

Chapter 7

Drug Resistance to Calicheamicin Conjugated Monoclonal Antibody Therapy

Miwa Adachi and Akihiro Takeshita

Abstract Many new agents have been introduced in the treatment of acute myeloid leukemia (AML), and around 80% of AML achieve complete remission (CR). However, a considerable number of patients relapse, which is mainly associated with drug resistance. Gemtuzumab ozogamicin (GO) is a conjugate of a cytotoxic agent, a calicheamicin derivative, linked to a recombinant humanized monoclonal antibody (mAb) directed against the CD33 antigen, which is expressed on leukemia cells from more than 90% of patients with AML. GO was approved with promising results from phase I and II studies. However, the initial phase III study failed to confirm the merit of GO compared to conventional chemotherapies. One of the reasons is explained by the drug resistance acquired in leukemia cells before and during the treatments. Several resistance mechanisms against GO have been proposed. Among them, the most important resistant mechanism is the multidrug resistant (MDR) P-glycoprotein (P-gp). Some MDR modifiers removed the resistance of GO *in vitro*. However, one of the MDR modifiers, cyclosporine A (CyA), did not improve the response rate or survival, despite considerable number of adverse effects. Several investigators have reported promising results with the use of GO in acute promyelocytic leukemia (APL), which commonly expresses a larger amount of CD33 and a lower amount of P-gp than that of AML. Recent results show the efficacy of GO in a favorable risk of AML, such as core binding factor leukemia and APL. Another calicheamicin immunoconjugate, inotuzumab ozogamicin (IO), also introduced in B cell malignancies, provides us with promising results. However, IO also reportedly has similar resistant mechanisms to GO.

Keywords Calicheamicin · Immunoconjugate · Gemtuzumab ozogamicin · Inotuzumab ozogamicin · Acute myeloid leukemia · B cell malignancies · P-glycoprotein

Abbreviations

AML Acute myeloid leukemia
mAb Monoclonal antibody

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R. S. Verma (ed.), *Resistance to Immunotoxins in Cancer Therapy*,
Resistance to Targeted Anti-Cancer Therapeutics 6, DOI 10.1007/978-3-319-17275-0_7

CR	Complete remission
GO	Gemtuzumab ozogamicin
MDR	Multidrug resistant
P-gp	P-glycoprotein
CyA	Cyclosporine A
APL	Acute promyelocytic leukemia
IO	Inotuzumab ozogamicin
OS	Overall survival
DFS	Disease-free survival
MRP1	MDR-associated protein 1
CRp	Complete remission with thrombocytopenia
RFS	Relapse-free survival
OR	Overall response
SOS	Sinusoid obstructed syndrome
HSCT	Hematopoietic stem cell transplantation
Ara-C	Cytosine arabinoside
DNR	Daunorubicin
RAEB	Refractory anemia with excess blasts
EFS	Event-free survival
pBzRs	Peripheral benzodiazepine receptors
HiDAC	High dose AraC
ETP	Etoposide
WHO	World Health Organization
ATRA	All- <i>trans</i> retinoic acid
Am80	Tamibarotene
ATO	Arsenic trioxide
MR	Molecular remission
ITAM	Immunoreceptor tyrosine activation motifs
ITIM	Immunoreceptor tyrosine inhibitory motifs
BCR	B-cell antigen receptor
CLL	Chronic lymphocytic leukaemia
NHL	Non-Hodgkin lymphoma
CBF	Core binding factor

7.1 Introduction

Acute myeloid leukemia (AML), one of the most representative hematological malignancies [1], constitutes approximately 25–30% of adult leukemias in the Western countries. The age-adjusted incidence rate of AML is approximately 3–4 per 100,000 people, and the incidence increases with aging. AML is characterized by the clonal proliferation of hematopoietic precursor cells and impairment of normal hematopoiesis. Many agents have been introduced in the treatment of AML, and around 80% of AML cases achieve complete remission (CR) [2, 3]. However, a

considerable number of patients relapse, and as a result, the 5-year-overall survival (OS) and disease-free survival (DFS) remain at around 40 and 20%, respectively. The reasons have been explored mainly by the genomic methods, which showed that AML was genetically more heterogeneous than expected. Moreover, the specificity of molecular diagnosis does not necessarily result in a specific molecular targeted therapy. Several promising agents have failed to win through randomized trials in AML [4, 5]. Monoclonal antibody therapy against CD33 was also introduced and developed despite such a background.

