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Depletion of Autoreactive Plasma Cells and Treatment of Lupus Nephritis in Mice Using CEP-33779, a Novel, Orally Active, Selective Inhibitor of JAK2

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Accumulating evidence suggests that autoreactive plasma cells play an important role in systemic lupus erythematosus (SLE). In addition, several proinflammatory cytokines promote autoreactive B cell maturation and autointo body production. Hence, therapeutic targeting of such cytokine pathways using a selective JAK2 inhibitor, CEP-33779 (JAK2 enzyme IC₅₀ = 1.3 nM; JAK3 enzyme IC₅₀/JAK2 enzyme IC₅₀ = 65-fold), was tested in two mouse models of SLE. Age-matched, MRL/lpr or BWF1 mice with established SLE or lupus nephritis, respectively, were treated orally with CEP-33779 at 30 mg/kg (MRL/lpr), 55 mg/kg or 100 mg/kg (MRL/lpr and BWF1). Studies included reference standard, dexamethasone (1.5 mg/kg; MRL/lpr), and cyclophosphamide (50 mg/kg; MRL/lpr and BWF1). Treatment with CEP-33779 extended survival and reduced splenomegaly/lymphomegaly. Several serum cytokines were significantly decreased upon treatment including IL-12, IL-17A, IFN-α, IL-1β, and TNF-α. Antinuclear Abs and frequencies of autoantigen-specific, Ab-secreting cells declined upon CEP-33779 treatment. Increased serum complement levels were associated with reduced renal JAK2 activity, histopathology, and spleen CD138⁺ plasma cells. The selective JAK2 inhibitor CEP-33779 was able to mitigate several immune parameters associated with SLE advancement, including the protection and treatment of mice with lupus nephritis. These data support the possibility of using potent, orally active, small-molecule inhibitors of JAK2 to treat the debilitating disease SLE. The Journal of Immunology, 2011, 187: 000–000.
Several novel therapies are in clinical development that target key cytokine pathways or cell types thought to be involved in SLE progression (20). B cells are thought to be one of the key cells types involved in the pathogenesis of SLE. However, depletion of these cell types in three separate, placebo-controlled, phase III trials using anti-human CD20 mAbs, rituximab and ocrelizumab, did not result in significant disease treatment, and safety was compromised as opportunistic infections increased with treatment over time (21).

The rationale for targeting the survival of LL-PCs in the treatment of SLE using proteasome inhibitors, anti-BAFF/BLyS mAbs, and anti–IL-6 or IL-6R Abs has emerged as alternative therapy for the treatment of SLE (22). As described above, JAKs play a critical role in transducing signals from the IL-6R, and IL-6 is involved in both SLE and the maintenance of the pool of potentially autoreactive plasma cells. Therefore, blockade of JAK signaling using a selective and potent JAK2 inhibitor could weaken the supportive effects of IL-6 on sustaining autoreactive plasma cells in SLE. In support of this hypothesis, the current report describes use of a novel, orally active, selective, ATP-competitive JAK2 inhibitor, CEP-33779, which abolishes SLE disease in two different mouse models of SLE: MRL/lpr and NZB/W F1 (BWF1) hybrid. In addition, CEP-33779 reduces several serum cytokines, circulating antinuclear Abs (ANAs), long-lived plasma cells, and autoreactive Ab-secreting cell types in secondary lymphoid organs. This selective JAK2 inhibitor was also effective in ameliorating glomerulonephritis in the BWF1 hybrid model of SLE, suggesting use in later-stage nephritis patient populations. These preclinical studies suggest the potential therapeutic utility of selective modulators of JAK/STAT signaling in the management of early-stage and progressive manifestations of SLE.

Materials and Methods

Animals and facilities

The in-life portions of these experiments were performed at Cephalon (West Chester, PA). Six-week-old SLE-prone female MRL/lpr (no. 000485; The Jackson Laboratory, Bar Harbor, ME) and non-SLE-prone female control MRL/MpJ (no. 000486; The Jackson Laboratory) mice and 15-wk-old female SLE (NZB×NZW)F1 hybrid (NZB/WF1/lpr, or BWF1 as it will be referred to in this article; cat. no. 100008; The Jackson Laboratory) and non-SLE female control NZW/LacI (cat. no. 001058) mice were maintained on a 24-h light-dark cycle, with food and water available ad libitum. All experimental animal procedures were approved by and in accordance with the regulations of the Institutional Animal Care and Use Committee of Cephalon. Body mass for each animal was determined using a Mettler AT260 balance (Mettler-Toledo, Columbus, OH) at 10–12 wk. Animals were housed in a negative-pressure, laminar-flow isolator facility, in 10 cages of 4 mice each, 2 cages of 2 mice each, and 4 cages of 1 mouse each (CO2, 4%, O2, 21%, relative humidity, 30–70%, and temperature, 21–23°C). All animals were provided with standard lab chow (PMI 5017, PMI Nutrition International, St. Louis, MO) and water ad libitum.

Study designs

MRL/lpr study. See Supplemental Fig. 1A for complete experimental design layout and group definitions. Eight-week-old MRL/lpr age-matched mice were randomized, ear tagged for identification, and initial baseline measurements including body mass and serum were collected before dosing. Mice were orally treated with CEP-33779 in PEG 400 at 30 mg/kg, 55 mg/kg, or 100 mg/kg twice daily. Dexamethasone was provided as standard of care at 1.5 mg/kg, i.p., three times a week. Vehicle was provided orally, twice daily, as PEG 400 only. Mice were treated from 8 to 11 and 18 to 21 wk of age with a dosing holiday for both CEP-33779 and dexamethasone from 11 to 18 wk of age (i.e., total of 7 wk without treatment). Serum and urine were collected throughout the study. Assay design and other specifics concerning this model are described elsewhere (25).

BWF1 study. Study design and readouts are presented in Supplemental Fig. 1B. Age-matched, female, BWF1 or NZW/LacI mice were matured for the study of SLE nephritis for a total of 210 d, at which time urine was collected for proteinuria detection. Mice with 0.5–1.0 mg/ml of urine protein as determined by an OD-based total protein precipitation assay or 30–300 mg/dl of protein as determined by a stick assay were considered proteinuria positive and selected for study entry. Groups were normalized to contain a Gaussian distribution of proteinuria-positive animals (i.e., one-third low proteinuria, one-third medium, one-third high), then randomized between groups before ear marking and taking baseline measurements including urine and serum collections. A total of five mice from the total population were randomly selected for baseline kidney histology evaluation. Treatments and tests for each group are described in Supplemental Fig. 1. Treatments started on day of age 212. Some mice died shortly after treatment, but all animals regardless of health status were counted against the total group size from time of dosing. It is important to note that all graphs shown in this report show day 0 as being the “start of treatment,” which represents 212 d of age. Day 98 or “end of treatment/study” represents 310 d of age for the BWF1 animals. All graphs show time as “day on study” with day on study starting at 212 d of age and ending at 310 d of age. Assay design and other specifics concerning this model are described elsewhere (25).

