# Characterizing Disease Progression in Preclincal Systemic Lupus Erythematosus Using Multimodality In Vivo Ultrasound and Fluorescent Imaging

Julie Braza, Jen-Chieh Tseng, Alexis Stanley, Craig McMannus, and Jeffrey D. Peterson

## 1 Abstract

Systemic Lupus Erythematosus (SLE) in humans is a complex multigenic, systemic autoimmune disorder that can cause acute or chronic inflammation of multiple organ systems, involving autoantibody production, lymphoid activation/hyperplasia, splenomegaly and nephritis. There are multiple mouse models that capture some of the important hallmarks of the human disease, and the MRL MpJ Fas-lpr /J (MRL/lpr) mouse is one of the most commonly used, developing lymphoproliferation by 12-14 weeks, as well as progressive proteinuria and renal disease, lymphadenopathy, and skin lesions, typically dying around 20 weeks of age.

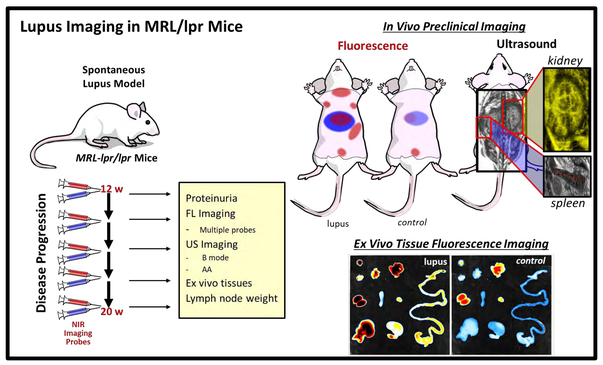
As fluorescent (FL) imaging using injectable NIR fluorescent probes has the potential for the sensitive detection of biological changes associated with disease, we selected a panel of 6 probes for monitoring lupus progression in MRL/lpr mice. We used IVISense™ fluorescent probes (Cat K 680 FAST [CK680], Transferrin Receptor 750 [TF750], Integrin Receptor 750, [IR750], Pan Cathepsin 680 [PC680], Cat B 750 FAST [CB750], and Vascular 680 [VAS680]) [Revvity], to detect, visualize, and quantify biological changes in various organ systems noninvasively in living MRL/lpr mice as compared to AKR control mice. Furthermore, ultrasound images of the kidney and spleen were also acquired on the Vega® (Revvity) to characterize size and density changes associated with disease progression. MRL/lpr and control mice were assessed at 12, 14, 16, 18 and 20 weeks for increased levels of proteinuria and lymphadenopathy.

In diseased mice, proteinuria assay readings ranged from 100 to 2000 mg/dL, and lymphadenopathy was of brachial and inguinal lymph nodes, ranged from <0.5 cm to >1 cm. AKR mice showed no signs of lymphadenopathy or increased proteinuria. Fluorescent imaging probes were injected IV (2 nmol/mouse) in depilated MRL/lpr and control mice every two weeks. Ventral and dorsal epifluorescence images were acquired on the IVIS® SpectrumCT (Revvity) at each timepoint, focusing on in vivo signal increases in the liver and kidneys. Changes in the kidneys with CK680, CB750, and VAS680 were evident, and liver changes were detected using PC680, CK680, and TF750. Ex vivo assessment of tissues allowed easier quantification of multiple organ systems, with CK680 revealing significant increases liver, lymph nodes, kidneys, thymus and spleen. TF750 revealed mostly changes in the liver, consistent with in vivo imaging results. Ultrasound imaging was a powerful tool for monitoring changes in spleen and kidney size, quantifying a doubling in kidney volume and a 300% increase in calculated spleen volume by 18 weeks of age in MRL/lpr mice, with minimal changes seen in AKR spleens and kidneys. Further, ultrasound acoustic angiography detected significant changes in kidney vascularity.

Recent studies have implicated the mammalian target of rapamycin (mTOR) signaling pathway to be of importance so we used non-invasive fluorescence and ultrasound imaging technologies to assess the effects of rapamycin treatment on the progression of lupus. Both Cathepsin K and the Transferrin Receptor are regulated by mTOR activity and have been identified as relevant biomarkers, as well as targets, for lupus. Treatment significantly reduced FL signal in all disease related tissues, including liver, kidneys, lymph nodes, and spleens. In addition, ultrasound imaging showed that rapamycin prevented or reversed changes in kidney and spleen size.

