The role of gemtuzumab ozogamicin in acute leukaemia therapy

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Abstract

Gemtuzumab ozogamicin (GO) is an immunoconjugate that binds to CD33 on the surface of acute myeloid leukaemia (AML) blasts and, after internalisation, releases a cytotoxic drug, calicheamicin. GO is approved by the US Food and Drug Administration for the treatment of CD33-positive AML at first relapse in patients 60 years and older who are not candidates for other cytotoxic therapy. GO as a single agent has low antileukaemic activity. When given to patients meeting the criteria noted above, it produces a complete response (CR) rate of only 12%, with another 12% achieving CR with inadequate platelet recovery (CRp). The median survival of patients treated with GO monotherapy is 11–2 months. GO therapy at 9 mg/m² is complicated with hepatic veno-occlusive disease in 15–25% of patients, particularly prior to or following stem cell transplantation. GO at lower doses combined with chemotherapy as induction or postremission therapy is promising, however, and phase III trials are ongoing. GO is probably most active in acute promyelocytic leukaemia (APL). It is used for induction regimens in high-risk APL and for the elimination of minimal residual APL. Case reports suggest that GO also has activity in CD33-positive acute lymphoblastic leukaemia. In conclusion, single agent GO can induce responses in patients with CD33-positive AML in first recurrence. The future of GO is its use in combination with other cytotoxic agents. Ongoing clinical trials may better define the role of GO combinations, particularly in untreated AML.

Keywords: gemtuzumab, leukaemia, acute, acute myeloid leukaemia, therapy

In acute myeloid leukaemia (AML), high-dose cytarabine and anthracycline combination regimens induce remission in 70–80% of patients younger than 60 years of age, but with intensive postremission therapy, the 5-year leukaemia-free survival (LFS) rate is only 25–35% (Burnett, 1998). In patients older than 60 years, response rates are <50% and the long-term LFS rate is 0–15% (Stone et al., 2004). The poor outcome of older patients with AML reflects a higher incidence of abnormal leukaemia cell cytogenetics, multidrug resistance mechanisms (such as P-glycoprotein-mediated drug efflux), a low bone marrow reserve that prevents or delays recovery of haematopoietosis after treatment and co-morbidities.

In the past decade there has been interest in the development of therapies directed against antigens on the surface of malignant cells. These therapies aim to target leukaemic blasts but not affect normal haematopoietic stem cells or non-haematopoietic tissues. Gemtuzumab ozogamicin (GO) was the first such agent approved by the US Food and Drug Administration (FDA) for the treatment of AML (Bross et al., 2001).

This review focuses on current and emerging data on the use of GO in the treatment of acute leukaemias.

CD33 antigen

The CD33 antigen is a 67-kDa type 1 transmembrane glycoprotein that belongs to the immunoglobulin gene superfamily of sialic acid-binding immunoglobulin-related lectins (siglec-s; siglec-3) (Freeman et al., 1995). It was identified by the murine monoclonal antibody anti-MY9 (Griffin et al., 1984). The human CD33 gene has been mapped to chromosome 19q13.3 (Peiper et al., 1988) and most closely resembles the genes for two adhesion receptors, the myelin-associated glycoprotein and the B-cell antigen CD22 (Simmons & Seed, 1988).

CD33 is expressed on early multilineage haematopoietic progenitors, myelomonocytic precursors, and more mature myeloid cells, macrophages, monocytes and dendritic cells (Andrews et al., 1983, 1986; Griffin et al., 1984). It is highly expressed on granulocyte precursors, such as promyelocytes and myelocytes, but its expression decreases with maturation and differentiation, and little is found on mature granulocytes. CD33 is also expressed by hepatocytes (Tchilian et al., 1994; Gao et al., 2001) but is absent on normal, and leukaemic, pluripotent haematopoietic stem cells (Robertson et al., 1992).

Approximately 85–90% of adult and paediatric AML cases and 15–25% of acute lymphoblastic leukaemia (ALL) cases are considered CD33-positive, as defined by the presence of antigen on at least 20–25% of leukaemic blasts (Griffin et al., 1984; Dinndorf et al., 1986; Scheinberg et al., 1989; Terstappen et al., 1992; Putti et al., 1998). AML blasts contain large
amounts of CD33; flow cytometry studies showed a mean of 10 380 CD33 molecules per cell in the bone marrow and 9175 CD33 molecules per cell in the peripheral blood (Jilani et al., 2004).

The presence of CD33 on AML blasts motivated the development of monoclonal antibodies against this myeloid cell-surface antigen. The first anti-CD33 monoclonal antibody investigated in the treatment of AML was the radioiodinated M195 (Scheinberg et al., 1989, 1991). This drug that was rapidly and specifically delivered to the bone marrow and efficiently internalised into target cells, showed activity in patients with relapsed AML (Scheinberg et al., 1991; Jurcic et al., 1995). Subsequently an anti-CD33 antibody was combined with cytotoxic calicheamicin to produce CMA-676, later known as GO (Bernstein, 2000; Hamann et al., 2002).

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin (Mylotarg™, CMA-676; Wyeth Laboratories, Philadelphia, PA, USA) consists of a recombinant humanised immunoglobulin G4 (IgG4) anti-CD33 monoclonal antibody (hP67/6) conjugated to the antitumour antibiotic calicheamicin-γ1, N-acetyl-γ1-calicheamicin dimethyl hydrazide (Zein et al., 1988; Sievers et al., 1999). Calicheamicins are very potent DNA-binding agents that cause site-specific double-stranded cleavage (Zein et al., 1988). In addition, GO retains the immunoreactivity of the unmodified antibody and has specific cytotoxicity towards antigen-positive tumour cells in vitro and in vivo (Hinman et al., 1993).

