ADVANCES IN LLM

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Inotuzumab: The Most Active Single Agent in Acute Lymphoblastic Leukemia?

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H&O What is inotuzumab ozogamicin, and what is its mechanism of action?

DT Inotuzumab ozogamicin is a monoclonal antibody targeting the surface antigen CD22 that is bound to calecheamicin, a toxic natural product of *Micromonospora echinospora*.[1] Inotuzumab ozogamicin has subnanomolar binding affinity, is rapidly internalized, and delivers the conjugated calecheamicin intracellularly. Calecheamicin then binds to the minor DNA groove and causes breaks in double-stranded DNA in a sequence-specific and thiol-dependent manner, leading to cell apoptosis. CD22, among the most frequently expressed surface antigens in B-lymphoblastic leukemia/lymphoma, is present on both immature and mature B-lymphoblasts, but not hematopoietic stem cells.[2] It is a member of the sialoglycoprotein family of adhesion molecules that regulate B cell activation and the interaction of B cells with T cells and antigen presenting cells. After binding, intracellular CD22 is phosphorylated, resulting in down regulation of CD19 and the B-cell receptor.[3] Preclinical work with inotuzumab demonstrated significant activity against REH acute lymphoblastic leukemia (ALL) cell lines, including induction of tumor regression and cures in leukemia-bearing mice.[4]

H&O Can you discuss your phase II study of inotuzumab in patients with refractory and relapsed ALL?

DT A phase II study of inotuzumab was conducted in relapsed or refractory B-lymphoblastic leukemia patients who had an Eastern Cooperative Oncology Group (ECOG) performance status score of 3 or better, adequate hepatorenal function, adequate cardiac function (New York Heart Association disease classification less than class III or ejection fraction at least 45%), and CD22 expression of at least 20% in the absence of known infection with hepatitis B virus, pregnancy, or allogeneic stem cell transplantation (SCT) within the prior 4 months.[5] The first 3 adult patients and the first 3 pediatric patients (age <16 years, enrollment allowed after treatment of at least 10 adults) were treated with inotuzumab 1.3 mg/m^2 intravenously (IV) every 3 weeks for up to 8 cycles. Once the safety was established, patients were treated with inotuzumab 1.8 mg/m^2 IV every 3 weeks, derived from the recommended phase II dose established for inotuzumab in indolent and aggressive non-Hodgkin lymphoma trials, where the predominant dose-limiting toxicity was thrombocytopenia.[6,7] In CD20-positive cases, the anti-CD20 monoclonal antibody rituximab (Rituxan, Genentech/Biogen Idec) could be incorporated (375 mg/m^2 IV) beginning with the third cycle in nonresponders after 2 cycles of single-agent inotuzumab.

H&O What were the response rates, and how do those rates compare to current standard treatments?

DT Forty-nine patients were treated with inotuzumab. The median age was 36 years (range, 6–80 years); 3 patients (6%) were aged 16 years or younger and 12 patients (24%) were at least 60 years of age. Nearly three-quarters of the

Table 1. Monoclonal Antibody Therapy for Acute Lymphoblastic Leukemia

Agent	Surface Antigen	Description	Single-Agent Efficacy Data	Status
Rituximab[11,12,22]	CD20	IgG1 humanized	Case reports	Phase II clinical trials (ongoing) Phase III clinical trials (ongoing)
Alemtuzumab[13,14]	CD52	IgG1 humanized	0/6 (adult) 2/13 (pediatric)	Phase I-II clinical trials (ongoing)
Epratuzumab[16]	CD22	IgG1 humanized	ORR, 7%	Phase II clinical trial (pediatric)
Inotuzumab ozogamicin[5]	CD22	IgG1 humanized + calecheamicin	ORR, 57%	Phase II clinical trials (weekly dosing) Phase III clinical trial (planned)
Moxetumomab pasudotox[17]	CD22	Variable domain fused to 38 KDa truncated form of *Pseudomonas* exotoxin A	ORR, 29%	Phase I clinical trial (pediatric)
Blinatumomab[21]	CD19 CD3 (T cell)	Bispecific T-cell engaging	ORR, 67%	Phase II clinical trials (ongoing) Phase III clinical trial (planned)
SAR3419[19]	CD19	IgG1 humanized + mytansine	No data	Phase I clinical trial (ongoing)

ORR=overall response rate.

patients received inotuzumab ozogamicin as second or later salvage treatment. Poor-risk karyotypes of t(9;22) or t(4;11) were noted in 7 (14%) and 5 (10%) cases, respectively. Seven patients (14%) had previously undergone allogeneic SCT. All patients expressed CD22 on at least 50% of the lymphoblasts; CD22 expression exceeded 90% in 28 patients (57%).

