Strategy to Assess the Pharmacokinetics of a Proprietary Human Acid α-Glucosidase Containing High Mannose 6-Phosphate For Its Development As a Potential Next-Generation Treatment for Pompe Disease

Elfrida Benjamin[1], Rick Hamler[1], Richie Khanna[1], Deborah Hilliard[1], Su Xu[1], Ken Valenzano[1], Russell Gotschall[1], Hung Do[1], and Franklin K. Johnson[1]

[1]Amicus Therapeutics, Cranbury, NJ, USA

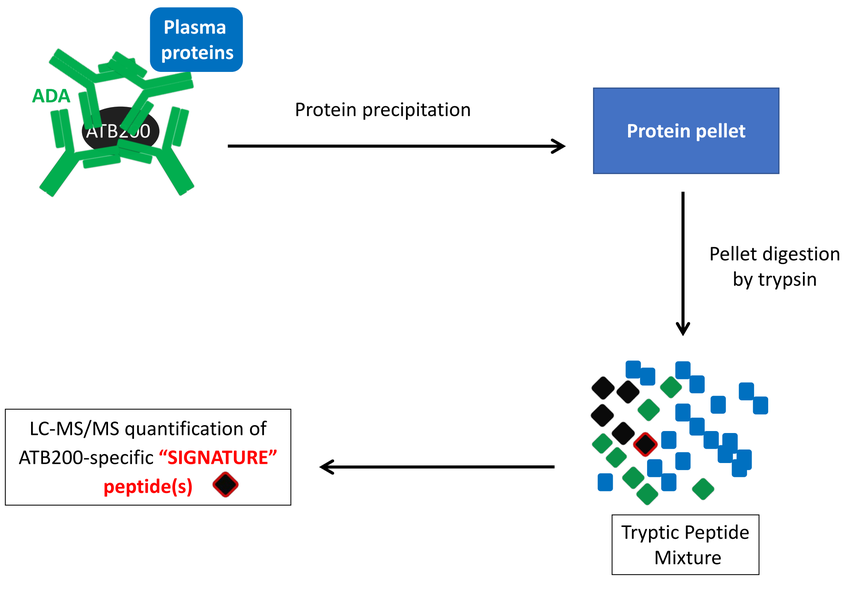
Introduction

Pompe disease is a lysosomal storage disorder that results from deficiency in acid α-glucosidase (GAA) activity, and is characterized by lysosomal glycogen accumulation. The current treatment is enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA). The targeting and uptake of rhGAA into lysosomes requires mannose-6-phosphate (M6P), a specialized carbohydrate that binds to cation-independent M6P receptors (CIMPR) at the cell surface. The quality and quantity of M6P on existing ERT is not optimal, which limits lysosomal targeting. While existing ERTs provide some clinical benefit, unmet needs still exist due to inadequate muscle uptake.

We have developed a novel rhGAA enzyme with significantly higher M6P content compared to existing products. This new rhGAA (designated as ATB200) binds the CIMPR with high affinity *in vitro*, and is more efficiently internalized by skeletal muscle myoblasts. Co-administration of a pharmacological chaperone to stabilize the ERT and prevent inactivation provides additive benefits.

To support the development of ATB200, a comprehensive approach to assess its pharmacokinetic profile was developed. The measurement of circulating active enzyme by a GAA activity assay is complemented with an absolute quantitative method to measure total rhGAA protein. The latter utilizes LC-MS/MS quantification of ATB200-specific "signature" peptides after trypsin digestion of proteins present in plasma samples.

