The clinical potential of inotuzumab ozogamicin in relapsed and refractory acute lymphocytic leukemia

Ther Adv Hematol

2015, Vol. 6(5) 253–261

DOI: 10.1177/2040620715596715

Musa Yilmaz, Samantha Richard and Elias Jabbour

***Abstract*:** Antibody–drug conjugates (ADCs) are likely to make a significant contribution in the treatment of acute lymphoblastic leukemia (ALL) by combining the cytotoxicity of chemotherapy with the specificity of monoclonal antibodies. CD22, an endocytic receptor expressed by the majority of B cells, is an excellent target for ADCs. Inotuzumab ozogamicin (INO) is an ADC that consists of a cytotoxic moiety (derivative of calicheamicin) attached to a humanized monoclonal anti-CD22 antibody. As a single agent, INO, was shown to be effective with an objective response rate of 50% in the treatment of relapsed and refractory CD22 positive ALL patients. Clinical trials investigating the combination of INO with the conventional chemotherapies are ongoing. This review summarizes the clinical potential of INO in treatment of relapsed and refractory ALL, based on currently available data in the literature.

Keywords: acute lymphoblastic leukemia, inotuzumab ozogamicin, refractory, relapsed, salvage

Introduction

Multiagent chemotherapy regimens induce complete response (CR) in 80–90% of acute lymphoblastic leukemia (ALL) patients [Kantarjian *et al.* 2004]. However, nearly half of these patients relapse eventually and absence of effective salvage regimens significantly limits patients' survival. Typically, 5-year overall survival (OS) for patients with relapsed and refractory ALL is less than 10% [Fielding *et al.* 2007]. Thus, there is a constant need to improve treatment strategies in ALL.

Leukemic cells express lineage-specific antigens which are utilized to make diagnosis and define immunologic subtypes. These surface and intracellular antigens also function as a target for novel treatment strategies with monoclonal antibodies. CD19, CD20, CD22 and CD52 are the main antigens expressed in B-ALL cases [Bene *et al.* 1998]. Among others, CD22 is one of the most commonly expressed antigens on mature and immature B cells, but not on pluripotent hematopoietic stem cells [Piccaluga *et al.* 2011]. It thus becomes an attractive target for immunotherapeutic approach.

CD22 is a member of a homologous family of sialic-acid-binding immunoglobulin-like lectins (siglecs), which comprise a group of receptors that are restrictedly expressed in immune cells [O'Reilly and Paulson, 2009]. Siglecs are endocytic receptors where cytotoxic agents conjugated to an antibody can bind and effectively carried into the cell without shedding into the extracellular environment [Shan and Press, 1995]. Inotuzumab ozogamicin (INO) is an antibody–drug conjugate (ADC) that consists of a derivative of calicheamicin (a potent DNA-binding cytotoxic agent) attached to an engineered humanized monoclonal immunoglobulin G4 (IgG4) antibody targeting CD22 [Ricart, 2011]. The therapeutic effect of INO has initially been shown in non-Hodgkin lymphoma (NHL) [Advani *et al.* 2010]. Herein, we review the properties and current clinical applications of INO in relapsed and refractory ALL.

Correspondence to:
Elias Jabbour, MD
Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Box 428, 1515 Holcombe Blvd., Houston, TX 77030, USA
ejabbour@mdanderson.org

Musa Yilmaz, MD
Department of Hematology and Oncology, Baylor College of Medicine, Houston, TX, USA

Samantha Richard, Advanced Prac. Registered Nurse
Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Inotuzumab ozogamicin (INO)

Description

INO consists of a semisynthetic derivative of *N*-acetyl γ-calicheamicin 1, 2-dimethyl hydrazine dichloride (NAc γ-calicheamicin DMH), a potent DNA-binding cytotoxic antibiotic, attached to a humanized monoclonal IgG4 antibody, G544, directed against the CD22 antigen present on B cells in all patients with mature B-ALL and most patients (>90%) with precursor B-ALL [Piccaluga *et al.* 2011]. Anti-CD22 monoclonal antibody without conjugated cytotoxic drug has shown to have no antitumor activity in preclinical models; instead conjugation with cytotoxic agent provided potent dose-depending cellular damage [Dijoseph *et al.* 2004]. IgG4 antibodies alone poorly fix complement and therefore cannot cause apoptosis *via* complement-mediated and antibody-dependent cytotoxicity [Advani *et al.* 2010].

