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Inotuzumab ozogamicin for the treatment of acute lymphoblastic leukemia

J. Michael Savoy, Mary Alma Welch, Patrice E. Nasnas, Hagop Kantarjian and Elias Jabbour

Abstract: Therapy for adult acute lymphoblastic leukemia (ALL) with multiagent cytotoxic chemotherapy has not been as successful as that for pediatric patients. The advent of targeted monoclonal antibodies against common cell surface antigens (i.e. CD19, CD20, and CD22) has resulted in improved outcomes without additional toxicities. Inotuzumab ozogamicin is an anti-CD22 antibody–drug conjugate approved for the treatment of relapsed or refractory B-cell precursor ALL. It improved outcomes compared with standard salvage chemotherapy. Its combination with low-intensity chemotherapy in the relapse setting and in frontline elderly patients is promising.

Keywords: Acute lymphoblastic leukemia, inotuzumab, monoclonal antibody

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Correspondence to:
Elias Jabbour
The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit # 428, Houston, TX 77030, USA
ejabbour@mdanderson.org

J. Michael Savoy
Department of Pharmacy, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Mary Alma Welch
Hagop Kantarjian
Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Patrice E Nasnas
Lebanese American University Gilbert and Rose-Marie Chagoury School of Medicine, Byblos, Lebanon

Introduction

Acute lymphoblastic leukemia (ALL) is characterized by the dysregulated growth and accumulation of lymphoid cells in the bone marrow and extramedullary tissues.[1,2] It has a bimodal age distribution with the majority of cases (~55%) diagnosed before age 20 years, and approximately 20% of cases diagnosed after age 50 years. This presents unique challenges to designing treatment regimens, as patient- and disease-specific factors must be considered. For the past 40 years, treatment of B-cell ALL has consisted largely of the same multiagent chemotherapy backbone. Among pediatric patients, dose and schedule intensification have yielded cure rates approaching 90%; however, the adaptation of these principles to adults has not produced the same success. While modern intensive chemotherapy regimens produce complete remission (CR) rates of 80–90% in adults, the 5-year survival rate is only 30–40%. This disparity is due to the increased incidence of high risk features and comorbidities in adults.[1–3] Until recently, salvage regimens for adults with relapsed/refractory (R/R) B-cell ALL produced CR rates of only 10–40%, resulting in a dismal 5-year overall survival (OS) rate of 10%. As a result of the low CR rates, few adults with R/R B-cell ALL (5–30%) are able to proceed to stem cell transplantation (SCT), the only potentially curative option in this setting.[2,4–6] Thus, novel therapies to improve disease-free survival in frontline adult patients and CR rates in the salvage setting are needed.

Antibody-based modalities are an exciting development for the treatment of adult ALL. Monoclonal antibodies are engineered to target tumor-specific antigens, such as CD19, CD20 or CD22, that are expressed on the leukemia cell surface. Monoclonal antibodies exert cytotoxicity *via* several mechanisms, including antibody-dependent cytotoxicity, complement-dependent cytotoxicity, and direct induction of apoptosis. In addition, a potent cytotoxic agent can be linked to an antibody targeting a leukemia-specific antigen, resulting in an additional mechanism of action. Antibody-mediated delivery of cytotoxic agents such as calicheamicin may help maximize their antitumor effect while avoiding the potential toxicity associated with traditional chemotherapy.[5,7,8] Inotuzumab ozogamicin (InO) is a CD22-targeted monoclonal antibody conjugated to a derivative of calicheamicin. Initial studies in non-Hodgkin's lymphoma (NHL) yielded promising results, which led to its investigation for treatment of R/R ALL. The promising results therein led to its approval by the US Food and Drug Administration (FDA) in 2017 for this patient population. In an

effort to improve on outcomes seen in single-agent trials, studies of InO in combination with low-intensity cytotoxic chemotherapy in the salvage and frontline setting were undertaken and produced positive outcomes.[9,10] Herein, we review the pharmacology and safety profile of InO and discuss its evolving role in the treatment of adult B-cell ALL.

