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MONOGRAPH

VELTUZUMAB

Rec INN; USAN

Humanized Anti-CD20 Monoclonal Antibody
Oncolytic

IMMU-106
hA20

Immunoglobulin G_1, anti-(human CD20 (antigen)) (human-mouse monoclonal hA20 heavy chain), disulfide with human-mouse monoclonal hA20 κ-chain, dimer

Immunoglobulin G_1, anti-(human B-lymphocyte antigen CD20 (membrane-spanning 4-domains subfamily A member 1, Leu-16, Bp35)); [218-arginine,360-glutamic acid,362-methionine]humanized mouse monoclonal hA20 γ1 heavy chain (224-213')-disulfide with humanized mouse monoclonal hA20 κ light chain (230-230'':233-233'')-bisdisulfide dimer

Immunoglobulin G_1, anti-[Homo sapiens CD20 (MS4A1, membrane-spanning 4-domains subfamily A member 1, B lymphocyte surface antigen B1, Leu-16, Bp35)] humanized monoclonal IMMU-106 (or hA20); γ1 heavy chain [humanized VH (Homo sapiens FR/Mus musculus CDR) [8.8.14]-Homo sapiens IGHG1*03] (224-213')-disulfide with κ light chain [humanized V-KAPPA (Homo sapiens FR/Mus musculus CDR) [5.3.9]-Homo sapiens IGKC*01]; (230-230'':233-233'')-bisdisulfide dimer

CAS: 728917-18-8

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SUMMARY

Veltuzumab is a humanized second-generation anti-CD20 monoclonal antibody (MAb). It was constructed recombinantly using the same human IgG framework as epratuzumab (anti-CD22 MAb) and provides the advantage of having less acute infusional toxicities compared with rituximab, without demonstrating any apparent impairment of biological activity. Veltuzumab displays similar mechanisms of action as rituximab, including apoptosis, antibody-dependent cellular cytotoxicity (ADCC) and cell-mediated cytotoxicity (CMC). In vivo studies in different lymphoma models have shown promising results and comparative studies have demonstrated improved survival with veltuzumab compared to rituximab. Clinically, veltuzumab is being evaluated in patients with B-cell neoplasms and autoimmune diseases. Four weekly doses of veltuzumab i.v. at up to 750 mg/m^2 were well tolerated and objective responses were achieved, including durable complete responses (even at low doses). The only significant toxicity was transient mild to moderate infusion reactions. Clinical results with subcutaneous injections in follicular non-Hodgkin's lymphoma (NHL) are also encouraging (e.g., convenient, well tolerated and clinically active). On the basis of these encouraging findings, veltuzumab is being pursued further. Prospective, randomized clinical trials are needed to delineate the role it will play in the future management of lymphoma.

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PREPARATION*

The $V_κ$ and V_H genes from A20, a murine monoclonal antibody against CD20, are isolated from hybridoma cells by reverse transcription-polymerase chain reaction (RT-PCR). In order to humanize the $V_κ$ and V_H sequences, the complementarity determining regions (CDRs) are grafted. For light-chain CDR grafting, the framework regions (FRs) from the human REI antibody are used, and for the heavy chain, human FR1, 2 and 3 from the human EU antibody and FR4 from the human NEWM antibody are used. Each variable chain is constructed in two parts (A and B) and each half is produced by PCR amplification of a single-strand synthetic oligonucleotide; they are then ligated and cloned into different vectors. In order to obtain a vector encoding for the whole antibody, the $V_κ$ sequence is cloned into a pdHL2 vector, which encodes for the human IgG_1 C1, C2, C3 and hinge regions and the human κ chain Cκ, obtaining hA20VκpdHL2. Then, the humanized V_H sequence is subcloned into the hA20VκpdHL2, obtaining the hA20-1pdHL2 vector encoding for veltuzumab. The heavy chain of veltuzumab contains nine changes from the human EU framework and the light chain contains seven changes from the REI framework. The plasmid hA20-1pdHL2 is digested with SalI and transfected into Sp2/0-Ag14 cells by electroporation to generate the hA20-producing clones. The selection of clones is then performed by adding methotrexate to the medium and assaying the human antibody secretion by ELISA. The positive clones are expanded and veltuzumab is finally purified from the supernatant by a combination of affinity chromatography (protein A columns) and size-exclusion chromatography (1).