Calicheamicin is natural product of *Micromonospora echinospora* and considered to be intolerantly toxic when not bound to the antibody [Kantarjian *et al.* 2012b]. Calicheamicin is linked to the antibody through 4-(4-acetylphenoxy) butanoic acid (acetyl butyrate), which provides stability in physiologic pH and successful calicheamicin release inside the acidic environment of the lysosomes [Hamann *et al.* 2002]. INO binds to the CD22 receptor on the surface of B cells and the CD22 receptor–INO complex is internalized forming an endosome. Subsequently, the CD22 receptor–INO complex containing endosome fuses with lysosomes. This is followed by intracellular release of calicheamicin. Calicheamicin binds to the minor groove of DNA in a sequence specific manner and breaks double-stranded DNA, resulting in cell death.

Pharmacokinetics and pharmacodynamics

The maximum tolerated dose (MTD) was determined as 1.8 mg/m^2 by a first-in-human clinical trial evaluating the safety of INO in 79 relapsed and refractory NHL patients [Advani *et al.* 2010]. The following dose escalation schedules were evaluated: 0.4, 0.8, 1.34, 1.8 and 2.4 mg/m^2 intravenously (as a 1-hour infusion) once every 3 weeks in the MTD lead-in cohort (36 patients). Escalation stop criteria were met as 2 of 6 cohort patients had dose-limiting toxicities (one grade 4 neutropenia, one grade 4 thrombocytopenia) at 2.4 mg/m^2. Thus, 1.8 mg/m^2 was established as the MTD.

Reversible thrombocytopenia is one of the main (90%) side effects of INO and it led to a number of treatment delays. Therefore, the declared MTD was evaluated in the once every 4 weeks schedule to allow platelet recovery. Dose-limiting toxicity was not observed in the six patient cohort of 1.8 mg/m^2 once every 4 weeks. As a result, this regimen was used in the extended MTD cohort.

Pharmacokinetic samples were available for INO, anti-CD22 antibody, free and total calicheamicin derivatives. The data showed that INO disposition was nonlinear with number of dose or increasing dose. Nonlinearity is seen commonly with other antibodies as well [Lobo *et al.* 2004]. It is due to target-mediated drug disposition, in which elimination and distribution are affected by the antibody and target cell interaction [Cao and Jusko, 2014]. Mean end of infusion peak concentrations for 2.4 mg/m^2 once every 3 weeks and 1.8 mg/m^2 once every 3 weeks could not be distinguished from each other. For the INO and total calicheamicin, increases in area under the curve extrapolated over dosing interval (AUC_T) with period and increases in AUC_T with dose were observed. Anti-CD22 antibody and total calicheamicin followed a similar elimination trend and the free calicheamicin concentration remained less than 1 ng/ml over time, suggesting that the acetyl butyrate linker is noticeably stable in plasma [Advani *et al.* 2010].

In humans, the mean molecular equivalents of soluble fluorophore expression of CD19 lymphocytes that were CD22-phycoerythrin-positive before and after INO infusion were reported [Advani *et al.* 2010]. Reductions in mean CD22 fluorescence intensity by flow cytometry were observed shortly after the first dose of INO and its intensity was even more reduced after the second dose.

Gemtuzumab ozogamicin (GO), another ADC of NAc γ-calicheamicin DMH, is directed against the CD33 antigen, and has been used in treatment acute myeloid leukemia (AML) [Larson *et al.* 2005]. However, drug resistance had a negative impact on the clinical outcome of patients treated with GO [Matsui *et al.* 2002]. It is proposed that the drug resistance was associated with P-glycoprotein (P-gp) expression. P-gp is a membrane glycoprotein that actively pumps cytotoxic agents out of the cells and reduces the intracellular concentration of the drug [Takeshita *et al.* 2005]. As with GO, INO was also found to be

affected with the same resistance mechanism. In a study, INO had no effect on CD22-positive malignant cell lines with P-gp expression compared with parental cells [Takeshita *et al.* 2009]. In clinical samples, the toxic effect of the INO was inversely related to the amount of P-gp ($p = 0.003$). In contrast, the cytotoxicity of INO correlated positively with the amount of CD22 ($p = 0.010$).

Clinical studies with INO in relapsed and refractory ALL

Dosing schedule of INO (single dose or weekly dose) has been studied in two separate phase II clinical trials [Kantarjian *et al.* 2012a, 2013]; most recently, the preliminary results of another weekly dosing schedule have been reported in relapsed and refractory ALL patients [Advani *et al.* 2014] (Table 1). INO and low intensity chemotherapy combination has also been shown to be tolerable and effective in the salvage setting [Jabbour *et al.* 2014b].

