Subcutaneous Veltuzumab, a Humanized Anti-CD20 Antibody, in the Treatment of Refractory Pemphigus Vulgaris

Christoph T. Ellebrecht, MD; Eun J. Choi, BS; David M. Allman, PhD; Donald E. Tsai, MD, PhD; William A. Wegener, MD, PhD; David M. Goldenberg, ScD, MD; Aimee S. Payne, MD, PhD

Pemphigus vulgaris (PV) is a potentially fatal autoimmune blistering disease caused by antibodies to the keratinocyte adhesion protein desmoglein (Dsg) 3. B-cell depletion with the chimeric anti-CD20 antibody rituximab is effective in PV, with 95% to 100% of patients achieving short-term healing of mucocutaneous lesions and approximately 50% experiencing complete remission of disease off therapy.\(^3\) However, more than 80% of the patients experience relapse, suggesting that most patients with PV treated with rituximab may require multiple cycles of therapy. Additionally, intravenous administration of rituximab is expensive, and neutralizing human antichimeric antibodies to rituximab can occur.\(^4\) Therefore, the development of alternative anti-CD20 therapies is desirable.

We report successful treatment in a patient with refractory PV using veltuzumab, a novel second-generation humanized anti-CD20 antibody, administered by subcutaneous injection. Subcutaneous veltuzumab was safe and effective, resulting in complete remission of disease off therapy and no serious adverse events during 35 months of follow-up.

**Report of a Case**

A woman in her late 20s developed intermittent oral lesions initially attributed to herpes simplex virus. Two years later she developed vaginal erosions and focal areas of skin blistering. A skin biopsy demonstrated suprabasal acantholysis, and direct immunofluorescence analysis showed intercellular staining of IgG and complement C3, establishing a diagnosis of PV. The patient’s disease cleared with prednisone, 40 mg/d, but her mucosal disease flared when the dose was tapered. Adjunctive immunosuppression with azathioprine, 150 mg/d (2.25 mg/kg/d), for 3 months was unsuccessful, and dapsone was not tolerated because of cytopenias. Treatment with mycophenolate mofetil, 3000 mg/d (45 mg/kg/d), allowed prednisone to be tapered to 5 mg/d but not lower. The patient received her first cycle of rituximab (four 375-mg/m² weekly intravenous doses), resulting in incomplete remission. Prednisone therapy was tapered to 3 mg/d while the mycophenolate mofetil dose remained 3000 mg/d, but mucosal blisters recurred 6 months after treatment.

A compassionate-use investigational new drug protocol was approved to administer veltuzumab, a second-generation humanized anti-CD20 antibody, to a patient with refractory PV. Veltuzumab was administered as two 320-mg (188 mg/m²) subcutaneous doses 2 weeks apart, resulting in complete remission of disease off therapy. The disease relapsed 2 years after treatment. A second cycle of subcutaneous veltuzumab, using the same dosage regimen, again induced complete remission off therapy, which remained at 9 months. No serious adverse events occurred during 35 months of follow-up. Serum veltuzumab levels were 22 and 29 μg/mL 2 weeks after the first dose of each cycle, and the drug remained detectable in the serum for longer than 3 months. Relapse and response to veltuzumab generally correlated with desmoglein 3 enzyme-linked immunosorbent assay index values. Shortly after a relapse that occurred after a long-term remission, the patient demonstrated an elevated naive (CD19^+CD27^-) to memory (CD19^+CD27+) B-cell ratio of 19.5 and transitional (CD19^+CD24^-CD38^+) B-cell frequency of 12.5%.

**Conclusions and Relevance**

Subcutaneous veltuzumab may be a safe, effective, and more economical alternative to intravenous rituximab for PV therapy. Clinical trials of subcutaneous veltuzumab for PV are warranted.

**Importance**

B-cell depletion with the anti-CD20 antibody rituximab is highly effective for pemphigus vulgaris (PV) treatment. However, most patients experience relapse, and intravenous rituximab infusions are expensive. Therefore, cost-effective anti-CD20 therapies are desirable.

