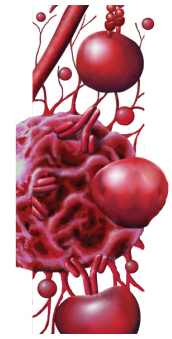
Drug Evaluation

Immunotherapy



Role of inotuzumab ozogamicin in the treatment of relapsed/refractory acute lymphoblastic leukemia

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Inotuzumab ozogamicin is a humanized anti-CD22 monoclonal antibody bound to a toxic natural calicheamicin, which is under investigation for the treatment of relapsed/refractory acute lymphoblastic leukemia. CD22 is commonly expressed in 90–100% of malignant mature B-lymphocyte lineage. The first Phase II study with inotuzumab ozogamicin conducted by Kantarjian *et al*. gave the opportunity for heavily pretreated patients with acute lymphoblastic leukemia to go for allogeneic stem cell transplant. Inotuzumab is well-tolerated with the exception of veno-occlusive disease. Overall inotuzumab ozogamicin is potentially an encouraging and promising therapy for patients.

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Better understanding of disease biology has led to significant advances in the treatment of acute lymphoblastic leukemia (ALL) [1]. In adults, complete response rates of 80–90% are routinely achieved with chemotherapy; however, 5-year overall survival of newly diagnosed patients with ALL is only around 40%, and overall survival at 5 years after disease relapse is dismal at <10% [2–5]. Intensification of chemotherapy has not been successful secondary to high toxicity rates [6]. Novel targeted therapies have been shown to improve outcomes in patients with adult ALL such as the use of tyrosine-kinase inhibitors in Philadelphia-chromosome positive ALL [7–9] and rituximab in CD20$^+$ ALL [10]. Antibody–drug conjugates (ADCs) represent a major advance in the treatment of ALL [11]. ADCs employ two important treatment principles: antibody specificity for the tumor antigen; and toxicity from the attached cytotoxic drug. Inotuzumab ozogamicin (IO), a CD22-targeted ADC is the focus of this review.

Mechanism of action

CD22 is a transmembrane glycoprotein specific to B-cell lineage and found in >90% with precursor B cell ALL and mature B-ALL [11,12] and 90–100% of normal and malignant cell of the mature B-lymphocyte lineage [13]. Memory and activated B cells highly express CD22 whereas lower levels are expressed in immature B cells [14–16]. CD22 is not expressed on nonlymphoid lineages, normal tissues, hematopoietic stem cells, hematopoietic precursor of B lymphocytes or any other nonhematopoietic lineage – thus making it a good target for monoclonal antibody (mAb) and overcoming the side effects of nontargeted chemotherapy [12–14,16–22]. CD22 therapy is not expected to affect tissue not expressing CD22, especially the generation of new B cells from the hematopoietic progenitors [13]. CD22 is a better internalizing molecule in comparison to other B-lymphoid lineage-specific surface antigen [13,23–27]. The true function of CD22 is not completely clear but it is considered to regulate B-cell

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migration, B-lymphocyte survival, signal transduction of surface immunoglobulin receptors on B cells, B-cell homing and cellular adhesion [14,16,28].

Inotuzumab is a humanized IgG4 G5/44, specific anti-CD22 mAb bound covalently to calicheamicin DMH (N-acetyl-γ-calicheamicin dimethyl hydrazide); a toxic natural product derived from soil microorganism actinomyces *Micromonospora echinospora,* subspecies *calichensis* [26,29–30]. Inotuzumab, previously referred to as CMC-544, is an ADC. ADC consists usually of three parts, a targeting antibody, an effector molecule and a linker joining the effector to the antibody. Unlike rituximab, which is an unconjugated antibody, an ADC, does not use ADCC or complement-dependent cytotoxicity, as anti CD22 mAb is unable to effectively mediate Fc-mediated functions. Cellular death induced by IO is solely mediated by cytotoxic payload-induced apoptosis, and not CD22 signaling [13,20,25]. The antibody entity binds to the protein antigen expressed on the tumor cell, leading to localized delivery of the payload within malignant tissue, thus allowing targeted therapy and minimizing potential toxicity to antigen negative cells [13,27]. Inotuzumab has subnanomolar binding affinity and is rapidly internalized via endocytosis into the cytoplasm with a half-life <1 h. When the anti-CD22 mAb binds to the leukemia cell, it delivers the conjugated calecheamicin intracellularly to the cytoplasm, antibody linker is released by hydrolysis, the calicheamicin moves to the nucleus, binding to the minor DNA groove, causing break in the double stranded DNA and arrest in cell cycle at G2/M Phase, followed by cell death [26,29,31–33] . Inotuzumab, unlike gemtuzumab ozogamicin (a CD33 antibody covalently linked to calicheamicin) neither need prolonged maximal saturation of the target antigen nor renewed expression of CD22 for efficient IO-induced cell death but rather saturation was dependent on IO concentration and not CD22 expression. Cell lines with low CD22 expression yielded high IO levels owing to no direct relation between CD22 expression and efficiency of IO; interestingly, cell lines with low- and high-CD22 expression benefited higher from continuous exposure of IO (48 h) rather than pulse exposure (1, 6, 24 h) for IO-induced cell death [20,30,34–36].

