Gemtuzumab ozogamicin: an anti-CD33 immunoconjugate for the treatment of acute myeloid leukaemia

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Background: Gemtuzumab ozogamicin consists of a semisynthetic derivative of calicheamicin, a potent cytotoxic antibiotic, linked to a humanized anti-CD33 monoclonal antibody. Objectives: To describe the pharmacology of gemtuzumab ozogamicin and to provide an overview of clinical trials in acute myeloid leukaemia. Methods: Review and summary of publications on gemtuzumab ozogamicin indexed in the PubMed electronic database. Results/conclusions: Gemtuzumab ozogamicin has shown moderate activity as a single agent in patients with CD33-positive refractory or relapsed acute myeloid leukaemia, with more promising results in acute promyelocytic leukaemia. The side effect profile may be an improvement on conventional chemotherapy, except for a higher frequency of veno-occlusive disease or sinusoidal obstructive syndrome, especially after a subsequent haematopoietic stem cell transplantation. Because of the different mechanisms of action and non-overlapping toxicities, the integration of this immunoconjugate with standard chemotherapy is a rational approach, and Phase III trials are ongoing both in the induction and in the post-remission settings.

Keywords: acute myeloid leukaemia, acute promyelocytic leukaemia, gemtuzumab ozogamicin, immunoconjugate, monoclonal antibodies

1. Introduction

Acute myeloid leukaemia (AML) is the most common type of acute leukaemia in adults and accounts for ~ 80% of all cases of acute leukaemia [1]. Overall incidence of AML is ~ 3.5 in 100,000 in western countries, and increases with age from 1 in 100,000 in people younger than 35 years to 15 in 100,000 in those aged > 75 years [2,3]. Rates are slightly higher in males and in whites.

In the absence of treatment, AML is typically fatal within a few weeks to months from the time of diagnosis. Treatment for AML is aimed at eradicating the leukaemic clone and allowing reestablishment of normal haematopoiesis, and has changed little over the last 20 years. With the possible exception of etoposide in patients aged < 55 years [4,5], large randomized trials failed to demonstrate the superiority of adding new cytotoxic agents to cytosine arabinoside (ara-C) and anthracycline-based regimens. With these conventional regimens, a complete remission (CR) is achieved in 60 – 80% of patients aged < 60 years [3]. Only 30 – 40% of those who achieve remission, however, can expect to enjoy long-term disease-free survival [3]. Results in older adults are even more disappointing, with 20% dying of complications during induction, CR achievable in 45 – 55%, and no more than 10 – 20% of those patients in initial remission living 3 years beyond their diagnosis [6].
Micromonospora echinospora calichensis calicheamicin DMH, a derivative of calicheamicin (NAc-gamma-[11]). Conjugation with the antibody is obtained by covalent linkage (condensation) of a bifunctional linker, 4-(4-acetylphenoxy)butanoic acid (AcBut linker), which allows the most favourable balance between hydrolytic stability in physiological buffers (pH 7.4) and efficient drug release at the pH of lysosomes (~4) [9]. GO has approximately 50% of the antibody loaded with 4 – 6 mol calicheamicin per mol of antibody. The remaining 50% of the antibody is not linked to the calicheamicin derivative [9]. Therefore, the average loading of calicheamicin on the antibody is 2 – 3 mol/mol. GO has a molecular weight of 151 – 153 kDa.

2.2 Pharmacodynamics

The CD33 antigen is a 67-kDa sialic acid-dependent adhesion protein that is specific for myeloid cells. CD33 is expressed in approximately 90% of AML cases, as defined by the presence of the antigen on > 20% of the leukemic blasts but not on normal CD34+ pluripotent hematopoietic stem cells or non-hematopoietic tissues [12].

In vitro data indicate that when GO binds the CD33 antigen, the complex is rapidly internalized [13,14]. Upon internalization, the calicheamicin derivative is released inside the lysosomes of the myeloid cell by acid hydrolysis. The released calicheamicin derivative binds to DNA in the minor groove resulting in DNA double-strand breaks and cell death by apoptosis.

GO is cytotoxic to the CD33+ HL-60 human leukaemia cell line and produces significant inhibition of colony formation in cultures of adult leukemic bone marrow cells [9]. The cytotoxic effect on normal myeloid precursors leads to substantial myelosuppression but this is reversible because pluripotent hematopoietic stem cells are spared. In preclinical animal studies, gemtuzumab ozogamicin demonstrates antitumour effects in the HL-60 human promyelocytic leukaemia xenograft tumour in athymic mice [9].

The existence of a quantitative relationship between CD33 expression levels and in vitro response to GO has been demonstrated by use of lentivirus-mediated gene transfer to manipulate CD33 expression in myeloid cell lines that normally lack CD33 or have very low levels of CD33 [15]. Resistance to GO has been correlated with function of the multi-drug resistance (MDR) phenotype in leukemic blasts and may be reversed in vitro by various agents [16,17]. Furthermore, AML blasts of responders were found to have a significantly higher mean CD33 level and lower P-glycoprotein (Pgp) activity compared with non-responders, with CD33 expression and Pgp activity showing an inverse correlation [18].

