Clinical and experimental efficacy of gemtuzumab ozogamicin in core binding factor acute myeloid leukemia

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Abstract

Leukemia-initiating cells of core binding factor (CBF) acute myeloid leukemia (AML) likely derive from early committed hematopoietic precursors expressing CD33. As such, targeting CD33 could ameliorate the chance of cure of CBF AML patients. We compared 12 CBF AML patients treated with Fludarabine, Cytarabine, Idarubicin and Gemtuzumab Ozogamicin (FLAI-GO regimen) with 25 CBF AML patients treated with the same schedule, but without GO. With the limit of small numbers, we observed a consistent trend toward better overall survival, disease free survival and event free survival in the FLAI-GO group. We also demonstrated the ability of GO to induce the disappearance *in vitro* of the AML1-ETO molecular transcript in a polymerase chain reaction-positive graft without decreasing the clonogenic potential of CD34+/CD38- cells. This represent the proof of principle for using GO in a purging strategy before autologous stem cell transplantation. Therefore, our data argue in favor of the reinstitution of GO in the therapy of CBF AML.

Brief Report

Core Binding Factor (CBF) Acute Myeloid Leukemia (AML) patients experience treatment failure in the order of 40-50%.[1] As such, efforts have been made to increase the dose intensity of first line treatment over the standard Daunorubicin/Cytarabine *3+7* induction, under the assumption that better survival could be achieved by obtaining a deeper early clearance of blasts before clonal evolution of the disease. Among other attempts, the addition of a third chemotherapeutic drug, such as Fludarabine, has been tested in various clinical trials,[2,3] with conflicting results regarding Overall Survival (OS), but, in most cases, with an increase in the rate of complete remission (CR) and Disease-Free Survival (DFS).[2,3] Moreover, novel agents with compatible safety profile have been tested in addition to chemotherapy; among these, the immunoconjugate Gemtuzumab Ozogamicin (GO), which combines in a single agent a monoclonal antibody targeting CD33 with the DNA damaging toxin calicheamicin. Following initial studies, GO has been combined to chemotherapy to improve efficacy, particularly in the case of CBF AML, in which blasts highly express the target antigen.[1,3] Three-drug Fludarabine-based regimens combined with GO proved successful mainly in the setting of cytogenetically favorable, such as CBF AML, or intermediate-risk AML,[3,4] whereas results have somehow been disappointing in adverse risk patients.[3,4] Despite this, the clinical development of GO has suffered from concerns raised by an increased incidence of hepatotoxicity and veno-occlusive disease when GO was used at the dose of 9 mg/m^2 twice during induction,[5] and from the early results of a randomized phase-3 trial that showed no advantage in OS and a significant increase of Treatment-Related Mortality (TRM) (5% *vs* 1%) in the GO-treated group.[6] This ultimately led to the withdrawal of the drug from the market in 2010. Later studies[3,7,8] showed, on the opposite, an unequivocal survival benefit by GO, even if mainly restricted to CBF AML, at the lower schedule of 3 to 6 mg/m^2, without neither increased hepatotoxicity nor higher TRM.[3,7,8] This led to the revaluation of GO, which, unfortunately, has not yet resulted in the reinstitution of the drug to clinical practice.[5]

In order to address the role of GO in the treatment of CBF AML, we retrospectively reviewed 12 CBF AML patients [t(8;21) n=8; inv(16) n=4] treated from 2006 to 2009 with the FLAI-GO regimen (Fludarabine 30 mg/m^2 on days1-5; Cytarabine 2 gr/m^2 on days1-5; Idarubicin 10 mg/m^2 on days 1,3,5; GO 3 mg/m^2 on day 6),[4] and consolidated with two high-dose cytarabine (HiDAC)-based cycles (overall dose 24 gr/m^2/cycle). We applied a regimen that was previously described in an independent series of AML patients.[4] Patients with c-KIT tyrosine kinase domain mutation at codon 816 (TKD^{816}) at diagnosis (n=2) or with the persistence of molecular transcript, assessed by sequential polymerase chain reaction (PCR), at the end of consolidation (n=5), *i.e.* Minimal Residual Disease (MRD)-positivity, were then consolidated with either allogeneic or autologous hematopoietic stem cell transplantation (HSCT) based on the availability of a donor. We decided to further intensify the treatment of KIT mutated patients early on, based on initial studies showing an adverse prognosis of these patients as compared to KIT wild-type CBF

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AML.[9] Conversely, the persistence of the molecular transcript at the end of consolidation was considered a predictor of adverse prognosis based on previous experiences,[10,11] as well as our own unpublished data.