Preclinical studies with inotuzumab ozogamicin in acute lymphoblastic leukemia

Immunoconjugates of calicheamicin have previously shown to be beneficial in acute myeloid leukemia using gemtuzumab ozogamicin (mylotarg) targeting CD33 [37]. *In vitro* studies have shown that IO has high cytotoxic effect at subnanomolar concentration on CD22-positive B-cell lymphoma in a dose-dependent manner, which is superior to unconjugated calicheamicin [13]. In preclinical models of ALL and non-Hodgkin lymphoma (NHL), IO was shown to be 39-times more potent than unconjugated calicheamicin [13]. ALL cell lines showed a greater sensitivity to both IO and free calicheamicin in comparison to B-cell lymphoma cell lines, showing the intrinsic differences in the NHL and ALL response to DNA damage [20,23,38]. *In vitro*, CD22-mediated intracellular delivery of calicheamicin elicits more cytotoxic effect than untargeted uptake of calicheamicin through the cell membrane. The antitumor effect of inotuzumab ozogamicin requires both the targeting antibody component and the toxin calecheamicin [13]. Inotuzumab was efficacious in mice against CD22 lymphoma xenograft via intraperitoneal (ip.) and intravenous (iv.) route. Inotuzumab had sustained antitumor effects at 160 μg/kg ip., q4dx3 against large established B-cell lymphoma (approximately 10% of body weight) causing near complete regression both Ramos (Burkitt lymphoma cell line) and RL (non-Hodgkin lymphoma cell line). The effect was sustained for >50 days in large Ramos lymphomas and in RL B lymphoma, which remained suppressed for nearly 30 days prior to tumor growth. Thus, confirmed IO can cause regression of large established B-cell lymphoma xenografts. Antitumor effects of IO were evaluated in systemically disseminated Ramos B-cell lymphoma model, which was grown in severe combined immunodeficient mice; IO was administered at 40 μg/kg, q4dx2 or at q4dx3, similar protection was seen in the mice against disseminated disease with the mice surviving beyond 100 days [13].

In vitro studies were done to understand the efficiency of IO in combination with chemotherapeutic regimen CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)/CVP (cyclophosphamide, vincristine, prednisone) in established RL or Ramos B-cell lymphoma xenografts. Inotuzumab was administered q4dx3 ip. at 80 μg/kg in the Ramos model and 160 μg/kg in the RL model of calicheamicin equivalents. The CHOP regimen did cause a significant suppression of lymphoma in both the models initially, but 3 weeks later, the tumor grew at a similar rate of the vehicle-treated group. The effects of IO were complete and sustained in both the models for >100 days of the experiment. This illustrates, IO has longer lasting and sustained antitumor effect in comparison to CHOP [38,39].

Further studies were done to learn the effects of IO with CHOP either sequentially or concurrently in the Ramos B-cell lymphoma xenograft. Concurrent administration of CVP (at minimum tolerated dose [MTD] of its individual components) and IO did cause regression of the established Ramos xenografts and increased the relapsed-free survival, although combination of IO with CHOP in similar settings showed evidence of toxicity in the mice along with antitumor

activity. Antitumor effects of IO were assessed in relapse/refractory in B-cell lymphoma xenografts previously treated to CHOP/CVP; IO retained their susceptibility, although they continued not to regress to subsequent CHOP or CVP therapy [24].