The cytotoxicity of GO can be increased by exposing AML blast cells to G-CSF, and this effect is not related to activation of cell division or an increase in CD33-binding sites on the cell membrane [19]. Furthermore, G-CSF induced dose-dependent inhibition of Pgp function in both GO-sensitive cell lines and in primary blasts from AML patients [19]. The histone deacetylase inhibitor valproic acid (VPA) also sensitizes AML cells to GO [20]. The effects of VPA treatment have been found to involve the DNA intercalation of calicheamicin and enhanced DNA degradation. Synergy between GO and VPA was restricted to GO-sensitive cell lines and in primary blasts from AML patients [19]. The histone deacetylase inhibitor valproic acid (VPA) also sensitizes AML cells to GO [20]. The effects of VPA treatment have been found to involve the DNA intercalation of calicheamicin and enhanced DNA degradation. Synergy between GO and VPA was restricted to GO-sensitive cell lines and in primary blasts from AML patients [19].
Figure 1. Schematic structure of gemtuzumab ozogamicin (GO). GO is composed of a humanized monoclonal antibody (hP67.6) joined to N-acetyl-γ calicheamicin dimethyl hydrazide via 4-(4-acetylphenoxy)butanoic, a bifunctional linker.
CD33 saturation levels were strongly related to reduced cell killing. The hypothesis is that high CD33-antigen loads in blood consume GO and thereby limit its penetration into bone marrow [21]. Consequently, CD33 saturation in bone marrow is reduced, which hampers efficient cell killing. Therefore, GO would probably be more effective if administered at higher or repeated doses, or, preferably, after reduction of the leukaemic cell burden by conventional chemotherapy.

2.3 Pharmacokinetics and metabolism
GO is administered by intravenous infusion over 2 h. Pharmacokinetic parameters of the immunoconjugate were reported for patients with AML recruited for Phase I/II clinical trials [22], and are characterized by separate assays of the antibody portion of the conjugate as well as calicheamicin (total and unconjugated) in plasma. The elimination half-life of the hP67.6 antibody was highly variable after intravenous administration of the 9 mg/m² dose and ranged from 67 ± 37 h to 88 ± 58 h from dose period 1 to dose period 2 [23]. The mean Cₘₐₓ of hP67.6 antibody following the first dose for patients who received 9 mg/m² GO was 3.0 mg/l, with values in the range of 0.4 – 18.3 mg/l. The Cₘₐₓ increased to 3.6 mg/l (0.3 – 10.6 mg/l) after the second dose, and the increase was believed to be due to a decrease in clearance by CD33⁺ blast cells, a result of the reduced tumour burden following the first dose [22]. Although highly variable between individuals, changes in concentrations could not be linked to age, sex, weight, body surface area or ethnicity [24]. The pharmacokinetics of gemtuzumab ozogamicin in paediatric patients followed the profile and variability of adult patients and mean pharmacokinetic parameters were similar to values reported in adults [25].

Studies in animals have demonstrated that unconjugated calicheamicin derivatives represent less than 4% of total derivatives in plasma, suggesting that calicheamicin remains linked to gemtuzumab ozogamicin in serum [23]. Calicheamicin metabolites are detectable transiently in the serum of patients receiving GO therapy; the clinical significance of such exposure has not been established [22]. Total calicheamicin elimination half-life and AUC were 39 ± 25 h and 2.1 ± 1.8 mg/h/l, respectively, after the first dose period of gemtuzumab ozogamicin administration, and both were increased in the second dose period (63 ± 63 h and 4.7 ± 4.1 mg/h/l, respectively) [23].

Animal studies indicate that gemtuzumab ozogamicin undergoes hepatobiliary elimination; however, the route of elimination has not been studied in humans [23]. Based on various in vitro assays, it was found that several enzyme systems in human liver microsomes, hepatocytes, and cytosol are involved in the activation/metabolism of the non-antibody active moiety of gemtuzumab ozogamicin, NAc-γ calicheamicin DMH. Enzyme systems involved include esterases, carbonyl reductase, and the involvement of CYP3A4 in the oxidative metabolism steps [23].

3. Clinical efficacy

3.1 Single-agent studies
A Phase I dose-escalation study indicated that 9 mg/m² is the maximum tolerated dose (MTD), and CD33 receptor site saturation data further supported the choice of 9 mg/m² as the appropriate dose for Phase II studies [26]. The dosing interval of 14 days was based on the half-life of the antibody. A dose would be expected to be cleared from the body in 4 – 5 half lives, or ∼ 12 – 15 days. Some of the patients with relapsed or refractory AML in this study had clearance of bone marrow blast cells with incomplete platelet recovery. This response was designated as CRp, and met all the criteria for CR except recovery to 100,000 platelets/µl. The CRp patients had to have sufficient bone marrow recovery to be platelet-transfusion-independent for at least 1 week. This was supposed to be a clinically meaningful standard, in that patients who are platelet-transfusion-independent are expected to be at a lower risk of bleeding than those who are not.

GO monotherapy in patients with AML in first relapse was first investigated in three pivotal Phase II trials multicenter trials in North America and Europe (0903B1-201-US/CA, 0903B1-202-EU, 0903B1-203-US/EU). Because of the similarity in study designs, objectives, patient demographics, and dosing schedules, data from the three studies were pooled to attain a larger efficacy population (277 patients). The initial report [27] was followed by a detailed subset analysis [28] and a final report [29]. The main inclusion criteria included CD33⁺ AML patients in first relapse (> 5% leukaemic blasts as determined by central flow cytometry laboratory tests) and are summarized in Table 1. Patients with a median age of 61 years received GO (9 mg/m² as a 2-h intravenous infusion in 2 doses separated by 2 weeks). Further consolidation therapy (hematopoietic stem cell transplantation (HSCT) or other chemotherapy) was allowed, 30 days after the bone marrow clearing of blasts indicating remission. Hydroxycarbamide use was used to reduce the peripheral white blood cell (WBC) count to < 30,000/µl before GO use if the initial WBC count was ≥ 30,000/µl, thereby minimizing the risk of tumour lysis syndrome.

The primary efficacy end point was the number of patients attaining a CR. The secondary end points were the rates of CRp, relapse-free survival (from initial documentation of remission and post-HSCT), total survival (from first dose of study drug and post-HSCT) and time to platelet- and time to absolute neutrophil count (ANC) recovery.

