An Ongoing Phase 2a Study to Investigate the Effect of AT2220 (Duvoglustat HCl) on the Pharmacokinetics of Acid α-Glucosidase in Subjects with Pompe Disease: Preliminary Results

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Introduction

Pompe disease is caused by mutations in the gene that encodes the lysosomal enzyme acid α-glucosidase (*GAA*), which hydrolyzes glycogen. AT2220 (1-deoxynojirimycin [DNJ] hydrochloride [HCl]) is an iminosugar that is a potent, competitive inhibitor of GAA. We have shown that AT2220 can increase GAA enzyme activity and protein processing in cell lines derived from Pompe patients and in COS-7 cells that transiently express various mutant forms of GAA. AT2220 has also been shown to stabilize human recombinant GAA (rhGAA, alglucosidase alfa) *in vitro* and co-administration of AT2220 with rhGAA results in significant increases in muscle GAA levels and decreases in glycogen levels in a mouse model of Pompe disease.

Objectives

Primary Objectives

- To evaluate the safety of single ascending oral doses of AT2220 administered 1 hour before administration of alglucosidase alfa in patients with Pompe disease
- To evaluate the effect of single ascending oral doses of AT2220 on the plasma pharmacokinetics of rhGAA

Secondary Objectives

- To assess rhGAA enzyme activity and protein levels in skeletal muscle at Day 3 and Day 7 following a single intravenous infusion with alglucosidase alfa alone and after pre-administration of single ascending oral doses of AT2220
- To evaluate the concentration of AT2220 in skeletal muscle on Day 3 and Day 7

Study Design and Methods

AT2220-010 is an ongoing, open-label, non-randomized, fixed-sequence, single-ascending dose study comprised of 2 periods per dose level in patients with Pompe disease.

- Period 1: IV infusion of rhGAA alone
- Period 2: AT2220 orally administered 1 hour prior to IV infusion of rhGAA (at the same dose and infusion duration as in Period 1)

Each period is separated by a minimum 14-day rhGAA dosing interval. AT2220 dose cohorts 1 – 4 (N of 4 to 6 patients per cohort) evaluated: 50 mg, 100 mg, 250 mg, and 600 mg administered as an oral solution.

Preliminary results are available for Cohorts 1, 2, and 3 (50 mg, 100 mg, and 250 mg) for Periods 1 and 2:

- Subjects receive their current dose and regimen of rhGAA alone as an IV infusion (approximately 20 mg/kg for 3 - 6 hrs) followed by oral AT2220 administered one hour prior to the next rhGAA infusion.
- IV infusions of rhGAA are balanced each period for dose and duration of infusion

Further details regarding study design and methods are presented in Poster #244.

Preliminary Results

Patient Disposition and Demographics

Four patients from Cohort 1 (50 mg), 6 patients from Cohort 2 (100 mg), and 6 patients from Cohort 3 (250 mg) with plasma and muscle rhGAA activity, total protein levels, and AT2220 concentrations from Periods 1 and 2 were evaluated.

- All patients received rhGAA alone during Period 1; all patients were co-administered with either 50 mg, 100 mg, or 250 mg AT2220 1 hour prior to initiation of rhGAA infusion during Period 2.
- Cohort 1 patients are identified as A, B, C, and D; Cohort 2 patients are identified as E, F, G, H, I, and J; and Cohort 3 patients are identified as K, L, M, N, O, and P.
- Six subjects were male and 10 were female with Pompe Disease aged 33-66 years, weight ranged from 55.8 - 109 kg, and estimated eGFR ranged from 75-250 mL/min.

The genotype, including nucleotide and amino acid changes, for each subject (where available) is presented in Table 1.

