Treatment of SLE Patients with Zetomipzomib (KZR-616), a Selective Inhibitor of the Immunoproteasome, Results in Circulating Gene Expression, Protein Level, and Immune Cell Phenotypic Changes with Potential Correlations to Clinical Response

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Introduction

- Systemic lupus erythematosus (SLE), a severe multiorgan autoimmune disease, involves dysfunction of multiple immune system components.
- In preclinical studies, zetomipzomib (KZR-616), a first-in-class small molecule selective inhibitor of the immunoproteasome, demonstrated pleiotropic immunomodulatory functions encompassing both innate and adaptive immune pathways[1,2].
- In the recently completed part 1 (phase Ib) portion of MISSION (NCT 03393013) trial in SLE patients, zetomipzomib exhibited encouraging safety, tolerability, and early efficacy[3].
- Preliminary biomarker analysis of the first two cohorts was previously reported[4]. Here, we present complete biomarker results from all cohorts in this study.

Methods

- The MISSION trial part 1 (phase 1b) was a 25-week open-label trial of zetomipzomib administered subcutaneously once weekly at doses ranging from 30-75 mg for 13 weeks (W) with a 12 W follow-up period.
- 47 SLE patients with or without nephritis were enrolled and 35 completed all 25 W including 2 patients had active lupus nephritis (LN).
- Clinical response at W13 or later was defined as a ≥4 reduction of SLE Disease Activity Index 2000 (SLEDAI-2K) over baseline (BL) for responders (R) or otherwise non-responder (NR).
- Biomarker samples included whole blood in PAXgene® RNA tubes, cryopreserved peripheral blood mononuclear cells (PBMCs), plasma and urine (24-hour pooled urine, LN patients only).
- Whole blood RNA sequencing (RNA-Seq) was performed using Illumina TruSeq®. Differential expression (DE) was modelled using DESeq2. Fast pre-ranked gene set enrichment analysis (FGSEA) was performed with gene sets derived from published literature. Type I interferon gene signature (IFNGS) status (high or low) was determined based on mean $\log_2$ expression of 4 genes (IFI27, IFI44, IFI44L, RSAD2).
- Cryopreserved PBMCs were analysed by flow cytometry to profile immune cell subtypes. Plasma proteins were measured by Meso Scale Discovery (MSD) kits and ELISA.

Biomarker sample	Complete sets
Whole blood RNA-Seq	31
Plasma cytokine	32
Cryopreserved PBMC (flow cytometry)	15
Urine	2

Results

Table 1. Zetomipzomib treatment was associated with minimal expression changes at the gene level

Comparison	# of DE genes (absolute $\log_2$FC>1.33; P_{adj}≤0.05)	
	Up	Down
All Patients: W5 vs. BL	5	16
All Patients: W17 vs. BL	0	3
All Patients: W25 vs. BL	0	0
SLEDAI R vs. NR at BL	4	1

Abbreviation: DE, differentially expressed; FC, fold of change; P_{adj}, adjusted P-value

Results (cont'd)

Figure 1. Gene module differences over time and between SLEDAI R and NR at baseline

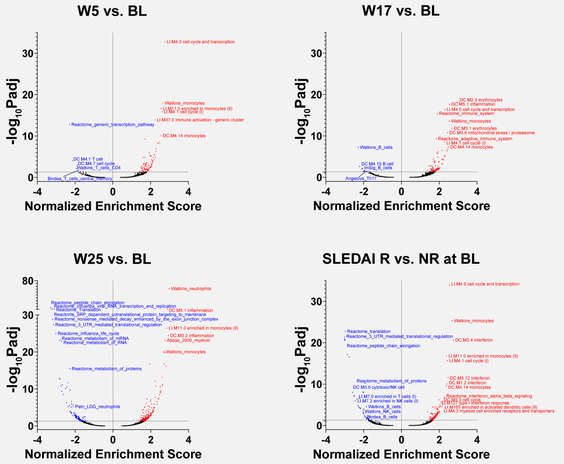
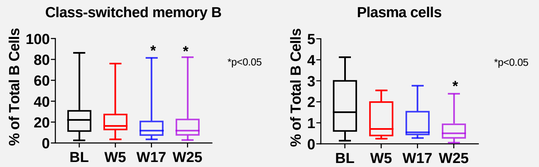
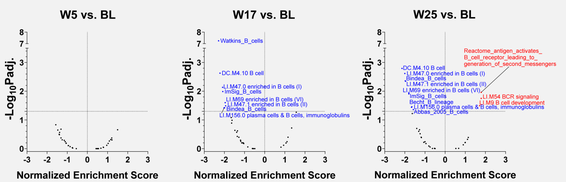


Figure 2. Class-switched memory B cells and plasma cells trended down after zetomipzomib treatment

