SUPPLEMENTARY MATERIALS AND METHODS

MRL/lpr mouse model

Animals

Four-week-old female MRL/MpJ-Faslpr/J mice (Jackson Laboratories) were group-housed under climate-controlled conditions with a 12-hour light / dark cycle, a standard temperature of 20 ± 3°C and appropriate environmental enrichment (cardboard, tissues, red polycarbonate houses, and seeds) in the cages. Mice had free access to food and drinking water ad libitum.

All experimental procedures were conducted in accordance with the Swiss animal welfare ordinance and Idorsia Animal Welfare policy on the use of experimental animals. The study was approved by the Basel-land Cantonal Veterinary Home Office.

Study procedure

Cenerimod (ACT-334441, ((S)-3-(4-(5-(2-cyclopentyl-6-methoxypyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)propane-1,2-diol, Idorsia Pharmaceuticals Ltd) was prepared as a mix in the regular food pellet (Granovit AG) at a final concentration of 200 mg/kg food. At this concentration and based on a daily food intake of 2-4g per animal, the mice would receive a daily dose of 20-40 mg/kg/day, which is 2–4 times the dose needed to obtain maximal constant lymphopenia at all times (10 mg/kg). The food pellet alone was used as the control treatment (vehicle).

At 6 weeks of age, mice were randomly assigned to the vehicle or cenerimod group (n=20 per group) based on body weight (treatment week 0) and started receiving vehicle or cenerimod treatment one week later (treatment week 1). Treatment continued until the end of the study, which was predefined as the time point when at least 20% morbidity/mortality was reached in one group (treatment week 11).

Blood was collected at treatment week 11 into EDTA tubes and plasma prepared with centrifugation for 5min at 14000rpm at 4°C (Eppendorf Centrifuge 5417R). Urine samples were collected over 24 hours one week before sacrifice (treatment week 10) when 20% mortality was reached in one group.

At treatment week 11 (17 weeks of age), all mice were euthanized with an overdose of intraperitoneal 150 mg/kg injection of pentobarbital natricum (Streuli Pharma SA), perfused with PBS at 37°C (Bioconcept AG) and necropsied. Organs were collected and weighed.

All *in vivo* readouts and histological data analyses were performed in a blinded fashion.

Immunophenotyping

Leukocytes were collected from peripheral blood. Erythrocytes from blood were lysed using RBC Lysis buffer (Biolegend) according to the manufacturer's instructions and single-cell suspensions were reconstituted in PBS.

Suspensions were incubated with a purified anti-mouse CD16/32 antibody (Fc Block, 2.4G2) for 10 min followed by staining for 30 min with an antibody cocktail specific for the different leukocyte subsets including B cells and T cells. The following antibody conjugates were purchased from Biolegend: PE-Cy7 anti-mouse β TCR (H57-597) and AF700 anti-mouse CD19 (6D5). The following antibodies were purchased from BD Bioscience: purified anti-mouse CD16/32 (Fc Block) (2.4G2), AF488 anti-mouse CD45R (B220), PerCP-Cy5.5 anti-mouse CD8a (53-6.7), PB anti-mouse CD4 (RM4-5). The cells were washed and stained with propidium iodide (Calbiochem) to identity dead cells. The cells were acquired on a Gallios Cytometer (Beckman Coulter) and absolute number determined using Trucount Tubes (BD Bioscience).

Analysis was performed using Kaluza analysis 1.5a software. For analysis, only live cells were gated followed by doublet exclusion. B cells were defined as CD19+B220+. T cells were defined as TCRβ+CD19- and the T-cell subpopulations as CD4+CD8- (CD4+ T cells), and CD4-CD8+ (CD8+ T cells).

Urine protein measurements

Albumin concentration was determined by mouse urinary albumin detection kit according to the manufacturer’s protocol (Chondrex). Urine samples were diluted in buffer, at five-fold serial dilutions starting at 1:1000 to 1:78125000; 4 dilutions/sample were added to

the plate. A minimum of 2 dilutions (mean) was used to determine the albumin concentration.

To normalize albumin concentration, the creatinine concentration was also measured using Creatinine Colorimetric Detection Kit according to the manufacturer's protocol (Enzo Life Sciences). Urine samples were diluted 1:20 in distilled water. The absorption of both assays was measured at 490 nm using Spectromax 384 microplate reader (Spectromax Molecular Devices).

