Anti-CD22 ^{90}Y-epratuzumab tetraxetan combined with anti-CD20 veltuzumab: a phase I study in patients with relapsed/refractory, aggressive non-Hodgkin lymphoma

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ABSTRACT

A lingering criticism of radioimmunotherapy in non-Hodgkin lymphoma is the use of cold anti-CD20 antibody along with the radiolabeled anti-CD20 antibody. We instead combined radioimmunotherapy with immunotherapy targeting different B-cell antigens. We evaluated the anti-CD22 ^{90}Y-epratuzumab tetraxetan with the anti-CD20 veltuzumab in patients with aggressive lymphoma in whom at least one prior standard treatment had failed, but who had not undergone stem cell transplantation. Eighteen patients (median age 73 years, median of 3 prior treatments) received 200 mg/m^2 veltuzumab once-weekly for 4 weeks, with ^{90}Y-epratuzumab tetraxetan at planned doses in weeks 3 and 4, and ^{111}In-epratuzumab tetraxetan in week 2 for imaging and dosimetry. Veltuzumab effectively lowered levels of B cells in the blood prior to the radioimmunotherapy doses. No significant immunogenicity or change in pharmacokinetics of either agent occurred in combination. ^{111}In imaging showed tumor targeting with acceptable radiation dosimetry to normal organs. For ^{90}Y-epratuzumab tetraxetan, transient myelosuppression was dose-limiting with 6 mCi/m^2 (222 MBq/m^2) x 2 being the maximal tolerated dose. Of 17 assessable patients, nine (53%) had objective responses according to the 2007 revised treatment response criteria, including three (18%) complete responses (2 relapsing after 11 and 13 months, 1 continuing to be clinically disease-free at 19 months), and six (35%) partial responses (1 relapsing after 14 months, 5 at 3 – 7 months). Responses occurred in patients with different lymphoma histologies, treated at different ^{90}Y dose levels, and with a predicted risk of poor outcome, most importantly including five of the six patients treated with the maximal tolerated dose (2 of whom achieved durable complete responses). In conclusion, the combination of ^{90}Y-epratuzumab tetraxetan and veltuzumab was well-tolerated with encouraging therapeutic activity in this difficult-to-treat population.

Introduction

Immunotherapy targeting B-cell antigens continues to play a central role in the treatment of non-Hodgkin lymphoma (NHL). Since the rates of complete responses to anti-CD20 antibodies alone are low, these antibodies are often combined with chemotherapy or given afterwards as maintenance therapy.[1-5] However, radiolabeled anti-CD20 antibodies are more potent, with a single course of radioimmunotherapy being capable of generating substantially higher response rates, including complete responses.[6-10] CD22 is another B-cell antigen expressed by most histological types of NHL. CD22 is internalized rapidly into cells after binding with epratuzumab, a humanized anti-CD22 antibody which also has therapeutic activity.[11-17] For radioimmunotherapy, enhanced delivery and retention of the radioisotope at tumor sites should further improve outcome, so epratuzumab was conjugated with 1,4,7,10-tetraazacyclododecane-N,N′,N′′,N′′′-tetraacetic acid (DOTA), an improved chelator for 90-yttrium (^{90}Y) binding.[18] The resulting radiolabeled antibody, ^{90}Y-epratuzumab tetraxetan, was well tolerated and active in initial studies in relapsed/refractory NHL.[19,20] Following theoretical considerations,[21] ^{90}Y-epratuzumab tetraxetan was administered in fractionated doses once-weekly for 2-3 consecutive weeks, allowing delivery of higher cumulative doses with favorable response rates compared to anti-CD20 radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan or ^{131}I-tositumomab.[22]

Combining immunotherapy with the more potent radioimmunotherapy is also an attractive prospect for NHL. ^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumomab are both administered with additional anti-CD20 antibody (~900 mg). This cold anti-CD20 antibody prevents sequestration of the radiolabeled anti-CD20 antibody by the normal B-cell antigen sink thus improving biodistribution,[23-25] but could also act competitively to reduce uptake of the more potent radiolabeled antibody at tumor sites of malignant B lymphocytes.[26,27] Since CD20 and CD22 are distinct antigens on B cells, the possibility of blocking tumor uptake would be eliminated if ^{90}Y-epratuzumab tetraxetan were to be used instead. In this case, cold anti-CD20 antibody could then potentially be given at single-agent dose levels for full therapeutic effects on its own.[28]

Based on the above considerations, we combined ^{90}Y-epratuzumab tetraxetan with veltuzumab, a humanized anti-