LC-MS/MS Signature Peptide Assay Principle



ADA: Anti-drug antibodies

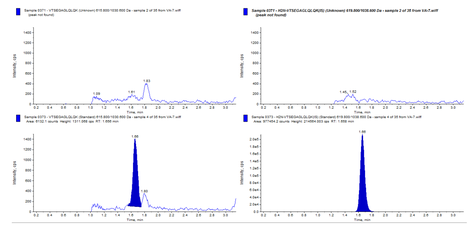
Signature Peptides

- Two signature peptides are monitored

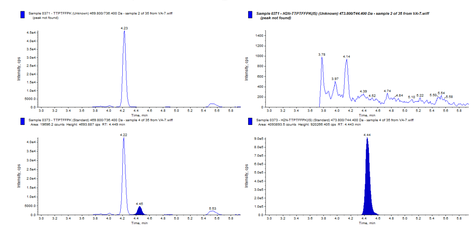
Experimental Method

- Analyte: ATB200, after digestion, signature peptides quantified by LC-MS/MS analysis
  - T50: VTSEGAGLQLQK
  - T9: TTPTFFPK
- Stable Isotope Labeled (SIL) Internal Standards:
  - T50: VTSEGAGLQLQK-$^{13}C_6{}^{15}N_2$
  - T9: TTPTFFPK-$^{13}C_6{}^{15}N_2$
- Sample Volume: 20 µL rat or monkey K_2 EDTA plasma
- Sample pretreatment: Protein precipitation followed by pellet digestion
- ATB200 Standards prepared in rat or monkey K_2 EDTA plasma
- Assay range: 0.500 – 500 µg/mL
- Quality controls prepared in rat or monkey K_2 EDTA plasma
- Calibration Curve: Linear regression with $1/x^2$ weighting
- Quantitation: Peak area ratio of signature peptide to internal standard
- Chromatography:
  - Waters Acquity UPLC system
  - Column: Acquity UPLC HSS (high strength silica) T3 (C18) (50x2.1 mm, 1.8 um particle size)
  - Mobile phases:
  - A: 0.1% formic acid in water
  - B: 90:10 / acetonitrile:DMSO (v:v)
  - Elution:
    - Time mL/min %A
    - Initial 0.800 100
    - 3.00 0.800 100
    - 3.05 0.800 95.0
    - 6.25 0.800 95.0
    - 6.30 0.800 50.0
    - 7.00 0.800 50.0
    - 7.10 0.800 10.0
    - 8.00 0.800 10.0
    - 8.10 0.800 100
- Detection: Mass spectrometer Triple Quad 6500 (ABSciex)
  - Ionization: positive electrospray
  - Analysis performed in the selected reaction monitoring mode
  - Transitions:
    - T50: 615.9 > 1030.6
    - SIL T50: 619.9>1038.6
    - T9: 469.8 > 736.4
    - SIL T9: 473.8 > 744.4
- Assay Validation Results (Both Peptides):
  - Intra-day accuracy (% bias) range: -4.5 to 8.4% and -5.7 to 9.2% for rat and monkey, respectively
  - Inter-day accuracy (% bias) range: -1.6 to 7.1% and 0.1 to 5.4% for rat and monkey, respectively
  - Intra-day precision (% coefficient of variation) range: 1.8 to 5.4% and 1.7 to 5.0% for rat and monkey, respectively
  - Inter-day precision (% coefficient of variation) range: 0.0 to 4.6% and 0.0 to 6.0% for rat and monkey, respectively
  - Selectivity in matrix ± high concentration anti-ATB200 antibodies, dilutional linearity, stability and other assay validation parameters met pre-specified acceptance criteria

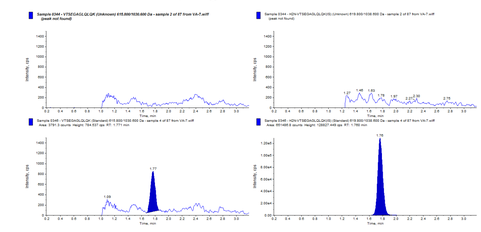
Rat Plasma / Blank and LLOQ Sample / **T50 Peptide**
Left Panel: Compound / Right Panel: SIL Internal Standard



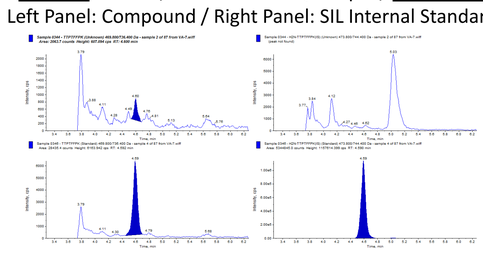
Rat Plasma / Blank and LLOQ Sample / **T9 Peptide**
Left Panel: Compound / Right Panel: SIL Internal Standard



Monkey Plasma / Blank and LLOQ Sample / **T50 Peptide**
Left Panel: Compound / Right Panel: SIL Internal Standard

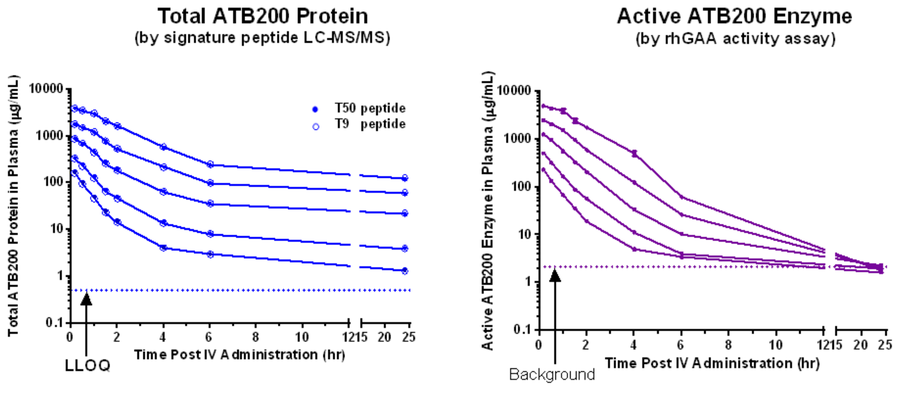


Monkey Plasma / Blank and LLOQ Sample / **T9 Peptide**
Left Panel: Compound / Right Panel: SIL Internal Standard