Single agent INO

In a phase II clinical trial, INO was tested for the first time in relapsed and refractory B-ALL and it showed significant clinical activity as a single agent (Table 1) [Kantarjian *et al.* 2012a]. A total of 49 patients were treated with 1.8 mg/m^2 INO every 3–4 weeks. Patients received a median of 2 (range 1 to 5) INO courses. A total of 82% of the patients received $\geqslant$2 cycles and 47% received $\geqslant$3 cycles of INO. In all patients, CD22 was expressed in $\geqslant$50% of blasts, and for 28 patients (57%) $\geqslant$90% of blasts.

The overall response rate (ORR) was 57% (28 of 49 patients); of these 28 patients, nine patients had complete marrow response (CR), 14 patients had CR without platelet recovery (CRp) and five patients had CR with incomplete recovery of peripheral blood cells (CRi). Patients with nondiploid karyotype [t(4;11), Philadelphia chromosome or others] were less likely to respond compared with patients with diploid karyotype. A total of 10 of 12 (83%) patients with diploid karyotype and 18 of 37 (49%) patients other karyotype achieved a response, but this difference was not statistically significant ($p = 0.08$). A total of 27 patients who achieved complete morphological response were assessed for minimal residual disease (MRD) and 63% (17 of 27) of patients was found to have MRD negative status. Most responses occurred early in the course of treatment; among nine patients who achieved CR, eight of these did so after one cycle, and one patient after two cycles. Among 14 patients who achieved CR without platelet recovery, 6 (43%) did so after one cycle and 8 (57%) after three cycles. Lastly, four of five patients achieved CR with incomplete count recovery of peripheral blood counts after two cycles of treatment and one after three cycles. Median OS was 5.1 months and 7.9 months, in all patients and 28 responders, respectively. Among nine patients who achieved CR, the estimated survival at 1 year was 78%. Patients undergoing allogeneic stem-cell transplant (allo-SCT) were censored for duration of response at the time of transplant, and censored data demonstrated similar median OS (5.2 months) in 22 (45%) patients who received SCT. After allo-SCT, 23% (five of 22) of patients had clinical evidence for veno-occlusive disease (VOD). Drug-related fever and hypotension were commonly observed during the first 1–2 days of infusion. Fever was reported in 29 (59%) patients and was grade 3–4 in 9 (31%) of these patients. Grade 1–2 hypotension was reported in 12 (24%) patients and grade 3–4 hypotension was observed in only one (2%) patient. Other commonly seen adverse event was elevated liver transaminases. Grade 1–2 elevations were observed in 27 of 49 (55%) patients and only one grade 3–4 event occurred.

Based on preclinical studies suggesting that more frequent but lower dose may improve efficacy and reduce toxicities, a weekly (0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on days 8 and 15 every 3 and 4 weeks) dose schedule of INO was investigated (Table 1) [Kantarjian *et al.* 2013]. A total of 41 patients were enrolled. Overall, 24 (59%) patients responded to weekly dose INO; 8 (20%) patients had CR, 13(32%) patients had CRp (3%) and 3 (7%) had CRi. These response rates are found to be similar to single-dose INO which is given every 3–4 weeks [Kantarjian *et al.* 2012a]. Receiving INO earlier led better outcomes. Median OS for patients who received INO as salvage 1, 2 or 3 were 9.2, 4.3 and 6.6 months, respectively ($p = 0.002$). MRD negative disease was achieved in 17 of 24 (70%) patients who had responded to INO. In the same study, MRD negative rates with weekly or single-dose on an every 3–4 week schedule were evaluated and no statistically significant difference was observed. A total of 14 (34%) patients were able to undergo allo-SCT, and 9 of 14 (65%) was alive and in remission at the last follow up. Fever and hypotension were less common with weekly INO.

Table 1. Summary of clinical trials for relapsed and refractory ALL patients treated with inotuzumab ozogamicin (INO) alone or in combination with chemotherapy.

