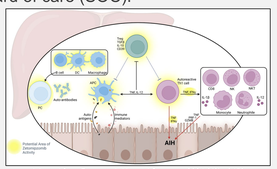
Analysis of Circulating Biomarkers in the Randomized, Double-Blind, Placebo-Controlled PORTOLA Phase 2a Study Evaluating Zetomipzomib, a Selective Immunoproteasome Inhibitor, in Patients with Autoimmune Hepatitis

Janet L. Anderl, Neel K. Anand, David Bade, Eric Lowe, Kathryn Ray, Brian Tuch, Jennifer A. Whang
Kezar Life Sciences, South San Francisco, CA, USA

Introduction

- Zetomipzomib (zeto) is a first-in-class, selective inhibitor of the immunoproteasome that has exhibited the ability to reduce inflammatory cytokine production and modulate immune effector cell gene expression and circulating populations in clinical studies in systemic lupus erythematosus (SLE) ± lupus nephritis (LN)[1-2].
- In PORTOLA (NCT05569759), a Phase 2a, randomized, double-blind, placebo-controlled study in active autoimmune hepatitis (AIH), zeto demonstrated disease-modifying activity, including steroid-sparing complete biochemical remission (CR, in 35.7% of patients entering on daily steroids), in a population with relapsed disease or inadequate response to standard of care (SOC).
- Pathogenesis of AIH is still poorly understood but involves a complex interplay of immune effector cells. Peripheral blood-derived biomarker changes following zeto administration in PORTOLA are presented here.



Adapted from Reau et al. *Hepatol Commun*. 2024;8(6):e0458 and Herkel et al. *J Hepatol*. 2020;73(2):446-448.

Methods

- In the double-blind treatment period of PORTOLA, patients (n = 24) received placebo (PBO) or 60 mg of zeto (1:2 randomization) subcutaneously once weekly for 24 weeks in addition to SOC with glucocorticoid (GC, with protocol-suggested taper) and up to two immunosuppressants.
- CR was defined as normalization of liver enzymes (ALT/AST) and immunoglobulin G (IgG, if elevated at baseline) with GC dose not higher than baseline dose.
- Whole blood was collected at Weeks (W) 0, 8 (except peripheral blood mononuclear cells [PBMCs]), 16, and 24 for biomarker analysis via the following methodologies:
  - Whole blood RNA sequencing to determine gene expression was performed using the Illumina TruSeq® Stranded mRNA kit. Differential expression was modeled using DESeq2. Fast pre-ranked gene set enrichment analysis (FGSEA) was performed with gene sets derived from published literature.
  - Plasma proteomics samples were prepared utilizing Mag-Net™-based extracellular vesicle enrichment (ReSyn Biosciences) and analyzed with tandem mass spectrometry.
  - Plasma proteins were quantified by electrochemiluminescent immunoassay (Meso Scale Discovery).
  - Immune cell profiling of cryopreserved PBMCs was performed via spectral flow cytometry.
  - Clinical laboratory values were obtained from K2 EDTA whole blood (absolute neutrophil count) and serum (IgG).

Results

Figure 1. Zeto Treatment Decreased B Cell-Related Whole Blood Gene Expression (A), Immunoglobulins (B, C), and Peripheral Cell Populations (D) and Increased Plasma BAFF Levels (E). Reduced Serum IgG and Elevated BAFF were Associated with CR.

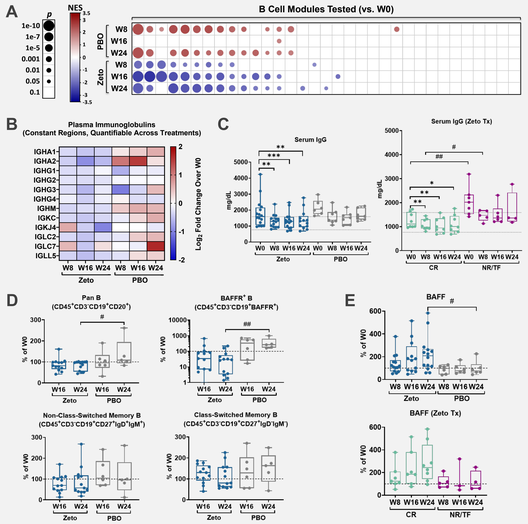
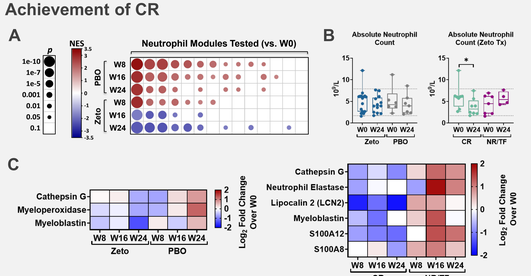


Figure 2. Decreases in Neutrophil-Related Whole Blood Gene Expression (A), Absolute Neutrophil Count (B), and Plasma Proteins (C) were Associated with Zeto Treatment and/or Achievement of CR



Results (cont'd)

Figure 3. Zeto Treatment Increased Natural Killer (NK) Cell-Related Whole Blood Gene Expression (A) and Peripheral Cell Populations (B). Lower Baseline NK Gene Expression (C) and Cell Populations (D) were Associated with Response.