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CD20 antibody with structural and functional differences from rituximab.[29,30] In NHL clinical trials, veltuzumab demonstrated single-agent activity comparable to rituximab even at low doses.[31,32] Preclinical studies showed that ^{90}Y-epratuzumab tetraxetan with veltuzumab substantially improved therapeutic responses compared to either agent alone.[33] As such, we hypothesized that both agents could be combined clinically for maximum therapeutic benefit without interfering with tumor targeting or increasing toxicity over that reported previously when the agents were given separately. Patients with aggressive NHL in whom frontline therapy has failed and who are not eligible for or refuse stem cell transplantation remain those most in need of effective therapies. This multicenter, open-label, phase I study was undertaken to determine an acceptable dose of ^{90}Y-epratuzumab tetraxetan in this population for use in combination with 200 mg/m^2 doses of veltuzumab, which in our prior studies had good therapeutic activity and achieved B-cell depletion.[31]

Methods

Adults with aggressive B-cell NHL in whom one or more standard chemotherapy regimen had failed were eligible if they had at least one lesion ≥1.5 cm (but none >10 cm) detectable by computed tomography (CT), Eastern Cooperative Oncology Group score 0-1, hemoglobin ≥10 g/dL, neutrophil count ≥1.5×10^9/L, platelet count ≥100×10^9/L, serum creatinine and bilirubin levels ≤1.5 x institutional upper limits of normal (IULN), and aspartate and alanine transaminases ≤2.5 x IULN. Patients who had undergone a prior stem cell transplant, received NHL therapy within the preceding 4 weeks, taken corticosteroids within the preceding 2 weeks, had an infection requiring antibiotics within the preceding 5 days, had central nervous system or ≥25% lymphomatous bone marrow involvement or pleural effusion, had ≥25% irradiation of red marrow, prior radioimmunotherapy, or prior radiation >3000 cGy to the liver or >2000 cGy to lungs or kidneys were excluded.

Patients received four doses of 200 mg/m^2 veltuzumab (days 1, 8, 15, and 22) and two doses of ^{90}Y-epratuzumab tetraxetan (days 15 and 22). For diagnostic studies, epratuzumab tetraxetan was radiolabeled with 111-indium (^{111}In) and administered on day 8. Veltuzumab was infused over approximately 1 hour. Radiolabeled epratuzumab tetraxetan was then infused over several minutes. Premedication included antihistamines and antipyretics; corticosteroids were not administered routinely. Adverse events and laboratory abnormalities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 3.1, with dose-limiting toxicity (DLT) defined as grade 2 immediate-type allergic/hypersensitivity reaction; non-hematologic grade 3 or 4 toxicity of any duration; anemia, neutropenia, or thrombocytopenia either grade 4 for more than 7 days or not recovered to grade 1 by 12 weeks after treatment. ^{90}Y dose levels started at 15 mCi/m^2/dose, a safe dose for 2 weekly infusions given as a single agent,[22] with dose escalation to continue if none of three or one of six patients developed DLT, but de-escalated if two patients had DLT. The maximum tolerated dose (MTD) was the highest ^{90}Y dose level with six patients treated and one or no DLT.

Baseline assessments included lymphoma classification[34] and International Prognostic Index (IPI) scores.[35] CT images, with FDG-positron emission tomography (PET) or PET/CT optional, at baseline, 4 and 12 weeks after treatment, then every 3 months until progression, were evaluated by local radiologists and classified according to 2007 revised treatment response criteria[36] as showing a complete response, partial response, stable disease, or progressive disease. Other study data collected included vital signs, information from physical examination, serum chemistry, complete blood count (once weekly, increased in the event of cytopenias), urinalysis, serum immunoglobulins, and B- and T-cell blood levels (based on CD19 and CD3, respectively). Serum veltuzumab levels and any human anti-veltuzumab or anti-epratuzumab antibodies were measured by enzyme-linked immunosorbent assay. Veltuzumab pharmacokinetics was analyzed by Phoenix WinNonlin v6.3 software (Cetera, L.P., St. Louis, MO, USA). Following ^{111}In-epratuzumab tetraxetan, ^{111}In scintigraphic images and ^{111}In blood levels were obtained over 7 days. Antibody tumor targeting and biodistribution were evaluated qualitatively by investigators from the images. Pharmacokinetics and normal organ dosimetry for ^{90}Y-epratuzumab tetraxetan were determined quantitatively by the MIRD methodology,[37] with red marrow dosimetry according to Sgouros,[38] and using OLINDA v1.1 software (Vanderbilt University, Nashville, TN, USA).

In the absence of DLT or human anti-veltuzumab or anti-epratuzumab antibodies, retreatment was allowed at the discretion of the investigator any time after hematologic toxicity had recovered to grade 1 levels. The institutional review board at each site approved the study, with written informed consent obtained from all patients.

Table 1. Patients' demographics and baseline characteristics.

