# Selective Inhibition of the Immunoproteasome with KZR-616 Blocks Multiple Cell Signaling Pathways, Plasma Cell Signatures and Myeloid Cell Associated Damage in the NZB/W Lupus Nephritis Model



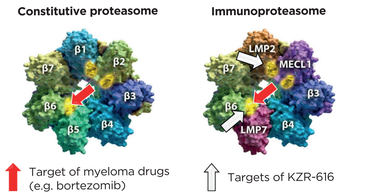
Tony Muchamuel, Janet L Anderl, R Andrea Fan, Henry W B Johnson, Dustin L McMinn, Christopher J Kirk, Brian Tuch

Kezar Life Sciences, South San Francisco, CA

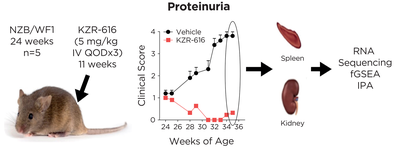
## BACKGROUND

- Proteasome inhibitors such as bortezomib, used to treat multiple myeloma, target both forms of the proteasome **(Figure 1)**.
- Bortezomib has been used successfully to treat patients with systemic lupus erythematosus (SLE) and lupus nephritis (LN).[1, 2]
- KZR-616 selectively inhibits the LMP7 and LMP2 subunits of the immunoproteasome, is in development for the treatment of LN, and has completed Phase 1b studies in patients with SLE (ACR Abstract 2520).[3]
- We previously demonstrated that KZR-616 treatment effectively blocks the progression of lupus in the NZB/W mouse model of LN, including reducing serum levels of anti-dsDNA antibodies.[4]
- Here we describe changes to global gene expression in the spleens and kidneys of diseased mice following KZR-616 treatment.

### Figure 1. Proteasome subunit composition




### Figure 2. Methods



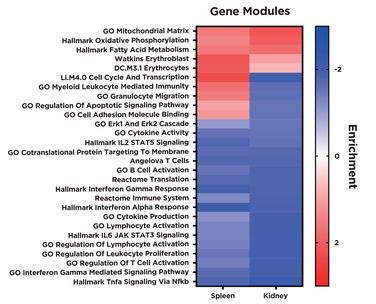

fGSEA: Fast Gene Set Enrichment Analysis; IPA: Ingenuity Pathway Analysis; SC: subcutaneous; QOD: every other day.

## METHODS

- 24 week old female NZB/W F1 mice were administered vehicle (veh) or 5 mg/kg KZR-616 intravenously three times per week for 13 consecutive weeks. At the end of the treatment period, spleens and kidneys were harvested and processed for histology, flow cytometry, IgG deposition, and RNA extraction **(Figure 2)**.
- RNA sequencing analysis was performed on both the spleen and kidney RNA (n=5 in each group).
- Raw data were processed with RSEM software. Differential expression was modeled using DESeq2 software, and a variety of gene modules examined using fGSEA. Canonical pathways and disease functions were extracted using Ingenuity Pathway Analysis (IPA) software (http://www.ingenuity.com).[4,5,6]

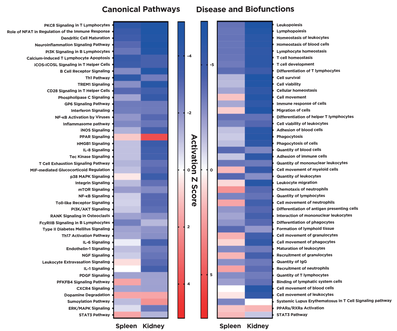
## RESULTS

### Figure 3. Significantly altered gene modules in the spleen or kidney of KZR-616-treated NZB/WF1 mice as determined by fGSEA



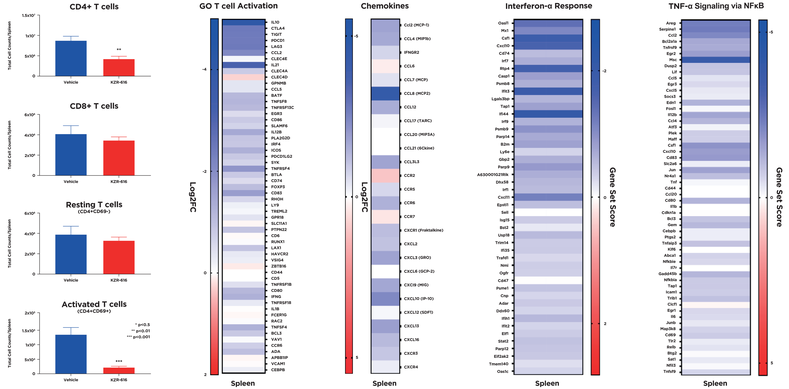

- KZR-616 down regulates multiple lymphocyte modules associated with activation.
- Reduction in cytokine and IFN-a responses.
- Increases in metabolic activity, erythropoiesis and granulocyte migration.

