Profiling of Gene Expression, Immune Cell Subtypes, and Circulating Biomarkers in Systemic Lupus Erythematosus Patients Treated with the Selective Immunoproteasome Inhibitor, KZR-616

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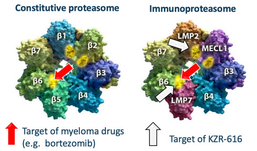
BACKGROUND

- KZR-616 is a selective inhibitor of the immunoproteasome, the form of proteasome found predominantly in immune cells. In nonclinical studies, KZR-616 blocked acute production of inflammatory cytokines, modulated T- and B-cell activation and differentiation *in vitro* and was efficacious in murine SLE models (*Muchamuel et al., ACR 2019 , Abstract No. 85*).[1]
- Recently, we presented initial safety and efficacy data from the first 3 cohorts from the open-label portion of the MISSION study of KZR-616 in SLE patients (pts) (*Furie et al., ACR 2019, Abstract No. 2520*.[2] Here we describe their baseline (BL) profiles and post-treatment changes in transcriptomic and phenotypic analyses of circulating immune cells and plasma biomarker levels.

METHODS

- Patients (N=24) received KZR-616 subcutaneously at 45 or 60 mg weekly for 13 weeks with follow-up through Week (W)25. Disease assessments were performed at BL and W5, 9, 13, 17, 21, and 25. Biomarker samples were collected at BL, W5, 17, and 25.
- Proteasome activity was measured by a subunit active site binding assay (ProCISE).[3] RNA sequencing was performed using Illumina TruSeq® with whole blood collected in PAXgene® RNA tubes. Raw data was processed with RSEM. Differential expression was modeled using DESeq2, and a variety of gene modules[4] examined using fGSEA. Cryopreserved PBMCs were analyzed by flow cytometry to profile immune cell subtypes. Plasma cytokines/proteins were quantified by electrochemiluminescent assays (Meso Scale Diagnostics, MSD) and colorimetric ELISAs.

Figure 1. Proteasome subunit composition



Muchamuel et al., ACR 2019, Abstract No. 85

Figure 2. Study design and response data

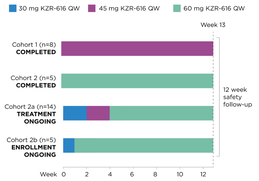
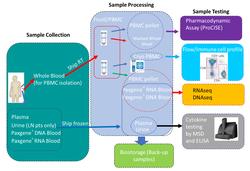


Figure 3. Sample Processing and Testing



Clinical Score*	Mean change in score from BL		
	W5 (N=16)	W13 (N=16)	W25 (N=13)
SLEDAI (BL mean: 9.9)	-1.3	-3.3	-2.2
CLASI (BL mean: 4.4)	-1.7	-2.6	-2.3
28 TJC (BL mean: 13.1)	-4.8	-6.3	-9.3
28 SJC (BL mean: 9.8)	-2.6	-6.1	-10.0
PhGA (BL mean: 54.1)	-11.9	-17.4	-19.7
PtGA (BL mean: 59)	-7.7	-20.8	-21.4
PtP (BL mean: 58.8)	-4.9	-16.1	-22.6

*SLEDAI-SLE Disease Activity Index, CLASI: Cutaneous Lupus Erythematosus Disease Area and Severity Index; TJC: Tender Joint; SJC: Swollen Joint Count; PhGA: Physician Global Assessment; PtGA: Patient Global Assessment; PtP: Patient Assessment of Pain.

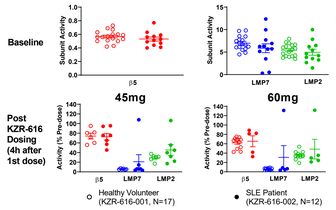
Adapted from Furie et al., ACR 2019, Abstract No. 2520

RESULTS

Table 1. Summary of available biomarker samples

	HV	SLE				
		BL (W1)	W5	W17	W25	Complete set (all 4 timepoints)
PD (pre and post dose)	17	12	NA			
Whole Blood RNAseq	6	19	20	13	11	10 (2 ongoing pts)
Plasma Cytokine	32	25	18	14	12	9 (3 ongoing pts)
Flow Cytometry	6	20	14	10	10	6 (1 ongoing pts)

Figure 4. Baseline proteasome subunit composition (top) and inhibition upon KZR-616 administration (bottom) in SLE pts and HV

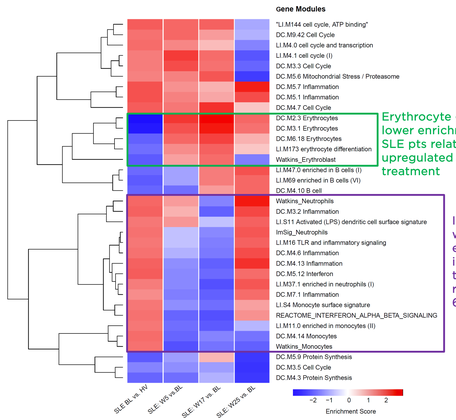


Peripheral blood baseline proteasome subunit composition in SLE pts is not significantly different from HV.

