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## Review paper

# Gemtuzumab Ozogamicin (CMA-676, Mylotarg) for the treatment of CD33$^+$ acute myeloid leukemia

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**Gemtuzumab Ozogamicin (GO, CMA-676) is a monoclonal antibody against the cellular surface antigen CD33 conjugated with the cytotoxic antibiotic calicheamicin. In the beginning of 2000 it obtained US Food and Drug Administration approval for the treatment of refractory acute myeloid leukemia (AML) expressing CD33 in patients older than 60 years who are not candidates for other chemotherapy. After ligation with the CD33 on the cell surface, GO is internalized and hydrolyzed. Its two components are released into the cytoplasm and calicheamicin enters the nucleus where it associates with the DNA, causing double helix breaks and finally cell death. GO is in general well tolerated. The most frequent adverse effect observed is myelotoxicity, with prolonged neutropenia and thrombocytopenia. Veno-occlusive disease of the liver is a less frequent but severe adverse effect. A phase II study points towards a percentage of overall hematologic response around 30% in the setting of refractory or relapsed disease. Future phase III trials will show the most suitable place of GO in the treatment of AML. [© 2002 Lippincott Williams & Wilkins.]**

*Key words:* Acute myeloid leukemia, CMA-676, Gemtuzumab Ozogamicin, immunoconjugates, monoclonal antibodies, veno-occlusive disease.

## Introduction

Gemtuzumab Ozogamicin (GO, CMA-676) is a monoclonal antibody immunoconjugate developed for the treatment of acute myeloid leukemia (AML).[1] It is comprised of an antibody against the CD33 molecule conjugated with the potent anti-neoplastic antibiotic calicheamicin. At the beginning of 2000, GO received approval by the US Food and Drug Administration for the treatment of refractory to standard chemotherapy or relapsing AML expressing the CD33 antigen in patients older than 60 years.[2]

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## The CD33 molecule

CD33 is a glycoprotein belonging to the sialoadhesin or siglecs (sialic acid-binding Ig-like lectins) family, and is expressed on the surface of 90% of myeloid leukemic blasts and on the surface of normal primitive hematopoietic cells, but not on less-committed hematopoietic stem cells or on cells of other tissues.[3] The CD33 gene is situated on chromosome 19q13.[4] The genes for other siglecs such as sialoadhesin (siglec 1), B lymphocyte antigen CD22 (siglec 2), receptor p75/AIRM (siglec 7) and MAG (myelin-associated glycoprotein, siglec 4) are located in the same area of the genome. Although all siglecs bind sialic acid, specificity of the inhibitory signal is provided by both the structure bearing the sialic acid and the fact that different subsets of hematopoietic cells express different siglecs on their surface.[5]

CD33's normal function after sialic acid ligation is the inhibition of cellular proliferation.[6,7] It also has a regulatory role in the development of myeloid cells.[8] CD33 has in its intracytoplasmic portion two domains of the immunoreceptor tyrosine-based inhibitory motif (ITIM) type which associate with the tyrosine phosphatases SH2-containing phosphatases 1 and 2 (SHP-1 and SHP-2) and lead to the transduction of an inhibitory signal.[9,10] This is a widespread phenomenon in hematopoietic cells in which ITIM-bearing receptors inhibit the activation of cells by recruiting phosphatases and dephosphorylating various protein substrates involved in the propagation of activating signals.[11]

In the $CD34^+$ myeloid precursors, CD33 ligation inhibits the differentiation towards dentritic cells,[8] and leads to apoptosis unless other anti-apoptotic and proliferative signals such as c-kit ligation or increased expression of *bcl-2* prevent this event (Figure 1).[12]

## Pharmacology and pharmacodynamics

The monoclonal antibody technology developed by Kohler and Milstein[13] is used in clinical oncology in three forms.[14–17] Non-conjugated monoclonal antibodies against antigens expressed on the tumor cell surface lead to the destruction of those cells by an antibody-dependent cellular cytotoxicity (ADCC) mechanism, by complement-dependent cytotoxicity[18] and induction of apoptosis.[19] Antibodies conjugated with an ionizing radiation emitting element kill their targets by irradiation. Finally, antibodies conjugated with a toxin function by driving the toxin to the target cells that express the matching antigen.