ANA ELISA assays

The measurement of serum anti-dsDNA and anti-Smith Abs was performed by an in-house–developed custom ELISA assay described elsewhere (25). Chromatin-coated plates were purchased from Inviva Diagnostics. Purified bovine thymus dsDNA (Sigma) or purified bovine Smith Ag (GenWay, San Diego, CA) were used as coating Ag for the detection of anti-dsDNA and anti-Smith Ag Ab, respectively. ELISA development details: coated plates were washed with borate sulfate saline and blocked with borate sulfate saline containing 1% BSA and 0.1% Tween 20 detergent. Standard curves were generated using mouse anti-chromatin Ab (2B1; Sigma) or 25-wk-old MRL/lpr serum. Mouse anti-dsDNA Ab (Abcam, Cambridge, MA) or mouse anti-Smith Ab (Abcam) were used as standards for each assay. Secondary Ab was purchased from Abcam (goat anti-mouse pAb-HRP), and the substrate tetramethylbenzidine was purchased from Rockland (Gilbertsville, PA), and stop reaction buffer was generated by diluting 1 ml concentrated sulfuric acid into 20 ml dH2O. Developed plates were read using a Victor-X spectrophotometer reading at 450 nm with a reference wavelength of 570 nm.

Ab-secreting B cell ELISPOT assays

Complete media consisted of RPMI 1640, 1% Pen-Strep, 1% l-Glu, 1% nonessential amino acids, 2-mercaptoethanol, and 10% FBS (all from Cellgr, Manassas, VA). B cell ELISPOT components were ordered from MabTech (Nacka Strand, Sweden) and nitrocellulose immunoprecipitation filter plates obtained from Millipore (Billerica, MA). ELISPOT wells were coated with either purified bovine thymus dsDNA (Sigma), purified bovine Smith Ag (GenWay), or boiled filtered purified chicken chromatin from lysed chicken RBCs (Rockland) at 10 µg/ml. Spleens were processed using glass homogenization, filtered through a 60-µm sterile cell strainer, and RBCs lysed using BioLegend lysis buffer (BioLegend, San Diego, CA). Processed splenoocytes were added to each well in culture medium. Anti-mouse pan-IgG was used as a positive control for total IgG-producing Ab-secreting cells (ASCs) and used to normalize results.

Luminex analysis of serum cytokine samples

For the processing of serum samples for cytokine analysis, frozen plasma at −80°C was thawed on ice, vortexed, and centrifuged for 10 min to remove debris and aggregates. A total of 25–50 µl serum was used for Luminex assays following the manufacturer’s instructions. Cytokines were measured using the mouse cytokine 10-plex (MRL/lpr) or 20-plex (BWF1) bead kit [no. LMC0001 (10-plex), no. LMC0006 (20-plex); Invitrogen, Carlsbad, CA]. Assay details: filter plates (no. MAIPSWU10; Millipore) were pre-wet with 200 µl wash solution (kit component) and 25 µl beads was added per well. Serum samples were diluted 1:10 in assay diluent. A total of 50 µl was added per well (i.e., 25 µl sample serum plus 25 µl assay diluent). Plates with beads were incubated for 2 h at room temperature on an orbital shaker in the dark. At the end of the incubation, plate(s) were
washed twice in buffer, and secondary biotinylated Ab was added at a 1:10 dilution (100 µl) in biotin diluent provided with the kit. Plates were incubated at room temperature for 1 h in the dark then washed twice in buffer. Streptavidin in assay diluent was added at 100 µl per well, then incubated for 30 min at room temperature in the dark. The plates were washed three times, then 100 µl wash solution was added and agitated for 2–3 min at room temperature in the dark. Plates were run immediately after this incubation period on a Luminex xMAP 200 U with data acquisition and analysis software (no. MAP200; Invitrogen, San Diego, CA). All bead washing was performed using a vacuum manifold unit (no. 5017; Pall, Ann Arbor, MI). For all cytokine Luminex assays, values below the limit of detection set by the lowest point along the standard or set by the manufacturer were considered out of range and were not estimated but assumed the lowest point along the standard curve for that particular assay.

Flow cytometry

Washed, RBC-lysed splenocytes were stained for plasma cell markers as previously used in published reports (14, 26). End of study analyses from long-term lupus experiments using the MRL/lpr and BWF1 lupus mice (Fig. 6, Supplemental Fig. 4) gated plasma cells as live using forward scatter and side scatter gates, CD19 and B220/CD45R double-negative, CD38- and CD138-positive events. A total of 300,000–500,000 events were collected per tube/sample. Flow staining protocol was as follows: briefly, cells were suspended in complete medium (a medium without Fc receptors, and 5 µg of anti-CD16/CD32 (FcBlock) with fluorochrome-conjugated Abs (see later). After 20 min of staining on ice, samples were washed then fixed for flow cytometry. All samples were replicated with appropriate, matched isotype controls as described later. Abs used for flow cytometry consisted of anti-mouse CD19–FITC, anti-mouse CD38–PE, anti-mouse B220/CD45R double-negative, anti-mouse CD138–allophycocyanin, rat IgG1–allophycocyanin isotype control, and rat IgG2a–PE isotype control (all from eBioscience, San Diego, CA). For the long-lived/short-lived plasma cell experiment using BrdU labeling in BWF1 mice after only 2 wk of CEP-33779 inhibition in BWF1 mice, spleen and bone marrow were individually processed to isolate leukocytes for flow staining. Cells were surface stained with anti-CD19–phycocyanin isotype control and CD38- and CD138-positive events. A total of 300,000–500,000 events were collected per tube/sample. Flow staining protocol was as follows: briefly, cells were suspended in complete medium (a medium without Fc receptors, and 5 µg of anti-CD16/CD32 (FcBlock) with fluorochrome-conjugated Abs (see later). After 20 min of staining on ice, samples were washed then fixed for flow cytometry. All samples were replicated with appropriate, matched isotype controls as described later. Abs used for flow cytometry consisted of anti-mouse CD19–FITC, anti-mouse CD38–PE, anti-mouse B220/CD45R double-negative, anti-mouse CD138–allophycocyanin, rat IgG1–allophycocyanin isotype control, and rat IgG2a–PE isotype control (all from eBioscience, San Diego, CA). For the long-lived/short-lived plasma cell experiment using BrdU labeling in BWF1 mice after only 2 wk of CEP-33779 inhibition in BWF1 mice, spleen and bone marrow were individually processed to isolate leukocytes for flow cytometry. Cells were surface stained with anti-CD19–Cyc, anti-CD38–PE, and anti-CD138–allophycocyanin, and the BrdU kit included anti-BrdU–FITC. Analysis included gating on live by forward scatter/forward scatter populations, CD19-negative, CD38-positive, and gating on CD138-positive, BrdU-negative (noncycling, long-lived plasma cells, region R2) or BrdU-positive (cycling, short-lived plasma cells, region R1) as shown in Fig. 7E. All samples were analyzed using an Accuri C6 Flow Cytometer. Complete media (R10) was used for all experiments depending on the experiment and tested hypothesis. A data acquisition. Mann–Whitney non-parametric and one- or two-way ANOVA were used as statistical tests where noted in the figure legends depending on the experiment and tested hypothesis. A p value <0.05 was considered significant. Statistical software used was GraphPad Prism (version 5.01), and calculations were performed using the 2003 Office Professional version of Microsoft Excel.

