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# Granulocyte-macrophage colony stimulating factor (GM-CSF) in the treatment of hematological malignancies

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**Acute myeloid leukemic (AML) cells not only express receptors for various cytokines but also produce hemopoietic growth factors themselves. Thus, they are able to stimulate their own activation and proliferation, a phenomenon known as autocrine growth. The cell cycle kinetics of AML blast cells are susceptible to stimulation by a variety of cytokines, including granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-3, granulocyte colony stimulating factor and stem cell factor. GM-CSF and IL-3 can markedly enhance the cytotoxicity of chemotherapeutic agents by increasing the proportion of AML cells in S-phase at any given time and by altering the metabolic status of the AML cells. A number of clinical studies involving the use of GM-CSF in association with chemotherapy in patients with AML are currently ongoing. In the next few years the first clinical results will become available indicating whether the use of cytokines holds any benefit for patients with AML.**

*Key words:* Acute myeloid leukemia, autocrine growth, cell cycle kinetics, chemotherapy, granulocyte-macrophage colony stimulating factor, interleukin-3.

## Introduction

Acute myeloid leukemia (AML) is a disease of transformed hemopoietic progenitors that are unable to mature. Among the principal biological features that characterize the hematological nature of the neoplasm are the expression and function of receptors for the major hemopoietic growth factors. The role of hemopoietic growth factors in the evolution of the disease is further supported by the occurrence of autocrine growth.

High affinity receptors for a variety of cytokines are expressed on the cell surface of AML blasts. Of these, the receptors for granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-3, granulocyte colony stimulating factor (G-CSF) and stem cell factor (also known as mast cell growth factor and c-*kit* ligand) are considered to be the most important and the cell cycle kinetics of AML blast cells are susceptible to stimulation by these molecules. The effects of using stem cell factor in combination with various other cytokines are shown in Figure 1.[1] Stem cell factor produces markedly positive effects on the proliferation rate of human AML cells in culture when it is used in combination with IL-3, GM-CSF and G-CSF. Therefore, in AML there is a unique opportunity for therapeutic intervention because the major growth factors which determine the course of the disease are known. There is no other human tumor that provides a model situation for the use of growth factors to modify the natural course of the disease.

### Autocrine growth in AML

AML cells not only express receptors for various cytokines but they also produce hemopoietic growth factors themselves. Thus, AML cells are able to stimulate their own activation and proliferation, as illustrated schematically in Figure

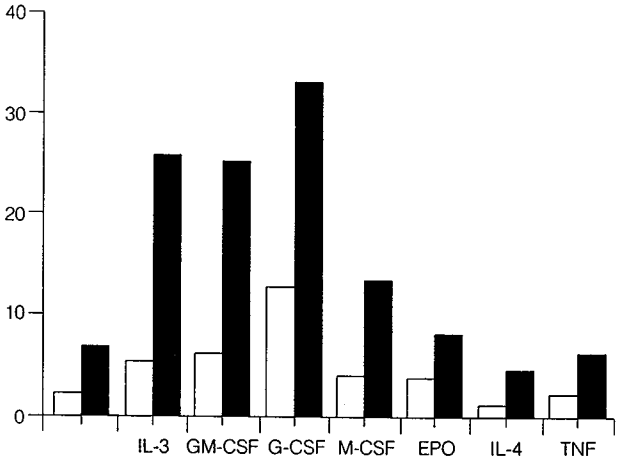


**Figure 1.** Colony formation by human AML blasts in the presence of various cytokines with or without stem cell factor (or mast cell growth factor, MGF). ■, +MGF; □, −MGF.

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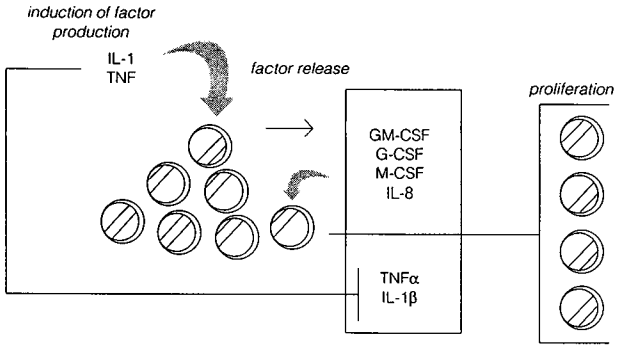


