

Treatment of Acute Promyelocytic Leukemia with Gemtuzumab Ozogamicin

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Abstract: Acute promyelocytic leukemia (APL) is a form of acute myeloid leukemia characterized by peculiar biologic features and a unique sensitivity to differentiation therapy with all-trans retinoic acid (ATRA). Modern treatment approaches to APL include simultaneous combination of ATRA and anthracycline-based chemotherapy. Gemtuzumab ozogamicin is a calicheamicin-conjugated monoclonal antibody directed against CD33, a cell surface antigen highly expressed on APL cells. Engagement of CD33 by gemtuzumab results in immunoconjugate internalization and hydrolytic release of calicheamicin, which, in turn, causes irreversible DNA damage and cell death. A number of preliminary reports have highlighted the sensitivity of APL to gemtuzumab given alone or in combination with other agents. Several reasons may account for the efficacy of gemtuzumab in APL, including: (1) CD33 is detectable in virtually 100% of APL cases; (2) calicheamicin belongs to the anthracycline family, a group of chemotherapeutic agents known to be highly effective in APL; and (3) the APL blast cells lack the multidrug resistance glycoprotein 170.

Due to the availability of other highly effective agents (ATRA, arsenic trioxide), relatively few APL patients have been treated thus far with gemtuzumab, and their follow-up is still short. However, it is conceivable that the use of this agent in APL will increase in the near future in light of its capability to induce molecular remission even in advanced disease. Furthermore, the use of low doses of gemtuzumab in high-risk patients might be relevant in order to reduce treatment toxicity due to conventional anthracyclines.

This review summarizes the mechanism of action and toxicity profile of gemtuzumab as well as the published experience with this compound in patients with newly diagnosed and relapsed APL.

Until recently, treatment of hematologic malignancies has been based on conventional chemotherapy, radiotherapy, and stem cell transplantation (SCT). Although implementation of these strategies and improved supportive care have resulted in higher response and survival rates, disease recurrence and severe toxicity still represent major problems leading to frequent treatment

Keywords

Acute promyelocytic leukemia, gemtuzumab ozogamicin, molecular remission

failure. In particular, conventional cytotoxic chemotherapy and allogeneic SCT are associated with significant morbidity and mortality.

Among the innovative treatment approaches developed in recent years, the use of monoclonal antibodies (mAbs) has emerged as an effective strategy in a number of hematologic malignancies, including leukemia and non-Hodgkin lymphoma (NHL).^{1,2} Two main classes of mAbs for therapy have been developed and tested in clinics. The first consists of unconjugated mAbs, where the antibody itself mediates cell killing. The other class is represented by antibodies conjugated to a chemotherapeutic agent, immunotoxin, or radioactive particle. Examples include anti-CD20—rituximab (Rituxan, Genentech)—and anti-CD52—alemtuzumab (Campath, Ilex)—mAbs used in chronic lymphoproliferative disorders, and anti-CD33 mAbs used in acute myelogenous leukemia (AML) and acute promyelocytic leukemia (APL).³⁻⁷

APL is a form of AML characterized by specific biologic and clinical features that require a tailored approach for treatment.^{8,9} Unlike all other hematopoietic tumors, APL is highly responsive to differentiative therapy with all-trans retinoic acid (ATRA) and other biological agents that also act as apoptosis inducers such as arsenic trioxide (ATO). Current consensus on APL treatment indicates that a simultaneous combination of ATRA and chemotherapy is the optimal front-line approach.^{8,9} Anthracyclines have long been shown to have striking efficacy in this leukemia even if used as single agents, and they are therefore included in association with ATRA in most APL protocols worldwide.⁸ As we will discuss, the newly developed anti-CD33 conjugate gemtuzumab ozogamicin (Mylotarg, Wyeth) offers a highly attractive approach for the treatment APL as it allows a potent intercalating agent to be delivered directly to leukemia blasts. Several recent studies have shown the efficacy and tolerability of gemtuzumab as a single agent or in combination with other agents for the treatment of APL.

The CD33 Antigen

The CD33 antigen is a 67 kD glycosylated transmembrane protein. It is a member of the sialic acid-binding, immunoglobulin-like lectin family, and contains two tyrosine residues (Y340 and Y358) on its cytoplasmic tail. Each tyrosine is followed, after three amino acids, by hydrophobic residues, similar to the immunoreceptor tyrosine-based inhibitory motif (ITIM) configuration of many inhibitory receptors.¹⁰ Phosphorylation of these tyrosine residues allows recruitment and activation of the tyrosine phosphatases SHP-1 and/or SHP-2.¹¹ The ability of CD33 to be phosphorylated and to recruit SHP-1 and SHP-2 suggests that it would function as an inhibitory

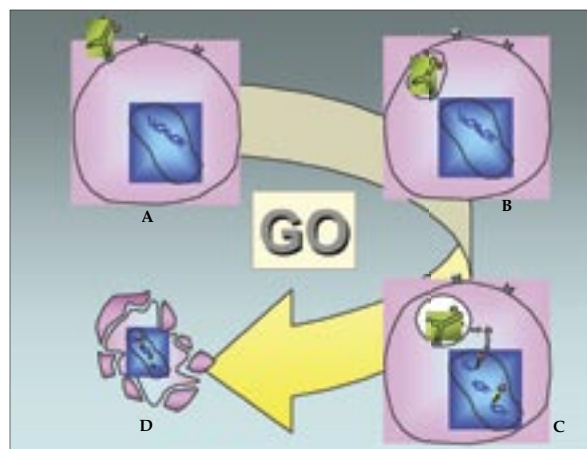


Figure 1. Mechanism of action of gemtuzumab ozogamicin (GO): (A) CD33-GO binding; (B) internalization of the immunocomplex GO-CD33; (C) calicheamicin release and DNA double strand breaks; (D) cell death.

receptor in the myeloid compartment probably suppressing, like other inhibitory receptors, signals generated by immunoreceptor tyrosine-based activation motif (ITAM)-containing receptor systems.