Patients treated	18
Sex (male/female)	12/6
Age, years, median (range)	73 (52-84)
ECOG score (0,1)	7, 11
Histology	
Diffuse large B-cell lymphoma	9
Mantle cell lymphoma	5
Transformed follicular lymphoma	4
Stage	
I	1
II	2
III	6
IV	9
Bone marrow involvement	
Positive	2†
Negative	16
IPI risk group	
Low	5
Intermediate-low	4
Intermediate-high	7
High	2
Prior treatments, median (range)	
Number	3 (1-7)
Months from last	9 (1-38)
Prior chemotherapy regimens*	
R-CHOP	18
Bendamustine plus rituximab	6
Bortezomib-containing	3
Lenalidomide-containing	3
ICE	3
Baseline hematology, median (range)	
Hemoglobin, g/dL	12.8 (9.7-15.6)
Platelets, ×10^9/L	186 (101-401)
Neutrophils, ×10^9/L	4.3 (1.6-11.9)

†*Leukemic infiltration < 5% for both cases;* **Chemotherapy regimens received by more than one patient*

Results

A total of 18 patients were enrolled between October 2010 and October 2012. They met all protocol entry criteria except for three waivers granted for oral antibiotic use (for an asymptomatic urinary tract infection), prior radioimmunotherapy 5 years earlier (patient met bone marrow requirements), and <10 g/dL hemoglobin (9.7 g/dL in a patient with stable chronic anemia). The patients' demographics and baseline characteristics are summarized in Table 1.

Treatment

All 18 patients completed their scheduled treatment doses. Three patients had brief interruptions (5 - 30 minutes) of veltuzumab infusions because of mild-moderate reactions that resolved spontaneously (mild itching, chills) or in response to intravenous steroids (pruritis/hives). The median (range) durations of the veltuzumab infusions were 2.0 (1.1 – 2.9), 1.3 (0.9 – 2.2), 1.3 (0.8 – 2.2), and 1.2 (0.7 – 2.2) hours for the four administrations, respectively. For ^{90}Y-epratuzumab, radiolabeling was efficient (3.9% median unbound ^{90}Y) and all infusions were administered within 3 - 13 min without adverse events.

Thombocytopenia and neutropenia were dose-limiting, resulting in repeated de-escalation of the ^{90}Y dose level as successive cohorts were enrolled (Table 2). In the initial cohort at the starting ^{90}Y dose level of 15 mCi/m^2, all three patients developed grade 3 or 4 thrombocytopenia or neutropenia, with hematologic DLT in two patients (one with grade 4 thrombocytopenia >7 days, the other with grade 3 thrombocytopenia but not recovered to grade 1 by 12 weeks). In the second cohort treated at the reduced dose level of 12 mCi/m^2, all three patients again developed grade 3 or 4 thrombocytopenia or neutropenia, with hematologic DLT in two patients (both with grade 4 thrombocytopenia >7 days, one also with platelet counts not recovering to grade 1 level by 12 weeks.). In the third cohort, five of six patients treated at a dose of 9 mCi/m^2 x 2 developed grade 3 or 4 thrombocytopenia or neutropenia, two patients with DLT (both with grade 4 neutropenia >7 days, one also with grade 4 thrombocytopenia >7 days). At a further reduced ^{90}Y dose level of 6 mCi/m^2, two of six patients had grade 3 or 4 thrombocytopenia or neutropenia, but there were no DLT. As such, by protocol, 6 mCi/m^2 x 2 (222 MBq/m^2 x 2) was declared the MTD with this dosing regimen.

Adverse events

Five patients had serious adverse events. Two patients (12 and 15 mCi/m^2 dose levels) were hospitalized with pneumonia symptoms and pulmonary infiltrates 4 weeks after treatment. One patient with multiple co-morbidities developed transient grade 4 neutropenia at that time, while the other patient with a history of pneumonia and with pulmonary tumors at study entry had only grade 2 neutropenia. These events were considered at least possibly treatment-related and eventually classified as atypical pneumonia/pneumonitis, resolving after treatment including empirical antibiotics although no infectious agents could be identified. The other serious adverse events were unrelated events 1 – 3 months after treatment (syncope with fracture, upper respiratory infection with clinical deterioration, atrial tachycardia and hypercalcemia).

Treatment-related events occurred in 15 (83%) of the 18 patients; except for cytopenias, which are a known consequence of radioimmunotheapy and the two cases of pneumonitis/atypical pneumonia, these were all grade 1-2 events with only fatigue, nausea and anorexia occurring in more than one patient. *Online Supplementary Table S1* summarizes the most frequent adverse events.

Besides the two cases of pneumonitis/atypical pneumonia, the only infections were grade 1-2 events in four patients which were considered unrelated to treatment (two upper respiratory tract infections, herpes zoster, monilial skin infection). One patient had an unrelated self-limited episode of vitreous hemorrhage attributed to central retinal vein occlusion; otherwise, the only bleeds were minor events in two patients (bruising, bleeding gums).

