A Next-Generation Fabry Enzyme Replacement Therapy: A Proprietary Recombinant Human α-Galactosidase A Coformulated With a Pharmacological Chaperone, AT1001, Shows Greater Substrate Reduction Than Standard of Care in Fabry Mice

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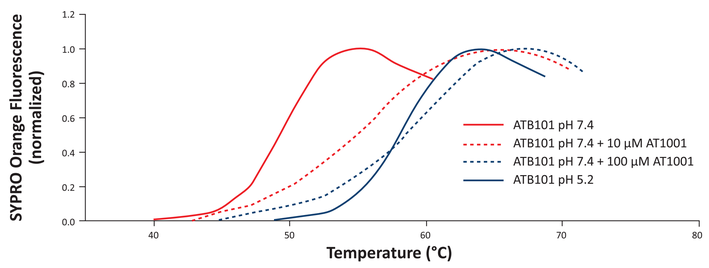
INTRODUCTION

- Fabry disease is an X-linked lysosomal storage disorder caused by a deficiency in α-galactosidase A (α-Gal A) activity, leading to progressive accumulation of lysosomal globotriaosylceramide (GL-3) in multiple tissues
- Although enzyme replacement therapy (ERT) with manufactured human α-Gal A, namely agalsidase beta and agalsidase alfa, has brought many therapeutic benefits to patients, the infused enzymes have potential limitations, including low physical stability, short circulating half-lives in blood, and variable uptake into different disease-relevant tissues, which may impact efficacy and tolerability
- Previously, we demonstrated that the pharmacological chaperone AT1001 (migalastat) improves the pharmacological properties of the manufactured enzymes via binding and stabilization
- A proprietary recombinant human α-Gal A (rhα-Gal A), ATB101, has recently been developed and is coformulated with AT1001 (designated as ATB101/AT1001). The coformulated ATB101/AT1001 as a single intravenously administered product is aimed to improve the pharmacological properties of the enzyme and result in improved substrate clearance compared with the standard of care. This concept was tested in preclinical studies using a Fabry mouse model (*Gla* knockout [KO])

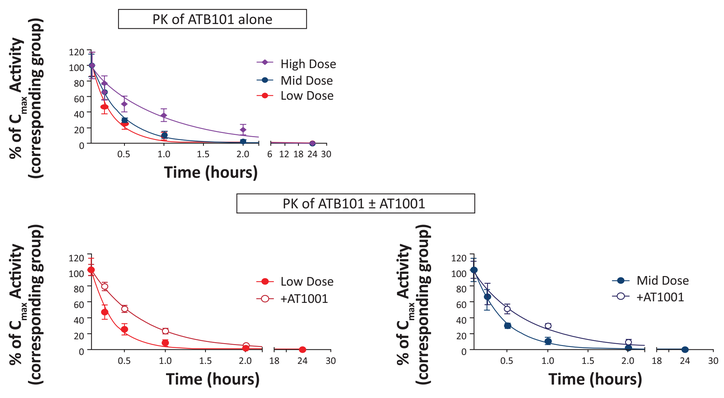
RESULTS

Figure 1. AT1001 Stabilizes ATB101 In Vitro



The thermal stability of ATB101 was assessed using a fluorescence-based thermal denaturation assay as described previously.[1,2] The thermal stability scans were performed in the absence and presence of 10 and 100 µM AT1001 at pH 7.4 and in the absence of AT1001 at pH 5.2. Data were normalized to the minimum and maximum fluorescence in each sample. As expected for any lysosomal enzyme at neutral pH, ATB101 was significantly less stable (melting temperature [T_m]=48.9°C) than at acidic pH (T_m=57.8°C). Coincubation with AT1001 at neutral pH resulted in a concentration-dependent stabilization of ATB101, with 10 µM AT1001 shifting the T_m to 54.6°C and 100 µM AT1001 shifting the T_m to 58.4°C. The latter was similar to the T_m observed for ATB101 alone at acidic pH.

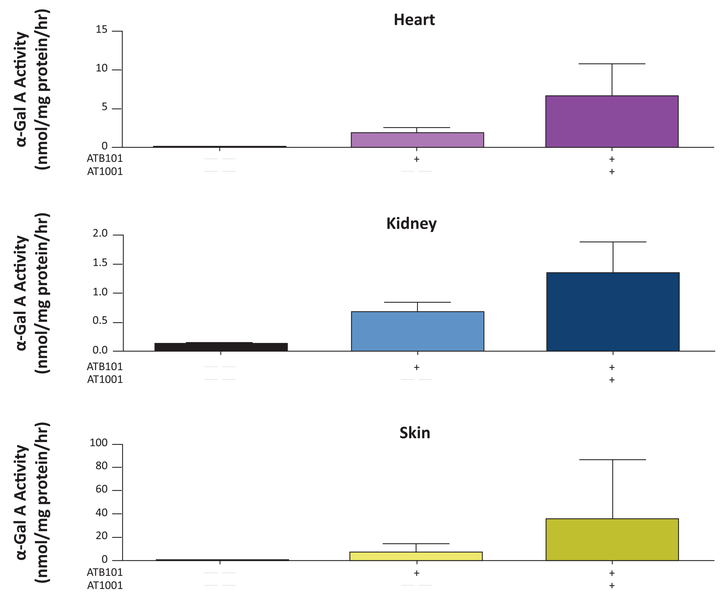
Figure 2. AT1001 Coformulation Increases the Circulating Levels of ATB101 in *Gla* KO Mice