Gemtuzumab ozogamicin entry into the cell depends on internalisation of the antibody. Such internalisation is a function of the number of CD33 molecules on the cell surface. The internalisation of antibody-bound CD33 is largely controlled by the CD33 cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (Walter et al., 2005). Mutations in these motifs prevent effective internalisation and reduce GO-induced cytotoxicity (Walter et al., 2005). A CD33-specific and a CD33-independent mechanism, which occurs in malignant cells with endocytic capacity, may be involved in GO internalisation (Jedema et al., 2004). After entering the cell, calicheamicin is released intracellularly (Hinman et al., 1993), causing G2 arrest and Chk1/Chk2 phosphorylation and/or induction of apoptosis through caspase activation (Amico et al., 2003).

The half-life of GO is 67 h, and a 2-week interval between doses of GO was chosen to prevent drug accumulation (Berger et al., 2002). Pharmacokinetic studies in adults and children with AML (Dowell et al., 2001; Sievers et al., 2001; Buckwalter et al., 2004) demonstrated that GO concentrations increase after the second dose, probably because of reduced tumour burden (Dowell et al., 2001). Although GO was not extensively distributed beyond the plasma compartment, no relationship was found between plasma concentration and response (Dowell et al., 2001). Pharmacokinetics were similar in children and adults (Buckwalter et al., 2004).

Gemtuzumab ozogamicin is approved by the FDA for treatment of patients with CD33-positive AML in first relapse who are 60 years or older and are not considered candidates for other cytotoxic chemotherapy. The approved dose is 9 mg/m² infused intravenously (i.v.) over 4 h and repeated in 14 days (Bross et al., 2001). However, GO is also administered in a 2-h infusion.

Phase I study of GO in AML

Forty patients with relapsed or refractory AML were entered into a phase 1 study (Sievers et al., 1999). Eight doses of GO (0·25, 0·5, 1, 2, 4, 5, 6 and 9 mg/m²) were evaluated. The 5 mg/m² dose saturated CD33 binding sites, but the maximum tolerated dose was 9 mg/m², at which there was >75% saturation of CD33 sites on leukaemic blasts and no severe non-haematological toxicity. Eight per cent of patients had grade 3 and 4 infusion-related fever, chills or hypotension, and 22% had grade 3 and 4 elevation of hepatic enzymes, particularly at the higher dose levels (33% at 5, 6 or 9 mg/m²). Leukaemic blasts were eliminated in 20% of patients, and blood counts were normalised in three (7·5%) patients [complete response (CR) = 2, CR with inadequate platelet recovery (CRp) = 1]. Clinical response was associated with low in vitro efflux of the P-glycoprotein (P-gp) substrate 3,3′-diethyloxacarbocyanine iodide (DiOC₂), indicating that resistance to GO is mediated by P-gp (Sievers et al., 1999).

Phase II studies of GO monotherapy in relapsed or refractory AML

Phase II clinical trials of GO monotherapy have recorded remarkably similar results, with an approximate CR rate of 13% and a CRp rate of 13% in patients with primary refractory or relapsed AML (Table I). Three phase II multicentre trials in the USA and Europe were the first to investigate GO monotherapy in patients with AML in first relapse (Sievers et al., 2001). A total of 142 patients were treated at a dose of 9 mg/m² on days 1 and 15. The median age was 61 years. Among 97 patients with available cytogenetic data at the time of relapse, 39% were in the poor-risk group and 5% were in the favourable-risk group. The median duration of first CR (CR1) before GO treatment was 11 months (range, 3–117 months) and 94% of patients had received postremission therapy following CR1. Twenty per cent of patients did not receive the recommended second dose (day 15) of GO because of disease progression and/or infection. GO induced a CR in 16% of patients, and approximately 13% of patients achieved a CRp, defined as fewer than 5% bone marrow blasts, recovery of neutrophils to at least 1500/µl, and platelet count <100 000/µl, although patients were platelet- and red blood cell-transfusion independent (Sievers et al., 2001). The median time to remission was 60 days. Multivariate analysis suggested that higher response rates were associated with higher baseline haemoglobin levels, lower circulating blast counts, lack of...
CD13 expression and lower levels of baseline multidrug resistance efflux. Multivariate analysis for survival suggested that favourable performance status, longer duration of CR1, lower circulating blast counts and lack of CD34 expression predict longer survival. First infusion-related toxicity occurred in 34% of patients (second infusion, 12%) and hypotension occurred in 4% of patients several hours after the infusion. Myelosuppression was noted in nearly all patients (grade 3 and 4 neutropenia, 93%; grade 3 and 4 thrombocytopenia, 99%). Twenty-eight per cent of patients developed grade 3 and 4 infections. Notably, grade 3 and 4 elevations in bilirubin and hepatic enzymes occurred in 23% and 17% of patients, respectively, after a median time of 8 and 20 days, respectively; two deaths were associated with these complications (Sievers et al, 2001). On the basis of these results, the FDA approved GO monotherapy in 2000 (Bross et al, 2001).