**Observations**

A compassionate-use investigational new drug protocol was approved to administer veltuzumab, a second-generation humanized anti-CD20 antibody, to a patient with refractory PV. Veltuzumab was administered as two 320-mg (188 mg/m²) subcutaneous doses 2 weeks apart, resulting in complete remission of disease off therapy. The disease relapsed 2 years after treatment. A second cycle of subcutaneous veltuzumab, using the same dosage regimen, again induced complete remission off therapy, which remained at 9 months. No serious adverse events occurred during 35 months of follow-up. Serum veltuzumab levels were 22 and 29 μg/mL 2 weeks after the first dose of each cycle, and the drug remained detectable in the serum for longer than 3 months. Relapse and response to veltuzumab generally correlated with desmoglein 3 enzyme-linked immunosorbent assay index values. Shortly after a relapse that occurred after a long-term remission, the patient demonstrated an elevated naive (CD19^+CD27^-) to memory (CD19^+CD27+) B-cell ratio of 19.5 and transitional (CD19^+CD24^-CD38^+) B-cell frequency of 12.5%.

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**Author Affiliations:** Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Ellebrecht, Choi, Payne); Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Allman); Department of Hematology/Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Tsai); Immunomedics Inc, Morris Plains, New Jersey (Wegener, Goldenberg).

**Corresponding Author:** Aimee S. Payne, MD, PhD, Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, 421 Curie Blvd, Room 1009 Biomedical Research Bldg, Philadelphia, PA 19104 (aimee.payne@uphs.upenn.edu).

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**Author Affiliations:** Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Ellebrecht, Choi, Payne); Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Allman); Department of Hematology/Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Tsai); Immunomedics Inc, Morris Plains, New Jersey (Wegener, Goldenberg).

**Corresponding Author:** Aimee S. Payne, MD, PhD, Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, 421 Curie Blvd, Room 1009 Biomedical Research Bldg, Philadelphia, PA 19104 (aimee.payne@uphs.upenn.edu).
The patient received a second cycle of the same dosage of rituximab 7 months after the first cycle, again resulting in incomplete remission; prednisone was discontinued and mycophenolate mofetil, 3000 mg/d, was continued, but the mucosal blisters recurred 6 months later. She received a third cycle of rituximab, with a modified regimen of two 1000-mg intravenous infusions 2 weeks apart, 8 months after the second cycle, with no response. Owing to the patient’s fatigue, the mycophenolate mofetil dose was decreased to 2000 mg/d, requiring a prednisone dose increase to 7.5 mg/d to achieve disease control, and doses could not be lowered further without disease flare. Infusion reactions and human antichimeric antibodies to rituximab were not observed.

Veltuzumab is a humanized anti-CD20 antibody that differs in sequence from rituximab, resulting in favorable pharmacokinetics and potent anti–B-cell activity in preclinical studies. Veltuzumab is currently under clinical development for the treatment of B-cell lymphomas and autoimmune diseases. A compassionate-use investigational new drug protocol to provide veltuzumab treatment for our patient with refractory PV was approved by the US Food and Drug Administration and a local institutional review board. The patient, in her early 40s at the time of treatment with veltuzumab (month 0 in Figure 1), received two 320-mg (188 mg/m²) subcutaneous doses of veltuzumab 2 weeks apart. She was observed for 1 hour after each injection without incident. No injection site reactions or serious adverse events were observed during long-term follow-up of 35 months.

After veltuzumab treatment, prednisone was successfully tapered to discontinuation, followed by mycophenolate mofetil, achieving the clinical end points of complete remission on minimal therapy at 13 months and complete remission off therapy at 22 months. At 24 months, her mucosal PV relapsed. She received a second cycle of veltuzumab 26 months after the first treatment, using the same veltuzumab dosing regimen without adjunctive use of daily systemic immuno-
She achieved complete remission of disease off therapy within 2 months, which remains ongoing at 35 months follow-up. Figure 1A details the patient’s clinical course and treatments. Human anti-veltuzumab antibodies were not detected during 35 months of follow-up (data not shown; eMethods in the Supplement). At 0.5 and 3.0 months, serum veltuzumab levels were 22 and 23 μg/mL, respectively, with the first cycle of treatment and 29 and 4 μg/mL, respectively, with the second cycle (Figure 1B). A modified Dsg3 enzyme-linked immunosorbent assay (1:1500 dilution to increase sensitivity by using the linear range of the assay) showed that Dsg3 index values generally correlated with treatment and disease activity (Figure 1C). Serum autoantibody levels decreased before the first cycle. Veltuzumab treatment correlated with a sustained decrease in Dsg3 index values. Relapse was preceded by increased autoantibody levels, and complete remission after the second treatment cycle correlated with reduced Dsg3 index values. Serum tetanus IgG remained stable above the protective limit of 0.1 IU/mL (Figure 1D). Epitope mapping experiments demonstrated that autoantibodies recognized Dsg3 extracellular domains 1 to 3, with no change in targeted epitopes throughout remission and relapse (data not shown).11

