blood advances

Inotuzumab: from preclinical development to success in B-cell acute lymphoblastic leukemia

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Inotuzumab ozogamicin (InO) is a recently US Food and Drug Administration–approved antibody–drug conjugate for the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia (ALL). InO consists of a CD22-targeting immunoglobulin G4 humanized monoclonal antibody conjugated to calicheamicin. Although initially developed for the treatment of non-Hodgkin lymphoma (NHL) because of activity in preclinical models and high response rates in indolent lymphomas, a phase 3 trial was negative and further development focused on $CD22^+$ ALL. Although results in NHL were disappointing, parallel testing in early-phase trials of $CD22^+$ ALL demonstrated feasibility and efficacy. Subsequently, the randomized phase 3 Study Of Inotuzumab Ozogamicin Versus Investigator's Choice Of Chemotherapy In Patients With Relapsed Or Refractory Acute Lymphoblastic Leukemia trial showed that InO was superior to standard of care regimens with a significantly improved complete remission (CR) rate in patients with relapsed/refractory disease (80.7% vs 29.4%, $P < .001$). Patients achieving CR with InO also had a significantly higher rate of undetectable minimal residual disease compared with chemotherapy (78.4% vs 28.1%, $P < .001$). InO-specific side effects, including veno-occlusive disease, have been an ongoing area of concern, and consensus guidelines for minimizing toxicities are now available. Ongoing trials are investigating the combination of InO with other agents in the relapse setting and the addition of InO to frontline therapy. This review details the preclinical and clinical development of InO, focusing on how best to use it and future directions for further development.

Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is a neoplastic proliferation of B-cell lymphoblasts that primarily affects the bone marrow, blood, and lymph nodes. Although B-ALL is the most common cancer in children, it represents only 2% of adult lymphoid malignancies.[1,2] Standard treatment of B-ALL in adults generally consists of multiagent chemotherapy with some variation in regimens based on factors including age, cytogenetics, and molecular genetics. Despite improved success over the past 30 years, up to 10% of patients will have disease that is refractory to initial treatment and 40% to 70% will relapse.[3] Standard chemotherapy offers few good options for patients with relapsed disease. However, the recent approval of inotuzumab ozogamicin (InO), blinatumomab, and tisagenlecleucel has significantly improved treatment outcomes for these patients.[4] Unlike the CD19-targeting therapies blinatumomab and tisagenleucel, InO offers the novel approach of an antibody–drug conjugate specifically delivering the highly potent chemotherapy agent calicheamicin to CD22-expressing cells.[5] Although initially developed to treat any $CD22^+$ B-cell malignancy, studies have shown that InO appears to be most active in B-ALL. Here, we review the preclinical development of InO with specific emphasis on drug development, preclinical testing, and results of initial clinical trials. We then discuss future directions and questions that can be addressed during the design of the next generation of antibody-dependent conjugates (ADCs).

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Preclinical development

CD22

CD22 is a type I transmembrane protein comprising 7 extracellular immunoglobulin (Ig)–like domains and an intracellular immunoreceptor tyrosine-based inhibitory motif.[6] CD22 is solely expressed on cells belonging to the B-cell lineage and negatively modulates B-cell receptor signaling though its immunoreceptor tyrosine-based inhibitory motif domain.[7] As a member of the Siglec family of cell surface receptors, it also binds to sialic acid molecules on various cell surface molecules. Despite high expression on malignant B cells, naked antibodies that target CD22 alone have had little activity in clinical trials.[8] However, CD22 is an attractive targeting molecule for an ADC. Rather than being shed into the extracellular environment following ligand binding or antibody crosslinking, CD22 is rapidly internalized, a characteristic conducive to specific delivery of ADCs.[9]

Calicheamicin is a highly potent chemotherapeutic drug belonging to the enediyne class of DNA-damaging cytotoxic agents derived from the soil bacterium *Micromonospora echinospora* subsp. *calichensis*.[10] It has a unique thiol-dependent mechanism of action, binding to the DNA minor grove with a sequence preference for T-C-C-T and resulting in double-stranded DNA breaks and inhibition of transcription.[11,12] To increase drug stability and to improve therapeutic utility, calicheamicin was optimized by acetylation of the aminosugar and by conversion to a more stable disulfide derivative.[13] This form, known as *N*-acetyl-γ-calicheamicin DMH, is the current formulation used for further drug development. Studies using calicheamicin have demonstrated potent killing of malignant cells but at a low therapeutic index because of nonspecific mechanisms of action. Specific delivery of this agent to target cells by means of an ADC offers a promising solution, allowing for preservation of potency while limiting the off-target effects.[14]