The initial report (Sievers et al, 2001) was followed by a detailed subset analysis (Larson et al, 2002) and a final report (Larson et al, 2005). In the latter report, 277 patients were treated with standard doses of GO (9 mg/m², 2-h i.v. infusion on days 1 and 15) (Larson et al, 2005). The median age was 61 years. In patients over 60 years of age, the CR rate was 12% and the CRp rate was 12%. In younger patients (<60 years) the response rates were slightly higher (CR = 13% and CRp = 14%). The median survival duration of patients treated with GO monotherapy was 11-2 months. The median recurrence-free survival duration was 6-4 and 4-5 months for patients who achieved CR and CRp respectively. Superior survival rates, but in some cases severe hepatotoxicity, were noted with subsequent allogeneic or autologous stem cell transplantation (SCT). Non-haematological toxicities included grade 3 or 4 hyperbilirubinaemia (29%) and hepatic transaminase elevations (Larson et al, 2005).

An Italian group has also conducted a phase II trial in 24 patients with relapsed or refractory AML, including five patients with myeloid sarcomas of the skin or bones (Piccaluga et al, 2004). The median age was 63 years (range, 20–75 years). Four patients had favourable karyotypes, 16 had intermediate and four had unfavourable karyotypes. Standard doses of GO were given for a maximum of three doses with 2-week intervals. Owing to toxicities, eight patients needed dose reductions to 6 mg/m² (n = 7) or 1.5 mg/m² (n = 1). The CR rate was 13% and the CRp rate 8%; the median response duration was 6 months. GO had activity in patients with myeloid sarcomas: four of these patients experienced tumour regression, and two patients also had clearance of the bone marrow blasts. The most common non-haematological toxicities were infections and elevation of bilirubin and hepatic enzymes. Hepatic veno-occlusive disease (VOD) occurred in one patient. Severe bleeding occurred in 21% of patients (Piccaluga et al, 2004).

Attempts to increase response rates to GO monotherapy prompted the sequential use of granulocyte-colony stimulating factor (G-CSF) and GO therapy in eight elderly patients with relapsed or refractory AML (Leone et al, 2004). Priming with rhG-CSF in vivo induced an increase in the proportion of CD33-positive cycling blasts, and responses were noted in six patients (CR = 4, partial response = 2) (Leone et al, 2004).

Clinical significance of CRp

The term CRp was first used to better describe patients treated with GO who did not meet the criteria for CR (Sievers et al, 2001). The clinical relevance of CRp has been questioned, and inferior clinical outcomes were reported in patients with CRp as opposed to those with CR in a final report of the three phase II multicentre trials in 277 patients (Larson et al, 2005). In this report, among 35 responders to GO treatment who received no further therapy, the relapse-free survival duration was 3-8 months in patients who achieved a CR (n = 17) compared with 2-4 months in patients who achieved a CRp (n = 18) (P = 0.007). Twenty-five of the 71 patients (35%) in the CR and CRp groups underwent subsequent SCT. When all responders to GO therapy were considered, irrespective of subsequent therapy, no significant difference in survival was shown between patients who achieved a CR and those who had a CRp, probably due to subsequent SCT in some patients (Larson et al, 2005). In our series, the survival duration of

<table>
<thead>
<tr>
<th>Group</th>
<th>No. pts</th>
<th>Age (years)</th>
<th>Median age (years)</th>
<th>Disease status</th>
<th>% CR</th>
<th>% CRp</th>
<th>Median survival (months)</th>
<th>Median LFS* (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piccaluga et al (2004)</td>
<td>24</td>
<td>20–75</td>
<td>63</td>
<td>Relapsed or refractory</td>
<td>13</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>EORTC/GIMEMA, Amadori et al (2005)</td>
<td>40</td>
<td>&gt;60</td>
<td>76</td>
<td>Untreated</td>
<td>10</td>
<td>7</td>
<td>4–3</td>
<td>6–1 (CR), 2–2 (CRp)</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukaemia; CR, complete response; CRp, complete response with inadequate platelet recovery; LFS, leukaemia-free survival; EORTC/GIMEMA, European Organization for Research and Treatment of Cancer/Gruppo Italiano Malattie Ematologiche dell’Adulto; pts, patients.

*Among responders.
patients who achieved a CRp was shorter than that of patients in CR but superior to that of non-responders (Estey et al, 2005).

Phase II studies of GO monotherapy in previously untreated patients with poor-risk AML

Gemtuzumab ozogamicin monotherapy was used as induction, consolidation and maintenance therapy in 12 patients 65 years or older (Nabhan et al, 2005) (Table I). Standard doses of GO were used as induction therapy. Consolidation therapy consisted of GO at 6 mg/m² given 45–60 d after induction upon recovery of the peripheral counts. Subsequently, GO was given four times at 3 mg/m² every 4 weeks for maintenance therapy. The response rate was 27%, and the median response duration was 7.6 months. The authors reported acceptable toxicities, but grade 3 and 4 cardiotoxicity was noted in 25% of patients (Nabhan et al, 2005).

The European Organization for Research and Treatment of Cancer – Leukaemia Group (EORTC-LG)/Gruppo Italiano Malattie Ematologiche dell’Adulti (GIMEMA) group has reported results of a standard-dose GO study in 40 patients older than 60 years who were not eligible for cytotoxic chemotherapy owing to advanced age or poor performance status (Amadori et al, 2005). The response rate was 33% in patients aged 61–75 years old but only 5% in older patients, and all induction deaths occurred in this group. This study suggested that reduced-dose GO should be used in patients older than 75 years (Amadori et al, 2005).

Phase II studies of GO and chemotherapy combination regimens in previously untreated patients with AML

Results of the initial phase II trials (Sievers et al, 2001) encouraged us to incorporate GO into induction combination regimens in untreated AML (Estey et al, 2002a; Tsimberidou et al, 2003a) (Table II).