We compared this group with 25 CBF AML patients [t(8;21) n=13; inv(16) n=12] treated according to the same criteria and with the same schedule, but without GO, in the years 2003-2006 and 2010-2013. The two groups were comparable in all clinical and laboratory features (Table 1). In the latter group, autologous HSCT was performed in 5 patients because of MRD-positivity, and allogeneic HSCT was performed in 2 patients because of the lack of cytogenetic response after induction therapy.

Patients in the two groups reached a comparable rate of CR after induction (n=12/12 *vs* 22/25, P=0.540); no death in induction was observed, and all patients completed their therapeutic schedule. At median follow-up of 69.2 months, 3 patients in the FLAI-GO group relapsed at 8, 14 and 42 months after the achievement of CR; of these, two out of three achieved second CR following rescue therapy and underwent allogeneic HSCT. Conversely, at median DFS 14.7 months, 11 patients of the FLAI group relapsed; among these, 7 out of 10 achieved second CR and were consolidated with allogeneic HSCT.

As shown in Figure 1, we observed a consistent, yet non-statistical trend towards better OS, DFS and, most importantly in evaluating the efficacy of first line therapy, Event Free Survival (EFS) in the FLAI-GO group (5-yrs OS 69.4% *vs* 48.6%, P=0.202; 5-yrs DFS 54.7% *vs* 42.4%, P=0.327; 5-yrs EFS 54.7% *vs* 36.9%, P=0.136; Figure 1). Besides, patients tended to relapse later when treated with FLAI-GO (median DFS: unreached *vs* 14.7 months; Figure 1). We believe these differences did not reach the statistical significance mainly due to small numbers. The achievement of MRD-negativity (FLAI-GO=100% of the 4 patients analyzed; FLAI=63% of the 13 patient analyzed), assessed by PCR, was of pivotal prognostic importance (P<0.001 for either OS, DFS and EFS).

The potential of GO in the treatment of CBF AML is based on strong biological bases: first of all, most t(8;21) AML blasts do not express the transporter P-glycoprotein (Pgp),[12] *i.e.* the Multi-Drug Resistance 1 (MDR1) gene product, and this seems related to a selective repression of the promoter of MDR1 by AML1-ETO.[13] Several studies have pointed out how GO extrusion by Pgp affects clinical response.[14] Moreover, CBF fusion transcripts promote leukemogenesis by inducing the initial expansion of a preleukemic myeloid cell compartment predisposed to secondary mutations and characterized by CD33+ early committed myeloid precursors incorporating the AML1-ETO or CBFB-MYH11 transcripts.[15] According to this model, founding Leukemia-Initiating Cells of CBF AML, differently from other types of AML, would arise from these early committed myeloid precursors rather than the more immature Hematopoietic Stem Cells,[15] and therefore be more sensitive to GO therapy because of their markedly higher expression

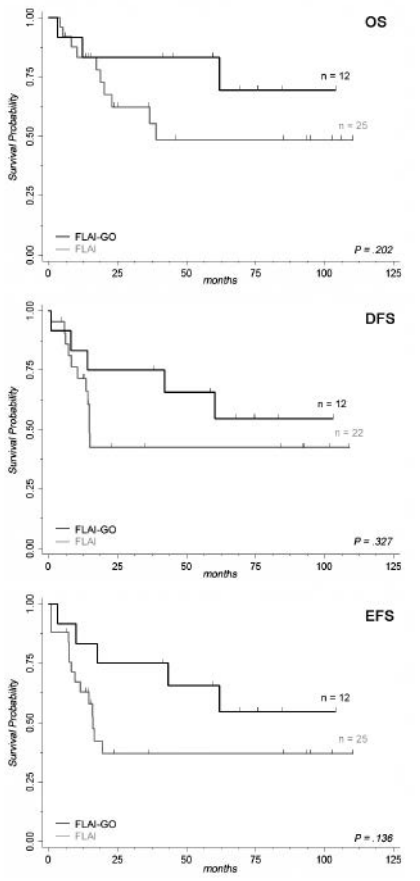

Figure 1. Survival by treatment with Gemtuzumab Ozogamicin (GO). Patients receiving FLAI-GO induction therapy showed a consistent, yet nonstatistical, trend towards better Overall Survival (OS), Disease-Free Survival (DFS) and, most importantly in determining the effect of first line treatment, Event-Free Survival (EFS), as compared to a group of patients treated with FLAI. Numbers of the two groups are showed as n; only patients achieving complete remission after induction therapy were considered in determining DFS. Inclusion criteria and treatment schedule was the same in the two groups apart from the addition of GO at 3 mg/m^2 to induction therapy at day 6 of the FLAI-GO regimen.