BACKGROUND

CD20 (human B-lymphocyte-restricted differentiation antigen) is a 35-kDa transmembrane protein that regulates an early step in the activation process for cell cycle initiation and differentiation, and

possibly functions as a calcium ion channel (2). This antigen is an appealing molecular target for B-cell-based therapy because of its restricted expression on mature B cells. CD20 is not detected on embryonic stem or pro-B cells, but is expressed on the surface as B cells mature, and expression essentially ceases upon terminal differentiation of B cells into plasma cells, thus facilitating post-anti-CD20 MAb therapy B cell recovery and maintaining host immunoglobulin production. The structure of the CD20 molecule crosses the plasma membrane four times, leaving two extracellular loops, which serve as ideal binding targets for anti-CD20 MAb-based therapy for B-cell neoplasms and B-cell-related autoimmune diseases. This antigen has several properties which make it an ideal target for antibody therapy (3, 4): 1) CD20 does not circulate freely in the plasma, thereby limiting the possibility of competition of free antigen for anti-CD20 antibody (5); 2) it has no known naturally occurring ligand; and 3) it is not internalized, down-modulated or shed from the cell membrane (2, 5-9). These characteristics allow for sustained recruitment of natural effectors, and subsequently, persistent immunological attack for as long as effectors are available (6).

Rituximab, a human–mouse chimeric anti-CD20 MAb, has revolutionized the treatment of B-cell lymphomas. Rituximab was initially approved for the treatment of relapsed or refractory indolent $CD20^+$ B-cell non-Hodgkin's lymphoma (NHL) (10). The addition of rituximab to chemotherapy regimens has improved overall outcomes (i.e., both progression-free and overall survival) in B-cell NHL (11). However, even with its demonstrated efficacy in the treatment of B-cell malignancies, many patients have diseases that are primarily refractory to rituximab therapy; approximately 50% of patients with relapsed/refractory $CD20^+$ follicular lymphoma (FL) previously treated with chemotherapy fail to respond to initial treatment with rituximab (10). Also, about 60% of prior chemotherapy-treated patients with FL who were previously responsive to rituximab become resistant to this therapy upon repeat treatment with rituximab monotherapy (12). Furthermore, patients with increased circulating lymphoma cell counts (≥ 25,000 cells/mL) or high tumor burden (i.e., bulky disease with tumors ≥ 10 cm) are at an increased risk of severe acute infusion-related adverse events with rituximab (11).

Based on the success of rituximab and the advantages offered by targeting the CD20 antigen, efforts are under way to introduce improved anti-CD20 antibodies into the clinic (6, 13-17). Several of these novel anti-CD20 MAbs under development have been engineered to have characteristics that may limit the "resistance" seen with rituximab and/or offer greater efficacy than that observed with rituximab therapy. Most of these new MAbs are constructed to reduce the amount of murine components while enhancing Fcγ receptor- or complement-mediated functions (6, 18), in an attempt to reduce toxicity and immunogenicity and permit more rapid administration, while maintaining or increasing antitumor activity (17, 19-23).

One of the initial second-generation MAbs under development is veltuzumab, previously termed hA20 or IMMU-106 (Immunomedics). Early data suggest that veltuzumab provides the advantage of a shorter infusion time, while maintaining high complete response rates when compared historically with rituximab (24, 25). Veltuzumab is a humanized anti-CD20 MAb with both structural and functional differences from rituximab (13, 26). This humanized MAb was constructed recombinantly using the same human IgG framework as epratuzumab (hLL2; Immunomedics), a humanized MAb directed against the B-cell antigen CD22 (27). Key murine residues were retained in the framework regions (FRs) to maintain the binding specificity and affinity of veltuzumab for CD20, similar to those of the parental murine antibody (A20). Veltuzumab has identical variable κ light chain CDRs, identical CDR1-V_H and CDR2-V_H, but a different CDR3-V_H compared to rituximab: a single amino acid change in CDR3-V_H (Asp101 instead of Asn101), which is believed to be responsible for some of the differences seen between veltuzumab and rituximab (28).

