Review article

Granulocytic colony-stimulating factors in the management of patients with acute myeloid leukemia

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Abstract

In the early stages of the development of granulocytic colony-stimulating factors (G-CSF and GM-CSF) in oncology and hematology, myeloid malignancies were considered to be a contraindication to their use. In fact, myeloid leukemic cells bear specific receptors for G-CSF and GM-CSF and these CSFs induce an *in vitro* proliferation in primary blast cells of most patients with acute myeloid leukemia (AML). In addition, autocrine or paracrine loops of stimulation have been demonstrated in some cases. Despite these theoretical risks of blast proliferation, G-CSF and GM-CSF have been extensively tested in patients with AML or myelodysplastic syndromes. Major objectives were the correction of acquired or chemotherapy-induced neutropenia, but also the reinforcement of the antileukemic efficacy of cytotoxic agents. Recently, G-CSF has also been used to mobilize hematopoietic progenitors in the peripheral blood. Major results of several double-blind clinical trials are the demonstration of the safety of CSF administration in these patients, since no risk of *in vivo* blast cell regrowth has been observed, and their efficacy to shorten the duration of chemotherapy-induced neutropenia. However, no significant reduction in the treatment-related mortality and no survival improvement were afforded by the use of these CSFs. From another point of view, the search for AML-specific CSF-receptor or CSF-receptor associated molecule abnormalities represents a new promising area to try to understand the mechanisms of leukemogenesis.

In the early stages of the development of granulocytic colony-stimulating factors (CSFs) in hematology, myeloid malignancies were considered to be a contraindication to their use owing to the risk of inducing the proliferation of malignant myeloid cells.

Paradoxically, despite this theoretical risk, granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been extensively tested in patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML).

Two factors, directly related to the nature of these diseases and the toxicity of the drugs employed to treat the patients, may explain this rapid development. First, the period of neutropenia induced by intensive courses of induction and consolidation in AML patients may be as long as four to six weeks and responsible for a significant number of deaths from infection, especially in patients of advanced age. Secondly, the stimulation of malignant cell growth by these CSFs, which could be seen as a limiting factor, can also be considered as a characteristic facilitating combined therapies designed to improve the antileukemic efficacy of cytotoxic agents.

The aim of this review is to provide an updated summary of results obtained with G-CSF and GM-CSF in AML clinical trials.

AML cells and granulocytic colony-stimulating factors: pre-clinical studies

CSF-induced cell proliferation

AML cells express high affinity surface receptors for G-CSF and GM-CSF. The quantity and affinity of these receptors on the surface of primary leukemic cells from most AML patients are similar to those of receptors found on normal myeloid progenitors [4, 17, 18, 23, 45, 60, 61, 65, 68, 70].
The responsiveness of AML cells from different patients to CSFs has been reported extensively, either with respect to a single type of CSF or with different combinations [27].
Proliferative responsiveness was evaluated using ^{3}H-TdR incorporation in a liquid suspension culture [69], and by blast cell colony formation after short-term and long-term cultures [14, 43, 59, 69, 71, 86]. In summary, these reports produced the following observations:
- The spontaneous *in vitro* proliferation of AML blasts and their growth response to each CSF exhibit high case-to-case variations.
- The number and affinity of receptors for granulocytic CSFs on the AML cell surface is not *per se* predictive of proliferation in the presence of exogenous CSFs.
- AML cell response to GM-CSF appears to be more frequent than a response to G-CSF [59, 69, 86]. *In vitro* exposure of these cells to GM-CSF gives rise to proliferation in about 75% of the cases.
- There is no clear relationship between *in vitro* proliferative responsiveness and AML subtypes in the French-American-British classification or AML cell surface phenotypes.
In addition, primary AML cells express and secrete active CSFs and cytokines including G-CSF, GM-CSF, macrophage colony-stimulating factor (M-CSF), interleukin-1b (IL-1beta), interleukin-6 (IL-6), and tumor necrosis factor (TNF-alpha) [55]. This expression is generally increased after the *in vitro* treatment of AML cells with IL-1beta or TNF-alpha [52].
However, whether an autocrine loop of stimulation has a role in the development of leukemia remains an open question. The presence and level of autocrine stimulation is variable in AML patients.
Four groups have been identified by the Nottingham group, based upon their proliferative *in vitro* characteristics [14, 71]: 1) cells which fail to proliferate spontaneously or in response to CSFs; 2) cells which proliferate only in the presence of exogenous CSFs; 3 and 4) cells which show an autonomous proliferation, that can be enhanced by exogenous CSFs in Group 3 cells, but not in Group 4 cells which proliferate in a CSF-independent manner. The clinical relevance of this classification for disease prognosis and outcome is yet to be determined.
Finally, the presence and level of autonomous growth has been correlated with the ability of AML blasts to produce GM-CSF and IL-1beta. However, this IL-1beta dependent loop of stimulation may be regulated by paracrine production of IL-1beta by more differentiated cells.