InO

Development of InO began by testing a panel of murine monoclonal antibodies for high-affinity binding to and internalization of CD22.[15] The candidate m5/44 best satisfied these criteria, removing >90% of CD22 from the cell membrane. This antibody was then further optimized to its g5/44 form by removal of a glycosylation site and a reactive lysine from its variable domain and then humanized to a human IgG4 backbone. ADCs are dependent on an optimal chemical linker to bridge the chemotherapeutic drug to the antibody and to release it at the appropriate location. Three chemical linkers were added to m5/44, 2 acid-labile linkers, 4-(4′-acetylphenoxy) butanoic acid (AcBut) and (3-acetylphenyl) acetic acid, and an acid-stable amide linker. Although both types of linkers demonstrated greater activity when compared with unconjugated calicheamicin or unconjugated antibody, the activity of the acid-labile linkers were far superior with a fivefold to eightfold increase in potency. Moreover, when tested on previously established lymphoma xenografts, the AcBut linked calicheamicin was able to render 6 of 7 mice tumor free, whereas all mice treated with the amide linker had eventual tumor regrowth. Based on these results, the humanized g5/44 AcBut linked calicheamicin construct, renamed CMC-544 and now known as InO, was selected for further development.

InO was evaluated in several B-cell lymphoma cell lines, yielding in vitro 50% inhibitory values that ranged from 6 to 300 pM (calicheamicin equivalent).[16] The conjugated calicheamicin consistently demonstrated increased potency in all reported CD22-expressing cell lines when compared with its unconjugated counterpart. In vivo testing of subcutaneous xenografts showed that InO inhibited growth of Ramos and RL tumors in a dose-dependent manner and that higher doses could render the mice tumor free. Importantly, control experiments showed that the unconjugated CD22 targeted antibody, G5/44, had no effect on tumor growth.

InO was also found to have activity in lymphoma models of disseminated disease. If these mice remain untreated, they develop hind-limb paralysis from involvement of the vertebral bone marrow and meninges or die of lymphomatous involvement of other organs. Interestingly, DiJoseph et al showed that, although anti-CD33–conjugated calicheamicin was similar to vehicle, InO was able to treat disseminated disease in >70% of the treated mice, with none of the mice developing hind-limb paralysis.[17] Moreover, InO remained protective even when given 3 weeks after lymphoma cell inoculation. This finding was in contrast to rituximab treatment, which was able to prevent death when given within 3 days of injection but was ineffective when given at later times. Of note, the investigators observed significantly decreased survival at the highest dose of InO used. When further examined, the mice were free from disease but had likely died of side effects of treatment, although the exact cause was not determined.

Additional preclinical studies focused on evaluating the efficacy of combining InO with other agents. DiJoseph et al also found the combination of InO and rituximab to be significantly more effective against subcutaneous or disseminated lymphoma than either InO or rituximab alone.[18] Combination treatment was further assessed with chemotherapy by using cyclophosphamide, hydroxydaunorubicin, Oncovin, and prednisone or cyclophosphamide, vincristine, and prednisone with InO.[19] Unsurprisingly, this combination was more effective when given together than when given alone.

InO was also tested against several B-ALL lines. In vitro testing of ALL cell lines revealed an order of magnitude increase in sensitivity in these cells to unconjugated calicheamicin compared with B-cell non-Hodgkin lymphoma (B-NHL) or acute myeloid leukemia (AML) cell lines, although the reason for this increased sensitivity has not been elucidated.[20] In vivo experiments with ALL xenografts also supported this finding because lower doses of InO were capable of preventing the growth of subcutaneous or disseminated disease in these models. Moreover, treatment with the CD33-targeted CMA-676 demonstrated significant activity in subcutaneous xenografts of REH B-ALL despite the lack of CD33 on the target tissue. In comparison, no effect was seen when CMA-676 was used to treat NHL cell lines in either subcutaneous or systemic xenografts. The efficacy of passive targeting by CMA-676 likely reflected the increased sensitivity of ALL cells to unconjugated calicheamicin. This differential sensitivity was even greater in primary ALL samples with reports that ALL was 3500 or 15 750 times more sensitive to unconjugated calicheamicin than either primary AML cells or normal bone marrow cells, respectively.[21]