Gemtuzumab ozogamicin (GO), whose development code was CMA676, is a conjugate of a calicheamicin derivative and a recombinant humanized antibody (IgG₄) directed against the CD33 antigen [6]. Calicheamicin is a highly potent anti-tumor antibiotic [7–10], which binds to DNA, breaks double-stranded DNA, and induces cell death. It is classified under the same category as toxin-conjugated antibody against surface antigen of tumor cells. Here, we try to understand the action and resistant mechanism of calicheamicin immune-conjugates by GO. In addition, we introduce several means to overcome the drug resistance.

7.2 CD33

The CD33 antigen, a 67-kDa trans-membrane glycoprotein, belongs to the immunoglobulin (Ig) superfamily subgroup of sialic acid-binding Ig-like lectins (siglecs) [6, 11, 12]. It consists of two Ig-like extracellular domains and two cytoplasmic domains, [13] which have tyrosine residues similar to the immune-receptor tyrosine-based inhibitory motifs. Several protein tyrosine phosphatase inhibitors or the bridge formation by immunoglobulins result in phosphorylation of the tyrosine. While the molecular reaction stream after the phosphorylation of the tyrosine and the precise function of CD33 have not been well elucidated, it has been thought to be associated with cell adhesion and interaction. It could suppress cell proliferation and function, and induce apoptosis *in vitro* [14], but these functions have not been clarified *in vivo*.

CD33 is normally expressed on myelocyte and myelomonocytic precursor cells, as well as mature myeloid lineage cells, macrophages, monocytes, and dendritic cells [15–17]. The amount of CD33 reaches highest in promyelocytes and myelocytes, and decreases with maturation of the myeloid lineage. CD33 is also expressed on erythroblasts, megakaryoblasts, and Kupffer cells at some level, [11, 12] but not on normal hematopoietic stem cells and lymphocytes [18, 19].

Eighty to 90% of AML are reportedly considered as CD33-positive [17, 20–22]. The amount of CD33 on AML cells is estimated at 10,000–20,000 copies/cell, which is 3–5 times more than normal bone marrow cells [23]. CD33 is sometimes determined on acute lymphoblastic leukemia (ALL), but the amount is relatively smaller (5–26%) than AML [22, 24] and differs among the ALL subtypes. These facts suggest that CD33 is a useful target for the development of therapeutic agents for AML and limited ALL.

Fluorescence conjugated with anti-CD33 antibody, hP67.8, which was detected on the cell surface just after incubation, moved to intracellular location after 3–5 h and disappeared after 24 h [25]. The data supports that CD33 is rapidly internalized after anti-CD33 antibody binding, and then moved to the lysosome where the immunoconjugates undergo degradation and quenching of the fluorochrome. The internalization process indicated that antibody-cytotoxic agent complexes can effectively be taken up by CD33 positive leukemia cells. Consequently, radio- and toxin-conjugated anti-CD33 antibodies have been developed, such as conjugates of radioisotopes, calicheamicin, gelonin, and ricin [26–29]. Of these, GO has drawn attention with the encouraging results.

Many surface antigens are reportedly co-expressed on CD33-positive AML cells [24]. However, only CD34 reportedly relates to the efficacy of GO. In the previous study, GO was less effective on CD34-positive leukemia cells, even when they expressed a sufficient amount of CD33; this effect was independent of the amount of CD34 [30]. Sievers et al [31] reported in their clinical study that the expression of CD34 was associated with a shorter survival after treatment with GO. These might be explained by that CD34-positive cells have more defensive mechanisms including P-glycoprotein (P-gp) than CD34-negative cells.

7.2.1 *Gentuzumab Ozogamicin (GO)*

GO is a humanized IgG4 anti-CD33 monoclonal antibody (hP67.6) conjugated to NAc-gamma calicheamicin DMH, a hydrazide derivative of calicheamicin (Fig. 7.1) [32]. Approximately half of antibodies are conjugated by calicheamicin, with an average load of 4–6 molecules of calicheamicin per antibody. Calicheamicin, a hydrophobic enediyne antibiotic agent, was first isolated from the actinomycete *Micromonospora echiospora ssp. Calichensis* [7, 8]. The hydrazone function in the AcBut linker, which links the antibody and calicheamicin, releases calicheamicin divertive from its conjugated state under acidic conditions.