In a randomised trial, patients 65 years or older with untreated AML or high-risk myelodysplastic syndrome (MDS) were randomised to receive GO with or without interleukin-11 (IL-11) (Estey et al, 2002a). GO was given at 9 mg/m² on days 1 and 8 or on days 1 and 15. In patients randomised to the combination arm, IL-11 was given at 15 µg/kg/d on days 3–28. The response rate was 8% (two of 26 patients) with GO monotherapy and 36% (nine of 25 patients) with GO and IL-11 combination therapy (P = 0.02), but survival was not significantly different between the two groups (median, 8 and 15 weeks respectively). Results were compared with those achieved with an idarubicin and high-dose cytarabine (IA) regimen in similar patients; IA therapy was associated with a CR of 48% and superior survival (P = 0.03) (Estey et al, 2002a).

<table>
<thead>
<tr>
<th>Group</th>
<th>Therapy</th>
<th>No. pts</th>
<th>Age (years)</th>
<th>Median age</th>
<th>Disease status</th>
<th>% CR</th>
<th>% CRp</th>
<th>Median LFS (months)*</th>
<th>Median survival (months)</th>
<th>% CRp</th>
<th>% CR</th>
<th>Median LFS (months)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDACC, Estey et al (2002b)</td>
<td>GO versus GO + IL-11</td>
<td>26 vs. 25</td>
<td>65</td>
<td>71</td>
<td>Untreated (AML 73%)</td>
<td>8 vs. 36</td>
<td>N/A, N/A</td>
<td>2 vs. 4</td>
<td>N/A, N/A</td>
<td>34 vs. 14</td>
<td>18</td>
<td>28 vs. 53</td>
</tr>
<tr>
<td>MDACC, Tsimberidou et al (2003a)</td>
<td>GO + BIDFA + CsA</td>
<td>59</td>
<td>27–76</td>
<td>57</td>
<td>Untreated (AML 66%)</td>
<td>45</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>18</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>MDACC, Tsimberidou et al (2003a)</td>
<td>GO + BIDFA + CsA (2003a)</td>
<td>32</td>
<td>&gt;18</td>
<td>57</td>
<td>First relapse or primary refractory</td>
<td>28</td>
<td>6</td>
<td>5</td>
<td>35</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EORTC/GIMEMA, Amadori et al (2004)</td>
<td>GO → MICE</td>
<td>57</td>
<td>61–75</td>
<td>68</td>
<td>Untreated</td>
<td>35</td>
<td>19</td>
<td>N/A</td>
<td>1-yr survival rate = 34%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AML, acute myeloid leukaemia; CR, complete response; CRp, complete response with inadequate platelet recovery; LFS, leukaemia-free survival; GO, gemtuzumab ozogamicin; IL, interleukin; CsA, cyclosporine; MICE, mitoxantrone, cytarabine and etoposide; EORTC/GIMEMA, European Organization for Research and Treatment of Cancer Group Malattie Ematologiche dell’Adulti; MDACC, MD Anderson Cancer Center; MICE, mitoxantrone, cytarabine and etoposide. * Among responders.

Included patients with AML and high-risk MDS; no difference in outcome by diagnosis.

In a phase II study of patients with untreated AML or high-risk MDS, GO was combined with fludarabine, cytarabine and ciclosporin (CsA) as a potential P-gp modifier (Tsimberidou et al, 2003a). Treatment consisted of GO (6 mg/m² i.v. on day 1), fludarabine and cytarabine (15 mg/m² and 0.5 g/m², respectively, twice daily on days 2–6) and CsA (6 mg/kg loading dose before GO, followed by 16 mg/kg continuous i.v. infusion on days 1 and 2). Sixty patients with prognostically intermediate-risk and unfavourable cytogenetics were treated. Their median age was 57 years (range, 27–76 years), 66% had AML and 34% had MDS. The response rate was 48% (CR = 46%, CRp = 2%), but the median survival duration was only 8 months, and the 1-year LFS rate was 27%. Response rates were similar in patients with AML and high-risk MDS.

This study demonstrated the feasibility of adding CsA to a GO and cytotoxic chemotherapy combination regimen. However, clinical outcomes and toxicities were unsatisfactory. Infections occurred in 38% of the courses of chemotherapy, grade 3 and 4 hyperbilirubinaemia occurred in 31% of patients, and elevation of hepatic enzymes occurred in 7%. Four patients (7%) developed VOD, as determined by the Seattle and Baltimore criteria (Bearman, 2000). Two of these four patients had predisposing risk factors, suggesting pre-existing liver pathology. Autopsy in one patient demonstrated hepatic VOD as the only evident pathology (Tsimberidou et al, 2003a).

The EORTC/GIMEMA group investigated GO followed by mitoxantrone, cytarabine and etoposide combination therapy in patients with untreated AML, aged 61–75 years (Amadori et al, 2004). The response rate appeared to be similar to that of cytotoxic therapy in untreated AML (CR = 23%; CRp = 12%), and this sequential therapy is being investigated in a phase III trial (Amadori et al, 2004).

Two recent studies suggested that the addition of GO to intensive chemotherapy may be associated with higher CR rates than intensive chemotherapy alone (De Angelo et al, 2003; Kell et al, 2003). Whether this improvement will result in the prolongation of LFS remains to be proven.

### Pilot and phase II studies of GO and chemotherapy combination regimens in relapsed or refractory AML

Several pilot and phase II studies of GO and cytotoxic chemotherapy combination regimens have been conducted as postremission (Tsimberidou et al, 2003b) or salvage (Cortes et al, 2002; Alvarado et al, 2003; Apostolidou et al, 2003; Tsimberidou et al, 2003c) therapy in AML (Table II).