Histology and immunohistochemistry (IHC)

After sampling, the right half brain and the right kidney were fixed in 10 % neutral buffered formalin (4 % formaldehyde) (J.T. Baker Inc.) at room temperature for 24 hours, paraffin embedded, processed to 4 µm sections, stained with Hematoxylin (J.T. Baker Inc.) and Eosin (HE), and morphologically assessed by bright field microscopy. The severity of lesions was graded using a 5-degree system: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked and 5 = severe.

For immunohistochemical analyses, the staining approach consists of serial application of TSA-amplified immunofluorescence labels (PerkinElmer) on the Leica Bond RX (Leica Biosystems).

Briefly, slides were deparaffinized using Bond Dewax solution RX (Leica Biosystems) on the Leica Bond-RX autostainer; epitope retrieval was performed with HIER (Heat-Induced Epitope Retrieval) (citrate-based pH 6.0 solution; Leica Biosystems). After blocking endogenous peroxidase (Leica Biosystems), staining was performed using primary antibodies against CD3 (SP7) (Abcam) followed by an HRP-conjugated secondary antibody (Jackson ImmunoResearch). Slides were counterstained with DAPI (Merck) and mounted with BrightMount/Plus Mounting Medium (Abcam). FFPE sections without primary antibodies were used as negative control in each batch of IHC staining.

Fluorescence images were acquired (x40) using the NanoZoomer S60 Digital slide scanner (Hamamatsu). The data from multispectral camera were accessed by Orbit Imaging software (http://www.orbit.bio). The immune cell populations were characterized and quantified using a tissue classification model.

A whole kidney section per animal and the choroid plexus area of two brain levels were manually annotated as region of interest (ROI) to be quantified. A classification model was applied to define the total number of CD3+ T cell area (mm^2) normalized against the total tissue area in mm^2.

Anti-dsDNA antibody titers

Anti–double-stranded DNA (dsDNA) antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) using a modified version of a previously described method (Taylor Meadows, 2018, PMID 29608575).

Briefly, calf thymus DNA sodium salt (Sigma Aldrich) was used to coat 96-well plates and 2% BSA/PBS (KPL) to block the plates. Serial plasma dilutions were added (3-6 dilutions per sample), starting from 1:200 to 1:25600. A standard curve was generated from a pool of ten 18-week-old MRL/MpJ-Faslpr/J mice from a previous study run with a known high concentration of anti-dsDNA antibodies. After plasma incubation, anti-mouse polyvalent immunoglobulins (IgG, IgM) - alkaline phosphatase antibody (Sigma Aldrich) was added and the reaction developed with Alkaline Phosphate Yellow Liquid substrate (Sigma Aldrich). The absorption was read at 405nm using a Spectromax 384 microplate reader (Spectromax Molecular Devices).

Results are expressed with an arbitrary unit (aU) as aU/L, referring to the standard curve obtained with a pool of plasma from the previous study for which an arbitrary value of 400 aU/L was given. A minimum of 2 dilutions (mean) was used to determine the quantity of anti-dsDNA antibodies.

Immunoglobulin isotyping

Plasma immunoglobulin isotyping was performed using the Milliplex Mouse Immunoglobulin Isotyping Magnetic Bead kit (Millipore) following the manufacturer's protocol. Plasma samples were diluted 1:100000 and run on a Luminex 200 system using Luminex xPONENt software.

Total IgG in the brain was measured by Quantitative ELISA using the Mouse IgG total Ready-SET-Go kit (Affymetrix) according to the manufacturer's protocol. Brain

homogenate samples were diluted 1:1000 in Assay Buffer. The absorption was measured at 450 nm using Spectromax 384 microplate reader (Spectromax Molecular Devices).

Protein biomarker analysis

After sampling and weighing, half brain was placed into 1 ml Tris 50mM/NaCl 0.1M/Triton 0.1% buffer containing 1% protease inhibitor cocktail (Sigma Aldrich) in a lysing Matrix D tube (MP Biomedicals). Samples were homogenized on a FastPrep 24 5G (MP Biomedicals) and centrifuged for 10 minutes at 14000 rpm at 4°C (Eppendorf Centrifuge 5417R). Supernatants were used for cytokine determination.

