Study design

This case series aimed to evaluate the tolerability and efficacy of bortezomib as induction therapy in patients with active and potentially life threatening SLE despite intensive previous conventional therapies. Patients were recruited at three German centres: Charité – University Medicine Berlin, University of Erlangen-Nuremberg and University of Cologne, and were prospectively followed after bortezomib treatment. All patients provided written informed consent after being informed about the nature of the “off-label” therapy and the potential risks of the treatment. The local Institutional Review Board (IRB) was notified in each individual patient and approved the analysis of samples for research (EA1/124/09). All work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Patients and treatment schedule

From May 2008 to November 2012, twelve SLE patients who met the ACR classification criteria[1] and had active disease (SLE disease activity index (SLEDAI-2K)[2] \(\geq 10\)) and elevated anti-dsDNA antibody titres despite immunosuppressive treatment or lack of tolerance to conventional therapy were administered intravenous bortezomib (Velcade®, Janssen-Cilag) at doses of 1.3mg/m\(^2\) on days 1, 4, 8, and 11 along with 20mg dexamethasone with each bortezomib injection followed by a 10-day treatment-free interval according to the approved protocol for multiple myeloma.[3] Patient characteristics and treatment regimens are described in Table S1. Immunosuppressive treatment was discontinued for a median of 31 days before the first bortezomib injection; only antimalarial drugs and oral corticosteroids were continued. In most cases, infection prophylaxis consisted of acyclovir (200mg daily) and cotrimoxazole (960mg every three days). After termination of bortezomib treatment, maintenance treatment was reintroduced, selected individually by the treating physician based on the patient’s organ
manifestations, tolerability and treatment history. Another four SLE patients with active disease received bortezomib 1.3mg/m² without co-administration of dexamethasone and were evaluated separately (Table S2).

**Safety and efficacy assessments**

Safety was assessed by monitoring adverse events (AEs), laboratory values, and vital signs at each visit. Efficacy was rated using the SLEDAI-2K scoring system.[2] Serum anti-dsDNA antibody and complement levels were determined on the first and last day of each bortezomib cycle, as well as 1 and 3 months after the last bortezomib cycle. In patients receiving bortezomib at Charité – University Medicine Berlin (n=8), total immunoglobulin levels, protective antibody titres in serum were analysed and peripheral blood lymphocyte subsets investigated by flow cytometry. Bone marrow biopsies were performed in one patient before and after four cycles of bortezomib treatment, and the samples were analysed by immunohistology.

**Statistical analysis**

Generalized linear mixed models[4] were used to investigate SLEDAI scores, serum (auto)antibodies concentrations and PC subsets over time and statistical inference. Several models with different structural assumptions regarding the change over time were tested by linear, quadratic, and piecewise change parameters. All models were compared by the Bayesian information criteria (BIC). Statistical inference was based on the model with the lowest BIC. Nonparametric regression technique was used for the estimation of a possible breakpoint in the change of biomarkers over time.[5] The breakpoint partitioned the regression line into two separate segments differing in the increase/decrease. Sensitivity analyses were performed by the last observation carried forward method. Percentage change of biomarkers was estimated for each patient, and the median percentage change was calculated for each parameter. All statistical analyses were conducted using STATA software (version 11.1; Stata Corp.).
Serologic analysis

Serum anti-dsDNA antibodies and autoantibodies to extractable nuclear antigens (ENAs) were analysed by ELISA (EUROIMMUN AG, Germany). Vaccine titres for tetanus toxoid, measles and mumps were analysed by ELISA.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were freshly isolated from heparinised blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia Biotec). The phenotypes of the cells were determined by flow cytometry using the following antibodies: anti-CD19 (SJ25C1, DAKO), anti-CD20 (2H7), anti-CD3 (UCHT1), anti-CD169 (Siglec-1, 7-239, AbD Serotec), anti-CD14 (MφP9), and anti-CD27 (2E4), obtained from BD Biosciences. Cells were washed before acquisition (FACSCanto™ flow cytometer, BD Biosciences) and analysis (FlowJo Software; TreeStar, San Carlos, CA). Quantification of peripheral blood lymphocyte subsets was performed with the TruCount™ system (BD Biosciences).

Immunohistology

Bone marrow biopsies were fixed in 4% formalin, decalcified and embedded in paraffin. 1-2 μm sections were cut and deparaffinised before incubation with anti-CD138 antibody (MI15, DAKO). Nuclei were counterstained with hematoxylin. Images were acquired using a fluorescence microscope (AxioImager Z1). Quantification of CD138+ PCs in 10 high-power fields (0.237 mm²) relative to total cell counts in the bone marrow was performed using the AxioVision 4 Module AutMess Plus (Carl Zeiss MicroImaging, Inc.).

References
1. Hochberg MC Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725-