In a phase II study in CD33-positive primary refractory or relapsed AML, the GO, fludarabine, cytarabine and CsA combination regimen resulted in a CR rate of 28% and a CRp rate of 6%. The overall median survival duration was 5.3 months (Tsimberidou et al, 2003c). The most serious toxicity was hepatotoxicity. Grade 3 and 4 hyperbilirubinaemia occurred in 44% of patients, and grade 3 and 4 elevation of hepatic enzymes occurred in 18% of patients. Three patients (9%) developed hepatic VOD (Bearman, 2000) and died. When compared with other salvage regimens used between 1991 and 2000, the GO and fludarabine, cytarabine and CsA combination regimen was associated with a higher CR rate (30% vs. 17–20%) in primary refractory patients whose CR1 lasted less than a year (Tsimberidou et al, 2003c). The same GO-containing regimen was used as postremission therapy in patients with AML who were in CR1 after a GO-based induction regimen (Tsimberidou et al, 2003b). Interestingly, no VOD was noted in this setting, probably because these patients were in CR and the tumour load was low (Tsimberidou et al, 2003b).

The GO and IA combination therapy – with intermediate-dose cytarabine – resulted in a response rate of 43% (CR = 21%, CRp = 21%) (Alvarado et al, 2003). In another pilot study, GO, liposomal daunorubicin, cytarabine and CsA therapy resulted in an overall response rate of 18% (Apostolidou et al, 2003). Similar activity and significant toxicity was shown with a GO, topotecan and cytarabine combination regimen (Cortes et al, 2002). These pilot studies showed that although GO and cytotoxic chemotherapy combination regimens are feasible and effective, they are associated with significant toxicities, particularly hepatotoxicity (Alvarado et al, 2003) (Apostolidou et al, 2003) (Cortes et al, 2002).

### Studies of GO in acute promyelocytic leukaemia

Acute promyelocytic leukaemia (APL) cells typically express large amounts of CD33, and GO has been successfully used in the treatment of APL (Petti et al, 2001; Estey et al, 2002b; Lo-Coco et al, 2004; Tsimberidou et al, 2004). We have investigated GO in combination with all-trans retinoic acid (ATRA), with or without idarubicin, in untreated APL (Estey et al, 2002b). Patients received ATRA at 45 mg/m²/d until CR and GO at 9 mg/m² on day 5 (and on day 1 if the baseline white blood cell count was >10 000/μl). Patients with initial white blood cell counts of more than 30 000/μl also received idarubicin at 12 mg/m²/d on days 1–3. Patients received postremission therapy with ATRA on a 2-weeks-on, 2-weeks-off schedule and GO at 9 mg/m² every 4–5 weeks for eight courses; idarubicin was added only for persistent or recurrent polymerase chain reaction (PCR) positivity for PML-retinoic acid receptor (RAR)-α. The CR rate was 84%. Twelve of the 12 patients tested were negative for PML-RAR-α on PCR 2–4 months from the CR date; seven of the seven patients evaluated subsequently remained PML-RAR-α negative, with a median follow up in CR of 5 months. Repeated administration of GO was feasible, without significant hepatotoxicity, as in other AML subtypes (Estey et al, 2002b).

The GO monotherapy is highly active in patients with APL at molecular relapse, but even in the setting of very advanced disease (Lo-Coco et al, 2004). In the study by Lo-Coco et al, patients were treated with GO at 6 mg/m² for two doses, and those achieving molecular remission, verified by reverse
transcription-PCR testing for PML-RAR-α, received a third dose. Molecular remission was demonstrated in nine of 11 patients tested after two doses and in 13 (100%) of 13 patients tested after the third dose. One additional patient had a molecular remission after one GO administration and received no further therapy owing to hepatotoxicity. Two patients had disease progression during treatment (Lo-Coco et al, 2004).

The high level of GO activity in APL has been attributed to low levels of P-gp function in leukaemic promyelocytes (Paietta et al, 1994). Additional in vitro studies have shown that GO has antileukaemic effects against ATRA- or arsenic trioxide (ATO)-resistant APL cells that do not express P-gp and that resistance to GO is mediated by mechanisms that differ from mechanisms of resistance to ATRA or ATO in APL cells (Takeshita et al, 2005).

We have noted that, in contrast to other subtypes of AML, GO therapy in APL is not complicated by significant hepatotoxicity. Although this may reflect the small number of patients with APL treated with GO, it also may be due to GO binding to circulating CD33, which prevents calicheamicin-induced damage of the CD33-bearing Kupffer cells in the liver. Based on the activity of GO and the lack of significant non-haematological toxicities, we have incorporated GO in our induction combination regimens for untreated and relapsed APL. GO therapy is given to patients with high-risk APL with presenting white blood cell counts >10 000/μl and as first therapy for APL in molecular relapse.

GO in paediatric leukaemia

Gemtuzumab ozogamicin has been used in relapsed or refractory paediatric AML and in some cases of paediatric ALL. In a study of the compassionate use of GO, children with relapsed or refractory AML were treated (n = 15). The median age was 9 years (range, 1–17 years). Patients received GO at 4–9 mg/m² for one to three courses. Five (33%) children achieved a CRp, and three additional children had reductions in bone marrow blasts to <5%. Six of the responding patients underwent SCT (Zwaan et al, 2003a). Two patients were in CR 6 and 9 months after SCT, and four patients died from progressive disease (n = 2) or sepsis (n = 2). The most common severe non-haematological toxicity was grade 3 and 4 hyperbilirubinaemia or liver toxicity (three patients), including a patient who died of deterioration of pre-existing VOD (Zwaan et al, 2003a).