Flow Cytometry

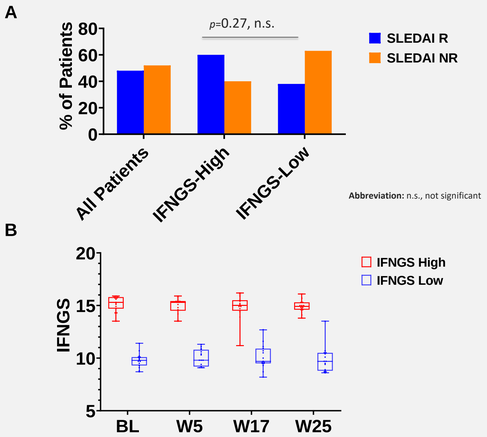


B-cell gene module enrichment



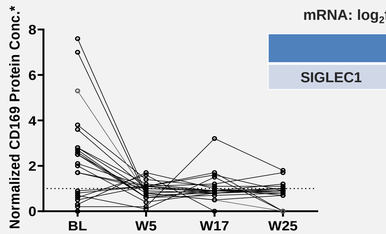
Results (cont'd)

Figure 3. Analysis of type I IFN gene signature (IFNGS) and SLEDAI responders



Abbreviation: n.s., not significant

Figure 4. Plasma CD169/SIGLEC1 protein level but not mRNA level was reduced following zetomipzomib treatment



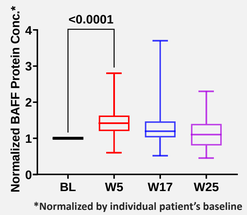
*Normalized over median level of all patients at BL (dotted line)

mRNA: $\log_2$fold-change over BL**

	W5	W17	W25
SIGLEC1	-0.10	-0.12	0.02

**p value n.s.

Figure 5. Plasma BAFF protein level but not mRNA level was transiently increased following zetomipzomib treatment



*Normalized by individual patient's baseline

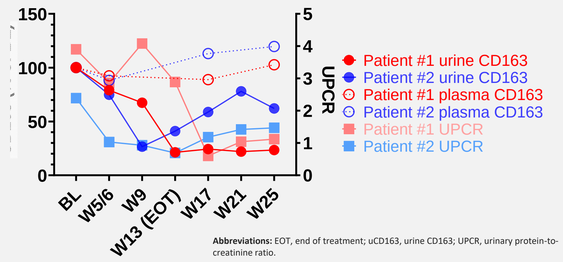
mRNA: $\log_2$fold-change over BL**

	W5	W17	W25
BAFF/TNFSF13B	0.10	0.09	0.10
TACI/TNFRSF13B	-0.08	-0.33	-0.23
BAFF-R/TNFRSF13C	-0.01	-0.25	-0.23
APRIL/TNFSF13	0.09	-0.01	0.12

**p value n.s.

Results (cont'd)

Figure 6. Reduction of urine CD163 levels following zetomipzomib treatment in 2 LN patients



Abbreviations: EOT, end of treatment; uCD163, urine CD163; UPCR, urinary protein-to-creatinine ratio.

Conclusions

- Multiple gene modules involved in immune effector cell function were found to be altered following zetomipzomib treatment.
- Circulating class-switched memory B cells and IgG-producing plasma cells were reduced over time following treatment and corresponded with reduced expression of several related gene modules. It is also consistent with previously reported reduction of circulating anti-dsDNA antibody titers in patients[3].
- Expression of a 4-gene type I interferon module at baseline showed trend of enrichment in SLEDAI R vs NR.
- Plasma protein levels of BAFF were significantly increased following treatment at W5 and returned to BL levels by W17. However, no effects on gene expression were seen for BAFF or other genes encoding related proteins (TACI, BAFF-R, APRIL).
- Plasma protein levels of CD169/ SIGLEC1, a monocyte activation marker, were reduced following treatment.
- Consistent with clinical improvements in UPCR, 2 patients with active proliferative nephritis showed a reduction in levels of urine CD163 while plasma levels of this marker were stable.

Discussion

- Our integrative analysis indicates that zetomipzomib treatment is associated with potent effects on multiple immune pathways in SLE patients.
- Potential new biomarkers (gene, gene module, circulating protein) that may be useful for prediction of patient response were identified.
- Biomarker analysis will be conducted for the MISSION Phase 2 and additional trials, including placebo-controlled trials to further our understanding of zetomipzomib mechanism of action and prediction of patient responses.

References

1. Kirk, C.J. et al., Cells 2022, 11(1):9 2. Muchamuel T, Arthritis Rheumatol. 2019; 71 (suppl 10). 3. Furie R et al., Annals of Rheumatic Diseases 2021;80:595-596. 4. Fan R, et al., Arthritis Rheumatol. 2019; 71 (suppl 10).

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