Table 1. *GAA* Genotypes

Patient ID	Nucleotide Change	Amino Acid Change
A	c.-32-13T>G	Splicing mutation
B	Not Available	Not Available
C	Not Available	Not Available
D	c.1222A>G / c.-32-13T>G	M408V / Splicing mutation
E	c.-32-13T>G	Splicing mutation
F	Not Available	Not Available
G	c.-32-13T>G / c.1445C>G	Splicing mutation / P482R
H	Not Available	Not Available
I	c.692+5G>T / c.1211A>G	Splicing mutation / D404G
J	Not Available	Not Available
K	Not Available	Not Available
L	c.-32-13T>G / c.1143delC	Splicing mutation / Thr381fsX9
M	c.-32-13T>G / c.876C>T / c.1143delC	Splicing mutation / Silent mutation/ Thr381fsX9
N	Not Available	Not Available
O	c.-32-13T>G / c.2481+102 / c.2646+31del	Splicing mutations
P	c.-32-13T>G	Splicing mutation

Disclosure Statement: Authors footnoted #1 – 11 above are currently participating investigators in the ongoing clinical trial, AT2220-010. Authors footnoted #12 are full-time employees of Amicus Therapeutics and are stockholders.

Plasma rhGAA Activity

Figures 1, 2, and 3. Plasma AUC rhGAA Activity vs. Treatment with Alglucosidase alfa for Cohorts 1, 2, and 3, respectively

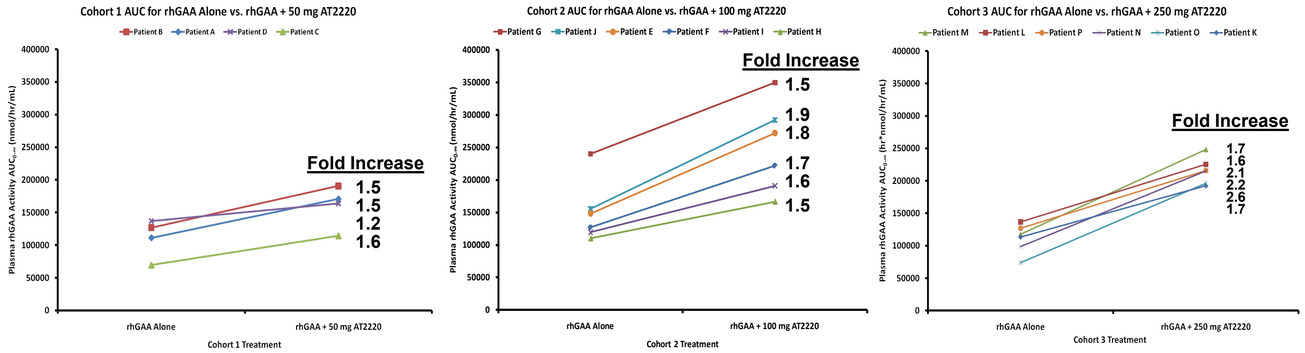


Table 2. Mean (SD) Plasma rhGAA Activity PK Summary for Cohorts 1, 2, and 3

Period/Treatment	Cohort	Period	C_{max}[a] (nmol/hr/mL)	T_{max}[b] (hr)	AUC_{0-T}[a] (hr*nmol/hr/mL)	$AUC_{0-\infty}$[a] (hr*nmol/hr/mL)	AUC Ratio[a]	$t_{1/2}$[c] (hr)
rhGAA Alone (N=4)	1	1	16441 (31.6)	5.0 (4 - 5)	105834 (30.7)	107707 (31.1)	-	3.9 (0.6)
rhGAA + 50 mg AT2220 (N=4)	1	2	19922 (24.4)	5.0 (4 - 6)	152207 (21.3)	157162 (22.4)	1.5 (13.2)	4.2 (0.8)
rhGAA Alone (N=6)	2	1	22785 (18.0)	4.0 (3 - 5)	142415 (30.4)	144953 (31.5)	-	3.8 (0.6)
rhGAA + 100 mg AT2220 (N=6)	2	2	28607 (13.9)	4.0 (3 - 6)	229434 (25.3)	241203 (27.5)	1.7 (10.1)	4.9 (0.8)
rhGAA Alone (N=6)	3	1	18986 (18.7)	4.0 (4 - 4)	107774 (19.7)	109165 (20.0)		3.6 (0.5)
rhGAA + 250 mg AT2220 (N=6)	3	2	22651 (9.0)	4.0 (4 - 4)	201224 (8.8)	214751 (9.5)	2.0 (19.7)	5.3 (1.1)

[a]Geometric mean (CV%)
[b]Median (range)
[c]Arithmetic mean (SD)