Using the 1988 criteria of the National Cancer Institute (protocol-defined criteria) [30], 13% of patients achieved a CR and 13% achieved a CRp. However, an additional analysis performed using the 2003 International Working Group (IWG) criteria [31] showed CR and CRp rates of 15 and 19%, respectively (pers. commun.). Over 50% of patients who achieved either a CR or a CRp had blast cell
Table 1. Key inclusion criteria of studies 0903B1-201-US/CA, 0903B1-202-EU, 0903B1-203-US/EU.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Study 201 (n = 84)</th>
<th>Study 202 (n = 95)</th>
<th>Study 203 (n = 98)</th>
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<tr>
<td>CD33 positive AML in first relapse</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Age, years</td>
<td>≥ 60</td>
<td>≥ 60</td>
<td>≥ 60</td>
</tr>
<tr>
<td>Duration of first remission, months</td>
<td>≥ 6</td>
<td>≥ 6</td>
<td>≥ 3</td>
</tr>
<tr>
<td>Prior HSCT</td>
<td>Not permitted</td>
<td>Permitted*</td>
<td>Not permitted</td>
</tr>
<tr>
<td>ECOG performance status 0 – 2</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Baseline serum creatinine</td>
<td>≤ 2.0 mg/dl (176.8 µmol/l)</td>
<td>≤ 2.0 mg/dl (176.8 µmol/l)</td>
<td>≤ 3.0 mg/dl (265.2 µmol/l)</td>
</tr>
<tr>
<td>Baseline serum total bilirubin</td>
<td>≤ 1.5 mg/dl (25.65 µmol/l)</td>
<td>≤ 1.5 mg/dl (25.65 µmol/l)</td>
<td>≤ 2.0 mg/dl (34.2 µmol/l)</td>
</tr>
<tr>
<td>No myelodysplastic syndrome (MDS)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>No secondary AML</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Originally not permitted but protocol 202 was amended to allow prior HSCT.
AML: Acute myeloid leukaemia; ECOG: Eastern Cooperative Oncology Group; HSCT: Hematopoietic stem cell transplantation.

clearance after the first dose of gemtuzumab ozogamicin. GO was found to be equally effective in patients aged ≥ 60 years (overall response [OR] rate 26%) and in younger patients (OR rate 34%). Furthermore, the adverse effects were similar in the two age groups. The duration of first complete remission (< or > 1 year) and cytogenetics (poor, intermediate or high risk) had no effect on the rate of response to GO.

The median overall survival was 4.8 months (5.3 and 4.5 months for patients aged < 60 and ≥ 60 years, respectively). The early death rate (death within 28 days) was 16%. Median survival for responders (CR + CRp) was 12.5 months, in part a reflection of consolidation therapy, since it was 18.1 months for those receiving consolidation versus 11.0 months for those receiving no consolidation.

Remission durations ranged between 4.5 (CRp patients) and 6.4 months (CR patients). A significant difference in remission duration was observed between patients younger than 60 and patients aged 60 and older (p = 0.008). This was possibly affected by postremission treatment options, especially HSCT. It is noteworthy that among patients who received neither HSCT nor additional chemotherapy after GO treatment, the overall recurrence-free survival differed significantly between those who achieved a CR (median, 3.8 months) and those who achieved a CRp (median, 2.4 months) [29]. These findings would suggest that the level of cytoreduction of leukaemic cells in patients with CRp is less than that of patients achieving true CR.

Based on these efficacy data and a reasonable safety profile (see below), the pivotal Phase II data, in May 2000 the US FDA approved the use of GO for patients with relapsed AML who are older than 60 years of age and who are considered unfit for conventional cytotoxic therapy [23]. The approval of GO by the FDA was conditional upon the conduct of studies of regimens combining it with standard anti-AML chemotherapy [23]. As a matter of fact, additional reports on the effectiveness of single-agent GO in unselected older patients with newly diagnosed AML have been rather disappointing (Table 2), with overall response rates (CR/CRp) seldom exceeding 25 – 30% [32-38]. The immunoconjugate therapy appears particularly toxic in patients aged > 75 years, for whom a dose reduction has been proposed [34]. The administration of fractionated doses of GO (3 mg/m² on days 1, 4 and 7 for one course) demonstrated an excellent efficacy/safety profile, and may represent a valuable alternative for frailer patients [36].

GO has been used in relapsed or refractory pediatric AML as well. In three European trials the immunoconjugate was used on a compassionate-use basis and at variable doses, showing limited but significant activity in aggregate results (CR or CRp in 11/39) [39-41]. In an American open-label dose-escalation study, 29 children and adolescents, 1 – 16 years of age, with refractory and relapsed AML received GO (6 – 9 mg/m² in two doses) [42]. The maximum tolerated dose was determined to be 6 mg/m², with 8 out of 29 patients achieving CR/CRp (28%).

3.2 Combination regimens
A number of Phase II studies (Table 3) in relapsed/refractory cases and in patients with previously untreated AML have assessed the feasibility of treatment protocols integrating GO and chemotherapy [43-53]. The results of these trials are quite heterogeneous, reflecting not only the variable activity of the regimens used but also the different characteristics of the patient populations.