Pharmacology

CD22 is a transmembrane sialoglycoprotein within the immunoglobulin superfamily thought to be involved in signal transduction of the B-cell receptor, B-cell migration, and maintenance of peripheral B-cell tolerance. Its expression is isolated to B-lymphocytes, and it is not present on nonlymphoid tissues or hematopoietic stem cells. Its expression increases as B-lymphocytes mature, but it is lost upon terminal differentiation into plasma cells. CD22 expression is greater than 90% on mature and precursor B-cell ALL blasts. Ligand or anti-CD22 antibody binding to CD22 triggers a cycle of rapid internalization, degradation, and renewed cell surface expression. Furthermore, it is not released into the extracellular milieu. These factors make it an amenable target for antibody-directed therapy.[8,11,12]

Calicheamicin is a prodrug produced by the bacterium *Micromonospora echinospora* and is the most potent member of the enediyne class of DNA-damaging cytotoxins. It binds to the minor groove of DNA before undergoing thiol-dependent reduction at the enediyne moiety to form a diradical intermediate. The diradical leaches hydrogen atoms from the phosphodiester backbone of the opposite DNA strand causing a double-stranded break and subsequent apoptosis. Unlike other tubulin-binding cytotoxic agents, which are most effective in actively replicating cells, the cytotoxicity of calicheamicin is cell cycle-independent. A chemically modified, more stable form, N-acetyl-γ-calicheamicin dimethyl hydrazide (CalichDMH), is used in the design of antibody–drug conjugates (ADCs). CalichDMH is covalently attached to a monoclonal antibody *via* an acid-labile linker group. Once internalized, the linker is hydrolyzed in the acidic lysosome, thus releasing the calicheamicin prodrug.[11]

InO is an ADC composed of a humanized IgG4 anti-CD22 antibody covalently linked, *via* a butanoic acid linker, to CalichDMH. The monoclonal antibody portion binds with high affinity to CD22, and conjugation to the calicheamicin derivative does not affect its binding affinity. The anti-CD22 antibody itself induces no cytotoxicity; thus, the antitumor activity of InO is mediated solely by CalichDMH. Additionally, the cytotoxic effect of CalichDMH was more pronounced with CD22-targeted intracellular delivery than with passive uptake.[7,11]

Pharmacokinetics

InO demonstrates a dose- and time-dependent cytotoxicity. In preclinical studies, ALL cell lines appeared to be more sensitive to CalichDMH compared with B-cell lymphoma cell lines. The cytotoxic effect was enhanced 2- to 6-fold with InO compared with unconjugated CalichDMH, confirming the important role of targeting CD22. Unlike gemtuzumab ozogamicin (GO), an anti-CD33 antibody conjugated to CalichDMH, InO's cytotoxicity is independent of its ability to saturate CD22. Instead, efficacy relies on sensitivity to CalichDMH and efficient internalization of the ADC.[12] Furthermore, *in vitro* data demonstrate InO is able to prevent dissemination of ALL to the central nervous system (CNS), a sanctuary site important in disease relapse.[8]

Although *in vitro* sensitivity of ALL cells to InO was higher, the initial dose used in clinical trials was borrowed from early data in NHL, which established 1.8 mg/m^2 intravenously (IV) every 3–4 weeks as the maximum tolerated dose. Clinical studies in ALL showed higher peak levels of InO with single-dose administration of InO 1.8 mg/m^2 compared with the same total dose administered in a fractionated, weekly fashion; however, area under the curve (AUC) levels were similar. While higher peak levels did not correlate with responses, higher cumulative AUC was associated with significantly higher response rates and less toxicity.[13] As a result of these observations, the phase III INO-VATE trial used the fractionated dose schedule, which became the US FDA-approved dose. Optimized dosing strategies for InO in combination with other cytotoxic agents are under investigation.