Results

CEP-33779 inhibition of JAK2 can protect MRL/lpr SLE mice from developing glomerulonephritis

Studies were performed according to the study design in Supplemental Fig. 1A. Because of the hyperproliferation of T and B cells, both splenomegaly (spleen swelling) and lymphomegaly (lymph node swelling) are common in MRL/lpr mice. MRL/lpr mice treated with CEP-33779 exhibited reduced lymphomegaly at the 55 mg/kg and 100 mg/kg dose levels but not at the 30 mg/kg dose level (100% without lymphomegaly for 100 mg/kg and 55 mg/kg, 0 for vehicle; p < 0.05) (Fig. 1A). Reduced splenomegaly (i.e., spleen mass) was also observed at the 100 mg/kg and 55 mg/kg doses (p < 0.001 and p < 0.05, respectively) compared with vehicle (49.4% decrease for 55 mg/kg and 74.1% decrease for 100 mg/kg).
versus vehicle) (Fig. 1A). The highest dose of CEP-33779 tested, 100 mg/kg, decreased spleen mass to the level of that of non-SLE control animals (MRL/Mp; 98 mg; Fig. 1B, top panel) used for comparison. Similar values were recorded for spleen number, as size was proportionate to total cell number for treated MRL/lpr mice (Fig. 1B, bottom panel). Both the 55 mg/kg and 100 mg/kg doses of CEP-33779 reduced total leukocyte counts below that of vehicle (p < 0.05) but not as low as that of the non-SLE MRL/Mp animal (Fig. 1B, bottom panel). The rate of body weight gain did not change significantly for any group tested (Fig. 1C). Serum IL-12 could be modulated depending on the treatment period, as IL-12 cytokine levels fell only during treatment phases from 8 to 11 wk of age and from 18 to 21 wk of age but returned to baseline levels during treatment holidays, 11 to 18 wk of age (Fig. 1D; *p < 0.05, **p < 0.01, 56.1% decrease for 100 mg/kg and 50.7% decrease for 55 mg/kg compared with vehicle). Compound levels were similar in plasma, spleen, and kidney of diseased MRL/lpr animals 4 h after oral administration of a single dose of CEP-33779 (Supplemental Fig. 2). Dose-dependent increases in plasma levels of CEP-33779 were observed, and greater than dose-proportional increases were measured in the tissues between 55 and 100 mg/kg.

In SLE, the level of complement C3 in the serum is inversely associated with the magnitude or extent of systemic inflammation, and polymorphisms in complement or receptors are highly linked to SLE risk factors (29–31). Treatment with either 55 mg/kg or 100 mg/kg CEP-33779 significantly increased serum C3 concentration relative to vehicle (52.5% increase for 100 mg/kg and 51.4% increase for 55 mg/kg over vehicle; *p < 0.05) (Fig. 2A). End-stage SLE nephritis was evaluated by histopathology and scored by a board-certified pathologist for the assessment of total renal damage in diseased animals. Decreases in both cellular infiltration/glomerulonephritis and glomerular size were noted in H&E-stained renal sections from all three CEP-33779 treatment groups compared with both the dexamethasone and vehicle treatment groups (Fig. 2B). At the highest dose of CEP-33779, significant reductions in histopathology scores relative to vehicle controls were reported for glomerular cellularity (33.3% decrease; p < 0.01), interstitial infiltration (40% decrease; p < 0.01), and vasculitis (46.1% decrease; p < 0.01), similar to the profile for dexamethasone (Table I). Interstitial infiltration in the 55 mg/kg treatment group decreased significantly compared with that in vehicle-only (33.3% decrease compared with vehicle; *p < 0.05) (Table I). Inhibition of p-STAT3 was used as a downstream pharmacodynamic marker of JAK2 inhibition. Significant reductions in renal p-STAT3 were observed at both the 55 mg/kg and 100 mg/kg doses of CEP-33779 in MRL/lpr mice 4 h after final.
FIGURE 2. CEP-33779 treatment protects MRL/lpr mice from developing SLE nephritis. A, Serum samples from weekly samples from treated MRL/lpr mice were analyzed using a C3 complement ELISA. Graph shows mean ± SEM. One-way ANOVA used as statistical test. B, EOS (day 98) H&E-stained paraffin wax-embedded kidney sections from treated MRL/lpr mice. Arrows indicate enlarged glomerulus and leukocytic infiltrates/glomerulonephritis. Representative images shown from n = 12 samples that were later scored, with results displayed in Table I. PAS and trichrome stains not shown but were used for scoring. C, Phosphorylated STAT3 levels from kidney extracts from treated mice at EOS day 98, 4 h after oral dosing, were determined using Luminex kits. Graph shows mean ± SEM. Mann–Whitney two-tailed statistical test was used to determine p value (p < 0.05 was considered significant). Dex, dexamethasone.

dosing of drug at end-of-study (EOS) day 147 of age (day 91 of study) (Fig. 2C). Thus, treatment of MRL/lpr mice with CEP-33779 over time was able to slow and/or prevent the development of SLE nephritis in SLE-prone mice.