Total rhGAA Protein by Western Blot

Figures 4, 5, and 6. Plasma AUC Total rhGAA Protein vs. Treatment with Alglucosidase alfa for Cohorts 1, 2, and 3, respectively

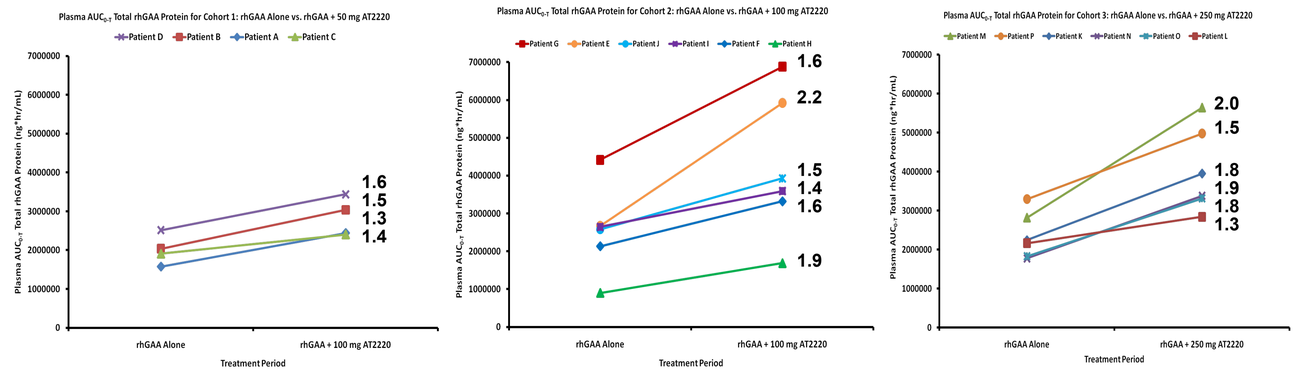


Table 3. Mean (SD) Plasma Total rhGAA Protein PK Summary for Cohorts 1, 2, and 3

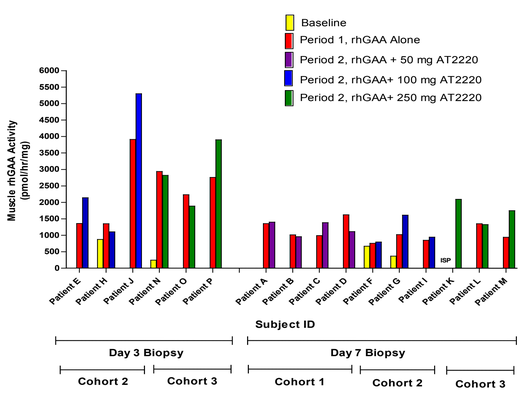
Cohorts 1, 2, and 3 Plasma rhGAA Protein PK Summary

Period/Treatment	Cohort	C_{max}[a] (nmol/hr/mL)	T_{max}[b] (hr)	AUC_{0-T}[a] (hr*nmol/hr/mL)	AUC_{0-T}[a] Ratio	$AUC_{0-\infty}$[a] (hr*nmol/hr/mL)	$T_{1/2}$[c] (hr)
rhGAA Alone (N=4)	1	283118 (45.9)	5.0 (5 - 5)	1979695 (19.4)	-	2172404 (12.8)	4.8 (3.7)
rhGAA + 50 mg AT2220 (N=4)	1	354035 (47.3)	5.5 (4 - 6)	2796515 (17.7)	1.4 (9.2)	3124625 (13.1)	4.8 (2.2)
rhGAA Alone (N=6)	2	399623 (25.8)	4.0 (4 - 5)	2315249 (44.3)	-	2862876 (86.8)	3.9 (4.5)
rhGAA + 100 mg AT2220 (N=6)	2	430738 (30.5)	4.0 (4 - 7)	3841773 (44.5)	1.7 (18.5)	5123518 (42.3)	6.1 (3.2)
rhGAA Alone (N=6)	3	405543 (10.7)	4.0 (4 - 5)	2291797 (25.3)	-	2411605 (29.0)	1.5 (0.8)
rhGAA + 250 mg AT2220 (N=6)	3	438795 (7.3)	4.5 (4 - 7)	3899573 (26.9)	1.7 (15.1)	4216417 (24.8)	3.0 (1.5)

[a]Geometric mean (CV%)
[b]Median (range)
[c]Arithmetic mean (SD)

Muscle rhGAA Activity

Figure 7. Muscle rhGAA Activity on Days 3 and 7



ISP = Insufficient rhGAA protein in muscle sample to determine activity.