In a dose-escalation study, 29 children 1–16 years old with refractory (n = 10) or relapsed (n = 19) AML were treated with GO (Arceci et al, 2005). GO was given at 6–9 mg/m² over 2 h on days 1 and 15 (Arceci et al, 2005). All patients experienced myelosuppression. The rates of grade 3 and 4 hyperbilirubinemia and elevation of hepatic transaminases were 7% and 21% respectively. One patient treated at 9 mg/m² died of VOD; therefore, the dose-limiting toxicity was defined as 6 mg/m² for two doses. GO treatment was followed by SCT in 13 patients, <3.5 months after the last GO infusion. The CR rate was 14%, and the CRp rate was 14%. Grade 3 and 4 infusion-related adverse events and grade 3 and 4 elevations in hepatic enzymes were each noted in 28% of patients. The overall incidence of VOD was 24% (Arceci et al, 2005). The AML Committee of the International Berlin–Frankfurt–Münster Study Group is conducting a phase II trial of GO as salvage treatment in children with refractory or relapsed AML (Zwaan et al, 2003a).

Some case reports suggest that GO also has activity in CD33-positive paediatric AML (Balduzzi et al, 2003; Cotter et al, 2003; Zwaan et al, 2003b). The rationale for using GO in AML is based on studies showing activity of GO in ALL cells and expression of CD33 in 15–25% of patients with ALL (Golay et al, 2005). In a pilot study, GO induced a CR in one of three treated children and in vitro cellular resistance studies demonstrated that ALL cells were significantly more sensitive to single-agent calicheamicin (P < 0.0001) than AML cells, suggesting that the antileukaemic effect of GO in patients with ALL was attributable to calicheamicin activity (Zwaan et al, 2003b). Another CR with GO monotherapy was reported in a 7-year-old patient with relapsed CD33-positive AML (Cotter et al, 2003). GO was given at 7.5 mg/m² on days 1 and 15 with concurrent intrathecal methotrexate on days 1, 15 and 29 (Cotter et al, 2003). In a case report of a 3-month-old infant with CD33-positive ALL and a t(4;11) translocation who had a relapse after SCT, GO treatment in combination with donor lymphocyte infusion resulted in remission, which lasted for at least 6 months (Balduzzi et al, 2003).

GO-related toxicities

The incidence of grade 3 and 4 infusion-related adverse events (chills, fever, hypotension, nausea and hypertension) is approximately 30% with the first infusion and decreases to 10% after the second dose (Larson et al, 2005). These reactions can be reduced with i.v. corticosteroids (Giles et al, 2003). Although GO has considerably less extramedullary toxicity than typical treatments for relapsed or refractory AML, such as high-dose cytarabine, extramedullary GO-induced toxicities that can be severe are hepatic VOD (Giles et al, 2001; Neumeister et al, 2001; Cohen et al, 2002; Wadleigh et al, 2003; Nabhan et al, 2004) and sinusoidal obstructive syndrome (Rajvanshi et al, 2002). In most reports, VOD is defined by the Seattle and Baltimore criteria, according to which hyperbilirubinemia is accompanied by painful hepatomegaly and fluid retention (e.g. ascites or a sudden weight gain of more than 5% of the patient’s pretreatment weight) in the absence of other causative factors (Bearman, 2000). In patients with a high tumour load, the incidence of hepatic VOD after GO therapy at 9 mg/m² is 5–10%. However, GO at lower doses combined with chemotherapy is less toxic. VOD was absent in one study when GO was given as postremission therapy (Tsimberidou et al, 2003b). In patients undergoing SCT, prior GO therapy is an independent risk factor for VOD (Wadleigh et al, 2003). This risk is higher in patients not exposed to


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GO who have undergone SCT (Wadleigh et al, 2003). However, if 3–5 months have elapsed between GO therapy and SCT, the incidence of hepatic VOD is significantly reduced (Wadleigh et al, 2003). Attempts to prevent or reverse VOD with the single-stranded polydeoxyribonucleotide defibrotide have been successful, as indicated in case reports (Saviola et al, 2003; Verslyus et al, 2004), in contrast with ursodiol which failed to prevent this complication (Giles et al, 2002).

The high incidence of hepatotoxicity with GO therapy may reflect the metabolism of the unbound drug and calicheamicin-induced damage to hepatic sinusoidal endothelial cells upon its separation from the anti-CD33 antibody, infiltration of the liver by leukaemic blasts (Scheimberg et al, 1995) or, less likely, damage to Kupffer and sinusoidal cells, which are CD33 positive (Cohen et al, 2002). However, even CD33-negative human hepatocytes can metabolise GO (Cohen et al, 2002). Until effective preventive therapies for hepatic VOD are developed, GO should be avoided in patients with pre-existing hepatic pathology (e.g. history of alcohol abuse, chronic hepatitis or concurrent azole antifungal therapy or thioguanine administration) (Kell et al, 2003) or a recent history of SCT (Wadleigh et al, 2003). All patients treated with GO should be closely monitored for hepatotoxicity. Less toxic anti-CD33 monoclonal antibodies, such as HuM195/rGel (HuM195 conjugated to the recombinant plant toxin gelonin), are expected to better deliver the monoclonal antibody, sparing patients from calicheamicin-associated toxicities (Talpaz et al, 2003).