PRECLINICAL PHARMACOLOGY

The antigen-binding specificity and affinity of veltuzumab, as evaluated by cell-surface competitive and direct saturation binding assays, were shown to be the same as rituximab in both human Raji NHL and Daudi cells (13, 26). In vitro studies (26) estimated the half-life of veltuzumab to be an average 2.7-fold longer than that of rituximab ($P < 0.001$), but indistinguishable from that of cA20, a chimeric form of veltuzumab ($P > 0.2$). In contrast, the D101N antibody, a mutant form of veltuzumab, dissociated from Raji cells with an off rate two- and sixfold more rapidly than rituximab and veltuzumab, respectively. These results suggest that the single amino acid change of asparagine to aspartic acid at position 101 is responsible for the slower dissociation rate of veltuzumab, and thus, the prolonged half-life. This possible extension of half-life may prove beneficial in clinical practice by extending dosing intervals.

In vitro growth inhibition of NHL cell lines showed specific inhibition of proliferation in Burkitt and non-Burkitt lymphoma cell lines (13). However, inhibition of proliferation was not directly related to antigen density. Whereas CD20 expression is on the order SU-DHL-6 > RL > Raji > Daudi, sensitivity of proliferation to both anti-CD20 MAbs rituximab and veltuzumab was on the order SU-DHL-6 > Daudi > Raji > RL SU-DHL-4, and SU-DHL-10 cells express low levels of CD20 and were found to be relatively insensitive to both anti-CD20 MAbs. No significant differences in potency were observed between veltuzumab and rituximab within an individual cell line (26).

Veltuzumab demonstrates similar mechanisms of cytotoxicity as rituximab, including apoptosis, antibody-dependent cellular cytotoxicity (ADCC) and cell-mediated cytotoxicity (CMC) (13). Comparison of the activity of rituximab and veltuzumab showed a consistently increased CMC-mediated killing of Daudi lymphoma cells with veltuzumab as compared to rituximab. However, no differences between veltuzumab and rituximab were observed with CMC results in the other two cell lines, Raji and Ramos. Also, no differences were observed between veltuzumab and rituximab with respect to ADCC results (26).

In vivo studies in different lymphoma models have also shown promising results. In severe combined immunodeficiency (SCID) mice bearing systemic Raji tumors, while control mice died of disseminated disease with a median survival time of 16.5 days after tumor inoculation, the median survival was extended to 98 days in the rituximab group and to 70 days for the veltuzumab group ($P < 0.0001$). No statistically significant difference was observed between veltuzumab and rituximab (13).

Disseminated Burkitt's lymphoma xenograft-bearing mice were treated with single i.p. or s.c. injections of veltuzumab at three different dose levels (26). At all dose levels, regardless of the route administered, significantly increased survival was seen in comparison to the control groups ($P < 0.001$). Comparisons between equivalent doses administered i.p. and s.c. did not yield significant differences. Even at the lowest dose of 5 µg or 0.25 mg/kg in each i.p./s.c. group, these mice had a > 3.2-fold increase in mean survival time (MST) compared with controls. In these murine studies, a dose–response was observed, but no significant difference between the i.p. or s.c. routes was noted. Since a single 5-µg dose of veltuzumab proved to be effective in this Daudi disseminated NHL model, lower doses (0.5, 0.25, 0.1 and 0.05 µg) were examined. Remarkably, all four dose levels significantly improved survival ($P < 0.001$) when compared with saline control mice. Even the lowest tested dose of 0.05 µg (50 ng, or 0.0025 mg/kg) increased the MST by more than twofold over the saline-treated controls.

In disseminated follicular cell lymphoma (WSU-FSCCL) xenografts (26), 5 days after tumor inoculation, mice were administered a single dose of veltuzumab at four different dose strengths (35, 3.5, 0.35 and 0.035 µg i.p.). All four doses significantly improved survival of the mice ($P < 0.001$) compared to the saline controls. The MST of mice administered the higher 35-µg dose (44.3 ± 4.9 days) was not significantly different from that of the 3.5-µg group (39.5 ± 4.6 days), but was significantly ($P < 0.021$) longer than that of the groups treated with the lower doses of 0.35 and 0.035 µg (35 ng or 0.002 mg/kg) (40.5 ± 1.6 days and 33.3 ± 2.1 days, respectively). These effective doses are lower than those reported for rituximab in animal models.