After GO binds to CD33 on the cells, CD33-antibody complexes are rapidly internalized and transferred into lysosomes [25]. The calicheamicin derivative is released via hydrolysis in the acid environment of the lysosome. Then it moves to the nucleus, and binds to the minor groove of DNA in a sequence-specific manner. It cleaves single and double-stranded DNAs by the removal of specific hydrogen atoms from the deoxyribose rings of DNAs [9]. DNA damage leads to apoptotic or non-apoptotic cell death due to mitochondrial damage [33–35]. Naito et al [36] observed cell morphology after the incubation of GO by video-microscopy, which revealed some cells exhibited apoptotic changes, while the remaining cells showed non-apoptotic features. The cytotoxic mechanism of GO is the same as that of free calicheamicin, except for the internalization via CD33. Cells incubated with calicheamicin undergo either temporary or permanent cell cycle arrest depending on the concentration [31, 36]. Transient G₂/M arrest was observed prior to the increase of the hypodiploid portion in cell lines incubated with GO. Several molecular pathways, such as Chk1 and Chk2 phosphorylation and caspase 3, reportedly played roles in this process [37].

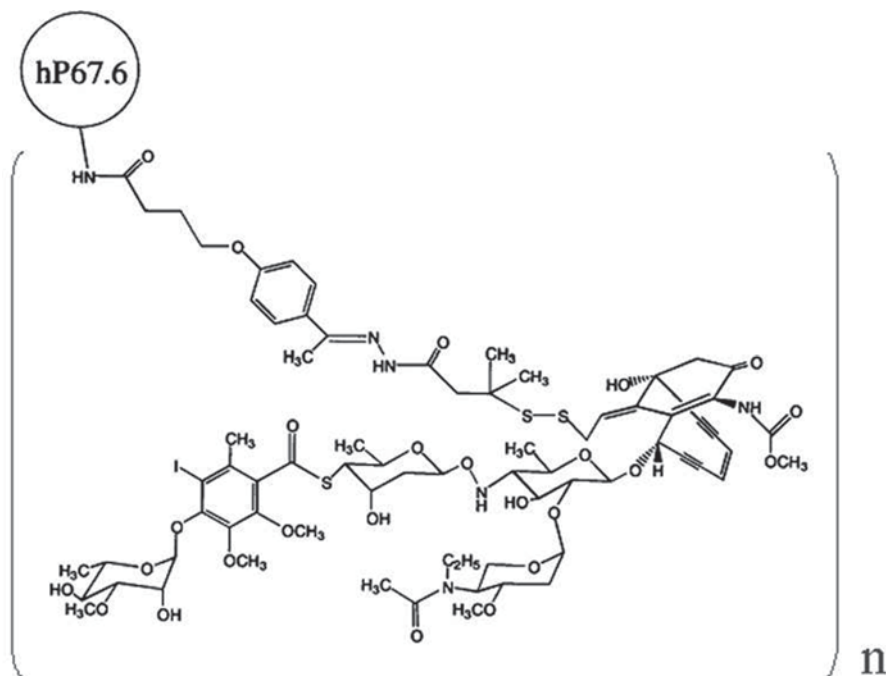


Fig. 7.1 GO is a humanized IgG4 anti-CD33 monoclonal antibody (hP67.6) conjugated to NAC-gamma calicheamicin DMH, a hydrazide derivative of calicheamicin

Cells expressing higher levels of CD33 were reportedly more susceptible to GO [38]. On the other hand, several patients with CD33-negative leukemia have also responded to GO [39]. Several studies have tried to explain the efficacy of GO on CD33-negative leukemia. One proposed explanation is that GO is partially moved into cell by CD33-independent endocytosis [39]. Another is that CD33-negative leukemia cells may have a sub-threshold low amount of CD33, which reacts substantially with GO [40].

7.2.2 GO Monotherapy, Phase I Study

In a phase I study conducted in the U.S., 40 patients with relapsed or refractory (relapsed/refractory) AML were treated by GO (0.25–9 mg/m²) [41]. Leukemia cells were eliminated from the blood and bone marrow of 8 (20%) of the 40 patients. Neutrophil counts recovered in five of these eight patients, but platelet count recovered in only three. Patients who achieved complete remission (CR) without recovering the platelet count more than $100 \times 10^9/L$ were entered to the concept of CR with thrombocytopenia (CRp), which has been subsequently used in the evaluation of GO.

