MRL/lpr Model of Systemic Lupus Erythematosus (SLE) in the Vium Digital Vivarium

INTRODUCTION
Systemic Lupus Erythematosus (SLE) is a chronic, inflammatory autoimmune disease that affects multiple organ systems. Common characteristics include kidney disease, skin eruptions, joint pain, and neuropsychiatric complications (1). Lupus nephritis is a significant cause of morbidity and mortality in patients (2).

MRL/MpJ-Faslpr (MRL/lpr), a commonly used mouse model of SLE, possesses a spontaneous genetic mutation in the Fas TNF family of receptors. Similar to patients, mutant mice show accelerated mortality, lymphadenopathy, nephritis, and elevated levels of autoantibodies, including anti-dsDNA (3).

In SLE preclinical research, therapies are evaluated against several reliable standard measures, including proteinuria, anti-dsDNA, skin lesions, splenomegaly, nephritis, and survival. These current measures can inadvertently cause stress to the animals during collection. In addition, they also lack the sensitivity to detect ongoing disease and fall short in capturing diverse behavioral changes manifested by SLE patients, such as fatigue, pulmonary involvement, and sleep disturbances (4,5).

We hypothesize that continuous monitoring of behavioral and physiological parameters will provide additional meaningful data to assess disease and efficacy in genetic rodent models of disease, including SLE. To address this hypothesis, the objectives of this study were: 1) To investigate behavioral and physiological characteristics of MRL/lpr mice using a low-touch, continuous monitoring platform, and 2) To evaluate and compare the effects of standard of care (SOC) compounds on conventional disease measures as well as behavioral and physiological phenotypes in MRL/lpr mice.

METHODS
Animals
Singly-housed, female MRL/lpr and MRL/MpJ (Control) mice (Jackson Laboratories, Bar Harbor, ME) were evaluated starting at 6 weeks old, treated with cyclophosphamide (CP), dexamethasone (DEX), or vehicle (Table 1) starting at 10 weeks, and euthanized at 22 weeks. Experiments were conducted in Vium’s AAALAC-accredited facility in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Vium.
CASE STUDY
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Table 1: Treatment Study Groups

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRL/MpJ (Control)</td>
<td>Saline</td>
<td>--</td>
<td>IP</td>
<td>3x/week</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>CP</td>
<td>25 mg/kg</td>
<td>IP</td>
<td>1x/week</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>Vehicle for CP</td>
<td>--</td>
<td>IP</td>
<td>1x/week</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>DEX</td>
<td>2 mg/kg</td>
<td>IP</td>
<td>3x/week</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>Vehicle for DEX</td>
<td>--</td>
<td>IP</td>
<td>3x/week</td>
</tr>
</tbody>
</table>

Breathing Rate and Motion
Subjects were housed within the Vium Digital Vivarium, where intelligent sensors and HD cameras allow for continuous and minimally invasive monitoring of animals, as well as collection of automated metrics including motion and breathing rate, in the home cage. All study data is available in real-time and accessible via the online Research Suite.

Body Weight and Proteinuria
Body weights were measured weekly. Urine was collected every-other week. Urine proteinuria was evaluated using Multistix 10 SG Reagent Strips (Siemens, Washington DC, USA) and the following criteria: 0= 0 mg/dL to 29 mg/dL; 1= 30 mg/dL to 99 mg/dL; 2= 100 mg/dL to 299 mg/dL; 3= 300 mg/dL to 1999 mg/dL; 4= over 2000 mg/dL.

Terminal Pathology
At study end, spleen and kidneys were weighed. Left and right kidneys fixed in 10% neutral buffered formalin were sent for histopathology analysis and scored for glomerulus crescents, tubular protein casts, interstitial inflammation, and vasculitis (Bolder BioPATH, Boulder, CO).

Statistical Analysis
One or two-way ANOVAs with Tukey’s multiple comparison tests were used to compare groups on specific or across study days, respectively. P values less than 0.05 were considered statistically different.