CSF-induced cell differentiation and apoptosis

Several observations suggested that some AML patients may benefit from granulocytic CSF therapy because of an *in vivo* CSF-induced differentiation effect and/or through an *in vivo* CSF-induced apoptosis of the leukemic cells:
- In certain murine or human AML cell lines and clones, maturation towards terminally differentiated myeloid cells, without a capacity for self-renewal, can be induced by interleukin-3 (IL-3), GM-CSF, and G-CSF [5, 49, 50, 56].
- Longer survival was reported among SJL mice with myeloid leukemia treated by multiple courses of cyclophosphamide followed by G-CSF as compared to control mice treated without G-CSF [52].
- Prolonged survival associated with the induction of apoptosis in the leukemic cells was also observed after G-CSF therapy in C3H/He mice inoculated with AML cells [7, 8].
- It has also been reported that apoptosis may occur in growth factor-dependent AML clones maintained in culture by addition of exogenous growth factors, as well as in normal myeloid precursors, after the withdrawal of exogenous growth factors [51].
Unfortunately, the terminal maturation resulting in non-dividing differentiated cells may not be initiated in most human primary AML cells, even when CSFs are used in combination or with all-trans retinoic acid [52].
There have only been a few reports of complete or partial remissions induced by G-CSF or GM-CSF used as the sole antileukemic agent in AML patients [3, 81, 42]. Nevertheless, two AML subtypes might be particularly sensitive to a CSF-induced differentiation signal:

- A possible *in vivo* differentiation of acute promyelocytic leukemia (APL) cells by G-CSF therapy was reported for one APL patient treated with G-CSF alone [84]. In addition, the differentiation of APL cells induced by all-trans retinoic acid is accelerated in the presence of G-CSF *in vitro*.
- *In vitro*, G-CSF induces the granulocytic differentiation of AML cells carrying the t(8;21) chromosomal translocation [82]. Enhancement of the spontaneous granulocytic maturation of these leukemic cells by G-CSF therapy has also been observed *in vivo*, in AML patients carrying the t(8;21) translocation [31].