The overall response rate (ORR) was 57% (95% confidence interval [CI], 42–71), including 9 (18%) complete remissions (CR), 14 (29%) CR with incomplete platelet recovery, and 5 (10%) incomplete recovery of peripheral blood counts (CRi). Most responses were observed within 1–2 cycles of therapy; only 1 patient responded (CR) after 3 courses of treatment. Response rates were lower in the poor-risk karyotype subsets [43% for t(9;22) and 20% for t(4;11)]. Absence of detectable minimal residual disease (MRD) by multiparameter flow cytometry did not correlate with improved outcomes. Of the 9 cases (18%) where rituximab was incorporated, only 1 response was noted after the fourth cycle. Inotuzumab ozogamicin levels were measured immediately after infusion, 3 hours after infusion, on days 7–9, and on days 14 or 15. Of the 9 patients whose concentrations were greater than 100 ng/mL 3 hours after infusion, 8 (89%) achieved CR; whereas only 5 of 15 patients (33%) with concentrations less than 100 ng/mL attained CR (P=.008). Early mortality occurred in 2 patients (4%). The median overall survival was 5.1 months (95% CI, 3.8–6.4). Median survival for the 28 responders was 7.9 months (95% CI, 5.3–10.5). Estimated survival at 12 months was 78% for the 9 patients who achieved CR. Twenty-two patients (45%) subsequently underwent allogeneic SCT, and had similar overall survival rates to those who did not.

The results are remarkable, considering that this population was heavily pretreated. Expectations regarding response after salvage therapy partially depend on salvage status (first, second, or later) and duration of first CR if the salvage attempt was the first one.[8,9] In patients with first CR duration greater than 1 year undergoing a first salvage attempt, the historical response expectations with various intensive multi-agent chemotherapy regimens include CR rates ranging from 35–40%. In those undergoing a second salvage attempt, the best expected CR rates range from 15–30% with multi-agent combination therapy regimens. The expected CR rates with miscellaneous single agents in these settings (first and second salvage) would be 8% and 4%, respectively. Bearing in mind that the dose-limiting toxicity of inotuzumab is thrombocytopenia, the respective response rates (CR+CRp+CRi) with this agent in the setting of first, second, and third or later salvage attempts were 69%, 47%, and 67%, respectively.[5]

H&O Are there any similar studies in support of inotuzumab?

DT There are 2 ongoing phase I/II clinical trials of inotuzumab in patients with relapsed/refractory CD22-positive B-lymphoblastic leukemia. An extension of our phase II trial

includes the exploration of weekly dosing of inotuzumab (0.8 mg/m^2 IV on day 1, then 0.5 mg/m^2 IV for 2 doses); accrual is ongoing, with results to be presented at the upcoming annual meeting of the American Society of Clinical Oncology (ASCO; ClinicalTrials.gov identifier NCT01134575). A separate multicenter phase I trial is exploring the use of weekly dosing of inotuzumab for a total dose of 0.8–2 mg/m^2 per course (ClinicalTrials.gov identifier NCT01363297). After the recommended phase II dose of inotuzumab is confirmed, a randomized study of inotuzumab versus investigator's choice (FLAG, fludarabine, cytarabine, filgrastim; HIDAC, high-dose cytarabine; or mitoxantrone/cytarabine) is planned (ClinicalTrials.gov identifier NCT01564784). A phase II trial of inotuzumab (single dose per cycle) in combination with "mini" hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cycles of methotrexate and cytarabine) with or without rituximab in older patients at least 60 years old with de novo CD22-positive B-lymphoblastic leukemia is currently accruing at our institution (ClinicalTrials.gov identifier NCT0137630).

H&O What other novel agents are showing promise in ALL treatment, and how do they compare to inotuzumab?

DT Several other monoclonal antibodies are being studied in ALL (summarized in Table 1). Rituximab, a nonconjugated monoclonal antibody directed against CD20, has predominantly been studied in combination with chemotherapy regimens.[10] Its efficacy as monotherapy has been limited to case reports in childhood Burkitt leukemia/lymphoma. When added to first-line chemotherapy with either the hyper-CVAD regimen or in the GMALL (German Multicenter Study Group for Adult ALL) study 07/2003 for de novo CD20 positive B-lymphoblastic leukemia, rituximab conferred a survival benefit.[11,12] Alemtuzumab, a monoclonal antibody directed against CD52, has limited single-agent activity.[13,14] It has been incorporated into first-line consolidation therapy as a single agent module in the Cancer and Leukemia Group B (CALGB) 10102 study, with encouraging disease-free and overall survival observations in the phase I portion of the regimen, resulting in continuation of the module for the phase II portion.[15]