Treatment response

Treatment response was assessed using revised criteria,[36] incorporating results from CT imaging as well as additional FDG-PET studies conducted in six patients. One patient withdrew early from the study after developing pneumonia-like symptoms and was unable to be assessed for response. At the first evaluation, 4 weeks after treatment, of the other 17 patients, one had a complete response, eight had partial responses, four had stable disease and four had progressive disease including two with possible mixed responses (one with lower extremity lesions substantially improved but lymphomatous bowel involvement worsened according to PET imaging; the other with multiple index lesions all unchanged by CT but several new lesions). One patient with a partial response

Table 2. Hematologic toxicity.*

^{90}Y dose level (mCi/m^2)	N	Neutrophils			Platelets		
		Grade 3-4	Grade 4 >7 days	Not grade 1 by 12 Weeks	Grade 3-4	Grade 4 >7 days	Not grade 1 by 12 weeks
15	3	2	0	0	3	1	1
12	3	2	0	0	3	2	1
9	6	2	2	0	5	1	0
6	6	1	0	0	2	0	0

**For neutrophils and platelets, number of patients at each dose level with maximum toxicity grade 3 or 4, those with grade 4 levels continuing >7 days, and those not recovered to grade 1 levels within 12 weeks of last treatment dose. For hemoglobin, results not shown since only one patient at the 12 mCi/m^2 dose level had a borderline grade 3 value which recovered by next evaluation.*

improved, achieving a complete response at the next scheduled evaluation at 12 weeks. Another patient with a partial response continuing at 12 weeks and considered a good candidate for retreatment by the investigator received a second course of 6 mCi/m^2 x 2 at that time, subsequently obtaining a complete response (Figure 1). Based on best response, the rate of overall objective responses (complete + partial responses) was 53% (9/17) and the complete response rate was 18% (3/17), with four other patients (24%) achieving stable disease. Table 3 lists response rates by histology, dose level, and IPI status.

The median progression-free survival from treatment initiation was 6.2 months for all 17 assessable patients. Of the three patients with complete responses (all with diffuse large B-cell lymphoma), one initially treated at 6 mCi/m^2 relapsed after 13.1 months with development of a single, new area of subcutaneous involvement. The patient was then retreated twice at the same dose, with stabilization of the lesion the first time and a partial response the second time, lasting 3 months (total progression-free survival, 6 months). Another patient treated at 12 mCi/m^2 was still disease-free at the 12-month CT evaluation but developed Parkinson disease that prevented further CT imaging; this patient continues to have no clinical evidence of disease 19 months after treatment. The third patient, initially with a continuing partial response after treatment at 6 mCi/m^2, was retreated with the same dose, achieving a complete response which lasted 6 months until the development of a single new liver lesion (total progression-free survival, 11.3 months). Of the six patients with partial responses as best response, one with diffuse large B-cell lymphoma treated at 9 mCi/m^2 was also long-lived (progression-free survival, 13.7 months). The other five partial responses progressed earlier (progression-free survival, 3.4 – 7.6 months) as did the four patients with stable disease as best response (progression-free survival, 3.4 – 8.5 months).

Biodistribution and radiation dosimetry

Based on ^{111}In scintigraphic imaging over 7 days, all patients had a normal biodistribution with no evidence of accelerated clearance from the circulation, splenic sequestration or altered organ uptake or redistribution. Although the planar ^{111}In images for dosimetry are not optimal for

Table 3. Treatment response.*

	N	OR (%)	CR (N)	PR (N)	SD (N)
All evaluable patients	17	53%	3	6	4
Histology					
Diffuse large B-cell lymphoma	8	50%	3	1	1
Mantle cell lymphoma	5	20%	0	1	3
Transformed follicular lymphoma	4	100%	0	4	0
^{90}Y Dose level					
12 and 15 mCi/m^2	5	60%	1	2	1
9 mCi/m^2	6	17%	0	1	3
6 mCi/m^2	6	83%	2	3	0
IPI scores					
High-intermediate/high (3-4)	8	25%	1	1	3
Low/low-intermediate (0-2)	9	78%	2	5	1

**Percent of patients with an objective response (OR) for all 17 assessable patients and among subgroups based on lymphoma histology, ^{90}Y dose level (12 and 15 mCi/m^2 groups combined due to small numbers), and International Prognostic Index (IPI) scores. The numbers of patients achieving a complete response (CR), partial response (PR) or stable disease (SD) as best response are also shown.*