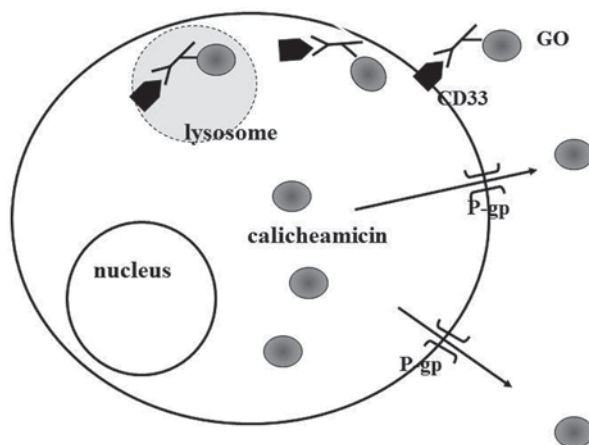
7.2.3 Phase II Study

Phase II trials with GO were started at a dose of 9 mg/m² (2-week intervals for two doses) [42]. A total of 142 patients with AML in first relapse were enrolled in the study. Of these, 30% achieved overall response (OR), including CR and CRp. The median relapse-free survival (RFS) was 5.3 months [43]. Grade 3 or 4 bilirubinemia was observed in 23%, and hepatic transaminitis in 17%. Hepatic sinusoid obstructed syndrome (SOS) was observed in seven patients (3%), and three of these were fatal. Five patients, who received hematopoietic stem cell transplantation (HSCT) before the treatment of GO, did not have apparent SOS. However, 3 of 27 patients, who received HSCT after the treatment of GO, died of SOS. Based on these results, the Food and Drug Administration of U.S. approved GO for relapsed CD33-positive AML in patients 60 years of age or older [45].

7.2.4 Drug Resistance via P-glycoprotein

MDR is a phenomenon in which malignant cells acquire cross-resistance to a variety of unrelated cytotoxic drugs. P-gp, one of the most potent MDR mechanisms, is a membrane glycoprotein that actively pumps cytotoxic agents out from cells, and decreases intracellular drug accumulation [44, 45]. Various agents have been introduced to overcome P-gp-associated drug resistance. They include calcium blocker, quinidine, cyclosporine, cepharantin, carotenoids and soforth. Naito et al [36] analyzed the cytotoxic effect of GO on NOMO-1 and NB4 cell lines as well as their multidrug resistant sublines, NOMO-1/MDR and NB4/MDR. They analyzed it by a video-microscopic system, DNA fragmentation, dye exclusion and ³H-thymidine uptake after analysis of CD33, CD34 and P-gp expressions. A concentration-dependent cytotoxic effect of GO was observed in cell lines that expressed CD33. Sensitive cells were temporally arrested at the G2/M phase of the cell cycle before undergoing morphological changes. GO was not effective on the multidrug-resistant sublines compared with the parental cell lines. MDR modifiers, MS209 and PSC833, restored the cytotoxic effect of GO in P-gp-expressing sublines. They concluded that calicheamicin derivatives, which are internalized with GO via CD33 and detached from GO in lysosomes, could be pumped out by P-gp from the cells (Fig. 7.2) [36]. Matsui et al [31] continuously analyzed the *in vitro* effects of GO on leukemia cells from 27 AML patients in relation to the amount of P-gp, MDR-associated protein 1 (MRP1), CD33 and CD34. The effect of GO, estimated by the amount of hypodiploid portion on the cell cycle, was inversely related to the amount of P-gp estimated by the MRK16 monoclonal antibody, and to the P-gp function assessed by intracellular rhodamine-123 accumulation in the presence of MDR modifiers. They showed that MDR modifiers reversed GO resistance in P-gp-expressing CD33⁺leukemia cells. GO was less effective on CD33⁺CD34⁺ than CD33⁺CD34⁻ cells. Interestingly, similar results were obtained in studies using inotuzumab ozogamicin (IO), a calicheamicin-conjugated anti-CD22 antibody, for lymphoid malignancies [46, 47]. It will, herein, subsequently be described in detail. Another study showed the cells that

Fig. 7.2 After GO binds to CD33 on the cells, CD33-antibody complexes are internalized and transferred into lysosomes, in which calicheamicin is detached. Intracellularly released calicheamicin derivatives are pumped out via P-gp in multidrug-resistant cells. MDR modifiers recover the effect of GO



were persistently exposed to low-dose GO acquired resistance to GO and expressed P-gp [48]. GO-sensitive HL-60 cells, which were persistently exposed to low concentrations of GO, changed to GO-resistant HL-60 (HL-60/GOR) cells. P-gp was significantly expressed in HL-60/GOR cells, but not in parental HL-60 cells.

