Acute Myeloid Leukemia

Molecular response in two children with relapsed acute myeloid leukemia treated with a combination of gemtuzumab ozogamicin and cytarabine

Phase I/II studies of gemtuzumab ozogamicin (GO) in pediatric refractory/relapsed acute myeloid leukemia (AML) have been reported. We present the cases of two children with relapsed AML who were treated with GO plus cytarabine, leading to a decrease of minimal residual disease down to levels not previously obtained. The toxicity profile of this treatment was relatively mild, except for severe but manageable myelosuppression.

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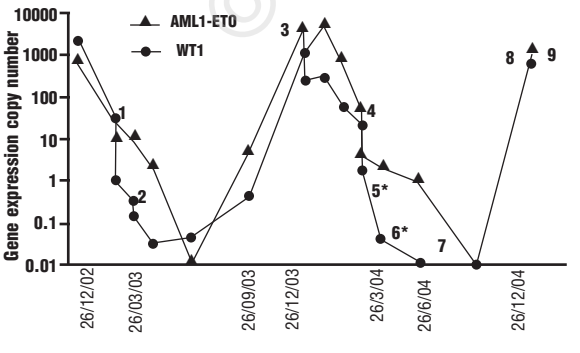
The 5-year event-free survival of children with acute myeloblastic leukemia (AML) is about 50%. Since approximately 85% of cases express the CD33 surface antigen,[1] the use of gemtuzumab ozogamicin (GO), a monoclonal anti-CD33 antibody linked to the cytotoxic calicheamicin,[2] has been investigated in AML. The feasibility and efficacy of GO have been demonstrated in phase I/II trials in adults with relapsed AML[3,4] and in children with refractory/relapsed AML.[5,6] The response rates are 20 to 30%. Severe myelosuppression is commonand sinusoidal obstructive syndrome (SOS) may occur in 5 to 36% of the cases.[3,4,7] So far there are no reports of GO in association with chemotherapy in children. We report here on two children with very early relapsed AML treated with GO plus cytarabine. These children were chosen because of the severity of their disease together with a molecular tool allowing the efficacy of this treatment to be assessed.

Patient #1. A 13-year old girl was diagnosed with *AML1-ETO* positive AML. Minimal residual disease (MRD) remained detectable after completion of conventional chemotherapy based on the French ELAM02 protocol (Figure 1). A bone marrow relapse occurred 8.6 months after the girl had achieved first complete remission. A second complete remission was obtained with high-dose cytarabine plus amsacrine. The same course gradually reduced the level of *AML1-ETO* transcripts. Because of leukemic cell surface positivity, for CD33, the girl was given two courses of a combination of cytarabine (3 g/m^2/12h, days 1 and 2) and GO (3 mg/m^2, day 3). The only serious adverse event was the expected grade IV myelosuppression. For the first time, *AML1-ETO* fusion transcripts became undetectable. She underwent intensified therapy based on autologous stem cell transplantation. Mild SOS occurred on day 20 but resolved in less than a week. Unfortunately, a second morphological relapse occurred 6 months after the autologous transplant. Interestingly, the duration of the second complete remission was longer than that of the first (10.0 vs 8.6 months).

Patient #2. A 13-year old boy was diagnosed with *TLS/FUS-ERG* positive AML, which was refractory to mitoxantrone-cytarabine induction therapy. A first complete remission was obtained after a second course containing high-dose cytarabine. Two months after achieving first complete remission he underwent a stem cell transplant from a matched unrelated donor. MRD was still detectable just before the transplant (Figure 2) and increased rapidly after it; morphological relapse was documented 5.5 months after the stem cell transplant. After confirming that the leukemic blasts, were positive for surface CD33 the boy was given a combination of GO (three doses of 3 mg/m^2, days 1, 4 and 7) and cytarabine (100 mg/m^2/day on 7 consecutive days). Complete response without total platelet recovery was obtained on day 38. As no serious adverse event occurred, except grade IV myelosuppression, a second course of GO (one dose of 3 mg/m^2) and cytarabine (same regimen as above) was given without unexpected toxicity.