Clinical development

NHL

The initial phase 1 dose-identifying trials of InO were conducted in patients with relapsed/refractory (R/R) NHL.[22] Doses were given once every 3 or 4 weeks, starting at 0.4 mg/m^2 and increased to 2.4 mg/m^2. The maximally tolerated dose (MTD) was determined to

be 1.8 mg/m^2 because some patients receiving 2.4 mg/m^2 developed grade 4 cytopenias. A total of 79 patients were treated in this trial, which yielded an overall response rate (ORR) of 39% (Table 1). Patients with follicular lymphoma (FL) fared better with an ORR of 68% vs 15% in patients with diffuse large B-cell lymphoma. Common side effects were thrombocytopenia (90%), asthenia (67%), nausea (51%), and neutropenia (51%). One patient developed veno-occlusive disease (VOD), but had also been treated with an autologous stem cell transplant and radiation therapy to the liver. Similar results were seen in a small phase 1 study of Japanese patients with FL.[23] A phase 2 study of advanced indolent lymphoma confirmed the phase 1 results with an ORR of 67%.[24] Phase 1/2 studies also tested the combination of rituximab and InO, which determined that the combination was well tolerated and had significant activity with ORR of 87% for FL and 74% for diffuse large B-cell lymphoma (DLBCL), but only 20% for refractory aggressive NHL.[25] Similar results were seen in a small phase 1 study of Japanese patients.[26]

A phase 3 study comparing rituximab and InO (R-InO) to rituximab and chemotherapy was conducted in patients with R/R aggressive B-NHL.[27] Patients were eligible if they had R/R CD20$^+$/CD22$^+$ aggressive B-NHL and were not candidates for high-dose chemotherapy with or without transplant. Because of entry criteria, most patients had DLBCL (91%) and most (68%) were >65 years of age. The study enrolled 338 patients and randomized them to either R-InO or to investigators choice of rituximab plus bendamustine or rituximab plus gemcitabine. The study was stopped early when an interim analysis showed futility. The results were recently published and demonstrated an ORR of 41% for R-InO vs 44% for investigators choice. Adverse events were similar to previous studies. However, this study did report 3 patients who developed VOD in the R-InO arm vs no cases in the chemotherapy arm. The lower response rate in the study might reflect the older age of the population and the combination of relapse and refractory DLBCL, whereas previous studies had separated relapsed from refractory disease.

These results show that, although InO has activity in CD22-expressing NHL, it was most effective in indolent lymphomas and in less heavily treated patients. Patients with aggressive, chemotherapy-refractory NHL tended to have lower response rates to InO than do chemotherapy-sensitive patients. These results suggest that in the appropriate clinical context, InO could be beneficial for treating patients with NHL. One ongoing trial seeks to answer this question by focusing on treatment-naïve patients with DLBCL who are ineligible for anthracyclines to determine if replacing doxorubicin with InO will be efficacious (NCT01679119).

ALL

Preclinical studies investigating InO showed that it had strong in vitro activity and was capable of curing mice injected with various ALL cell lines. Investigation of InO in the ALL clinical arena began with a single-institution phase 1 trial that evaluated its efficacy in CD22$^+$ ALL patients with relapsed or refractory disease.[28] This study enrolled 49 patients (Table 2) ranging from 6 to 80 years of age and included patients with Philadelphia chromosome positive (Ph$^+$) ALL, although the latter did not continue on tyrosine kinase inhibitors (TKIs). Based on the previous MTD in NHL, the goal dosing was 1.8 mg/m^2 administered once every 3 to 4 weeks. However, the first 3 adults and the first 3 pediatric patients were initially given 1.3 mg/m^2 to evaluate tolerability. Treatment with InO