Inotuzumab studies were combined with rituximab in the preclinical setting, as rituximab is an established therapy for NHL. *In vitro* studies were done to compare the efficiency of rituximab with IO in antitumor effects. Rituximab was able to suppress the growth of developing B-cell lymphoma RL and Ramos xenograft as long as it was administered but the tumor regrew on discontinuation. Inotuzumab dosed at 160 µg/kg of calicheamicin equivalents q4dx3 IP rendered total inhibition in the development of B-cell lymphoma, showing a potent antitumor activity, while rituximab was noted to be ineffective or with modest activity in established B-cell lymphoma xenograft. Combination antitumor activity of IO (160 µg/kg q4dx3) and rituximab (2 mg/kg q4d3) were studied *in vitro* in developing Ramos B-cell lymphoma xenografts and disseminated Ramos B-cell lymphoma model. IO and rituximab showed synergistic effect in the developing Ramos B-cell lymphoma model in comparison to administered individually. In the disseminated Ramos model, 90% of the mice treated with the combination of IO (4 µg/kg q4dx3) and rituximab (1 mg/kg q4dx3) survived >125 days, showing an additive effect, while in the disseminated Ramos model treated separately with the similar dosing schema, only 60% of the IO treated mice and 20% of the rituximab treated mice survived at 125 days. Rituximab administered concurrently or prior to IO did not dilute the anticancer effects of IO [25] and pretreatment with IO did maintain increased CD20 levels, which is needed for the therapeutic effect of rituximab [25].

As IO had good results with NHL, it was investigated *in vitro* in REH precursor B cell ALL xenograft growing subcutaneously or as disseminated tumors. Inotuzumab inhibited the growth of ALL cell lines more efficiently *in vitro* in comparison to Ramos B-lymphoma cells. When testing for disseminated disease, tumor cells were injected systemically via iv. route in the lateral tail in severe combined immunodeficient mice and allowed to disseminate; causing the mice to develop hind-limb paralysis. Inotuzumab at the dose of 80 µg/kg q4dx3 produced complete survival of the treated mice over the observation time of 127 days, whereas the mice not treated with IO had disseminated disease by day 77 with an average survival of 55 days and hind-limb paralysis was prevented with treatment with IO. Femur bone marrow of the vehicle-treated mice with disseminated disease showed human CD45$^+$ leukemia cells by flow cytometry, verifying engrafted ALL cells, while the mice treated with IO showed a significantly reduced ALL engraftment [23].

Pharmacokinetics of inotuzumab ozogamicin

Pharmacokinetics profiles of IO are developed using specific ELISA assays. The analytes measured in serum included total anti-CD22 mAb G544 (conjugated and unconjugated), IO (conjugated mAb), total and unconjugated calicheamicin derivatives [38]. Advani *et al.* in a single agent trial of 79 patients with IO in B-cell NHL, serum measures were available for IO, G544, free calicheamicin and total calicheamicin. Concentration measures were deduced at the dose expansion and dose-escalation cohort at the MTD. At dosing of 1.8 mg/m^2 once Q4 weeks and 2.4 mg/m^2 once Q3 weeks, the mean end infusion peak concentration (C_{max}) could not be distinguished from each other. Inotuzumab exhibited a nonlinear distribution with increasing treatment cycle; this is apparently mediated by the target-mediated disposition. Mean end-of-infusion concentration and AUC_{tau} (area under the curve extrapolated over the dosage interval) increased with dose. AUC_{tau} also increased with treatment duration. For total calicheamicin and G544, similar trends of elimination were observed but longer half-life was seen in comparison to IO. The latter observation was attributed to the limitation in assay sensitivity or differential binding of the cleaved payload to plasma protein components [38,40].

In studies by Kantarjian *et al.*, slower clearance rate and a higher AUC of IO were associated with a higher bone marrow complete response (CR) whereas, a higher plasma concentration of IO was not associated with higher response rate on the single-dose schema. The IO was measured 3 h post infusion and on days 7 and 8 [41].