CEP-33779 inhibition of JAK2 can reverse SLE nephritis disease in proteinuria-positive BWF1 mice

Studies were performed according to the study design in Supplemental Fig. 1B. NZB/W F1, or New Zealand Merged (BWF1), mice were matured and monitored via both serum and urine for the presence of SLE-related biomarkers, the presence of ANAs in serum, and degree of proteinuria. Mice were screened monthly for proteinuria levels above 0.5 mg/ml of total protein or 30–300 mg/dl by Uristix urine stick assays (both assays were performed on all samples and cross-checked for consistency). When >90% of the mice reached the total urine protein levels of ≥0.5–1.0 mg/ml (or 30–300 mg/dl as defined by Uristix), animals were randomized, and baseline urine and serum measurements were collected before initiation of treatment. Survival of BWF1 mice treated with 55 mg/kg or 100 mg/kg CEP-33779 was significantly extended over that of vehicle (70% survival for 100 mg/kg and 90% survival for 55 mg/kg at EOS; p < 0.01) and comparable with that of standard-of-care agent CTX (70% survival at EOS; p < 0.001 versus vehicle) (Fig. 3A). The presence of anti-dsDNA Abs is used as a clinical biomarker associated with a poor prognosis of SLE and is strongly associated with developing, and often fatal, lupus nephritis (32, 33). Treatment with CEP-33779 reduced the overall levels of serum anti-dsDNA ANAs throughout the course of disease (73.9% decrease for 100 mg/kg, 67.0% decrease for 55 mg/kg compared with the vehicle; p < 0.01) and was on par with standard-of-care agent CTX (65.3% decrease compared with the vehicle; p < 0.001) (Fig. 3B). Changes in other ANAs were also observed, including significant reduction

Table I. Renal scores from CEP-33779–treated MRL/lpr mice

<table>
<thead>
<tr>
<th>MRL/lpr SLE Model Group</th>
<th>Glomerular Cellularity</th>
<th>Glomerular Necrosis</th>
<th>Glomerulosclerosis</th>
<th>Interstitial Infiltration</th>
<th>Tubular Atrophy</th>
<th>Interstitial Fibrosis</th>
<th>Vasculitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.60 ± 0.55</td>
<td>2.60 ± 0.55</td>
<td>2.40 ± 0.89</td>
<td>3.00 ± 0.71</td>
<td>2.00 ± 0.00</td>
<td>2.00 ± 0.00</td>
<td>2.60 ± 0.55</td>
</tr>
<tr>
<td>CEP-33779 30 mg/kg</td>
<td>2.60 ± 0.55*</td>
<td>1.80 ± 0.45</td>
<td>1.60 ± 0.55</td>
<td>2.20 ± 0.45</td>
<td>1.20 ± 0.45</td>
<td>1.60 ± 0.55</td>
<td>1.80 ± 0.45</td>
</tr>
<tr>
<td>CEP-33779 55 mg/kg</td>
<td>3.20 ± 0.45</td>
<td>1.80 ± 0.45</td>
<td>2.00 ± 0.00</td>
<td>2.00 ± 0.71*</td>
<td>2.20 ± 0.45</td>
<td>1.20 ± 0.45</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>CEP-33779 100 mg/kg</td>
<td>2.40 ± 0.55**</td>
<td>1.8 ± 0.45</td>
<td>2.20 ± 0.84</td>
<td>1.80 ± 0.84**</td>
<td>1.40 ± 0.55</td>
<td>1.40 ± 0.55</td>
<td>1.40 ± 0.55**</td>
</tr>
<tr>
<td>Dexamethasone 1.5 mg/kg</td>
<td>3.00 ± 0.71</td>
<td>2.00 ± 0.00</td>
<td>2.00 ± 0.00</td>
<td>1.60 ± 0.55**</td>
<td>1.20 ± 0.45</td>
<td>1.20 ± 0.45</td>
<td>1.40 ± 0.55**</td>
</tr>
</tbody>
</table>

Pathologist scored H&E-, PAS-, and trichrome-stained paraffin-embedded kidney sections displayed as mean ± SD, n = 5 mice per group shown. Pathologist scoring method and definitions can be found in Materials and Methods. Statistics were performed using a two-tailed Mann–Whitney t test.

*p < 0.05, **p < 0.01, ***p < 0.001 (compared with vehicle).
Table II. Renal scores from CEP-33779–treated BWF1 mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glomerular Cellularity</th>
<th>Glomerular Necrosis</th>
<th>Glomerulosclerosis</th>
<th>Interstitial Infiltration</th>
<th>Tubular Atrophy</th>
<th>Interstitial Fibrosis</th>
<th>Vasculitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.22 ± 0.83</td>
<td>2.11 ± 0.60</td>
<td>2.55 ± 1.33</td>
<td>2.44 ± 1.33</td>
<td>2.66 ± 1.22</td>
<td>2.44 ± 1.33</td>
<td>1.20 ± 0.44</td>
</tr>
<tr>
<td>CEP-33779 55 mg/kg</td>
<td>1.33 ± 0.50***</td>
<td>1.22 ± 0.44</td>
<td>1.00 ± 0.0*</td>
<td>1.11 ± 0.33</td>
<td>1.22 ± 0.44</td>
<td>1.0 ± 0.0*</td>
<td>1.11 ± 0.33</td>
</tr>
<tr>
<td>CEP-33779 100 mg/kg</td>
<td>1.44 ± 0.52***</td>
<td>1.44 ± 0.72</td>
<td>1.22 ± 0.66</td>
<td>1.22 ± 0.44</td>
<td>1.33 ± 0.50</td>
<td>1.22 ± 0.44</td>
<td>1.44 ± 0.52</td>
</tr>
<tr>
<td>CTX 50 mg/kg</td>
<td>2.0 ± 0.70*</td>
<td>1.77 ± 0.83</td>
<td>2.11 ± 1.16</td>
<td>1.55 ± 1.01</td>
<td>2.00 ± 1.32</td>
<td>1.55 ± 1.01</td>
<td>1.33 ± 0.50</td>
</tr>
<tr>
<td>Dexamethasone 1.5 mg/kg</td>
<td>3.0 ± 0.81</td>
<td>3.0 ± 0.81</td>
<td>3.42 ± 1.27</td>
<td>2.71 ± 0.47</td>
<td>3.14 ± 1.46</td>
<td>2.71 ± 1.25</td>
<td>2.42 ± 1.13</td>
</tr>
<tr>
<td>BWF1 7-mo baseline</td>
<td>2.50 ± 1.00</td>
<td>1.50 ± 0.57</td>
<td>2.00 ± 0.81</td>
<td>1.50 ± 1.00</td>
<td>2.00 ± 1.41</td>
<td>2.00 ± 1.41</td>
<td>1.75 ± 0.50</td>
</tr>
<tr>
<td>Non-lupus control</td>
<td>1.40 ± 0.89**</td>
<td>1.20 ± 0.44</td>
<td>1.20 ± 0.44</td>
<td>1.20 ± 0.44</td>
<td>1.40 ± 0.89</td>
<td>1.20 ± 0.44</td>
<td>1.20 ± 0.44</td>
</tr>
</tbody>
</table>