A Milliplex Mouse Cytokine/Chemokine Magnetic kit (Millipore) was used to quantify the Cytokine/Chemokine concentration in plasma and brain homogenates. Plasma samples were diluted 1:2 in assay buffer. Brain homogenates were used undiluted.

Samples were run on a Luminex 200 system using Luminex xPONENt software.

BAFF, IFN-α, and Galectin-9 concentrations

BAFF concentrations in plasma and brain homogenate were measured by Quantitative ELISA using the Mouse BAFF/BLyS/TNFSF13B kit (R&D Systems) according to the manufacturer's protocol. Plasma samples were diluted in calibrator Diluent at 1:30, 1:90, and 1:270. Brain homogenate samples were used undiluted. A minimum of 2 dilutions (mean) was used to determine the plasma BAFF concentration. The absorption was measured at 450 nm using Spectromax 384 microplate reader (Spectromax Molecular Devices).

IFN-α concentrations in plasma and brain homogenate were measured with a high sensitivity ELISA using a mouse pan-IFN-α kit (Pbl assay science) according to the manufacturer's protocol. Plasma samples were diluted 1:2 in sample diluent. Brain homogenate samples were used undiluted. The absorption was measured at 450 nm using Spectromax 384 microplate reader (Spectromax Molecular Devices).

Galectin-9 levels in EDTA plasma and brain homogenate were determined using an internally developed electrochemiluminescence assay. Briefly, capture antibody (RnD Systems) was coated to a small spot 96 wells plate (MSD) overnight at 4 °C. All following

incubation steps were done in assay buffer (PBS1x, 1% of milk powder) at room temperature on a plate shaker (800 rpm) and were preceded by three wash steps. The plate was blocked with PBS / 3% of milk powder for 1h. The standard (RnD Systems) (10 to 40,000 pg/ml) or 1/20 diluted samples were added to the plate and incubated for 2h. Plate was then incubated for 1h with the biotinylated detection antibody (RnD Systems). Afterward, MSD SULFO-TAG labelled streptavidin (MSD) was added and incubated for 1h. As a last step, ECL read buffer (MSD) was added and the ECL signal measured using the MSD Sector Imager 600 plate reader. The values of each standard and sample was subtracted from the blank value (assay buffer alone). A standard curve was generated in a log/log curve-fit using SoftmaxPro (Molecular Device). The analyte concentrations (pg/mL) were obtained from the interpretation of the electrochemiluminescence signal from the standard curve.

Statistical analyses

GraphPad Prism Software version 7.04 for Windows was used for all data analysis and graphical illustrations. Comparisons between groups were made using a Mann-Whitney test. Statistical analysis of the survival curve was performed using a Mantel-Cox test; p-values <0.05 were considered statistically significant. The effect of cenerimod on the inflammatory phenotype was assessed in heatmaps after calculating the z-score for each biomarker.

The non-interventional exploratory study and the phase 2 SLE study

In the non-interventional exploratory study, 10 patients (SLEDAI ≥ 6-31), and 10 matched healthy subjects were recruited. Patients with SLE were predominantly female (8/10), between 19-63 years old, and treated with antimalarials (7/10) and corticosteroids (7/10). In the phase 2 SLE study, 60 patients (modified PD set) were included (Hermann V. *et al.* Lupus Sci Med. 2019 PMID 31798918). In brief, the phase 2 SLE study part A consisted of a placebo group (n=11), 0.5 mg (n=12), 1 mg (n=10), and 2 mg cenerimod treatment groups (n=13); the phase 2 SLE study part B consisted of a placebo group (n=5) and 4 mg cenerimod treatment group (n=9).