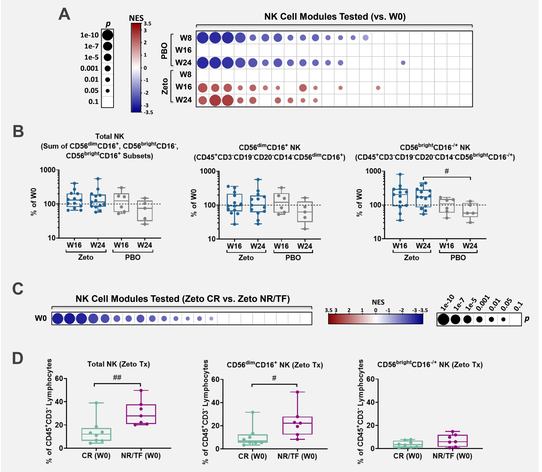
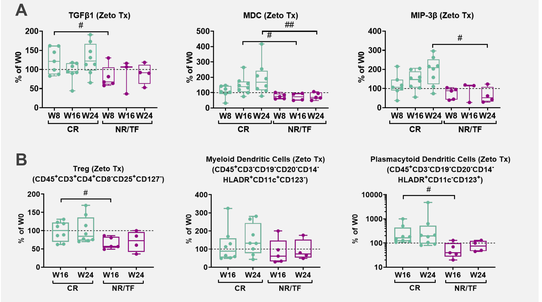
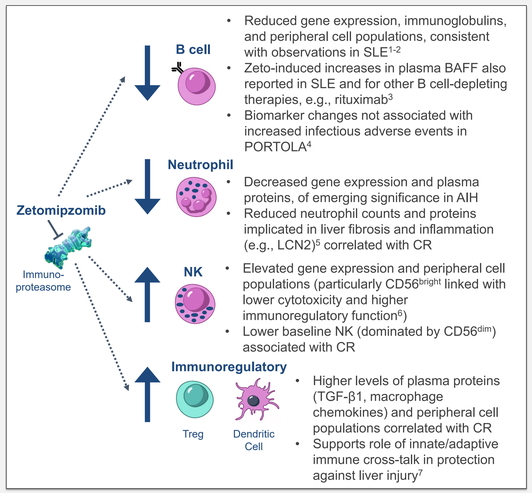


Figure 4. Higher Levels of Immunoregulatory Plasma Proteins (A) and Peripheral Cell Populations (B) were Associated with Attainment of CR Following Zeto Treatment



Summary



- Reduced gene expression, immunoglobulins, and peripheral cell populations, consistent with observations in SLE[1-2]
- Zeto-induced increases in plasma BAFF also reported in SLE and for other B cell-depleting therapies, e.g., rituximab[3]
- Biomarker changes not associated with increased infectious adverse events in PORTOLA[4]
- Decreased gene expression and plasma proteins, of emerging significance in AIH
- Reduced neutrophil counts and proteins implicated in liver fibrosis and inflammation (e.g., LCN2)[5] correlated with CR
- Elevated gene expression and peripheral cell populations (particularly CD56^{bright} linked with lower cytotoxicity and higher immunoregulatory function[6])
- Lower baseline NK (dominated by CD56^{dim}) associated with CR
- Higher levels of plasma proteins (TGF-β1, macrophage chemokines) and peripheral cell populations correlated with CR
- Supports role of innate/adaptive immune cross-talk in protection against liver injury[7]

Conclusions

- In the PORTOLA Phase 2a study, zeto administration resulted in notable alterations in biomarker activity across both the innate and adaptive immune systems that correlated with clinically relevant biochemical responses in AIH.
- Circulating biomarker changes following zeto treatment were consistent with previous reports from clinical studies in patients with SLE ± LN.
- These findings provide intriguing new insights into AIH biology for further mechanistic studies and validation in the AIH population.

References

1. Fan et al. 2022 EULAR Congress. **2**. Furie et al. 2024 ACR Convergence. **3**. Vallerskog et al. *Arthritis Res Ther*. 2006, 8(6):R167. **4**. Lammert et al. 2025 AASLD TLM. **5**. Chen et al. *Sci Rep*. 2020, 10(1):15558. **6**. Liu et al. *Front Immunol*. 2021, 12:622306. **7**. Koda et al. *J Clin Invest*. 2019, 129(8):3201.

Author Disclosures and Acknowledgements

JLA, **NKA**, **DB**, **EL**, **KR**, and **JAW** are employees and shareholders of Kezar. **BT** is a consultant and shareholder of Kezar.
The authors express their gratitude to the patients and families that participated in the PORTOLA trial.

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAFF, B cell activating factor; BAFFR, B cell activating factor receptor; CD, cluster of differentiation; CR, complete (biochemical) remission; FGSEA, fast pre-ranked gene set enrichment analysis; GC, glucocorticoid; HLADR, human leukocyte antigen-DR isotype; IgG, immunoglobulin G; K2 EDTA, dipotassium ethylenediaminetetraacetic acid; LCN2, lipocalin 2; LN, lupus nephritis; MDC, macrophage-derived chemokine; MIP-3β, macrophage inflammatory protein 3β; NES, normalized enrichment score; NK, natural killer; NR, no response; PBMC, peripheral blood mononuclear cell; PBO, placebo; RNA, ribonucleic acid; SLE, systemic lupus erythematosus; SOC, standard of care; TF, treatment failure; TGFβ1, transforming growth factor β1; Treg, regulatory T cell; Tx, treatment; W, week; zeto, zetomipzomib.

Statistical Analyses: *p < 0.05, **p < 0.01, *** p < 0.001 by Wilcoxon matched-pairs signed-rank test;
#p < 0.05, ##p < 0.01, ###p < 0.001 by Wilcoxon rank-sum (Mann-Whitney U) test

Presented at AASLD TLM 2025 • Washington, DC, USA • November 10, 2025

KEZAR LIFE SCIENCES