Pompe disease is an inherited lysosomal storage disease that results from deficiency in acid β-glucosidase (GAA) activity, and is characterized by accumulation of lysosomal glucoglycan primarily in heart and skeletal muscles. While recombinant human GAA (rhGAA; alglucosidase alfa; Lumizyme; Genzyme-Sanofi) provides some clinical benefits, the infused enzyme tends to be unstable at neutral pH/body temperature, shows insufficient uptake in key tissues, and can elicit immune responses that affect tolerability and efficacy. The pharmacological chaperone AT2220 (3-deoxyinosinimycin HCl, divulgostat HC) has been shown to improve the pharmacological properties of rhGAA via direct binding and stabilization, leading to increased enzyme uptake and glycogen reduction in Gaa knock-out (KO) mice when co-administered (prior to rhGAA) or co-formulated with rhGAA. Liquid chromatography/tandem mass spectrometry quantitation of AT2220 in animal plasma and tissues is challenging due to the compound’s polarity, low molecular weight, and high baselines observed in mass spectra. A LC-MS/MS method has been developed for the quantitation of AT2220 in rodent plasma, and the disease-relevant tissues heart and skeletal muscle (quadriceps). This method has enabled in vivo preclinical studies of AT2220 co-formulated or co-administered with rhGAA.

3. Representative Calibration Curve of AT2220 in Rat Plasma

- Heparinized plasma from male Sprague Dawley rats was spiked with known concentrations of AT2220 in order to generate an 11-point calibration set. After SPE extraction and LC-MS/MS analysis, the resulting AT2220 concentrations were plotted using linear regression with 1/x weighting.
- For rat plasma, the correlation coefficient (R) is R = 0.9982.
- The rat plasma AT2220 assay range is 1.00 - 2000 ng/mL.
- Inter-day mean accuracy (NRbias) ranged from 1.06 – 15.7 and inter-day precision (%CV) ranged from 3.91 – 11.1.
- The assay range for mouse heart and quadriceps tissue homogenate is 1.00 – 2000 ng/mL (6.13 nM - 12.3 μM) and 2.00 – 2000 ng/mL (12.3 nM - 12.3 μM). This equates to 0.01 – 16000 ng/g tissue (0.01 nM – 98.1 μM) and 16.0 – 16000 ng/g tissue (98.1 nM – 98.1 μM), respectively.
- R values, mean accuracy, and precision were similar for the mouse heart and quadriceps tissue analysis (data not shown).

4. LC-MS/MS Chromatograms of AT2220 Extracted from Rat Plasma and Disease-Relevant Mouse Tissues

- AT2220 was detected up to 8 hrs after administration as co-formulation or co-administration with rhGAA.
- For disease-relevant tissue samples, AT2220 levels were measured by LC-MS/MS AT2220 levels were detected in all tissue analyzed.

5. AT2220 Levels were Detected in Rat Plasma and Disease-Relevant Mouse Tissues

- AT2220 was detected in all tissue analyzed.
- AT2220 levels were detected up to 8 hrs after administration as co-formulation or co-administration with rhGAA.
- AT2220 levels were detected in all tissue analyzed.

6. Co-formulation or Co-administration of AT2220 Increases the Circulating Levels of Active rhGAA in Rats

- AT2220 increases the physical stability of rhGAA at neutral pH and body temperature in vitro.
- AT2220 maintains rhGAA in a stable active form ex vivo in human whole blood at neutral pH and body temperature.
- A LC-MS/MS method was developed for the quantitation of AT2220 in rodent plasma and disease-relevant heart and quadriceps tissue. The method showed good linearity, dynamic range, accuracy and precision, allowing reliable quantitation.
- AT2220 levels were measured in plasma up to 8 hours from rats given a single tail vein bolus administration of rhGAA (20 mg/kg) co-formulated with AT2220 (10 mg/kg) or rhGAA (20 mg/kg) 30 minutes following an AT2220 (10 mg/kg) oral co-administration.
- AT2220 levels were measured in heart and quadriceps tissue from wild-type mice given a single oral dose (30 mg/kg) and were not detectable (BLG) after oral dose, flash frozen, and AT2220 levels were measured by LC-MS/MS AT2220 levels were BLG for all 72 hour samples.

7. Co-formulation or Co-administration of AT2220 Leads to Greater Enzyme Uptake Compared to rhGAA Alone in Disease-Relevant Tissues of Gaa KO Mice

- AT2220 increases the physical stability of rhGAA at neutral pH and body temperature in vitro.
- AT2220 maintains rhGAA in a stable active form ex vivo in human whole blood at neutral pH and body temperature.
- A LC-MS/MS method was developed for the quantitation of AT2220 in rodent plasma and disease-relevant heart and quadriceps tissue. The method showed good linearity, dynamic range, accuracy and precision, allowing reliable quantitation.
- AT2220 levels were measured in plasma up to 8 hours from rats given a single tail vein bolus administration of rhGAA (20 mg/kg) co-formulated with AT2220 (10 mg/kg) or rhGAA (20 mg/kg) 30 minutes following an AT2220 (10 mg/kg) oral co-administration.
- AT2220 levels were measured in heart and quadriceps tissue from wild-type mice given a single oral dose (30 mg/kg) and were not detectable (BLG) after oral dose, flash frozen, and AT2220 levels were measured by LC-MS/MS AT2220 levels were BLG for all 72 hour samples.

Conclusions

- AT2220 increases the physical stability of rhGAA at neutral pH and body temperature in vitro.
- AT2220 maintains rhGAA in a stable active form ex vivo in human whole blood at neutral pH and body temperature.
- A LC-MS/MS method was developed for the quantitation of AT2220 in rodent plasma and disease-relevant heart and quadriceps tissue. The method showed good linearity, dynamic range, accuracy and precision, allowing reliable quantitation.
- AT2220 levels were measured in plasma up to 8 hours from rats given a single tail vein bolus administration of rhGAA (20 mg/kg) co-formulated with AT2220 (10 mg/kg) or rhGAA (20 mg/kg) 30 minutes following an AT2220 (10 mg/kg) oral co-administration.
- AT2220 levels were measured in heart and quadriceps tissue from wild-type mice given a single oral dose (30 mg/kg) and were not detectable (BLG) after oral dose, flash frozen, and AT2220 levels were measured by LC-MS/MS AT2220 levels were BLG for all 72 hour samples.