CD33 is normally expressed on multilineage and myelomonocytic precursors, and its expression is down-regulated on granulocytes.¹² Its expression is highly frequent on leukemic blasts from AML and myelodysplastic syndrome (MDS) patients (>90% of cases), while outside the hematopoietic system. CD33 is expressed by Kupffer cells in the liver and in the microglia.¹³ Many of the cells that express CD33 also express the CD64 antigen, a high-affinity receptor for IgG whose signal transduction activity involves ITAM.¹³

The precise function of the CD33 molecule is still unclear. According to a recently proposed model, CD33 is phosphorylated on tyrosine by an Src-family kinase activated by the CD64 cross-linking. Upon phosphorylation and subsequent recruitment of the SHP phosphatases, CD33 inhibits CD64-mediated signals leading to monocytic activation. This allows CD33 bright cells to ignore stimuli that would otherwise result in monocytic activation. As the myeloid lineage develops with the downregulation of the CD33, the cells become more responsive to stimulation through CD64 and/or other activation receptors.¹⁴ The ITIM-like domains of CD33 also seem to play a role in intracellular trafficking of anti-CD33 immunoconjugates. In fact, recent *in vitro* studies show that modification of these domains impair the internalization of gemtuzumab-bounded CD33 and gemtuzumab-induced cytotoxicity.¹⁵ The latter observation suggests that structural variations of the ITIM-like

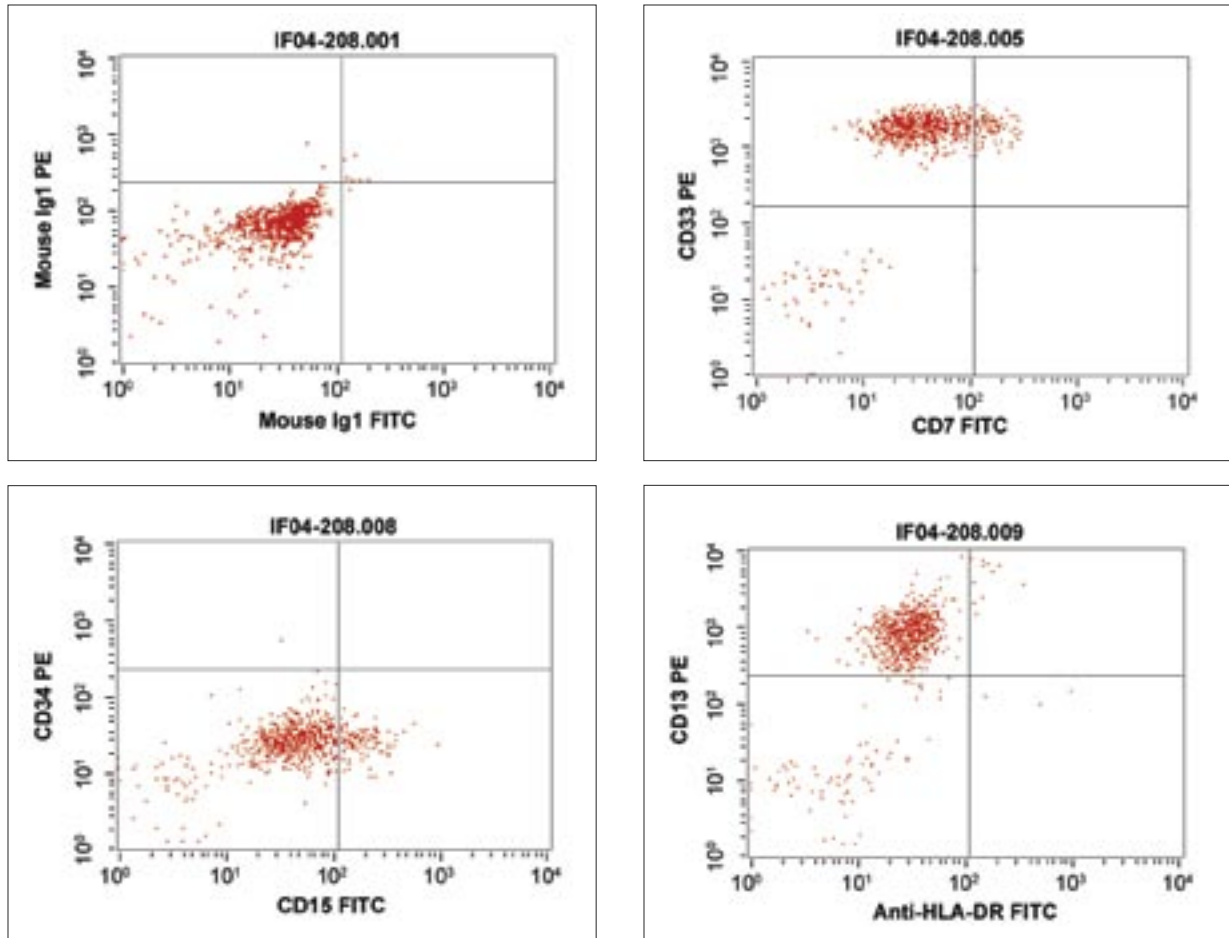


Figure 2. Antigen expression profile in a case of acute promyelocytic leukemia.

configuration may produce changes in the efficacy of anti-CD33 therapies modifying the rate of internalization of antibody-bound CD33.