Epratuzumab, a nonconjugated monoclonal antibody directed against CD22, has limited activity when given as monotherapy prior to combination chemotherapy in relapsed/refractory pediatric B-lymphoblastic leukemia.[16] When given in combination therapy, the CR rates were similar to historical expectations of the regimen without epratuzumab, albeit with higher rates of MRD negativity with epratuzumab. A Southwest Oncology Group study using epratuzumab in combination with clofarabine and cytarabine for adults with relapsed/refractory B-lymphoblastic leukemia is currently accruing (ClinicalTrials.gov identifier NCT00945815). Moxetumomab pasudotox (CAT-8015) is an anti-CD22 immunotoxin with encouraging activity in a phase I trial in relapsed/refractory pediatric (up to age 25 years) B-lymphoblastic leukemia, with reduction in frequency of capillary leak syndromes after implementing concurrent use of dexamethasone.[17]

Anti-CD19 monoclonal antibody conjugates (eg, ricin) previously exhibited minimal efficacy in B-lymphoblastic leukemia.[18] However, antibody conjugates directed against CD19 using novel agents, such as high-potency tubulin inhibitors (eg, SAR3419), may be more efficacious.[19] A promising dual epitope CD19-directed agent, blinatumomab, uses a bispecific T-cell engaging (BiTE) mechanism to recruit polytypic cytotoxic CD3-positive T-cells, which become activated on binding CD19-positive lymphoblasts. An initial study of blinatumomab in de novo B-lymphoblastic leukemia with persistent MRD by polymerase chain reaction at 16 weeks from the start of first-line therapy (expected relapse in 94% in this setting) showed efficacy as measured by eradication of the MRD in approximately 80% of the cases, resulting in durable remissions with or without allogeneic SCT.[20] In a subsequent phase II study of single-agent blinatumomab in adults with relatively minimally treated (predominantly first salvage attempts) relapsed B-lymphoblastic leukemia, an overall response rate of 67% was observed after 2 cycles of therapy.[21] Confirmatory phase II clinical trials are under way.

H&O What does the future hold for inotuzumab, and for ALL treatment approaches in general?

DT Owing to its promising single-agent activity in relapsed/refractory ALL, inotuzumab clearly offers a salvage strategy with the potential to induce significant cytoreduction of disease, which can facilitate allogeneic SCT. Confirmatory phase I/II single-agent trials are under way. In our phase I/II study, inotuzumab was well tolerated, with the predominant side effect of fever within the first 24–48 hours after administration, despite premedication. Protracted use may be limited by hepatotoxicity (elevations in bilirubin and/or hepatic transaminases) incurred by the calecheamicin component. Although in most cases, hepatic dysfunction was mild and reversible, there were a few cases of more severe hepatic injury pattern, including periportal fibrosis on liver biopsy. In the 22 patients (45% of the study population) who successfully underwent allogeneic SCT following therapy with inotuzumab, 5 cases (23%) of veno-occlusive disease (VOD) were observed, although these were confounded by the

preparative regimens (containing hepatotoxic agents thiotepa or clofarabine) and/or status as second allogeneic SCTs. A lower-dose weekly schedule of inotuzumab may potentially reduce the incidence of hepatotoxicity owing to lower peak levels of calecheamicin; the 2 ongoing phase I/II trials are exploring this approach. Preliminary findings of our trial suggest similar efficacy rates with a lower incidence of hepatotoxicity.

Unfortunately, despite the high response rates, the majority of responses were not durable (median duration, 6 months). A response to inotuzumab did not significantly impact survival rates compared with historical expectations, despite facilitation of allogeneic SCT in approximately half of the patients. However, those who achieved CR and/or underwent therapy as first salvage had 1-year survival rates of at least 70%. As single-agent therapy, inotuzumab could potentially be used to eradicate persistent MRD after frontline chemotherapy. Combination therapy strategies will likely be needed in order to improve the durability of responses in the salvage setting. The incorporation of inotuzumab into frontline therapy ("mini hyper-CVAD with or without rituximab") is currently being explored in the setting of elderly patients with de novo CD22-positive B-lymphoblastic leukemia in an effort to mimic the improvement in outcomes observed with the addition of rituximab to hyper-CVAD in older patients with de novo Burkitt leukemia/lymphoma and in younger patients with de novo CD20-positive B-lymphoblastic leukemia.[11,22] The use of inotuzumab in the treatment of CD22-positive B-lymphoblastic leukemia appears to be a very promising therapeutic strategy. Inotuzumab and other promising monoclonal antibodies have the potential to significantly improve outcomes for patients with ALL.

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