These *in vitro* results were confirmed in previously mentioned phase I studies of GO [40, 41]. Good responders were more frequently observed in leukemia patients characterized by low dye efflux *in vitro*. Any kind of screening tests for P-gp before the treatment of GO might be helpful to have a better clinical outcome. Naito et al [36] suggested that the combination use of GO and MDR modifiers may be an ideal therapeutic approach for P-gp-expressing leukemia, assuming that the hematologic and non-hematologic toxicities are not worsened. This idea has been tried clinically in relapsed/refractory AML.

7.2.5 GO Treatment with MDR Modifier, CyA

Cyclosporin A (CyA), which has been easily-available and widely used as an immunosuppressant, has a considerable effect as an MDR modifier on the other hand. It has, in fact, been administered as an adjunct to GO-containing chemotherapy in the treatment of AML (Table 7.1) [49–51]. Apostolidou et al [49] treated with GO (6 mg/m² on day 6), cytosine arabinoside (Ara-C) (1 g/m² on days 1–5), liposome-encapsulated daunorubicin (DNR) (75 mg/m² on days 6–8) and CyA (on day 6) (MDAC regimen) for 11 patients with relapsed/refractory AML. One (9%) patient achieved a transient CR, and one achieved CRp. Grade 3/4 toxicities included sepsis in 7 patients (63%); hyperbilirubinemia in 6 (54%), and mucositis in 3 (27%).

Tsimberidou et al [50] evaluated the efficacy and toxicity of a combination regimen of GO (6 mg/m² on day 1), fludarabine (15 mg/m² on days 2–6), AraC (0.5 g/m² on days 2–6) and CyA (6 mg/kg on days 1 and 2) (MFAC regimen) in 59 patients with previously untreated AML, refractory anemia with excess blasts (RAEB), or

RAEB in transformation (RAEBT): 39 patients (66%) were AML and 20 patients (34%) were RAEB/RAEBT. CR was achieved in 27 patients (46%) and CRp was achieved in patient (2%). The 1-year OS was 38% and the event-free survival (EFS) in patients with CR/CRp was 27%. Grade 3/4 toxicity included hyperbilirubinemia in 31% and transaminitis in 7% of the patients. Four patients (7%) developed SOS. They conducted a Phase II study of the MFAC regimen in 32 patients with resistant/relapsed AML [51]. Nine (28%) patients achieved CR, and 2 (6%) CRp. The 1-year OS was 19%. Fourteen patients (44%) developed grade 3/4 hyperbilirubinemia, 6 (18%) grade 3/4 hepatic transaminitis, and 3 (9%) SOS.

CyA did not improve the response rate nor survival, although SOS was observed in a considerable number of patients. The unsuccessful attempt of the treatment may be explained by the possibility that CyA ablates the function of P-gp, which is widely distributed across critical organ systems, resulting in increased adverse effects, and that the clinical outcome from the P-gp negative cases assumed influence on the non-significance of the results [52]. Several transporters other than P-gp have also been suggested. MRP1, another well-known transporter protein, is sometimes expressed in AML [53]. However, the clinical importance of MRP1 was relatively limited among the mechanisms of resistance to GO [54]. Other transporters reportedly have further limited effects.

7.2.6 Drug Resistance Other Than P-glycoprotein

The roles of β cl-2 and β cl-x, anti-apoptotic proteins, in the resistance to GO have been reported [55, 56]. GO induced proapoptotic activation of Bak and Bax and stress-activated protein kinase in sensitive AML cells, but not in resistant ones, KG1a AML cells. The effect of GO was enhanced by β cl-2 antisense oligonucleotide, oblimersen sodium, but reduced by over-expression of β cl-2 and β cl-x. Bax, Bak and stress-activated protein kinase may play a role in resistance to GO [57]. The resistance mechanism is not specific for GO, but considerable. Oblimersen (7 mg/kg, days 1–7 and 15–21) was administered with GO (9 mg/m² on days 4 and 18) in 48 elderly patients with relapsed AML (Table 7.1) [55]. Twelve patients (25%) achieved OR. The median OS for all patients enrolled was 2.3 months. Grade 3/4 toxicities were sepsis (12%) urinary tract infection (8%), pneumonia (6%) and respiratory events (31%).