The MD Anderson Cancer Center group has been particularly active in investigating combination regimens with GO. In one study, GO at a dose of 9.0 mg/m² (intravenous) over 2 h on day 1 was combined with ara-C at a dose of 1.0 g/m² over 2 h (intravenous) on days 1 – 5 and topotecan at a dose of 1.25 mg/m² by continuous
intravenous infusion on days 2 – 5. Seventeen patients (nine patients with primary resistant disease and eight patients with recurrent disease) received 20 courses of therapy. The median age was 55 years (range, 20 – 70 years). Two patients (12%) achieved a CR. The median survival was 8.2 weeks. Five patients (29%) developed Grade 3 or 4 transaminitis, including 1 patient (6%) who died from veno-occlusive disease (VOD) of the liver. In another study, GO (Mylotarg) was combined with idarubicin and ara-C (MIA) in patients with refractory AML. GO was given at a dose of 6.0 mg/m² (intravenous) over 2 h on days 1 and 15, idarubicin was given at a dose of 9.0 mg/m² by continuous intravenous infusion on days 2 – 5. The of the 14 patients treated, 4 (29%) had primary refractory AML, and 10 (71%) had recurrent disease. Seven patients were aged ≥ 60 years. MIA induced CR in three patients (21%) and CRp in three patients (21%). The median survival was 8 weeks (range 2 – 64 weeks), and the median failure-free survival of complete responders was 27 weeks (range 11 – 64 weeks). All patients developed grade 3/4 myelosuppression, with severe sepsis occurring in 10 patients (71%). Other grade 3/4 non-haematological toxicities included transaminitis, oral mucositis and diarrhoea. Two patients (14%) developed hepatic VOD. A report from the same group concerned the MDAC regimen (ara-C 1.0 g/m² (intravenous) over 2 h daily on days 2 – 5; GO 6.0 mg/m²

### Table 2. Phase II studies of GO monotherapy in acute myeloid leukaemia.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Number of patients</th>
<th>Median age (years) (range)</th>
<th>AML status</th>
<th>CR/CRp (%)</th>
<th>Induction deaths (%)</th>
<th>Incidence of veno-occlusive disease (%)</th>
<th>Median overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larson et al. [29]</td>
<td>277</td>
<td>61 (20 – 87)</td>
<td>First relapse</td>
<td>13/13</td>
<td>NR</td>
<td>5.3</td>
<td>4.9 months (all patients)</td>
</tr>
<tr>
<td>Roboz et al. [32]</td>
<td>43</td>
<td>62* (19 – 84)</td>
<td>7, untreated 29, relapsed 2, RAEBT 14, secondary AML 5, CML blast phase</td>
<td>9/5</td>
<td>14</td>
<td>0</td>
<td>4 months for CR and CRp patients</td>
</tr>
<tr>
<td>Estey et al. [33]</td>
<td>51</td>
<td>71 (65 – 89)</td>
<td>37, untreated 6, RAEBT, 8, MDS</td>
<td>8 – 36/NR</td>
<td>37</td>
<td>15.7</td>
<td>2 months in the no IL-11 group</td>
</tr>
<tr>
<td>Amadori et al. [34]</td>
<td>40</td>
<td>76 (61 – 89)</td>
<td>Untreated</td>
<td>10/7</td>
<td>17</td>
<td>2.5</td>
<td>4.3 months (all patients)</td>
</tr>
<tr>
<td>Nabhan et al. [35]</td>
<td>12</td>
<td>75 (33 – 79)</td>
<td>Untreated</td>
<td>27/0</td>
<td>0</td>
<td>0</td>
<td>NR</td>
</tr>
<tr>
<td>Taksin et al. [36]</td>
<td>57</td>
<td>64 (22 – 80)</td>
<td>First relapse</td>
<td>26/7</td>
<td>7</td>
<td>0</td>
<td>8.4 months (all patients)</td>
</tr>
<tr>
<td>van der Heiden et al. [37]</td>
<td>38‡</td>
<td>58 (27 – 77)</td>
<td>15, untreated 17, relapsed 6, refractory</td>
<td>18/13</td>
<td>NR</td>
<td>2.6</td>
<td>NR</td>
</tr>
<tr>
<td>Piccaluga et al. [38]</td>
<td>24</td>
<td>63 (20 – 75)</td>
<td>18, relapsed 6, refractory</td>
<td>13/8</td>
<td>12</td>
<td>4.2</td>
<td>2 months (all patients)</td>
</tr>
</tbody>
</table>

*Mean age.
‡19 patients received GO alone and 19 received GO in combination with standard intensive chemotherapy.
AML: Acute myeloid leukaemia; CML: Chronic myeloid leukaemia; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; MDS: Myelodysplastic syndrome; NR: Not reported; RAEBT: Refractory anemia with excess blasts in transformation.