Comparative therapeutic effects of veltuzumab and rituximab studied in three different SCID mouse models, Daudi, WSU-FSCCL and Raji tumor models, showed statistically significantly improved survival with veltuzumab compared to rituximab (26). The MST of single doses of 0.05 and 0.1 µg (0.0025 and 0.005 mg/kg, respectively) given 1 day after grafting was 28 and 35 days, respectively, versus 24 and 28 days for veltuzumab and rituximab ($P = 0.001$) in the Daudi Burkitt's lymphoma, and also at the single low dose of 0.035 µg (0.0021 mg/kg) given 5 days after transplant of the WSU-FSCCL model ($P = 0.005$). In these studies involving three lymphoma models in SCID mice, comparisons of low and high, single or multiple doses showed a significantly increased survival time after veltuzumab compared with rituximab treatment.

Combining MAbs recognizing different tumor-associated antigens can potentially augment antitumor activity. To investigate this prospect, the potential of combining veltuzumab with epratuzumab was studied in vitro by evaluating effects on cell proliferation in culture and in vivo in SCID mice bearing disseminated Raji tumors (13). The combination of the two naked MAbs appeared to be more effective than either MAb alone. Veltuzumab alone caused a 53% inhibition of the proliferation of SU-DHL-6 cells, and epratuzumab alone had no effect. However, the combination of the two MAbs increased the inhibition of proliferation to 83% ($P < 0.001$). In SCID mice bearing disseminated Raji cells, veltuzumab administered alone improved median survival to 25 days and the combination of veltuzumab and epratuzumab yielded a slight increase in median survival; however, prolonged survival was observed in 30% of the mice given the combination of veltuzumab plus epratuzumab compared with veltuzumab monotherapy alone. Mechanisms of enhancement of efficacy may include upregulation of antigen levels, as well as synergy between two different signaling pathways. The effect on receptor expression was examined by studying the CD22 and CD20 antigen density on cultured B-cell lines after incubation of the cells with epratuzumab or veltuzumab. Overnight incubation with veltuzumab increased CD22 expression by 33%, whereas incubation of cells with epratuzumab did not have a similar effect.

The benefit of combining unconjugated MAbs with radiolabeled MAbs against different antigens has been described previously (29, 30), and there are many reports of drug combinations with radiolabeled or unlabeled MAbs (31). Based on this, the combination of veltuzumab with a radiolabeled anti-CD22 MAb (epratuzumab) was evaluated (32). In nude mice grafted with the Ramos human B-cell lymphoma, the combination of ^{90}Y-anti-CD22 MAb and the unconjugated anti-CD20 MAb veltuzumab provided a markedly enhanced therapeutic effect compared with either of the MAbs administered alone. ^{90}Y-Epratuzumab alone had a substantial effect, with essentially all of the tumors regressing to a small size, but all of these tumors regrew in a few weeks. In comparison, veltuzumab alone did not have a substantial effect, as most of the tumors continued to grow despite veltuzumab administration. When unconjugated veltuzumab was given with ^{90}Y-epratuzumab, 80% (12 of 15) of animals were cured, with no visible tumors over the observation period. One possible explanation for these findings is the induction of a higher level of expression of the CD22 antigen by veltuzumab (13), which leads to an increase in the uptake of ^{90}Y-epratuzumab in the tumor.