ATB200 Single-dose Rat Study

- Study Design:
  - ATB200 doses of 10, 20, 50, 100, and 200 mg/kg were administered via bolus tail vein injection to Sprague Dawley rats
  - Plasma samples for active ATB200 (measured by GAA activity assay) and total ATB200 concentrations (measured by T09 and T50 signature peptide LC-MS/MS assay) were collected up to 24 hours post-dose
- Results:
  - Total ATB200 protein concentrations closely matched those of active ATB200 at time points up to 4 hours
  - At later time points, the concentrations of active ATB200 were progressively lower compared to total ATB200 protein



ATB200 Single-dose Rat Study: Pharmacokinetic Analysis

Dose (mg/kg)	ATB200	C_{max} [a] (µg/mL)	C_{24h} [b] (µg/mL)	AUC_{0-t} [a] (µg*hr/mL)	$AUC_{0-\infty}$ [a] (µg*hr/mL)	$t_{½}$ [b] (hr)	CL_T [b] (mL/hr/kg)	V_{ss} [b] (mL/kg)
10	GAA Activity	230 (4.9)	1.66 (0.792)	237 (5.7)	258 (11.5)	10.1 (6.57)	38.9 (4.45)	244 (121)
10	T09	162 (1.7)	1.31 (0.053)	174 (0.97)	200 (0.64)	13.5 (1.30)	50.1 (0.320)	438 (34.3)
10	T50	170 (4.4)	1.37 (0.062)	184 (0.79)	210 (1.7)	13.7 (1.05)	47.5 (0.823)	414 (24.8)
20	GAA Activity	504 (7.6)	1.97 (0.469)	512 (3.5)	523 (2.7)	5.5 (2.9)	38.3 (1.04)	113 (22.8)
20	T09	329 (7.7)	3.87 (0.340)	454 (0.8)	515 (6.0)	11.3 (4.6)	38.9 (2.34)	328 (107)
20	T50	339 (7.2)	4.06 (0.556)	480 (1.2)	544 (7.6)	11.4 (5.0)	36.8 (2.72)	309 (111)
50	GAA Activity	1262 (4.1)	2.31 (0.365)	1515 (7.1)	1535 (6.7)	6.2 (0.8)	32.6 (2.13)	71.9(10.3)
50	T09	865 (6.9)	21.8 (0.774)	1714 (3.1)	1942 (11.8)	9.0 (5.6)	25.9(2.88)	232 (73.6)
50	T50	881 (5.2)	21.9 (2.03)	1747 (3.4)	1978 (14.2)	8.9 (5.7)	25.4 (3.34)	225 (72.1)
100	GAA Activity	2471 (4.5)	2.04 (0.805)	3737 (2.8)	3745 (2.9)	3.3 (0.68)	26.7 (0.766)	46.0 (2.67)
100	T09	1790 (8.4)	61.1 (10.7)	4605 (2.9)	5133 (1.9)	7.9 (3.24)	19.5 (0.361)	168 (27.8)
100	T50	1791 (9.2)	59.3 (11.8)	4567 (2.9)	5058 (0.9)	7.6 (2.85)	19.8 (0.179)	165 (22.1)
200	GAA Activity	4937 (5.1)	1.80 (0.338)	9307 (10.2)	9310 (10.2)	2.1 (0.03)	21.6 (2.15)	36.6 (3.34)
200	T09	3899 (5.4)	124 (6.03)	11391 (2.5)	12049 (2.6)	5.3 (0.04)	16.6 (0.436)	109 (1.97)
200	T50	3893 (4.5)	121 (4.37)	11211 (2.1)	11846 (2.2)	5.3 (0.03)	16.9 (0.369)	110 (2.58)

[a] Geometric mean (CV%); [b] Arithmetic mean (SD); LLOQ<0.500 µg/mL

- The ATB200 systemic exposures (AUC_{0-t} and C_{max}) as measured by the T09 and T50 peptide LC-MS/MS and GAA activity assays were similar
- Systemic exposure increased in a greater than dose-proportional manner

Conclusions

- To support the development of ATB200, the measurement of circulating active enzyme by GAA activity assay was complemented with an absolute quantitative method to measure total ATB200 protein.
- The assay of total ATB200 protein utilized LC-MS/MS quantification of ATB200-specific "signature" peptides after trypsin digestion of protein present in plasma samples. The method has been analytically validated in rat and monkey plasma.
- In a single-dose research study in rats, total ATB200 protein levels measured in plasma after bolus tail vein IV injection closely matched those of active ATB200 at time points up to 4 hours.
- In the same study, the concentrations of active ATB200 were progressively lower compared to total ATB200 protein at later time points.
- The ATB200 systemic exposures (AUC_{0-t} and C_{max}) as measured by T09 and T50 peptide LC-MS/MS and GAA activity assay were similar.
- ATB200 systemic exposure increased in a greater than dose-proportional manner.
- These data demonstrate that characterization of the pharmacokinetics of both active and total ATB200 is relevant for this high M6P rhGAA, a potential next-generation treatment for Pompe disease.

Acknowledgements: The authors wish to thank PRA Health Sciences (Assen, The Netherlands) who performed the development and GLP-validation of the total ATB200 assays.