Table 4. Muscle rhGAA Activity Summary

SubjID	Biopsy Day	Period 2 / Period 1 Ratio	% Change from Period 1	% Change from Baseline Period 1 / Period 2
Cohort 1: Period 1 rhGAA Alone, Period 2 rhGAA + 50 mg AT2220				
Patient A	7	1.0	3.4	ND
Patient B	7	0.9	-5.4	ND
Patient C	7	1.4	39.9	ND
Patient D	7	0.7	-31.6	ND
Mean	**7**	**1.0**	**1.6**	-
Cohort 2: Period 1 rhGAA Alone, Period 2 rhGAA + 100 mg AT2220				
Patient E	3	1.6	57.8	ND
Patient H	3	0.8	-18.1	54.8 / 26.7
Patient J	3	1.4	35.5	ND
Mean	**3**	**1.3**	**25.0**	-
Patient F	7	1.0	5.0	13.6 / 19.2
Patient G	7	1.6	57.8	178 / 339
Patient I	7	1.1	11.5	ND
Mean	**7**	**1.2**	**24.8**	-
Cohort 3: Period 1 rhGAA Alone, Period 2 rhGAA + 250 mg AT2220				
Patient N	3	1.0	-3.9	1100 / 1053
Patient O	3	0.8	-15.5	ND
Patient P	3	1.4	41.4	ND
Mean	**3**	**1.1**	**7.3**	-
Patient K	7	ISP	ISP	ND
Patient L	7	1.0	-2.1	ND
Patient M	7	1.9	86.2	ND
Mean	**7**	**1.4**	**42.1**	-

ND = Optional baseline biopsy not done.
ISP = Insufficient Period 1 sample rhGAA protein for activity determination

Plasma and Muscle AT2220 Concentrations

Figure 8. Mean (SD) Plasma AT2220 Concentrations

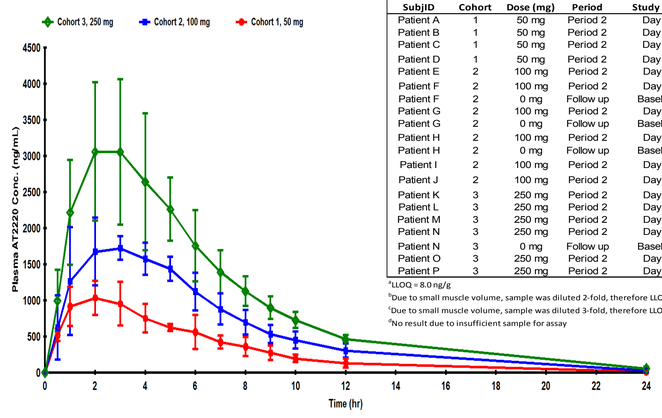


Table 5. Individual Muscle AT2220 Concentrations

AT2220-010 Muscle AT2220 Conc for Cohorts 1, 2, and 3

SubjID	Cohort	Dose (mg)	Period	Study Day	Muscle AT2220 Conc (ng/g)[a]
Patient A	1	50 mg	Period 2	Day 7	11.8
Patient B	1	50 mg	Period 2	Day 7	10.0
Patient C	1	50 mg	Period 2	Day 7	13.1
Patient D	1	50 mg	Period 2	Day 7	BLQ[b]
Patient E	2	100 mg	Period 2	Day 3	17.8
Patient F	2	100 mg	Period 2	Day 7	BLQ[b]
Patient F	2	0 mg	Follow up	Baseline	BLQ
Patient G	2	100 mg	Period 2	Day 7	BLQ
Patient G	2	0 mg	Follow up	Baseline	BLQ
Patient H	2	100 mg	Period 2	Day 3	BLQ[c]
Patient H	2	0 mg	Follow up	Baseline	BLQ
Patient I	2	100 mg	Period 2	Day 7	BLQ[b]
Patient J	2	100 mg	Period 2	Day 3	BLQ[c]
Patient K	3	250 mg	Period 2	Day 7	ISV[d]
Patient L	3	250 mg	Period 2	Day 7	28.1
Patient M	3	250 mg	Period 2	Day 7	27.9
Patient N	3	250 mg	Period 2	Day 3	BLQ
Patient N	3	0 mg	Follow up	Baseline	BLQ[c]
Patient O	3	250 mg	Period 2	Day 3	54.4
Patient P	3	250 mg	Period 2	Day 3	61.9