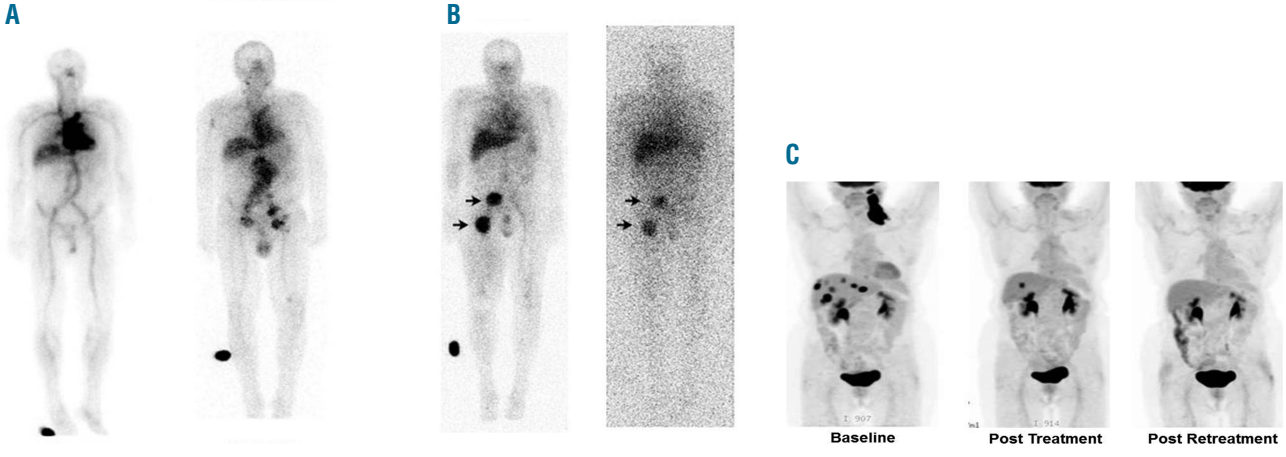

Figure 1. Imaging examples. (A) Anterior whole-body scintigraphic images in a patient with mantle cell lymphoma. Images acquired 30 minutes after intravenous infusion of ^{111}In-epratuzumab tetraxetan (left) and then 1 week later (right) show antibody uptake over this period at sites of known tumor involvement in the mid-abdomen and bilateral iliofemoral lymph nodes. (B) Anterior whole-body scintigraphic images in a patient with diffuse large B-cell lymphoma. ^{111}In image acquired 1 week after intravenous infusion of ^{111}In-epratuzumab tetraxetan (left) compared to bremstrallung image acquired 1 week after first intravenous infusion of ^{90}Y-epratuzumab tetraxetan (right). Arrows denote sites of known lymphomatous involvement. Although bremstrallung imaging has poorer spatial resolution, the pattern of uptake with ^{90}Y-epratuzumab tetraxetan appears similar to that with ^{111}In-epratuzumab tetraxetan. (C) ^{18}F-FDG-PET images in a patient with diffuse large B-cell lymphoma who had a partial response to treatment as determined by CT and after retreatment achieved a complete response by CT. Of the intense uptake seen at sites of a known large left cervical mass and multiple liver metastases at baseline (left), uptake occurs only at the site of one liver metastasis following initial treatment (center), with no uptake seen following retreatment (right).

visualizing smaller tumors, 13 patients showed evidence of antibody targeting with uptake localized at one or more lymphoma sites identified on CT (Figure 1). Radiation dose estimates for normal organs with this treatment regimen, calculated for solid organs from the serial images and for red marrow from serial ^{111}In blood counts, were acceptable at all ^{90}Y dose levels (*Online Supplementary Table S2*).

Pharmacokinetics

Veltuzumab serum levels increased with initial infusions, then declined (Figure 2) with an analysis of the pharmacokinetics (mean ± SD) after the last infusion showing a maximum concentration of 190.1±43.3 μg/mL, terminal half-life of 21.1±6.0 days, area under the curve of 4117±1671 day-μg/mL, and clearance of 59.5±34.5 mL/day/m^2. Serum levels of ^{111}In-epratuzumab tetraxetan following administration decreased over 1 week (Figure 2) with a clearance half-life of 96.5±21.0 hours.

Laboratory assessments

Blood counts obtained at least weekly showed dose-dependent thrombocytopenia and neutropenia with little anemia following treatment (Table 2). Platelet and neutrophil levels started declining by the second dose of ^{90}Y-epratuzumab tetraxetan, with nadirs occurring 3-5 weeks after treatment for platelets and 4-7 weeks for neutrophils. Platelet and neutrophil counts then generally improved to grade 1 levels over several weeks and reached normal levels by 9-12 weeks after treatment. Serum chemistry evaluated on the last day of treatment, then 4 or 12 weeks later, showed no evidence of renal or liver abnormalities (*Online Supplementary Table S3*).

At study entry, B-cell blood levels were variable and five patients had levels too low to be measured. As shown in Figure 3, B-cell levels depleted following the first dose of veltuzumab remained decreased at ^{90}Y-epratuzumab tetraxetan administrations, and at 4 and 12 weeks after treatment. Subsequent follow-up data for B-cell recovery