**Gemtuzumab ozogamicin**

**Intravenous infusion on days 1 – 5**. Seventeen patients (nine patients with primary resistant disease and eight patients with recurrent disease) received 20 courses of therapy. The median age was 55 years (range, 20 – 70 years). Two patients (12%) achieved a CR. The median survival was 8.2 weeks. Five patients (29%) developed Grade 3 or 4 transaminitis, including 1 patient (6%) who died from veno-occlusive disease (VOD) of the liver. In another study, GO (Mylotarg) was combined with idarubicin and ara-C (MIA) in patients with refractory AML. GO was given at a dose of 6.0 mg/m² (intravenous) over 2 h on days 1 and 15, idarubicin was given at a dose of 9.0 mg/m² by continuous intravenous infusion on days 2 – 5. Of the 14 patients treated, 4 (29%) had primary refractory AML, and 10 (71%) had recurrent disease. Seven patients were aged ≥ 60 years. MIA induced CR in three patients (21%) and CRp in three patients (21%). The median survival was 8 weeks (range 2 – 64 weeks), and the median failure-free survival of complete responders was 27 weeks (range 11 – 64 weeks). All patients developed grade 3/4 myelosuppression, with severe sepsis occurring in 10 patients (71%). Other grade 3/4 non-haematological toxicities included transaminitis, oral mucositis and diarrhoea. Two patients (14%) developed hepatic VOD. A report from the same group concerned the MDAC regimen (ara-C 1.0 g/m² (intravenous) over 2 h daily on days 2 – 5; GO 6.0 mg/m²...
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(intravenous) over 2 h after day 6; ciclosporin A (CSA), loading dose 6.0 mg/kg over 2 h on day 6 followed by 16.0 mg/kg as a continuous intravenous infusion on days 6 – 8; and liposome-encapsulated daunorubicin 75.0 mg/m² daily as a continuous intravenous infusion on days 6 – 8) in patients with refractory AML [45]. Among 11 patients 1 (9%) achieved a CR, and a second patient achieved a CRp. Grade 3 or 4 toxicities included sepsis (63%), hyperbilirubinemia (54%), mucositis (27%) and transaminitis (9%). Ciclosporin A has also been combined with GO, fludarabine, and ara-C (MFAC) in the de novo and recurrent settings [46,47,54]. The activity of the MFAC regimen as induction therapy has been evaluated in 39 patients with previously untreated AML and 20 patients with refractory anemia with excess blasts (RAEB), or RAEB in transformation (RAEBT) [46]. Their median age was 57 years (range, 27 – 76 years). The MFAC regimen induced CR in 27 patients (46%) and CRp in 1 patient (2%). The median overall survival was 8 months. At 12 months, the survival rate was 38% and the event-free survival rate in patients with CR/CRp was 27%. Infections complicated 38% of the courses of chemotherapy. Grade 3/4 toxicity included hyperbilirubinemia in 31% and transaminitis in 7% of the patients. Four patients (7%) developed hepatic VOD. The MFAC regimen was also evaluated as post-remission therapy in patients with AML after a GO-containing induction regimen [54]. Patients in CR commenced idarubicin and ara-C (IA) alternating with MFAC or vice versa for 9 months from the date of CR. Idarubicin was administered at 8 mg/m² on days 1 and 2 and ara-C at

<table>
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<tr>
<th>Ref.</th>
<th>Number of patients</th>
<th>Median age, years (range)</th>
<th>AML status</th>
<th>Chemotherapy agents (besides GO)</th>
<th>CR/CRp (%)</th>
<th>Induction deaths (%)</th>
<th>Incidence of veno-occlusive disease (%)</th>
<th>Median overall survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortes et al. [43]</td>
<td>17</td>
<td>55 (20 – 70)</td>
<td>AML, relapsed AML, refractory</td>
<td>Topotecan, Ara-C</td>
<td>12</td>
<td>29</td>
<td>5.9</td>
<td>8.2 weeks</td>
</tr>
<tr>
<td>Alvarado et al. [44]</td>
<td>14</td>
<td>61 (34 – 74)</td>
<td>AML, relapsed AML, refractory</td>
<td>Idarubicin, Ara-C</td>
<td>21/21</td>
<td>43</td>
<td>14.3</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Apostolidou et al. [45]</td>
<td>11</td>
<td>37 (16 – 67)</td>
<td>AML, relapsed AML, refractory</td>
<td>Ara-C, DNX, CSA</td>
<td>9/9</td>
<td>18</td>
<td>0</td>
<td>3 months</td>
</tr>
<tr>
<td>Tsimberidou et al. [46]</td>
<td>59</td>
<td>57 (27 – 76)</td>
<td>AML, untreated MDS, untreated</td>
<td>Fludarabine, Ara-C, CSA</td>
<td>46/2</td>
<td>25</td>
<td>6.8</td>
<td>8 months</td>
</tr>
<tr>
<td>Tsimberidou et al. [47]</td>
<td>32</td>
<td>53 (18 – 78)</td>
<td>AML, relapsed AML, refractory</td>
<td>Fludarabine, Ara-C, CSA</td>
<td>28/6</td>
<td>NR</td>
<td>9.4</td>
<td>5.3 months</td>
</tr>
<tr>
<td>Kell et al. [48]</td>
<td>64</td>
<td>46.5 (18 – 59)</td>
<td>AML, untreated</td>
<td>DNR, Ara-C, 6-Tg or Fludarabine, Ara-C, Idarubicine</td>
<td>84</td>
<td>9</td>
<td>10.9</td>
<td>78% at 8 months</td>
</tr>
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<td>Amadori et al. [49]</td>
<td>57</td>
<td>68 (61 – 75)</td>
<td>AML, untreated</td>
<td>Mitoxantrone, Ara-C, VP-16</td>
<td>35/19</td>
<td>14</td>
<td>8.8</td>
<td>10.4 months</td>
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<td>Piccaluga et al. [53]</td>
<td>9</td>
<td>63 (50 – 71)</td>
<td>5, untreated 2, relapsed 2, refractory</td>
<td>Ara-C</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>6 months</td>
</tr>
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<td>Chevallier et al. [50]</td>
<td>17</td>
<td>54 (21 – 68)</td>
<td>13, relapsed 4, refractory</td>
<td>Mitoxantrone, Ara-C</td>
<td>70/6</td>
<td>11.7</td>
<td>5.8</td>
<td>11 months</td>
</tr>
<tr>
<td>Clavio et al. [51]</td>
<td>46</td>
<td>66 (60 – 80)</td>
<td>Untreated</td>
<td>Fludarabine, Ara-C, Idarubicin</td>
<td>52.1</td>
<td>2.1</td>
<td>0</td>
<td>8 months</td>
</tr>
<tr>
<td>Specchia et al. [52]</td>
<td>21</td>
<td>52 (36 – 68)</td>
<td>10, relapsed 11, refractory</td>
<td>Mitoxantrone, Ara-C</td>
<td>9.5/9.5</td>
<td>19.0</td>
<td>0</td>
<td>7 months for CR/CRp patients</td>
</tr>
</tbody>
</table>