RESULTS
Compared with MRL control mice, vehicle-treated MRL/lpr mutant mice showed increased body weight as early as days 73-87 (~10-12 weeks of age). Treatment with cyclophosphamide (CP) or dexamethasone (DEX) rescued this phenotype starting ~13 weeks (Figs. 1A and 2A). Vehicle-treated MRL/lpr mice also showed increased proteinuria scores as early as 15-17 weeks of age. Treatment with CP or DEX rescued this phenotype around 16 and 17 weeks, respectively (Figs. 1B and 2B).

When assessed using Vium’s breathing rate metric, vehicle-treated MRL/lpr mutant mice showed increased breathing rate as early as 16 weeks of age (Figs. 1C and 2C). Treatment with CP or DEX attenuated this phenotype, with CP-related and DEX-related reductions detected as early as 14 and 15 weeks of age, respectively. On average throughout the study, vehicle-treated MRL/lpr mice generally showed lower activity levels compared to MRL controls, and this phenotype was attenuated in CP- and DEX-treated mice (Figs. 1D and 2D).
Histopathology corroborated these results. Compared with MRL controls, MRL/lpr mice showed increased spleen and kidney weights, as well as kidney histopathology scores at study end. CP- and DEX-treated mice showed attenuated organ weights and histopathology scores (Table 2).

Table 2. Organ weight and pathology results

<table>
<thead>
<tr>
<th>Measurement</th>
<th>MRL Controls</th>
<th>MRL/lpr CP Treatment</th>
<th>MRL/lpr DEX Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>CP</td>
<td>Vehicle</td>
</tr>
<tr>
<td>SPLEEN WEIGHT (g)</td>
<td>0.42 ± 0.06</td>
<td>0.54 ± 0.30</td>
<td>0.25 ± 0.08 ^</td>
</tr>
<tr>
<td>KIDNEY WEIGHT (g)</td>
<td>0.46 ± 0.02</td>
<td>0.56 ± 0.05</td>
<td>0.47 ± 0.05 ^</td>
</tr>
<tr>
<td>KIDNEY HISTOPATHOLOGY SCORE</td>
<td>0.42 ± 0.14</td>
<td>6.62 ± 2.50</td>
<td>4.25 ± 3.09 ^</td>
</tr>
</tbody>
</table>

Values represent Means ± SEM. N=3-13 per group. ^ P<0.05 MRL/lpr Vehicle vs. MRL Control Vehicle and * P<0.05 MRL/lpr DEX or CP vs. MRL/lpr Vehicle.
DISCUSSION AND FUTURE DIRECTION

We demonstrate that continuous automated detection of breathing rate and motion in the home cage provides useful information for evaluating disease progression in the MRL/lpr mouse model for systemic lupus erythematosus (SLE). More specifically, changes in breathing rate followed the course of disease severity and was rescued by treatment with reference compounds. Histopathology results confirmed splenomegaly and renomegaly, as well as interstitial inflammation, vasculitis, and tubular protein casts in the kidney.

SLE is a chronic and heterogeneous autoimmune disease; patients present with multiple behavioral and physiological disease symptoms (1,2,4,5). We demonstrate that the Vium Digital Vivarium platform can provide insight into physiologically relevant measures, which track disease and reflect drug efficacy, such as motion and breathing rate—both of which provide a holistic overview of disease state in SLE patients. We also observed an approximately 20% increase in breathing rate in mutant mice, a phenotype not normally assessed in this mouse model. Increased breathing rate may be indicative of pulmonary edema or acute reversible hypoxemia secondary to renal failure, interstitial pneumonitis increased cell infiltrates in lungs, or a general immune-mediated response, which have all been observed in SLE patients (6,7). Although its pathophysiological relevance to disease requires further investigation, breathing rate may provide an additional read-out for assessing disease.

In addition to providing more physiologically relevant metrics to complement standard measurements, a low-touch, continuous monitoring platform can also be used to minimize stress and inherent human impact during experimentation, especially in a model wherein disease etiology results from a combination of genetic and environmental factors.

REFERENCES