$S1P_1$ receptor staining and internalization

Fresh blood was collected in EDTA blood collection tubes (BCT) (BD Bioscience) and lymphocytes were subsequently isolated using isolation kits (Miltenyi). Cenerimod was added in serial dilution to cells and incubated at 37°C for 30min. Cells were subsequently stained. For B cells (A488 CD20 clone 2H7, PE CD27 clone M-T271, PE/Cy7 CD38 clone HIT2, BV421 CD69 clone FN50, BV510 IgD clone IA6-2, BV785 CD19 clone HIB19, Biolegend, eFluor660 CD363 clone SW4GYPP, ThermoFisher, isotype control mouse IgG1k eFluor660 clone P3.6.2.8.1, eBioscience) and T cells (FITC CD8 clone RPA-T8, PE CCR7 clone G043H7, PE/Cy7 CD4 clone RPA-T4, BV421 CD69 clone FN50, BV510 CD3 clone OKT3, BV785 CD45RA clone HI100, Biolegend, eFluor660 CD363 clone SW4GYPP, ThermoFisher, isotype control mouse IgG1k eFluor660 clone P3.6.2.8.1, eBioscience) and measured in a NovoCyte (ACEA Biosciences Inc) and analyzed using Flowjo software (Becton Dickinson) and prism (graphpad).

Biomarker analysis in the non-interventional exploratory study and the phase 2 SLE study

Immunophenotyping in the non-interventional exploratory study

Fresh blood was collected in EDTA BCT (BD Bioscience) and stained using the following staining panels. T cell panel: anti-CD3 BV510 clone OKT3, anti-CD4 APC Cy7 clone RPA-T4, anti-CD8 PE Cy5 clone RPA-T8, anti-CD45RA AF488 clone HI100, anti-CCR6 PE Cy7 clone G034E3, anti-CXCR5 PE clone J252D4, anti-CD25 BV605 clone BC96, anti-CD45 BV570 clone HI30, anti-CCR7 BV785 clone G043H7 (Biolegend); anti-CXCR3 BV421 clone 1C6, anti-CD127 AF647 clone HIL-7R-M21 (BD Bioscience). B cell panel: Anti-CD20 APC Cy7 clone 2H7, anti-CD19 BV510 clone HIB19, anti-IgD FITC IA6-2, anti-CD27 APC clone M-T271, anti-CD38 BV650 clone HB-7, anti-CCR6 PE Cy7 clone G034E3, anti-CXCR5 PE clone J252D4, anti-CD45 BV570 clone HI30 (Biolegend); anti-CXCR3 BV421 clone 1C6 (BD Bioscience). Monocyte / neutrophil panel: Anti-CD14 APC clone HCD14, anti CD16 FITC clone 3G8, anti-CD88 PE clone S5/1, anti-CD11b PE Cy7 clone ICRF44, anti-CD62L BV650 clone DREG-56, anti-CD25 BV421 clone BC96, anti-CD45 BV570 clone HI30 (Biolegend). pDC panel: anti-CD3 APC clone OKT3, anti-CD303 FITC clone 201A, anti-CD123 PE clone 6H6, anti-CD4 PE Cy7 clone RPA-T4, CD19

BV510 clone HIB19, anti-CD45 BV570 clone HI30 (Biolegend). Stained samples were measured on a NovoCyte (ACEA Biosciences Inc) and analyzed using Flowjo software (Becton Dickinson) and Graphpad Prism.

Immunophenotyping in the phase 2 SLE study

Blood was taken using 5 ml Cyto-Chex® BCT tubes. Blood samples were shipped at ambient temperature to the analysis lab and analyzed within 6 days after collection. All staining antibodies were purchased from BD Bioscience. Staining antibodies were mixed to obtain two staining cocktails to phenotype B cell and T cell subsets.

B cell subset panel: anti-CD19 clone SJ25C1 PECy7, anti-CD20 clone L27 APC H7, anti-IgD clone IA6-2 FITC, anti-CD27 clone M-T271 BV421, anti-CD38 clone HB7 PE, anti-CXCR3 clone 1C6 APC, anti-CD138 clone MI15 PerCP Cy 5.5, anti-CD45 clone HI30 BV510. T cell subset panel: anti-CD3 clone SK7 FITC, antiCD4 clone SK3 APC H7, anti-CD8 clone SK1 PerCP Cy5.5, anti-CD45RA clone L48 PE-Cy7, anti-CD25 clone 2A3 PE, anti-CD127 clone HIL-7R-M21 AF647, anti-CD45 clone HI30 BV510 and anti-CCR7 clone 150503 BV421 added separately to the blood.

