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.

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/m² 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 $10^9/L$ either before or after in vivo priming with rhG-CSF [median leukocyte count 3.1 (range 0.7-7.3) and $4.1\times10^9/L$ (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,
while 2 other patients obtained only a transient hemato-
logic 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 devel-
oped hyperbilirubinemia (a bilirubin increase of 8– and 3-
fold the normal value), which was transient and complete-
ly 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
dause 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 devel-
oped 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 tar-
get 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. Subse-
quent 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 old-
er 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 prolif-
erative 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 responded to this nov-
el 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 Kupffer cells,
and possibly by hepatocytes near portal areas and sinusoidal
endothelial cells,6 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 prim-
ing 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.

Giuseppe Leone, Sergio Rutella, Maria Teresa Voso,
Luana Fianchi, Alessandra Scardocci, Livio Pagano
Istituto di Ematologia, Università Cattolica
del Sacro Cuore, Rome, Italy

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.

Correspondence: Giuseppe Leone, M.D., Istituto di Ematologia,
Università Cattolica del S. Cuore, Largo F. Vito 1, 00168 Rome,
Italy. Fax: international +39.06.3051343. E-mail: gleone@rm.uni-
catt.it

Table 1. Characteristics of patients.

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<thead>
<tr>
<th>Sex (M/F)</th>
<th>5/3</th>
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<tr>
<td>Median Age (range) y.o.</td>
<td>72 (61-77)</td>
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<td>Phase of AML</td>
<td>7</td>
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<td>Duration (weeks) of first remission (mean and range)</td>
<td>60 (6-132)</td>
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<td>CD33 expression (median, range)</td>
<td>Before rhG-CSF 42 (8-71) P=0.0016 After rhG-CSF 47 (33-96)</td>
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<td>CD34 expression (median, range)</td>
<td>Before rhG-CSF 21.8 (0.7-43) After rhG-CSF 43 (1.7-44) P=0.0115</td>
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<td>CD33/34 expression (median, range)</td>
<td>Before rhG-CSF 70 (0.6-81) P=0.0001 After rhG-CSF 82 (1.6-99)</td>
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<td>Haematological recovery from GO [median days (range)]:</td>
<td>22.5 (11-59) (&gt;0.5×10^9/L) 24 (16-43) (&gt;50×10^9/L)</td>
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<td>Outcome</td>
<td>CR 4 (50%) Hematological improvement 2 (25%) Resistant 2 (25%)</td>
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<td>CR duration (in 4 patients in CR) weeks</td>
<td>19 (11-33)</td>
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References