KZR-616 selectively inhibits the immunoproteasome to similar levels in SLE pts and HV post KZR-616 dosing of 45 or 60 mg

*Whole Blood (Constitutive Proteasome, β5 subunit), PBMC (immunoproteasome, LMP7 and LMP2 subunits)

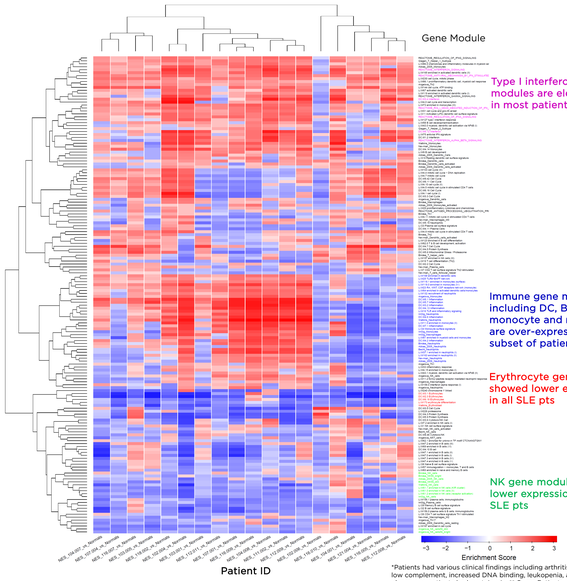
Figure 5. Gene module analysis comparing HV and SLE patients after KZR-616 administration



Erythrocyte gene modules with lower enrichment/expression in SLE pts relative to HV are upregulated after KZR-616 treatment

Immune gene modules with higher enrichment/expression in SLE pts BL relative to HV are down-regulated after KZR-616 treatment

Figure 6. Heterogeneity in gene expression across 19 patients



Type I interferon gene modules are elevated in most patients

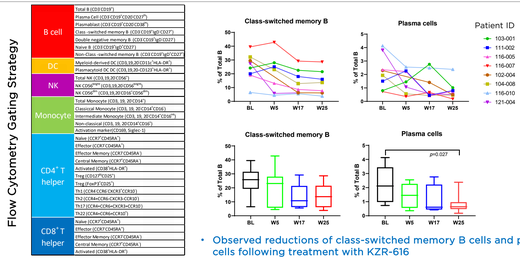
Immune gene modules including DC, BAFF, monocyte and neutrophil are over-expressed in a subset of patients

Erythrocyte gene modules showed lower expression in all SLE pts

NK gene modules showed lower expression in most SLE pts

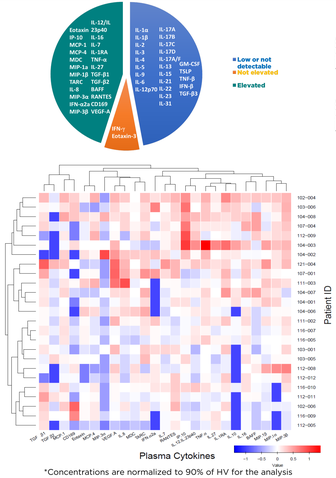
*Patients had various clinical findings including arthritis, rash, alopecia, low complement, increased DNA binding, leukopenia, and mucosal ulcers; one patients had proteinuria. ** Some Patients were taking antimalarials, prednisone.

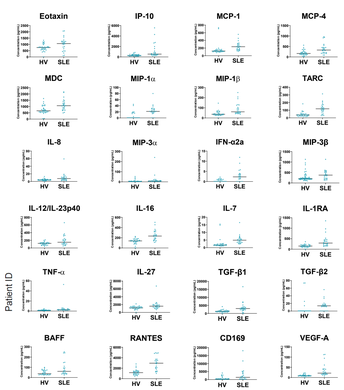
Figure 7. Immune cell profile changes with KZR-616 treatment



- Observed reductions of class-switched memory B cells and plasma cells following treatment with KZR-616

Figure 8. Baseline plasma biomarker protein profiling in SLE pts (left) and comparison with HV (right)





- Many plasma biomarkers are elevated in SLE, some cytokines were reduced in a subset of patients (not shown)

CONCLUSIONS

- We have established a workflow for sample collection and analysis of pharmacodynamic activity as well as downstream transcriptomic, proteomic, and cellular changes in patients with SLE and other autoimmune disorders treated with KZR-616.
- Basal levels of proteasome subunit composition and immunoproteasome inhibition following KZR-616 SC administration is similar in SLE patients and HV.
- Despite heterogeneity in baseline gene expression modules in SLE patients, several immune related gene modules with higher enrichment vs. HV were downregulated after treatment with KZR-616 in SLE patients.
- Erythrocyte gene modules which showed lower enrichment in SLE pts vs. HV were upregulated in patients with treatment.
- By flow cytometry, we detected reductions of class-switched memory B cells and plasma cells following treatment with KZR-616.
- We noted an array of cytokines in plasma with higher levels of expression in SLE pts vs HV.
- Our results are consistent with nonclinical data with KZR-616 and show broad anti-inflammatory activity across T, B, and innate immune effector cells at the transcriptomic, cellular, and proteomic levels.
- Analyses of additional cohorts are underway to further elucidate these initial findings and may inform future patient stratification based on molecular or cellular diagnostic criteria.

References

1. Muchamuel et al. ACR 2017; 69 (suppl 10)., ACR 2019 Abstract 85 **2.** Furie et al. EULAR 2019. Abstract #FR0196; ACR 2019, Abstract No. 2520 **3.** Lickliter et al. ACR. 2017; 69 (suppl 10). **4.** Chaussabel et. al., Immunity. 2008 29(1):150

Author Contributions

Substantial contributions to study conception/design, or acquisition/analysis/interpretation of data: RAF, JLA, BT, DB, NG, CJK; Drafting of the publication or revising it critically for important intellectual content: RAF, JLA, BT, DB, NG, CJK; Final approval of the publication: RAF, JLA, BT, DB, NG, CJK.

Author Disclosures

RAF, JLA, DB, CJK are employees of Kezar Life Sciences. BT: Consultancy fees from Kezar Life Sciences; NG: Shareholder of Kezar Life Sciences (currently with Caduceus Biomedical Consulting, LLC). This study was funded by Kezar Life Sciences.

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