1. ABSTRACT

Antibody-drug conjugates (ADCs) represent a major advance in the treatment of acute lymphoblastic leukemia (ALL). CD22 expression, seen in majority of patients with B-lineage ALL, is an ideal target for ADCs. Inotuzumab ozogamicin is an ADC comprised of a humanized IgG4 monoclonal antibody covalently linked to calicheamicin. Inotuzumab ozogamicin has shown promising single-agent activity in patients with ALL and in CD22 positive non-Hodgkin lymphoma. Studies exploring the combination of inotuzumab ozogamicin with chemotherapy are ongoing.

2. INTRODUCTION

The outcomes of patients with acute lymphoblastic leukemia (ALL) have improved substantially with the introduction of multi-drug combination chemotherapy with cure rates of >80-90% in children (1-3). However, even with combination chemotherapy regimens, the clinical outcomes in adults are suboptimal with long-term survival achieved in only 30-40% of patients (1). Thus, there is a need to develop novel therapeutics for adults with ALL. In the era of personalized medicine and targeted therapies, new insights regarding the molecular pathogenesis of ALL has afforded opportunities to develop
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novel molecularly-targeted agents. A variety of targeted monoclonal antibodies are currently being explored as therapy for ALL. These include unconjugated monoclonal antibodies (such as rituximab and ofatumumab (both anti-CD20), alemtuzumab (anti-CD52), epratuzumab (anti-CD22)), conjugated antibodies to cytotoxic agents or toxins (such as inotuzumab ozogamicin, SAR3419, moxetumomab pasudotox), and novel class of bispecific T-cell engager (BITE®) antibodies (4).

3. ANTIBODY-DRUG CONJUGATES

Antibody-drug conjugates (ADCs) represent a major advance in the treatment of ALL (4). ADCs encompass two distinct but important treatment principles: 1) antibody specificity for the tumor antigen providing a ‘targeted’ approach, and 2) cellular toxicity from the attached cytotoxic drug (5). Major strides in development of ADCs for lymphoid malignancies were initially achieved in diseases such as Hodgkin’s lymphoma (HL), non-Hodgkin lymphoma (NHL). Brentuximab vedotin, an anti-CD30 chimeric antibody conjugated to monomethyl auristatin E (MMAE) has been shown to induce durable responses in patients with relapsed HL and anaplastic large cell lymphoma and has been approved by the United States Food and Drug Administration for the treatment of these entities (6).

Another ADC monoclonal antibody previously developed in acute myeloid leukemia (AML), gemtuzumab ozogamicin (a CD33-targeted immunoconjugate of calicheamicin), was initially approved for the treatment of relapsed/refractory disease but was subsequently withdrawn from the market owing to purported lack of benefit in the frontline setting. Recent emerging data, however, from several randomized clinical trials indicates that gemtuzumab ozogamicin does improve outcomes for patients with AML, particularly the core-binding factor subset, indicating that ADCs have the potential to offer survival benefit when added to chemotherapy (7-10).

B-cell lymphoblasts express a variety of surface antigens that have been explored as clinical targets. CD20 is expressed in up to 90% of patients with B-lymphoblastic leukemia and nearly all cases of Burkitt’s leukemia/lymphoma. The addition of the anti-CD20 monoclonal antibody rituximab (Rituxan®) to intensive chemotherapy has been shown to improve survival outcomes for these subgroups (11-13). Two other surface antigens highly expressed on B-lymphoblasts, CD19 and CD22, are targeted by monoclonal antibodies which are being actively pursued in clinical trials. SAR3419, a humanized IgG1 anti-CD19 monoclonal antibody conjugated to a maytansine derivative, has reported encouraging results in NHL and in early phase I-II clinical trials in ALL (14-16). Inotuzumab ozogamicin, an antibody-drug conjugate targeting CD22 is the focus of this review.

