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*Recent Advances in the Cytobiology of Leukemias**

POTENTIAL CLINICAL APPLICATIONS OF rhGM-CSF IN ACUTE MYELOID LEUKEMIA BASED ON ITS BIOLOGIC ACTIVITY AND RECEPTOR INTERACTION

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ABSTRACT

Background and Objective. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multilineage hemopoietic growth factor that stimulates proliferation, differentiation, and survival of progenitor cells, enhances the functional activities of mature myeloid effector cells, and plays a key role in host defense and the inflammatory process. Although the clinical use of rhGM-CSF in patients affected by lymphoid malignancies is widely accepted, its utility and safety in the management of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) is still controversial. The three main schedules adopted for clinical application of GM-CSF in AML are as follows: A) post-chemotherapy, in order to shorten the duration of neutropenia and/or monocytopenia; B) pre-chemotherapy to recruit blast cells into active cell cycle phases, and to increase their sensitivity to cell cycle-dependent cytotoxic drugs; C) as a mobilizing agent to induce the release of progenitor cells from bone marrow into circulation (peripheral blood progenitor cell transplantation – PBPC). The objective of this paper is to analyze the potential clinical applications of rhGM-CSF in AML.

Evidence and Information Sources. The material examined in the present review includes several personal papers in this field and articles and abstracts published in journals covered by the Science Citation Index.

State of the Art. Based on current knowledge, it may be argued that rhGM-CSF should be used only in a subset of AML patients at high risk of infection mortality, including elderly subjects, and/or in those AML patients who relapse or are resistant to induction treatment. However, the risk of stimulating the leukemic clone following GM-CSF therapy should be kept in mind when using this growth factor in the clinical setting, even though the great majority of the reported papers on this subject have shown that GM-CSF therapy does not affect relapse rates, frequency of remissions or patient life expectancy.

Perspectives. It is likely that new data from controlled clinical trials will clarify the therapeutic role of GM-CSF in myeloid-derived malignancies, allowing the establishment of consensus guidelines for its use.

Key words: acute myeloid leukemia, rhGM-CSF, GM-CSF receptors, blast cell recruitment, neutrophil recovery, peripheral blood progenitor cell transplantation

Use of rhGM-CSF in AML

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multilineage hemopoietic growth factor that stimulates proliferation, differentiation, and survival of progenitor cells, enhances the functional activities of mature myeloid effector cells (monocytes and granulocytes), and plays a key role in host defense and the inflammatory process.[1-5]

GM-CSF was purified in 1977 and molecularly cloned in 1984; it is now available for clinical use in at least three different forms: molgramostim, sargramostim, and regramostim, that are synthesized in bacteria, yeast, and mammals, respectively. The main clinical application of human recombinant GM-CSF (rhGM-CSF) is to stimulate the recovery of neutrophils and/or monocytes following myeloablative chemotherapy, radiotherapy, and/or bone marrow transplantation.[4]

Another attractive use of rhGM-CSF in acute myeloid leukemia (AML) is related to the possibility of increasing the cytotoxicity of cell cycle-specific agents through induction of substantial dose-dependent enhancement of proliferation and clonogenic growth of leukemic cells.[6]

A new area of investigation of GM-CSF in cancer patients derives from the insights into its physiological role revealed by studies on GM-CSF-deficient mice, GM-CSF transgenic mice, and mice overexpressing GM-CSF. This research has shown that GM-CSF is not essential for baseline or emergency granulopoiesis, whereas it plays a key role in pulmonary physiology and host defense since GM-

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CSF-deficient mice develop alveolar proteinosis, peribronchovascular lymphoid hyperplasia, and bacterial and/or fungal infections. Knockout mice studies have also demonstrated that rhGM-CSF may stimulate the development of specific antibodies and thus confer active immunity against a specific disease or infectious agent (vaccine adjuvant activity); it ameliorates the immune response of the host through a number of biological activities, and may have a therapeutic role in the treatment of skin ulcers when injected locally in damaged skin areas.

