Acute Myeloid Leukemia

In vivo priming with granulocyte colony-stimulating factor possibly enhances the effect of gemtuzumab-ozogamicin in acute myeloid leukemia: results of a pilot study

Eight elderly patients with relapsed or refractory acute myeloid leukemia were treated sequentially with recombinant human granulocyte colony-stimulating factor with rhG-CSF and Mylotarg. Priming with rhG-CSF *in vivo* induced an increase in the proportion of CD33$^+$ cycling blasts. Four patients (50%) achieved a complete remission, 2 patients had a partial remission and the other 2 were resistant.

haematologica 2004; 89:634-636
(*http://www.haematologica.org/journal/2004/5/634)*

Mylotarg (gemtuzumab-ozogamicin (GO), recently approved in the USA and in Europe for the treatment of elderly patients with relapsed acute myeloid leukemia (AML), administered as a single agent results in overall response rates of about 30%.[1,2] GO selectively targets CD33$^+$ cells. Although nearly 80% of AML cells express the CD33 antigen, the intensity of expression is variable. CD33$^-$ blast cells may escape killing by agents such as GO. A substantial increase in the percentage of CD34$^+$/CD33$^+$ cells was found by several groups including ours in CD34$^+$ peripheral blood cells, mobilized by rhG-CSF in healthy donors, while in patients treated with chemotherapy and rhG-CSF, up to 95% of mobilized CD34$^+$ cells are CD33$^+$.[3,4]

We administered a sequential treatment with rhG-CSF and GO to relapsed elderly AML patients in order to increase CD33 expression on the surface of AML cells and ultimately to improve the cytotoxic effect of GO in these poor prognosis patients.

Between November 2002 and August 2003, 8 patients (5 males and 3 females), with a median age of 71 years (range 61-79 years), who had relapsed or refractory AML received 5 mg/kg rhG-CSF subcutaneously for 3 days (days 1 to 3), followed on day 5 by 9 mg/m^2 GO as induction treatment. The same protocol was repeated on day 21 or later according to the patients clinical conditions and following peripheral blood and bone marrow evaluation.

A total of 16 courses of combined therapy were administered. Bone marrow and peripheral blood were examined on days 0, 5, 21 and then every week to quantify the percentage of blasts and their CD34 and CD33 expression.

To determine whether the rhG-CSF-induced expression of CD33 was associated with significant cell expansion, AML blast cells were loaded with the fluorescent probe CFDA-SE before cytokine treatment. Of interest, incubation of AML blast cells with exogenous rhG-CSF was associated with the onset of cell proliferation (Figure 1). Collectively, these data suggested that CD33 antigen is significantly up-regulated on AML blasts exposed to rhG-CSF and that CD33$^+$ AML cells are particularly sensitive to the growth-promoting effect of rhG-CSF.

The clinical characteristics of patients are reported in Table 1. None of the patients had a leukocyte count higher than 10×10^9/L either before or after *in vivo* priming with rhG-CSF [median leukocyte count 3.1 (range 0.7-7.3) and 4.1×10^9/L (0.9-6.3), respectively].

An infusion-related reaction was observed in only 1 patient. All patients developed profound and prolonged bone marrow aplasia. The median duration of neutropenia (defined as neutrophil counts < 0.5×10^9/L) after the first treatment course was 22.5 days (range 11-59), while the median duration of thrombocytopenia (defined as platelet counts < 50×10^9/L) was 24 days (range 16-43). The second therapy course was administered at a median of 37.5 days after the first course (range 19-60).