In summary, NIR fluorescent imaging strategies using validated IVISense fluorescent imaging probes, as well as ultrasound imaging, provided novel means for the monitoring of relevant biomarkers and physiologies in preclinical lupus progression. In addition, expansion of this approach to other relevant biomarkers is ongoing to identify other critical biological mechanisms involved in lupus development or progression.

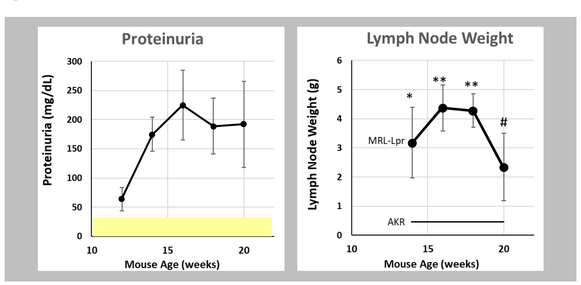
## 2 Murine Spontaneous Lupus Protocol




| Probe | Specification | Probe | Specification |
|---|---|---|---|
| IVISense Cat B 750 FAST | Activated by lysosomal cathepsin B as a biomarker of a variety of inflammatory cell types. | IVISense Pan Cathepsin 680 | Activated by the family of lysosomal cathepsins which are biomarkers of inflammatory cells |
| IVISense Cat K 680 FAST | Activatated by secreted cathepsin K from osteoclasts and some inflammatory macrophage populations | IVISense Vascular 680 | Targets Annexin V expression associated with cell death (early necrosis and apoptosis) |
| IVISense Integrin Receptor 750 | Activated by the family of MMPs secreted by a variety of cells associated with inflammation and fibrosis | IVISense Transferrin Receptor 750 | Target the transferrin receptor involved in iron transport into cells |

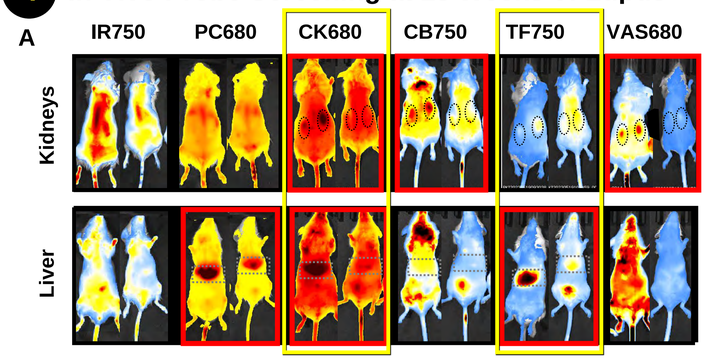
MRL MpJ Fas-lpr /J (MRL-lpr) and related AKR mice (Jackson Laboratories) were monitored longitudinally between 12 and 18-20 weeks of age by standard metrics (proteinuria, lymphadenopathy). Mice were monitored by NIR Fluorescence using select in vivo imaging probes as biomarkers, as described in the table. Ultrasound imaging was also used to assess changes in spleen and kidney size associated with disease.

## 3 Lupus General Metrics

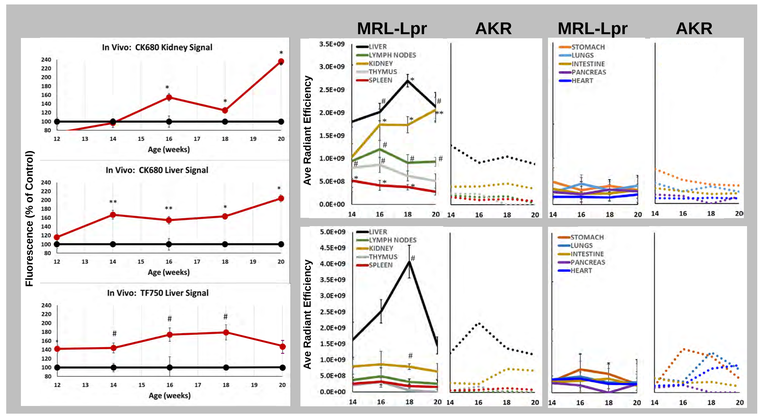



MRL MpJ Fas-lpr /J (MRL-lpr) and related AKR mice (Charles River Labs) were weighed weekly, and lymphadenopathy was assessed at 12-16 weeks in representative mice by excising and weighing lymph nodes. Proteinuria was measured using Albustix Reagent Strips (Siemens Healthineers, Lowell MA).