Pathologist scored H&E-, PAS-, and trichrome-stained paraffin was embedded kidney sections displayed as mean ± SD, n = 10 mice per group shown (except for non-SLE control mice = 4 mice only). Pathologist scoring method and definitions can be found in Materials and Methods. Statistics were performed using a two-tailed Mann–Whitney test.

*p < 0.05, **p < 0.01, ***p < 0.001 (compared with vehicle).
FIGURE 4. CEP-33779 treatment reverses lupus nephritis in BWF1 mice. Paraffin wax-embedded H&E-stained sections of 10-mo-old BWF1 mice treated with CEP-33779 and standards of care. Representative images from 10 to 11 samples per group; scoring of images shown in Table II. A, Kidney, arrows mark enlarged glomeruli, hypercellularity, and leukocytic infiltrates and/or glomerulonephritis. B, Representative images from H&E-stained paraffin wax-embedded lungs from treated BWF1 mice at the end of the study. Arrows point to examples of pulmonary infiltrates and vasculitis. In some instances, adenomas were present in vehicle-treated samples. Samples were not scored, pathologist’s comment withheld. C, p-STAT3 levels from 10-mo-old BWF1 mouse kidney extracts analyzed by Luminex 4 h post dosing. Mann–Whitney test: **p < 0.01.

CEP-33779 decreases several SLE-associated proinflammatory cytokines and reduces levels of a bone resorption biomarker associated with increased osteoclast activity

As a systemic autoimmune disease, lupus involves both the innate and adaptive immune arms. Thus, both early and late proinflammatory cytokines are thought to be involved and can be monitored over time during disease treatment. Reduction or modulation in these cytokine profiles can provide a favorable benefit for the patients and thus were monitored in the BWF1 mouse model as an indirect indicator of disease resolution. Treatment of proteinuria-positive BWF1 mice with CEP-33779 significantly reduced several serum cytokines associated with SLE progression (Table II, Supplemental Fig. 3). Both doses, 100 mg/kg and 55 mg/kg, of CEP-33779 significantly reduced IL-12 (99.4 and 99.9% decrease; p < 0.001; Fig. 5A), IL-17A (79.1 and 86.2% decrease; p < 0.001; Fig. 5B), IL-6 (98.2 and 97.0% decrease; p < 0.001; Fig. 5C), CCL3/MIP-1α (94.5% decrease for both 100 mg/kg and 55 mg/kg; p < 0.01; Fig. 5D), CXCL10/IP-10 (47.3 and 80.0% decrease; p < 0.01; Fig. 5E), CXCL9/MIG (83.7 and 85.7% decrease; p < 0.001; Fig. 5F), IL-4 (p < 0.01; Supplemental Fig. 3A), IL-13 (p < 0.001; Supplemental Fig. 3B), TNF-α (p < 0.001; Supplemental Fig. 3C), and KC/IL-8 (p < 0.001; Supplemental Fig. 3D) compared with vehicle. Of note, several cytokines dropped significantly compared with those in vehicle controls in response to both standards of care, CTX and dexamethasone, evaluated in this study. The effect of treatment with CEP-33779 on several cytokines mirrored that of treatment with CTX and dexamethasone (Fig. 5B, 5D, 5F). Reductions in splenomegaly (i.e., spleen mass) followed cytokine profiles, in that both CEP-33779 doses of 55 mg/kg and 100 mg/kg significantly reduced total EOS spleen mass of treated BWF1 animals (73.5% decrease for 100 mg/kg and 71.8% decrease for 55 mg/kg; p < 0.001). The magnitude of reduction seen with CEP-33779 was comparable with that of CTX as compared with vehicle (data not shown). No change in body mass was observed for either dose of CEP-33779 evaluated (data not shown).

CEP-33779 inhibition of JAK2 decreases autoantibody-producing plasma cells in SLE-prone MRL/lpr and lupus nephritis-prone BWF1 mice

With prior knowledge that the IL-6R is primarily controlled by the JAK–STAT pathway (i.e., JAK2/STAT3) and that IL-6 plays a major role in the survival of LL-PCs, CEP-33779 was used to test the effect of blockade of JAK2 on the development of autoantibody-producing cell types, most importantly plasma cells (defined in this article as CD19+, CD45R/B220+, CD138hi, CD38hi) (26, 34, 35). Treatment of MRL/lpr mice with CEP-33779 resulted in a decrease in spleen plasma cells at the highest dose tested, 100 mg/kg (65.6% decrease; p < 0.01; Supplemental Fig. 4A). Reduction in the frequency of antichromatin ASCs in the spleens of treated MRL/lpr SLE mice was observed for only the 100 mg/kg dose of CEP-33779 (89.1% decrease; p < 0.001; Supplemental Fig. 4B). Treatment of BWF1 mice recapitulated the results of the MRL/lpr model in that spleen plasma cells were reduced upon twice-daily oral treatment with both 100 mg/kg and 55 mg/kg CEP-33779 (47.1% and 57.0% decrease, respectively; p < 0.05; Fig. 6A). As expected, reduced levels of ASCs specific for chromatin were observed for both doses of CEP-33779 and were on par with CTX (97.4, 100, and 100% decrease, respectively; p < 0.05; Fig. 6B). These results suggest that the targeting of autoantibody-producing cells, in particular that of plasma cells, may be sensitive to JAK2 blockade and that this may be an alternative or additional therapeutic target for the treatment of SLE.