6-TG: 6-Tioguanine; AML: Acute myeloid leukaemia; APL: Acute promyelocytic leukaemia; Ara-C: Cytosine arabinoside; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; CSA: Ciclosporin A; DNX: Liposome-encapsulated daunorubicin; MDS: Myelodysplastic syndrome; NR: Not reported; VP-16: Etoposide.
1.5 g/m$^2$ on days 1 and 2. A total of 22 patients received 76 courses of MFAC (35 courses alternating with IA (41 courses) or vice versa. The interval between courses, and degrees of myelosuppression, were equivalent in the alternating regimens. Failure-free and survival rates at 12 months were 32 and 55%, respectively. Grade 3/4 toxicities, including sepsis, neutropenic fever and nausea/vomiting, were equivalent with MFAC and IA. These findings indicate that postremission therapy with MFAC is feasible and well tolerated in patients with AML. Finally, MFAC was used in 32 patients with primary resistant AML (34%) or recurrent AML (66%) [47]. Nine patients (28%) achieved a CR, and two patients (6%) achieved a CRp. The median survival was 5.3 months, and the 12-month survival rate was 19%. Forty patients (44%) developed Grade 3 or 4 hyperbilirubinemia, six patients (18%) had Grade 3 or 4 transaminisits, and three patients (9%) had hepatic VOD.

Other cooperative groups have published the results of their pilot trials in patients with untreated AML, and have used these results to design prospective randomized trials. The EORTC/GIMEMA group investigated the effects of standard intensive chemotherapy, with or without GO administered frontline, as induction and consolidation therapy in patients aged 61 – 75 years [49]. The overall response rate to the entire induction sequence was 54.4% (31/57), with CR in 35.1% and CRp in 19.3%. An initial response to GO was documented in 20 patients (35.1%), with CR in 22.8% and CRp in 12.3%. This sequential regimen is being investigated in a Phase III trial (AML-17). A second trial (AML-19) includes GO monotherapy versus standard supportive care in patients older than 75 years with untreated AML who are not candidates for intensive chemotherapy.

The Medical Research Council (MRC) evaluated the feasibility of combining lower doses of GO (3 mg/m$^2$) with three different intensive chemotherapy regimens as first-line treatment in 72 AML patients aged 17 – 59 years [48]. They obtained CR rates of 86 – 91%, with a higher frequency of Grade 4 liver toxicity and sinusoidal obstructive syndrome in tioguanine-containing schedules. The experience of this pilot trial was used to design the MRC AML15 study, which randomly assigned patients aged < 60 years to receive induction chemotherapy ± GO/consolidation ± GO. A preliminary analysis on 1115 patients indicated that the use of GO (3 mg/m$^2$ on day 1) results in a significant reduction in relapse risk (37 versus 52% at 3 years, p = 0.01) and improvement in DFS (51 versus 40% at 3 years, p = 0.008) [55]. A subset analysis showed that GO is beneficial for patients with favorable or intermediate-risk cytogenetics but not for those with adverse cytogenetics. Subsequent follow-up has even shown a significant overall survival benefit in the favorable and intermediate groups [7]. Interestingly, in this trial P-glycoprotein expression, which is known to be inversely related to calicheamicin activity in vitro, appeared to have little effect on outcome. Also, the risk of hepatoxicity in patients who subsequently went on to transplantation did not appear increased.

Other Phase III studies that incorporate GO as part of standard induction chemotherapy in newly diagnosed patients with AML are ongoing (see [56] for additional details). The Southwest Oncology Group (SWOG) is carrying out a randomized study (SWOG106) in which GO is added to a conventional cytosine arabinoside and daunorubicin induction regimen. Patients then undergo a second randomization to receive or not receive GO as maintenance. The Eastern Cooperative Oncology Group (ECOG) is conducting a trial (ECOG E1900) in which patients in CR after two cycles of intensive consolidation chemotherapy are randomized to a single dose (6 mg/m$^2$) of GO prior to autologous HSCT. Finally, the Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) is testing the benefits of GO as maintenance therapy in older patients (HOVON 43).

Combination regimens are also being actively investigated in pediatric AML. The Children’s Oncology Group has completed a pilot study of GO in combination with chemotherapy [57] and is currently accruing children and young adults with de novo AML to a randomized Phase III trial of combination chemotherapy with or without GO [58].

### 3.3 Trials in acute promyelocytic leukaemia

Acute promyelocytic leukaemia (APL) is an ideal model to test the efficacy of GO. In fact, APL blasts typically have high and homogeneous expression of the CD33 antigen and lack of, or have very low levels of, Pgp [59]. In addition, resistance mechanisms to GO have been shown to differ from those reported to explain resistance to all-trans retinoic acid (ATRA) or arsenic trioxide (ATO) [60]. The GO monotherapy is highly active not only in patients with APL at molecular relapse [61] but even in the setting of overt or very advanced disease [62-64].

In the study by Lo Coco et al., patients were treated with GO at 6 mg/m$^2$ for two doses, and those achieving molecular remission received a third dose [61]. Molecular remission was demonstrated in 9 of 11 patients tested after two doses and in 13 of 13 patients tested after the third dose. One additional patient achieved a molecular remission after one GO administration and received no further therapy owing to hepatotoxicity.

Estey et al. administered GO 9.0 mg/m$^2$ with ATRA to 19 patients with previously untreated APL [63]. Once they achieved CR, patients were to receive 8 courses of GO (9 mg/m$^2$ every 4 – 5 weeks) and ATRA; idarubicin was added only for persistent or recurrent reverse transcription polymerase chain reaction (RT-PCR) positivity. Sixteen patients (84%) achieved a CR. All 12 patients who were tested at the time of the report had negative PCR results at 4 – 6 months after they achieved a CR; none of seven patients who were evaluated subsequently reverted to
positive PCR results (median follow-up in CR, 5 months; maximum, 14 months). GO was not associated with clinically significant hepatotoxicity.