Staining cocktail was directly added to Trucount tubes (BD Bioscience). For the T cell subsets, blood was pre-incubated with the anti-CCR7 antibody for 10 min at 37 °C. Afterwards blood was added to the dedicated Trucount tubes and gently mixed and incubated for 30 min at room temperature. Red blood cells were lysed using Lysis buffer (BD Bioscience) and samples were acquired within 1 h after sample preparation on a FACS Canto II (BD Bioscience). The cell populations of interest and Trucount beads were gated during the acquisition using the Diva software and corresponding event counts were exported from the Diva Software to an Excel format. Absolute cell count per µl of blood were calculated from the event count using the number of Trucount beads. Cell counts were either analyzed as absolute values or normalized to percent baseline using GraphPad prism. For testing statistical significance in two group comparisons the Mann-Whitney t-test was applied. For testing statistical significance in multiple group comparisons, the Kruskal-Wallis with Dunn's multiple comparison test was applied. Correlation analysis were carried out using the Spearman's rank correlation coefficient.

List of lymphocyte subsets including the employed cell phenotype.

Population	Abbreviation	Cell phenotype
B cells	B cells	CD45+/CD19+
Antibody secreting cells	ASC	CD45+/CD19+lo/CD27+/IgD-/CD20-/CD38++
Naïve T cells	naïve	CD45+/CD3+/CD4+orCD8+/non-Treg/CCR7+/CD45RA+
Central memory T cells	CM	CD45+/CD3+/CD4+orCD8+/non-Treg/CCR7+/CD45RA-
Effector memory T cells	EM	CD45+/CD3+/CD4+orCD8+/non-Treg/CCR7-/CD45RA-
Effector memory RA+ T cells	EMRA	CD45+/CD3+/CD4+orCD8+/non-Treg/CCR7-/CD45RA+
Monocytes		SSC/CD45+/CD14+/CD16-
Neutrophils		SSC/CD45+/CD16+
Eosinophils		SSC/CD45+/FSC/CD16-
pDCs		CD45+/CD19-/CD3-/CD4+/CD123+/CD303+

Unbiased analysis of flow cytometry data from the phase 2 SLE study

Data were analyzed using R version 3.4.4. FCS files from the B cell subset flow cytometry analysis of the phase 2 SLE study A after 12 weeks of cenerimod treatment were processed using FlowSOM package from Bioconductor 3.4. Cells were assigned to a neuron based on self-organizing maps. Hierarchical clustering was used on the grid of 1'000 neurons to build the final clusters. The number of clusters was determined using

sum of squares errors. Cenerimod affected clusters were identified based on the placebo vs 2 mg contrast using a Wilcoxon test with Benjamini-Hochberg multiplicity adjustment.

Protein biomarker analysis in plasma samples from the non-interventional exploratory study and the phase 2 SLE study

Fresh blood was collected in EDTA BCT following the instructions given by the provider. Plasma samples were kept frozen at -80°C until further analysis. CXCL10 was measured in EDTA plasma using a human CXCL10 V-plex kit from Mesoscale discovery (MSD). IFN-γ and TNFα were measured in EDTA plasma using a human V-plex kit from MSD. CXCL9 was measured in EDTA plasma using a prototype human triplex kit from MSD. Pan-IFN-α and Galectin-9 were measured in EDTA plasma using commercial ELISA kits from PBL assay and RnD Systems, respectively. IL6 and IL10 were measured in EDTA plasma using human SMC high sensitivity immunoassay kits from EMD Millipore. All MSD assays were acquired on a Sector imager from MSD. Raw data were analyzed using SoftmaxPro to derive the biomarker concentration. All ELISA plates from were acquired on an Envision reader from Perkin Elmer. Raw data were analyzed using SoftmaxPro to calculate the biomarker concentration. SMC immunoassays were acquired on an Erenna reader (Merck Millipore). Biomarker concentrations were automatically generated by the Erenna system.

Results were further analyzed using GraphPad prism either as absolute values or normalized as percent of baseline value. For testing statistical significance in two group comparisons the Mann-Whitney t-test was applied. Correlation analysis were carried out using the Spearman's rank correlation coefficient. Patient heterogeneity for the inflammatory phenotype was assessed in heatmaps after calculating the z-score for each biomarker. IFN-associated plasma biomarker from the phase 2 SLE study were normalized as z-score and plotted as heatmaps. Biomarkers were ranked according to the difference between placebo and cenerimod treatment.