Clinical efficacy

Single agent

Based on the early trials in lymphoma, investigators at The University of Texas MD Anderson Cancer Center (MDACC) conducted a phase II,

single-center study to assess the efficacy of InO in adult and pediatric patients with R/R B-cell ALL. Since this was the inaugural study in ALL, the first six patients received InO at an initial dose of 1.3 mg/m^2 intravenously (IV) during the first cycle to ensure safety, and then 1.8 mg/m^2 every 3–4 weeks during subsequent cycles. The remaining 43 patients received InO 1.8 mg/m^2 IV every 3–4 weeks. Patients who were CD20-positive and had stable disease after two courses of InO could receive rituximab 375 mg/m^2 IV once per course. The primary endpoint was overall response. A total of 49 patients were treated; 73% were in second or later salvage, and 14% had previously undergone allogeneic SCT. Baseline adverse cytogenetic features, including Philadelphia chromosome (Ph)-positive, translocation *t*(4;11), and complex karyotype, were present in 42% of patients. The overall response rate (ORR) was 57%, of which 18% were CR. Addition of rituximab in nine patients was of little benefit and resulted in only one additional response. Median OS was 5.1 months and was higher among responders compared with nonresponders (7.9 months *versus* 2.4 months). Nearly 50% of patients proceeded to allogeneic SCT. Infusion-related reactions, such as fever and hypotension, were common, as were hyperbilirubinemia and elevated liver enzyme concentrations. Veno-occlusive disease, or sinusoidal obstruction syndrome (SOS), was the most serious adverse event, occurring in 23% of patients after allogeneic SCT.[14]

Pharmacokinetic data suggesting a more optimal dosing strategy led to an amendment to the trial incorporating lower weekly InO doses in an effort to improve efficacy and minimize toxicity. An additional 41 patients were enrolled and received the same cumulative dose of InO per cycle (1.8 mg/m^2) administered in weekly doses: 0.8 mg/m^2 IV on day 1 followed by 0.5 mg/m^2 IV on days 8 and 15. Rituximab was not allowed in patients receiving weekly InO. As opposed to the monthly schedule, the weekly cohort had significantly more patients in salvage 1 with first CR (CR1) duration $<$12 months (29% *versus* 6%, $p = 0.003$) than in salvage 2 (24% *versus* 49%, $p = 0.016$). Treatment with weekly InO resulted in an ORR of 59% (CR: 20%), median survival was 7.3 months, and 34% proceeded to allogeneic SCT. Although efficacy was similar between single-dose and weekly InO, the latter was associated with fewer drug-related adverse effects, such as fever and hypotension, hyperbilirubinemia, and elevated liver enzymes. Among the 14 patients receiving weekly InO who proceeded to allogeneic SCT, only 1 developed SOS. In a multivariate analysis, a dual-alkylator conditioning regimen was the only significant factor correlating to higher incidence of SOS.[13]

Overall, the response rate among all the patients in the R/R cohort treated with single-agent inotuzumab was 58% (CR: 19%). Median survival for the entire cohort was 6.2 months. Survival was significantly better for patients achieving CR (13.1 months) and those treated in salvage 1 (9.2 months). Complete cytogenetic response and minimal residual disease (MRD)-negative status was achieved in 90% and 72% of responders, respectively; however, neither correlated with improved survival. Patients with Ph-positive ALL and translocation *t*(4;11), those in salvage 2 or later, and those with increased tumor burden (defined as high absolute peripheral blast count) had lower response rates.[13,14]