Rationale for the use of combined CSF, CSF/cytotoxic, or CSF/inhibitor therapy

Preclinical data demonstrated synergistic effects of various hematopoietic growth factors including stem cell factor (SCF), IL-3, GM-CSF, G-CSF, IL-1beta, IL-6, and erythropoietin (EPO) on primitive hematopoietic progenitors [58]. Similar synergistic effects have been reported using G-CSF + GM-CSF, IL-3 + GM-CSF, IL-3 + G-CSF, or IL-3 + G-CSF + GM-CSF combinations on AML cells [69, 86]. These combinations of growth factors may be of potential value in future clinical trials.
To date, the most widely tested combination in AML patients has been the simultaneous administration of CSF and cytosine arabinoside (Ara-C), in order to improve the antileukemic activity of Ara-C alone.
Cytosine arabinoside is one of the principal cytotoxic drugs employed in AML therapy. This and other agents are effective in actively cycling cells. However, certain leukemic precursors belong to a non-cycling leukemic population and may escape the cell death induced by these drugs.
Although patient-to-patient heterogeneity is high, a relatively low percentage of leukemic blasts is found in the S or G2 + M phases of the cell cycle (about 5 to 10%). The recruitment of quiescent leukemic cells into the cell cycle by granulocytic CSFs may thus enhance Ara-C cytotoxicity against AML cells. Such an effect was observed *in vitro* with G-CSF, GM-CSF, and IL-3, and was stronger in AML cells from newly diagnosed patients than in those from relapsed or refractory patients [79-80].
Enhancement of Ara-C cytotoxicity induced by G-CSF or GM-CSF ± IL-3 administration appeared to be lower in normal myeloid progenitors as compared to leukemic precursors [10, 79].
As there is no clear correlation between the CSF-induced increase in the proliferative activity of leukemic precursors and the enhancement of Ara-C cytotoxicity, other mechanisms must be involved. It has been shown that GM-CSF also increases the level of the intracellular Ara-CTP metabolite in most AML cases and stimulates the activity of DNA polymerases, which are essential for DNA nucleotide incorporation [91].
Several antileukemic drugs, including nitrogen mustards, cisplatin, topoisomerase II inhibitors, mitoxantrone, Ara-C and fludarabine, have been reported to induce apoptosis in malignant cells. Among these drugs, Ara-C and fludarabine have been shown to cause apoptosis in AML cells [37, 83]. Interestingly, it has been demonstrated that G-CSF may potentiate Ara-C or Ara-C + fludarabine-induced AML cell apoptosis [11, 83]. A similar effect has also been reported with the GM-CSF/IL-3 fusion protein [12].
On the other hand, enhancement of the myelopoietic response to GM-CSF administered after cytosine arabinoside (Ara-C) chemotherapy was observed in mice which had previously received a negative hematopoietic regulator which protected the stem cell compartment during chemotherapy [13].

Clinical applications

Given these preclinical data, granulocytic CSFs were initially administered after chemotherapy in high-risk AML patients only, because of the potential for *in vivo* stimulation of residual AML cells. The principal objectives were to reduce the duration of chemotherapy-induced neutropenia, the incidence of severe infections and early mortality following the induction course of chemotherapy.
Investigators rapidly realized that it might also be possible to test these growth factors as direct antileukemic agents: by providing a proliferative stimulus for leukemic cells, resulting in their recruitment into the cell cycle prior to the initiation of chemotherapy regimens containing Ara-C; and by providing a differentiating stimulus and/or inducing apoptosis of leukemic cells.
On the other hand, G-CSF and GM-CSF may be used in AML patients after achieving a complete remission (CR) for peripheral blood progenitor cell (PBPC) mobilization prior to therapeutic intensification and autotransplantation.

Correction of chemotherapy-induced neutropenia

In 1990, Ohno et al published the results of the first Japanese randomized, controlled study of G-CSF administered after the completion of induction chemotherapy in a heterogeneous population of patients with refractory or relapsed acute leukemia [62]. Each patient received an individualized, response-oriented induction course of mitoxantrone, etoposide, and behenoylcytosine arabinoside. Mitoxantrone and occasionally etoposide doses were increased in cases of persistent blast cells in the bone marrow examination on Day 8, Day 10, and sometimes Day 12. Only patients achieving a severely hypoplastic bone marrow after chemotherapy were randomized to receive G-CSF or placebo. Even though patients treated with G-CSF received higher doses of chemotherapy than those treated with placebo, their neutrophil counts recovered significantly earlier to a level higher than 500/mm^3 or 1000/mm^3. The duration of neutropenia was decreased by about one week. The incidence of documented infections was significantly lower in the G-CSF group. There was no difference between the two treatment groups in terms of leukemic regrowth with G-CSF/placebo therapy. Furthermore, there was a trend towards a higher CR rate in the G-CSF group (50% compared to 36% in the placebo group; P = 0.16). Remission durations were similar in both groups.
The safety of GM-CSF administration after induction therapy has also been reported in poor-prognosis AML patients by the MD Anderson Group [32].
In 1991, Büchner et al reported the results of a comparative study of GM-CSF following induction therapy in thirty elderly or relapsed AML patients [15]. GM-CSF was only administered in patients with an aplastic bone marrow after the completion of chemotherapy. A historical control group of similar patients who had not received GM-CSF was used for comparison. The duration of neutropenia was reduced by 6 to 9 days in patients treated with GM-CSF. The early death rate was significantly reduced (14 vs 39%) and there was a trend towards more complete remissions in the GM-CSF group (50 vs 32%). Remission durations were identical in both groups. Two patients experienced marked leukemic regrowth with GM-CSF therapy; however, this was totally reversible in one of them once GM-CSF had been discontinued.
Since these early promising reports, several large prospective, randomized, controlled trials have assessed the efficacy of G-CSF [30, 36, 40, 48, 16, 39, 53, 54, 73, 77, 90, 92] in newly diagnosed AML patients (Tables 1and 2). Some of these studies only randomized older patients [30, 36, 53, 73, 77, 90].