Table 1. Baseline patient characteristics.

	FLAI5	My-FLAI5	P
Median age	41.3 (18-66)	46.3 (29-67)	0.2602
Sex	12 M + 13 F	6 M + 6 F	0.909
Secondary acute myeloid leukemia	0	0	NA
Hepatomegaly	4	2	1.0
Splenomegaly	3	2	1.0
Sarcoma	0	1	1.0
Hemoglobin gr/dL	8.4 (4.2-11)	8.5 (5-13.6)	0.9100
White blood cells $\times 10^3$/ L	19.0 (1.6-95)	18.7 (4.5-45.5)	0.9706
N $\times 10^3$/ L	1.86 (0.33-6.35)	2.58 (0.45-11.36)	0.3680
Mo $\times 10^3$/ L	0.95 (0.01-2.56)	0.58 (0.01-2.68)	0.1330
Ly $\times 10^3$/ L	2.67 (0.50-6.80)	2.86 (0.60-7.73)	0.8016
Blasts $\times 10^3$/ L	10.9 (0.01-65.55)	11.6 (0.22-32.0)	0.9074
Platelets $\times 10^3$/ L	60.66 (8-255)	73.72 (6-531)	0.7150
Elevated LDH	18	10	0.638
DIC	2	2	0.305
Acute renal failure	0	1	0.314
t(8;21)/inv(16)	13/12	8/4	0.491
FLT3-ITD	3	2	1.0
NPM1 mutated	0	0	NA
KIT TKD ^{816} mutated	1	1	1.0
Packed BM (>80%)	12	5	0.717
Additional cytogenetic abnormalities	None: 14 pts; 1: 7 pts; 2: 3 pts; 3: 1 pts	None: 4; 1: 5 pts; 2: 1 pts; 3: 2 pts	0.387

of CD33. The distinctive chemosensitivity shown by CBF AML[1] could also be explained by these biological differences.[15]

As such, autologous HSCT could be used to increase the dose intensity of first line therapy in selected patients, improving OS, as shown by some studies.[16] Results are best when MRD-negativity in the bone marrow and in the products of leukapheresis is obtained before transplantation.[17] Recently, a 5-year EFS of 93% was reported in a small series of CBF AML patients undergoing autologous HSCT with a PCR-negative graft, with the disappearance of CBF transcripts after HSCT in 8 out of 10 previously MRD-positive patients.[18] Nonetheless, in a previous series we observed that clinical outcome of patients could be improved also when autologous HSCT had been performed with CD34+ grafts with persistent molecular transcripts.[16] We explain this finding by the higher overall dose-intensity achieved by first line treatment by including autologous HSCT.

The achievement of MRD-negativity has been related to a better prognosis by many trials.[19] Autologous HSCT, performed at the end of first line treatment, might be beneficial for selected CBF AML patients to achieve the disappearance of MRD, especially when performed using MRD-negative Peripheral Blood Stem Cells (PBSC).[17] Monoclonal antibodies are thus being tested to provide *in vivo* purging of PBSC. This approach resembles the use of Rituximab in the therapy of patients affected by CD20+ lymphomas and undergoing autologous HSCT. Concerns are there, though, that treatment with GO might affect hematopoietic reconstitution by reducing the number of hematopoietic long-term repopulating cells collected by leukapheresis.

We therefore evaluated the clonogenic growth of PBSC collected at the end of consolidation therapy from five patients with CBF AML. Briefly, mononuclear cells were cultured in RPMI medium in the presence or absence of GO at a concentration of 5 µg/mL for two hours, a previously *in vitro* dose proved able to induce the near-complete saturation of the CD33 antigen sites.[20] Cells were then collected, GO removed by centrifugation, and cells reseeded in Petri dishes containing semisolid MethoCult GF-H4434 medium. The number of Colony-Forming Units (CFU) was determined after 14 days incubation.