A multicenter phase I/II study evaluated safety and efficacy of several InO dose cohorts in 72 R/R ALL patients aged at least 18 years of age with CD22-positive disease (defined as $\geqslant$20% CD22 blasts). Patients enrolled in the phase II portion of the study were in salvage 2 or later. Patients with Ph-positive who had failed treatment with at least one tyrosine kinase inhibitor were allowed. The phase I dose cohorts were as follows: InO 1.2 mg/m^2 per cycle (0.8 mg/m^2 on day 1 and 0.4 mg/m^2 on day 15); InO 1.6 mg/m^2 per cycle (0.8 mg/m^2 on day 1 and 0.4 mg/m^2 on days 8 and 15); InO 1.8 mg/m^2 per cycle (0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on days 8 and 15). The recommended phase II dose was InO 1.8 mg/m^2 per cycle with a dose-reduction to 1.6 mg/m^2 per cycle for patients achieving CR/CR with incomplete marrow recovery (CRi). The primary phase II endpoint was CR/CRi. Among all treated patients, 78% had received at least two prior therapies and 32% had undergone prior SCT; 21% had complex cytogenetics; and 22% had Ph-positive disease. During the phase 1 portion, dose-limiting toxicity (grade 4 elevated lipase) occurred in only one patient at the 1.8 mg/m^2 per cycle dose level. CR/CRi rates for the 1.2, 1.6, and 1.8 mg/m^2 per cycle cohorts were 67% ($n = 2/3$), 75% ($n = 9/12$), and 89% ($n = 8/9$), respectively. Of the 35 patients treated in the phase II portion, 69% achieved CR/CRi ($p < 0.001$). Among all patients who received InO, 68% achieved CR/CRi and 84% of responders achieved MRD-negativity. Median duration of response was relatively short (4.6 months). Median progression-free survival

(PFS) and OS were 3.9 and 7.4 months, respectively, and 33% proceeded to SCT. The most common grade ⩾3 treatment-related adverse effects (⩾10% of patients) were thrombocytopenia, neutropenia, febrile neutropenia, and anemia. Treatment-related hepatic adverse effects, including elevated transaminase and hyperbilirubinemia, of grade ⩾3 occurred in <5% of patients; however, four patients (5%) developed treatment-related SOS (one during treatment, one during follow up, and two following post-study SCT). Overall, three cases of SOS were treated with defibrotide. There were two patients that had SOS ongoing at the time of death due to pneumonia and progressive disease, while the other two, both receiving defibrotide, recovered.[15]

The positive results observed in phase II trials led to a multicenter, randomized phase III study comparing InO monotherapy with intensive chemotherapy among patients with R/R ALL. In the INO-VATE trial, 326 patients with R/R ALL in first or second salvage were randomly assigned in a 1:1 ratio to receive either InO or investigator's choice of chemotherapy. Patients were stratified by duration of first remission, phase of salvage treatment and age. InO was administered weekly at a cumulative dose of 1.8 mg/m^2 IV per cycle (0.8 mg/m^2 IV on day 1, and 0.5 mg/m^2 IV on days 8 and 15) until patients achieved CR/CRi, at which point the day 1 dose was reduced to 0.5 mg/m^2 IV. Standard of care (SOC) therapy included fludarabine, cytarabine, and granulocyte colony-stimulating factor, mitoxantrone plus continuous-infusion cytarabine, or high-dose cytarabine. The coprimary objectives were CR/CRi and OS. Baseline characteristics were well balanced.[16] Response rates were significantly higher in the InO group compared with the SOC group, including CR rates (Table 1).[17] The rate of MRD-negativity among responders was also significantly higher for InO than SOC (78% *versus* 28%, $p < 0.001$).[16] With the exception of patients with Ph-positive and $t(4;11)$, InO produced significantly higher CR rates compared with SOC in all subgroups, including the salvage setting (1 and 2), age (age < 55 years and age ⩾ 55 years), and level of CD22 expression (<90% and ⩾90%).[16,18,19] Among patients treated with InO, higher response rates were observed for patients treated in salvage 1 than salvage 2 (88% *versus* 69%) with similar rates of MRD-negativity.[20] Significantly more patients proceeded to allogeneic SCT after InO treatment than SOC (41% *versus* 11%, $p < 0.001$).[16]

Median PFS was significantly longer among patients receiving InO (5 months *versus* 1.8 months, $p < 0.001$). Although not statistically significant, an improvement in survival was also noted (7.7 months *versus* 6.2 months), with a hazard ratio for death of 0.77 [95% confidence interval (CI), 0.58–1.03].[17] Due to departure of the survival data from the proportional hazard assumption, a *post hoc* analysis of restricted mean survival demonstrated significantly longer mean survival in the InO group than in the SOC group (13.9 *versus* 9.9 months, $p = 0.05$). Aside from ORRs, levels of CD22 did seem to impact other clinical outcomes. While patients with CD22 expression ⩾90% treated with InO had significantly longer PFS, duration of response, and OS, the statistically significant difference in median PFS was lost and median duration of response and OS inverted for patients with CD22 expression <90%.[18] Younger patients (age < 55 years) receiving InO had significantly longer median OS compared with older patients (age ⩾ 55 years). Patients treated with InO who achieved CR/CRi and then proceeded to allogeneic SCT had a significantly longer median OS, regardless of age, compared with those who did not receive allogeneic SCT (11.9 *versus* 5.7 months, $p = 0.0004$).[19]

