000 neutrophils/μL translated into a modest reduction in the duration of hospitalization in some studies. Comparisons among the studies are difficult because criteria for discontinuation of antibiotics and hospital discharge are usually not provided and are left to the discretion of the treating physician. However, presumably, the blinded nature of most (but not all) of the studies should correct for any bias in the management of patients receiving either placebo or growth factor. Indeed, the necessity of placebo control was shown in the large trial CALGB trial in which approximately one third of the patients were removed from treatment in each arm because of the perception by the physicians that a variety of symptoms that in retrospect may have been due to the AML and the side effects of treatment were attributable to the GM-CSF. The results in this trial were not different when the analyses were performed for the entire group (intention to treat) or when the subgroup of patients who actually completed the GM-CSF were analyzed. Other studies using nonglycosylated (E coli) GM-CSF have also noted some adverse consequences attributed to the growth factor, including a significant delay in the interval before beginning the second course of chemotherapy, more patients removed from study due to side effects than in the placebo arm, and increased duration of fever and more pronounced fluid retention. Side effects may be lower using G-CSF, and the single study using glycosylated (yeast-derived) GM-CSF did not report an increase in side effects in the growth factor recipients. More details are necessary from reports published in complete form to make more definitive assessments.

Although the potential economic impact of shortening of hospitalization is obvious (depending on whether this savings is overcome by the cost of the 10 to 20 days of growth factor), it is not clear whether the decision to discharge patients was made simply on the achievement of a particular threshold neutrophil count or whether the patients in the growth factor arm were actually clinically improved and in better shape than those in the control arm. As with other studies of myeloid growth factors in patients receiving intensive or ablative therapy, the count nadirs were not eliminated but were rather shortened, with the rate of recovery accelerated. Most clinicians are comfortable with discontinuing antibiotics at granulocyte counts of greater than 500/μL, but it is probable, albeit unproven, that this could also be performed safely at lower neutrophil counts as soon as there is some evidence of neutrophil recovery. It is clear that, at low WBC levels, the circulating neutrophil count is an underestimate of the available neutrophil pool. It is quite common, for example, to identify granulocytes at areas of infection in patients beginning to recover neutrophil counts even with neutrophil counts of less than 100/μL. These levels can now be detected with automated cell counters. Studies of indium-labeled granulocytes transfused to neutropenic patients have shown immediate migration of transfused cells to infected sites, suggesting that newly produced neutrophils also do not wait around in the blood to be counted but, rather, obey their suicidal, chemotactic instincts and perform their job description. Lastly, it has been shown that neutrophils can be detected in abundance even in severely neutropenic patients by assays quantifying the number of cells migrating across the buccal mucosa. Thus, it is quite possible that many patients in the control arm of CSF studies could have been discharged from the hospital earlier and with equivalent safety to those discharged with somewhat higher neutrophil counts, thereby decreasing the magnitude of this possible benefit of CSF use.

The available clinical trials did not show an obvious ad-
verse impact of the use of growth factors. With the exception of a small study by the EORTC/GIMEMA groups in which only about 25 patients were included in each of the arms, CR rates were equivalent or sometimes slightly higher in patients receiving growth factors, and long-term outcome appeared similar. This does not mean that no patient was hurt by the use of growth factors in terms of leukemia stimulation. Large randomized trials only provide information as to the boundaries of expected benefit in the overall population of patients and, particularly when the differences between the groups are small, do not afford predictions about behavior of individual patients. Also, unless each case is exquisitely examined by the principle investigator, they do not necessarily detect subgroups or individuals who may have had more subtle benefit from the therapeutic maneuver being evaluated. Nonetheless, it is quite comforting that all but one of the randomized trials in which the growth factors were administered after the completion of chemotherapy showed no apparent detrimental effect on leukemia response.

This is of particular importance when considering the safety of growth factors in patients receiving intensive consolidation therapy. Such patients in remission already have a lower leukemia burden and would presumably be even less likely to suffer an adverse effect due to stimulation of the residual clone, particularly because the growth factors are administered after the completion of a course of consolidation. These patients also always start with normal neutrophil counts and generally are uninfected and not receiving antibiotics at the initiation of chemotherapy. One might therefore predict that shortening of neutropenia would be more apparent and of greater benefit for such individuals. Not all of the clinical trials extended the randomization to patients in remission. Surprisingly, the ECOG study, which was the most positive during induction, failed to show any shortening of neutropenia by GM-CSF in the smaller group (49 patients) who continued consolidation therapy (median duration of absolute neutrophil count [ANC] <500/µL was 13.5 days in patients receiving GM-CSF and 14 days in placebo recipients). GM-CSF was not begun until 4 to 5 days after the completion of high-dose ARA-C in this study. In contrast, the large Amgen-sponsored G-CSF trial that began the growth factor the day after completion of postremission chemotherapy did show shortening of neutropenia, although further details are not given in the abstract (7- and 5.5-day reduction in ANC <500/µL in patients receiving standard-dose and high-dose cytarabine consolidation, respectively). Postremission data are not available from the SWOG study using G-CSF.