To investigate this question further, whether in fact inhibition of JAK2 using CEP-33779 can significantly impact the levels of short-versus long-lived plasma cells, additional studies were performed using the traditional method of in vivo BrdU incorporation as previously performed in published lupus studies (26). Fifteen-week-old BWF1 lupus-prone mice were treated with 30, 55, or 100 mg/kg CEP-33779, orally, twice daily, standard of care reference agent CTX, 50 mg/kg, once a week, or vehicle, PEG 400,
Table III. Comparisons of renal scores, anti-dsDNA Ab titers, and proteinuria levels for treated BWF1 mice

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Table III. (Continued)

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**Mean ± SD**

|    | 284.9 ± 40.9 | 30.9 ± 22.2 |

Dexamethasone 1.5 mg/kg, three times per week

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**Mean ± SD**

|    | 261.4 ± 37.6 | 42.7 ± 51.0 |

*Age at full life or EOS date.

Scores for individual mice for kidney sections stained with H&E, PAS, and trichrome stains: –, no disease; +, mild; ++, moderate; ++++, high; +++++, severe disease. See Materials and Methods for full description of each score and pathologist definitions.

Proteinuria concentrations from total urine protein assays. Values >1 mg/ml are set in boldface. Null values such as “0.00” were considered “below quantifiable levels” for the assay.

**p < 0.01, ***p < 0.001 (versus vehicle, two-tailed Mann–Whitney t test).**

ND, not determined.
orally, twice daily, for a total of 2 wk. Thymidine analogue BrdU was provided at 2 mg, i.p., in saline 24 h before the end of the study. Spleen and bone marrow were processed for live, CD19-negative, CD38/CD138 double-positive plasma cells that were detected as cycling (BrdU+, short-lived, region R1) or noncycling (BrdU−, long-lived, region R2) cells. No reduction in short-lived spleen plasma cells was observed, only a significant reduction for CTX-treated mice (*p < 0.05). Reduction in bone marrow short-lived plasma cells for 100 mg/kg (65.3% reduction, ***p < 0.001) was on par with that of the CTX treatment (Fig. 7B). All doses of CEP-33779 could reduce the levels of spleen long-lived plasma cells (57–70% reduction, *p < 0.05), but only the highest dose of CEP-33779 at 100 mg/kg could impact long-lived plasma cell levels in the bone marrow (69% reduction, *p < 0.05) most likely due to the protective environment of the bone marrow (36).

Discussion

Patients with SLE suffer a broad range of symptoms that primarily stem from systemic autoimmunity leading to several rheumatologic and often painful outcomes such as malaise, arthritis, and in severe cases cardiac, pulmonary, renal, and CNS involvement. Current treatments work, however not without side effects, and maintenance therapy with high-dose glucocorticoids helps temporarily to relieve symptoms but over time can lead to severe bone loss and physical impairment. Because many SLE patients suffer from debilitating symptoms that limit their ability frequently to access clinics to receive treatment, an orally available drug taken twice a day to relieve SLE-associated symptoms and possibly slow the progression of the underlying disease would be advantageous and improve patient compliance with therapy. This report describes the successful treatment of SLE disease in two mouse models using a highly selective, orally active JAK2 inhibitor, CEP-33779. Most importantly, established SLE-associated glomerulonephritis could be mitigated without observable toxicity. Several SLE-associated immune parameters were decreased upon treatment, including several proinflammatory cytokines, ANAs, and ASCs. These results demonstrate that a selective JAK2 inhibitor has the potential of treating connective tissue diseases such as SLE and suggests that the inhibition of JAK2 may have further implications in the treatment of similar autoimmune diseases where humoral immunity is exaggerated such as Sjögren’s syndrome or myasthenia gravis (37, 38).
To date, no other specific JAK kinase inhibitors have been reported for their effects on SLE. Results of published studies using nonselective pan-JAK kinase, and general pan-kinase, inhibitors (e.g., AG490) have been tested in the MRL/lpr or related models (39, 40). To date, most of the JAK-targeting activities have been centered on rheumatoid arthritis, psoriasis, myeloproliferative diseases, and cancer (41–45). Targeting cytokine/growth factor pathways important for plasma cell generation and SLE development is supported by the literature, and targeting the IL-6 pathway and receptor for SLE treatment is currently being tested (13, 46, 47). Targeting of the BAFF pathway has been successful, and studies in the field are under way to look at the role of APRIL in plasma cell generation (48). A possible next step is to target the IL-6R pathway via the blockade of JAK2 to better target the development and survival of autoreactive, pathogenic plasma cells during early SLE.

Using the MRL/lpr model as an acute-lupus system, glomerulonephritis could be prevented via the treatment of mice with the lowest dose of CEP-33779 tested, 30 mg/kg. It is possible that frequent low doses of a JAK2 inhibitor may be enough to prevent or slow glomerulonephritis, as p-STAT3 renal levels (an indicator of JAK2 activity) inversely correlated with rising C3 complement levels in the plasma of treated mice indicating that a pharmacological inhibition of JAK2 at the local level does translates into an effect on SLE at the systemic level. However, treatment of existing renal disease is more clinically relevant to SLE than protection, and this report clearly demonstrates that inhibition of JAK2 using CEP-33779 not only can protect mice from advancing to end-stage renal disease but also can treat spontaneous SLE mice with active glomerular disease (i.e., BWF1 mice). As shown in histology sections and pathologist scores from CEP-33779–treated mice, the levels of immune infiltrates and glomerular capsule size were both reduced, including the incidence of vasculitis and pulmonary infiltrates (Fig. 4A, Supplemental Fig. 2). These findings corresponded well with the reduced levels of p-STAT3 in affected kidneys similar to the findings for the MRL/lpr model. Treatment of BWF1 mice with CEP-33779 additionally protected mice from developing pulmonary adenomas, as observed in both the vehicle- and dexamethasone-treated mice (data not shown).