Table 1. Select trials in NHL

Trial	clinicaltrials.gov identifier	Disease	InO schedule	No. of patients	ORR, % (CR, %)	VOD	Notes
Phase 1 MTD[22]		R/R NHL	0.4-2.4 mg/m^2 once per cycle	79	39	1/79	Determined MTD of 1.8 mg/m^2
Phase 1[23]	NCT00717925	R/R Follicular NHL	1.3-1.8 mg/m^2 once per cycle	13	85 (54 CR)	None	Tested in Japanese population
Phase 1 combining InO and rituximab[26]	NCT00724971	R/R NHL	1.8 mg/m^2 once per cycle	10	80	None	Tested in Japanese population
Phase 1/2 combining InO and rituximab[25]	NCT00299494	Relapsed FL, relapsed DLBCL, refractory aggressive NHL	1.8 mg/m^2 once per cycle	118	87% FL, 74% DLBCL, 20% refractory aggressive NHL	None	Tested combination therapy
Phase 2[24]	NCT00868608	Refractory indolent NHL	1.8 mg/m^2 once per cycle	81	67 (31)	None	
Phase 3 combining InO and rituximab vs R-chemo[27]	NCT01232556	R/R aggressive NHL	1.8 mg/m^2 once per cycle	338	41 vs 44 (R-InO vs R-chemo)	3/338	Compared with R-bendamustine or R-gemcitabine; stopped early for futility

R-, rituximab.

Table 2. Selected trials in ALL

Trial	clinicaltrials.gov identifier	Disease	InO schedule	No. of patients	CR, %	VOD	Notes
Phase 1/2[30]	NCT01363297	R/R B-ALL, Ph^+ included	1.2-1.8 mg/m^2 per cycle given on days 1, 8, 15; 1.8 for phase 2	72	68	4	
Phase 2[28,29]	NCT01134575	R/R B-ALL, Ph^+ included	1.3-1.8 mg/m^2 once per cycle (n = 49). 1.8 mg/m^2 per cycle given on days 1, 8, 15 (n = 41)	90	58 (CR + CR_p + CR_i)	6/90, 6/36 transplant patients	Noted reduced adverse events with weekly dosing
Phase 2 combing InO with chemotherapy[41]	NCT01371630	R/R B-ALL, Ph^+ excluded	1.3-1.8 mg/m^2 cycle 1, 1.0-1.3 mg/m^2 subsequent cycles, all dosed once per cycle	59	78 (CR + CR_p + CR_i)	9/59	InO combined with mini-HCVD noted to have increased VOD rate
Phase 2 combining InO with chemotherapy[42]	NCT01371630	Newly diagnosed B-ALL, Ph^+ excluded	1.3-1.8 mg/m^2 cycle 1, 1.0-1.3 mg/m^2 subsequent cycles, all dosed once per cycle	52	98 (CR + CR_p + CR_i)	4/52	InO combined with mini-HCVD; first to show high response rate of InO in frontline setting
Phase 3 INO-VATE comparing InO with chemotherapy[31]	NCT01564784	R/R B-ALL, Ph^+ included	1.8 mg/m^2 per cycle given on days 1, 8, 15; 1.5 mg/m^2 once in CR	326	80.7 vs 29.4	11% vs 1%	Phase 3 trial that compared single-agent InO to chemotherapy regimens; notably increased rate of VOD

CR_p, complete remission with incomplete platelet recovery.

produced an ORR of 57% in this heavily pretreated population (73% of patients were salvage 2 or higher). MRD^- status was attained in 63% of the patients but was not associated with improved survival. Despite the deep responses, the responses were short-lived, with a median duration of 6.3 months. Adverse events included worsening cytopenias, drug-related fevers, drug-related hypotension, and elevated liver function tests. Twenty-two of the patients proceeded to allogeneic stem cell transplant, with 23% developing clinical evidence of VOD after transplant.