Patient characteristics	INO alone			INO + chemotherapy
	Single dose* *n* = 49 (%)	Weekly dose$ *n* = 41 (%)	Weekly dose‡ *n* = 35 (%)	Single dose with mini-hyper-CVD§ *n* = 35 (%)
Median age	36	NR	34	35
Salvage status				
Salvage 1	13 (27)	16 (39)	NR	19 (54)
Salvage 2	24 (49)	10 (24)	NR	8 (23)
Salvage 3 or more	12 (24)	15 (37)	NR	8 (23)
Karyotype				
Diploid	12 (24)	9 (22)	3 (9)	8 (23)
t(9;22)	7 (14)	8 (20)	9 (26)	*NR*
t(4;11)	5 (10)	3 (7)	*NR*	5 (14)
Other	25 (51)	21 (51)	18 (51)	22 (63)
Prior allo-SCT	7 (14)	3 (7)	15 (43)	*NR*
Response				
Overall response rate	27 (57)	24 (59)	23 (66)	25 (71)
CR	9 (18)	8 (20)	11 (32)	18 (51)
CRi	19 (39)	16 (39)	12 (34)	7 (20)
No response	19 (39)	15(37)	12 (34)	4 (12)
Early death	2 (4)	2 (5)	*NR*	6 (17)
MRD negativity	19 (39)	17 (41)	18 (51)	*NR*
Proceeded to allo-SCT	22 (45)	14 (34)	7 (20)	12 (34)
Median follow up, months	21	4	4.4	10
Overall survival, months	5	7.3	7.4	7
All grades				
Hyperbilirubinemia	14 (29)	2 (5)	*NR*	*NR*
Elevated transaminase	28 (57)	11 (27)	2 (6)‖	*NR*
Veno-occlusive disease	5 (10)	1 (2)	3 (9)	4 (11)

Outcomes of the above mentioned trials should not be compared with each other directly.
ALL, acute lymphoblastic leukemia; allo-SCT, allogeneic stem-cell transplantation; CR, complete response; CRi, complete response with incomplete count recovery; MRD, minimal residual disease, NR, not reported.
*Kantarjian *et al.* [2012a]: INO dose, 1.8 mg/m^2 every 3–4 weeks.
$Kantarjian *et al.* [2013]: INO dose, weekly (0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on days 8 and 15), every 3–4 weeks.
‡Advani *et al.* [2014]: INO dose, weekly (0.8 mg/m^2 on day 1, and 0.5 mg/m^2 on days 8 and 15), every 3–4 weeks.
§Jabbour *et al.* [2014b]: mini-hyper-CVD (no anthracycline, cyclophosphamide and dexamethasone (50% dose reduction), methotrexate (75% dose reduction), cytarabine (0.5 mg/m^2 × 4 doses)). Rituximab was given during first four cycles. INO was administered on day 3 of each of the first 4 cycles (1.8 mg/m^2 on first cycle and 1.3 mg/m^2 on subsequent cycles).
‖Data only for ⩾ grade 3 events.

Drug-related fever within 1–2 days of infusion was seen in nine of 41 (21%) patients treated with weekly dose compared with 29 of 49 (59%) patients with the single-dose INO. Liver toxicity was also less common with weekly dose INO. Only 11 of 41 (27%) patients had elevated liver transaminases with weekly INO compared with 28 of 49 (57%) with single-dose INO. VOD was observed in only one patient amongst who underwent allo-SCT.

The preliminary results of another phase II trial were reported recently (Table 1) [Advani *et al.* 2014]. A total of 35 patients with relapsed and refractory CD22 + B-ALL were treated with a total dose of 1.8 mg/m^2 per cycle as starting dose followed by dose reduction to 1.6 mg/m^2 upon achievement of CR, CRp or CRi with a 28-day schedule of up to 6 courses. ORR (CR, CRi, CRp) was 66% and 78% (18 of 23) of patients with response achieved MRD negative status.

Overall, median time to achieve remission and MRD negativity was 25 days. A total of 34% (12 of 35) of patients were able undergo allo-SCT, and six of these were alive and in remission at the last follow up. INO was discontinued in five patients due to adverse events and the elevated liver transaminases were the most common reason (⩾ grade 3 in two patients, 6%). The incidence of grade 3 or more transaminitis with weekly INO appears to be comparable with single dose INO schedule (2%) [Kantarjian *et al.* 2013]. However, grade 1 or 2 transaminitis is much less common with weekly dose schedule. Other relevant INO related grade ⩾3 events were as follows: thrombocytopenia (31%), neutropenia (26%) and neutropenic fever (20%).