The treatment-related effect on SLE with CEP-33779 is more evident when looking at individual mouse proteinuria levels over time compared with those of vehicle controls (data not shown). Several mice entered studies with urine protein levels above those of the age-matched, non-SLE control mice, NZW/LacJ mice (Fig. 6A, top graph, dotted line), however, upon treatment with CEP-33779, fewer mice died (Fig. 5A), and many resolved renal issues as their levels of urine protein decreased to below those of the non-SLE controls. This reduction in proteinuria is less evident in the

**FIGURE 6.** CEP-33779 treatment decreases circulating plasma cells and ASCs. A. Top panel, Flow cytometry analysis of whole splenocytes stained with CD19–FITC, CD45R/B220–Cy, CD38–PE, CD138–allophycocyanin and plotted as a percentage of live size-gated lymphocytes. Bottom panel, Representative flow cytometry dot plots of live cell events gated on CD19^+ CD45R/B220^+ cells from splenocytes of CEP-33779–treated BWF1 mice. Plots show gating strategy and serve as representative examples of data analyzed. B. Top panel, ELISPOT analyses of ASCs from the spleens of BWF1 mice treated with CEP-33779 or standards of care. Graph shows counted spots per million splenocytes. ELISPOT wells coated with 10 μg/ml of chromatin Ag; media-alone spot numbers subtracted from final results. Bottom panel, Representative well images from ELISPOT data graphed in top panel. ELISPOTs were scanned on an Immunospot C.T.L. scanner and analyzed using spot analysis software. All graphs show mean ± SEM. One-way ANOVA or Mann–Whitney used as statistical test is indicated on plots, *p < 0.05. Dex, dexamethasone.
dexamethasone- and vehicle control-treated animals. It is important to note that this improvement observed with CEP-33779 was on par with that of one of the current treatments for lupus nephritis, CTX. Matching of individual mice with anti-dsDNA ANA levels (Ab titers directly associated with the development of lupus nephritis), histopathology scores, and urine protein levels, it was evident that as mice were treated with CEP-33779, their renal pathology resolved, an event associated with an increased life span and decreased proteinuria (Table III). The strength of these data is the consistent homogeneous response observed in the CEP-33779–treated mice, strongly suggesting that the efficient and potent blockade of JAK2 can effectively treat spontaneous SLE mice (closest to the human condition) with renal manifestations with favorable tolerability, thus extending disease-free survival.

Modulation of cytokine levels in BWF1 mice was investigated because of the overlapping role JAK2 plays in controlling and
interacting with several different cytokine pathways (49). Levels of several SLE-associated and promoting cytokines, including IFN-α, IL-12, IL-6, and CXCL10/IP-10, were decreased upon treatment with CEP-33779. This is important because these cytokines are involved in the augmentation of flares and ongoing immune responses to self-antigens perpetuating the disease (50–52). Reduction or modulation in these cytokine profiles can provide a favorable benefit for patients and may offer an indirect indicator of disease resolution. In addition, impact on T and B cell responses was expected, as well as impact on plasma cell differentiation and survival resulting in a decrease in Ab production. Decreases in anti-Smith, anti-dsDNA, and antichromatin ANAs were all observed in CEP-33779–treated BWF1 mice, consistent with this hypothesis. One of the most important findings is the impact of JAK2 inhibition on the formation of autoantibody-producing plasma cells in the spleens of treated animals. In addition, JAK2 inhibition was able to reduce the long-lived, usually radioresistant and chemoresistant, population of plasma cells from BWF1 lupus-prone mice. Using both ELISPOT assay and flow cytometry, consistent depletion of plasma cells was observed in both models when mice were treated with CEP-33779 (Figs. 5A, 7, Supplemental Fig. 4A). It is important to note that spleen plasma cell proportions decreased to the levels observed in non-SLE control mice but not below, suggesting that blockade of JAK2 with CEP-33779 restored the normal proportions of plasma cell frequencies. Reductions in ASCs were also observed for antichromatin splenocytes for both MRL/lpr and BWF1 models treated with CEP-33779 (Fig. 5, Supplemental Fig. 4A). Difference in susceptibility to different doses of CEP-33779 may partially be attributable to strain and mechanism of disease difference between the two models, as the MRL/lpr model is more dependent on the lack of the Fas molecule, whereas the BWF1 system is primarily a genetic model dependent on a variety of factors including environmental influences (1, 53). Long-lived plasma cells primarily reside in the bone marrow but can also be found at sites of inflammation and in the spleen (54). Targeting long-lived plasma cells is important because these cells are difficult to kill, are usually chemoresistant and radioresistant, and can survive for many decades within the bone marrow architecture, and produce the majority of autoantibodies during SLE. As Fig. 7 shows, both cycling (short-lived, region R1) and noncycling (long-lived, region R2) CD138+ plasma cells decrease upon treatment with CEP-33779. Only the 100 mg/kg upper dose of CEP-33779 could impact short- and long-lived plasma cells in the bone marrow possibly due to the niche survival environment that the bone marrow supports (36). All three doses were able to impact long-lived plasma cells in the spleen but not short-lived plasma cells. This suggests that two different mechanisms of plasma cell death may explain the differences in response between the two locations. For example, in the spleen, differentiation to long-lived plasma cells may be stunted because of the lack of appropriate helper cytokine signals (55).

The specific blockade of JAK2 could also help treat several of the pathological manifestations of SLE, including arthritis and dermatitis, and may even help prevent or treat some comorbidities, such as atherosclerosis, as JAK2 has demonstrated a role in the development of atherosclerotic plaques and is necessary for erythropoietin function in megakaryocytes (49, 56, 57). Cerebritis (CNS inflammation) is another major concern for SLE patients, and therapies for treatment are also very limited to treat these manifestations. The use of a brain-penetrant (data not shown), JAK2 inhibitor such as CEP-33779 may also have implications for the treatment of cerebritis, as this chronic inflammation in the brain is dependent on IL-6 and IFN-α (e.g., cytokines found in CSF of SLE patients with CNS involvement) (58). In conclusion, these studies in two distinct models of SLE indicate that the potent and selective JAK2 inhibitor CEP-33779 significantly mitigated multiple immune parameters associated with SLE development and progression, including the protection and management of mice with lupus nephritis. These data support the possibility of using potent, orally active, small-molecule inhibitors of JAK2 to treat the debilitating clinico-pathological manifestations of SLE.

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Disclosures
All authors of this study are employees of Cephalon, Inc.

References
17. Yu, C. C., A. A. Mamchak, and A. L. DeFranco. 2003. Signaling mutations and progression, including the protection and management of mice with lupus nephritis. These data support the possibility of using potent, orally active, small-molecule inhibitors of JAK2 to treat the debilitating clinico-pathological manifestations of SLE.
A. CEP-33779 Test in MRL/lpr Lupus Model Experiment

**Age:** 8 wks  
**Randomize cages:**  
**Stop Treatment Check ANAs**  
**Age:** 11 wks  
**Treatments:**  
1. MRL/lpr + CEP-33779-high (12)  
2. MRL/lpr + CEP-33779-med (12)  
3. MRL/lpr + CEP-33779-low (12)  
4. MRL/lpr + DEX (12)  
5. MRL/lpr + Vehicle (12)

**Groups**
1. MRL/lpr + CEP-33779-high (12)
2. MRL/lpr + CEP-33779-med (12)
3. MRL/lpr + CEP-33779-low (12)
4. MRL/lpr + DEX (12)
5. MRL/lpr + Vehicle (12)

**Treatments**
1. CEP-33779 = 100 mg/kg, b.i.d., p.o.
2. CEP-33779 = 55 mg/kg, b.i.d., p.o.
3. CEP-33779 = 30 mg/kg, b.i.d., p.o.
4. DEX = Dexamethasone = 1.5 mg/kg, 3x wk, i.p.

