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ORIGINAL ARTICLE

Gemtuzumab ozogamicin with cytarabine and mitoxantrone as a third-line treatment in a poor prognosis group of adult acute myeloid leukemia patients: a single-center experience

G. Specchia · D. Pastore · P. Carluccio · G. Spinosa · M. Giannoccaro · R. Rizzi · A. Mestice · V. Liso

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Abstract We analyzed the safety and efficacy of gemtuzumab ozogamicin (GO) combined with cytarabine and mitoxantrone in the treatment of 21 patients with acute myeloid leukemia (11 refractory and 10 in second relapse). Patients' median age was 52 years (range 36–68); all patients had previously been treated with anthracycline-containing regimens (daunorubicin and idarubicin). GO at a dosage of 3 mg/m^2 was administered as a 2-h intravenous infusion on days 1 and 14, cytarabine at 100 mg/m^2 on days 1–7, and mitoxantrone at 12 mg/m^2 on days 1–3. Infusion-related events were observed in 15 of 21 (71.4%) patients. The incidence of grade 1 or 2 elevations of bilirubin and hepatic transaminases was 4 of 21 (19%) and 3 of 21 (14.2%). In response to chemotherapy, 2 of 21 (9.5%) achieved complete remission and 2 of 21 (9.5%) achieved complete remission with incomplete platelet recovery, with an overall remission rate of 4 of 21(19%); median survival of these 4 patients was 7 months. Four of 21 patients (19%) died during aplasia after chemotherapy; no veno-occlusive disease occurred. No treatment-related cardiotoxicity or cerebellar toxicity was observed. In our experience, the addition of GO to mitoxantrone and cytarabine is feasible in refractory or second relapse acute myeloid leukemia patients but yields a low response rate when used as a third-line treatment.

Keywords Acute myeloid leukemia · Refractory · Relapsed · Gemtuzumab ozogamicin

G. Specchia (✉) · D. Pastore · P. Carluccio · G. Spinosa · M. Giannoccaro · R. Rizzi · A. Mestice · V. Liso
Hematology Section, DAP, University of Bari,
Piazza G. Cesare 11,
70124 Bari, Italy
e-mail: g.specchia@ematba.uniba.it

Introduction

Patients with refractory acute myeloid leukemia (AML) after two courses of chemotherapy or in second relapse have very poor prognosis [1–3, 14]. Most patients have already been exposed to intensive multiagent chemotherapy, and most reinduction regimens in current use cause substantial toxicity; such patients are not always eligible for intensive chemotherapy. The goal of therapy varies from temporary prolongation of life and palliation of symptoms to achievement of complete remission (CR). Antibody-targeted chemotherapy is presumed to be less toxic than conventional chemotherapy and has been developed for treatment of CD33-positive AML [4]. The antibody-targeted chemotherapy gemtuzumab ozogamicin (GO; Wyeth Laboratories, Philadelphia, PA, USA) consists of a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin; this antibiotic is believed to be released inside the lysosomes of the myeloblast, binding to DNA in the minor groove and causing DNA double-strand breaks and ultimately cell death [4–6]. The CD33 antigen is expressed on approximately 90% of AML blasts, including leukemic clonogenic precursors as well as normal myeloid precursor cells, but not on CD34+ pluripotent hematopoietic stem cells [11, 18]; nevertheless, a recent report showed that many human hematopoietic stem cells express myeloid markers, overturning the dogma that hematopoietic stem cells are devoid of lineage-associated markers [15]. So GO may be a targeted treatment for patients with CD33-positive AML because the antibody component of the molecule targets calicheamicin [4]. Several pilot and phase II studies of GO and cytotoxic chemotherapy combination regimens as second-line treatment have been conducted in relapsed/refractory AML patients, with different results (20–42% CR) [1, 3, 6, 16]. We conducted a study of GO combined

with mitoxantrone and cytarabine as third-line treatment in a homogeneous "poor prognosis" group of patients with refractory/relapsed AML to evaluate the safety and toxicity of the drug in this group of patients.