The outcome of relapsed and refractory ALL patients who received single dose (n = 49 patients) or weekly dose (n = 41 patients) INO was compared with historical data which consisted of 292 ALL patients with similar clinical characteristics treated with conventional chemotherapy [Faderl *et al.* 2013]. The median age was 39 (4–84) and 37 (14–81) years in the INO and chemotherapy groups, respectively. The overall CR/CRi rate was 49% for INO and 29% for chemotherapy group. The CR/CRi rate for patients treated with INO or chemotherapy, as first salvage, was 66% and 40%, respectively (p = 0.007). Response rates were also superior as second salvage (44% *versus* 16%, $p < 0.001$), third salvage (46% *versus* 19%, p = 0.03) and forth salvage (27% and 9%, p = 0.01) therapy. The median OS appeared to be better in patients treated with INO, but it was not statistically significant except in the third salvage group; salvage 1 (9.2 months *versus* 6.2 months, p = 0.06), salvage 2 (4.3 months *versus* 2.5 months, p = 0.74), salvage 3 (6.6 months *versus* 2.6 months, p = 0.01) and salvage 4 (7.4 months *versus* 1.9 months, p = 0.09).

Combination of INO with chemotherapy

The preliminary results of the first clinical trial combining INO with nonmyelosuppressive chemotherapy have recently been reported [Jabbour *et al.* 2014b]. A total of 35 patients with relapsed and refractory CD22 + B-ALL received lower intensity chemotherapy, which was referred to as mini-hyper-CVD (Table 1). INO was administered on day 3 of each of the first 4 cycles. A total of 46% (16 of 35) of patients received INO as their second or later salvage regimen. The ORR was 71%; 18 (51%) patients had CR, 6 (17%) patients had CRp and 1 (3%) had CRi. The median OS for responders (CR, CRp, CRi) was 14 months; it was less than 1 month in nonresponders. Median OS was not reached for all patients. Grade 3–4 nonhematologic adverse events included infections, elevated liver transaminases and VOD (1 patient with prior SCT during study and 3 patients after study during SCT developed VOD).

INO and mini-hyper-CVD combination has also been tested in the frontline setting in elderly B-ALL patients [Jabbour *et al.* 2014a]. A total of 26 patients with a median age of 68 years old (range 60–79) were treated and ORR was 96%; 21 patients had CR and 4 patients had CRp. All patients with CR achieved MRD negative status as well. Median follow up was 13 months, and at last follow up, 20 (74%) patients were alive and in remission. Most common grade 3–4 adverse events included infections (85%), thrombocytopenia (65%), hyperglycemia (44%), hyperbilirubinemia (22%), intracranial hemorrhage (15%) and elevated transaminases (11%). No dose-limiting toxicity was reported.

Prognostic factors for outcome in patients treated with INO

Baseline clinical and disease related factors affecting the outcome of INO treatment have been investigated. A total of 89 relapsed and refractory B-ALL patients treated with single dose (n = 49) or weekly (n = 40) INO schedule were analyzed [Jabbour *et al.* 2015]. ORRs (CR, CRp, CRi) were similar with single dose or weekly schedule (57% and 61%, respectively). The presence of complex karyotype, t(4;11), t(9;22), chromosome 17 abnormality, salvage 2 or beyond, high peripheral blood absolute blast count [ABC ($\geqslant 1.0 \times 10^9$/l)] and thrombocytopenia ($<100 \times 10^9$/l) were associated with lower probability of marrow CR achievement by univariate analysis (Table 2). Baseline thrombocytopenia and high ABC were found to be independently associated with lower probability of marrow CR achievement by multivariate analysis. By univariate analysis, patients with complex karyotype, t(4;11), t(9;22) or chromosome 17 abnormality had a median OS of 5 months compared with 44 months for others or diploid karyotype ($p < 0.001$) (Table 2). Patients who received INO as salvage 1 had superior median OS compared with salvage 2 or later. Baseline thrombocytopenia and high ABC were also

Table 2. Clinical parameters associated with response and survival post inotuzumab ozogamicin therapy.*