GO belongs to this third type. It is constituted by a monoclonal antibody with a specificity against the CD33 molecule, linked to the antibiotic calicheamicin.[20] The monoclonal antibody is of the IgG4 class and is humanized, the constant murine sequences being replaced by human ones. Calicheamicin is a potent antineoplastic antibiotic belonging to the family of enediyne antibiotics originating from the microorganism *Micromonospora echinospora calichensis*. It consists of two parts, an aryltetrasaccharide part, which is responsible for the ligation with the DNA, and an enediyne portion, which produces DNA ruptures.[21] Half of the antibody is conjugated with calicheamicin in the pharmacologic preparation of GO, the other half being free. An average of four to six molecules of calicheamicin correspond to each antibody molecule.[2]

GO is attached by its antibody part to the CD33 molecule. After ligation the complex is internalized in the cytoplasm where the two GO components are dissociated by cellular hydrolases. Free calicheamicin enters the nucleus and intercalates in the DNA on specific sequences of the minor groove. DNA bending induced by the antibiotic is important for the association[22] which causes multiple DNA double breaks finally leading to apoptosis.[23] Given the physiologic role of CD33 in the inhibition of cell proliferation, its ligation by GO may provide additional antileukemic effects than those resulting from targeting calicheamicin, but this remains uncertain at present.[24]

Pharmacokinetic studies in patients with AML showed that GO has a half-life of 72 h after the first dose of 9 $mg/m^2$.[25] Those studies confirmed also that calicheamicin remains conjugated to the antibody and is delivered to the leukemic cells. After the second dose of GO, increased plasma concentrations are observed as compared with the first dose, possibly because of reduced tumor burden. Nevertheless, no correlation of drug clearance with the number of blasts was found.[2] There are no differences between sexes or in older persons in GO pharmacokinetics.[26,27] The main route of elimination of GO is hepatobiliary,[2] although it must be noted that the data are from animal models and no elimination studies in humans have been done thus far.

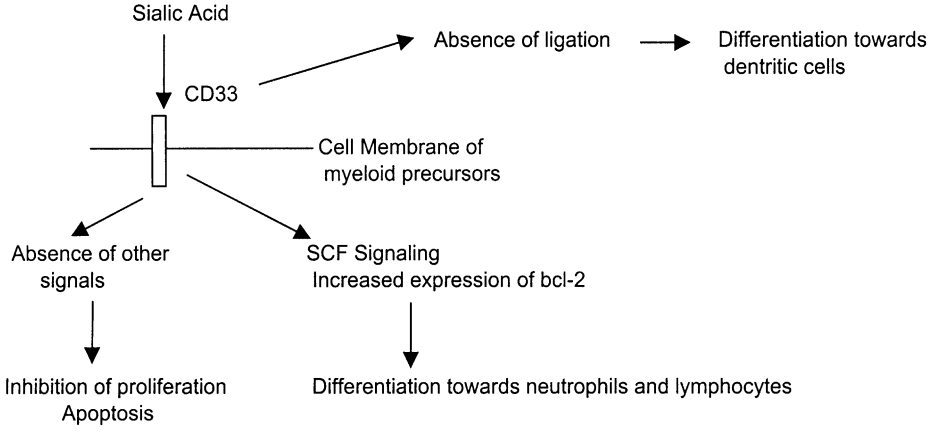


**Figure 1.** Proposed role of the CD33 antigen in the differentiation of myeloid precursor cells. In the absence of ligand, cells differentiate towards dentritic cells. Sialic acid ligation of CD33 leads to the inhibition of cell proliferation and apoptosis except if cells receive other anti-apoptotic signals in the marrow environment, permitting survival and differentiation to neutrophils and lymphocytes.

## Preclinical and clinical studies

*In vitro* cellular cultures have shown that GO is almost $10^5$ times more toxic for cells expressing the CD33 antigen than for those lacking it.[28] Leukemic blasts from patients with AML internalize the immunoconjugate after fast saturation of antigenic sites.[29] The proliferation of cell lines expressing CD33 was inhibited in phase $G_2$/M of the cellular cycle after GO treatment.[28] In those experiments, DNA laddering, an apoptosis sign,[30] was not observed. In contrast, apoptotic death was seen in other studies.[23] Those discrepancies are due to different methods used to detect cell death *in vitro*. *In vivo* GO certainly causes apoptotic death of leukemic blasts as inflammation the hallmark of necrotic death is absent.[31] Cell lines that do not express CD33 were not affected by GO treatment. CD33-expressing sublines that also expressed P-glycoprotein (P-gp or multidrug resistance) displayed resistance to GO, probably because calicheamicin is a P-gp substrate. Indeed, it was shown that blasts from patients resistant to GO exhibited a higher percentage of dye efflux *in vitro* in comparison with blasts from responding patients.[32] This efflux was blocked by the P-gp inhibitor cyclosporin A. In addition, *in vitro* treatment of resistant blasts with cyclosporin A increased the rate of their GO-induced apoptosis as measured by an Annexin V-based assay.[32]