## 4 In Vivo Probe Screening at 18 Weeks of Lupus

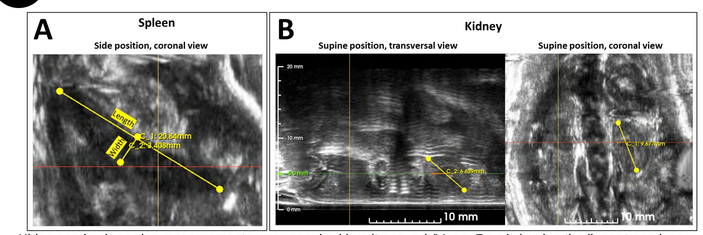



**B In Vivo Quantification** **C Ex Vivo Tissue Quantification**



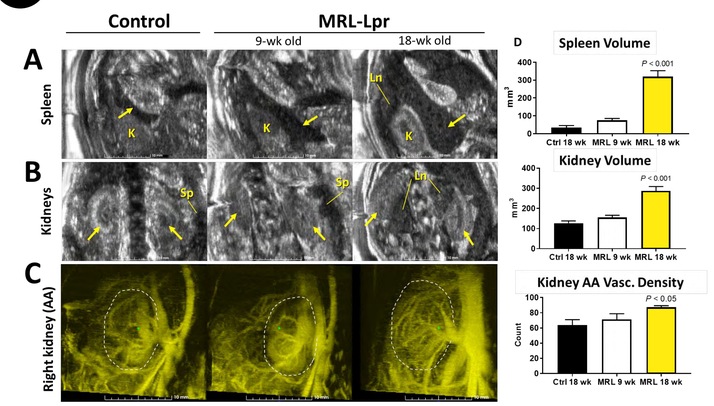

MRL MpJ Fas-lpr /J (MRL-lpr) and related AKR mice (Charles River Labs) were monitored longitudinally at 14, 16, 18, and 20 weeks by regular NIR Fluorescence imaging using 6 imaging probes as biomarkers of inflammation, protease activity, liver metabolism, and vascular changes. At each imaging time, a small number of mice were selected for ex vivo tissue assessment of fluorescent signal in a range of organs/tissues, providing access to difficult to detect tissues as well as improving sensitivity of quantification. P-value: student t-test against control [# p<0.05, *p<0.01, ** p<0.001].

## 5 Vega Ultrasound of Kidneys and Spleen: Method



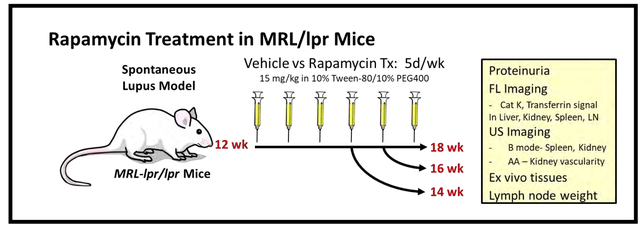

Kidney and spleen size measurements were acquired by ultrasound (Vega, Revvity) using the linear transducer array in B-mode. Mice were placed either supine (kidneys) or left side down (spleen). Spleen length was measured along the longest axis and width at the mid-point of the spleen. (B) Kidney width was determined from the base of renal vein to the apex of the capsule in the opposite direction. Volumes were estimated using the formula $0.5 \times L \times W^2$.