Clinical features	*n*	Marrow CR, *n* (%)	UVA	MVA		Median survival, months	UVA	MVA	
			p	OR	*p*		*p*	HR	*p*
Platelets, × 10^9/l									
<100	71	35 (49)	<0.001	11.5	0.03	5.0	<0.001	NA	NS
⩾100	18	17 (94)				NR			
ABC, × 10^9/l									
<1.0	58	43 (74)	<0.001	6.8	<0.001	7.8	<0.001	1.7	0.02
⩾1.0	31	9 (29)				4.3			
Dose schedule									
Weekly	40	24 (60)	0.79	NA	NA	9.5	0.03	NA	NS
Single dose	49	28 (57)				5.0			
Treatment status									
Salvage 1	29	22 (76)	0.02	NA	NS	10.2	<0.001	2.3	0.007
>Salvage 1	60	30 (50)				4.8			
Karyotype									
Diploid	17	NS	0.05	NA	NS	44.0	<0.001	2.9	0.01
Other	10								
Complex	24					5.0			
Abnormal Ch 17	11								
Ph-positive	17								
Translocation (4;11)	9								

*Jabbour *et al.* [2015]
ABC, peripheral blood absolute blast count; Ch: chromosome; CR, complete response; HR, hazard ratio; MVA, multivariate analysis; Ph: Philadelphia chromosome; NA, not applicable; NR, not reported; NS, not significant; OR, odds ratio; UVA, univariate analysis.

associated with worse survival. By multivariate analysis, the following clinical characteristics were found to be associated with worse survival: disease salvage 2 or beyond, adverse cytogenetics [complex karyotype, t(4;11), t(9;22), chromosome 17 abnormality] and high ABC. Each of these three clinical features has relatively similar impact on survival. Thus, an arbitrary value of 1 was assigned for each adverse feature. Patients with 3 (n = 22), 2 (n = 35), 1 (n = 25) or 0 (n = 6) adverse features had a median OS of 2.4, 7.4, 7.6 and 39 months, respectively.

Feasibility of allogeneic stem-cell transplant following INO

Currently, allo-SCT is the most effective therapeutic approach for durable disease control in relapsed and refractory ALL patients [Fielding *et al.* 2007]. The OS rate was 0–4% for patients who received chemotherapy alone, compared with 16–23% for patients who received allo-SCT [Kozlowski *et al.* 2012]. Use of an effective targeted antibody therapy with nonoverlapping toxicity allows more patients to undergo SCT in the salvage setting. In several clinical trials, single agent INO allowed large number (20–45%) of multiply refractory B-ALL patients to proceed with allo-SCT.

The outcomes of 26 patients who received allo-SCT following single dose or weekly INO have been reported [Kebriaei *et al.* 2013]. Patients had a median of three salvage regimens before allo-SCT. A total of 24 patients who achieved at least marrow CR underwent allo-SCT directly, whereas 2 patients who did not respond to INO received chemotherapy before starting the allo-SCT conditioning regimen. The majority of the patients (77%) received a matched related or unrelated donor transplant with myeloablative conditioning regimens (85%). A total of 15 (58%) patients had no MRD at the time of allo-SCT; five patients died on day 30 (four had organ failure, one had disease progression) and one patient died of severe graft *versus* host disease (GVHD) and was not assessed before his death. Median follow-up duration was

13 months and OS survival at 1 year was reported as 20%. None of the patients with MRD positive disease at the time of allo-SCT survived beyond 1 year and 42% of MRD negative patients were alive at 1 year ($p = 0.04$). Adverse events were within the expected range of allo-SCT related complications except liver toxicity. A total of 7 patients (27%) had grade 3–4 elevated transaminases. In this study, 5 patients (19%) had fatal liver VOD at a median 23 days post-transplant. The rates of post-SCT VOD vary widely, ranging from 0to 38% [Carreras *et al.* 1998, Johnson *et al.* 2012].

Historically, regimens containing total body irradiation (TBI), thiotepa combined with other alkylating agents have been associated with high VOD rates [Lee *et al.* 1999]. Two of five patients had received prior allo-SCT and four patients received preparative regimens containing two alkylating agents (thiotepa combined with melphalan or busulphan) regimens and three patients received TBI based regimens. Calicheamicin containing ADCs have also been shown to be associated with high incidence of VOD. However, the interval between INO administration and start of preparative regimen did not affect the risk of developing VOD (median, 36 days in non-VOD group *versus* 40 days in VOD group). Cumulative nonrelapse mortality (NRM) at 1 year was 60%. A total of 11 deaths were due to NRM and refractory disease accounted for 8 deaths. GVHD development does not appear to be associated with INO administration.