Mechanism of Action of Gemtuzumab

Gemtuzumab is a conjugated mAb designed to deliver a cytotoxic agent to target cells. Direct delivery of the cytotoxic compound to tumor cells through CD33-antibody ligation should minimize side effects to nontargeted normal cells that do not express the antigen. The cytotoxic agent of gemtuzumab is N-acetyl-gamma calicheamicin dimethyl hydrazide, a derivative of gamma calicheamicin.⁵ Binding of gemtuzumab to the CD33 antigen is followed by endocytosis, cleavage of the covalent link between the mAb and calicheamicin in lysosomes by acid hydrolysis, and release of calicheamicin. Glutathione reduction produces a reactive intermediate of calicheamicin that in turn causes DNA double strand breaks (Figure 1).¹⁶⁻²¹ The cytotoxicity of calicheamicin is thought to be approxi-

mately 1,000 times greater than that of doxorubicin, a widely used antibiotic chemotherapy agent. This has precluded clinical studies using calicheamicin as a free nonconjugated agent.^{22,23}

Calicheamicin metabolites are detectable in the serum from patients receiving gemtuzumab and this may at least in part explain some of the reported adverse effects. However, gemtuzumab toxicity is limited to certain organs, such as liver and bone marrow,^{24,25} suggesting a specificity for gemtuzumab independent of CD33. Indeed, in addition to CD33-dependent internalization, some investigators have proposed an alternative mechanism of action of gemtuzumab that enables it to kill CD33-negative leukemic cells via CD33-independent endocytosis internalization mechanisms.²⁶ Both gemtuzumab uptake mechanisms are influenced by the cell cycle status, with cells in G1, S, or G2/M phase being sensitive to gemtuzumab and resting cells in G0 phase being resistant. This may also partially explain the limited toxic effects of gemtuzumab observed in extrahemopoietic organs.²⁶

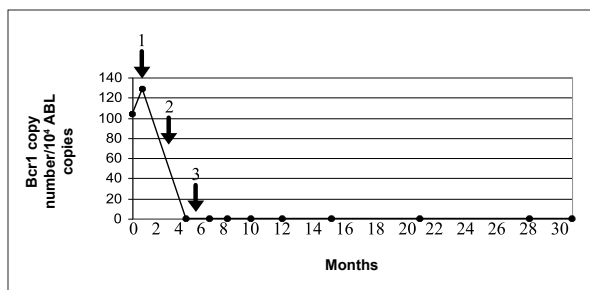


Figure 3. Real-time quantitative polymerase chain reaction evaluation of the response to GO in acute promyelocytic leukemia, showing a decline of the PML/RAR α transcript copy number after the first GO dose. Arrows represent GO administrations (6 mg/m²).

GO = gemtuzumab ozogamicin.

Rationale for Use of Gemtuzumab in APL

Although thus far there have been few published reports on the use of gemtuzumab in the treatment of APL, it is likely that its use in this subset of leukemia patients will grow in the near future, and most investigators believe that APL represents an ideal target disease for gemtuzumab.

Several reasons may account for the high efficacy of anti-CD33 antibody-based treatment in APL. First, compared to other AMLs, APL is characterized by a more consistent immunophenotypic profile, consisting of negative staining for HLA-DR and CD34 and expression of CD13 and CD33 (Figure 2). Of note, CD33 is detectable in virtually 100% of cases and shows a highly homogeneous expression pattern.^{27,28} This may explain the excellent response seen with both conjugated and nonconjugated anti-CD33 antibodies. Furthermore, the chemotherapy agent vehicled in gemtuzumab, calicheamicin, is a potent intercalator, similar to anthracyclines, which are known to be highly effective in APL. Finally, lack of or very low expression of the multidrug resistance glycoprotein 170 (Pgp) in APL blasts accounts for the striking sensitivity of APL to anthracyclines and calicheamicin.^{29,30}

Gemtuzumab for Relapsed APL

Petti and colleagues³¹ reported in 2001 on the prolonged hematologic and molecular complete response (CR) in a heavily pretreated APL patient who was treated in third hematologic relapse with two doses of gemtuzumab at 9 mg/m². This patient had previously received front-line ATRA plus anthracycline (AIDA regimen), salvage chemotherapy followed by autologous SCT for first relapse, and ATO for second relapse. Hence, gemtuzumab appeared highly effective in advanced disease, and this observation

prompted the Italian investigators to expand the use of this agent in APL. Besides treatment of molecular relapse, the GIMEMA group is conducting a randomized study comparing a two-dose treatment course of gemtuzumab with 2 years of maintenance therapy with low-dose chemotherapy and ATRA after front-line induction and consolidation.