CD22 expression occurs in >90% of patients with B-lineage ALL (17-20). The CD22 antigen is a 135-kDa type I transmembrane sialoglycoprotein expressed on the surface of mature B-lymphocytes and in the majority of B-cell malignancies. CD22 is thought to play a role in regulation of B-cell function (21). It is not expressed on non-B lineage cells or hematopoietic stem cells (22-24). In addition, CD22 is rapidly internalized after binding of the anti-CD22 antibody and is not shed in the extracellular environment, features that make it an attractive target for ADCs (25,26). CD22 receptor has previously been targeted by a recombinant immunotoxin (BL22) and was shown to induce complete remission in majority of patients with relapsed/refractory hairy cell leukemia (27), supporting the choice of CD22 as a target antigen. In addition, CD22 has been targeted by the unconjugated humanized IgG1 antibody epratuzumab and by the radiolabeled conjugate 90Y-epratuzumab (28-30).

Inotuzumab ozogamicin is an ADC comprised of a humanized IgG4 monoclonal antibody (G5/44) that recognizes CD22 antigen and, similar to gemtuzumab ozogamicin, is covalently linked via an acetyl butyrate linker to N-acetyl-γ-clecheamicin dimethyl hydraza (CalichDMH), a semisynthetic derivative of calicheamicin (31). Clecheamicin is a toxic natural product of actinomycete Micromonospora echinospora calichensis (32,33). Inotuzumab ozogamicin binds to CD22 on the surface of B-cells and within 30 minutes, the CD22 receptor-inotuzumab complex is internalized into the endocytic compartment of the cell. This is followed by fusion of the inotuzumab ozogamicin containing endosome with a lysosome. Acidic pH of the lysosomes leads to hydrolysis of the acetyl butyrate linker chain and subsequent intracellular release clecheamicin. Clecheamicin binds to minor groove of the DNA, undergoes structural changes to generate a di-radical (1,4-dehydrobenzene) that extracts hydrogen from phosphodiester DNA backbone (32-34). This induces double-strand DNA breaks resulting in G2/M cell cycle arrest and finally apoptotic cell death.

4. PRECLINICAL STUDIES

Inotuzumab ozogamicin has been extensively studied in preclinical models of NHL and ALL. It has been shown that conjugation of anti-CD22 antibody to clecheamicin does not adversely affect the binding of the antibody to the CD22 antigen (31). Inotuzumab ozogamicin has been shown to have cytotoxicity at subnanomolar concentration against B-cell lymphoma cell lines. In addition, inotuzumab ozogamicin is reported to be 1.5 to 39 fold more potent than unconjugated native clecheamicin in CD22+ B-cell lymphoma cell lines, indicative of efficient CD22-mediated intracellular delivery of clecheamicin (31). Inotuzumab ozogamicin was also demonstrated to cause regression of tumors in BCL xenograft models at a dose less than one-tenth the maximal toxic dose. Notably, native unconjugated antibody (IgG4 G5/44 antibody) has no effect on the growth of B-cell lymphoma cell lines as well as in xenograft models. This is likely because human IgG4 antibodies fix complement poorly and thus, cannot mediate apoptosis via complement-dependent cytotoxicity (CDC), and do not mediate antibody-dependent cellular cytotoxicity (ADCC). Thus, inotuzumab ozogamicin induced cellular death is completely mediated by clecheamicin-induced apoptosis and not via CD22
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signaling (31,35). This is in contrast to gemtuzumab ozogamicin where CD33 signaling contributes to induction of cell death (36). The anti-tumor effect of inotuzumab ozogamicin requires both the targeting antibody and calecheamicin. Unconjugated antibody, unconjugated calecheamicin, or their admixture has no cytotoxicity both in vitro and in vivo (31). Inotuzumab ozogamicin is also active against systemically disseminated B-cell lymphoma in SCID mice (37). Interestingly, CD22+/ALL cell lines (REH, RS4;11), despite having lower CD22 surface expression than B-cell lymphoma Ramos cell lines, were reported to be 2-7 fold more sensitive to growth inhibition when treated with inotuzumab ozogamicin in vitro (38). The efficacy of inotuzumab ozogamicin is reported to be highly dependent on calecheamicin sensitivity and CD22/inotuzumab internalization capacity of ALL cell lines rather than surface CD22 saturation levels. This is in contrast to the data in gemtuzumab ozogamicin in AML cell lines where apoptosis induction is dependent on prolonged and maximal saturation of the CD33 receptor (35,39). Inotuzumab ozogamicin has shown superior cytotoxicity in vitro in B-cell lymphoma cell lines when compared with combination chemotherapy (CVP or CHOP). Enhanced cytotoxicity was observed when inotuzumab ozogamicin was combined with chemotherapy (40). These studies provide a strong preclinical rationale to evaluate inotuzumab ozogamicin in B-lineage ALL and NHL.