Four patients achieved a CR (50%) after the first course,

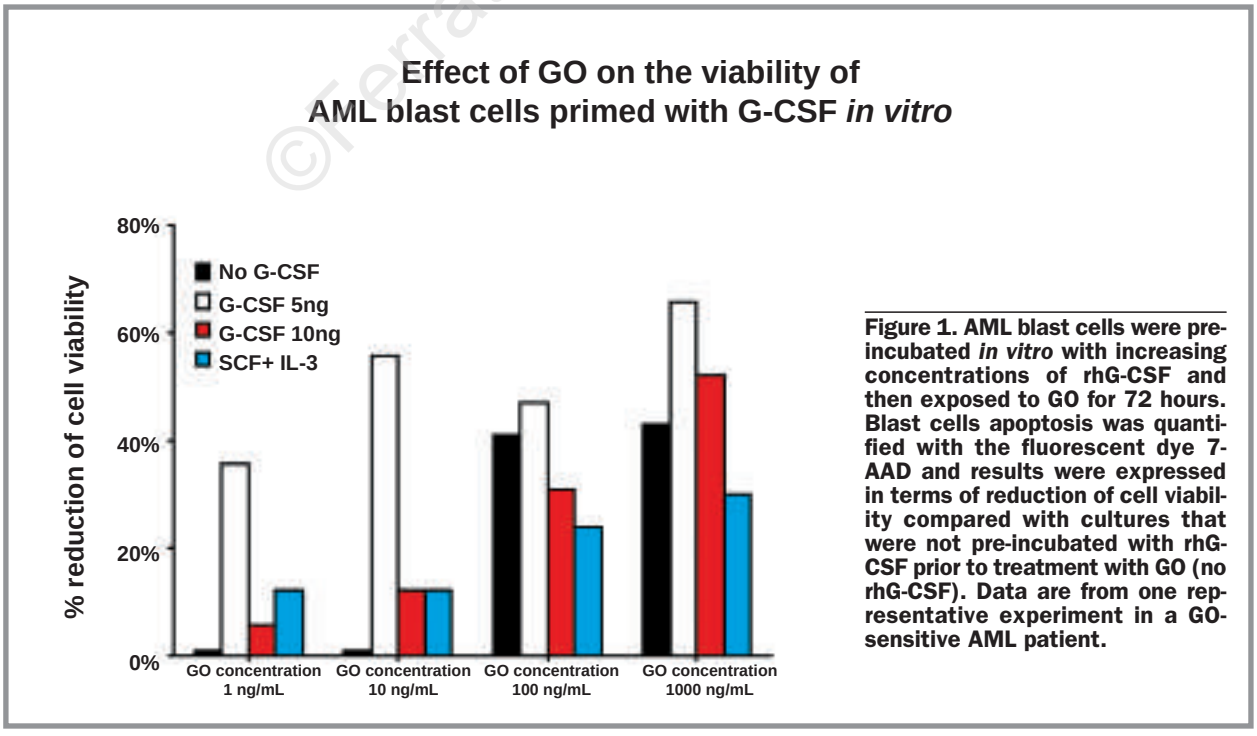

Figure 1. AML blast cells were pre-incubated *in vitro* with increasing concentrations of rhG-CSF and then exposed to GO for 72 hours. Blast cells apoptosis was quantified with the fluorescent dye 7-AAD and results were expressed in terms of reduction of cell viability compared with cultures that were not pre-incubated with rhG-CSF prior to treatment with GO (no rhG-CSF). Data are from one representative experiment in a GO-sensitive AML patient.

Table 1. Characteristics of patients.

Sex (M/F)	*5/3*	
Median Age (range) y.o.	72 (61-77)	
FAB-subtype:		
M0	1	
M1	1	
M2	4	
Post-MDS	2	
Phase of AML		
Relapse	7	
Duration (weeks) of first remission (mean and range)	60 (6-132)	
Resistant	1	
CD33 expression (median, range)		
Before rhG-CSF	42 (8-71)	*P*=0.0016
After rhG-CSF	47 (33-96)	
CD34 expression (median, range)		
Before rhG-CSF	21.8 (0.7-43)	
After rhG-CSF	43 (1.7-44)	*P*=0.0115
CD33/34 expression (median, range)		
Before rhG-CSF	70 (0.6-81)	*P*=0.0001
After rhG-CSF	82 (1,6-99)	
Haematological recovery from GO [median days (range)]:		
neutrophils recovery (>0.5×10^9/L)	22.5 (11-59)	
platelets recovery (>50×10^9/L)	24 (16-43)	
Outcome		
CR	4 (50%)	
Hematological improvement	2 (25%)	
Resistant	2 (25%)	
CR duration (in 4 patients in CR) weeks	19 (11-33)	

while 2 other patients obtained only a transient hematologic improvement, characterized by a peripheral increase of all hematologic parameters and by a 30% reduction of the bone marrow blast count. Two further patients were unresponsive and died of leukemia progression after the second course of Mylotarg. All patients but the one who died in CR of VOD, relapsed and the median CR duration in responsive patients was 19 weeks (range 11-33).

Toxicity was evaluated according to the WHO grading. The main extrahematologic toxicity involved the liver and was observed in 5 patients. Two of these 5 patients developed hyperbilirubinemia (a bilirubin increase of 8- and 3-fold the normal value), which was transient and completely resolved without any specific therapy after 17 and 5 days, respectively. The other 3 patients developed veno-occlusive disease (VOD) (37.5%) and in all of them it was the main cause of death. In fact one patient died, still in CR, of liver failure. All patients had, either during the first or second treatment course, fever of unidentified origin that required broad-spectrum antibiotics. Two patients who had developed a pulmonary aspergillosis during the induction of the first CR, showed fungal infection reactivation and required anti-mycotic treatment. All patients recovered from the infectious complications without any sequelae. No major hemorrhagic complications were observed. At present 5 patients are still alive, and the median overall survival is of 17 weeks (5-36).

This pilot study suggests that the efficacy of targeted drugs may be increased by specific modulation of the target antigen. Myeloid blast cell CD33 expression was up-regulated by rhG-CSF *in vitro*, and priming with rhG-CSF *in vivo* induced an increase in the proportion of CD33$^+$ blasts in a cohort of elderly patients with relapsed AML. Subsequent GO administration resulted in a notable CR rate.

Cytokines have been extensively used in AML prior to chemotherapy to sensitize leukemic blasts to the cytotoxic effects of S-phase-specific drugs, with good results in older patients treated with low-dose chemotherapy.[5,6] In our leukemic patients, rhG-CSF increased, *in vivo* and *in vitro*, the proportion of CD33-positive blasts, with a high proliferative potential *in vitro*.

From a clinical point of view the association of rhG-CSF and GO gave encouraging results. Despite their previous extensive treatment, 50% of patients responsed to this novel therapeutic approach. Unfortunately the consistent CR percentage obtained was not followed by prolonged disease-free survival. Furthermore liver toxicity was a major problem. In the normal human liver CD33 is expressed by Küpffer cells, and possibly by hepatocytes near portal areas and sinusoidal endothelial cells,[7] G-CSF could increase CD33 expression also at this level, thus increasing the hepatic toxicity.

In conclusion, our preliminary *in vitro* and *in vivo* results, although based on only a few cases, suggested that priming with rhG-CSF could increase the efficacy of GO by up-regulating the proportion of CD34$^+$CD33$^+$ cycling AML blast cells. Nevertheless, therapy with the GO alone appears not to be able to eradicate the leukemic clone. Combining an antiproliferative drugs (e.g. Ara-C) with lower doses of GO could enhance the CR duration without increasing toxicity.

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Funding: This work was supported by a grant from M.U.R.S.T. (Ministero dell'Università e della Ricerca Scientifica e Tecnologica).

Key words: acute myeloid leukemia, elderly, gentuzumab-ozogamicin, rhG-CSF.

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