*In vivo* experiments in the mouse showed that GO had the ability to inhibit the proliferation of transplanted human tumor xenografts.[16]

The first phase I clinical study of GO concerned patients with refractory or relapsed AML expressing CD33.[33] Forty patients, 18 of whom had previously received an autologous or allogeneic bone marrow transplantation (BMT), were included in this study. A dose escalation from 0.25 to 9 mg/m$^2$ was used. The non-myeloid toxicity was considered moderate, the more frequent adverse effects being infusion reactions and reversible hepatic enzymes elevations. Hematologic toxicity, prolonged neutropenia and thrombocytopenia were observed mainly with the higher dose of 9 mg/m$^2$. Since a significant number of patients were cytopenic even before treatment, the exact role of GO was considered uncertain at that point. Eight patients (20%) had a major response and three of them satisfied the complete remission criteria. In 79% of patients with circulating blasts in the beginning of treatment, a drop in blast counts was observed and 19% showed complete clearance of peripheral blasts after treatment. Five patients (12.5%) had normalization of neutrophil counts above 1500/μl and three of them also had platelet count normalization.

A single phase II study has been reported, representing the compilation of three smaller phase II studies.[34] One-hundred forty-two patients with AML in first relapse were treated with GO at the dose of 9 mg/m$^2$ for up to three doses 2–4 weeks apart. AML cases were considered as CD33 positive if, after anti-CD33 fluorescence-conjugated antibody staining, more than 80% of leukemic blasts displayed a CD33 staining at least 4 times above the irrelevant control antibody. The majority of patients were caucasian (94%), men (59%), had received post-remission chemotherapy (94%) and had poor- or intermediate-risk cytogenetics (95% of the cases where cytogenetics were known). The median age was 61 years (range 22–84) and the median duration of the first complete remission was 11.1 months (range 3–117). Twenty-three patients of the 142 (16%) fulfilled the complete response (CR) criteria. Nineteen other patients (12%) fulfilled all CR criteria except for a persistent thrombocytopenia of less than 100 000/μl. This category of patients, designated $CR_p$, had a median relapse-free survival not statistically different from patients with CR (7.2 months for CR and 4.4 months for $CR_p$, $p=0.624$). Thus the two responder groups were pooled together giving an overall response (OR) rate of GO in this phase II study of 30%. The overall survival (OS) depended heavily on treatment after GO, but was better in patients who had previously responded to GO. OR patients who subsequently received a hematopoietic stem cell transplantation had a median OS of more than 14.5 months.[34,35] OR patients who received no further treatment after GO had a median OS of 12.8 months, while non-responders who received no further treatment survived for a median of 2.5 months. Based on this efficacy and a reasonable safety profile (see below), GO was approved by the FDA for relapsed AML in patients over the age of 60 and not candidates for conventional chemotherapy.[2] GO was found to be equally effective in patients 60 years old or over (OR rate 26%) and in younger patients (OR rate 34%). Furthermore, the adverse effects were similar in the two age groups.[36] The duration of first complete remission (less than or more than 1 year) and cytogenetics (poor, intermediate or high risk) had no effect on the rate of response to GO.[34]

A retrospective comparison of patients with refractory AML treated with GO or high-dose aracytine-based chemotherapy showed that patients older than 60 years and with a first remission of less than 10.5 months had a greater probability of obtaining a

second remission when treated with GO.[37] Younger patients with a first remission of more than 19 months responded better to a treatment by high-dose aracytine-based chemotherapy. Those results are not discrepant with the above-mentioned data indicating that GO is equally effective in younger than 60 years old and older patients,[34] and are probably due to the fact that patients with a shorter remission duration are inherently resistant to conventional chemotherapy[38] and older patients tolerate this chemotherapy less well. In fact, independently of age, patients with a short response to a given treatment would be expected to respond better to a different treatment rather than to the same treatment they had been resistant to.