However, although this cytokine has powerful effects on several hemopoietic cells and is effective in many clinical areas, its clinical efficacy and safety in the management of AML and myelodysplastic syndromes (MDS) is still the object of debate. Most of the data reported so far in the literature refer to the use of GM-CSF in patients affected by lymphoid malignancies [acute lymphoblastic leukemias (ALL), multiple myeloma (MM), malignant lymphomas (ML), Hodgkin's disease (HD)], where its therapeutic role is well known and widely accepted. As far as the utilization of GM-CSF in AML is concerned, current clinical data derived from controlled and uncontrolled *in vivo* studies have demonstrated the safety of this growth factor in this clinical setting. Over the last decade it has been shown that rhGM-CSF could be administered to AML patients without increasing relapse rates, reducing the frequency of remissions, or shortening patient life expectancy.[7-12] Based on this clinical evidence, it is believed that the changes which stimulate the leukemic clone following GM-CSF therapy are very slight, even in patients with myeloid malignancies.[7-12] Therefore it can be postulated that rhGM-CSF could be used in a subset of AML patients at high risk of dying of infections, including elderly subjects (older than 60 years), and/or in those AML patients who relapse or are resistant to induction treatment. In these categories rhGM-CSF could be proposed for *in vivo* use to prevent post-chemotherapy infectious diseases, or as supplementary treatment to conventional antibiotics for febrile patients who experience severe bacterial or fungal infections.

The schedules adopted for the *in vivo* use of GM-CSF in AML are as follows: A) during and/or post-chemotherapy; B) pre and/or during chemotherapy; C) as a mobilizing agent to induce the release of progenitor cells from bone marrow into circulation (peripheral blood progenitor cell transplantation – PBPC).

Use of rhGM-CSF after chemotherapy

The aim of this strategy is to shorten the duration of chemotherapy-induced neutropenia/monocytopenia, and to reduce the incidence and the severity of infectious diseases in neutropenic patients.[4, 7-12]

Although almost all patients with lymphoid-derived malignancies can benefit from this treatment modality, there is limited information on AML patients (Table 1). From a theoretical point of view, GM-CSF has the capacity to stimulate AML cell growth via its interaction with specific receptors which are expressed at different levels on the blast surface, and thus there is a risk of increasing the incidence of disease relapse.[13]

However, a careful analysis of reported data seems to support the concept that the combined application of GM-CSF and chemotherapy in AML is not associated with an increase in resistance or relapse rates.[7,14-16] Only one recent study found that AML patients receiving GM-CSF after induction chemotherapy showed a significantly lower com-

Table 1. Controlled and uncontrolled clinical trials dealing with the use of rhGM-CSF IN AML after chemotherapy.

Author	*No. of pts. study design*	*Schedule*	*Results*
Estey et al. *Blood 1990; 75:1766*	12 pts *de novo* uncontrolled	120 μg/m²/d after CHT	no difference
Buchner et al. *Blood 1991; 78:1190*	36 pts (>65 yrs) (*de novo*, resistant) uncontrolled	250 μg/m²/d after CHT	↑ PMNs, ↑ CR rate, ↓ infections, ↓ early death
Zittoun et al. *Blood 1994; 84:231*	102 adult pts GIMEMA-EORTC randomized	5 μg/kg/d during-after CHT	↓ CR rate, ↑ resistant cases
Baro et al. *Bone Marrow Transplant 1995; 15:721*	20 pts uncontrolled	5 μg/kg/d after CHT	↑ CR rate, ↓ relapse rate, ↑ NK activity
Stone et al. *N Engl J Med 1995; 332:1671*	388 elderly pts, *de novo* randomized	5 μg/kg/d after CHT	↓ duration of neutropenia
Heil et al. *Leukemia 1995; 9:3*	80 adult pts randomized	250 μg/m²/d during-after CHT	↓ platelet recovery
Rowe et al. *Blood 1995; 86:457*	124 pts (55-70 yrs) randomized	250 μg/m²/d after CHT	↑ CR rate, ↑ PMNs, ↓ therapy-related toxicity, ↓ infections, ↑ survival

CHT: chemotherapy; PMN: neutrophil granulocytes; CR: complete remission.

plete remission rate and an increased frequency of resistant disease.[17] In addition, some studies have demonstrated that GM-CSF can lower the duration and the cost of hospitalization,[10] as well as reduce the death rate in elderly and/or relapsed or resistant AML patients at high risk of dying of infections. As a result it can be postulated that the survival rates of a subset of AML could be improved by this treatment modality.[7-12] All these clinical and biologic aspects should be carefully evaluated when rhGM-CSF is given to AML patients.