Conclusion

Historically, CR with conventional chemotherapy in the relapsed and refractory ALL setting has been reported in only one-third of the patients [Thomas *et al.* 1999; Fielding *et al.* 2007; Faderl *et al.* 2013]. In phase I/II studies, single agent INO has been shown to induce CRs in up to two-thirds of the patients. Despite the high response rates and improved MRD negativity, the responses were not durable and OS was limited; 20–45% of patients treated with single agent INO were able to proceed with allo-SCT. Thus, proper adjustments of the conditioning regimens and perhaps addition of chemotherapy to INO before allo-SCT may allow longer survival. Currently, a randomized phase III clinical trial comparing single-agent INO with investigator choice of chemotherapy (FLAG: fludarabine, cytarabine, and G-CSF, HIDAC: high dose cytarabine, or cytarabine and mitoxantrone) in patients with relapsed and refractory ALL is ongoing. INO is further being evaluated in a randomized trial in patients with refractory ALL in combination with nonintensive chemotherapy in elderly patients and in patients with Philadelphia-positive ALL. Future trials assessing the role of INO in patients with positive MRD and in the frontline setting in younger patients are planned.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

Advani, A., Coiffier, B., Czuczman, M., Dreyling, M., Foran, J., Gine, E. *et al.* (2010) Safety, pharmacokinetics, and preliminary clinical activity of inotuzumab ozogamicin, a novel immunoconjugate for the treatment of B-cell non-Hodgkin's lymphoma: results of a phase I study. *J Clin Oncol* 28: 2085–2093.

Advani, A., Stein, A. and Kantarjian, H. (2014) A phase II study of weekly inotuzumab ozogamicin (INO) in adult patients with CD22-positive acute lymphoblastic leukemia (ALL) in second or later salvage. *56th ASH Annual Meeting and Exposition*, 6–9 December, San Francisco, abstract 2255.

Bene, M., Kolopp Sarda, M., El Kaissouni, J., De March Kennel, A., Mole, C., Kohler, C. *et al.* (1998) Automated cell count in flow cytometry: a valuable tool to assess CD4 absolute levels in peripheral blood. *Am J Clin Pathol* 110: 321–326.

Cao, Y. and Jusko, W. (2014) Incorporating target-mediated drug disposition in a minimal physiologically-based pharmacokinetic model for monoclonal antibodies. *J Pharmacokinet Pharmacodyn* 41: 375–387.

Carreras, E., Bertz, H., Arcese, W., Vernant, J., Tomas, J., Hagglund, H. *et al.* (1998) Incidence and outcome of hepatic veno-occlusive disease after blood or marrow transplantation: a prospective cohort study of the European Group for blood and marrow transplantation. European Group for Blood and Marrow Transplantation Chronic Leukemia Working Party. *Blood* 92: 3599–3604.

Dijoseph, J., Armellino, D., Boghaert, E., Khandke, K., Dougher, M., Sridharan, L. *et al.* (2004) Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. *Blood* 103: 1807–1814.

Faderl, S., Jain, N., O'Brien, S. and Thomas, D. (2013) Inotuzumab ozogamicin (CMC-544) compared to chemotherapy in patients (pts) with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL): a retrospective comparison. *J Clin Oncol* 31 (Suppl.): abstract 7095.

Fielding, A., Richards, S., Chopra, R., Lazarus, H., Litzow, M., Buck, G. *et al.* (2007) Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood* 109: 944–950.

Hamann, P., Hinman, L., Hollander, I., Beyer, C., Lindh, D., Holcomb, R. *et al.* (2002) Gemtuzumab ozogamicin, a potent and selective anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. *Bioconjug Chem* 13: 47–58.

Jabbour, E., O'Brien, S., Huang, X., Thomas, D., Rytting, M., Sasaki, K. *et al.* (2015) Prognostic factors for outcome in patients with refractory and relapsed acute lymphocytic leukemia treated with inotuzumab ozogamicin, a CD22 monoclonal antibody. *Am J Hematol* 90: 193–196.

Jabbour, E., O'Brien, S., Thomas, D. and Kantarjian, H. (2014a) Inotuzumab ozogamicin in combination with low-intensity chemotherapy (Mini-Hyper-Cvd) as frontline therapy for older patients (⩾60 years) with acute lymphoblastic leukemia (ALL). 56th ASH Annual Meeting and Exposition, 6–9 December, San Francisco, abstract 794.

Jabbour, E., O'Brien, S., Thomas, D. and Kantarjian, H. (2014b) Inotuzumab ozogamicin in combination with low-intensity chemotherapy (mini-hyper-CVD) as salvage therapy for adult patients with refractory/relapse (R/R) acute lymphoblastic leukemia (ALL). 56th ASH Annual Meeting and Exposition, 6–9 December, San Francisco, abstract 964.