Adverse events occurred with similar frequency in the two arms. Although InO was associated with less grade ⩾3 thrombocytopenia and febrile neutropenia, patients experienced more hepatotoxicity, including SOS.[16] Patients treated in salvage 2 had significantly higher incidence of hepatoxicity compared with those in salvage 1. In addition, the number of prior therapies and prior SCT were associated with increased incidence of SOS.[20] Serious adverse effects, including thrombocytopenia, febrile neutropenia, and infections, were more frequent among older patients. Specifically, post-transplant SOS was more prevalent among older patients (41% *versus* 17%).[19] A multivariate analysis revealed only dual-alkylator conditioning regimen to be significantly associated with SOS ($p = 0.04$).[16]

Long-term results of INO-VATE remain consistent with those previously reported; the 2-year survival was significantly longer for InO (23%, 95% CI, 16.7–29.6%) compared with SOC (10%, 95% CI, 5.7–15.5).[17]

Combination therapy

The combination of InO with low-intensity cytotoxic chemotherapy to improve on the single-agent

Table 1. Outcomes from phase III INO-VATE Trial.[16, 19–21, 22].

	Inotuzumab (*n* = 164)	**Standard of care (*n* = 162)**
ORR†	73%	31%
CR†	33%	16%
CRi†	40%	15%
ORR by age		
<55 years†	75%	28%
⩾55 years†	70%	37%
ORR by salvage*		
Salvage 1†	88%	29%
Salvage 2†	67%	31%
MRD	78%	28%
MRD by salvage**		
Salvage 1	78%	N/A
Salvage 2	79%	N/A
PFS†, median (months)	5.0	1.7
OS, median (months)	7.7	6.2
CR (MRD-negative)	14.1	N/A
CR (MRD-positive)	7.2	N/A
No CR	2.6	N/A

CR, complete response; CRi, complete response with incomplete recovery of peripheral blood counts; MRD, minimal residual disease; N/A, not available; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.
†p value < 0.05; *$n = 218$ (inotuzumab $n = 109$, standard of care $n = 109$); **$n = 108$.

outcomes was investigated in R/R and frontline elderly ALL patients. InO was first combined with an attenuated version of the intensive hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) regimen. Referred to as mini-HCVD, it eliminates doxorubicin, and consists of vincristine, plus 50% dose reductions of hyper-fractionated cyclophosphamide and dexamethasone, a 75% reduction of methotrexate and an 83% reduction of cytarabine. InO was given during the first four cycles, initially as a single dose of 1.3–1.8 mg/m^2 in cycle 1, followed by a single dose of 1.0–1.3 mg/m^2 IV in subsequent cycles. Patients whose blasts expressed ⩾20% CD20 received rituximab for the first four cycles.

The efficacy and safety of this regimen was evaluated in a cohort of 70 patients with Ph-negative, R/R ALL. The majority of patients were treated in first or second salvage. The ORR was 77% (CR: 59%); 81% of patients achieved MRD-negativity (Table 2). Patients in salvage 1 appeared to derive the greatest benefit with ORR and CR rate of 93% and 89%, respectively. After a median follow up of 20 months, median relapse-free survival (RFS) and OS were 16 and 11 months, respectively, with an estimated 2-year RFS and OS rates of 49% and 35%, respectively. The survival results were also more pronounced for salvage 1, with a 2-year OS rate of 49%.[21] Patients who became MRD-negative did better than those who did not, with 1 year OS 64% *versus* 31% and

Table 2. Results from inotuzumab combination trials.