A single phase I study of GO in children is available and shows a tolerance profile similar to that of adults.[39] In this study, 18 children aged 1–16 years old with refractory or AML in relapse were included. Four of 11 children (36%) who received the dose of 9 mg/m$^2$ for two doses obtained a response with less than 5% marrow blasts.

GO was effective in a case of AML in relapse expressing CD33 and similtaneously positive for the *bcr–abl* t(9;22)(q34;q11) translocation.[40] After treatment with GO the real-time polymerase chain reaction (PCR) for *bcr–abl* diminished by 3 logs and the patient received an allogeneic bone marrow transplantation. Unfortunately she died of an intracranial hemorrhage 10 days after the transplant.

The efficacy of GO was also documented in a case of AML M3 (acute promyelocytic leukemia) in third relapse and refractory to all-*trans* retinoic acid, standard chemotherapy, high-dose chemotherapy with autologous stem cell transplantation and arsenic trioxide.[41] This patient remained in molecular remission for 11 months with negative PCR after treatment with GO. Notably thrombocytopenia with platelets between 20 and 60 $\times$ 10$^3$/$\mu$l persisted during the entire remission period.

Finally, it should be mentioned that in AML cases where there is an indication for CNS treatment, GO should be combined with conventional chemotherapy drugs used in this indication because it does not penetrate the blood–brain barrier[42].

## Resistance to GO

Many mechanisms could theoretically play a role in the lack of response to GO of a significant percentage of AML patients (Table 1), but only the contribution of the multidrug resistance phenotype has been documented experimentally in blasts from patients who failed to respond to GO treatment,[32] as mentioned earlier.

**Table 1.** Partial list of potential mechanisms of resistance to GO

| |
|---|
| Multidrug resistance (MDR) phenotype |
| Increased serum protein binding |
| Down-regulation of CD33 |
| Inherent leukemic cell resistance to apoptosis |

The plasma concentration of GO is increased after a second or subsequent doses compared with the first dose and this difference does not correlate with a decreased tumor burden.[2] This increase could be due to decreased blast uptake but also to additional mechanisms mediating drug resistance such as increased plasma protein binding and decreased clearance. Increased plasma protein binding occurs in the case of other drugs such as the new kinase inhibitor imatinib mesylate.[43]

Loss of the target surface molecule has been reported in cases of lymphoma after treatment with the anti-CD20 monoclonal antibody rituximab.[44] Whether a similar mechanism could mediate resistance to GO remains to be seen.

Finally, leukemic cells may be inherently resistant to GO-induced apoptosis because of activation of anti-apoptotic pathways or down-regulation of pro-apoptotic ones and increased ability to repair damaged DNA, all well-recognized causes of cancer cells resistance to chemotherapeutic drugs.[45]

## Adverse effects

As expected from the type of cells expressing the CD33 antigen, the most common adverse effect of GO treatment is neutropenia (Table 2) seen in almost all patients in the phase II study.[34] Moreover, 40 of the 142 patients (28%) in this study had grade 3 or 4 infections and two patients died of sepsis. Although only a small minority of megakaryocytes express CD33, thrombocytopenia was also an almost universal event after GO treatment. As already mentioned in a previous section, a prolonged thrombocytopenia was observed in some patients reaching all other criteria of CR, necessitating the designation of the special category CR$_p$. Five patients (3%) in the phase II study died of CNS hemorrhage. On the contrary, anemia was a less prominent side effect.

**Table 2.** Adverse effects of GO

| Adverse effect | Percent of treated patients |
|---|---|
| Neutropenia | 98 |
| Thrombocytopenia | 99 |
| Infusion-related symptoms (grade 3 or 4) | 34 |
| ARDS-like syndrome | rare |
| Reversible hepatic enzyme elevations | 35–50 |
| VOD | 4–5 |
| Tumor lysis syndrome | rare |
| Mucositis | 5 |

A constellation of infusion symptoms consisting of fever, chills, hypotension and dyspnea, were seen after GO infusion in a significant number of patients despite pre-medication with acetaminophen and diphenhydramine. This infusion syndrome encountered also with other monoclonal antibodies[14,46] was more common after the first infusion of GO (34% of patients experienced grade 3 or 4 symptoms) than after the second infusion (12% of patients) and could occur several hours after the completion of the infusion. Hypotension was reversible with i.v. fluids and in only one patient necessitated vasopressors. In the post-marketing surveillance, nine cases of severe hypersensitivity reactions have been reported, resulting in four deaths.[2]