Based on the high clinical response rate in patients with advanced ALL and concerns about the hepatic toxicities, the trial was expanded into a second stage. However, dosing for the next cohort of 41 patients was split into weekly doses of 0.8, 0.5, and 0.5 mg/m^2 based on higher preclinical activity and decreased toxicity with lower, more frequent dosing.[29] The response rate with weekly dosing was similar to that seen with single-dose InO, resulting in a combined response rate of 58% in the 90 enrolled patients. The median overall survival (OS) for patients who received InO was 6.2 months. This survival varied based on response to treatment with patients in CR having a median survival of 13.1 months, patients in complete remission with incomplete platelet recovery (CR_i) and complete remission with incomplete hematologic recovery having a median survival of 7.4 months, and patients with resistant disease having median survival of 3.1 months. Weekly dosing had a lower rate of drug-related fevers, drug-related hypotension, and elevated liver function tests. Only 14 patients proceeded to allogeneic stem cell transplant after weekly dosing, with only 1 developing VOD.

The ORR and safety in ALL patients was verified in a multicenter phase 1/2 trial that enrolled 72 patients with $CD22^+$ B-ALL.[30] The phase 1 component of the trial confirmed the safety/tolerability of 1.8 mg/m^2 split into 3 weekly doses (days 1, 8, and 15) of a 21-day cycle. Forty-nine patients attained CR or CR_i, with 84% of these patients having MRD^- disease. Similar to the previous study, weekly dosing was better tolerated. In this study, 4 patients experienced VOD, with 2 patients developing VOD following transplant and the remaining 2 in the absence of transplant.

The success in the these phase 1/2 trials led to the Study Of Inotuzumab Ozogamicin Versus Investigator's Choice Of Chemotherapy In Patients With Relapsed Or Refractory Acute Lymphoblastic Leukemia (INO-VATE) study, a randomized phase 3 trial of InO vs chemotherapy for R/R $CD22^+$ B-ALL in first or second salvage.[31] INO-VATE enrolled patients age $\geq$18 years with both Ph^+ and Ph^- ALL and randomly assigned them to InO or investigators' choice of standard chemotherapy in a 1:1 ratio. For those individuals randomized to chemotherapy, regimens included fludarabine, cytarabine, and granulocyte-colony-stimulating factor; cytarabine plus mitoxantrone; or high-dose cytarabine. Patients were stratified by 3 factors at the time of randomization: first remission $<$12 months vs $\geq$12 months, first vs second salvage, and age $<$55 years vs $\geq$55 years. Patients in the InO arm were treated with 0.8 mg/m^2 on day 1 and 0.5 mg/m^2 on days 8 and 15 of a 21-day cycle. Patients who achieved CR or CRi were then treated at a reduced dose of 0.5 mg/m^2 on days 1, 8, and 15 for subsequent cycles. Up to 6 cycles were administered, and patients were allowed to proceed to stem cell transplant at the investigator's discretion. Patients treated with InO had a significantly higher rate of CR than those receiving standard chemotherapy (80.7% vs 29.4%, $P < .001$). Moreover, of the patients who achieved CR, InO had a significantly higher rate of MRD

undetectable disease (78.4% vs 28.1%, $P < .001$) and longer remission duration (median, 4.6 vs 3.1 months, $P = .03$). Progression-free survival (PFS) was significantly improved in the InO-treated group, with a median PFS of 5.0 months vs 1.8 months (hazard ratio, 0.55; $P = .03$). However, median OS was not significantly different between the treatment groups (7.7 vs 6.7 months), although the hazard ratio suggested improved OS at 0.77 ($P = .04$), likely reflecting a separation of the 2 survival curves at later time points (2-year OS, 23% vs 10%). This late survival benefit was postulated to be due to the higher rate of stem cell transplant in the InO group (41% vs 11%, $P < .001$).

