Quantification of peripheral blood mononuclear cell (PBMC) glycogen as a novel biomarker for therapeutic intervention in Pompe Disease

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Introduction

Pompe disease is caused by mutations in the enzyme acid alpha-glucosidase and is characterized by pathological accumulation of glycogen that drives dysfunction in skeletal and cardiac muscle. As a potential novel treatment for Pompe disease, we have developed a small molecule inhibitor of GYS1, the primary glycogen synthase in muscle. A key challenge for all glycogen-targeted investigational therapies for Pompe disease is that reliable longitudinal measurement of muscle glycogen is limited by the difficulty of obtaining multiple muscle biopsies. Motivated by previous observations that PBMCs contain glycogen, we developed an assay to quantify PBMC glycogen for the purpose of monitoring GYS1 inhibition in humans[1].

Methods

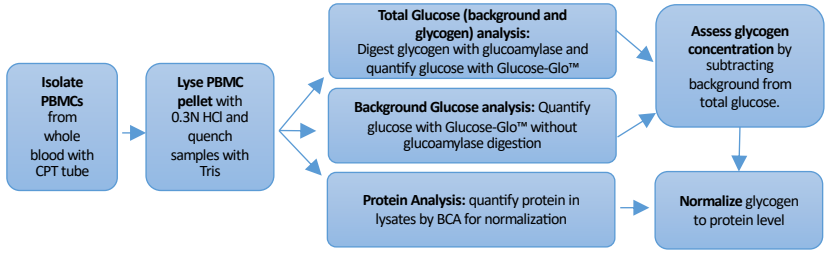
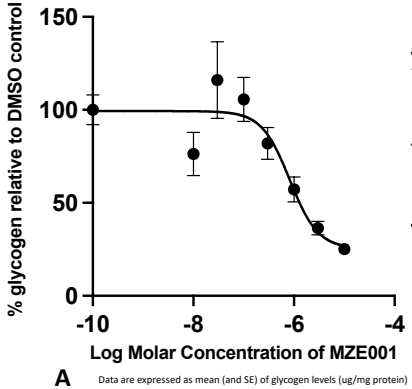


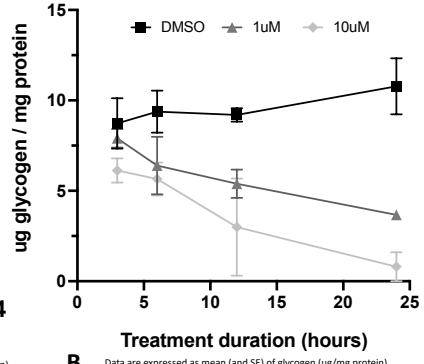
Figure 1. PBMC glycogen assay flow chart. Both Glucose-Glo™ measurements were performed on the same plate to avoid plate effects. Results were graphed using GraphPad Prism 9.

MZE001 reduces glycogen in human PBMCs *in vitro*

Figure 2. Pharmacological inhibition of GYS1 with MZE001 reduces glycogen in cultured human PBMCs in a dose- and time-dependent manner. Effects of MZE001 on PBMC glycogen in cultured human PBMCs isolated from healthy donors. **A.** MZE001 was added to cultured PBMC media at varying concentrations and incubated for 24 hours followed by glycogen quantification. **B.** MZE001 was added to cultured PBMC media at varying concentrations incubated for 3, 6, 12, or 24 hours followed by glycogen quantification.



A Data are expressed as mean (and SE) of glycogen levels (ug/mg protein) normalized to the DMSO controls.



B Data are expressed as mean (and SE) of glycogen (ug/mg protein) from PBMCs from two donors.

GYS1 SRT reduces glycogen in Pompe mouse and canine PBMCs *in vivo*

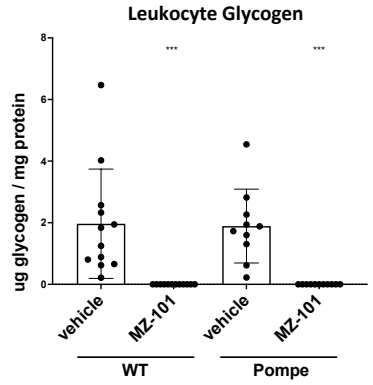
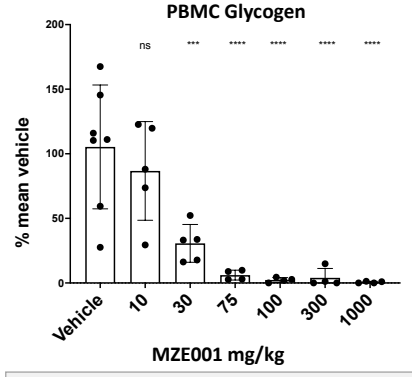
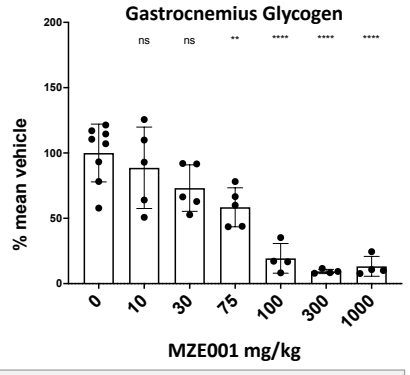


Figure 3 (top). MZ-101 orally dosed ad libitum via chow formulation reduced glycogen in leukocytes isolated from WT and Pompe mice. GYS1 inhibitor MZ-101 was formulated in rodent chow diet and given to male WT (B6129SF1/J) and Pompe (Gaa^{tm1Rabn}) mice ad libitum. Mouse leukocytes (PBMCs and granulocytes) were isolated at 4-weeks using a red blood cell lysis method and glycogen (ug/mg protein) was quantified. [Mean ± SEM; One-way ANOVA (WT vehicle vs WT MZ-101 and Pompe vehicle vs Pompe MZ-101) ***p<0.001; Two values were identified as outliers (Grubbs' test) and excluded]

Figure 4 (bottom). MZE001 orally dosed QD for 7 days reduces PBMC glycogen and muscle (gastrocnemius) glycogen in canines in a dose-dependent manner. Effects of MZE001 (10, 30, 75, 100, 300 and 1000 mg/kg QD on PBMC glycogen (ug/mg protein) in beagle dogs. PBMCs were isolated using Histopaque®-1077.