Tsimberidou and coauthors³² described an APL patient who relapsed with extramedullary disease 2.5 years after initial treatment with liposomal ATRA. The combination of ATRA plus ATO induced a second hematologic and molecular CR, but the patient underwent further relapse. This was managed successfully with six courses of gemtuzumab, given at single 9 mg/m² doses once per month, combined in this case with simultaneous ATRA. Molecular CR was achieved in this patient after the first gemtuzumab dose.

Schwarz and associates³³ reported a patient in second molecular relapse who was treated with a single dose of gemtuzumab at 9 mg/m² and maintained with ATRA (1 week on, 1 week off) for 12 months. Also in this case, the patient obtained molecular CR after a single gemtuzumab dose.

The efficacy of gemtuzumab as a single agent for treatment of molecular relapse was investigated in 16 APL patients who had first (8 patients), second (5 patients), third (2 patients), or fourth (1 patient) molecular relapse.⁶ Gemtuzumab was administered at 6 mg/m² for two doses and patients who achieved molecular remission (MR) were given a final third dose. Patients who remained positive for the PML/RAR fusion protein in the marrow according to polymerase chain reaction (PCR) were given additional gemtuzumab courses until PCR was negative or up to a total of six doses. MR was obtained in 91% of patients tested after two doses and in 100% of patients tested after the third gemtuzumab dose. Of the three remaining patients, one achieved MR after a single gemtuzumab administration and received no further therapy due to hepatic toxicity, and two showed disease progression during treatment. As shown by real-time quantitative PCR experiments (Figure 3), there was a remarkable decline (2 logs) of the PML/RAR transcripts already after the first gemtuzumab dose. Of 14 responders, seven remained in sustained MR for a median of 15 months while seven relapsed at 3–15 months. Significantly, gemtuzumab was administered again in two relapsing patients and both obtained a new MR. Observed toxicity in this study consisted of transient myelosuppression in all cases and an asymptomatic increase of hepatic aspartate aminotransferase in four cases. One patient developed severe hepatic toxicity (grade 3) without veno-occlusive disease (VOD). In summary, gemtuzumab was well tolerated and showed high antileukemic efficacy in advanced APL. However,

Table 1. Gemtuzumab Therapy in APL

Authors	Type of Gemtuzumab Administration	Number of Cases	Status of Disease Before Therapy	Result	Toxicity
Estey et al ⁷	Gemtuzumab + ATRA ± IDA	19	Diagnosis	MCR (84%)	Hepatotoxicity, neutropenia, thrombocytopenia
Lo Coco et al ⁶	Gemtuzumab	16	Molecular relapse	MCR (88%)	Hepatotoxicity, neutropenia, thrombocytopenia
Schwarz et al ³³	Gemtuzumab + ATRA	1	Molecular relapse	MCR	Pancytopenia, hepatotoxicity
Tsimberidou et al ³²	Gemtuzumab + ATRA	1	Molecular relapse	MCR	NA
Petti et al ³¹	Gemtuzumab	1	Hematologic relapse	MCR	Neutropenia, thrombocytopenia

ATRA = all-trans retinoic acid; IDA = idarubicin; MCR = molecular complete remission; NA = not assessed.

since a high proportion of patients receiving gemtuzumab as a single agent relapsed, the authors suggested that further treatment should be given in these cases after the achievement of MR.⁶

Experience With Gemtuzumab as Front-line Therapy of APL

A single study has been published reporting the use of gemtuzumab in combination with other agents as front-line therapy of APL.⁷ In this study, newly diagnosed patients were induced into remission with conventional doses of ATRA and gemtuzumab (9 mg/m²), given at day 5 if white blood cell count was less than 10,000 mm³ or at day 1 if greater than 10,000 mm³. Gemtuzumab was administered at day 1 together with idarubicin (12 mg/m² daily, days 1–3) in three patients with leukocyte counts at diagnosis higher than 30,000/mm³. Patients in hematologic CR were to receive eight additional monthly courses of gemtuzumab along with intermittent ATRA, and idarubicin only if they persisted or converted to PCR-positive for PML/RAR. The hematologic CR rate was 84% (16 out of 19) in patients treated with ATRA plus gemtuzumab with or without idarubicin, and 88% (14 out of 16) in patients treated only with ATRA plus gemtuzumab. All 14 patients remained PML/RAR PCR-negative at a median follow-up of 12–15 months from CR. No severe toxicities were reported. Although this preliminary investigation suggests that the ATRA–gemtuzumab combination is safe and effective in APL, long-term results in a larger patient population are needed to verify whether this approach is advantageous over more established associations including ATRA and conventional

anthracycline-based chemotherapy. A summary of results reported with gemtuzumab for APL is shown in Table 1.

