Pharmacology of Small Molecule Inhibitors of GYS1 in Canines and a Mouse Model of Pompe Disease

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Introduction

Pompe disease is a glycogen storage disease caused by mutations in the enzyme acid alpha-glucosidase, resulting in pathological accumulation of glycogen. Glycogen can accumulate in virtually all tissues, but the primary pathology affects skeletal and cardiac muscle. Current standard of care treatment for Pompe patients consists of enzyme replacement therapy (ERT) with human recombinant GAA (Byrne et al., 2011). ERT treatment has been life saving for infant-onset Pompe disease (IOPD) patients, and significantly slows the rate of disease progression in late-onset Pompe Disease (LOPD) patients. However, for both patient populations the beneficial effects of ERT generally plateau within a few years of treatment, after which the disease continues to progress; an unmet need remains for an effective and safe treatment for Pompe disease (Schoser, 2019, Meena et al., 2020).

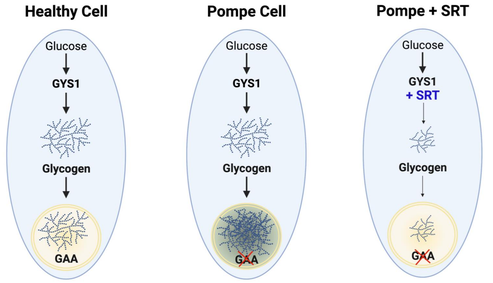
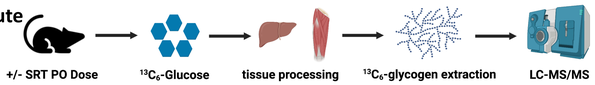


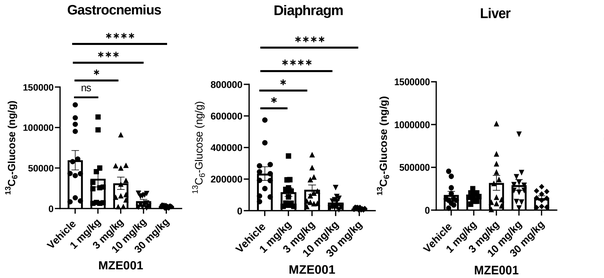
Figure 1. Schematic of restoration of glycogen homeostasis in Pompe cells after Substrate Reduction Therapy (SRT)

GYS1 is the rate-limiting enzyme in muscle glycogen biosynthesis. In mouse models of Pompe disease, reducing glycogen synthesis via genetic ablation of GYS1 attenuates glycogen accumulation and muscle pathology (Douillard-Guilloux et al., 2010). MZE001 and MZ-101 are novel, selective, small molecule inhibitors of muscle glycogen synthase 1 (GYS1) under development at Maze Therapeutics. We propose that by inhibiting glycogen synthesis, tissue glycogen content and its associated toxicity will be reduced in Pompe patients. Figure 1 illustrates our therapeutic hypothesis.

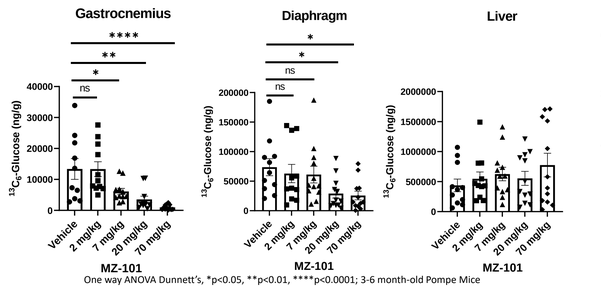
MZE001 and MZ-101 inhibit *de novo* glycogen synthesis *in vivo*

Figure 2. Schematic of acute $^{13}C_6$-glycogen synthesis study design





One way ANOVA Dunnett's, *p<0.05, ***p<0.001, ****p<0.0001; 7-10 week-old Pompe Mice



One way ANOVA Dunnett's, *p<0.05, **p<0.01, ****p<0.0001; 3-6 month-old Pompe Mice

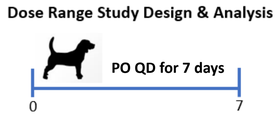
Figure 3. MZE001 and MZ-101 potently inhibit glycogen synthesis in skeletal muscle but not liver in Pompe Mice

Male Pompe mice were dosed as indicated in Figure 2.

MZE001 and MZ-101 potently inhibit glycogen synthesis in Pompe gastrocnemius and diaphragm in a dose-dependent manner, indicating on-target GYS1 inhibition *in vivo*. Glycogen synthesis in the liver is not altered by MZE001 or MZ-101.

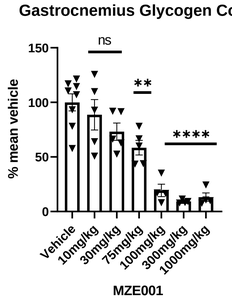
Values plotted as mean ± SEM.

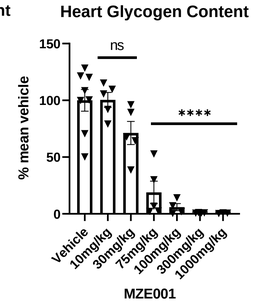
MZE001 reduces muscle glycogen & biomarkers in canines

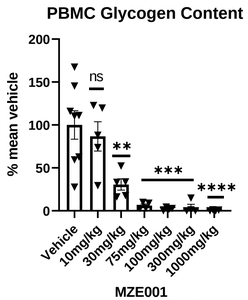


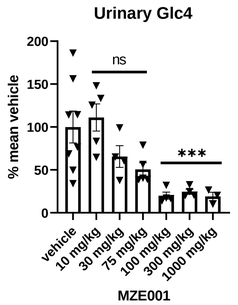
N=4-7 Male & Female Wild-Type beagle dogs

Data are combined from two separate studies









One way ANOVA Dunnett's, **p<0.01, ***p<0.001, ****p<0.0001

Figure 4. MZE001 QD for 7 days reduces PBMC glycogen, uGlc4 and muscle glycogen in dogs in a dose-dependent manner

MZE001 reduces glycogen content in the gastrocnemius and heart in a dose-dependent manner in dogs. Correlation in PBMC glycogen (collected 24 hours post day 7 dose) and uGlc4 (collected from day 7 necropsy) reductions is also observed.