Data from each study are expressed as mean (and SE) of glycogen levels normalized to the vehicle controls from the same study. Mean ± SEM; One-way ANOVA, Dunnett's post-hoc test vs vehicle; ns p>0.05, **p<0.01, ***p<0.001, ****p<0.0001; One value in the 75 mg/kg group was identified as an outlier (Grubbs' test) and excluded.





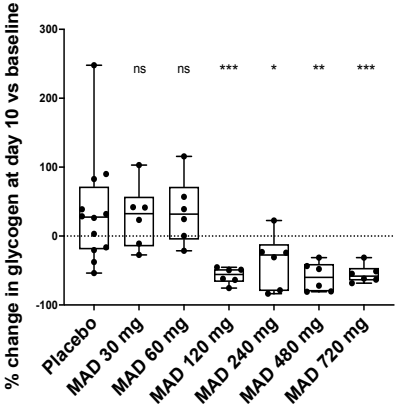
Fit-for-purpose qualification of clinical PBMC glycogen assay

Table 1. Excerpt of results of assay qualification demonstrate the PBMC glycogen assay's specificity, accuracy, precision and limits of detection. %RE and %TE were also assessed and are within 20%.

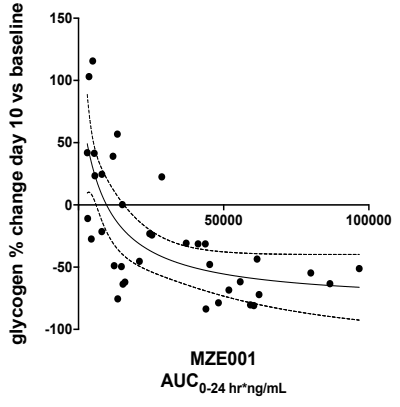
		Intra-Assay %CV:	Inter-Assay %CV:	
Standard Curve	Buffer Treated	0.6 – 8.7%	0.2 – 1.7%	Range: 50.0 – 0.512 µM
	Enzyme Treated	0.2 – 7.8%	0.3 – 1.0%	
HQC (40 µM)	Buffer Treated	2.0 – 4.2%	7.3%	Inter-assay %RE buffer vs enzyme treated: 1.7%
	Enzyme Treated	2.3 – 4.2%	6.0%	
MQC (25 µM)	Buffer Treated	2.3 – 4.4%	5.6%	Inter-assay %RE buffer vs enzyme treated: 2.3%
	Enzyme Treated	1.8 – 5.0%	7.3%	
LQC (10 µM)	Buffer Treated	1.4 – 3.6%	6.1%	Inter-assay %RE buffer vs enzyme treated: 1.7%
	Enzyme Treated	0.7 – 6.1%	6.3%	

MZE001 reduces PBMC glycogen in healthy human subjects

Figure 5. MZE001 leads to dose-dependent and exposure-dependent reduction in PBMC glycogen after 10 days of treatment with MZE001 in healthy volunteers.
PBMCs were isolated for glycogen quantification at baseline and day 10. **A.** Data are presented as % reduction vs baseline. **B.** Data are presented as individual values from all dosing cohorts on day 10. X-axis shows the AUC exposure of MZE001 on day 10. Y-axis shows the %change in PBMC glycogen on day 10 compared to baseline.



Min-max, median; Ranked ANCOVA vs Placebo ns p>0.05, *p<0.05, **p<0.01, ***p<0.001



3 parameter non-linear curve fit with 95% confidence intervals; R^2 = 0.4997

Conclusions

1. We developed a novel assay to quantify PBMC glycogen and used it in preclinical studies to demonstrate that pharmacological inhibition of GYS1 reduces glycogen in PBMCs from healthy human volunteers treated *in vitro*, and in PBMCs from healthy canines and WT and Pompe mice treated *in vivo*.
2. PBMC glycogen correlates with gastrocnemius glycogen levels in canines treated with varying doses of MZE001.
3. Fit-for-purpose qualification of the assay by KCAS Bioanalytical and Biomarker Services, Kansas, demonstrated the assay to be robust, accurate, precise and suitable for clinical use.
4. Phase I data demonstrate MZE001 drives dose-dependent and concentration-dependent reduction of PBMC glycogen in healthy volunteers with similar potency to preclinical models.

For more information please see: PRESENTATION *Late-Breaking Science* "Results from a first in human study of MZE001, an orally bioavailable inhibitor of glycogen synthase 1 and potential substrate reduction therapy for Pompe disease" (also presented in **POSTER LB-65**)

References

[1]D. Parisi, O. Musumeci, S. Mondello, T. Brizzi, R. Oteri, A. Migliorato, A. Cranni, T. Mongini, C. Rodolico, G. Vita, A. Toscano, Vacuolated PAS-Positive Lymphocytes on Blood Smear: An Easy Screening Tool and a Possible Biomarker for Monitoring Therapeutic Responses in Late Onset Pompe Disease (LOPD). Front Neurol. Volume 9, Article 880 (2018).

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