Use of rhGM-CSF before and/or during chemotherapy

Great attention has been focused on the use of growth factors (GM-CSF, G-CSF, interleukin 3, stem cell factor) aimed at sensitizing leukemic cells to chemotherapy.[6,11,12,14-26] This area of research has been rapidly expanding due to evidence that the administration of GM-CSF to AML patients before chemotherapy may be associated with recruitment of quiescent (G_0) blast cells into active cell cycle phases (S-G_2-M), together with increased activity of DNA polymerases, enzymes that are essential for nucleotide incorporation into DNA.[6] Using this approach, it has been demonstrated that more vulnerable S-phase blast cells can be killed by cell cycle-specific cytostatic drugs. In recent papers it has been shown that partial responses to chemotherapy may be related in part to the kinetic quiescence of AML blast cells.[6] However, although the sequential use of chemotherapy and GM-CSF may be associated both *in vivo* and *in vitro* with a significant increase in blast proliferation rate and in cell cytotoxicity, there is no clear evidence that this treatment strategy is of clinical benefit. The data reported failed to show any significant increase in the complete remission rates of AML patients treated with GM-CSF prior to or concomitantly with aggressive chemotherapy compared to those of patients receiving conventional chemotherapy without GM-CSF. The reasons for this are uncertain and need to be clarified in randomized clinical trials. Based on recently available data, it can be hypothesized that GM-CSF may be of clinical benefit only in a subset of AML cases with a low pre-treatment proliferative rate, while for the remaining AML patients there is no evidence of a positive effect for this growth factor[6] (Table 2). A recently reported randomized trial showed that rhGM-CSF can determine better disease-free survival and a lower relapse rate only in AML patients under 60 with a favorable karyotype.[26]

GM-CSF could also be used before – and after-chemotherapy to recruit AML cells into the cell cycle and to accelerate neutrophil recovery.[27-30]

Use of rhGM-CSF as a mobilizing agent to induce the release of progenitor cells from bone marrow into circulation (PBPC transplantation)

There is mounting evidence that rhGM-CSF may be used in patients suffering from a wide variety of malignancies with the scope of increasing the number of peripheral blood $CD34^+$ cells intended for PBPC harvest.[5,28,30] However, there is limited experience on the use of GM-CSF in complete remission AML patients to be submitted to PBPC transplant. Most of the mobilization regimens are in fact based

Table 2. Controlled and uncontrolled clinical trials dealing with the use of rhGM-CSF in AML before chemotherapy.

Author	*No. of pts.; study design*	*Schedule*	*Results*
Visani et al. *Ann Hematol 1991; 63:276*	15 adult pts (resistant, relapse) uncontrolled	5-10 µg/kg/d prior to, after CHT	↑ PMNs, ↑ blasts
Aglietta et al. *Leukemia 1991; 5:979*	6 pts uncontrolled	8 µg/kg/d before, during CHT	↑ blast proliferation, = duration of neutropenia
Bettelheim et al. *Blood 1992; 77:700*	18 adult pts (*de novo*) uncontrolled	250 ug/m^2/d before CHT (2d)	↑ blast proliferation
Estey et al. *Blood 1992; 79:2246*	56 adult pts uncontrolled	20-125 µg/m^2/d before, during CHT	↓ survival, ↓ CR
Puntous et al. *Leuk Lymphoma 1993; 12:95*	10 pts uncontrolled	3 µg/kg/d before CHT	↑ blast proliferation, ↑ PMNs, = CR
Bernell et al. *Leukemia 1994; 8:1631*	14 pts uncontrolled	3 µg/kg/d before CHT	↑ CR rate, ↓ duration neutropenia
Wiley et al. *Leukemia 1994; 8:181*	15 pts uncontrolled	5 µg/kg/d before CHT	↑ blast cell proliferation
Witz et al. *Blood 1994; 84:231a*	220 pts controlled	5 µg/kg/d before, during, after CHT	↑ disease-free survival, ↓ duration neutropenia, ↓ duration of antibiotics administration
Buchner et al. *Blood 88:214a*	114 pts controlled	250 µg/m^2/d before, during, after CHT	↑ disease-free survival, ↑ CR rate (good prognosis group)

CHT: chemotherapy; PMN: neutrophil granulocytes; CR: complete remission.