Values plotted as percent of mean of respective vehicle groups.

SRT/ERT combination therapy reduces PD biomarkers

Figure 5. Schematic of chronic SRT/ERT treatment in 6-9 week-old male 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. 20mg/kg ERT (Alglucosidase alfa) was given i.v. biweekly.

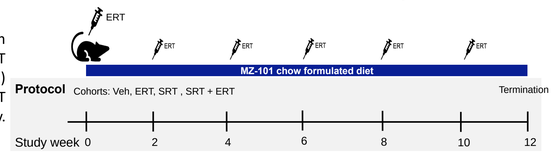
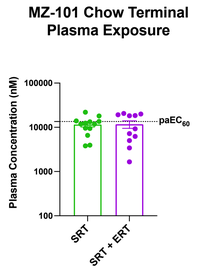
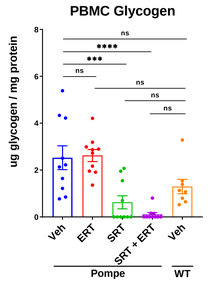
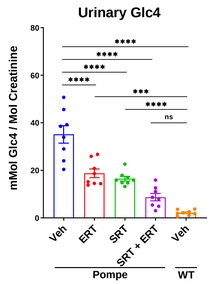


Figure 6. SRT mean plasma exposure of ~paEC$_{60}$ potently reduced PBMC glycogen and uGlc4 with additive benefit from combination SRT + ERT therapy



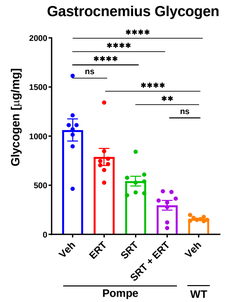
Value plotted as Mean ± SEM

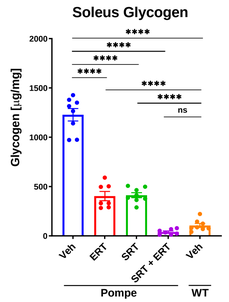


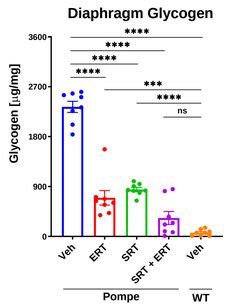


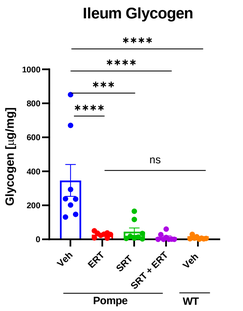
Value plotted as Mean ± SEM One way ANOVA Dunnett's, ***p<0.001, ****p<0.0001

SRT/ERT combination normalizes glycogen & cellular dysfunction









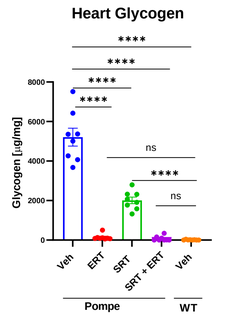
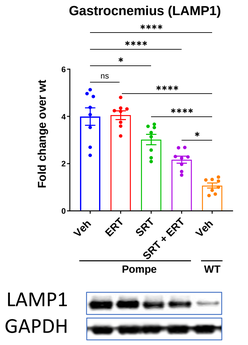


Figure 7. SRT/ERT combination normalized muscle glycogen levels in Pompe mice

ERT and SRT mono-therapy for 12 weeks significantly reduces glycogen content across all tissues. Combination treatment of ERT and SRT provides additive benefit in glycogen clearance and normalized glycogen levels in skeletal muscles.

Values plotted as mean±SEM.

One way ANOVA Dunnett's, **p<0.01, ***p<0.001, ****p<0.0001



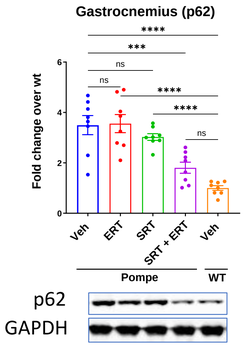


Figure 8. SRT/ERT combination therapy restored lysosomal and autophagasome stress in Pompe gastrocnemius muscle

12 weeks of combination treatment of ERT and SRT significantly improve markers of lysosomal (LAMP1) and autophagosome (p62) stress in the gastrocnemius compared to SRT or ERT mono-therapy.

Western blots analyzed by densitometry and plotted as fold change over vehicle treated WT mice. Representative samples from each treatment group shown.

One way ANOVA Dunnett's, *p<0.05, ***p<0.001, ****p<0.0001

Conclusions

1. MZE001 and MZ-101 potently and specifically inhibit GYS1 *in vivo*.
2. MZE001 reduces tissue glycogen, which strongly correlates with decreased biomarkers in dogs.
3. Chronic treatment with MZ-101 reduces elevated glycogen levels in Pompe mouse skeletal muscle and is associated with improvements in markers of cellular dysfunction.
4. Combination therapy of MZ-101 + ERT normalizes tissue glycogen and restores cellular homeostasis.

References & Acknowledgements

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4. Schoser B (2019). Pompe disease: what are we missing? Ann. Transl. Med., 7: 292.
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