Johnson, D. and Savani, B. (2012) How can we reduce hepatic veno-occlusive disease-related deaths after allogeneic stem cell transplantation? *Exp Hematol* 40: 513–517.

Kantarjian, H., Thomas, D., Jorgensen, J., Jabbour, E., Kebriaei, P., Rytting, M. *et al.* (2012a) Inotuzumab ozogamicin, an anti-CD22-calecheamicin conjugate, for refractory and relapsed acute lymphocytic leukaemia: a phase 2 study. *Lancet Oncol* 13: 403–411.

Kantarjian, H., Thomas, D., Jorgensen, J., Kebriaei, P., Jabbour, E., Rytting, M. *et al.* (2013) Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphocytic leukemia. *Cancer* 119: 2728–2736.

Kantarjian, H., Thomas, D., O'Brien, S., Cortes, J., Giles, F., Jeha, S. *et al.* (2004) Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer* 101: 2788–2801.

Kantarjian, H., Thomas, D., Wayne, A. and O'Brien, S. (2012b) Monoclonal antibody-based therapies: a new dawn in the treatment of acute lymphoblastic leukemia. *J Clin Oncol* 30: 3876–3883.

Kebriaei, P., Wilhelm, K., Ravandi, F., Brandt, M., De Lima, M., Ciurea, S. *et al.* (2013) Feasibility of allografting in patients with advanced acute lymphoblastic leukemia after salvage therapy with inotuzumab ozogamicin. *Clin Lymphoma Myeloma Leuk* 13: 296–301.

Kozlowski, P., Astrom, M., Ahlberg, L., Bernell, P., Hulegardh, E., Hagglund, H. *et al.* (2012) High curability *via* intensive reinduction chemotherapy and stem cell transplantation in young adults with relapsed acute lymphoblastic leukemia in Sweden 2003–2007. *Haematologica* 97: 1414–1421.

Larson, R., Sievers, E., Stadtmauer, E., Lowenberg, B., Estey, E., Dombret, H. *et al.* (2005) Final report of the efficacy and safety of gemtuzumab ozogamicin (mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. *Cancer* 104: 1442–1452.

Lee, J., Gooley, T., Bensinger, W., Schiffman, K. and Mcdonald, G. (1999) Veno-occlusive disease of the liver after usulfan, melphalan, and thiotepa conditioning therapy: incidence, risk factors, and outcome. *Biol Blood Marrow Transplant* 5: 306–315.

Lobo, E., Hansen, R. and Balthasar, J. (2004) Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* 93: 2645–2668.

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

O'Reilly, M. and Paulson, J. (2009) Siglecs as targets for therapy in immune-cell-mediated disease. *Trends Pharmacol Sci* 30: 240–248.

Piccaluga, P., Arpinati, M., Candoni, A., Laterza, C., Paolini, S., Gazzola, A. *et al.* (2011) Surface antigens

analysis reveals significant expression of candidate targets for immunotherapy in adult acute lymphoid leukemia. *Leuk Lymphoma* 52: 325–327.

Ricart, A. (2011) Antibody-drug conjugates of calicheamicin derivative: gemtuzumab ozogamicin and inotuzumab ozogamicin. *Clin Cancer Res* 17: 6417–6427.

Shan, D. and Press, O. (1995) Constitutive endocytosis and degradation of CD22 by human B cells. *J Immunol* 154: 4466–4475.

Takeshita, A., Shinjo, K., Naito, K., Matsui, H., Sahara, N., Shigeno, K. *et al.* (2005) Efficacy of gemtuzumab ozogamicin on atra- and arsenic-resistant acute promyelocytic leukemia (APL) cells. *Leukemia* 19: 1306–1311.

Takeshita, A., Shinjo, K., Yamakage, N., Ono, T., Hirano, I., Matsui, H. *et al.* (2009) CMC-544 (inotuzumab ozogamicin) shows less effect on multidrug resistant cells: analyses in cell lines and cells from patients with B-cell chronic lymphocytic leukaemia and lymphoma. *Br J Haematol* 146: 34–43.

Thomas, D., Kantarjian, H., Smith, T., Koller, C., Cortes, J., O'Brien, S. *et al.* (1999) Primary refractory and relapsed adult acute lymphoblastic leukemia: characteristics, treatment results, and prognosis with salvage therapy. *Cancer* 86: 1216–1230.