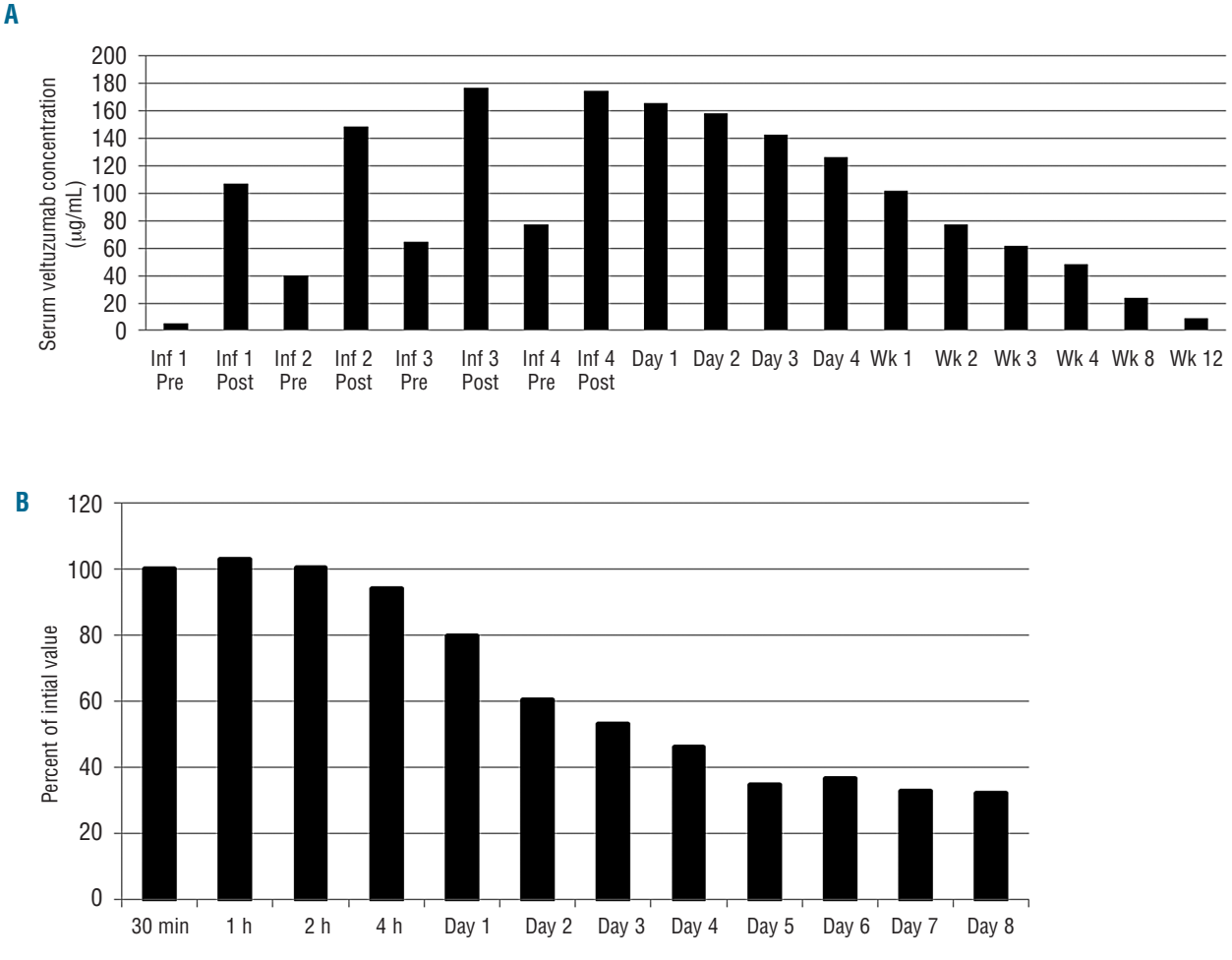

Figure 2. Pharmacokinetics. (A) Mean serum levels of veltuzumab. All patients received 200 mg/m^2 veltuzumab administered intravenously once a week for 4 consecutive weeks. Serum samples were obtained before and 30 minutes after each of the four infusions; 1, 2, 3 and 4 days after the last dose; and then 1, 2, 3, 4, 8, and 12 weeks later. Veltuzumab serum levels were determined by enzyme-linked immunosorbent assay with a minimum detectable level of 0.5 μg/mL. (B) Mean serum concentration of ^{111}In-epratuzumab tetraxetan. All patients received a 5 mCi diagnostic dose of ^{111}In-epratuzumab tetraxetan administered intravenously following the second weekly dose of veltuzumab. Serum samples were obtained 30 minutes, 1, 2 and 4 hours after infusion, and then 1, 2, 3, 4 or 5, and 6, 7 or 8 days later. At each time point, ^{111}In disintegration counts per unit volume were measured in local nuclear medicine departments using a calibrated gamma well counter, corrected for ^{111}In physical decay, and expressed as a percentage of the initial 30-minute value.

were not obtained in most patients in this study. T-cell levels were modestly decreased with treatment in most patients but improved by 12 weeks (median decreases from baseline: 33% on last treatment day; 39% at 4 weeks, 7.7% at 12 weeks). Changes in the quantity of serum immunoglobulins were variable and small, with median decreases from baseline at last treatment day, and 4 and 12 weeks later all remaining <10% for IgG and IgA and <20% for IgM.

Immunogenicity

Serum samples at baseline, 4 and 12 weeks after treatment were negative (<50 ng/mL) for both anti-epratuzumab and anti-veltuzumab antibodies, except one sample at 12 weeks, which was borderline positive only for anti-veltuzumab antibodies (60 ng/mL).