Toxicity of Gemtuzumab

Infusion-related adverse events after gemtuzumab administration occur mainly with the first dose and are similar to those observed with the infusion of other mAbs. These events may occur despite prophylactic treatment with acetaminophen and antihistamines and include fever, chills, cutaneous rash, hypotension, hypertension, hyperglycemia, dyspnea, nausea, emesis, and headache.³⁴

The most important gemtuzumab-associated toxicities are myelosuppression and hepatotoxicity.³⁵ The effect on myelopoiesis is likely due to the fact that although pluripotent progenitor cells do not express CD33, more differentiated multipotent hematopoietic cells are CD33-positive and may therefore represent a target for gemtuzumab. Indeed thrombocytopenia and neutropenia are constantly observed in patients treated with gemtuzumab-based regimens and represent a drug-related effect that may lead to a modification of the therapeutic schedule.^{5,36}

In phase II trials evaluating the efficacy and safety of gemtuzumab as a single agent in 142 AML patients in first relapse, there were 24 patients (17%) experiencing grade 3 or 4 aminotransferase increases and 33 (23%) with grade 3 or 4 hyperbilirubinemia.⁵ Twenty-seven patients received hematopoietic SCT, and three of them died of hepatic VOD. More recently, Wadleigh and colleagues³⁷ conducted a retrospective study of 62 patients with AML/MDS to determine whether gemtuzumab exposure prior to allogeneic SCT increased the incidence

of VOD. Nine (64%) of 14 patients who underwent SCT with prior gemtuzumab exposure developed VOD compared with four (8%) of 48 without prior gemtuzumab exposure. It is noteworthy that none of the 4 patients who underwent SCT more than 3.5 months from gemtuzumab administration developed VOD compared to 9 of 10 patients who underwent SCT less than 3.5 months following gemtuzumab administration. The authors concluded that prior treatment with gemtuzumab is a significant risk factor for VOD especially if the time interval between the two therapies is short.³⁷ Moreover, Rajvanshi and coauthors reported that 11 of 23 patients who received gemtuzumab following SCT developed liver injury, which in seven patients consisted of weight gain, ascites, and jaundice. Seven patients died of liver failure, and necroscopic examination of 5 patients showed sinusoidal injury with extensive sinusoidal fibrosis.³⁸ Nabhan and associates performed a retrospective analysis of 47 AML patients treated in a single institution with two doses of single-agent gemtuzumab 9 mg/m² given 14 days apart. Twenty-three (48%) patients experienced liver toxicity but only 1 patient developed VOD. The authors concluded that, when administered as a single agent and in absence of SCT, gemtuzumab has little association with VOD.³⁹ However in another cohort of 119 patients who received gemtuzumab without SCT, this agent was associated with the development of potentially fatal VOD.⁴⁰

As for gemtuzumab therapy for APL, to our knowledge no cases of VOD have been reported. In our series treated for molecular relapse, the most important toxicity was transient myelosuppression associated in a few cases with fever of unknown origin. Only 1 patient developed severe hepatic toxicity (grade 3) without VOD.⁶ In the APL study conducted by Estey and colleagues, 9 of 16 patients developed asymptomatic self-limited increases in serum glutamic-pyruvic transaminase (seven cases) or bilirubin (two cases). Here again, myelosuppression was observed in all cases while no patients developed VOD.⁷

The reasons underlying the apparent absence of VOD in APL patients receiving gemtuzumab are unclear. However, it is conceivable that the high CD33 expression level in promyelocytes may account for drug sequestration in the hemopoietic compartment avoiding its diffusion in other districts.

Mechanisms of Resistance to Gemtuzumab

The multidrug resistance (MDR) mechanism has been implied in resistance to gemtuzumab. The permeability glycoprotein (Pgp), a member of the adenosine triphosphate binding cassette superfamily, is the most extensively characterized peptide responsible for the efflux of a number of drugs and xenobiotics, including

anthracyclines. Calicheamicin is similar in structure and size to other substrates of Pgp,^{41,42} and studies on different AML cell lines showed that gemtuzumab cytotoxicity is influenced by Pgp expression.^{43,44} Furthermore, a phase II clinical trial with gemtuzumab in AML patients showed that Pgp expression correlates with treatment failure.⁴⁵

MDR modifiers are able to overcome gemtuzumab resistance and restore drug sensitivity in blasts derived from AML patients and in AML cell lines.^{43,46} The results obtained in vitro with MDR modifiers and the observation that Pgp expression correlates with clinical outcome led to pilot studies with gemtuzumab-based combination regimens and cyclosporine A as a Pgp inhibitor.⁴⁷⁻⁴⁹ The results showed that cyclosporine A can be safely used in combination with gemtuzumab but the response rate was not superior to that of other trials using gemtuzumab alone.

Multidrug resistance protein 1 (MRP1) is overexpressed in 7–30% of non-M3 AML cases, and in several studies its expression was higher at relapse compared to the time of diagnosis.^{50,51} However the effect of MRP1 on gemtuzumab resistance seems less important than that of Pgp on cells coexpressing both MRP1 and Pgp proteins.⁴⁴

Compared to other AML subsets, APL shows significantly lower expression levels of both Pgp and MRP1.^{29,30,52} Therefore, gemtuzumab cytotoxicity is probably more efficient in APL due to the virtual absence of this resistance mechanism. A recent study by Jedema and coauthors²⁶ pointed out the role of cell cycle on gemtuzumab sensitivity. In particular, similar to what is observed with conventional daunorubicin therapy, gemtuzumab toxicity appears to be specific for cells in G1, S, or G2/M phase of the cell cycle, with cells in resting G0 phase being resistant. Resting cells in G0 phase are not only less sensitive to the cytotoxic action of calicheamicin but also appear less efficient in taking up gemtuzumab regardless of CD33 expression level.