Adverse events were similar between the 2 arms with the exception of significant thrombocytopenia and hepatic toxicity. Although thrombocytopenia remained the most frequent hematologic side effect in both treatment arms, the percentage of patients with grade 3 or higher thrombocytopenia occurred less frequently in the InO-treated than the chemotherapy-treated group (37% vs 59%). Liver-related side effects occurred more commonly in the InO-treated group. Aspartate aminotransferase elevation was the most common (20% vs 10%) liver-related adverse event followed by increased GGT (17% vs 8%), hyperbilirubinemia (15% vs 10%), elevated alanine aminotransferase (14% vs 11%), and elevated alkaline phosphatase (12% vs 6%). Interestingly, elevated lipase was seen in the InO-treated group only (10% vs 0%). The rate of VOD was increased in the InO-treated population, with 11% of patients developing VOD vs 1% in the chemotherapy group. Of the 15 patients that developed VOD, 5 developed it during or shortly after treatment (2/5 had prior stem cell transplant). Of the 48 patients in the InO group who went on to transplant, 10 developed VOD (3/10 were second transplant). However, only 1 of 20 patients experienced VOD after transplant in the chemotherapy group. In a multivariate analysis, the sole factor that predicted VOD in transplanted patients was administration of a dual alkylator conditioning regimen ($P = .04$). An updated analysis of INO-VATE again showed this conditioning regimen was associated with VOD in addition to pretransplant bilirubin greater than the upper limit of normal.[32] A combined analysis of phase 3 trial patients treated with InO for ALL or NHL looked at hepatic toxicities and found that, although the VOD rate from InO alone was low at 1.5%, it increased to 27% in those that subsequently underwent allogeneic transplant.[33] This is compared with 0% and 9% for those treated with chemotherapy and chemotherapy/allogenic transplant, respectively. Although not all cases of VOD were severe, analysis of risk factors for the development of VOD were not able to differentiate people at risk for mild vs severe disease.

Taking the findings of the clinical trials discussed here into consideration, expert guidelines were recently published to assist in the management and prevention of InO-related adverse events.[34] Specifically, these address InO-related neutropenia, thrombocytopenia, infusion-related reactions, tumor lysis syndrome, prolonged QT syndrome, and VOD. To reduce the risk of VOD, the guidelines recommend avoiding stem cell conditioning regimens containing dual alkylating agents, thiotepa, or both. They also recommend prophylactic ursodiol, avoidance of hepatotoxic agents during conditioning, and limiting treatment with InO to 2 cycles in patients proceeding to stem cell transplant.

Predictors of response in ALL

Analysis of patient characteristics revealed several interesting findings. First, analysis of all 3 stratification factors favored treatment with InO. Second, patients from most cytogenetic groups fared better with InO therapy with the exception of patients with t(4;11) or Ph^+ disease. Although Ph^+ patients treated with InO had a remission rate of 78.6%, Ph^+ patients treated with chemotherapy did better (44.4% CR) than other cytogenetic groups. Therefore, in Ph^+ disease, this analysis seems to be skewed by improved efficacy of chemotherapy, possibly because of the lack of prior exposure to chemotherapy in these patients treated with TKIs, rather than inferior response to InO. However, the same cannot be said for t(4;11), in which there were similarly low rates of remission in both groups (33.3% vs 33.3%). Third, age was not a determining factor in response rate to InO. Patients <55 or ≥ 55 years of age had similarly high response rates to InO (80.3% vs 81.4%) and were superior to chemotherapy in both age groups (31.9% vs 25%). Fourth, CD22 expression on $>90\%$ of cells was not a significant determinant of InO response (79.2% vs 82.4%). Fifth, patients in first salvage had a higher rate of response vs second salvage (87.7% vs 66.7%). Last, patients with a high disease burden ($>50\%$ marrow blasts) still had a high response rate to InO treatment (86.7% vs 77.9%).

Mechanisms of action and resistance

The effective destruction of target cells by InO requires the completion of a multistep process. These steps include the successful delivery of antibody–drug conjugate to the tumor microenvironment, binding to surface CD22, receptor internalization, hydrolysis of the chemical linker, activation of calicheamicin by cytoplasmic thiols, and the action of calicheamicin on DNA before cellular efflux. To estimate which of these factors most influence InO efficacy, Betts et al produced a quantitative pharmacokinetic/pharmacodynamic model of InO built on preclinical experiments and clinical trial data.[35] This model suggested that a major pharmacokinetic difference between NHL and ALL arises from an estimated 100-fold difference in predicted intracellular calicheamicin concentration. It is unclear how much this difference might explain differences in efficacy observed for InO between NHL and ALL. In a sensitivity analysis that looked at which factors most influence InO efficacy, the authors found that tumor growth rate, drug efflux, and InO clearance were the most sensitive to changes in values. Although drug efflux has not been directly looked at for InO, previous work with gemtuzumab ozogamicin showed that increased expression of efflux pumps for calicheamicin reduced gemtuzumab efficacy.[36] Surprisingly, CD22 expression was the least sensitive parameter they examined.[35] This is consistent with the updated results from the INO-VATE trial, which suggested a small but not statistically significant decrease in efficacy when $<90\%$ of blasts were positive for CD22.[37] Loss or downregulation of CD22 expression, another potential mechanism for relapse that has been reported for patients receiving CD22 chimeric antigen receptor T-cell therapy,[38] has not yet been studied extensively following treatment with InO. A recent report, however, suggests that this may be true, at least in some cases.[39] A review of a limited number of patients who achieved CR on the INO-VATE trial demonstrated loss of CD22 expression on lymphoblasts at the time of relapse.[40]