5. CLINICAL STUDIES

Initial phase I clinical trials using inotuzumab ozogamicin were conducted in previously treated CD22+/NHL (41). Five dose levels were evaluated during dose-escalation: 0.4, 0.8, 1.3, 1.8, and 2.4 mg/m² every 3 weeks. Seventy-nine patients were treated and most patients had follicular lymphoma (FL) or diffuse large B-cell lymphoma (DLBCL). At the 2.4 mg/m² dose level, 2 of 6 patients had dose-limiting toxicities (DLTs) as defined by the protocol (one patient had grade 4 thrombocytopenia, and one patient had grade 4 neutropenia). Therefore, the maximum-tolerated dose (MTD) was established as 1.8 mg/m². Since several subjects demonstrated persistent thrombocytopenia prohibiting continuation of therapy with 1.8 mg/m² administered every 3 weeks, an every 4 weeks schedule was tested in the MTD expansion cohort. For the 49 patients treated at the MTD, the overall response rate (ORR) was 41% (68% for FL, 15% for DLBCL). The median progression-free survival (PFS) was 317 days for the 28 responding patients was 7.9 months. For the 28 responding patients was 7.9 months. For patients who achieved a CR, the estimated survival at 1 year was 78%. Seven patients with Philadelphia chromosome positive ALL were treated; 3 (43%) achieved complete cytogenetic remission without the use of concomitant tyrosine kinase inhibitor therapy. Philadelphia chromosome or t(4;11) karyotypes were associated with lower overall response rates, though the differences were not statistically significant. CD22 expression level in ALL blasts did not correlate with clinical responses as would be expected from analyzing the preclinical data. Drug-related fever was commonly reported during the first 1-2 days of receiving the study drug. Grade I-II elevations in hepatic transaminases were seen in 55% of the patients with grade III elevations in only 2% of patients. Forty-five percent of patients were able to undergo allogeneic stem cell transplantation (SCT). VOD was subsequently observed in 5 of the 22 transplanted patients (23%); but attribution was confounded by use of conditioning transplantation with radiation as part of the preparative regimen experienced veno-occlusive disease (VOD). Infectious complications were not increased above historical rates. Goy and colleagues reported preliminary results of a phase II study of single-agent inotuzumab ozogamicin in patients with CD22-positive indolent B-cell NHL that had progressed after 2 or more systemic therapies (42). Inotuzumab ozogamicin was administered at 1.8 mg/m² every 28 days for 4 to 8 cycles. Forty-three patients (35 patients with FL, 3 with marginal zone lymphoma, 3 with small lymphocytic lymphoma) were enrolled. An ORR of 53% was observed, with an ORR of 66% in the FL cohort. The most common adverse events included thrombocytopenia (65%), neutropenia (53%), elevated AST (48%), leukopenia (40%), nausea (40%), fatigue (35%), lymphopenia (35%), decreased appetite (33%), and elevated alkaline phosphatase (25%). Kantarjian and colleagues conducted the first phase II trial of single-agent inotuzumab ozogamicin in patients with relapsed/refractory ALL (43). Inotuzumab ozogamicin was given at 1.8 mg/m² intravenously over 1 hour every 3-4 weeks. Forty-nine patients were treated. The median age was 36 years (range 6-80 years). The median number of courses administered were 2 (range 1-5) and the median time between courses was 3 weeks (range 3-6). For patients with stable disease after two cycles of treatment, and with CD20 expression of at least 20%, standard dose rituximab (375 mg/m²) was added (one dose per cycle). The overall response rate was 57% (28 of 49 patients) (CR 18%, marrow CR 39%). Responses were seen irrespective of number of prior salvage treatments (salvage 1: 69%; salvage 2: 46%; salvage ≥ 3: 67%). Responses were usually achieved within first 1-2 cycles of treatment. Eighteen of the 28 responding patients had cytogenetic abnormalities at baseline and 16 of these 18 patients (89%) achieved cytogenetic remission. Multi-parameter flow-cytometry for minimal residual disease (MRD) showed that MRD negativity was achieved in 63% (17 out of 27) of evaluable patients. The median overall survival was 5.1 months. The median survival for the 28 responding patients was 7.9 months. For patients who achieved a CR, the estimated survival at 1 year was 78%. Seven patients with Philadelphia chromosome positive ALL were treated; 3 (43%) achieved complete cytogenetic remission without the use of concomitant tyrosine kinase inhibitor therapy. Philadelphia chromosome or t(4;11) karyotypes were associated with lower overall response rates, though the differences were not statistically significant. CD22 expression level in ALL blasts did not correlate with clinical responses as would be expected from analyzing the preclinical data. Drug-related fever was commonly reported during the first 1-2 days of receiving the study drug. Grade I-II elevations in hepatic transaminases were seen in 55% of the patients with grade III elevations in only 2% of patients. Forty-five percent of patients were able to undergo allogeneic stem cell transplantation (SCT). VOD was subsequently observed in 5 of the 22 transplanted patients (23%); but attribution was confounded by use of conditioning transplantation with radiation as part of the preparative regimen experienced veno-occlusive disease (VOD). Infectious complications were not increased above historical rates.
regimens containing known hepatotoxic drugs such as thiopeta and clofarabine.