### Figure 4. Top canonical and disease functions modulated in the spleen and kidney of KZR-616-treated NZB/W F1 mice via IPA analysis

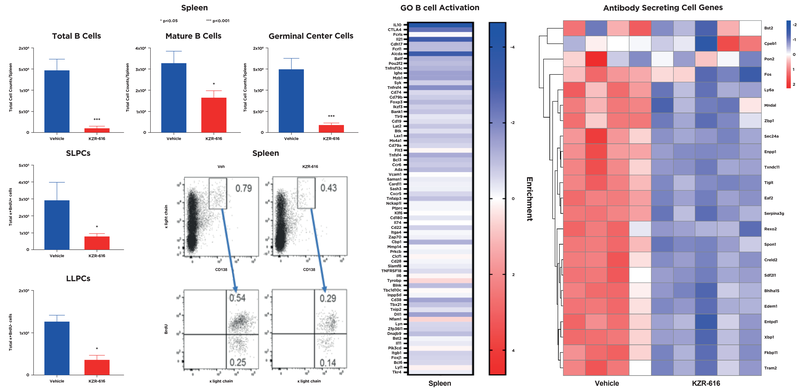



- Broad reduction in multiple signaling pathways.
- Strong decrease in immune effector cell functions in the kidney.
- Up regulation of cellular migration functions in the spleen.

### Figure 5. Reduced presence of multiple immune effector cell components following KZR-616 administration

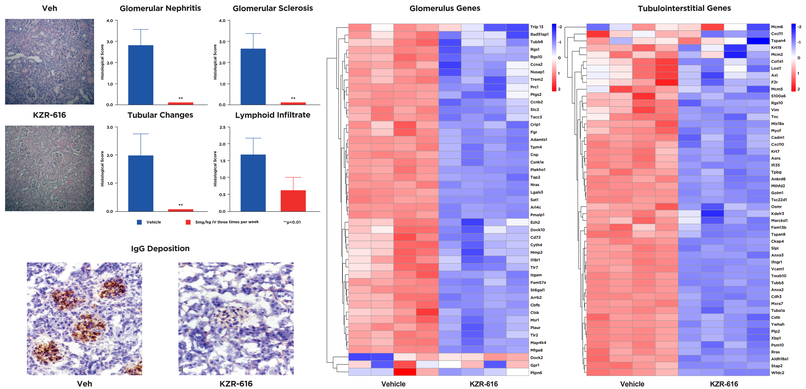



### Figure 6. KZR-616 reduces multiple B and plasma cell populations in association with down regulation of B cell activation and plasma cell differentiation

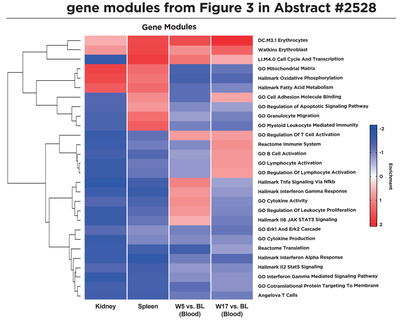



LL: long-lived, PC: plasma cells, SL: short-lived.

### Figure 7. Administration of KZR-616 prevents renal damage and reduces expression of genes associated with tissue damage in the glomerulus and tubulointerstitium of LN patients




### Figure 8. Significantly altered gene modules in the kidney or spleen of KZR-616-treated NZB/W mice vs patients treated with KZR-616 as determined by fGSEA gene modules from Figure 3 in Abstract #2528




- Patients received KZR-616 subcutaneously at 45 or 60 mg weekly for 13 weeks with follow-up through Week (W) 25. Whole blood (Paxgene RNA tube) samples were collected at BL (Baseline), W5 (Early Tx) and 17 (Late Tx).
- RNA sequencing analysis was performed on whole blood RNA (n=13–20 in each group).
- Raw data were processed with RSEM. Differential expression was modeled using DESeq2, and a variety of gene modules examined using fGSEA and compared to the analogous mouse results.

## CONCLUSIONS

- KZR-616 is highly active in the NZB/W mouse model of SLE.
- KZR-616 effectively blocks disease progression in a mouse model of SLE by regulating expression of genes that are involved in immune response and effector cell function, plasma cell differentiation and antibody secretion, and glomerular and tubulointerstitial renal pathology.
- These experimental data support the ongoing clinical evaluation of KZR-616 in patients with LN and other autoimmune diseases.

### References

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### Author Contributions

All authors contributed to the acquisition, analysis or interpretation of data, critically revised the poster, and approved the final content.

### Author Disclosures

BT: Consultancy fees from Kezar Life Sciences; all other authors are employees of Kezar Life Sciences.

### Acknowledgments

This study was funded by Kezar Life Sciences. Editorial services by Emma Philips, DPhil, Costello Medical, Cambridge, UK, funded by Kezar Life Sciences.