Reference Year	CSF	Modalities of CSF administration	N evaluable patients	Median age (protocol range)
Büchner [16] 1994	*E. coli*-derived GM-CSF	before, during, and after courses 1 to 5	96	49 years (15-75)
Zittoun [92] 1994	*E. coli*-derived GM-CSF	various, after the 1st course (4 treatment arms[a])	102	NA (15-60)
Witz [90] 1995	*E. coli*-derived GM-CSF	during and after the 1st course	232	67 years (55-75)
Heil [39] 1995-1	*E. coli*-derived GM-CSF	before, during, and after courses 2 to 4	80	50 years (15-75)
Stone [77] 1995	*E. coli*-derived GM-CSF	after the 1st course	388	69 years (>60)
Löwenberg [53] 1995-1	*E. coli*-derived GM-CSF	during and after courses 1 and 2	316	68 years (>60)
Löwenberg [54] 1995-2	*E. coli*-derived GM-CSF	various, after courses 1 to 3 (4 treatment arms[a])	253	42 years (18-60)
Rowe [73] 1995	Yeast-derived GM-CSF	after courses 1 and 2	117	64 years (55-70)
Heil [40] 1995-2	Non-glycosylated G-CSF	after courses 1, 2, and ± 3	521	NA (>15)
Godwin [36] 1995	Non-glycosylated G-CSF	after courses 1 and 2	193	66 years (>55)
Dombret [30] 1995	Glycosylated G-CSF	after the 1st course	173	71 years (>65)
Link [48] 1996	Glycosylated G-CSF	after 1 to 4 courses	103	NA

Table 1
Randomized trials with granulocytic colony-stimulating factors (CSFs) in patients with acute myelogenous leukemia: trials and patients characteristics

[a] These EORTC-GIMEMA and HOVON-4A trials comprised four groups of randomization (no GM-CSF, GM-CSF after chemotherapy, GM-CSF before and during chemotherapy, GM-CSF before, during and after chemotherapy). The comparisons between the two groups without GM-CSF after the chemotherapy and the two groups with GM-CSF after the chemotherapy are indicated.
NA, not available

Reference Year	Duration of neutropenia	Incidence of infections	Mortality	CR rate	DFS, EFS	Survival
Büchner [16] 1994	*reduced*[a,b]	NA	similar	similar (81% vs 84%)	no change	NA
Zittoun [92] 1994	NA	similar	similar	*decreased* (47% vs 75%)	*decreased*[c]	NA
Witz [90] 1995	*reduced*	similar	similar	similar (62% vs 61%)	*increased*	similar
Heil [39] 1995-1	similar[b]	similar	similar	similar (81% vs 79%)	similar	similar
Stone [77] 1995	*reduced*	NA	similar	similar (51% vs 54%)	NA	similar
Löwenberg [53] 1995-1	*reduced*	similar	similar	similar (56% vs 55%)	similar	similar
Löwenberg [54] 1995-2	*reduced*	NA	similar	similar (77% vs 77%)	similar	similar
Rowe [73] 1995	*reduced*[b]	*decreased*[d]	similar	similar (60% vs 44%)	similar	NA[e]
Heil [40] 1995-2	*reduced*	NA	similar	similar (69% vs 68%)	similar	similar
Godwin [36] 1995	*reduced*	similar	NA	similar (42% vs 49%)	NA	similar
Dombret [30] 1995	*reduced*	similar	similar	*increased* (70% vs 47%)	similar	similar
Link [48] 1996	*reduced*	similar	similar	similar (62.5% vs 47%)	NA	NA

Table 2
Randomized trials with granulocytic colony-stimulating factors (CSFs) in patients with acute myelogenous leukemia: results