Recently, Aribi et al. investigated the efficacy of a combination of ATO, ATRA and GO in eight patients with APL in first recurrence [65]. All patients had received previous treatment with ATRA either alone or in combination with other agents. All seven patients with haematological recurrence achieved both a haematological and a molecular CR, and the single patient with a molecular relapse also achieved a molecular response. Six of eight patients remained in second CR for longer than the duration of their first CR.

At present, no cases of VOD have been reported in APL; this may relate to the GO binding to the high level of CD33 in circulating promyelocytes, which avoids calicheamicin-induced damage of hepatic cells.

### 3.4 Safety

In the pivotal Phase II studies, adverse events were categorized as either infusion-related (those that occurred on the day of GO administration) or those that occurred during the remainder of the treatment period [29]. The incidence of Grade 3 or 4 infusion-related adverse events included chills (8%), fever (6%), hypotension (4%), nausea (3%) and hypertension (2%). Hypotension occurred several hours after completion of infusion and was transient and reversible with intravenous fluid administration in most patients. The incidence of infusion-related symptoms was significantly lower on repeat administration: 30% of patients experienced Grade 3 or 4 infusion-related events after the first dose, whereas only 10% experienced such events after the second dose (p < 0.0001). Preventive therapy with corticosteroids in addition to acetaminophen and diphenhydramine has been reported to eliminate or greatly reduce infusion-related toxicities [66].

Myelosuppression was reported in virtually all patients and was expected because CD33 is expressed on normal myeloid cells beyond the progenitor cell stage. Grade 3 or 4 neutropenia occurred in 98% of patients. Patients with CR and CRp had recovery of their absolute neutrophil count (ANC) to 500/µl in a median of 40 and 43 days, respectively, from the first dose of GO. There was no significant difference in the median time to ANC recovery to 500/µl between younger and older patients. The incidence of Grade 3 or 4 thrombocytopenia was 99%. Patients who achieved CR or CRp had recovery of platelet counts to 25,000/µl in a median of 36 and 51 days, respectively, from the first dose of GO.

During the treatment period, Grade 3 or 4 non-haematological adverse events that occurred in ≥ 5% of patients included sepsis (17%), fever (13%), chills (9%), nausea or emesis (10%), pneumonia (8%), dyspnea (8%), hypertension (8%), hypotension (8%), asthenia (6%), increased lactate dehydrogenase (6%) and neutropenic fever (6%). Of potential importance with respect to intercurrent infections, gut toxicity usually did not occur.

Grade 3 or 4 hepatic toxicity was manifested primarily by transient elevations of liver transaminases (18%) or hyperbilirubinemia (29%). Liver toxicity was usually transient, and required no medical intervention. However, some patients developed liver sinusoidal injury manifest as features similar to veno-occlusive disease (VOD) of the liver. Among 200 patients who received GO treatment without undergoing prior or subsequent HSCT, the incidence of hepatic VOD was 0.9%. However, the incidence rate increased to 19% in patients who had undergone HSCT prior to GO chemotherapy, and to 17% in those who had received GO before undergoing HSCT. In a retrospective study, the incidence of VOD was 64% (9 of 14) when GO was administered to patients with AML, before undergoing myeloablative allogeneic HSCT [67].

A report by the MD Anderson group indicates that GO is associated with a high risk of developing a potentially fatal VOD even in patients who had not received HSCT [68]. The authors hypothesized that in Phase II studies the incidence of VOD was under-reported because, prior to the use of GO, the occurrence of VOD outside the HSCT setting had been very uncommon [69]. They also noted that in the APL trials or in the MD Anderson studies on the MFAC regimen as postremission therapy, no patient developed hepatic VOD. To explain these findings, they speculated that there was a correlation between the circulating tumour load, tumour load in the liver, or circulating soluble CD33 levels and ensuing GO-associated SOS and comparative rates of SOS without GO. SOS developed at a median of 10 days following GO administration for patients who did not undergo prior or subsequent HSCT, the incidence of GO-associated SOS was 0.9% and increased to 19% in those who had undergone HSCT and who had received GO before undergoing HSCT. Among 200 patients who received GO treatment without undergoing prior or subsequent HSCT, the incidence of GO-associated SOS was 0.9% and increased to 19% in those who had undergone HSCT and who had received GO before undergoing HSCT. In a retrospective study, the incidence of GO-associated SOS was 64% (9 of 14) when GO was administered to patients with AML, before undergoing myeloablative allogeneic HSCT [67].

McKoy et al. reviewed safety reports for GO included in reports of clinical trials and observational studies, interim reports from an FDA-mandated Prospective Observational Registry, and the FDA’s Adverse Event Reporting System [73]. Medline searches provided incidence estimates of GO-associated SOS and comparative rates of SOS without GO. SOS developed at a median of 10 days following GO administration for patients who did not undergo allogeneic stem cell transplantation (SCT) and 13 days following an allogeneic SCT for patients who had previously received GO. Among adult AML patients who...
Gentuzumab ozogamicin

received GO in clinical trials, SOS incidence was 3% at doses ≤ 6 mg/m² if administered as monotherapy or in combination with non-hepatotoxic agents versus 28% if administered with tioguanine and 15% when administered as monotherapy at a dose of 9 mg/m². Observational studies identified SOS rates of 15 – 40% if an SCT is performed within 3 months of GO administration. The FDA-mandated Prospective Observational Registry of patients who received care at 60 medical centres has identified GO-associated SOS rates of 14% if an SCT is performed and 9% otherwise.

A few studies have addressed the issue of the pharmacological prevention of GO-related SOS/VOD. Recently, the prophylactic use of defibrotide has been shown to prevent SOS/VOD in children who had undergone stem cell transplantation after GO exposure [74].