Figure 2. Immunophenotype of Repopulating B Cells 26 Months After the First Cycle of Veltuzumab Therapy

A, CD19+ peripheral blood B-cell depletion and repopulation during the second cycle of veltuzumab therapy. B-cell subsets were analyzed at 26 months, shortly after relapse from long-term remission. B, Long-term remission after the first cycle of veltuzumab correlates with the elevation of naive (CD19+CD27−) compared with memory (CD19+CD27+) B-cell frequencies. C, Long-term remission after the first cycle of veltuzumab correlates with the high levels (12.5%) of transitional (CD19+CD24−CD38+) B cells, as previously described.2

Multiparameter flow cytometry of whole blood was performed to evaluate the kinetics of B-cell depletion and repopulation (Figure 2A). Unexpectedly, the patient’s peripheral CD19+ B cells were almost undetectable just before the first veltuzumab treatment cycle, which precluded analysis of B-cell depletion during that cycle. The first veltuzumab injection occurred 10 months after the third cycle of rituximab, when the patient was taking 7.5 mg/d of prednisone and 2000 mg/d of mycophenolate mofetil. The levels of CD19+ B cells were 15/μL at 7 months and 294/μL at 25 months (during relapse). Two weeks after the first veltuzumab injection of cycle 2, peripheral blood CD19+ B-cell counts were undetectable, with repopulation first detected at 7 months after the second treatment cycle (42/μL). A previous study2 of patients with PV who were treated with rituximab reported B-cell repopulation characteristics for patients achieving long-term complete remission off therapy compared with patients in incomplete remission. In the present patient, 2 years after the first veltuzumab treatment and shortly after relapse, naive (CD19+CD27−) B cells constituted 95.1% of the total B-cell population, with a ratio of naive to memory (CD19+CD27+) B cells of 19.5 (absolute B-cell counts were 280/μL and 14/μL in the naive and memory pools, re-
subcutaneous veltuzumab. Veltuzumab is a second-generation anti-CD20 antibody with humanized framework regions and a single amino acid change in the heavy chain complementarity-determining region 3 compared with rituximab (aspartic acid at position 101 [D101] instead of asparagine). In preclinical studies, veltuzumab demonstrated a 2.7-fold higher half-life on the surface of human B-cell lines compared with rituximab, confirmed by site-directed mutagenesis studies to be due to the D101 substitution. Cynomolgus monkeys receiving low-dose subcutaneous veltuzumab demonstrated complete depletion of B cells in the peripheral blood, spleen, and mandibular and mesenteric lymph nodes, whereas rituximab effectively depleted B cells in the peripheral blood, but not consistently in the lymph nodes and/or spleen. Consistent with this enhanced anti-B-cell activity, low-dose subcutaneous veltuzumab has shown efficacy comparable to that of intravenous rituximab in phase 1 studies of patients with non-Hodgkin lymphoma and relapsed immune thrombocytopenia (ITP).

Treatment with subcutaneous veltuzumab in our patient with refractory PV was effective, resulting in peripheral blood B-cell depletion within 2 weeks of the first subcutaneous injection and complete remission of the disease off therapy. Given the peripheral blood B-cell depletion and decreasing Dsg3 enzyme-linked immunosorbent assay index values at the start of veltuzumab therapy, we cannot rule out a delayed effect of rituximab in response to the first cycle of veltuzumab treatment. However, peripheral blood B-cell depletion has been reported in patients receiving high-dose mycophenolate mofetil and prednisone therapy who have never received rituximab, so we cannot definitively attribute the baseline B-cell depletion to prior rituximab therapy.

Serum levels of veltuzumab were sustained in our patient at 2 weeks and 3 months for the first treatment cycle (22 and 23 μg/mL, respectively) but not the second treatment cycle (29 and 4 μg/mL). In comparison, the mean veltuzumab serum level in patients with ITP who were receiving the same dosing regimen were 20 and 2 μg/mL at 2 weeks and 3 months, respectively. Although speculative, the sustained serum level of veltuzumab after the first treatment cycle in this patient might reflect her low baseline peripheral blood B-cell count (ie, low CD20 target antigen to deplete circulating veltuzumab), which recovered by the time of the second treatment cycle to values similar to those observed in the ITP population.