The peripheral benzodiazepine receptors (pBzRs) locate in the multiprotein mitochondrial pore complex which regulates mitochondrial membrane potential. Bcl-2 and related anti-apoptotic proteins block apoptosis by keeping the pores closed, but pBzR ligands promote the opening of pores and induce apoptosis. The pBzRs ligand, PK11195, increased the sensitivity of AML cells to standard chemotherapeutics both by inhibiting P-gp and by promoting mitochondrial apoptosis [56]. It increased the sensitivity to GO in AML cells *in vitro*.

Rosen et al [58] reported that the activation of survival signaling pathways, such as PI3K/AKT, MEK/ERK and JAK/STAT, is reportedly associated with GO

Table 7.1 Treatment with GO in combination with multidrug resistant modifiers for the relapsed/refractory AML

Regimen	Combination therapy	GO (/m ²)	No. of cases	Median age	CR (CRp) %	Refractory case (%)	VOD (%)	Authors
MDAC	DNA, AraC, CyA	6 mg × 1	11	37 (16–67)	9 (9)	37	0	Apostlidou et al [49]
MFAC	F, AraC, CyA	4.5 mg × 1	32	53 (18–78)	28 (6)	34	9	Tsimberidou et al [50]
Anti- β cl2	Oblimersen	9 mg × 1	48	67 (>60)	10 (15)	0	0	Moore et al [55]

AraC cytarabine, *CyA* cyclosporine A, *DNR* daunorubicin, *F* fludarabine

resistance *in vitro* in AML cells. An AKT inhibitor, MK-2206, restored the resistance of GO and calicheamicin in resistant AML cells.

The transport of GO into the bone marrow may be important for intensifying the effect of GO [27, 38]. An excess of circulating CD33-positive cells decreased the effect of GO, and resulted in worse outcomes [26, 59]. GO may be spent in the circulation before it reaches the bone marrow [31, 32, 36]. This suggests that GO might be made more effective by the reduction of CD33 in peripheral blood by proceeding chemotherapy [46]. Therefore, GO is often managed several days after the start of induction chemotherapy. However, we understand that a high blast cell count is equally an adverse prognostic factor in leukemias treated with other anti-leukemic agents.

Several agents may also enhance the effect of GO. G-CSF increased the effect of GO, and induced AML cells to enter G₂/M and a hypodiploid phase [60, 61]. Valproic acid, a histone deacetylase inhibitor, strengthened the effect of GO [62]. However, the synergistic effect of GO with these agents has not been confirmed in clinical studies. Clinically, multiple mechanisms may simultaneously arise in the development of resistance to GO.

7.2.7 Phase III Study with GO for AML and Disappearance from the Market

The Southwest Oncology Group (SWOG) studyS0106 reported the benefit and toxicity of adding GO to standard therapy in 627 patients with *de novo* AML [63]. Patients were randomized to receive induction therapy with DNR (45 mg/m² on days 1–3) and AraC (100 mg/m² on days 1–7) and GO (6 mg/m² on day 4) (AD+GO) or standard induction therapy with DNR (60 mg/m² on days 1–3) and AraC (100 mg/m² on days 1–7) (AD). After patients achieved CR, they received consolidation therapy with 3 courses of high dose AraC (HiDAC). Patients in remission were re-randomized to the treatment of GO (5 mg/m² every 28 days, 3 doses) or observation.

The OR rate was 74% in both induction arms. The RFS was not significantly different between two arms. Adverse effects were significantly increased in the AD+GO arm. The results of SWOG-S0106 triggered Pfizer Corp. to voluntarily withdraw GO from the market in 2010.

7.2.8 *Subsequent Phase III Study for AML*

In a subsequent study, 238 patients with *de novo* AML and an intermediate karyotype were treated with standard chemotherapy with or without GO [64]. GO (6 mg/m²) was added to standard 3+7 induction, and to a consolidation of mitoxantrone (MIT) and AraC. The CR rate and early death rate were not different between both groups. Grade 3/4 hepatic toxicities were increased in the GO arm. The EFS and the OS were not changed in both treatment arms. In patients who did not receive HSCT, EFS was significantly higher in the GO arm (54 vs 27%) while OS was not improved.