Using unsorted cells, we observed a significant decrease in the number of CFU-GEMM (clonal efficiency 0.000697 *vs* 0.01037, P=0.016), CFU-GM (0.002606 *vs* 0.004071, P=0.038) and BFU-E colonies (0.003213 *vs* 0.004697, P=0.031) in the cells exposed to GO. We then performed the same experiments on samples from the same PBSC units after the enrichment in CD34+/CD38- cells by immunomagnetic sorting. The efficacy of this sorting was proved by either immunophenotypic analysis and functional assays, which resulted in significantly more numerous CFU-GEMM, CFU-GM, BFU-E (*data not shown*). This time, though, we did not observe a significant decrease in the clonogenic potential of CD34+/CD38- cells by the exposure to GO (0.01518 *vs* 0.02631, P=0.351), thus confirming the preservation of more immature hematopoietic precursors. Moreover, in one MRD-positive patient we could observe the disappearance of the AML1-ETO molecular transcript following *in vitro* incubation with GO at a concentration of 5 µg/mL for two hours (Figure 2).

Therefore, with the limit of small numbers, the results that we report suggest the possibility of using GO in a purging strategy that would possibly act on residual CBF AML cells without affecting the repopulating ability of PBSC. In order to avoid the limitation of *in vitro* purging, GO could be used *in vivo* before $CD34^+$ cell collection in MRD-positive patients.

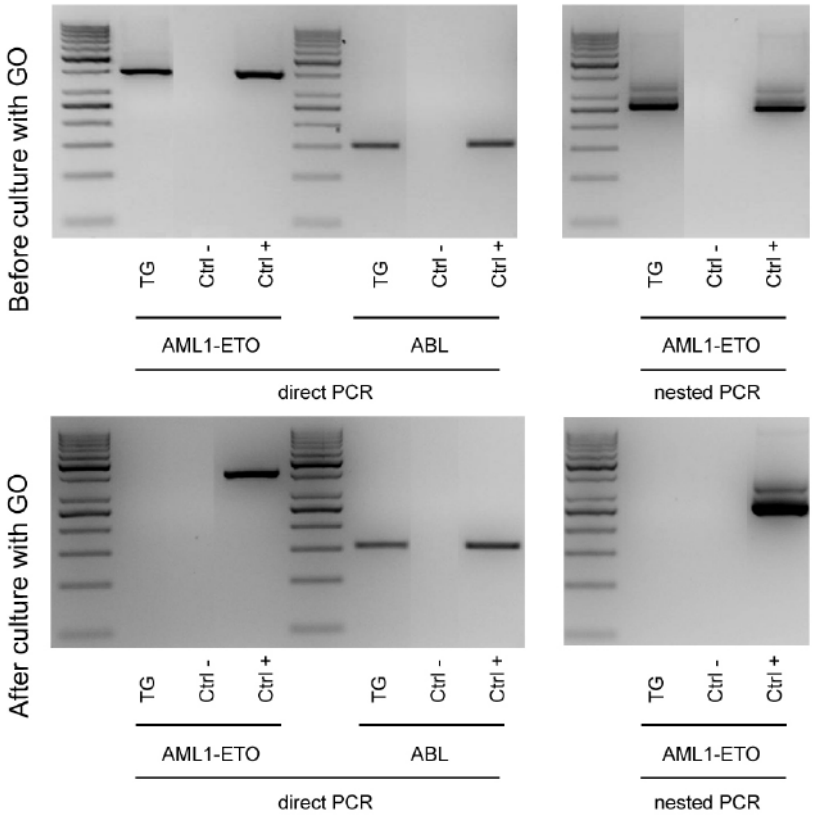

Figure 2. Disappearance of AML1-ETO molecular transcript following in vitro purging of PBSC with Gemtuzumab Ozogamicin. Samples from PBSC collected from 5 patients affected by CBF AML at the end of consolidation were cultured in the presence or absence of Gemtuzumab Ozogamicin at a concentration of 5 µg/mL for two hours. In one patient (*i.e.* TG) affected by t(8;21) AML with persistent AML1-ETO transcript at the end of consolidation and in the PBSC, incubation with GO obtained the disappearance of cells expressing AML1-ETO, as tested by either direct and nested PCR. Abelson (ABL) amplification was used as internal control. TG: initials of the patient's name; Ctrl-/Ctrl+: negative and positive controls.

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