Based on the superior activity demonstrated by the combination of veltuzumab and epratuzumab in a lymphoma xenograft model (13) and the enhanced anti-lymphoma efficacy seen in patients with the combination of rituximab and epratuzumab, without increased toxicity (33-35), bispecific MAbs against CD20 and CD22 were constructed and evaluated. Using a new platform technology, the dock-and-lock (DNL) method (36), two separate hexavalent anti-CD20/22 bispecific MAbs were constructed, one binding CD22 bivalently and CD20 tetravalently, and vice versa (37). These anti-CD20/22 bispecific MAbs were shown to have distinct properties compared to their parent MAbs, including improved anti-lymphoma activity in vitro and comparable efficacy in vivo, despite demonstrating shorter half-lives. Both bispecific MAbs also showed potent antiproliferative and apoptotic effects against B-lymphoma cells in vitro. Hence, these hexavalent, bispecific anti-CD20/CD22 antibodies have the potential of someday comprising a new, more potent class of anti-lymphoma antibodies.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetic analyses in cynomolgus monkeys (26) estimated the half-life of veltuzumab to be 5-8 days after i.v. administration and 6-13 days following s.c. injection. The t_{max} (time to maximum concentration, C_{max}) for the s.c. route was 2-5 days and 1-1.5 hours with i.v. administration. The AUC was greater for i.v. administration than for the s.c. route. This was thought to be likely related to the longer period required for the MAb to enter the blood via the s.c. route, with a similar rate of clearance. At all dose levels, the mean volume of distribution was greater after s.c. administration than after i.v. infusion. The dose-normalized AUC values showed accumulation of veltuzumab after i.v. infusion or s.c. injection at all

dose levels. Notably, rapid depletion of peripheral and splenic B cells occurred for veltuzumab at the lowest single dose of 6.7 mg/kg (equivalent to 80 mg/m^2 in humans), whether given by the i.v. or s.c. route.

In a multicenter phase I/II study, pharmacokinetics were analyzed for 72 of 82 patients with B-cell NHL (38). At all dose levels, mean serum levels of veltuzumab after the first infusion exceeded the 25 mg/mL value associated with maintained efficacy of rituximab (39-41). At 375 µg/m^2 (the most commonly used dose of rituximab), the mean peak and trough serum antibody levels achieved with veltuzumab were comparable to values reported at the same dose for rituximab (39). In addition, veltuzumab remained in the circulation after the last infusion, with half-lives that were similar at all dose levels and for at least as long as reported for rituximab in different studies (39, 40). Among follicular lymphoma patients, higher serum levels occurred in responders compared to nonresponders and in patients with less disease, as has been previously reported with rituximab.

An s.c. formulation of veltuzumab was evaluated in an open-label, multicenter phase I study (42). As compared to the peak concentrations seen immediately after i.v. infusion, following s.c. administration, veltuzumab was released slowly into the blood, achieving peak levels only after several days. Despite the low doses administered in this study on an every other week dosing schedule, sustained serum levels of veltuzumab were achieved across the treatment period and were still measurable for 4-8 weeks after the last dose. Although, as expected with the slower release of veltuzumab into the blood, the mean maximum antibody serum levels of 19, 25 and 63 µg/mL obtained at the doses of 80, 160 and 320 mg given weekly for a total of four doses, respectively, were generally lower than the maximum values that occurred with bolus delivery of higher i.v. veltuzumab doses (38). Nonetheless, s.c. dosing achieved serum levels that are close to or exceed the 25 µg/mL value associated with maintained efficacy of rituximab (41). Veltuzumab was pharmacologically active when given by s.c. injection. Similar to what was observed with higher i.v. doses of veltuzumab (38), B-cell depletion occurred after the first administration, even at a low dose of only 80 mg. The mean terminal half-lives for the three s.c. dose levels studied were similar following the last administration (12.4-13.9 days) and were comparable to half-lives previously reported with higher doses of i.v. veltuzumab (13.3-19.7 days). While these pharmacokinetic and pharmacodynamic findings generally support the use of s.c. dosing, serum levels of veltuzumab were very low or unmeasurable for two patients with leukemic involvement (one splenic marginal zone lymphoma [MZL] and one small lymphocytic lymphoma [SLL]). These two patients had elevated peripheral B-cell levels which decreased, but were not depleted, with low doses of 160 mg. Also, in an additional patient with FL treated at 80 mg who had extensive adenopathy (including masses > 10 cm), serum veltuzumab levels were very low. These results suggest that while s.c. treatment may be sufficient to cover the antigen sink in most indolent NHL patients, those with greater tumor burden (e.g., leukemic involvement with increased circulating lymphoma cells or bulky adenopathy) will likely require higher or more frequent dosing of the MAb.