**Phase III trials of GO and chemotherapy combination regimens in AML**

Four ongoing cooperative group phase III studies that combine GO with standard-dose chemotherapy in AML may provide some answers (Table III). The Southwest Oncology Group (SWOG) is investigating cytarabine and daunorubicin with or without GO followed by high-dose cytarabine followed by either GO or no additional therapy in patients with untreated de novo AML (SWOG trial S0106) (http://www.cancer.gov/clinicaltrials/SWOG-S0106, last accessed 14 September 2005). Patients aged 18–55 years are to receive daunorubicin i.v. on days 1–3, cytarabine i.v. continuously on days 1–7 and GO i.v. on day 4. Patients also receive filgrastim or sargramostim i.v. or subcutaneously (s.c.) daily, starting on day 15. Reinduction therapy consists of daunorubicin i.v. on days 1–3 and cytarabine i.v. continuously on days 1–7. Consolidation therapy consists of high-dose cytarabine i.v. on days 1, 3 and 5. Patients are randomised to receive consolidation therapy with GO or no additional therapy. GO courses are repeated every 28 days for three courses.

The Eastern Cooperative Oncology Group (ECOG) is studying daunorubicin dose intensification and GO consolidation therapy prior to autologous SCT in patients with AML, 16–60 years of age (E1900) (http://www.cancer.gov/clinicaltrials/ECOG-1900, last accessed 14 September 2005). Patients are randomised to a ‘3 + 7’ regimen of standard- or high-dose daunorubicin and continuous infusion of cytarabine. Postremission therapy consists of allogeneic SCT alone (if they have unfavourable cytogenetics or a high white blood cell count and a related donor is available) or GO consolidation therapy and autologous transplantation. Patients with intermediate-risk cytogenetics may undergo allogeneic SCT. Patients not eligible for allogeneic SCT receive two courses of high-dose cytarabine i.v. Autologous peripheral blood stem cells (PBSCs) are harvested. Subsequently, patients are randomised to receive autologous PBSCs in two arms. Arm 1 includes conditioning with a busulfan and cyclophosphamide preparative regimen. In arm 2, patients receive GO i.v. on day 1 and granulocyte macrophage-colony stimulating factor s.c. or i.v. on day 10 until blood count recovery. Within 2–3 weeks after blood

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**Table III. Ongoing phase III trials of cytotoxic chemotherapy with or without gemtuzumab ozogamicin in untreated AML**

<table>
<thead>
<tr>
<th>Study</th>
<th>Time (years)</th>
<th>Estimated number of patients</th>
<th>Age (years)</th>
<th>CD33 status</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWOG S0106 (<a href="http://www.cancer.gov/clinicaltrials/SWOG-S0106">http://www.cancer.gov/clinicaltrials/SWOG-S0106</a>)</td>
<td>5</td>
<td>684</td>
<td>18 to 55</td>
<td>Any</td>
<td>Daunorubicin + cytarabine ± GO → HDAC → GO or no further treatment</td>
</tr>
<tr>
<td>ECOG E1900 (<a href="http://www.cancer.gov/clinicaltrials/ECOG-1900">http://www.cancer.gov/clinicaltrials/ECOG-1900</a>)</td>
<td>5</td>
<td>830</td>
<td>16 to 60</td>
<td>Any</td>
<td>Daunorubicin + cytarabine ± GO → stem cell transplant</td>
</tr>
<tr>
<td>MRC AML15 (<a href="http://www.aml15.bham.ac.uk/trial/">http://www.aml15.bham.ac.uk/trial/</a>)</td>
<td>5</td>
<td>&gt;2500</td>
<td>&lt;60 to &gt;60</td>
<td>Any</td>
<td>(ADE versus DA versus FLAG-Ida) ± GO → MACE ± GO versus MidAC ± GO versus HDAC ± GO, etc.</td>
</tr>
<tr>
<td>EORTC/GIMEMA-06012, 2005 (<a href="http://www.cancer.gov/clinicaltrials/EORTC-06012">http://www.cancer.gov/clinicaltrials/EORTC-06012</a>)</td>
<td>3-75</td>
<td>450</td>
<td>61 to 75</td>
<td>+</td>
<td>GO → MICE versus MICE</td>
</tr>
</tbody>
</table>

AML, acute myeloid leukaemia; SWOG, Southwest Oncology Group; GO, gemtuzumab ozogamicin; HDAC, high-dose cytarabine; ECOG, Eastern Cooperative Oncology Group; MRC, Medical Research Council; ADE, daunorubicin, cytarabine, etoposide; DA, daunorubicin, cytarabine; FLAG, fludarabine and cytarabine; Ida, idarubicin; MACE, amsacrine, cytarabine, etoposide; MidAC, intermediate-dose cytarabine; EORTC, European Organization for Research and Treatment of Cancer; GIMEMA, Gruppo Italiano Malattie Ematologiche dell’Adulto; MICE, mitoxantrone, cytarabine and etoposide.  

count recovery, patients in arm 2 receive conditioning and undergo autologous PBSC transplantation as in arm 1 (http://www.cancer.gov/clinicaltrials/ECOG-1900, last accessed 14 September 2005).

In the Medical Research Council AML15 trial, patients with AML are randomised to receive two induction courses with cytarabine, daunorubicin and etoposide; daunorubicin and cytarabine; or fludarabine, cytarabine, idarubicin and G-CSF, with or without GO on day 1 of course 1 (six arms) (http://www.mrcl.bham.ac.uk/trial/, last accessed 14 September 2005). Patients are stratified according to cytogenetics and blast clearance. A consolidation randomisation compares arsamide, cytarabine, etoposide combination therapy and mitoxantrone/cytarabine with high-dose cytarabine. Patients who are allocated to the chemotherapy comparisons and have experienced no GO-related side effects are randomised to GO on day 1 of course 3. Patients with intermediate or high-risk AML and an available sibling donor are randomised to mini versus standard allogeneic SCT. GO is also incorporated in postremission therapy of patients with APL who are randomised to receive GO or no GO on day 1 of course 3 (http://www.mrcl.bham.ac.uk/trial/, last accessed 14 September 2005).