Another mechanism involved in gemtuzumab resistance that has been recently proposed relates to the CD33 antigen saturation status of peripheral blood (PB) and bone marrow (BM). In 92 AML patients who received gemtuzumab as salvage therapy, there was a significant inverse correlation between the PB CD33 load and the achievement of CR. Therefore, it was suggested that high PB CD33 loads reduce gemtuzumab efficacy, probably by drug sequestration and reduction of its availability for blast cell saturation in the BM. In six patients in whom BM CD33 load was evaluated, high CD33 expression levels were related to lower CD33 saturation, suggesting that CD33 load in BM also contributes to CD33 saturation and then to gemtuzumab efficacy.⁵²

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Together these findings indicate that gemtuzumab resistance is likely mediated by different mechanisms related both to intrinsic calicheamicin toxicity and the complex systems that regulate the CD33–gemtuzumab interactions.

Conclusion

Gemtuzumab has proven highly effective and relatively safe in APL, both as a single agent or in combination with other approaches. Because of its recent introduction in clinics, long-term results in patients receiving this treatment are still lacking. Due also to the small number of APL patients treated with gemtuzumab and the availability in this leukemia subset of other highly effective agents such as ATRA, ATO, and conventional anthracyclines, it is hard to establish at present the place of gemtuzumab in the therapy of APL. While long-term data on larger APL series are awaited, the use of gemtuzumab in advanced disease (second or further relapse) is advisable in light of its capability to induce molecular remission. Finally, gemtuzumab may represent a valid alternative to conventional chemotherapy in patients with severe cardiac dysfunction.