**Figure 2.** Schematic representation of autocrine cytokine pathways in AML.

2. Although the proliferation of human AML cells generally may be enhanced by exogenous growth factors, autocrine pathways apparently operate to activate a baseline level of growth. In the majority of cases, the leukemic cells show some spontaneous proliferative activity *in vitro*. Figure 3 illustrates that approximately one third of cases show high spontaneous growth. In a study in 114 AML patients of different phenotypes (different French-American-British (FAB) classification types and different cytogenetic abnormalities) it was found that individuals with a high spontaneous growth rate had a low probability of achieving complete remission and once complete remission has been attained these patients tend to relapse.[2] Therefore, the level of autocrine growth may have important implications for the duration of disease-free survival and the probability of relapse.

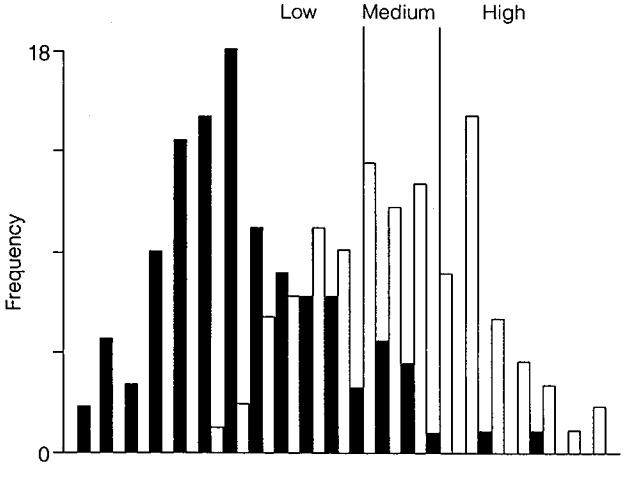


**Figure 3.** A total of 114 cases of AML with spontaneous DNA synthesis in culture. *x*-axis: thymidine uptake of AML blasts in non-stimulated culture (closed bars); *y*-axis: frequency of cases. Control values of pre-irradiated cells showing background thymidine uptake of the same AML cell specimens are also indicated for comparison (open bars).

## The use of growth factors to enhance the cytotoxicity of chemotherapeutic agents

Agents which increase the proportion of AML cells in cycle at any given time, or which can alter the metabolic status of AML cells, such as GM-CSF and IL-3, can have a profound effect on the cytotoxicity of the chemotherapeutic agents commonly used for the treatment of patients with AML. *In vitro* experiments by Hiddemann *et al.*[3] demonstrated that GM-CSF and IL-3 enhance the intracellular metabolism of cytosine arabinoside (ara-C) and its incorporation into the DNA of leukemic blasts, resulting in an average increase in cytotoxicity of ara-C of approximately 10-fold in clonogenic cells from patients with AML. GM-CSF and IL-3 increased the fraction of cells in S-phase as well. A clear relationship between the number of cells recruited into S-phase and the increase in the number of cells killed with ara-C has been demonstrated for various growth factors, and GM-CSF and IL-3 are among the most active molecules in this respect.[4–6]

These data create the basis for the clinical application of hemopoietic growth factors in patients with AML, with the objective of increasing the cytotoxicity of anti-leukemic agents by altering the cell cycle status and/or the metabolic status of the AML cells. Thus, hemopoietic growth factors such as GM-CSF and IL-3, given in association with chemotherapeutic agents, enhance the cytotoxicity of these agents, in addition to their use for reducing marrow aplasia after high-dose chemotherapy.

## Clinical studies in patients with AML

A number of clinical studies dealing with the use of GM-CSF in association with chemotherapy in patients with AML have been launched by various groups, based on the rationale discussed above. Most of the studies are still ongoing and so efficacy data cannot be presented this time. However, details of study designs, protocols and safety data are available.