	Mini-HCVD plus InO		InO plus bosutinib[23]
	R/R[21] (*n* = 70)	Frontline[10] (*n* = 52)	R/R (*n* = 14)
Patient characteristics			
Age, median (range)	35 (9–87)	68 (64–72)	62 (19–74)
Response			
ORR	77%	98%	79%
CR	59%	85%	
MRD-negativity*, %	81%	96%	73%
OS, months	11	NR	8.2
Salvage 1	25		
Salvage 2	6		
Salvage 3	7		

CR, complete response; InO, inotuzumab ozogamicin; mini-HCVD, mini hyper-fractionated cyclophosphamide, vincristine, and dexamethasone; MRD, minimal residual disease; NR, not reached; ORR, overall response rate; OS, overall survival; R/R, relapsed/refractory.

median survival of 25 months *versus* 9 months, respectively.[9] A *post hoc* analysis comparing outcomes of mini-HCVD plus InO with historical outcomes of InO alone revealed significantly higher OS (9 months *versus* 6 months, $p = 0.02$). A total of 38% of patients proceeded to allogeneic SCT.[21] Significant adverse events (grade ⩾3) included prolonged thrombocytopenia, hyperbilirubinemia, transaminitis, and SOS. All cases of SOS were associated with allogeneic SCT.[9,21]

In order to address the need for effective, less intensive chemotherapy regimens for elderly patients, the MDACC conducted a phase II trial to evaluate the efficacy and safety of mini-HCVD plus InO in the frontline setting. A total of 52 newly diagnosed patients aged 60 years and older with Ph-negative ALL were treated. The primary endpoint was PFS at 2 years. Median age was 68 years; 60% of patients were CD20-positive and received rituximab. Abnormal baseline cytogenetics were present in 51% of patients. The ORR was 98% (CR: 85%; Table 2). MRD-negativity among responders was achieved in 78% of patients at the time of morphologic response and in 96% within the first three cycles. All patients with baseline abnormal karyotype achieved a complete cytogenetic response. The estimated 2-year and 3-year PFS was 59% and 49%, respectively, with median PFS of 59 months. The estimated 2-year and 3-year OS was 66% and 56%, respectively, with median survival not reached at the time of analysis. When compared with historical survival data of patients treated with hyper-CVAD with or without rituximab, the 3-year survival was significantly higher with mini-HCVD plus InO (32% *versus* 56%, $p = 0.004$). Overall, the regimen exhibited acceptable tolerability, and no early mortality occurred. Thrombocytopenia was observed in 81% of patients. Infectious complications were noted in 52% and 69% of patients during induction and consolidation, respectively. Abnormal liver function tests grade ⩾3 were noted in 33% of patients. SOS developed in four patients; two died as a result of SOS, one died from deconditioning, and one recovered and completed therapy off-protocol.[10] Results for mini-HCVD plus InO in both the R/R and frontline settings require validation in larger randomized trials.

A phase I/II study of InO in combination with bosutinib is being conducted at MDACC to assess safety and maximal tolerated dose in patients with R/R, Ph-positive ALL or chronic myeloid leukemia (CML) in lymphoid blast phase (LBP). Bosutinib was given at three dose levels (300 mg daily, 400 mg daily, and 500 mg daily).

During cycle 1, InO was administered weekly to a cumulative dose of 1.8 mg/m^2 (0.8 mg/m^2 IV on day 1 and 0.5 mg/m^2 IV on days 8 and 15). For responders, the dose was reduced in subsequent cycles to a single 1 mg/m^2 IV dose per cycle. Of the 14 patients treated, 12 had R/R, Ph-positive ALL; 12 were in first or second salvage; and 6 patients had undergone previous allogeneic SCT. The ORR was 79% (Table 2). Among responders, rates of complete cytogenetic response and MRD-negativity were 91% and 73%, respectively; BCR-ABL was undetectable in 55%. Median event-free survival and OS were both approximately 8 months. Only one patient experienced grade 3 rash, and two patients experienced elevated alanine transaminase. Notably, no SOS was observed.[23]