A pulmonary syndrome resembling Adult Respiratory Distress Syndrome (ARDS) was also reported in eight cases in the post-marketing surveillance of GO and was fatal in five cases.[2]

Hepatic toxicity with elevation of bilirubin and liver enzymes is common after GO treatment, encountered in about one-third to half of the patients and is mostly reversible. Veno-occlusive liver disease (VOD) initially thought to be rare[47] and mostly seen in patients previously treated with BMT is a complication occurring in 4–5% of patients[2] or even more frequently.[48,49] Other predisposing factors such as amphotericin B treatment[50] are often present. The increased risk of VOD after BMT persists even if this therapy had been administered several months before GO treatment.[51] A role of acetaminophen in enhancing the hepatotoxic effect of GO has been proposed.[52] This is potentially important, given the fact that acetaminophen is recommended together with diphenhydramine as pre-medication before the GO administration to prevent hypersensitivity reactions. It should be stressed that VOD can happen even in patients treated with GO who had not previously been treated with high-dose chemotherapy and BMT. In a recent report,[53] 14 of 119 patients (12%) treated for AML, advanced myelodysplastic syndrome or blast phase of chronic myeloid leukemia by GO alone or in combination with other chemotherapy agents and who had not previously received BMT, developed VOD. Thus, patients treated with GO should be monitored for clinical and laboratory signs of VOD (jaundice, hepatic pain, weight gain and fluid retention, bilirubin and liver enzymes elevations) even if they had not been previously treated by BMT. Whether the same factors predicting the occurrence of VOD in patients who have received BMT[54] apply to patients after GO treatment remains unknown.

A tumor lysis syndrome can follow GO treatment in patients with high white blood cell counts (WBCs). Thus, reduction of WBCs to less than $30 \times 10^3/\mu$l with hydroxyurea before GO treatment is recommended.[2] Mucositis occurs in about 5% of patients,[2,55] while alopecia, cerebellar toxicity or cardiotoxicity are not associated with GO.[34] The lack of cardiotoxicity is remarkable given the relatedness of GO with anthracyclines and confirms the targeting of GO to CD33-expressing cells.

Development of antibodies against components of GO, which had been seen in rare patients in the phase I study[33] without clinical sequelae, was not seen in the phase II study.[34]

## The place of GO in the treatment of AML

GO is approved in the indication of CD33-expressing AML in patients over the age of 60 who are not candidates for other chemotherapeutic agents. This indication does not imply that GO is a drug that can be well tolerated by all elderly patients. Indeed, as already discussed, it has a significant potential for side effects which would preclude its use except in patients with a relatively good condition. One could consider GO in cases of older patients with a good performance status, relapsing shortly after conventional chemotherapy. In late relapses, re-treatment with conventional AML chemotherapy may be preferable. GO could be an option in those cases if clinical conditions such as borderline cardiomyopathy or neuropathy would limit further use of anthracyclines or aracytine.

For younger patients who are candidates for an allogeneic BMT in second remission, if they have a matched related or unrelated donor, the place of GO in the treatment scheme is not well defined. GO could be useful in those patients who are refractory

to other drugs for obtaining a response before transplantation.

The combination of GO with chemotherapy of aracytine/anthracycline or high-dose aracytine type would be difficult given the prolonged myelotoxicity seen with both GO and conventional chemotherapy alone. Use of a lower dose of GO in these combinations in an attempt to obtain an additive effect without excessive toxicity may be more feasible.

The combination of GO with less myelotoxic treatments such as the novel tyrosine kinase inhibitor STI571[56,57] in cases of AML expressing CD33 and positive for the *bcr–abl* translocation could be an avenue to explore.[55]

In cases of $CD33^+$ AML overexpressing P-gp, combining GO with P-gp inhibitors such as cyclosporine A (CsA) or quinine could be of interest, although great caution should be exerting because of the potential additive hepatotoxic effect of GO and CsA.

Although phase III studies that directly compare GO alone or in combinations, with standard chemotherapeutic treatments are not yet available, there are currently data confirming the efficacy of this targeted biologic treatment in a subset of patients with refractory AML. Nevertheless, one should always keep in mind that GO possesses a significant hepatotoxic potential. Patients with pre-existing liver or pulmonary disease or previous BMT deserve a particularly close follow-up.

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