SAFETY

The safety of veltuzumab was evaluated in 16 male and 16 female cynomolgus monkeys. Veltuzumab administered i.v. or s.c. as single or multiple doses was well tolerated overall, with no clinical or persistent laboratory test abnormalities noted other than B-cell depletion in the circulation and lymphatic organs (26). Postmortem changes in the lymphoid organs of animals receiving all doses were comprised of follicular lymphoid depletion of the spleen, as well as mandibular and mesenteric lymph nodes, at all doses. Temporary decreases in white blood cells, neutrophils, basophils and lymphocytes were noted, but only a rapid reduction in the number of peripheral blood B cells was observed. These effects occurred within 2 days of dosing irrespective of the route of administration, and were present at doses of 6.7 mg/kg or higher. The animals recovered at either 28 days when treated with a single dose or at 56 days when administered three doses.

In a multicenter phase I/II study in 82 patients with NHL (38), veltuzumab was well tolerated, with no serious infusion reactions or increases in the more common milder infusion reactions, although infusion times were shorter than for rituximab. The most common treatment-related events were transient grade 1-2 infusion-related symptoms, predominantly with the first infusion; these included, in decreasing order of frequency: fatigue (23%), pruritus (13%), fever (13%), headache (11%), asthenia (11%), dyspnea (10%) and cough (10%). Ten patients had serious adverse events (SAEs); however, all were attributed to complications of underlying disease or other medical conditions. Sixteen patients had infections; 4 of them had grade ≥ 3 infections requiring treatment with i.v. antibiotics or multiple oral antibiotics, but none of these events was attributed to veltuzumab. All other infections were grade 1-2 events and treated with oral medications. At all dose levels, B-cell depletion was almost complete after the first infusion and returned toward baseline after 9-12 months. No other major changes in laboratory parameters after treatment were noted, and no cases of delayed neutropenia occurred in the 41 patients who were monitored for at least 6 months. Quantitative serum immunoglobulin (Ig) and T-cell levels measured up to 12 weeks after treatment showed no significant decreases, with median changes from baseline being small at most time points (typically < 20% for IgM, < 5% for IgA and IgG, and < 15% for T cells). Post-treatment serum samples analyzed for immunogenicity (human anti-human antibodies, HAHA) were all negative (< 50 ng/mL).

In another open-label, multicenter phase I study, veltuzumab was administered s.c. (42) and 13 patients had 1 or more adverse events during the study. The most common events were injection-site reaction (35%), pain in extremities (24%), upper respiratory tract infection (18%), nausea (18%), chills (12%) and dyspnea (12%). One patient had a solitary pulmonary nodule on CT scan noted prior to study entry, which was confirmed as a squamous cell carcinoma while on study and not related to veltuzumab. There were no SAEs and all other adverse events were either grade 1 or 2. Most common events were injection-site reactions, which occurred in nine patients and included localized reactions such as pain (n = 8), tenderness (n = 7) and redness (n = 6) at the injection site; systemic injection reactions included chills, nausea, thoracic pain, general aches, headache, swollen tongue or rash. These injection-site reactions were predominantly only mild (grade 1) transient events that resolved either spontaneously or with the use of topical or oral medications (no steroid treatments were required). Interestingly,

no premedications were used in this study. Four patients had infections, all of which were mild grade 1 events that involved the upper respiratory tract or sinuses, and resolved with oral medication. No abnormal pattern of changes in hematology and serum chemistries was observed, and no cases of delayed neutropenia were noted (including 10 patients monitored for 6 months, and 6 patients for up to 1 year). Notably, two patients in this study had leukemic involvement with high circulating B-cell levels. The patient with splenic MZL had 36,820 cells/μL, which decreased by 42% to 21,387 cells/μL over the course of treatment, while one of the two patients with SLL had 2,845 cells/μL, which decreased by 98% to 43 cells/μL. For the other 15 patients with peripheral B-cell counts that were not elevated at baseline, B-cell depletion occurred after the first injection for all three dose groups, with recovery toward baseline observed by 9-12 months. Quantitative serum Ig and T-cell levels were measured at different time points. Overall, for IgG, IgA and T cells, median changes from baseline were variable but small at most time points (typically < 10%). Median IgM levels were consistently reduced, but not clinically significantly, with median decreases at each time point ranging between 16% and 37%. All samples analyzed for human anti-veltuzumab antibodies were negative (< 50 ng/mL).