In the MRC-AML15 trial, 1113 patients with *de novo* AML, excluding APL, were randomly assigned to receive either of the following 3 induction treatments: DNR and AraC; DNR, etoposide (ETP) and AraC; or fludarabine, IDA, AraC and G-CSF; with or without GO (3 mg/m²) [65]. After achieving remission, 948 patients were randomly assigned to GO (3 mg/m²) in combination with amsacrine, AraC and ETP or HiDAC (1.5 g or 3 g/m²). The CR rate or the OS were not significantly different between both groups. Survival benefit of GO was observed in patients with favorable cytogenetics, but not in patients with high-risk cytogenetics. GO did not increase toxicity.

In other results from the UK and Denmark, 1115 patients with AML or high-risk MDS were randomly assigned to receive induction chemotherapy with either DNR (50 mg/m² on days 1, 3, 5) and AraC (100 mg/m² twice a day on days 1–10) or DNR and clofarabine (20 mg/m² on days 1–5), with or without GO (3 mg/m²) [66]. The OR rates were not different between both groups. GO did not increase toxicity and mortality. Three-year cumulative incidence of relapse was significantly lower, and 3-year OS was significantly better in the patients treated with GO.

Two hundred and seventy-eight elderly patients with *de novo* AML received DNR (60 mg/m² on days 1–3) and AraC (200 mg/m² for 7 days) without (control group) or with GO (3 mg/m² on days 1, 4, and 7) [67]. The OR rate was not different between the two groups. The 2-year-EFS, OS, and RFS were significantly improved by the addition of GO. GO did not increase the risk of death from toxicity.

These recent results demonstrated some advantage for patients treated with GO. In addition, induction mortality was not increased in these studies. Efficacy was observed, typically in patients with favorable-risk, and sometimes in intermediate-risk. The reason for this has not been elucidated. However, multiple resistant mechanisms observed in high-risk could explain it.

7.2.9 *The Efficacy of GO for Acute Promyelocytic Leukemia (APL)*

APL, which is classified as AML-M3 in the FAB classification system and as APL with t(15;17)(q22;q12) and *PML-RARA* transcript within myeloid malignancies according to the World Health Organization (WHO) classification system [68]. This disease is characterized by differentiation arrest in myeloid precursor cells and their uncontrolled proliferation. All-*trans* retinoic acid (ATRA) has dramatically decreased these complications, and around 90% of newly-diagnosed patients achieved CR and more than 60% survived long-term with subsequent post-remission chemotherapy [69–72]. While ATRA combined with chemotherapy has been the standard treatment for patients with APL, approximately 20% undergo relapse [73–75]. Several salvage therapies, including tamibarotene (Am80), arsenic trioxide (ATO), and stem cell transplantation, have been introduced for the treatment of APL [76, 77]. GO was also administered to APL, and the successful outcome of this therapy has been reported for patients with newly diagnosed or relapsed APL [78–80].

Several reasons have been proposed to explain the efficacy of GO for APL [81, 82]. First, a large amount of CD33 is commonly expressed on the surface of APL cells. Second, the level of P-gp on the surface of APL cells is significantly lower than that of AML. Third, APL cells are highly sensitive for free calicheamicin. Lo-Coco et al [79] reported that 14 of 16 patients with molecularly relapsed APL achieved molecular remission (MR) after GO monotherapy (6 mg/m² at 2-week intervals for three doses). Of 14 responders, seven (50%) remained in sustained MR for a median of 15 months. GO was administered again in two patients with relapse, and both obtained a new MR.

Another study reported that two patients in a third morphologic relapse with a considerable number of APL cells were treated by GO monotherapy (9 mg/m² on days 1 and 15) and achieved CR [80]. One of the patients was treated with consolidation chemotherapy, but the other was not. Both patients had a considerably long CR. GO may represent another treatment option if stem cell transplantation is not being considered in APL.