Gene expression sequencing in whole blood and isolated ASC from the non-interventional exploratory study

PBMC were isolated from EDTA blood using a density gradient Histopaque 1077 (Sigma) in SepMate tubes (Stemcell technologies). After washing, isolated PBMC were stained with the described antibody panel and analyzed with a Sh800SE cell sorter (SONY). PBMCs staining panel: anti-CD3 PerCP Cy5.5 clone OKT3, anti-CD20 BV421 clone 2H7, anti-CD19 PE Cy7 clone HIB19, anti-CD27 APC clone M-T271, anti-CD38 PE clone HB-7 (Biolegend); anti-IgD BB515 clone IA6-2 (BD Bioscience). ASC and naïve B cells were gated based on their antigen expression and sorted directly in tubes containing Lysis buffer RL (Norgen) for RNA extraction.

Human whole blood, from 10 SLE patients and 10 matched healthy donors, was collected using PAXgene Blood RNA Tubes (Qiagen,) and stabilized 2 hours at RT before transferring to -20°C and subsequently to -80°C for long storage. Total RNA was isolated using PAXgene Blood RNA Kit (Qiagen) including a DNase treatment (Qiagen). Total RNA concentration was measured with Qubit BR RNA kit (Invitrogen) and RNA quality was assessed with RNA Nano LabChip on the Bioanalyzer (Agilent). The RNAseq Library was performed using NuGen Universal Plus mRNA-Seq (NuGen) from 200 ng of total RNA; including a polyA RNA selection and a Human Globin depletion. Final Library concentration was measured with Qubit HS dsDNA kit (Invitrogen) and RNA quality was assessed with Fragment Analyzer HS NGS kit (Agilent). In parallel qPCR quantification was performed using KAPA ABI prism Library Quant kit (Roche). qPCR quantification was selected to normalize each library to 2 nM and run RNAseq on NextSeq 500/550 following Illumina's instructions. The 26 samples were randomized across 2 flow cells NextSeq 500/550 High Output Kit v2.5 75 Cycles (Illumina).

Total RNA was extracted from sorted ASC or Naïve B cells using NORGEN Single cell RNA purification kit (Norgen) including a DNase treatment (Norgen). Total RNA concentration was measured with Qubit HS RNA kit (Invitrogen) and RNA quality was assessed with RNA Pico LabChip on the Bioanalyzer (Agilent) RNAseq Library was performed using NuGen TRIO RNAseq (NuGen) from 5 ng of total RNA; including a ribosomal depletion. Final Library concentration was measured with Qubit HS dsDNA kit

(Invitrogen) and RNA quality was assessed with Fragment Analyzer HS NGS kit (Agilent). In parallel qPCR quantification was performed using KAPA ABI prism Library Quant kit (Roche). qPCR quantification was selected to normalize each library to 2 nM and run RNAseq on NextSeq 500/550 following Illumina's instructions. The 58 samples were randomized across 8 flow cells NextSeq 500/550 High Output Kit v2.5 75 Cycles (Illumina).

Sequenced reads were aligned with STAR to the human Reference genome GRCh38.p13 and alignments per gene quantified with featureCounts using Ensembl gene annotations. All samples passed quality control (QC): > 20e6 reads; > 90% mapping rates and good on-target mapping rates for each protocol (reads mapped to annotated genes, > 80% for mRNA data and > 30% for total RNA). Genes expressing less than 10 reads per sample across x samples were omitted and differentially expressed genes calculated using edgeR, where x is the size of the smallest group in the study (10 for whole blood and 9 for FACS cells). Genes with an FDR > 0.05 and a fold change (FC) > 1.5 were deemed significant. A new metric for a genomic disease score (GDS) was introduced, which were used to correlate expression profiles with other biomarker data. In short, the dimensions of the expression data were reduced with PCA and the center of mass calculated for the control and disease groups. A line was fit to the center of mass of the two groups, and all points (samples) projected to this line. The distance between the two centers of mass were re-scaled to unit length with control at 0 and disease at 1, and the projections onto this scale used as the GDS values. The method generalizes to any dimension of principal components (PCs), but for the present study only the first two PCs were used for the GDS calculations. All analysis of the mapped reads, heatmaps and the GDS score was implemented in R.