Predictive factors for response

Patient- and disease-specific factors, as well as the number of previous therapies, impact outcomes for R/R ALL patients receiving InO. The MDACC analyzed the phase II data in order to identify factors contributing to response.[24] High absolute blast count ($\geq 1 \times 10^9$/l) and decreased platelet count ($< 100 \times 10^9$/l) were independent predictors of failure to achieve marrow CR. Median survival favored patients achieving marrow CR (9.2 *versus* 3.4 months). Adverse cytogenetics (t[4;11] and t[9;22], complex karyotype, and abnormal chromosome 17), high peripheral absolute blast count ($\geq 1 \times 10^9$/l), and salvage 2 or higher status correlated with shorter survival, regardless of dosing schedule. To identify patients who may derive the least benefit from single-agent InO, a scoring system was developed. Based on the number of adverse factors, patients were assigned a score of 0, 1, 2 or 3 corresponding to median survival of 39+, 7.6, 7.4, and 2.4 months, respectively. A model with similar predictive value was developed, although not validated, for patients achieving marrow CR. Identifying patients unlikely to benefit from single-agent InO might allow for early selection of patients for combination therapy.[24] Genomic assessment is ongoing to better evaluate the impact of InO in patients with baseline adverse features, such as MLL rearrangement and Ph-like phenotype.[25]

Safety

The most significant adverse effect observed with InO is hepatotoxicity, specifically SOS. The pathophysiology of SOS resulting from InO is not completely understood but is thought to be similar to that caused by GO, an anti-CD33 antibody conjugated to calicheamicin. Since the commonality between both compounds is the calicheamicin component, it has been implicated as the likely cause of hepatotoxicity, including SOS. Leading hypotheses suggest possible mechanisms may be exposure to free circulating calicheamicin, nonspecific uptake of the ADC by hepatic cells, or that, when exposed to InO or GO, CD22 and CD33 expressed on sinusoidal epithelial cells may facilitate intracellular delivery of the cytotoxin, causing direct injury to the sinusoids. Primate studies of antibody-calicheamicin conjugate effects on liver microvasculature and thrombocytopenia revealed microscopic findings consistent with hepatocyte atrophy and sinusoidal dilatation leading to fibrosis.[3,26] SOS has been described in patients receiving InO as a single agent for R/R disease and in combination with mini-HCVD in both the R/R and frontline settings. Data from INO-VATE and another single-agent InO study suggest an SOS incidence of 12%.[27] The majority of patients who developed SOS had undergone allogeneic SCT after InO therapy, suggesting a correlation between development of SOS and the time between InO and allogeneic SCT. However, analysis from the INO-VATE study showed the incidence of SOS to be irrespective of the time between last dose of InO and allogeneic SCT.[16] The incidence of SOS does appear to increase with the number of cycles received. Further analysis from INO-VATE revealed high rates of SOS among patients aged $\geq$65 years, those with last pre-SCT bilirubin concentrations greater than or equal to the upper limit of normal (ULN), and those receiving dual-alkylating SCT conditioning regimens. In a multivariate analysis, the latter two factors were strongly associated with development of SOS. Patients receiving pre-study SCT and those with last pre-SCT bilirubin concentration greater than or equal to the ULN were at increased risk for more severe (grade $\geq$3) SOS.[27] Consensus recommendations to mitigate the risk of SOS in patients considered for SCT suggest limiting the number of InO cycles, avoiding dual-alkylating agent conditioning regimens, and utilizing ursodiol as prophylaxis in all patients receiving InO. While not included in the Center for International Blood and Marrow Transplant Research (CIBMTR) SOS risk scoring system, patients receiving InO should be considered high risk and monitored accordingly.[28] Presently, defibrotide is the only US FDA-approved agent for the treatment of severe SOS

associated with kidney or lung toxicity following SCT. It has been used with mixed results in patients receiving InO who developed SOS.[16] New strategies, including a weekly schedule of lower doses of inotuzumab, the sequential use of blinatumomab [ClinicalTrials.gov identifier: NCT01371630], and selection of less hepatotoxic SCT preparative regimens may further improve outcomes and decrease the rates of SOS.