**Read-outs:**
- **TIME POINTS:** ANA antibodies (Sm, dsDNA, Chromatin)
- **TIME POINTS:** Urine protein – Assay and sticks
- **EOS:** Serum cytokines (Luminex)
- **EOS:** PK – Blood/Spleen/Kidney; PD – Spleen/Kidney
- **EOS:** Spleen B cell Elispots (B: dsDNA, Sm, Chromatin)
- **EOS:** Kidney pathology - fixed and frozen

8 wks old

Randomize cages  
Stop Treatment Check ANAs  
Start Treatment  
Stop Check Urine

---

B. CEP-33779 Test in NZBWF1 (BWF1) Lupus Model Experiment

**Proteinuria positive (30-100mg/dl)**

**Proteinuria negative**

**Randomization cages**

**Treatments**

**Study end**

**Initial measurements, bleeds, tissue sampling**

**Remove 5 mice for baseline**

**Groups**
1. BWF1 + CEP-33779 Group-high (10)
2. BWF1 + CEP-33779 Group-low (10)
3. BWF1 + CTX (10)
4. BWF1 + DEX (10)
5. BWF1 + Vehicle (11)

**Treatments**
1. CEP-33779 = 100 mg/kg, b.i.d., p.o.
2. CEP-33779 = 55 mg/kg, b.i.d., p.o.
3. CTX = Cyclophosphamide = 50 mg/kg, 1x wk. i.p.
4. DEX = Dexamethasone = 1.5 mg/kg, 3x wk. i.p.
5. Vehicle = Solutol/PBS/DMSO, 1x wk. i.p.

**Read-outs:**
- **TIME POINTS:** ANA antibodies (Sm, dsDNA, Chromatin), C3 levels (EUSA)
- **TIME POINTS:** Urine protein – Assay and sticks, Urine Biomarker
- **EOS:** Serum cytokines (20-plex Luminex), Serum CTX (EUSA)
- **EOS:** PD – Spleen/Kidney (pSTAT3)
- **EOS:** Spleen B cell Elispots: dsDNA, Sm, Chromatin, total IgG
- **EOS:** Spleen Plasma cells
- **EOS:** Kidney pathology - fixed, scoring
Figure S2

CEP-33779 PK at 4 hours

Concentration (ng/ml)

Plasma  Spleen  Kidney

CEP-33779 30 mg/kg
CEP-33779 55 mg/kg
CEP-33779 100 mg/kg
Figure S3

A. Serum IL-4

```
**p<0.01 vs. Vehicle; 1-way ANOVA
```

B. Serum IL-13

```
***p<0.001 vs. Vehicle; 1-way ANOVA
```

C. Serum TNFα

```
**p<0.01 vs. Vehicle; 1-way ANOVA
```

D. Serum KC/IL-8

```
***p<0.001 vs. Vehicle; 1-way ANOVA
```
Figure S4

A. Spleen Plasma Cells

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>CEP-33779 30 mg/kg</th>
<th>CEP-33779 55 mg/kg</th>
<th>CEP-33779 100 mg/kg</th>
<th>Dex 1.5 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>Percent of live lymphocytes</td>
<td><strong>p&lt;0.01 vs Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
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</table>

B. Anti-Chromatin ASCs

<table>
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<th>CEP-33779 30 mg/kg</th>
<th>CEP-33779 55 mg/kg</th>
<th>CEP-33779 100 mg/kg</th>
<th>Dex 1.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spots/million spleen cells</td>
<td>*p&lt;0.05; <strong>p&lt;0.001 vs Vehicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mouse Number

1. Vehicle
2. CEP-33779 100 mg/kg
3. CEP-33779 55 mg/kg
4. CEP-33779 30 mg/kg
5. Dex 1.5 mg/kg
Supplemental Figure Legends

Figure S1. Experimental study design of MRL/lpr and NZM treatments

(A) Treatment schedule of MRL/lpr mice with CEP-33779, dexamethasone and vehicle. Mice were treated from 8-11 and 18-21 weeks with a treatment holiday from 11-18 weeks of age. EOS: End of Study. (B) Treatment schedule of NZM mice with CEP-33779, cyclophosphamide, dexamethasone and vehicle. Seven month old NZM mice were treated for the entire length of the study. Observations and serum/urine collections were performed as described in figure.

Figure S2. PK of CEP-33779 in MRL/lpr Mice
Pharmacokinetics (PK) of CEP-33779 four hours post oral administration of 30, 55 and 100 mg/kg of compound in the plasma, spleen and kidney. Graph shows concentration of compound in ng/ml as mean±SEM, significance not determined.

Figure S3. Serum cytokines from treated NZM mice during SLE
Cytokine levels as determined by Luminex assays from serum samples of treated NZM mice. (A) IL-4, (B) IL-13, (C) TNFα, (D) KC/IL-8. 1-way ANOVA, graphs show mean±SEM, *p<0.05, **p<0.01, ***p<0.001.

Figure S4. CEP-33779 reduces plasma cells in MRL/lpr SLE mice
(A) Top panel, Flow cytometry analysis of whole splenocytes stained with CD19-FITC, CD45R/B220-Cyc, CD38-PE, CD138-APC and plotted as a percent of live size gated lymphocytes. Bottom panel, representative flow cytometry dot plots of live cell events gated on CD19- CD45R/B220- cells from splenocytes of isotype controls, vehicle-treated or CEP-33779-treated MRL/lpr mice. Plots show gating strategy and serve as representative examples of data analyzed. (B) Top panel, Elispot analyses of antibody secreting cells from the spleens of CEP-33779 treated MRL/lpr mice. Graph shows counted spots per million splenocytes. Elispot wells coated with 10 μg/ml of chromatin antigen, media alone spot numbers subtracted from final results. Bottom panel, representative well images from Elispot data graphed in top panel. Elispots were scanned on an Immunospot C.T.L. scanner and analyzed using spot analysis software. All graphs show mean±SEM, 1-way ANOVA or Mann-Whitney used as statistical tests were indicated on plots, *p<0.05, **p<0.01, ***p<0.001.