[a] The reduction in the duration of neutropenia was observed after the first course of chemotherapy only
[b] A prolongation in the duration of thrombocytopenia was observed in these two studies including multiple courses of chemotherapy with GM-CSF
[c] The increase in DFS was mainly observed in 55-65 year old patients
[d] A reduction in the incidence of grade 4/5 infections only was observed
[e] Only median comparisons are available, showing longer median DFS and median survival in the GM-CSF group
CR, complete remission; *DFS*, disease-free survival; *EFS*, event-free survival; *NA*, not available

Certain investigators restricted CSF administration to patients with a documented aplastic bone marrow after the induction course [36, 73], as used in early Japanese and German reports. In contrast, the growth factor was administered not only after the completion of chemotherapy, but also during and occasionally before the chemotherapy in some studies using GM-CSF [16, 39, 53, 90, 92].
Growth factor administration was sometimes repeated during consolidation therapy [16, 36, 40, 48, 53, 54, 73], or even limited to consolidation therapy [39].
Results (Table 2) have been described as disappointing, since no clear reduction in chemotherapy-related mortality was observed and overall survival was not significantly improved [38]. Furthermore, remissions continued to be of very short duration in elderly AML patients [30, 77].
Several important observations should, however, be noted and encourage further studies:
1) The period of neutropenia induced by the induction course was consistently reduced after the induction course, sometimes by as much as a week, although not after the consolidation courses [16, 39, 73]. On the contrary, an increase in the duration of thrombocytopenia was even observed after consolidation courses in the two studies with GM-CSF administered before, during, and after multiple courses of chemotherapy [16,

39].
2) No significant induction of leukemic regrowth was observed with either G-CSF or GM-CSF. Complete remission rates were similar or higher in the CSF-randomized groups than in the control groups in all studies but one, which was reported by the EORTC-GIMEMA Cooperative Group [92]. In that study, GM-CSF administration after the completion of induction chemotherapy and continued until myeloid recovery, was associated with lower CR rates and event-free survival (EFS), which was not due to a higher chemotherapy-related death rate.
3) A highly significant increase in the CR rate was observed in the first trial using glycosylated G-CSF [30]. This increase did not result from a reduction in treatment-related mortality from infections, but rather from a lower incidence of resistant patients. Furthermore, the benefit of G-CSF administration was mainly observed in AML patients with poor prognostic characteristics, such as unfavorable cytogenetics or marrow blasts persisting after the completion of induction chemotherapy [30]. Interestingly, the first interim analysis of a second trial using the same CSF in similar settings also showed a trend towards a higher CR rate in the G-CSF group [48].
This observation suggests that G-CSF may contribute to the antileukemic effect of chemotherapy. The possible mechanisms remain unclear but include a growth advantage for the normal hematopoietic elements [42], a differentiation effect, an induction of residual leukemic cell apoptosis, or a cytokine-mediated effect.
4) A trend towards longer disease-free survival or overall survival was observed in some studies [16, 73, 90], including those with multiple CSF/chemotherapy courses [16, 73].
In addition, an interesting randomized placebo-controlled study from the Japanese Adult Leukemia Study Group using macrophage colony-stimulating factor (M-CSF) in AML patients has been reported recently [64]. Macrophage colony-stimulating factor not only stimulates the production of mature monocyte-macrophages as well as their antibacterial and antifungal functions, but also induces the secretion of G-CSF, GM-CSF, IL-6, and IL-8 by stimulated cells. Human urinary M-CSF or placebo was administered after three courses of consolidation chemotherapy in 198 AML patients in first CR. Although no difference in DFS was observed between the two treatment groups, M-CSF significantly reduced the incidence and duration of febrile neutropenia and shortened the time required to complete the three courses of therapy.
Granulocytic colony-stimulating factor administration has also been recently reported after intensive chemotherapy in patients with MDS, AML evolving from prior MDS, and therapy-related AML [2, 6, 22, 34, 35, 87, 76].
High CR rates were observed [43, 22], but randomized trials are required to clearly evaluate the role of CSFs in this high-risk patient population, since similar CR rates have also been reported without the addition of any CSF [24, 25, 26, 57, 74].
One randomized study using GM-CSF before, during, and after sequential chemotherapy with intermediate dose Ara-C and mitoxantrone is ongoing in Germany [41]. Preliminary results of another randomized study have been recently reported showing a higher CR rate in the G-CSF group as compared to the placebo group (61% vs 48%, p=0.31) in 60 evaluable patients [66].