References

1. Tomblyn MR, Tallman MS. New developments in antibody therapy for acute myeloid leukemia. *Semin Oncol*. 2003;30:502-508.
2. Campbell P, Marcus R. Monoclonal antibody therapy for lymphoma. *Blood Rev*. 2003;17:143-152.
3. Cheson BD. Rituximab: clinical development and future directions. *Expert Opin Biol Ther*. 2002;2:97-110.
4. Moreton P, Hillmen P. Alemtuzumab therapy in B-cell lymphoproliferative disorders. *Semin Oncol*. 2003;30:493-501.
5. Sievers E, Larson R, Stadmauer E, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol*. 2001;19:3244-3254.
6. Lo-Coco F, Cimino G, Breccia M, et al. Gemtuzumab ozogamicin (Mylotarg) as a single agent for molecularly relapsed acute promyelocytic leukemia. *Blood*. 2004;104:1995-1999.
7. Estey EH, Giles FJ, Beran M, et al. Experience with gemtuzumab ozogamicin ("mylotarg") and all-trans retinoic acid in untreated acute promyelocytic leukemia. *Blood*. 2002;99:4222-4224.
8. Tallman MS, Nabhan C, Feusner JH, Rowe JM. Acute promyelocytic leukemia: evolving therapeutic strategies. *Blood*. 2002;99:759-767.
9. Sanz MA, Tallman MS, and Lo-Coco F. The tricks of the trade for the appropriate management of newly diagnosed acute promyelocytic leukemia. *Blood*. 2005;105:3019-3025.
10. Simmons D, Seed B. Isolation of a cDNA encoding CD33, a differentiation antigen of myeloid progenitor cells. *J Immunol*. 1988;141:2797-2800.
11. Taylor VC, Buckley CD, Douglas M, Cody AJ, Simmons DL, Freeman SD. The myeloid-specific sialic acid-binding receptor, CD33, associates with the protein-tyrosine phosphatases, SHP-1 and SHP-2. *J Biol Chem*. 1999;274:11505-11512.
12. Andrews RG, Torok-Storb B, Bernstein ID. Myeloid-associated differentiation antigens on stem cells and their progeny identified by monoclonal antibodies. *Blood*. 1983;62:124-132.
13. Angata T, Kerr SC, Greaves DR, Varki NM, Crocker PR, Varki A. Cloning and characterization of human Siglec-11: a recently evolved signaling that can interact with SHP-1 and SHP-2 and is expressed by tissue macrophages, including brain microglia. *J Biol Chem*. 2002;277:24466-24474.
14. Gillooly DJ, Allen JM. The human high affinity IgG receptor (Fc gamma RI) signals through the immunoreceptor tyrosine-based activation motif (ITAM) of the gamma chain of Fc epsilon RI. *Biochem Soc Trans*. 1997;25:215S.
15. Paul SP, Taylor LS, Stansbury EK, McVicar DW. Myeloid specific human CD33 is an inhibitory receptor with differential ITIM function in recruiting the phosphatases SHP-1 and SHP-2. *Blood*. 2000;96:483-490.
16. Walter RB, Raden BW, Kamikura DM, Cooper JA, Bernstein ID. Influence of CD33 expression levels and ITIM-dependent internalization on gemtuzumab ozogamicin-induced cytotoxicity. *Blood*. 2005;105:1295-1302.
17. Dedon PC, Salzberg AA, Xu J. Exclusive production of bistranded DNA damage by calicheamicin. *Biochemistry*. 1993;32:3617-3622.
18. LaMarr WA, Yu L, Nicolaou KC, Dedon PC. Supercoiling affects the accessibility of glutathione to DNA-bound molecules: positive supercoiling inhibits calicheamicin-induced DNA damage. *Proc Natl Acad Sci U S A*. 1998;95:102-107.
19. Yu L, Goldberg IH, Dedon PC. Enediynes-mediated DNA damage in nuclei is modulated at the level of the nucleosome. *J Biol Chem*. 1994;269:4144-4151.
20. Kumar RA, Ikemoto N, Patel DJ. Solution structure of the calicheamicin gamma II-DNA complex. *J Mol Biol*. 1997;265:187-201.
21. Zein N, Sinha AM, McGahren WJ, Ellestad GA. Calicheamicin gamma II: an antitumor antibiotic that cleaves double-stranded DNA site specifically. *Science*. 1988;240:1198-1201.
22. Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE, Upeslaci J. Preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel and potent family of antitumor antibiotics. *Cancer Res*. 1993;53:3336-3342.
23. Lode HN, Reisfeld RA, Handgretinger R, Nicolaou KC, Gaedicke G, Wrasidlo W. Targeted therapy with a novel enediynes antibiotic calicheamicin theta(1) effectively suppresses growth and dissemination of liver metastases in a syngeneic model of murine neuroblastoma. *Cancer Res*. 1998;58:2925-2928.
24. Rajvanshi P, Shulman HM, Sievers EL, McDonald GB. Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy. *Blood*. 2002;99:2310-2314.
25. Leopold LH, Berger MS, Feingold J. Acute and long-term toxicities associated with gemtuzumab ozogamicin (mylotarg(r)) therapy of acute myeloid leukemia. *Clin Lymphoma*. 2002;2(suppl 1):S29-S34.
26. Jedema I, Barge RMY, van der Velden VHJ, et al. Internalization and cell cycle-dependent killing of leukemic cells by gemtuzumab ozogamicin: rationale for efficacy in CD33-negative malignancies with endocytic capacity. *Leukemia*. 2004;18:316-325.
27. Paietta E. Expression of cell-surface antigens in acute promyelocytic leukaemia. *Best Pract Res Clin Haematol*. 2003;16:369-385.
28. Guglielmi C, Martelli MP, Diverio D, et al. Immunophenotype of adult and childhood acute promyelocytic leukemia: correlation with morphology and type of PML gene breakpoint: a cooperative Italian study on 196 cases. *Br J Haematol*. 1998;102:1035-1041.
29. Michieli M, Damiani D, Ermacora A, et al. P-glycoprotein (PGP), lung resistance-related protein (LRP) and multidrug resistance-associated protein (MRP) expression in acute promyelocytic leukaemia. *Br J Haematol*. 2000;108:703-709.
30. Paietta E, Andersen J, Racevskis J, et al. Significantly lower P-glycoprotein expression in acute promyelocytic leukemia than in other types of acute myeloid leukemia: immunological, molecular and functional analyses. *Leukemia*. 1994;8:968-973.
31. Petti MC, Pinazzi MB, Diverio D, et al. Prolonged molecular remission in advanced acute promyelocytic leukaemia after treatment with gemtuzumab ozogamicin (Mylotarg CMA-676). *Br J Haematol*. 2001;115:63-65.
32. Tsimberidou AM, Estey E, Whitman GJ, et al. Extramedullary relapse in a patient with acute promyelocytic leukemia: successful treatment with arsenic trioxide, all-trans retinoic acid and gemtuzumab ozogamicin therapies. *Leuk Res*. 2004;28:991-994.
33. Schwarz J, Markova J, Pekova S, Trnkova Z, Sponerova D, Cetkovsky P. A single administration of gemtuzumab ozogamicin for molecular relapse of acute promyelocytic leukemia. *Hematol J*. 2004;5:279-280.
34. Giles F, Estey E, O'Brien S. Gemtuzumab ozogamicin in the treatment of acute myeloid leukemia. *Cancer*. 2003;98:2095-2104.
35. Leopold LH, Berger MS, Feingold J. Acute and long-term toxicities associated with gemtuzumab ozogamicin (Mylotarg) therapy of acute myeloid leukemia. *Clin Lymphoma*. 2002(2 suppl 1):S29-S34.
36. Sievers EL. Antibody-targeted chemotherapy of acute myeloid leukemia using gemtuzumab ozogamicin (Mylotarg). *Blood Cells Mol Dis*. 2003;31:7-10.
37. Wadleigh M, Richardson PG, Zahrieh D, et al. Prior gemtuzumab

ozogamicin exposure significantly increases the risk of veno-occlusive disease in patients who undergo myeloablative allogeneic stem cell transplantation. *Blood*. 2003;102:1578-1582.

38. Rajvanshi P, Shulman HM, Sievers EL, McDonald GB. Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy. *Blood*. 2002;99:2310-2314.

39. Nabhan C, Rundhaugen L, Jatoi M, et al. Gemtuzumab ozogamicin (Mylotarg™) is infrequently associated with sinusoidal obstructive syndrome/veno-occlusive disease. *Ann Oncol*. 2004;15:1231-1236.

40. Giles FJ, Kantarjian HM, Kornblau SM, et al. Mylotarg™ (gemtuzumab ozogamicin) therapy is associated with hepatic venoocclusive disease in patients who have not received stem cell transplantation. *Cancer*. 2001;2:406-413.

41. Tsuruo T. Mechanisms of multidrug resistance and implications for therapy. *Jpn J Cancer Research*. 1998;79:285-296

42. Ueda K, Taguchi Y, Morishima M. How does P-glycoprotein recognize its substrates? *Semin Cancer Biol*. 1997;8:151-159.