on the use of G-CSF. rhGM-CSF may be given to AML patients together with chemotherapy and mobilized circulating progenitor cells can be collected with a leukapheresis machine. This therapeutic approach is applicable in complete remission AML patients and is aimed at determining whether further eradication of residual blast cells can lead to a significant improvement in disease-free survival. However, it must be kept in mind that this procedure may be associated with early disease relapse due the presence of contaminating leukemic cells in both CD34-positive and CD34-negative cell fractions which have been infused into the patient. At present, we believe that PBPC harvest can only be performed in AML patients who have achieved a *true* complete remission following induction-consolidation chemotherapy, as evaluated by morphology, cytogenetics, immunophenotype and molecular biology analysis. Another point which needs to be clarified is related to the degree of the tumor contamination of the mobilized peripheral blood progenitors of AML patients compared to that of bone marrow graft.[31] Whether the use of PBPC could result in faster neutrophil and platelet recovery, lower transfusion requirement, and shorter hospitalization time for AML patients than autologous bone marrow transplantation is under investigation. A number of factors may be involved, e.g. the level of leukemia contamination, the threshold dose of $CD34^+$ cells and/or CFU-GM colonies given to the patient, the optimal timing of peripheral blood collection, and the type and schedule of growth factors used to improve PBPC harvest.

Biological and clinical significance of measuring GM-CSF receptors on AML blasts

The activities of growth factors on hemopoietic cells depend on their binding to specific cell surface receptors which are expressed on both normal and leukemic cells.

Receptors for GM-CSF are high affinity receptors composed of alpha and beta subunits with molecular weights of approximately 80 and 130 kD, respectively. The α subunit binds the specific ligand with low affinity, while the β subunit (common to interleukin 3 and IL5 receptors) does not bind GM-CSF, but its association with the α subunit generates high affinity receptors.[34-41] At the 5th International Workshop on Leukocyte Differentiation Antigen held in Boston (USA), November 1993, GM-CSF/R was designated as CDw116.[38] GM-CSF/R is expressed on neutrophils, monocytes, bone marrow myeloid and monocytic precursors, and a subset of $CD34^+$ bone marrow progenitors committed to the myelo-monocytic lineage.[35,38,39,50,52,53] Personal data have shown that a subset of lineage-negative $CD34^+$ progenitor cells expresses small numbers of GM-CSF receptors.[49]

The biological effects deriving from the binding of GM-CSF to its receptor are numerous and depend on the concentration and the route of administration. In general, interaction between GM-CSF and its receptors induces cell proliferation and differentiation[41-45] (Table 3). Evidence of the role played by receptors for GM-CSF in the blast cell proliferation pathway comes from *in vitro* studies showing that most AML cell clones are directly stimulated by rhGM-CSF to proliferate in a dose-dependent way, thus confirming the preservation of these receptors on leukemic myeloid cells.

Several methods are utilized for the quantitation of GM-CSF receptors on hemopoietic cells (radioactive GM-CSF; combined use of flow cytometry and fluorescein-labelled GM-CSF MoAbs; FITC-coupled GM-CSF). The recent availability of quantitative microbead calibration standards has allowed precise and reproducible cytofluorimetric quantitation of the number of receptors or molecules per cell by means of monoclonal antibodies directed against cytokine receptors or other antigens.[46-48] The microbeads act as reference standards which allow a comparison between the fluorescence intensity on cells stained by a given MoAb and that of the four or five bead populations. The use of microbeads also permits comparisons of flow cytometry data over time and between laboratories.

Receptors for GM-CSF are detectable on 50-70% of AML cells. Blasts from FAB (French-American-British classification) M4 and M5 have the highest number of molecules per cell (25,000-40,000 antibody binding capacities – ABC).[9,10,49] By contrast, most ALL are GM-CSF/R negative,[49] and only sporadically show dim expression of GM-CSF receptors (< 1,000 ABC). The number of GM-CSF/R expressed by AML blasts in a few cases (5-10%) is higher (32,000-41,000) than that of normal hematopoietic cells (400-32,000), and thus leukemic cells can be distinguished from normal cells by their antigenic density for this cytokine receptor, allowing this marker to be employed for minimal residual disease. In addition, overexpression of GM-CSF receptors might account for the enhanced proliferation of leukemia cells. Preliminary results collected by our group have shown that receptors for GM-CSF can also be expressed by a significant number of leukemic cells from patients with biphenotypic leukemias, in which blasts are characterized by simultaneous expression of myeloid and lymphoid immunophenotypic markers on the same cells.[49]

The number of GM-CSF receptors expressed by normal hemopoietic cells is usually small (500-28,000 per single cell), and their affinity varies from cell to cell. As far as affinity status is concerned, at least three different types of GM-CSF/R have been identified, namely low, intermediate, and high affinity receptors.[48,50]

It is of potential importance to evaluate cellular

regulation of GM-CSF receptors after *in vivo* or *in vitro* exposure of normal and leukemic cells to GM-CSF. Recently, It was shown that either the affinity status or the number of receptors can be significantly modified after treatment with GM-CSF[50-52] (Table 3). In 60% of the cases GM-CSF downmodulates GM-CSF receptors and induces a decrease in their affinity. In the remaining 40% (including both normal $CD34^+$ cells and AML blasts) GM-CSF induces an upregulation of GM-CSF/R.