Moving forward: combination treatment in ALL

The success of InO in the relapsed setting has led to trials combining it with additional therapy. One such study added single-dose InO to mini–cyclophosphamide, methotrexate, vincristine, and dexamethasone

(HCVD) regimens for the treatment of R/R ALL, including those patients with R/R Ph^- B-ALL.[41] Despite not limiting the number of prior treatments, this regimen was able to obtain remission in 78% of patients, which is comparable to the observed response rate from the INO-VATE trial and higher than previous studies in multiply relapsed patients. The rate of VOD was similar to that seen in the INO-VATE trial, with 15% of patients developing VOD.

Frontline inotuzumab

Mini-HCVD with single-dose InO has also been tested as frontline treatment of Ph^- B-ALL in patients >60 years of age who were unlikely to proceed to transplant.[42] Instead, patients are typically consolidated with up to 3 years of dose-reduced Purinethol (6-mercaptopurine), Oncovin (vincristine sulfate), methotrexate, and prednisone. Treatment-naïve patients enrolled in this trial had an exceptional ORR of 98%. Importantly, responses were more durable than those seen in the relapsed setting with a median PFS of 35 months. Moreover, the 2- and 3-year OS rates were estimated at 66% and 56%, respectively. Only 3 of the 52 patients underwent stem cell transplant. The incidence of VOD was approximately one-half the rate observed in previous ALL studies, likely owing to the reduced exposure to transplant conditioning. These results show the great promise of using this treatment in the frontline setting, especially in patients that have not traditionally tolerated multiagent chemotherapy. One question that remains unanswered is the response rate of single-agent InO in treatment-naïve patient, but studies to address this are in development (see the following section).

Ongoing clinical trials, additional questions, and future directions

Several clinical trials are ongoing to improve our understanding of how and when to best use InO (Table 3). Some interesting questions that are being tested include the safety and efficacy of using a TKI and InO concurrently during treatment of relapsed Ph^+ ALL (NCT02311998) and the safety/efficacy of using InO in the upfront setting with an intensive pediatric regimen in older adolescent and young adult patients (NCT03150693) that explores the question of whether early introduction of InO can increase rates of undetectable MRD to enhance event-free survival. Other trials are testing whether InO can be combined with intensive chemotherapy in the frontline setting (NCT03488225) and the safety/efficacy of using InO to eliminate MRD (NCT03441061), a strategy recently used to obtain US Food and Drug Administration approval (4/18) of blinatumomab for treatment of MRD^+ postremission. In the relapsed setting, an ongoing trial is testing the efficacy of lower doses of weekly InO (NCT03094611). Finally, it is exciting to imagine a "chemotherapy-free" regimen; a proposal is pending to combine InO with blinatumomab for older adults with previously untreated ALL.

Beyond determining optimal dosing, timing, and sequencing for clinical use, there are several interesting observations from the published experience with InO and others calicheamicin ADCs that remain to be answered. First, what is the pathologic mechanism by which the calicheamicin-conjugated ADC inotuzumab and gemtuzumab induce VOD in treated patients? Some initial reports speculated that it might result from problems encountered during antibody recycling within the reticuloendothelial system.[22] It would be interesting to determine if the problem was due to inadvertent release of calicheamicin from decreased pH within recycling