CNS disease

While preclinical data suggest that InO may prevent dissemination of disease to the CNS,[8] the risk of CNS disease in patients with systemic relapse is high. The phase III INO-VATE trial strongly recommended the use of concomitant prophylactic intrathecal chemotherapy.[16] Additionally, frontline and R/R ALL patients who received mini-HCVD plus InO also received SOC prophylactic intrathecal chemotherapy.[9,10] While most single-agent InO trials excluded patients with active CNS disease, patients who developed CNS disease who derived benefit from InO therapy were permitted to continue InO while receiving CNS-directed intrathecal chemotherapy. Therefore, patients receiving InO therapy, as a single agent or in combination with other therapy, should receive prophylactic intrathecal chemotherapy to prevent CNS relapse. Patients with active CNS disease who may benefit from InO should be considered for InO therapy in addition to standard CNS-directed intrathecal chemotherapy.

Conclusion and future direction

InO has demonstrated promising results in both single-agent and combination therapy. InO therapy has resulted in response rates of 60–80% for patients with R/R ALL. In treatment-naïve elderly patients, the response rate with mini-HCVD plus InO approaches 100%. Additionally, it has improved survival and allowed 30–40% of patients with R/R ALL to proceed to allogeneic SCT. While combination therapy with mini-HCVD and other agents requires further study, results from the INO-VATE trial led to the US FDA approval as a single agent in August 2017.

Blinatumomab is a bispecific T-cell engager antibody that binds CD-3 positive T-cells and CD19-positive B-cells allowing the native cytotoxic T-cells to identify and eradicate the ALL blasts. It is also US FDA-approved for relapsed or refractory B-cell precursor ALL. The benefit of blinatumomab over SOC chemotherapy was confirmed in the phase III TOWER trial. Blinatumomab resulted in a significantly higher rate of CR/CRi (44% *versus* 23%) and OS (7.7 *versus* 4.4 months) compared with chemotherapy and allowed 24% of patients to proceed to SCT.[29] While direct comparison of results between blinatumomab and InO is not possible and beyond the scope of this review, InO and blinatumomab should be considered complimentary, as opposed to competitive, therapy. Both agents are currently under investigation in the frontline and R/R setting in novel combinations and sequences, including with each other. In the frontline setting, this may allow for reduced reliance on intensive chemotherapy while, hopefully, being able to overcome adverse disease features and eliminate MRD. Incorporation of novel combinations and sequences of InO and blinatumomab into the R/R setting may further improve on the percentage of patients able to proceed to SCT over that observed with either agent alone.

A number of trials are ongoing to determine the further utility of InO in various combinations. The previously discussed phase I/II study of InO plus bosutinib for patients with Ph-positive ALL and CML LBP [ClinicalTrials.gov identifier: NCT02311998] is ongoing. The combination of InO with cyclophosphamide, vincristine, and prednisone is also under investigation in patients with R/R CD22-positive ALL [ClinicalTrials.gov identifier: NCT01925131]. Maintenance therapy with InO in the post-SCT setting is also being explored [ClinicalTrials.gov identifier: NCT03104491]. Efficacy and safety of the combination of InO with the more intensive hyper-CVAD regimen is also underway in patients age $\geqslant$16 years with newly diagnosed B-cell ALL [ClinicalTrials.gov identifier: NCT03488225]. Efficacy of InO is also being investigated in combination with another pediatric-inspired regimen in young adults with frontline B-cell ALL [ClinicalTrials.gov identifier: NCT 03150693]. Furthermore, a trial examining the efficacy of InO in patients with MRD-positive disease after achieving CR from prior therapy is also underway [ClinicalTrials.gov identifier: NCT03441061]. The trial of InO with mini-HCVD in frontline patients is continuing and exploring the efficacy of sequential blinatumomab therapy with this combination [ClinicalTrials.gov identifier: NCT01371630]. The role of InO continues to evolve as these novel combinations are studied. In

the meantime, InO has generated promising results for patients with R/R disease and offers older patients a viable alternative to intensive chemotherapy.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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