Materials and methods

Between October 2004 and January 2006, after obtaining informed consent, 21 AML patients received GO, cytarabine, and mitoxantrone. Eleven patients were refractory after DCE (daunorubicin, cytarabine, and etoposide) as first-line therapy and FLAG-IDA (fludarabine, cytarabine, idarubicin and G-CSF) as second line; ten patients were in second relapse and were treated with DCE at onset of disease and with FLAG-IDA in first relapse [13]; this "poor prognosis" group was therefore homogeneous as regards previous chemotherapy regimens. In the group in second relapse, the median duration of first and second CR was 10 and 6 months, respectively; the median time in refractory patients from first- and second-line therapy to GO was 3 and 1 month, respectively. During the first-line therapy with DCE, the most severe non-hematological side effects were mucositis (70%), diarrhea (35%), and increased bilirubin (20%); during the second line with FLAG-IDA, the worst non-hematological side effects were mucositis (65%) and increased bilirubin (30%) [13]. The patient group included 12 men and 9 women with a median age of 52 years (range 38–68; Table 1); the median white blood cell (WBC) and marrow blast cell count was 13×10^9/l (range 3–25×10^9/l) and 38% (range 31–90), respectively; median CD33 positivity was 62% (range 22–95). According to the French-American-British classification, three patients had M1, ten M2, five M4 and three M5. According to Grimwade et al. [8], two patients were in the favorable cytogenetic group, four in the intermediate-risk group, and 15 in the poor-risk group. Eligibility for this study included CD33 expression on >20% of blasts, no hepatic disease or bilirubin >2 mg/dl, and WHO performance status 0–2. GO was administered at 3 mg/m^2 intravenously on days 1 and 14, mitoxantrone 10 mg/m^2 daily on days 1 through 3, and cytarabine as a continuous infusion 100 mg/m^2 daily on days 1 through 7; premedication before GO with acetaminophen and antihistamines was routinely given to prevent or reduce infusion reactions. CR was defined as a bone marrow containing fewer than 5% blast cells and a blood smear free of leukemic blasts; the absolute neutrophil count (ANC) had to be at least 1.5×10^9/l and the platelet count greater than 100×10^9/l. On the basis of phase 1–2 clinical results indicating that in some responders to GO a delay in platelet recovery may occur, the additional category of complete remission with incomplete platelet recovery (CRp) was introduced; patients with CRp met all CR parameters with the exception of full platelet recovery.

Table 1 Clinical and laboratory characteristics of 21 AML patients

	Data
Age (years) (range)	52 (36–68)
Sex (M/F)	12/9
Second relapse (%)	10 (47.6)
Refractory at second-line therapy (%)	11 (52.4)
Median WBC $\times10^9$/l (range)	13 (3–25)
% Median bone marrow blasts (range)	38 (31–90)
% Median CD33 positivity (range)	62 (22–95)
Karyotype	
Favorable risk (%)	2 (9.5)
Intermediate risk (%)	4 (19)
Poor risk (%)	15 (71.5)

Results

The overall CR rate was 4 of 21 (19%), 3 of 10 (30%) in second relapse and 1 of 11 (9%) in refractory patients (Table 2); CR and CRp were achieved in 2 of 21 (9.5%) and 2 of 21 (9.5%), respectively; of these 4 responder patients, one was in the favorable cytogenetic group [t(8,21)] and three were in the intermediate (two with normal karyotypes and one with +8); no response was achieved in the poor cytogenetic risk group. Four patients died during therapy (overall treatment-related mortality, 19%), two due to sepsis (*Pseudomonas aeruginosa*), one to cerebral hemorrhage, and one to acute respiratory distress syndrome. Baseline characteristics including age, type of AML, WBC count, and marrow blasts were not predictive of treatment in this study. Furthermore, the CR/CRp rate after GO, cytarabine, and mitoxantrone was not influenced by CD33 positivity. All patients experienced profound neutropenia ($<0.1\times10^9$/l); in patients achieving CR/CRp, the median time to reach ANC $>0.5\times10^9$/l and 1×10^9/l was 28 days (range 21–33) and 34 days (range 29–39), respectively. Grade III–IV anemia and thrombocytopenia were observed in all cases; a grade I/II bilirubin increase occurred in 4 of 21 cases (19%; Table 3); grade I/II ALT/AST elevation was documented in 3 of 21 cases (14.2%); grade I/II alkaline phosphatase elevation was observed in 3

Table 2 Results of treatment with GO, ARA-C, and MITOX of 21 AML patients

	Number of patients (%)
Overall survival	4 (19)
CR	2 (9.5)
CRp	2 (9.5)
Treatment-related mortality	4 (19)