The hematologist should be aware of such changes when giving GM-CSF to a patient with AML, even though the clinical implications of GM-CSF receptor modulation after growth factor administration are not yet fully understood. We believe that dynamic evaluation of receptor status on the blast and *supposedly* normal cell populations could represent a promising tool for optimizing GF treatment in patients with acute leukemias, and make it possible to critically analyze data from clinical trials based on the combined use of chemotherapy and GM-CSF.

In theory, a careful evaluation of receptor status could also help to distinguish AML patients suitable for GM-CSF treatment who need stimulation of neutrophil recovery from those eligible for a chemotherapy-based strategy for the recruitment of blast cells into the cell cycle. We propose treating with GM-CSF the group of patients whose cells contain few or no GM-CSF receptors that are downmodulated by GM-CSF, while those patients characterized by higher levels of GM-CSF receptors which are slightly and transiently downregulated after ligand binding should not be treated.

In acute leukemia, there are additional biological activities mediated by GM-CSF that need to be carefully evaluated in future investigations: 1) differentiation activity on blast cells; 2) stem cell protection; 3) interruption of autocrine/paracrine loops; 4) interference with the apoptotic process[55-58] (Table 4).

As far as differentiation activity is concerned, it has been demonstrated that some growth factors such as GM-CSF and G-CSF can be used as differentiating agents with the aim of exhausting the self-renewal potential of leukemic progenitors. In this context, however, G-CSF seems to be the most promising growth factor.

We know that AML and MDS blasts not only express receptors for GM-CSF but also synthesize GM-CSF themselves. As a consequence, they can stimulate their own proliferation (autocrine effect). This autocrine effect can be interrupted by the administration of exogenous growth factors. It has been demonstrated that the use of antisense oligonucleotides to GM-CSF inhibits *in vitro* proliferation of human AML blasts, thus confirming the key role of endogenous GM-CSF in the regulation of cell kinetics.

Table 3. Factors influencing the effects of GM-CSF on normal and leukemic cells.

1. Number and affinity status of GM-CSF receptors
2. Cellular regulation of GM-CSF receptors after exposure to GM-CSF
   a) down- or upregulation of receptor number
   b) receptor internalization
   c) changes in affinity status of receptors (depending on the schedule, route, and dosage of GM-CSF treatment)
3. Synthesis of autoantibodies directed against GM-CSF

Table 4. Putative biological activities exerted by GM-CSF in AML.

1. Recruitment of blast cells into active cell cycle phases
2. Shortening the period of neutropenia, monocytopenia and (probably) thrombocytopenia
3. Enhance differentiation of blast cells
4. Stem cell protection (decrease of S-phase progenitors)
5. Activation of monocyte and neutrophil function (increased resistance to infections)
6. Interruption of autocrine /paracrine loops
7. Protective effect against apoptosis

The possibility that patients with hematological disorders undergoing GM-CSF treatment may produce specific autoantibodies which can limit the biological activities of this growth factor must also be considered.[59]

Although the presence of point mutations of the GM-CSF gene is considered very rare in patients with hematologic malignancies,[60] it can be argued that in a small number of cases blast cells have a growth advantage over normal cells and their abnormal maturation ability may be determined by genetic anomalies involving the GM-CSF gene.

In conclusion, it has been several years since GM-CSF was first licensed for clinical use in hematological malignancies of lymphoid origin, and one year (1995) since the US FDA (Food and Drug Administration) approved its use in AML patients. However, although this cytokine has powerful effects on hemopoiesis and is effective in many clinical areas, its clinical utility and safety in the management of AML and MDS is still the object of debate, and therefore its application should be recommended only in a subset of AML patients at high risk of dying of infections, including elderly AML patients, or in those who relapse or are resistant to induction treatment. It is likely that new data from controlled clinical trials will clarify the therapeutic role of this growth factor in myeloid-derived malignancies, allowing the establishment of consensus guidelines for its use.

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