The Dutch HOVON 4A study examines the value of GM-CSF (Sandoz, Basel, Switzerland and Schering-Plough, Kenilworth, NJ, USA). In this study GM-CSF is given by continuous infusion,

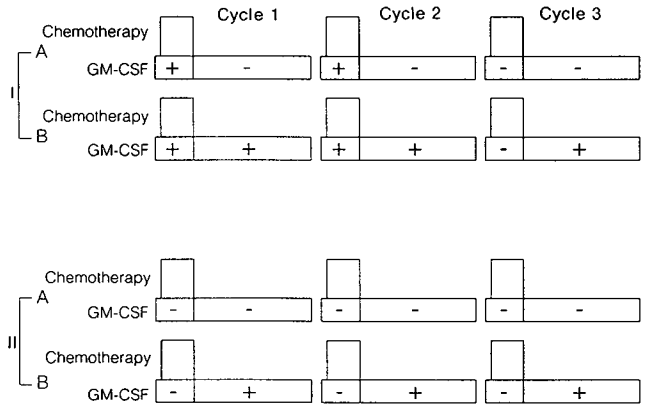


**Figure 4.** Outline of HOVON-4 AML study in adult AML. Patients are treated with GM-CSF according to four comparative schedules: GM-CSF is administered with chemotherapy (IA); GM-CSF is administered with chemotherapy and after chemotherapy (IB); no GM-CSF (IIA); and GM-CSF is administered only after chemotherapy (IIB).

either: IA—from 24 h prior to the start of chemotherapy (to prime the blasts) and continuing throughout chemotherapy; IB—from 24 h prior to the start of chemotherapy and continuing throughout and after chemotherapy; or IIA—not at all; or IIB—only after chemotherapy, to see which regimen gives the greatest therapeutic benefit. The study is designed so that administration of GM-CSF is repeated during successive cycles of chemotherapy (as summarized in Figure 4), so that if there is a difference between the treatment arms it should be maximized during the course of the study. The efficacy results are not yet available, but feasibility data from almost 100 patients drawn from several different centers was obtained at the last interim analysis in Spring 1992. For all patients (not divided by group) the period until recovery of white blood cells ($>1 \times 10^3/\mu l$), neutrophils ($>0.5 \times 10^3/\mu l$) and platelets ($>50 \times 10^3/\mu l$) increased with each successive cycle, indicating that the greater the cumulative dose of chemotherapy, the longer the bone marrow remains hypoplastic. Fluid retention was the main adverse effect which was seen more often with GM-CSF, especially when GM-CSF was given during or during and after chemotherapy.

Also ongoing is an EORTC study in elderly patients (mostly >60 years) with AML, receiving conventional therapy. This study aims to evaluate survival, complete remission (%), time to complete remission (months), number of cycles of chemotherapy until complete remission is achieved, disease-free interval, regeneration failures and deaths during bone marrow aplasia, number of nights spent in hospital and frequency of admission, and the number of days to hemopoietic recovery.

### *In vivo* activity of GM-CSF in AML patients

Are AML cells really activated when a hemopoietic growth factor, such as GM-CSF, is given to patients with AML? The *in vitro* evidence suggests that this is the case. If the hemopoietic growth factor is given starting one day before chemotherapy begins, then there will be 1 day during which AML blasts may be sampled, to see whether there is any change in cell cycle parameters solely due to the growth factor. Several studies have been performed by a number of different groups, which confirm that there is some increase in the percentage of AML blasts in S-phase following GM-CSF administration (Löwenberg, unpublished)[7] although the changes may be small. However, these studies have concentrated on overall AML blasts irrespective of whether they belong to the progenitor compartment or not. A more important consideration is whether the progenitors of AML can be recruited, as these are the cells which are believed to contribute to the expansion and maintenance of the disease.

We have analyzed the percentages of cells in S-phase before and after GM-CSF administration, showing that there is a small increase in the mature $BrdU^+$ cell population with GM-CSF. However, if the immature subpopulations are studied, using double marker analysis, a considerable increase of S-phase cells in the $CD34^+$ subset is seen. Therefore, although the overall results indicate that in some patients GM-CSF administration produces only small changes, when cells belonging to the progenitor compartment are considered, more dramatic increases are seen. It is not yet possible to say how these findings will correlate with clinical results but they confirm that GM-CSF does indeed produce profound effects on the leukemic blasts in the blood and in the marrow, when given *in vivo* to patients with AML.

## Conclusion

The development of biological therapy for AML is still at an early stage. GM-CSF appears to be a good candidate for use in this context, though other cytokines may also be used in the future, perhaps in combination with GM-CSF. In the next few years

the first clinical results will become available, indicating whether any benefit may be derived from the use of cytokines in patients with AML.

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