Half Life, Hours	Low Dose	Mid Dose	High Dose
ATB101 alone	0.19	0.26	0.61
ATB101/AT1001	0.44	0.49	N/A
Fold increase compared with enzyme alone	2.3	1.9	N/A

Approximately 6-month-old male *Gla* KO mice (n=5/group) were given a single intravenous (IV) bolus administration of low-, mid-, or high-dose (up to 10 mg/kg) ATB101 alone or ATB101 co-formulated with AT1001 (ATB101/AT1001) at low or mid enzyme dose. Blood samples were collected from each mouse using serial mandibular bleeds at 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, and 24 hours after IV administration, and α-Gal A activity in plasma was determined using an enzymatic method with 4-MU-galactopyranoside (4MU-Gal) as the substrate. The plasma activity was used to determine pharmacokinetic parameters using GraphPad version 6. For each group, the averaged activity of each timepoint was normalized to the averaged peak plasma α-gal A activity (C_{max}) of the corresponding group, and a plot was made using the normalized activity and the nominal time. The half-life of ATB101 activity following each dosing regimen was calculated using a one-phase decay model. The fitted curves are shown in the graphs, and the calculated half-lives are summarized in the table. When administered alone, ATB101 showed dose-dependent, nonlinear pharmacokinetics, as the half-lives increased with increasing doses. Coformulation with AT1001 increased circulating α-Gal A activity levels, with an up to 2.3-fold increase in ATB101 half-life. N/A=not applicable; PK=pharmacokinetics.

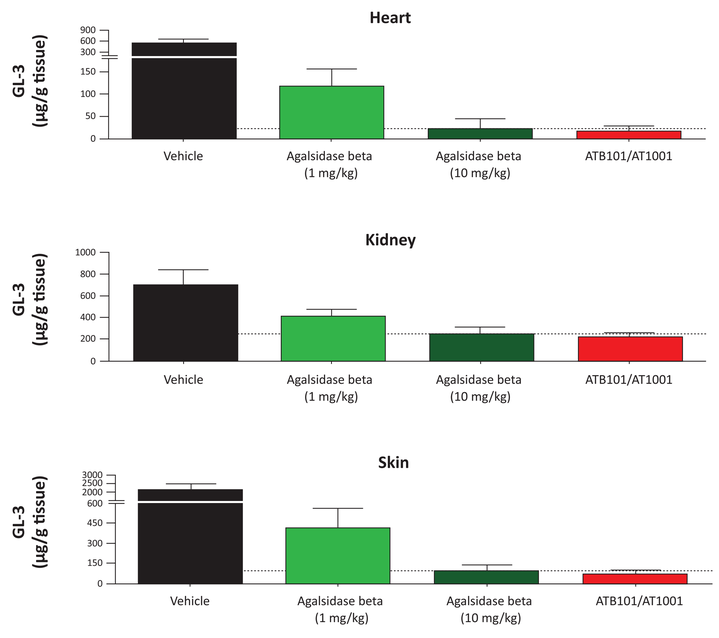
Figure 3. ATB101/AT1001 Coformulation Increases α-Gal A Activity in Tissues of *Gla* KO Mice



α-Gal A Activity, nmol/mg protein/hr (standard deviation)	Heart	Kidney	Skin
ATB101 alone	1.9 (0.7)	0.7 (0.2)	7.2 (7.3)
ATB101/AT1001	6.7 (4.1)	1.4 (0.5)	30.1 (50.5)
Fold increase compared with enzyme alone	3.6	2.0	4.2