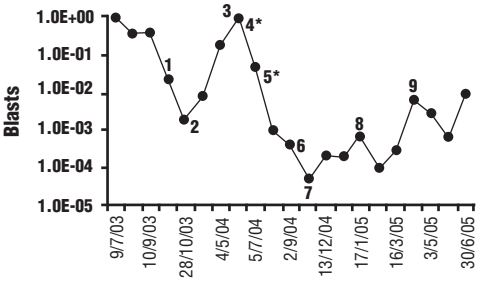


1. First complete remission
2. First course of consolidation therapy
3. Hematopoietic relapse 8.5 months after first complete remission
4. Second complete remission after high-dose cytarabine
5* and 6*. Two consolidation courses of gemtuzumab ozogamicin
8. Second relapse 6 months after autologous stem cell transplant
9. Death 5 months after relapse in progressive disease and with invasive aspergillosis

- Grade IV immunosuppression
- Sinusoidal obstruction syndrome day 20 after autologous stem cell transplant

Real time quantitative RT-PCR amplification of *AML/ETO* fusion transcripts was performed on a Light Cycler System (Hoffman-Roche) using the forward primer: CACCTACCACAGAGCCATCAAA, reverse primer: ATCCACAGGTGAGTCTGGCATT, and TaqMan probe: FAM-AACCTCGAAATCGTACTGAGAAGCACTCCA-TAMRA (*Gabert et al. Leukemia, 2003;17:2318-57).* Quantitative results were normalized as copy numbers of the target gene against copy numbers of the endegeneous control *TBP* x1000 (*Bièche I et al. Clin Chem; 1999; 45:1148-56*).

Figure 1. Case #1. Quantitative evaluation of *AML1-ETO* transcripts by real time polymerase chain reaction during treatment.



1. First complete remission
2. MUD-SCT n.1 (12 Gy TBI+cyclophosphamide); GVHD - II day 12
3. Hematologic relapse 7 months after first complete remission
4* and 5*. Induction and consolidation course n.1 . Containing GO +cytarabine
6. Crp2 and consolidation courses n. 2 and 3
7. MUD-SCT n. 2 (busulfan+cyclophosphamide); GVHD - II day 18
8 and 9. Chronic GVHD

- Grade IV myelosuppression
- No sinusoidal obstruction syndrome

CRp: complete remission without total recovery of platelets; MUD-SCT: matched unrelated donor stem cell transplantation; TBI: total body irradiation; GO: gemtuzumab ozogamycin; GVHD: graft-versus-host disease.

RTQ-PCR amplification of *TLS/FUS/ERG* fusion transcripts was performed on an ABI 7700 as described by *Gabert et al. Leukemia 2003;17:2318-57*, using the forward primer: 6/9 785 F: ATGAACCCAGAGGTCGTGGA, reverse primer: 6/9 935 R: TCTTGAACTCCCCGTTGGTG, Taqman probe: Erg6/9 889P: FAM-CAACTCCAGCTGCATCACCTGGGAA.TAMRA. RTQ-PCR data were normalized according to *ABL* gene expression (*Beillard E et al. Leukemia 2003;17:2474-86*) in the diagnostic sample. Follow-up samples were quantified in comparison with diagnostic tumoral blood sample and the results were expressed as dilution equivalents of the diagnostic sample.

Figure 2. Case #2. Quantitative evaluation of *TLS-ERG* transcripts by real time quantitative polymerase chain reaction (RTQ-PCR) during treatment.

The level of MRD fell far below that after any previous treatment. Thirteen months after the first dose of GO and 9 months after a second transplant from a matched unrelated donor, the boy was still in morphological second complete remission but MRD increased. Again, the duration of the second complete remission was longer than that of the first (12 vs 7.5 months).

To our knowledge, this is the first report on the use of GO combined with chemotherapy in pediatric AML. Both patients had a very poor prognosis due to a very early relapse of *AML1-ETO* positive AML or *TLS-ERG* associated AML.[8] Although the schedules and timing of therapy were different, MRD diminished rapidly, to levels far below those previously obtained with intensive multiagent chemotherapy and/or stem cell transplantation. The two children underwent further intensification therapy with stem cell transplantation. Not unexpectedly, a second morphological relapse was documented in the first case and a molecular relapse was documented in the second one.

The efficacy of combining GO with intensive chemotherapy as first-line treatment for AML has been assessed in 72 adult patients.[9] GO given as a single dose of 3 mg/m^2 on day 1 of induction chemotherapy produced complete remissions achieved CR in 91% of the patients, and 78% of these patients were in continuous complete remission at 8 months.

The concomitant infusions of GO and cytarabine were well tolerated. The only serious adverse event was severe myelosuppression, as expected. Hepatotoxicity had previously been described in patients receiving GO in addition to conventional chemotherapy and after stem cell transplantation.[10] The first child developed non life-threatening SOS 20 days after the conditioning regimen for stem cell transplantation, which resolved in a few days. SOS did not occur in the second patient, who received GO after a transplant from a matched unrelated donor, despite acute graft-versus-host disease involving the liver. This patient received a second transplant from an unrelated donor, and has not developed SOS.

In conclusion, in two children with relapsed AML, the combination of GO and chemotherapy gave better results in terms of blast clearance than previous high-dose chemotherapy and/or stem cell transplantation. Toxicity was manageable. Combination treatment with GO and chemotherapy deserves prospective evaluation in children with relapsed AML.

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