43. Naito K, Takeshita A, Shigeno K, et al. Calicheamicin-conjugated humanized anti-CD33 monoclonal antibody (gemtuzumab ozogamicin, CMA-676) shows cytotoxic effect on CD33-positive leukemia cell lines, but is inactive on P-glycoprotein-expressing sublines. *Leukemia*. 2000;14:1436-1443.

44. Walter RB, Raden BW, Hong TC, Flowers DA, Bernstein ID, Linenberger ML. Multidrug resistance protein attenuates gemtuzumab ozogamicin-induced cytotoxicity in acute myeloid leukemia cells. *Blood*. 2003;102:1466-1473.

45. Linenberger ML, Hong T, Flowers D, et al. Multidrug-resistance phenotype and clinical response to gemtuzumab ozogamicin. *Blood*. 2001;98:988-994.

46. Matsui H, Takeshita A, Naito K, et al. Reduced effect of gemtuzumab ozogamicin (CMA-676) on P-glycoprotein and/or CD34-positive leukemia cells and its restoration by multidrug resistance modifiers. *Leukemia*. 2002;16:813-819.

47. Tsimberidou A, Cortes J, Thomas D, et al. Gemtuzumab ozogamicin, fludarabine, cytarabine and cyclosporine combination regimen in patients with CD33+ primary resistant or relapsed acute myeloid leukemia. *Leuk Res*. 2003;27:893-897.

48. Tsimberidou A, Estey E, Cortes J, et al. Gemtuzumab, fludarabine, cytarabine, and cyclosporine in patients with newly diagnosed acute myelogenous leukemia or high-risk myelodysplastic syndromes. *Cancer*. 2003;97:1481-1487.

49. Apostolidou E, Cortes J, Tsimberidou A, Estey E, Kantarjian H, Giles FJ. Pilot study of gemtuzumab ozogamicin, liposomal daunorubicin, cytarabine and cyclosporine regimen in patients with refractory acute myelogenous leukemia. *Leuk Res*. 2003;27:887-891.

50. Schneider E, Cowan KH, Bader H, et al. Increased expression of the multidrug resistance-associated protein gene in relapsed acute leukemia. *Blood*. 1995;85:186-193.

51. Zhou DC, Zittoun R, Marie J-P. Expression of multidrug resistance-associated protein (MRP) and multidrug resistance (MDR1) genes in acute myeloid leukemia. *Leukemia*. 1995;9:1661-1666.

52. Del Poeta G, Venditti A, Aronica G, et al. P-glycoprotein expression in de novo acute myeloid leukemia. *Leuk Lymphoma*. 1997;27:257-274.

53. van der Velden VH, Boeckx N, Jedema I, et al. High CD33-antigen loads in peripheral blood limit the efficacy of gemtuzumab ozogamicin (Mylotarg) treatment in acute myeloid leukemia patients. *Leukemia*. 2004;18:983-998.

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Abstract 1079 A Phase I Trial of *Scutellaria Barbata* (BZL101) for Metastatic Breast Cancer

Tagliaferri M, Cohen I, Vogel C, et al.

Preclinical studies found BZL101, an aqueous extract from *Scutellaria barbata*, a member of the Lamiaceae family, to have in vitro growth inhibitory effects on four human breast cancer cell lines and one murine breast cancer cell line. Studies have shown that BZL101 arrests breast cancer cells in the G1 phase and induces apoptosis.

Tagliaferri et al conducted a phase I open-label study of BZL101 for metastatic breast cancer. A total of 21 patients were treated with BZL101 350 mL/day until disease progression, toxicity, or personal decision to stop therapy. The primary endpoints were safety, toxicity, and tumor response, as defined by RECIST criteria.

No grade 3 or 4 hematologic or nonhematologic adverse events were observed. The most frequently reported side effects were grade 1/2 nausea (43%), diarrhea (20%), headache (20%), flatulence (14%), vomiting (10%), constipation (10%), and fatigue (10%). Among the 16 patients who were evaluable for response, four had stable disease for greater than 90 days and three for greater than 180 days. Three patients experienced objective tumor regression, with one being 1 mm short of a partial response, as defined by RECIST criteria. The authors concluded that BZL101 is associated with a favorable tolerability profile and early encouraging clinical activity in this heavily pretreated population (median prior treatments, 3.6 [range, 0–10]).

Abstract 203 In Vitro Propagation and Characterization of Tumorigenic Breast Cancer Cells With Stem/Progenitor Cell Properties

Daidone MG, Ponti D, Sozzi G, et al.

Another compelling area covered at the Symposium was the search for recognizable gene expression patterns among breast cancer-initiating cells. Daidone et al compared the gene expression profiles from cells of three breast cancer lesions and from the established breast carcinoma cell line MCF-7 (MCF-S) with the gene expression profile of the MCF-7 parental cell line. Cytogenetic analyses were performed on MCF-S and MCF-7 cell lines.

The findings showed overlapping transcriptional profiles among breast tumorigenic cells with stem/progenitor properties that were propagated in vitro as nonadherent tumor spheres. These profiles derived from an established breast carcinoma cell line and were distinct from those exhibited by the parental cell line. There were fewer structural chromosomal aberrations detected in MCF-S compared with MCF-7, in keeping with the hypothesis that a protective mechanism favors the survival of cells with self-renewing properties. The authors conclude that long-term cultures of breast cancer-initiating cells represent a suitable in vitro model for the development of novel diagnostic and therapeutic approaches targeting these stem cells.

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