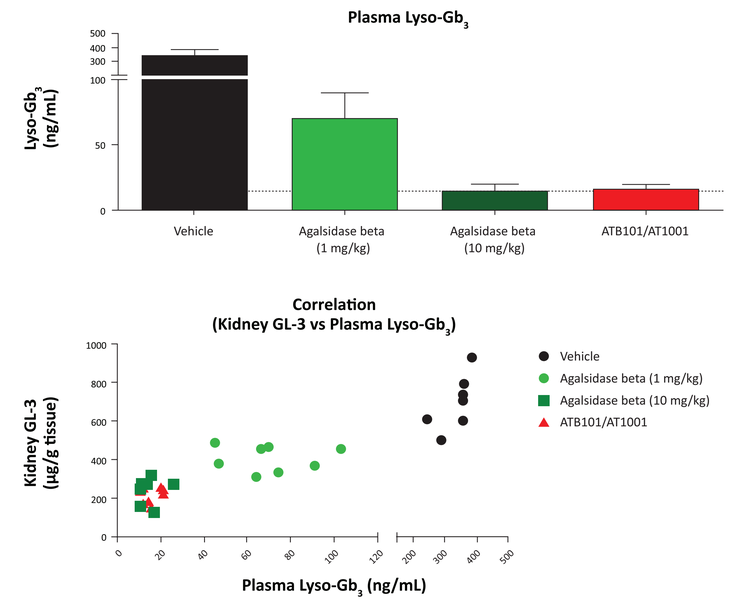
Approximately 16-week-old male *Gla* KO mice (n=8/group) were given two biweekly IV bolus administrations of either ATB101 alone (<10 mg/kg) or ATB101/AT1001. Seven days after the final drug administration, the α-Gal A activity in disease-relevant tissues was measured using an enzymatic method with 4MU-Gal as the substrate. Coformulation with AT1001 substantially increased α-Gal A activity in all tissues measured compared with enzyme alone.

Figure 4. ATB101/AT1001 Coformulation Improves the Tissue GL-3 Reduction in *Gla* KO Mice Over Standard of Care



Approximately 16-week-old male *Gla* KO mice (n=8/group) were given two biweekly IV bolus administrations of either 1 or 10 mg/kg of agalsidase beta or co-formulation of AT1001 with <10 mg/kg ATB101 (ATB101/AT1001). Disease-relevant tissues were collected 7 days after the last administration and GL-3 levels were determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). In all tissues tested, ATB101/AT1001 co-formulation achieved GL-3 reduction that was significantly greater (P<0.05) than agalsidase beta 1 mg/kg (standard of care). Importantly, the GL-3 reduction with ATB101/AT1001 co-formulation reached or exceeded the reduction seen with agalsidase beta 10 mg/kg, demonstrating substantially superior substrate clearance compared with the current standard of care. GL-3=globotriaosylceramide.

Figure 5. ATB101/AT1001 Coformulation Improves Plasma Lyso-Gb_3 Reduction in *Gla* KO Mice Over Standard of Care



In the same study described in **Figure 4**, plasma samples were collected 7 days after the last administration, and levels of globotriaosylsphingosine (lyso-Gb_3), an important biomarker for Fabry disease severity, were determined by LC-MS/MS. ATB101/AT1001 co-formulation achieved plasma lyso-Gb_3 reduction that was significantly better (P<0.05) than agalsidase beta 1 mg/kg and similar to the effects seen with agalsidase beta 10 mg/kg, once again demonstrating substantially superior efficacy compared with the current standard of care.

Plasma lyso-Gb_3 levels were correlated with kidney GL-3 (the most severely affected tissue in Fabry disease) using GraphPad version 6. As reported previously for patients with Fabry disease,[3] a strong correlation (p<0.0001) between plasma lyso-Gb_3 and kidney GL-3 was observed, indicating the utility and reliability of testing plasma lyso-Gb_3 in preclinical studies.

CONCLUSIONS

- AT1001 increased the physical stability of a proprietary rhα-Gal A, ATB101, currently in nonclinical development
- In mice, following IV administration, ATB101 showed dose-dependent, nonlinear pharmacokinetics, as the half-lives increased with increasing doses. Upon coformulation with AT1001, the half-life of active ATB101 in plasma increased up to 2.3-fold compared with enzyme alone
- In *Gla* KO mice, coformulated ATB101/AT1001 led to substantially increased α-Gal A activity in disease-relevant tissues compared with enzyme alone
- Importantly, under a repeat IV administration regimen, coformulated ATB101/AT1001 achieved robust GL-3 reduction in kidney, heart, and skin tissues, reaching or even exceeding the levels achieved with 10 mg/kg agalsidase beta (ie, 10 times the standard-of-care dose)
  - In plasma, a similar effect on the lyso-Gb_3 levels was observed, and levels correlated well with kidney GL-3
- Collectively, these results indicate that ATB101/AT1001 coformulation increased the stability of the enzyme, resulting in greater substrate reduction in preclinical models compared with the current standard therapy. Therefore, ATB101/AT1001 coformulation has the potential to represent a promising next-generation treatment for Fabry disease and warrants further investigation

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DISCLOSURES

Conflicts of Interest

All of the authors are employees of and hold stock in Amicus Therapeutics, Inc.

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