Clinical studies & results with inotuzumab ozogamicin in acute lymphoblastic leukemia

With the encouraging overall response rates using IO in Phase I trials in patients with relapsed/refractory CD22$^+$ NHL [40], Kantarjian *et al.* conducted the first Phase II clinical trial at MD Anderson Cancer Center (TX, USA) using single agent IO in patients with relapsed/refractory ALL, expressing CD22 in at least 20% of the blast cells, at the dosing regimen of established MTD of 1.8 mg/m^2 every 3–4 weeks, as used with the NHL trial [40,42]. In a 49 patient cohort with a median age of 36 years (range: 6–80 years), the overall response rate was 57%, CR was 18%, complete marrow CR was 39%, which includes marrow response without platelet recovery (CRp) and without peripheral blood count recovery (CRi). The median overall survival was 5.1 months while the median survival of the responding 28 patients was 7.9 months.

Of the responding 28 patients, 18 had chromosomal abnormalities at the start of treatment, of which 16 (89%) had complete cytogenetic response.

In patients who achieved CR, the 12 months survival was estimated at 78%. Eighteen of the 28 patients with a response had cytogenetic abnormalities at baseline, and 89% achieved cytogenetic remission. Of the nine patients who achieved CR, eight (89%) achieved it after a course, while the remaining one patient achieved it post two courses. Of the 27 evaluable patients, minimal residual disease (MRD) status was negative in 17 (63%) patients; although median overall survival was not superior in patients with MRD positive (7.7 months) to negative (7.3 months); p = 0.724, again keeping in mind that the cohort was heavily pretreated with poor prognostic factors and that MRD negativity in salvage therapy may not completely translate to an increased overall survival in comparison to patients who are MRD negative in first treatment remission.

Allogeneic stem cell transplant (ASCT) was performed in 22 (45%) of 49 patients, of which nine died of infection and four of veno-occlusive disease (VOD). VOD was seen in 5/22 patients post ASCT; four of five patients had received thiotepa and clofarabine [43] containing conditioning regimen, which are known to be hepatotoxic, causing an additive toxic effect [41,43].

Preclinical studies by DiJoseph *et al.* suggested a frequent lower, weekly dosing was more effective; hence a weekly dosing schema was pursued by Kantarjian *et al.* [13,41]. The dosing schema included weekly IO as a short iv. infusion, dose of 0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on day 8 and 15, for a total course of 1.8 mg/m^2 per course. The 1 h infusion was repeated every 3–4 weeks depending on recovery of counts and bone marrow results on day 21 and 28. If the bone marrow showed persistent leukemia or an increase in blast percentage on day 21 or 28, another course of IO was given in spite of peripheral counts, while an decrease in blasts or blasts <5% on days 21 through 28, another course of IO was administered only after count recovery. Patients who responded to treatment with CR or marrow CR post one or two courses were allowed a total of four courses. In the weekly schema, 41 patients were enrolled, CR was seen in eight of 41 patients (20%), CRp in 13/41 (32%) and CRi 3/41 (7%). There was no difference in the response rate between weekly and every 3 weeks IO dosing. Inotuzumab was the second or more salvage treatment for 25/41 (60%) patients, Philadelphia positivity was seen in 8 (20%) of patients while t (4; 11) in 3 (7%) of patients. CD22 was positive in >50% in the leukemia cells. Negative MRD was achieved in 17 of 41(41%) patients with the weekly schedule [41].

In the 90 patients cohort (cohort of weekly dose IO plus single dose IO), patients with Philadelphia chromosome positive ALL and translocation (4; 11) had a lower response rates of 40% and 38%, respectively; however, this was not statistically different from the responses seen with other chromosomal abnormalities including diploid cytogenetics (p = 0.47). The response rate was lower for patients getting IO as salvage 3 or more in comparison to salvage 1; 48–76% (p = 0.047). ASCT was performed on 14 of 41 patients of the weekly IO and 22 of 49 patients of the single dose IO. ASCT did not appear to benefit in the overall survival when censoring for the time to transplant. Patients with MRD negative status (n = 15) at time of stem cell transplant had a markedly better 1-year overall survival of 42% in comparison to MRD positive patients (n = 11), wherein there were no survivors at 1 year [44]; in a positive perspective, patients who were heavily pretreated were feasible for another ASCT post-treatment with IO [44]. VOD was seen in 1 (0.07%) of 14 patients on the weekly IO and 5 (23%) of 22 patients on the single dose IO, which could be secondary to the conditioning regimen prior to transplant. Preparative regimen that contained two alkylating agent (busulfan or melphalan combined with thiotepa) was associated with higher incidence of VOD (n = 5 of 13) versus getting one alkylating agent (n = 1 of 21; p = 0.02) [41,42,44]. Median from start of treatment to ASCT was 11 weeks (range: 2–14 weeks) and 5 weeks (range: 2–14) from the end of IO therapy to transplant [41].