Increase in the response to cytotoxic therapy

A different approach has been adopted by several investigators in an attempt to improve the results of conventional chemotherapy. The *in vitro* sensitivity of AML cells to granulocytic CSFs has been used to test the hypothesis that GM-CSF administered before the initiation of the chemotherapy may recruit leukemic cells into the cell cycle and thereby maximize leukemic cell kill by cell cycle-active cytotoxic agents. In all Phase I/II trials, GM-CSF administration was started one or more days before chemotherapy and then discontinued during [47] or on completion of chemotherapy [21, 33], or even later [6, 9, 85].
Certain investigators combined GM-CSF administration and timed-sequential chemotherapy [1, 19, 29]. The use of timed-sequential therapy was based on the hypothesis that initial cytoreduction stimulates the growth and sensitivity of residual leukemic cells to cycle-active agents. The observed change in the *in vivo* growth of residual cells was correlated with a clinical response to therapy [44].
Overall, these studies produced the following results:
- An *in vivo* recruitment of AML cells into the drug-sensitive phases of the cell cycle was demonstrated using several methods, including the evaluation of CFU-L growth, ^{3}H-TdR incorporation, Ara-CTP formation, and flow cytometric DNA/RNA, DNA/BrdU, or DNA/Ki67 content. An increase in the percentage of cells in S-phase

was clearly observed in some cases.
- Normal hematopoietic progenitors were probably not damaged by this combined therapy, since no increase in the duration of therapy-induced aplasia was observed.
- Decreases in CR rate and survival were reported when GM-CSF was administered for a long period prior to chemotherapy initiation [33], thus suggesting that caution should be exercised when applying this recruitment concept. This adverse outcome appeared to be less frequent with G-CSF [42].
Since these earlier reports, several randomized Phase III trials have been initiated to evaluate the effect of this CSF-induced recruitment of AML cells into the cell cycle. Some of these trials are still ongoing. Unfortunately, CSF administration has been continued after the completion of the chemotherapy in most studies [16, 39, 53, 63], so that few studies have evaluated the concept of recruitment as a single endpoint. To date, no significant difference in CR rate between treatment groups with or without attempted AML cell recruitment has been seen in two EORTC and HOVON-SAKK trials using GM-CSF administered according to a two-by-two randomization plan in newly-diagnosed AML patients [92, 54]. A French multicenter randomized trial is currently underway to assess GM-CSF administered between Days 4 and 8 only of a timed-sequential chemotherapy schedule in patients with resistant and relapsed AML.

PBPC autologous transplantation in AML patients

As in other malignancies, PBPC reinfusions have been used in AML patients after high-dose intensification therapy [46, 75], especially in older patients [20]. More recently, growth factor-primed PBPCs, shown to ensure earlier engraftment than marrow progenitor cells, have been used for autologous transplantation, with or without marrow progenitors, in AML patients and in patients with high-risk myelodysplastic syndromes [22, 28, 26, 88].
As unmanipulated CSF-primed PBPC may be contaminated by leukemic cells [67], major studies are needed to assess the risk of post-transplant AML relapse following PBPC reinfusion as well as the role of cell selection or purging in this setting.

Conclusion

The principal positive results obtained through the use of granulocytic growth factors in the treatment of AML are the safety of their use with respect to the risk of malignant clone stimulation and the reduction in the duration of neutropenia induced by the cytotoxic agents employed for treatment. However, no clear results have been obtained to date concerning any improvement in the survival of AML patients.
One research option could be to define patient subgroups most likely to benefit from the administration of CSFs. In fact, it is possible that the discordant results obtained in published randomized trials are due to differences in the profile of patients treated.
A second research option could be to test either the combined use of several growth factors or the sequential use of hematopoiesis-inhibiting factors and CSFs. Such combinations might achieve higher reductions in the duration of chemotherapy-induced neutropenias and consequently an improvement in rates of mortality from infections.

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