Epratuzumab doses

Following prior studies of ^{90}Y-epratuzumab tetraxetan alone, with each dose of radiolabeled epratzumab tetraxetan the initial seven patients (including all 6 patients at ^{90}Y dose levels ≥12 mCi/m^2) also received 1.5 mg/kg epratuzumab, but this was discontinued for the final 11 patients. Comparing pharmacokinetic, tumor targeting, biodistribution and dosimetry data, the serum half-life (mean ± SD) of ^{111}In-epratuzumab tetraxetan was modestly increased with added epratuzumab (114±11.8 hours *versus* 84.4±17.1 hours, respectively), while splenic uptake appeared moderately decreased on ^{111}In imaging, consistent with a lower estimated splenic radiation dose (18.8±11.5 cGy/mCi *versus* 37.0±19.2 cGy/mCi, respectively). However, there was no obvious effect upon tumor targeting with investigators able to visualize ^{111}In-epratuzumab uptake at one or more lesions identified on CT in five of the seven patients treated with additional epratuzumab compared to eight of the 11 patients not given additional epratuzumab.

Discussion

This treatment regimen of ^{90}Y-epratuzumab tetraxetan combined with veltuzumab was well tolerated in patients with relapsed/refractory aggressive lymphomas. Infusion reactions were infrequent, mild-moderate and transient, and there was no significant immunogenicity to either agent. Neutropenia and thrombocytopenia were dose-limiting, as expected, but transient and manageable at the 6 mCi/m^2 x 2 dose level established as the MTD. The only serious events considered at least possibly treatment-related were two cases of pneumonitis or atypical pneumonia occurring at dose levels above the MTD and resolving after antibiotic treatment. Importantly, there were no other significant infections or bleeding episodes associated with this combination approach, with other treatment-related clinical events being primarily limited to mild-moderate constitutional events (fatigue, nausea, anorexia, etc.).

At a dose level of 200 mg/m^2, veltuzumab effectively depleted peripheral blood B-cell levels prior to administration of the ^{90}Y-epratuzumab tetraxetan doses. The mean serum levels of veltuzumab exceeded the 25 μg/mL level often associated with maintained efficacy with rituximab. Comparing mean ± SD values, the 190±43 μg/mL maximum concentration achieved by the fourth dose and the subsequent clearance half-life of 21.1±6.0 days appear similar to the 155±60 μg/mL and 18.4±9.8 days previously obtained with 200 mg/m^2 veltuzumab given alone once-weekly for 4 weeks.[31] In spite of the depletion of B-cell blood levels by veltuzumab in this study, the clearance half-life of 96.5±21.0 hours for ^{111}In-epratuzumab tetraxetan was also comparable to the value of 85.0±34.0 hours determined previously when it was given alone.[19] Thus, the pharmacokinetics of either agent when given in combination remained largely unchanged compared to prior results obtained when given separately.

^{111}In imaging following the second veltuzumab dose showed tumor targeting with no evidence of splenic sequestration and with radiation dosimetry estimates for normal organs below accepted limits even at the highest ^{90}Y dose level. As expected with radioimmunotherapy, transient myelosuppression was dose-limiting with 6 mCi/m^2 ^{90}Y x 2 (222 MBq/m^2 x 2) determined as the MTD. This is lower than the 20 mCi/m^2 x 2 dose established for ^{90}Y-epratuzumab tetraxetan alone[22] or the 15 mCi/m^2 x 2 dose used for consolidation in diffuse large B-cell lymphoma patients 8 weeks after finishing R-CHOP chemotherapy.[39] Since veltuzumab depletes B cells, a

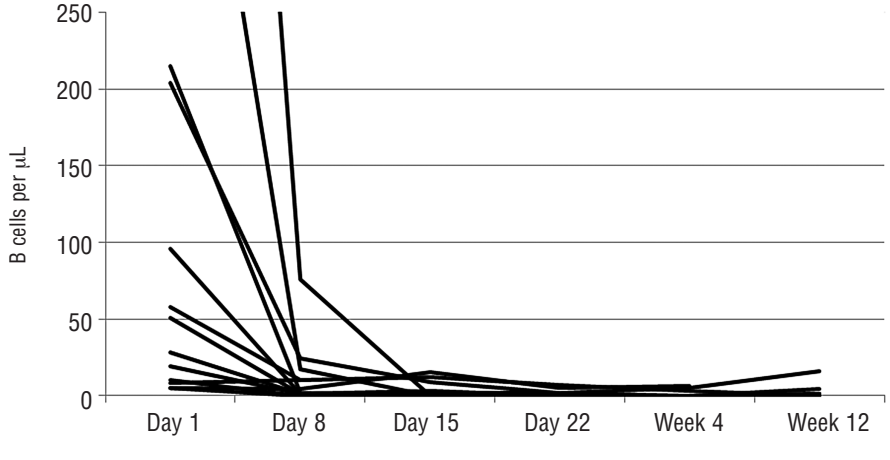

Figure 3. B-cell blood levels. All patients received veltuzumab on days 1, 8, 15 and 22 with ^{90}Y-epratuzumab administered on days 15 and 22. B-cell levels were obtained on treatment days and then 4 and 12 weeks later. Two patients had B-cell levels outside the display window on day 1 (544 and 1190 cells/μL).