Weekly IO schema was associated with less adverse events (AEs) in comparison to the single dose IO, which may be due to the fact that the weekly schedule did not reach a peak plasma concentration in comparison to the weekly IO schedule. Post-infusion fever and hypotension noted within the first 48 h were less frequent. Grade 1–2 bilirubin elevation was seen in only two patients, with no patient reaching grade 3 hyperbilirubinemia. Grade 3 elevated liver enzymes were seen in two patients. All AEs were reversible within 1–2 weeks of therapy; in contrary to single dose IO that had persistent liver function abnormalirty in two of 49 patients [41].

Rytting *et al.* published data on Phase II study with IO on five pediatric patients (<18 years) with relapsed B-cell ALL. Inotuzumab dosing was similar to the adult counterpart of 1.3 mg/m^2 every 3 weeks; in the later enrollments, the dose was increased to 1.8 mg/m^2 with a weekly schedule of 0.8, 0.5, 0.5 mg/m^2/week. One patient achieved CR while two achieved CRp (one had prior ASCT). Median cycles to respond were two. Responding patients had diploid cytogenetics and were taken to ASCT within 4 weeks of CR/CRp, although they were MRD positive at transplant, two patients relapsed and died post-transplant while the other patient relapsed 100 days after transplant. The patients achieving CRp received IO of 1.3 mg/m^2, while two patients (CR = 1, CRp = 1) received IO at 1.8 mg/m^2 dosing.

Of the nonresponding patients (n = 2), one had Li-Fraumeni syndrome. The therapy was tolerated well in the pediatric population. None developed grade 3–4 hepatic toxicity, although one of the transplanted patients developed VOD (conditioning regimen of busulfan and clofarabine); VOD resolved after therapy with defibrotide. Fever was reported in three of five patients with first IO infusion. Grade 2 ALT elevation was reported in two of five patients [45].

Jabbour *et al.* analyzed the entire treatment cohort (weekly + single dose) to elicit factors associated with clinical response. In a univariate analysis, complex karyotype, translocation (4;11), translocation (9;22), abnormal chromosome 17, salvage 2 and beyond, high white count of $\geq 4.0 \times 10^9$/l, high absolute peripheral blast count of $\geq 1.0 \times 10^9$/l and a platelet count of $<100 \times 10^9$/l were associated with a lower probability to achieve a marrow CR. In a multivariate analysis, high absolute blast count ($p < 0.001$) and low platelet count ($p = 0.03$) were independently associated with lower marrow CR, while treatment in salvage 1 or beyond did not affect marrow response. Prognostic factors by univariate analysis delineated poor survival for complex karyotype, translocation (4;11), translocation (9;22), abnormal chromosome 17; presenting with a worse median survival of 5.0 months to 44+ months with others ($p < 0.001$); salvage 2 and beyond had a median survival of 4.8 months in comparison to receiving treatment in salvage 1 with a median survival of 10.2 months ($p < 0.001$), high white count of $\geq 4.0 \times 10^9$/l, high peripheral blast count of $\geq 1.0 \times 10^9$/l and a platelet count of $<100 \times 10^9$/l. In univariate analysis, weekly IO schedule was better than the single dose schema ($p = 0.003$), but did not sustain its benefit in the multivariate analysis ($p = 0.11$). In the multivariate analysis, independent factors to poor survival were, complex cytogenetics, translocation (4;11), translocation (9;22), abnormal chromosome 17 ($p = 0.01$), treatment with IO beyond salvage 1 ($p = 0.007$) and high absolute blast count $\geq 1.0 \times 10^9$/l ($p = 0.02$). A prognostic scoring model was initiated based on the impact the three factors had on the survival outcome and marrow response. Each impact factor was given a value of 1. Patient that had 3 adverse factors had a worse median survival of 2.4 months in comparison to patients who had none had median survival of 39+ months. The prognostic model was validated by incorporating it to a historical cohort of 253patients with relapsed/refractory ALL treated at MD Anderson Cancer center [46].