Table 3. Selected ongoing trials

Trial	clinicaltrials.gov identifier	Disease	InO schedule	Patients	Open	Notes
Phase 1/2 testing safety and efficacy of bosutinib and InO	NCT02311998	R/R Ph^+ B-ALL, CML in lymphoid blast phase	1.8 mg/m^2 per cycle given on days 1, 8, 15	Adults 18+ y	Recruiting	Trial to formally evaluate MTD of TKI and InO
Phase 3 A041501: (C10403 ± InO)	NCT03150693	Newly diagnosed B-ALL	1.8 mg/m^2 per cycle given on days 1, 8, 15	Adults 18-39 y	Recruiting	Trial to test the addition of InO to improve frontline therapy in AYA patients
Phase 2 hyper-CVAD + InO	NCT03488225	Newly diagnosed B-ALL, Ph^+ excluded	0.6 mg/m^2 day 1 and 0.3 mg/m^2 day 8 of cycles 5-8	16 y and older	Recruiting	Plan to test addition of InO to hyper-CVAD backbone
Phase 2 testing InO for MRD	NCT03441061	B-ALL, Ph^+ excluded, in CR with MRD^+	0.6 mg/m^2 day 1 and 0.3 mg/m^2 days 8 and 15	Adults	Recruiting	Plan to the tolerability and efficacy of using InO to eliminate MRD^+ disease.
Phase 2 testing low-dose InO in relapsed disease	NCT03094611	R/R B-ALL, Ph^+ excluded	Cycle 1: 0.8 mg/m^2 day 1 and 0.5 mg/m^2 days 8 and 15 Subsequent cycles: 0.6 mg/m^2 day 1; 0.3 mg/m^2 day 8,	12 y and older	Recruiting	Plan to test efficacy and tolerability of a reduced dosing schedule for R/R disease
Randomized phase 2 in DLBCL patients ineligible for anthracyclines	NCT01679119	Frontline DLBCL, not anthracycline candidates	0.8 mg/m^2 day 2	Adult	Recruiting	Testing replacement of anthracycline by either InO or gemcitabine to R-CHOP regimen in patients who cannot safely receive standard treatment

AYA, adolescent and young adult; CML, chronic myeloid leukemia; hyper-CVAD, cyclophosphamide, vincristine, doxorubicin (Adriamycin), and dexamethasone.

endosomes or if fully conjugated antibody was unable to bind the neonatal Fc receptor for efficient recycling.[43] An alternative hypothesis is that liver sinusoids might be more sensitive to free calicheamicin liberated from the ADC. Understanding the mechanism of VOD could help to avoid this toxicity in future drug development. Another intriguing observation was that CD22 expression levels did not predict the rate of response in trials. This is a curious result, because the premise of ADC is to deliver drug specifically to the cells that express the extracellular target. However, we might be able to reconcile this by some interesting preclinical observations, namely that ALL cell lines and, even more so, primary blasts were exquisitely sensitive to free calicheamicin. This degree of sensitivity was such that even calicheamicin liberated from gemtuzumab had significant activity on $CD33^-$ ALL cell lines. This is in contrast to AML blasts and B-NHL cells, which have a higher intrinsic resistance to calicheamicin. Therefore, it is likely that ALL treatment benefits from an area effect such that InO bound to $CD22^+$ blasts is able to kill neighboring $CD22^-$ blasts because of this effect. This increased sensitivity to the calicheamicin payload likely explains the higher activity of InO in ALL vs NHL. Finally, it is important to determine why patients with t(4,11) are resistant to InO therapy. The number of t(4;11)-treated patients has been small, but it would be interesting to determine if they were refractory because of an outgrowth of $CD22^-$ blasts, lineage switch to a myeloid phenotype, or if the inherent calicheamicin sensitivity is more akin to AML blasts than ALL blasts.[44-46]

Conclusions

InO is a highly active antibody–drug conjugate for the treatment of patients with $CD22^+$ B-ALL. Its recent approval has greatly increased the ability to attain remission in patients with R/R disease and represents a significant advance in therapeutic options for treatment of relapsed ALL. However, the duration of response is short in relapsed/refractory disease and further work is needed to improve survival rates following InO with additional consolidation approaches or improving the safety of transplant strategies. Ongoing studies to expand testing of InO in the frontline setting, reduce its toxicity, and combine it with both standard ALL regimens and other new immunotherapeutic approaches are ongoing, and we eagerly await the results of these trials.

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Authorship

Contribution: W.S. designed, wrote, and edited the manuscript; and J.W. and D.W. wrote and edited the manuscript.

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