Based on higher in vitro efficacy with more frequent exposure, a weekly schedule (0.8 mg/m² day 1, 0.5 mg/m² days 8 and 15, every 3-4 weeks) was explored (44). With the weekly schedule (n=34), overall response rate of 53% was similar to the less frequent schedule; but the frequency of reversible grade 1-2 and 3-4 elevations in hepatic transaminases (21% and 6%, respectively) appeared lower with the weekly schedule indicating similar efficacy with improved toxicity profile.

6. COMBINATION THERAPIES

Based on the preclinical evidence that the combination of inotuzumab ozogamicin with rituximab was additive in in vitro and in vivo (45), a phase I/II study of the inotuzumab ozogamicin with rituximab was conducted in relapsed/refractory CD20+/CD22+ NHL (46). Patients with FL and DLBCL were treated with rituximab (375 mg/m² every 4 weeks) in combination with escalating doses of inotuzumab ozogamicin ranging from 0.8 to 1.8 mg/m² every 4 weeks. The MTD of inotuzumab ozogamicin (in combination with rituximab) was confirmed as 1.8 mg/m² every 4 weeks. The most common grade 3-4 adverse events were thrombocytopenia (31%) and neutropenia (22%). At the MTD, the objective response rates of 87% and 74% were noted in patients with relapsed FL and DLBCL, respectively. The 2-year PFS was 68% for patients with relapsed FL and 42% for patients with relapsed DLBCL. These promising early results indicated that combination therapy may further improve outcomes.

In vitro and in vivo data also suggests that inotuzumab ozogamicin can be combined with chemotherapeutics for improved clinical outcomes (40). The combination of inotuzumab ozogamicin with chemotherapy (‘mini’ hyper-CVD; dose-reduced cyclophosphamide, dexamethasone, methotrexate, cytarabine, no anthracycline) as frontline therapy for older patients with B-lymphoblastic leukemia is actively enrolling; preliminary results show significant efficacy and improved tolerance of the chemotherapy component. A randomized study of inotuzumab ozogamicin versus investigator choice chemotherapy for relapsed/refractory ALL in first or second salvage continues accrual.

7. CONCLUSIONS

The use of inotuzumab ozogamicin for the treatment of CD22+ ALL and NHL offers the potential to improve survival outcomes in the de novo setting. Response rates attained in phase I/II clinical trials of single agent inotuzumab ozogamicin are quite encouraging and appear superior to those observed with conventional chemotherapeutics. Not unexpectedly, responses are not durable with monotherapy with inotuzumab ozogamicin. Clinical trials are currently exploring incorporation of this agent with established chemotherapy regimens. The promising results observed with inotuzumab ozogamicin are changing the treatment paradigm for ALL and NHL.

8. REFERENCES


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