## 6 Ultrasound of Kidneys and Spleen in Lupus

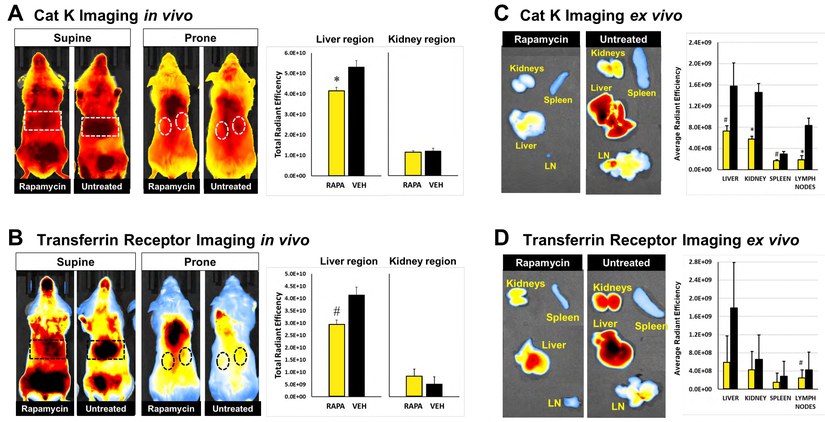



Linear array B-mode US imaging of the spleen and kidneys in the MRL and control AKR mice. Yellow arrows indicate spleen or kidney positions. (**A**) Splenomegaly is observed in 9-week old MRL mice, becoming more pronounced at 18-week. K indicates the adjacent left kidney and enlarged lymph nodes (Ln) can been seen in 18-week old MRL mice. (**B**) B-mode imaging of control kidneys shows distinctive layers for cortex, medulla and renal pelvis. In contrast, these features are generally lost in the MRL kidneys. Enlarged spleens (Sp) are also seen in the 18-week old MRL mice with advanced lupus. (**C**) Acoustic angiography (AA) of the kidneys reveals increased vasculature and perfusion as the disease progresses in the MRL mice. (**D**) Quantitative representation of spleen, kidney volumes and average kidney AA signal density in the MRL (n = 7 for both ages) and control mice (n = 3). P-value: student t-test against ctrl. Bar: s.e.m.

## 7 Lupus Treatment: Rapamycin inhibition of mTOR

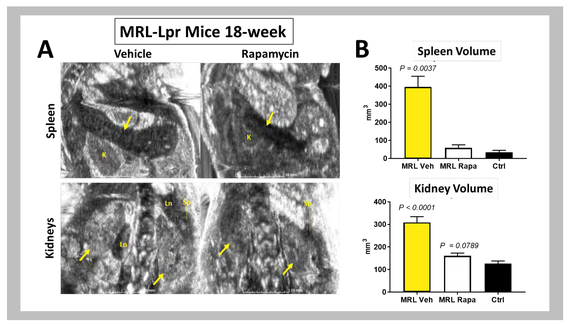



## 8 FL Imaging of Rapamycin Lupus Treatment




Rapamycin early treatment decreased disease-associated tissue fluorescence in vivo and ex vivo. At 12 weeks, 77% of mice showed some level of detectable proteinuria prior to enrollment into the treatment study. Noninvasive in vivo measurement of IVISense Cat K (A) and Transferrin Receptor (B) probe signal in livers and kidneys revealed trends of decrease (representative images shown). However, noninvasive imaging was not as sensitive or accurate as ex vivo imaging of excised tissue. CK680 signal was more consistent that TF750 signal, showing consistent decreases in liver, kidneys, spleen, and lymph nodes of rapamycin-treated MRL-Lpr mice. Treated mice returned to near normal signal in these tissues. (Vehicle: n = 5; Rapa: n = 5)

## 9 Ultrasound assessment of Rapamycin Efficacy




Rapamycin treatment suppresses spleen and kidney enlargement as visualized by linear array B-mode ultrasound imaging. Rapamycin treatment began when the MRL mice were 12-week old and B-mode imaging was performed at 18-week to evaluate the treatment efficacy. (A) B-mode imaging clearly show the drug treatment effectively suppress splenomegaly. The treated kidneys also show better structure integrity in the kidneys and no abnormal lymph node enlargement. (B) Quantitative representation of spleen and kidney volume measurements of the in response to rapamycin (Vehicle: n = 5; Rapa: n = 5; Ctrl: n = 3) . P-value: student t-test against ctrl. Bar: s.e.m.

## 10 Summary

The present studies provide evidence for the utility of fluorescence and ultrasound, using the IVIS Spectrum and Vega imaging systems, for the detection and quantification of spontaneous lupus in MRL MpJ Fas-lpr /J mice. IVISense Cath K and Transferrin Receptor NIR fluorescent imaging probes detected changes in liver, kidney, spleen, and/or lymph nodes associated with disease progression. Ultrasound imaging provided non-invasive means for also assessing pathological changes in spleen and kidney size. Early treatment of mice (starting at 12 weeks of age), effectively reversed early signs of proteinuria in addition to preventing gross physiologic changes to the spleen and kidney as assessed by ultrasound. Fluorescent imaging with the cathepsin K-activatable probe, in particular, efficiently detected a decrease in tissue fluorescence to levels near to normal mouse controls.

In conclusion, fluorescent imaging of relevant biomarkers and ultrasound measurements of spleen and kidney, can be performed quickly and easily to provide robust measurements of lupus progression and treatment efficacy.



Revvity, 940 Winter Street, Waltham, MA USA (800) 762-4000 or (+1) 203 925-4602 www.revvity.com