greater effective proportion of the administered ^{90}Y-epratuzumab tetraxetan might remain in circulation, both to radiate the bone marrow and target the tumor. Although this could explain why the MTD was lower but still therapeutically effective with the combination, the pharmacokinetic analysis does not support this hypothesis, since the serum half-life of radiolabeled epratuzumab tetraxetan was not noticeably prolonged in combination with veltuzumab. The patients here were a decade older than those in a prior study of ^{90}Y-epratuzumab tetraxetan alone,[22] with a median age of 73 *versus* 64 years, respectively. In addition, they were more heavily pretreated with a median of three prior treatment regimens for aggressive disease, compared to two regimens in the prior study of ^{90}Y-epratuzumab tetraxetan alone which was primarily for indolent disease, and only one regimen in the consolidation study.[39] Thus, the population here may not have been able to tolerate the same dose of ^{90}Y-epratuzumab tetraxetan as given previously alone due to the increased bone marrow damage sustained from the more intensive prior chemotherapy they had received. Finally, a more conservative hematologic DLT definition was used here, in which the occurrence of grade 4 cytopenias lasting >7 days was considered a DLT, instead of related clinical events (bleeding due to thrombocytopenia, neutropenic fever/infection) as had been used previously.[22] This more stringent definition may also have contributed to the lower dose determined to be acceptable in this study.

Nine (53%) of the 17 assessable patients achieved objective responses with this treatment regimen, including three (18%) complete responses and six (35%) partial responses. Of the three patients with complete responses, two remained progression-free until 11 and 13 months after treatment initiation, with the third still continuing without clinical evidence of progression at 19 months. One partial response was also particularly long-lasting, with the patient remaining progression-free for nearly 14 months. Several other patients with partial responses or stable disease as their best response remained progression-free up to 7 or 8 months. Since many patients in this study were older, heavily pretreated and at increased risk of poor outcome (50% with IPI scores 3-5), their treatment outcomes cannot be compared directly to those of the small number of patients with aggressive lymphomas in the prior study of ^{90}Y-epratuzumab alone,[22] or with other anti-CD20 based radioimmunotherapy studies conducted with ^{90}Y-ibrituzumab tiuxetan in aggressive disease.[40,41] However, in this study, responses occurred in all three subtypes of aggressive NHL, at all ^{90}Y dose levels and in patients with all IPI risk scores. Most importantly, five of six (83%) patients at the MTD achieved objective responses, including two patients with durable complete responses. Thus, this treatment regimen appears active in these populations, and further study of this combination approach in aggressive lymphoma appears warranted.

In prior studies of radioimmunotherapy with ^{90}Y-epratuzumab tetraxetan alone, unlabeled epratuzumab was also administered to prevent potential sequestration of the radiolabeled antibody by the B-cell pool in the spleen, blood or bone marrow. Interestingly, even with the addition of veltuzumab in this regimen, the same small dose of added epratuzumab received by the initial seven patients still had an apparent measurable effect on splenic uptake and serum circulation of the ^{111}In-epratuzumab tetraxetan. However, these findings are of questionable clinical significance, and since there was no apparent difference in tumor targeting, the added epratuzumab was discontinued as being unnecessary with this combination approach. Both veltuzumab and epratuzumab are therapeutically active, so the efficacy observed in this study may be partly due to the "naked" antibody administered. Finally, two patients were retreated at the investigator's discretion with no additional safety concerns: one with a partial response after initial treatment which converted into a complete response with retreatment, the other after initially relapsing following a complete response, achieving a partial resposne with two additional retreatments. Since both epratuzumab and veltuzumab are humanized antibodies, the expected lack of significant immunogenicity should allow not only repeated treatment cycles of combination therapy to be given, but extended maintenance dosing afterwards. These considerations remain areas for further exploration.

In conclusion, this combination approach has a sound rationale, addressing historical critical analyses of radioimmunotherapy regimens giving 'cold' antibody targeting the same antigen as the 'hot' antibody, and uses improved ^{90}Y chelation and a fractionated dosing schedule intended for better delivery and retention of the radioisotope at the tumor sites. This regimen would be well suited for the elderly or other patients with aggressive disease who relapse and are not candidates for stem-cell transplantation, and would also be ideal to combine with an oral agent such as ibrutinib, everolimus or lenalidomide. Whether this combination approach truly represents an advance over currently approved radioimmunotherapy regimens remains to be determined in a controlled setting. However, we observed encouraging therapeutic activity in difficult-to-treat patients with relapsed/refractory aggressive NHL and an acceptable ^{90}Y dose was determined for further studies. A phase 2 study is now in progress comparing the combination to ^{90}Y-epratuzumab tetraxetan alone.

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