Supplementary Online Content

Ellebrecht CT, Choi EJ, Allman DM, et al. Subcutaneous veltuzumab, a humanized anti-CD20 antibody, in the treatment of refractory pemphigus vulgaris. *JAMA Dermatol*. Published online August 13, 2014. doi:10.1001/jamadermatol.2014.1939.

eMethods. Clinical treatment, assessment, and laboratory analyses

This supplementary material has been provided by the authors to give readers additional information about their work.

Methods. Clinical treatment, assessment, and laboratory analyses

Clinical treatment and assessment

A compassionate-use investigational new drug protocol to treat a refractory PV patient with veltuzumab was approved by the United States Food and Drug Administration and local Institutional Review Board. The patient received two 320 mg (188 mg/m2) subcutaneous doses of veltuzumab two weeks apart, with each dose administered via 160 mg injections at two different sites. The patient was clinically assessed and blood was collected at follow up visits every 3-7 months.

ELISA

Dsg3 and tetanus-toxoid antibody titers were evaluated by ELISA (MBL International and IBL International, respectively) according to the manufacturer's recommendations. A modified Dsg3 ELISA, using a serum dilution of 1:1500, was used to better demonstrate changes in Dsg3 ELISA titers within the linear range of the assay[1]. For tetanus-toxoid, sample OD values (measured in duplicate) were compared to an anti-tetanus-toxoid standard of known concentration within the linear range of the assay.

Rituximab and veltuzumab serum analyses

Human anti-chimeric antibodies to rituximab were measured as previously described[2]. Serum veltuzumab levels and human-anti veltuzumab antibody levels were measured by Immunomedics, Inc.

Flow cytometry

EDTA-anticoagulated whole blood was subjected to red-blood-cell-lysis (FACSlyse, BD) for 15 minutes at room temperature. Cells (10^7/mL) were washed in PBS plus 5% fetal bovine serum, 1 mM EDTA. 10^6 cells were stained with monoclonal anti-human CD3, CD19, CD24, CD27, CD38, CD45 (BD and eBioscience) for 2 hours at 4°C. Samples were analyzed on an LSRII flow cytometer (Becton Dickinson), and computational analysis was performed with FlowJo software (Treestar). At each time point, whole blood samples from an unaffected sex- and age-matched person were analyzed as a control. Lymphocytes were detected first by a $CD45^+SSC^{lo}$ gate and further characterized as described.

Dsg3 epitope mapping

Dsg3 epitopes targeted by patient serum autoantibodies were determined as previously described, using Dsg3-Dsg2 extracellular domain-swapped constructs generously provided by Prof. Masayuki Amagai[3].

References

(1) Cheng SW, Kobayashi M, Tanikawa A, Kinoshita-Kuroda K, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. *Br J Dermatol* 2002;147:261-265.

(2) Lunardon L, Payne AS. Inhibitory human antichimeric antibodies to rituximab in a patient with pemphigus. *J Allergy Clin Immunol* 2012;130:800-803.

(3) Ohyama B, Nishifuji K, Chan PT et al. Epitope spreading is rarely found in pemphigus vulgaris by large-scale longitudinal study using desmoglein 2-based swapped molecules. *J Invest Dermatol* 2012;132:1158-1168.