CLINICAL STUDIES

Veltuzumab has been studied in over 150 patients with B-cell malignancies and autoimmune diseases. The initial administration of this antibody was as compassionate use in a patient with severe refractory systemic lupus erythematosus (SLE) with life-threatening cytopenia no longer responsive to standard medications or rituximab. Although the patient had extremely high serum levels (43,000 ng/mL) of anti-rituximab antibodies (human anti-chimeric antibodies [HACA]), veltuzumab given as four weekly doses of 375 mg/m^2 effectively depleted B cells and the patient responded rapidly, with improvement in counts and clinical status (43). This clearly indicated that patients with autoimmune diseases who may have high titers of neutralizing anti-rituximab antibodies may still respond to veltuzumab.

A multicenter phase I/II study in 82 patients with NHL (55 patients with FL, 27 patients with other B-cell lymphomas), which was briefly described in the previous "Safety" section, demonstrated that 4 weekly doses of veltuzumab given i.v. at doses of up to 750 mg/m^2 were well tolerated and clinically active. Responses occurred at all dose levels, even at 80 mg/m^2, which was the lowest dose level tested (38). Most patients (n = 65) had advanced disease (stage III-IV) at study entry. To prevent infusion-related reactions, patients were premedicated with antihistamines and antipyretics, but steroids were not given. The median first infusion times were 4.7 hours at the highest dose of 750 mg/m^2, 3.1 hours at 375 mg/m^2 and 1.8-2.4 hours at lower doses, with median times for subsequent infusions decreasing to 2.1-2.6 hours at 375 or 750 mg/m^2 and 1.2-1.5 hours at lower doses. Because the protocol limited further reduction of the rate of infusion in this study, it may be possible to achieve more rapid administration. Treatment responses, as classified by International Working Group response criteria (44), for the largest group of patients with FL, most of whom had prior exposure to one or more rituximab-containing regimens, were favorable, with a 44% overall response rate (ORR) and a 27% complete response/complete response unconfirmed (CR/CRu) rate, which is similar to the 40% ORR and the 11% complete response rate (CRR) reported for patients who had previously responded to rituximab monotherapy (12). The highest response rates with veltuzumab were observed in a very small subgroup of rituximab-naive FL patients (57% [4 of 7] ORR, 43% [3 of 7] CR/CRu). Encouragingly, responses, including CRs, even occurred in FL patients who were at increased risk of a less favorable outcome (e.g., higher Follicular Lymphoma International Prognostic Index [FLIPI] scores, elevated lactate dehydrogenase, tumor masses > 5 cm, etc.). For the overall group, the Kaplan–Meier median progression-free survival (PFS) was 6 months, but for the 24 patients with FL who achieved objective responses, the median duration of response was 10 months, which compares favorably to the 11 months reported in patients with relapsed/refractory, indolent NHL after their first exposure to rituximab (10, 45). Although a longer 15-month median duration of response has been reported in patients retreated with rituximab (12, 45), most had received one prior course of rituximab as a single agent, while patients in the veltuzumab study had often received multiple prior courses of rituximab given in combination with chemotherapy. Also, in this study, the patients with FL who achieved CR/CRu generally had durable responses (median duration of response and PFS of 20 and 24 months, respectively), and the response durability appears to be similar at variable veltuzumab dose levels. Among the patients with non-follicular histologies, ORRs varied by histology. In MZL, 5 of 6 patients had ORR, with 2 CR/CRu and 3 long-term responses (15-24 months). In diffuse large B-cell lymphoma (DLBCL), although all patients had previously received rituximab and CHOP, three of seven patients (one at 80 mg/m^2) had partial responses, yielding a 43% ORR, which is not inferior historically to the 37% ORR reported in rituximab-naive DLBCL patients treated with eight weekly doses of rituximab (46).

Veltuzumab showed less activity in patients with mantle cell lymphoma, SLL or lymphoplasmacytic lymphoma, as has been the experience with rituximab as a single agent in these histologies (10, 49-51).