Repopulation of B cells began 7 months after the first treatment cycle, although relapse of the disease did not occur until 24 months after treatment. Patients achieving long-term complete remission after rituximab therapy have been reported to have a significantly higher mean frequency of transitional B cells compared with patients in incomplete remission (8.1% vs 1.9%). Similarly, our patient achieving long-term complete remission demonstrated a 12.5% transitional B-cell frequency 2 years after treatment, shortly after disease relapse.

Veltuzumab was well tolerated by our patient, with no serious adverse events, injection site reactions, or constitutional symptoms following treatment. No serious adverse events or serious infections have been observed in phase 1 studies of subcutaneous veltuzumab use in 34 patients with ITP and 17 patients with non-Hodgkin lymphoma. The most common adverse event was mild to moderate injection site reactions, experienced by 41% of the patients with ITP and 35% of those with lymphoma.

Conclusions
This single-patient experience suggests that subcutaneous veltuzumab may be a safe and effective alternative to rituximab for the treatment of PV, even in refractory cases. Subcutaneous administration is more convenient and likely cost-effective as it avoids the substantial costs of intravenous infusion. Clinical trials of veltuzumab in PV are warranted.
White Shadows in a Dark Land

Rina M. Allawh, BS; Scott A. Norton, MD

The documentary In the Shadow of the Sun captures Josephat Torner, an advocate for Tanzania’s albino community, stating “It’s my dream in my life that people with albinism are respected and given all rights which other human beings are being given.” Since 2000, nearly 200 albinos in East Africa have been killed and dozens more mutilated by the intentional severing of limbs. Recent efforts from local and international albino advocacy organizations, along with the United Nations, have increased protections for albinos in East Africa and have prompted education, locally and globally, about the plight of albinos.

Albinism encompasses several inherited disorders of melanin biosynthesis characterized by various degrees of incomplete pigmentation of hair, skin, and eyes. Clinical manifestations and classification are based on the genes affected; OCA2 is the most common form in sub-Saharan Africans (OMIM 20320). In the United States, approximately 1 in 20,000 individuals have albinism, but this condition is 5 times more common in East Africa.1

The award-winning documentary, In the Shadow of the Sun, uncovers the social rejection, dehumanization, discrimination, and violence facing Tanzania’s albinos through the stories of 45-year-old Josephat Torner and Vedastus Zangule, a schoolchild. Filmed over 6 years, In the Shadow of the Sun is an 84-minute, small-budget film directed by British filmmaker Harry Freeland and produced by Inroads Films. It premiered at the International Documentary Film Festival in Amsterdam in November 2012 and then shown at 50 festivals in 28 countries, including Tanzania.2

With a passion for East African culture, Freeland traveled to Ukerewe, an island in Lake Victoria, where he met the force behind the film, Josephat, at a protest confronting the superstitions and myths about albinism.3 Persecution against albinos within Tanzanian society arises from an ancient spiritual belief, reinforced by traditional healers, that blood and body parts of albinos enhance the ability of potions, charms, and ritual practices to confer magical powers, prosperity, and good health to a believer.1,2

Are traditional healers the only perpetrators? Freeland’s interview with local fishermen reveals that anti-albino barbarism is a business, perhaps originally based on deep-rooted superstitions, but now fueled by poverty, involving “people with money and power.”3 By merging distressing footage with present-day interviews, Freeland reveals that traditional healers and community entrepreneurs are involved in these murders.1

Despite unpredictable dangers, Josephat and Vedastus are determined to overcome personal obstacles, thereby providing a sense of accomplishment and hope to albinos in East Africa. No longer hidden in the shadows, the voices of Josephat, Vedastus, and others experiencing discrimination are now heard worldwide. As a result of the global success of the documentary (which is available on DVD in England and Ireland and for downloading on movieler.com), Freeland has founded a frontline nonprofit organization, Standing Voice, that advances human rights for people with albinism in East Africa.1

Author Affiliations: George Washington University School of Medicine, Washington, DC (Allawh); Department of Dermatology, Children’s National Medical Center, Washington, DC (Norton).

Corresponding Author: Scott A. Norton, MD, Department of Dermatology, Children’s National Medical Center, 1111 Michigan Ave NW, Washington, DC 20010 (scottanorton@gmail.com).