Table 3 Treatment-related toxicity

	Grade 1	Grade 2	Grade 3	Grade 4
Infusion-related events	3	8	5	
Fever	3	5	5	
Dyspnea		2		
Arrhythmia		1		
Hepatic dysfunction	6	4		
Transaminases increase	2	1		
Bilirubin increase	2	2		
Alkaline phosphatase	2	1		
Neutropenic fever		14	5	
Infection			1	4
Hemorrhage	3	2	1	1
Nausea/vomiting	5	12		

of 21 (14.2%) cases; no veno-occlusive disease (VOD) occurred. The most common non-hematologic adverse events were infusion-related allergic reactions (16 of 21 or 76.1%), infection (5 of 21 or 23.8%), and febrile neutropenia (19 of 21 or 90.4%). The median age in the four patients who achieved CR and CRp was 50 years, versus 52 years in the refractory group. The four responder patients were treated with GO (3 mg/m^2 monthly for 3 months); three out of the four responding patients relapsed after 3, 4, and 6 months, respectively, and died after 5, 6, and 8 months, respectively; these patients did not reach allogeneic stem cell transplantation due to the lack of a human leukocyte antigen-compatible donor or comorbidity; the other patient, the only one to undergo allogeneic stem cell transplantation, is still in CR at 8 months after the administration of GO, cytarabine, and mitoxantrone.

Discussion

The prognosis of refractory AML after two courses of chemotherapy, or in second relapse, is very poor [1, 14]. The treatment of these patients continues to be a controversial issue because most of them have already been exposed to intensive multiagent chemotherapy, and refractory AML patients have a higher incidence of poor-risk cytogenetic abnormalities and expression of the multidrug resistance phenotype [1–4]. In this "poor prognosis" group, treatment options are limited and are associated with extensive adverse effects; thus, there is an urgent need for novel therapeutic options. In particular, monoclonal antibodies, as carriers of antileukemic drugs, have been proposed as potential therapeutic agents in AML. The CD33 antigen is expressed on approximately 90% of AML myeloblasts, including leukemic precursor as well as normal myeloid precursor cells [3, 5, 6]. Several pilot and phase II studies of GO and cytotoxic chemotherapy combination treatments have been conducted as second-line therapy in AML. In a phase II study [16] in CD33-positive primary refractory patients or in first relapse of AML, the GO, fludarabine, cytarabine, and Cyclosporine (CsA) combination regimen resulted in a CR rate of 28% and a CRp of 6%; in this study (with GO at 9 mg/m^2), the most serious toxicity was hepatotoxicity; grade 3 and 4 hyperbilirubinemia occurred in 44% of patients, grade 3 and 4 elevation of hepatic enzymes occurred in 18% of patients, and 3 of 32 (9%) developed hepatic VOD and died. In another study [1], the GO and idarubicin and cytarabine combination resulted in a response rate of 42% (CR 21%, CRp 21%) in primary resistant or in first relapse AML patients, with 43% treatment-related mortality. Other studies [3, 6] reported an overall CR of 12–18% with GO combined with chemotherapy (topotecan and cytarabine or liposomal daunoroubicin, cytarabine, and CsA) as second-line therapy in refractory AML. These pilot studies, with GO and chemotherapy as second line in primary refractory or AML in first relapse, showed that although GO and cytotoxic chemotherapy combination regimens are feasible and effective, they are associated with significant toxicity, particularly hepatotoxicity. To our knowledge, the combination of cytarabine-mitoxantrone with or without other drugs [7, 9, 10, 12, 17] has previously been used in refractory (after first-line therapy) or first relapse AML patients but not as third-line therapy. In our study, we examined a homogeneous "poor prognosis" group as regards previous chemotherapy (DCE and FLAG-IDA); all patients were refractory after two lines of chemotherapy or in second relapse, and we observed a lower CR rate with GO and chemotherapy as third line compared with other studies with GO and chemotherapy as second-line treatment [1, 3, 6, 16]. Treatment of patients with refractory AML or in relapse typically requires intensive supportive care; in the present study, neutropenia was more prolonged than after FLAG-IDA [10] in refractory or first relapse AML patients (19 and 28 days for ANC>0.5×10^9/l, respectively), suggesting that aplasia after GO lasts longer than after other therapy without GO, probably because of a particular cytotoxicity of GO to human stem cells [5, 15]. In our study, no VOD occurred, and we observed a lower hepatotoxicity compared with other studies [1, 3, 6, 16], probably because of the lower dosage of GO (3 mg/m^2 on days 1 and 14). The increasing GO dosage may, however, be complicated by increased severe side effects, including VOD; alternatively and preferably, GO could be administered after reducing the leukemic cell burden by conventional chemotherapy. In conclusion, we suggest that the addition of GO to mitoxantrone and cytarabine as third-line therapy is feasible in relapsed/refractory AML patients, showing acceptable toxicity and a 19% overall response rate. The possible role of GO combined with chemotherapy should be tested in terms of efficacy in larger series as second-line therapy. A better understanding of the GO mechanism of

action will hopefully contribute to the design of future treatment protocols that maximally exploit the potency of GO with low toxicity.

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