As a result of the observation that i.v. veltuzumab is active in patients with NHL even at doses as low as 80-120 mg/m^2 weekly for a total of four administrations, with complete responses demonstrated (38), a higher-concentration formulation was developed for delivery of low doses by s.c. injection. Safety data for s.c. veltuzumab were previously described in the "Safety" section from an open-label, multicenter phase I study (42). Seventeen patients with indolent NHL (14 patients with FL, 3 patients with other indolent histologies) were enrolled in this trial and received 80, 160 or 320 mg s.c. veltuzumab administered every other week for a total of 4 doses. Across all histologies, s.c. administration of veltuzumab was clinically active, with an ORR of 47% (24% CRR) in this small group of patients. The median time to onset of an objective response was 11 weeks from the start of treatment, which is close to the 3.3-month interval reported with i.v. veltuzumab, indicating that there is no apparent delay of treatment response associated with the s.c. route of administration. Objective responses occurred at all dose levels. Responses, including CR/CRu, occurred in treatment-naive, as well as previously treated, patients (including those exposed to rituximab-containing regimens). Four of the 8 responders had durable

responses of over 60 weeks, and 3 other patients who did not achieve an overall response by International Working Group criteria remained progression-free for at least 1 year; almost half of the patients are still in ongoing follow-up. These results with s.c. dosing in follicular NHL are particularly encouraging. While direct comparisons are limited by the small number of patients in this study, the results for the 11 previously treated FL patients (55% ORR, 27% CR/CRu) are similar to those obtained in previously treated FL patients who received 4 weekly i.v. doses of veltuzumab of 80-750 mg/m^2 (44% ORR, 27% CR/CRu) (38), as well as similar to historical data for rituximab therapy in FL (10, 12). Two FL patients with bulky disease (one received doses of 160 mg and the other 80 mg) did not achieve an objective response, again suggesting that higher doses or more frequent dosing may be required for patients with higher tumor burdens. Of the 3 patients with non-follicular lymphoma histologies, 1 with SLL achieved a complete response, which was ongoing at 72 weeks after treatment when last reported. The other two patients (one SLL, one MZL) had high levels of circulating B cells and failed to achieve an objective response. This has also been reported in patients with chronic lymphocytic leukemia (CLL) treated with i.v. veltuzumab. Although the same s.c. doses and dosing schedule of veltuzumab used failed to achieve meaningful clinical benefit, it did show evidence of pharmacological activity, with transient decreases in the high levels of circulating leukemic B cells (52). This again emphasizes that more frequent or extended dosing or combination therapy with other agents will likely be required to overcome the higher antigen burden in these settings.

Since veltuzumab, like rituximab, depletes normal B cells (38), its role in the treatment of immune thrombocytopenia (ITP) is being evaluated, especially by the s.c. route of administration (53).

CONCLUSION

Veltuzumab, a second-generation humanized anti-CD20 MAb, differs structurally from rituximab in one CDR by a single amino acid change, but also a different framework region. These structural differences appear to be functionally important, since veltuzumab showed antiproliferative, apoptotic and ADCC effects in vitro similar to rituximab (12), but with other qualitative differences, including slower off rates and increased CMC in several human lymphoma cell lines (26). Veltuzumab is being developed for the treatment of both oncological and autoimmune conditions. It is well tolerated despite rapid infusion and is effective even at low doses. In NHL and ITP, low s.c. doses were safe and demonstrated levels of activity comparable to those reported historically with higher doses of rituximab (41, 52). By avoiding prolonged i.v. administration and the need for dedicated infusion sites, convenient outpatient s.c. dosing of veltuzumab may offer benefits for both patients and the healthcare system.

On the basis of these encouraging preclinical and early clinical results, further studies of veltuzumab, given i.v. or, more conveniently, by s.c. injection, appear reasonable and are ongoing. Veltuzumab is being studied as a single agent, in combination regimens or as part of a recently described novel construct. Veltuzumab is being evaluated in phase II trials in combination with standard chemotherapy, as well as in combination with epratuzumab. In addition, a phase III study directly comparing veltuzumab plus chemotherapy to rituximab plus chemotherapy in first-line indolent NHL is planned.

SOURCES

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DISCLOSURES

The authors state no conflicts of interest.

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