DeAngelo *et al.* presented data on 37 patients with relapsed/refractory ALL with single agent IO in a Phase I dose escalation and expansion cohort to determine the optimum dose, safety and efficacy of a weekly schedule. Inotuzumab was administered every 28 days [47]. Median age was 56 years, 17 (46%) were in salvage 1, nine (24%) in salvage 2, ten (27%) in salvage 3 or more, seven (19%) had a prior ASCT, six (16%) had translocation (9;22); CD22 was present in 98% of median blast. Median follow-up of the surviving patients was 4.1 months (1–12.6 months). The dose escalation cohort (n = 24) response rate (CR + CRi) was 79% while the dose expansion cohort (n = 13) had a response rate of 46%, taking into consideration the dose expansion cohort had a higher number of high-risk cytogenetics (10/13 [77%] vs 11/24 [46%]) and peripheral blast count in comparison to the escalation cohort. The median time to remission and MRD negativity was 29 days (21–85) in the escalation cohort and 34 days (22–141) in the expansion phase. The response rate of the whole cohort (n = 37) was 25 (68%), while for MRD negative was seen in 22/25 (88%). Dosing schema for the escalation had three cohorts with different dosing regimen. The first cohort (n = 3) got a total of 1.2 mg/m^2 of IO – day 1 was 0.8 mg/m^2, day 8 was none and day 15 was 0.4 mg/m^2. Second cohort (n = 12) got a total 1.6 mg/m^2 – day 1 was 0.8 mg/m^2, day 8 was 0.4 mg/m^2 and day 15 was 0.4 mg/m^2. Cohort 3 (n = 9) got a total of 1.8 mg/m^2 – day 1 was 0.8 mg/m^2, day 2 was 0.5 mg/m^2 and day 15 was 0.5 mg/m^2 (Table 1). The median number of cycles were 4 (1–6), 2 (1–5) and 3 (1–4) for dose escalation cohort 1, 2 and 3 respectively. There were no dose limiting toxicities (DLTs) for cohort 1 and 2, but 1 DLT in cohort 3 of the expansion phase. Response rates (CR and CRi) of the escalation cohorts 1, 2, 3 were, 2/3 (67%), 9/12 (75%), 8/9 (89%), while MRD negativity of the response patients were, 2/2 (100%), 8/9 (89%), 8/8 (100%). The expansion cohort (n = 13) had a higher risk population with worse cytogenetics, two patients had translocation (9; 22) and two had translocation (4;11). The cohort got a total dose of 1.8 mg/m^2 for a 28 day cycle similar to the MD Anderson study. On day 1, were administered 0.8 mg/m^2, day 8 and day 15, 0.5 mg/m^2. Bone marrow to assess for response was done on day 21. Median number of cycles in the escalation cohort was 3 (range: 1–5), CR + CRi in 6/13 (46%), MRD negative on the response group was 4/6 (67%); median time to CR/CRi was 29 days (20–85) [47]. Inotuzumab was discontinued in 32 patients, of whom 11 proceeded to ASCT, 14 secondary to progression of disease, one to receive maintenance therapy, one for Grade 3 VOD, five had other AEs. The dose of IO was determined to be 1.8 mg/m^2 per cycle, similar to MD Anderson dosing schema [47].

Combination therapy of IO with low-dose chemotherapy was tested in elderly patient ≥60 years, as conventionally older patients have higher toxicity and deaths in CR with intense chemotherapy in comparison to the younger population. Results of low-intensity chemotherapy with IO were presented at American

Table 1. Results of single agent inotuzumab in relapsed/refractory acute lymphoblastic leukemia.