Lastly, in a nonblinded study comparing sequential cohorts of younger patients with AML receiving very intensive consolidation with daziquone and mitoxantrone, the CALGB noted an 11- to 12-day decrease in the duration of ANC <500/µL in patients receiving 5 µg/kg/d of G-CSF begun the day after chemotherapy was completed, compared with patients treated without the growth factor. A somewhat unique observation, there was a nearly statistically significant shortening of the duration of severe thrombocytopenia as well.

Rigorous information is not available on the effects of growth factors during repeated courses of postremission consolidation. However, it is of note that Büchner et al actually reported longer durations of neutropenia and thrombocytopenia in a group of patients receiving repetitive courses of chemotherapy with growth factors, suggesting that there may be limits to how many times and how frequently the marrow may be intensively flogged and stimulated with growth factors. However, further studies are necessary in this important clinical situation before any conclusions can be made. Because many of these patients are not hospitalized, it is important that the growth factor use not be accompanied by fever or other symptoms that would require hospitalization.

FUTURE STUDIES

Timing of growth factor administration. If, in fact, the major potential clinical impact occurs in the last few days of induction, when there may be a benefit to having a rapid rise in neutrophil count, might it be possible to begin the growth factor 1 week to 10 days later than was performed in the recent clinical trials, thereby substantially reducing the costs of treatment? Although this suggestion has some intuitive appeal, it has not been borne out in the available clinical trials in non-AML patients who were randomized to late administration of growth factor or placebo at the time neutropenic fever developed. The original schedule of growth factor administration immediately after completion of chemotherapy was chosen to accelerate all stages of myeloid differentiation and it is not at all clear whether late administration alone would achieve even the modest shortening of neutropenia noted in Tables 1 and 2. Ohno et al described a group of leukemia patients with severe infections complications who received late G-CSF as a means of supportive care. Most patients survived their infections and there was no apparent increase in the relapse rate. Some patients had already begun to manifest WBC recovery, and it is impossible to assess the effect of the G-CSF in these patients. Although the ASCO guidelines mention the late use of growth factors in severely infected patients, rigorous support for this approach is lacking. It would probably be of importance to more carefully analyze the last week of the clinical courses of the patients entered on the more recent studies before committing resources for a large clinical trial investigating this particular approach.

Dose of growth factor. Most studies used approximately 5 µg/kg/d of growth factor administered either subcutaneously or by intravenous infusions of different durations. Some of the older studies used the latter route, because the subcutaneous pharmacokinetic/pharmacodynamic data were not available at the time the studies were initiated and because of concerns about subcutaneous injections in patients who were thrombocytopenic. Might a higher dose of growth factor been more effective? It is obviously impossible to answer this with any assurance, but there are suggestions that the 5 µg/kg dose might actually be greater than what is needed, at least in more routine clinical situations with less prolonged neutropenia.

Priming studies: Should further randomized trials be conducted using different doses and schedules of growth factor and/or chemotherapy? It is very likely that the degree of
stimulation and possible chemotherapeutic sensitization is variable from patient to patient and probably within subpopulations of cells within the same patient. As noted above, even large clinical trials are likely to miss important phenomena occurring in small groups of patients. There are few studies correlating in vitro characteristics of leukemia cells from individual patients with subsequent clinical response. There are virtually no observations correlating the response to growth factors of clonogenic leukemia cells from individual patients with clinical outcome. These are cumbersome, nonstandardized, and highly variable assays fraught with technical difficulties. Indeed, difficulties with reliably culturing and maintaining clonogenic cells from leukemia patients has presented the major problem in performing assays of drug sensitivity and serial evaluations of in vitro parameters in individual patients. However, it is likely that it is the effects on subpopulations of clonogenic cells, rather than what may be more easily measured in the entire cell population, that are most important in influencing long-term outcome.24,61,62