The EORTC-LG is conducting a randomised phase III trial (AML-17) of standard intensive chemotherapy, with or without GO, as induction and consolidation therapy in patients aged 64–75 years with untreated AML (http://www.cancer.gov/clinicaltrials/EORTC-06012, last accessed 14 September 2005). Arm 1 consists of GO i.v. on days 1 and 15. Intensive chemotherapy starts on days 50–53 and consists of mitoxantrone, etoposide and cytarabine i.v. continuously on days 1–7 (MICE). Patients in CR proceed to consolidation therapy with GO, followed by idarubicin, etoposide and cytarabine i.v. continuously on days 1–5 (1 or 2 courses). Patients randomised to arm 2 receive induction with MICE followed by consolidation as in arm 1 but without GO (http://www.cancer.gov/clinicaltrials/EORTC-06012, last accessed 14 September 2005).

The EORTC-LG and GIMEMA groups are also conducting a phase II/III trial (AML-19) of GO monotherapy versus standard supportive care in patients with untreated AML older than 75 years of age who are not candidates for intensive chemotherapy (http://www.cancer.gov/clinicaltrials/EORTC-06031, last accessed 14 September 2005).

**GO as an adjunct to conditioning regimens for SCT**

The role of GO prior to SCT has been examined in several phase II studies. In general, prior exposure to GO significantly increases the risk of hepatic VOD in patients undergoing myeloablative allogeneic SCT, but this risk decreases if at least 3–5 months have elapsed since the last dose of GO (Wadleigh et al, 2003). In the final report on three phase II trials of GO in relapsed AML, 71 patients responded to GO. Of these 71 patients, 25 underwent allogeneic or autologous SCT, 11 received additional chemotherapy and 35 received no further therapy (Larson et al, 2005). Overall survival was significantly longer in patients who achieved remission after GO treatment and underwent subsequent allogeneic SCT (14 patients; median survival not reached at 18 months), compared to those who underwent autologous SCT (11 patients; median survival, 17 months), additional chemotherapy (11 patients; median survival, 12 months) or those who received no additional therapy (35 patients, median survival, 11 months; \( P = 0.007 \)) (Larson et al, 2005).

**Mechanisms of resistance to GO**

The GO delivery systems to leukaemic blasts play a major role in cytotoxicity (Linenberger, 2005; Walter et al, 2005). High levels of CD33 tumour load in the peripheral blood (van der Velden et al, 2001, 2004) and high levels of circulating CD33 confer drug resistance and are associated with worse outcomes, presumably because of GO consumption in the peripheral blood and poor delivery to the bone marrow (van der Velden et al, 2004). Alternative resistance mechanisms include altered pharmacokinetics, reduced GO-binding capacity to leukaemic blasts (van der Velden et al, 2001), antiapoptotic mechanisms independent of drug efflux (van der Kolk et al, 2002), bcl-2 antiapoptotic proteins (Walter et al, 2004) and resting state of cell cycle (Jedema et al, 2004).

As with other therapies for AML, the expression of P-gp (Naito et al, 2000; Linenberger et al, 2001; Matsui et al, 2002) and, to a lesser degree, of multidrug resistance protein (Walter et al, 2003, 2004) have been shown to mediate in vitro resistance to GO. Multidrug resistance inhibitors (Matsui et al, 2002), such as CsA (Linenberger et al, 2001) and the novel peripheral benzodiazepine receptor ligand PK11195, which promotes mitochondrial apoptosis, have been shown to increase in vitro GO sensitivity in AML cells (Walter et al, 2004). In the 1990s, several attempts to overcome multidrug resistance with the addition of P-gp inhibitors to standard chemotherapy for patients with AML failed to demonstrate reproducible clinical benefit. Nonetheless, the observation that P-gp mediates resistance to GO in vitro (Linenberger et al, 2001) encouraged us to conduct pilot studies incorporating the P-gp inhibitor CsA in GO-containing regimens as induction, salvage or postremission therapy in AML (Cortes et al, 2002; Alvarado et al, 2003; Apostolidou et al, 2003; Tsimeridou et al, 2003a,c). Although GO could be safely administered with CsA, the addition of CsA did not appear to increase rates of response and survival, and an increased incidence of VOD was noted in patients with high tumour loads.

**Conclusions**

As the first targeted therapy approved for CD33-positive AML at first relapse, in patients 60 years or older who are not candidates for other cytotoxic chemotherapy, GO initially
generated great interest. Complete remissions are achieved with GO monotherapy in 13% of patients in first relapse. At the approved dose (9 mg/m²), GO therapy is associated with increased rates of hepatotoxicity, resulting in fatal hepatic VOD in some patients. Therefore, GO should be avoided in patients with pre-existing hepatic pathology. To decrease the risk of VOD, a 4-month time period between GO therapy and SCT is also recommended. All patients should be closely monitored for hepatotoxicity. Although GO has been shown to have efficacy in CD33-negative malignancies with endoctic capacity, it is currently recommended only for CD33-positive AML. Ongoing cooperative group phase III clinical trials are investigating whether the addition of low-dose GO to cytotoxic chemotherapy will improve clinical outcomes in AML and may better define subgroups of patients who will benefit from GO therapy. The future of GO is clearly its use in combination with other cytotoxic agents. Further trials to optimise the dose and timing of GO administration in AML are warranted.

References