Therapy schedule and response rate	Dose escalation cohort (n = 24)			Dose expansion cohort (n = 13)	Dose escalation and expansion cohort
	1 (n = 3)	2 (n = 12)	3 (n = 9)	4 (n = 13)	Total (n = 37)
Total dose/cycle, mg/m^2	1.2	1.6	1.8	1.8	
Day 1, mg/m^2	0.8	0.8	0.8	0.8	
Day 8, mg/m^2	0	0.4	0.5	0.5	
Day 15, mg/m^2	0.4	0.4	0.5	0.5	
Day 21	Disease assessment				
CR + CRi rate, n (%)	2 (67)	9 (75)	8 (89)	6 (46)	25 (68)

CR: Complete response; CRi: Peripheral blood count recovery.

society of hematology in 2013 by Jain *et al.* and further updated with a longer follow-up presented by Jabbour *et al.* in American society of Hematology 2014. Elderly patients with newly diagnosed B-cell ALL were treated with low-intensity chemotherapy-mini Hyper-CVD (cyclosphosphamide, vincristine and dexamnethasone; 50% dose reduction of cyclophosphamide and dexamethasone, 75% dose reduction of methotrexate, cytarabine 0.5 mg/m^2 × 4 doses and no anthyracylines) which is a lower intensity therapy of the conventional Hyper-CVAD (cyclosphosphamide, vincristine, adriamycin and dexamnethasone). IO was administered on day 3 of each 28 day cycle for the first four courses. Patients (n = 15, 56%) with CD20 positive ALL ≥20% were given rituximab in the first four courses, while patients with CNS disease were given concurrent intrathecal therapy. The cohort of 27 patients had a median follow-up of 13 months and median age of 68 years. The first six patients of the cohort received IO at 1.3 mg/m^2 for cycle 1 followed by 0.8 mg/m^2 for subsequent cycles. The subsequent seven patients onward received IO at 1.8 mg/m^2. The response rate (CR + CRp) was 25 (96%) of the 26 evaluable patients (one patient was in CR with prednisone therapy), 21 for CRs and four for CRp. MRD negativity was achieved in all CR. There were seven (26%) deaths on the study; causes of deaths were primary refractory (n = 1), relapse from no therapy post second and third course myelosuppression (n = 2), one each from pneumonia complication, metabolic encephalopathy/renal failure, sepsis/multiorgan failure, gunshot wound; there were no DLT. None underwent ASCT. Six patients were switched to maintenance therapy early on secondary to persistent thrombocytopenia. Grade ≥3 nonhematological AEs included infections (n = 23; 85%), increased bilirubin (n = 6; 22%), increased ALT (n = 3; 11%) hyperglycemia (n = 12; 44%), intracranial hemorrhage (n = 4; 15%), headache (n = 1; 4%), cognitive disturbance (n = 1; 4%), hematuria (n = 2; 7%), 1 (7%) with ascites and diarrhea (n = 1; 7%). Nonhematological ≥3 toxicity was thrombocytopenia (n = 17; 65%). One year disease-free survival and overall survival were 86 and 81%, respectively [48].

A Phase III randomized study is currently ongoing (NCT01564784). It enrolls patients with relapsed or refractory B-cell ALL, aged 18 and older, in salvage 1 or 2. The study is to compare IO and investigators choice chemotherapy. Rates of CR/CRi and overall survival will be evaluated. Arm A would consist of IO administered to a maximum dose of 1.8 mg/m^2 every cycle; 0.8 mg/m^2 on week 1 followed by 0.5 mg/m^2 on week 2 and 3 for every 21–28 day cycle. Starting cycle 2 and onward, IO will be administered at a dose of 0.5 mg/m^2 on week 1, in patients with no peripheral blasts for a total of 1.5 mg/m^2 each cycle for a total of six cycles. Arm B patients would receive any of the following chemotherapy regimen: fludarabine, cytarabine, G-CSF for a total of four cycles; cytarabine and mitoxantrone for a total of four cycles or high-dose cytarabine for a total of 12 doses. The study does allow for ASCT for patients who are responding to therapy.

Safety of inotuzumab ozogamicin

In the first Phase II ALL study, the most frequent grade 3–4 AE was drug-related fever in 9/49 patients seen in the first 2 days of treatment, grade 3 AE of increased bilirubin in 2/49 patients, while other grade 3 AEs including mucositis, increased liver enzymes were seen in a patient respectively. These AEs were seen in the dosing regimen of 1.8 mg/m^2 every 3–4 weeks. VOD post-transplant was seen in 23% (5/22 pts), though this was considered to be secondary to the additive effect of using two alkylating agents as a conditioning regimen known to cause hepatotoxicity. The other frequent AEs of all grades were drug fever (59%), elevated liver enzymes (57%), elevated bilirubin (28%) and hypotension (26%). Most AEs were reversible within 1–2 weeks except for two patients. Grade 1–2 AE was hypotension seen in 24% of patients in the first 48 h [42]. Cohort of