[a]LLOQ = 8.0 ng/g
[b]Due to small muscle volume, sample was diluted 2-fold, therefore LLOQ = 16.0 ng/g
[c]Due to small muscle volume, sample was diluted 3-fold, therefore LLOQ = 32.0 ng/g
[d]No result due to insufficient sample for assay

Safety

To date, single doses of 50 mg, 100 mg, and 250 mg AT2220 have been found generally well-tolerated in patients with Pompe Disease. Only mild or moderate, transient AEs have been reported, none of which were related to AT2220. One serious AE was reported of citalopram-induced QTc prolongation. The QTc prolongation attenuated following citalopram dose reduction. Generally, urine hexose tetrasaccharide levels either did not change from baseline, or did not show any consistent trend following a single dose of AT2220. Additionally, CPK levels did not appreciably change from baseline in any dose cohorts.

Summary of PK Results

Plasma rhGAA activity and total protein AUC increased for all patients for all 3 co-administered doses relative to alglucosidase alfa alone (Figs. 1-6). Increases in AUC were primarily driven by prolonged plasma half-life due to increases in rhGAA active enzyme levels at post-T_{max} time points (Tables 2 and 3). The increases in plasma rhGAA activity and total protein AUCs suggests an increase in stabilized rhGAA is available for tissue distribution.

Muscle biopsies were taken on Day 7 for all four Cohort 1 patients, and on Day 3 or Day 7 for each of 3 of 6 Cohort 2 and 3 patients. Three patients from Cohort 2 and one patient from Cohort 3 had an optional Day 30 muscle biopsy that was used as a baseline for those patients. Generally, Day 3 biopsies showed significantly greater increases in rhGAA activity with or without AT2220 than Day 7, when rhGAA activity decreased to levels not much different to the observed baseline levels. Of Day 3 biopsies, Patients J and P demonstrated rhGAA activity levels approximately 29%-39% of normal (Fig 7, Table 4).

The pharmacokinetics of plasma AT2220 are approximately dose proportional from 50 mg to 100 mg doses however, exposure was less than proportional from 50 mg to 250 mg (1.9-fold and 3.2-fold increases in plasma AUC, respectively, Fig. 8). Muscle AT2220 concentrations from Day 7 biopsies had attenuated from 28.1 ng/g to below the limit of quantification (8 ng/g). However, it should be noted that in 3 patients with Day 7 biopsies, due to insufficient tissue sample, 2-fold dilutions were necessary that resulted in doubling of the lower limit of quantitation from 8 ng/g to 16 ng/g (Table 5).

Preliminary Conclusions

- AT2220 was generally well-tolerated.

Plasma

- rhGAA activity increased 20% to 60%, 50% to 90%, and 60% to 160% following single doses of 50 mg, 100 mg, and 250 mg AT2220, respectively.
- Plasma total rhGAA protein PK followed a similar trend to rhGAA activity PK.
- Dose-related increases in plasma AT2220 concentrations were observed. The plasma pharmacokinetics of AT2220 are nonlinear for the 3 doses evaluated to date.

Muscle

- Day 3 biopsies indicated rhGAA provided increases in activity in muscle relative to baseline levels (N=2). Co-administration with 100 mg or 250 mg AT2220 provided additional increases in muscle rhGAA activity compared to rhGAA alone for 3 of 6 patients.
- Day 7 biopsies showed muscle AT2220 levels from below the lower limit of quantification to 28.1 ng/g (20.1 ng/g above the LOQ).