Aribi et al [81] reported the efficacy of a combination therapy consisting of ATO, ATRA and GO in eight patients with APL in first relapse. Patients were treated with ATO until CR, and then received the consolidation therapy including ATO, ATRA and GO (9 mg/m²) once a month for 10 months. The second CR was longer than the first CR in 75%. Moreover, all patients achieved MR. Grade 3/4 non-hematological toxicities were not observed. These reports show that GO is effective for APL patients with molecularly relapsed and advanced relapsed forms of the disease. These data also support the use of GO treatment for APL, which usually have low levels of P-gp and high levels of CD33.

7.3 CD22

CD22, a 140 kD a transmembrane sialo-adhesion glycoprotein, is widely distributed in mature B cells.[83–85] CD22 is a member of the Ig super-family and has seven extracellular Ig-like domains, which mediate cell adhesion to sialic-acid-bearing ligands. The cytoplasmic regions of CD22 have the immune receptor tyrosine activation motifs (ITAM) and tyrosine inhibitory motifs (ITIM). CD22 ITAMs are phosphorylated after BCR activation, and enhance the recruitment of protein tyrosine phosphatases to CD22. The CD22-associated phosphatases then dephosphorylate BCR components resulting in the attenuation of BCR signaling. The function of CD22 is reportedly to modulate the B-cell antigen receptor (BCR) signaling and to regulate cell-cell interactions. The activation of CD22 by ligand binding and cross-linking send negative signals and result in cytotoxicity for B-cell lymphoma [86–89].

7.3.1 *Inotuzuma Bozogamicin*

Calicheamicin conjugated antibody-targeted chemotherapy strategy has been also applied to B cell malignancies. Because the expression of CD22 is restricted to the B cell lineage and CD22 has a characteristic of internalising molecules, anti-CD22 antibody can be used for targeted delivery of calicheamicin. IO is the calicheamicin conjugated to a humanized IgG4 anti-CD22 mAb, G544, with the linker containing an acid-labile hydrazone. Therefore, the action mechanisms of IO are similar to GO, except that these conjugates recognize distinct molecular targets. Clinical efficacies have been reported in several B cell malignancies [90–92].

7.3.2 *Drug Resistance of IO*

The reports about the resistant mechanism of IO have not be more frequently found than those of GO. However, the similar resistant mechanisms observed in the studies of GO can be found in IO. The effect of IO was analyzed in relation to CD22 and P-gp in B-cell chronic lymphocytic leukaemia (CLL) and non-Hodgkin lymphoma (NHL) *in vitro* [47]. The cell lines used were the CD22-positive parental Daudi and Raji and their P-gp positive sublines, Daudi/MDR and Raji/MDR. The effect of IO was analyzed by morphology, annexin-V staining, and cell cycle distribution. A dose-dependent, selective cytotoxic effect of IO was observed in cell lines that expressed CD22. CMC-544 was not effective on Daudi/MDR and Raji/MDR cells compared with their parental cells. The MDR modifiers, PSC833 and MS209, restored the cytotoxic effect of CMC-544 in P-gp-expressing sublines. In clinical samples, the cytotoxic effect of CMC-544 was inversely related to the amount of P-gp, and to intracellular rhodamine-123 accumulation. The effect positively correlated with the amount of CD22.

7.4 Conclusion

Antibody-targeted chemotherapy using immunoconjugates of calicheamicin is theoretically an effective therapeutic method in the treatment of cancers. They have improved the specificity and therapeutic effects. They have been used as a single agent or in combination with conventional chemotherapies or other molecular target therapies, and several successes have been reported. However, the immunoconjugates of calicheamicin also acquire drug resistance and, hence, it should be used with understanding of their characteristic features.

GO has introduced a new perspective into the treatment of AML. However, the second evaluation of this treatment did not yield positive results mainly due to MDR. Recent studies have shown the efficacy of GO in AML, with a favorable risk in APL as well. Subsequent evaluations should focus on the efficacy of GO in the core binding factor (CBF) leukemia and its mechanism of action, which may lead to the re-approval of GO. IO is a very potent agent against B cell malignancies. IO action and resistant mechanisms will be similar to GO. Combination therapies with other agents will be promising.

Conflict of Interest No potential conflicts of interest were disclosed.

Acknowledgements We would like to express sincere gratitude Dr. Ryuzo Ohno (Aichi Cancer Center, Nagoya, Japan) and Tomoki Naoe (National Hospital Organization Nagoya Medical Center, Nagoya, Japan) for their continuous advises of this work.

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