For example, a recent report indicated that a 72-hour infusion of G-CSF administered to newly diagnosed patients with AML produced increases in leukemia burden in 27 of 28 patients as assessed by serial evaluation of marrow or blood blasts or the percentage of cells in S phase.63 However, the mean change in S phase was only from 6% to 10%, and it is unknown whether this was a consequence of recruitment of cells from G0, or changes in the duration of the cell cycle or whether there are different effects (either positive or negative) on more resistant cells. These questions remain unanswered from other trials that performed in vitro assays showing similarly reproducible but apparently modest effects after cytokine priming.8,10,11,28 More detailed analyses of clinical trials evaluating priming should be available in the near future. Should these trials not provide discrete hypotheses, then it is unlikely that further empiric manipulations will be productive. Rather, careful in vitro studies are more likely to be illuminating than further large randomized trials. Given these issues, it will also be difficult to evaluate combinations of cytokines in clinical trials, as has been suggested by some in vitro studies.21,22,25,63

**Thrombopoietic factors.** With the exception of a nonrandomized study in patients receiving intensive consolidation,57 none of the studies showed a shortening of the duration of thrombocytopenia or a reduction in the number of platelet transfusions required. Even should a thrombopoietic agent be highly effective, a substantial fraction of transfusions are administered during the first 2+ weeks of induction therapy, at a time before thrombopoiesis can presumably be effectively stimulated. Although a number of cytokines such as interleukin-3 (IL-3), IL-6, and IL-11 have modest to moderate thrombopoietic activity, it appears that the "real thing" has recently been cloned in a number of laboratories.65,66 These thrombopoietic factors with a high degree of lineage specificity are entering clinical trial. Preliminary data in primates are quite encouraging in that platelet transfusion requirements can be eliminated in situations in which monkeys received near ablative treatment with radiation or chemotherapy.67 However, these models incorporated only a single day of treatment, as compared with the 7 to 10 days of treatment for AML induction, and the animals had normal marrow function at the initiation of treatment. Patients with AML represent probably the largest population who would stand to benefit from effective stimulation of platelet production and likely will figure prominently in the early stages of development of these compounds. Some of the hurdles noted in the trials of myeloid growth factors will also be relevant to these studies. In addition, it is unclear how the oncology community will respond to the accumulated data from the G-CSF and GM-CSF trials. Certainly, even before the availability of these data, G-CSF and GM-CSF were used extensively by many clinicians for patients with AML. Should this practice continue, then consideration will have to be given to measuring the combined effects of myeloid and thrombopoietic growth factors. Very preliminary evidence from the primate models suggests an absence of antagonism with regard to platelet production and perhaps some evidence of synergy in terms of granulocyte recovery.68 The next few years should provide some very exciting information about the effects of thrombopoietic agents in patients with acute leukemia.

**CONCLUSION**

So, what should physicians prescribe and patients receive? Overall, it must be concluded that the results using growth factors as adjuncts to therapy in patients with AML have been disappointing. Certainly there is no evidence from well-conducted clinical trials that pretreatment with growth factors to enhance sensitivity to chemotherapy has been effective, with at least some studies suggesting some deleterious effects. Similarly, the possibility of induction of differentiation as tantalizingly suggested by a few case reports is intriguing, but far from proven, and therefore suitable only for very unique experimental circumstances.

The decision with regard to using growth factors after induction therapy is less straightforward. The duration of neutropenia is modestly, but unequivocally shorter and this may translate, in some patients, into shortened hospitalization and decreased use of antibiotics. When considered in aggregate, with minor exceptions in both directions, there does not appear to be any significant effect on CR rate, CR duration, or overall survival. If one accepts that shorter hospitalization durations may be a consequence of more rapid neutrophil recovery in the absence of other more leukemia-specific benefits, the decision to use CSFs may, in fact, be governed by cost considerations. Enter the new discipline of pharmacoconomics and the less disciplined HMOs. Is a few days less hospitalization worth 20 days of growth factor? This is similar to the questions raised in patients with solid tumors that a variety of consensus groups have attempted to address. If the growth factors either cost nothing or were quite inexpensive, then it might be more reasonable to incorporate them into AML regimens, conceivably even in younger patients, in whom there is no supporting evidence from clinical trials. However, the growth factors are expensive and are not devoid of side effects. As with most drugs, new side effects will probably become more apparent as their usage in a given disease increases.
Unfortunately, the growth factors do not appear to be the answer that the many investigators who designed and participated in these many clinical trials hoped that they would be. Whether they will permit increased dose intensity in consolidation (and whether that, in turn, will be effective) remains to be seen. However, it would appear that any new major advances in AML therapy will come from elsewhere. New studies are involving modulation of the multidrug resistance phenotype as well as administration of immunomodulatory compounds such as IL-2. These will hopefully be succeeded by more directed molecular approaches as our understanding of the particular pathogenesis of leukemogenesis and drug resistance of different subtypes of AML become better understood.

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Hematopoietic growth factors as adjuncts to the treatment of acute myeloid leukemia

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