3.5 Regulatory affairs

Based on the efficacy data and safety data of the pivotal Phase II trials, marketing approval of GO was granted in May 2000 by the US FDA under the Accelerated Approval regulations [23]. GO was indicated for the treatment of patients with CD33⁺ AML in first relapse who are 60 years of age or older and who are not considered candidates for cytotoxic chemotherapy. The approval of GO by the FDA was conditional upon the conduct of studies of regimens combining it with standard anti-AML chemotherapy in patients with de novo AML [23]. The drug was approved with the same indications in July 2005 by Japanese authorities. In Europe it was granted orphan drug designation for the treatment of acute myeloid leukaemia in October 2000 [75] but it has been recently denied marketing authorization for reinduction treatment of CD33⁺ AML adult patients in first relapse who are not candidates for other intensive reinduction chemotherapy regimens [76].

4. Conclusion

GO is a potent myelosuppressive agent which has a limited in vivo activity when used in relapsed AML. Actually, the most promising application of single-agent therapy with GO appears the elimination of minimal residual disease in APL.

The cumulative experience with GO suggests that it is relatively well tolerated in patients of all ages receiving treatment for AML in first relapse. The safety profile does not differ significantly between patients < 60 years and patients ≥ 60 years of age. The main safety issues consist of severe myelosuppression and hepatotoxicity, including VOD/SOS and infusion-related events. Caution should be exercised when using GO in routine clinical practice, particularly if administered with other hepatotoxic agents, in those with pre-existing hepatic pathology, or within 3 months of a SCT procedure. 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 months have elapsed since the last dose of GO. The use of doses ≤ 6 mg/m² appears to be equally effective as the FDA-approved dose of 9 mg/m² and is characterized by the absence of significant side effects, particularly VOD.

Because of the different mechanisms of action and partly non-overlapping toxicities, particularly the lack of mucositis, the integration of GO with standard chemotherapy is a rational approach and is actively investigated. Ongoing randomized trials will better define the optimal dose and timing of GO administration in AML, as well as the subgroups of patients who will benefit from GO therapy.

5. Expert opinion

GO is an important new antileukemic agent that needs further clinical development to define its optimal use. There are uncertainties regarding the role of single-agent therapy with GO in overt relapsed AML. The study population of the pivotal Phase II studies is not representative of the target population for the claimed indication (patients ≥ 60 years of age in first relapse and unfit for intensive chemotherapy). In the pivotal trials 43% of patients had an age less than 60 years, and their performance status made them potentially eligible for intensive treatment. Some of the patients actually underwent high-dose chemotherapy and allogeneic HSCT afterwards. Furthermore, a number of agents other than GO have been used for salvage chemotherapy of AML in first relapse, and similar CR rates have been described with remission duration of typically 4 – 6 months [77]. On these grounds, the European Medicines Agency (EMEA) has recently issued a negative opinion regarding the marketing authorization for reinduction treatment of CD33⁺ AML adult patients in first relapse who are not candidates for other intensive reinduction chemotherapy regimens [76]. The EMEA experts argued that in the absence of randomized trials it is difficult to quantify the clinical benefits of GO in the context of other available treatment options. Nevertheless, further investigation of single-agent treatment with GO in a randomized trial in this setting has not been deemed of sufficient interest by the major cooperative groups, essentially for the lack of a real comparator in the elderly population. In fact, a trial comparing GO to supportive care or palliative chemotherapy is seen as unethical by many haematologists.

The exact population of patients with relapsed AML for which one would currently use single-agent GO remains very difficult to define, as this requires an individual patient’s assessment of available options, including various reinduction regimens and types of HSCT, which are not scientifically established. For instance, frail patients with important co-morbidities who cannot tolerate high-dose chemotherapy yet are sufficiently fit to tolerate the GO-associated toxicity might be considered for GO on a case-by-case basis. This would to a large extent depend on the haematologist’s attitude to using more or less aggressive
treatments, and is based mostly on expert judgment rather than hard evidence. Patients who are 60 – 75 years old with high-risk features, such as antecedent haematological disorder, unfavourable karyotype or poor performance status, would appear a reasonable target. Patients with these characteristics who receive conventional chemotherapy have very few chances to either attain a remission or survive the toxicity of treatment. Unfortunately, GO is not the long hoped-for ‘magic bullet’ for AML, and remissions are short-lived if additional treatment is not administered. In this regard, much of the future of GO depends also on the development of other targeted therapies with minimal toxicity that can be used either in combination with GO, or in a sequential fashion. There is an ongoing cooperative trial of tipifarnib (a farnesyl transferase inhibitor) maintenance in AML and trials of other agents (preferably oral) with favourable toxicity profiles should be initiated in the postremission setting. Recent data suggesting that the ataxia telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3 related (ATR)-checkpoint (chk)1/chk2 pathway is important in GO-mediated cytotoxicity may encourage the investigation of combinations of GO with non-cytotoxic modulators of this pathway [78].

As a matter of fact, the more realistic expectations for GO are the incorporation of the immunoconjugate in combination regimens or in more elaborate treatment algorithms. The non-overlapping systemic toxicity with conventional chemotherapy is clearly a characteristic that supports the use of the drug in complex protocols. The results of the MRC AML15 trial, although preliminary, indicate that the use of combination regimens can actually result in improved outcomes because of the better quality of the clinical remissions. On the other hand, the excellent activity shown in APL calls for randomized trials with the agent given upfront. In the setting of minimal residual disease of APL, a confirmatory Phase II study would probably be sufficient. In fact, treatment would be guided solely by the detection of the promyelocytic leukemia-retinoic acid receptor (PML-RAR) alpha gene rearrangement, a unique molecular marker of this disease. Further trials to optimize the dose and timing of GO administration in both AML and APL are warranted.

Declaration of interest

The author has no conflict of interest to declare and no fee has been received for preparation of the manuscript.

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