ALL patients on weekly regimen of modified fractionated IO, who continued to get a total of 1.8 mg/m^2 q3w/q4w, saw fewer AEs with comparable response rate to the weekly regimen. Grade 3–4 AEs were seen in 14% patients for drug fever and 4% patients for elevated liver enzymes. VOD occurred in one (0.07%) of 14 patients who underwent ASCT after weekly IO to five (23%) of 22 patients who underwent ASCT post single dose of IO. VOD was seen in five (38%) of 13 patients post-transplant treated with two alkylating conditioning regimen (busulfan or melphalan with thiotepa) while only one (0.04%) of 21 patients treated with one alkylating agent ($p = 0.02$) [41,44]. The time between IO administration and start of transplant preparative regimen did not increase the risk for VOD, median of 40 days in the VOD group to 36 days in the non-VOD group. Median number of IO courses administered in the VOD group versus non-VOD group was same [44]. Hepatic toxicity has to be addressed to improve overall survival; avoiding myeloablative double alkylator conditioning combinations, known to historically cause hepatic injury, could be one of the options. Furthermore, avoiding other hepatotoxic agents prior to transplant including antifungal azoles and adding ursodiol may avoid hepatic injury/VOD in the post-transplant setting [44]. Improvement in safety profile in the fractionated schedule may be associated to a lower peak level of IO, thus concurring a weekly dose is less toxic than every 3 weekly dosing with no added benefit in response rate [38,41].

Conclusion

Inotuzumab has shown impressive monotherapy activity in heavily pretreated patients, and in combination with low-dose chemotherapy for front-line treatment of elderly patients with ALL. It is well-tolerated with a better safety profile seen with the weekly regimen. Combination of IO with other therapies may potentially improve clinical outcomes.

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Executive summary

Mechanism of action

- CD22 is commonly expressed in 90–100% of malignant mature B-lymphocyte lineage.
- Inotuzumab is a humanized anti-CD22 monoclonal antibody bound to a toxic natural product calicheamicin.
- Inotuzumab is internalized by endocytosis into the cytoplasm.
- Calicheamicin moves to the nucleus, binds to minor DNA groove, causes break in the double-stranded DNA and arrest cycle in G2/M phase.

Preclinical studies

- Inotuzumab was efficacious against CD22 lymphoma xenograft having sustained antitumor effects and near complete regression of large established B-cell lymphoma.
- *In vitro* studies of inotuzumab ozogamicin (IO) with chemotherapy in established Ramos B-cell lymphoma xenografts increased the relapse-free survival.
- *In vitro* studies of IO in REH pre-B acute lymphoblastic leukemia xenografts produced a complete survival of the treated mice over 127 days while disseminated disease in mice not treated with IO.

Clinical studies

- First Phase II study conducted by Kantarjian *et al.* with inotuzumab gave the opportunity for heavily pretreated patients with acute lymphoblastic leukemia to go for allogeneic stem cell transplant.
- Jabbour *et al.* combined inotuzumab with dose-reduced chemotherapy (mini Hyper-cyclosphosphamide, vincristine and dexamnethasone) in the elderly patients unable to get standard chemotherapy. This combination rendered an overall response rate of 79%.

Safety & tolerability

- Generally well-tolerated, except for veno-occlusive disease, which can be fatal especially in the setting of using two alkylating agents as conditioning regimen prior to transplant.
- Better management of veno-occlusive disease, by moving to weekly inotuzumab dosing and avoiding two alkylating agent as conditioning regimen.

Dosing schedule

- Initial Phase II study by Kantarjian *et al.* dosed inotuzumab intravenously once every 3–4 weeks as treated in non-Hodgkin lymphoma to a total dose of 1.8 mg/m^2.
- Preclinical studies by Di Joseph *et al.* pointed toward improved bioavailability with weekly dosing, total dose remained unchanged of 1.8 mg/m^2; hence, inotuzumab dosing schema was modified with improved toxicity profile, while rendering comparable response rate.

Disclosure

In addition to the peer-review process, with the author(s) consent, the manufacturer of the product(s) discussed in this article was given the opportunity to review the manuscript for factual accuracy. Changes were made at the discretion of the author(s) and based on scientific or editorial merit only.

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