

# PHARMACOLOGICAL CHAPERONE THERAPY FOR THE TREATMENT OF GAUCHER DISEASE: AT2101 INCREASES ACID $\beta$ -GLUCOSIDASE LEVELS IN CELLS, MICE AND HEALTHY HUMAN SUBJECTS

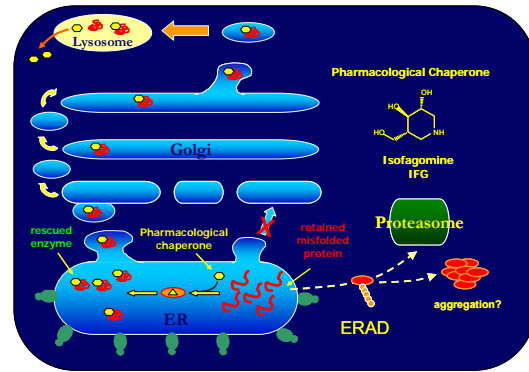
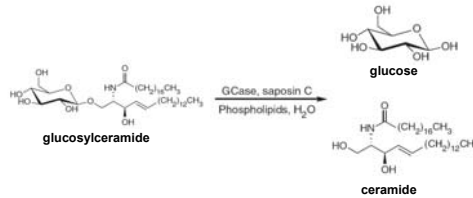
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## INTRODUCTION

Gaucher disease is caused by deficient acid  $\beta$ -glucosidase (GCase) activity. Over 90% of Gaucher patients have at least one allele with the N370S or L444P missense mutation. These GCase variants have impaired exit from the ER and a significant fraction is prematurely eliminated prior to reaching the lysosome. We have developed orally-administered small molecules called pharmacological chaperones as a means of increasing endogenous GCase activity. Pharmacological chaperones selectively bind to target proteins, increasing protein stability, proper protein processing and trafficking. In this study, we evaluated the effect of AT2101 (isofagomine tartrate) on N370S and L444P GCase in Gaucher fibroblasts and a Gaucher mouse model expressing murine L444P GCase. Our results show that AT2101 significantly increased N370S and L444P GCase levels in cells and L444P GCase levels in animals. The drug was well-tolerated and also increased wild type GCase levels in healthy human subjects. These results show that AT2101 may be an effective therapy for Gaucher disease.

## 1. Biological Roles of Acid $\beta$ -Glucosidase and Pharmacological Chaperones

Acid  $\beta$ -glucosidase (GCase) cleaves glucose from the glycolipid glucosylceramide in lysosomes (below). Genetic mutations lead to the production of unstable GCase proteins that are degraded prior to reaching lysosomes. The pharmacological chaperone AT2101 increases GCase levels by selectively binding and stabilizing GCase in the ER, preventing premature ER associated degradation, and promoting its trafficking to the lysosome (right).



## 2. AT2101 Binds the Active Site of GCase

AT2101 is a potent competitive inhibitor of GCase at neutral (ER) pH but is significantly less potent at acid (lysosomal) pH (see table). Co-crystallization studies confirmed that AT2101 binds the active site of GCase and forms novel hydrogen bonds with key residues.

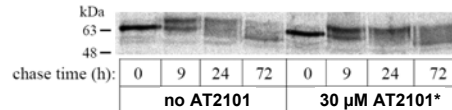
IC <sub>50</sub> values for AT2101		
	CTRL	N370S
pH 5.2	30 nM	128 nM
pH 6.4	9 nM	26 nM
pH 7.3	5 nM	18 nM

Steet et al. (2006) PNAS 103(37):13813

## 3. AT2101 Prevents Premature Degradation of GCase

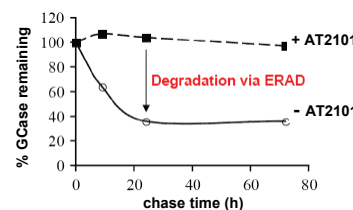
Pulse-chase experiments revealed that ~60% of N370S GCase is degraded (presumably due to degradation via ERAD) by 24 hrs in patient fibroblasts. In contrast, nearly all newly-synthesized N370S GCase remained with AT2101 treatment. Once in the lysosome, N370S GCase is stable for at least 72 hrs in the presence or absence of AT2101.

Autoradiograph of ER and lysosomal forms of GCase synthesized with or without GCase



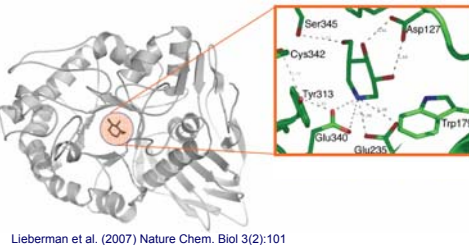
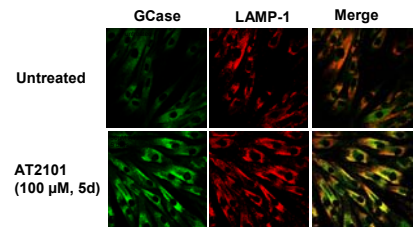
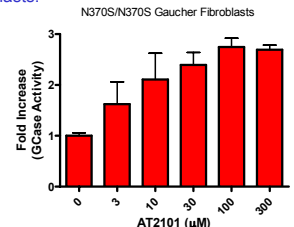
\*AT2101 was removed prior to the chase  
Steet et al. (2006) PNAS 103(37):13813

Densitometric quantification of autoradiograph (above)



## 4. AT2101 Increases N370S GCase Levels in Patient-Derived Cells

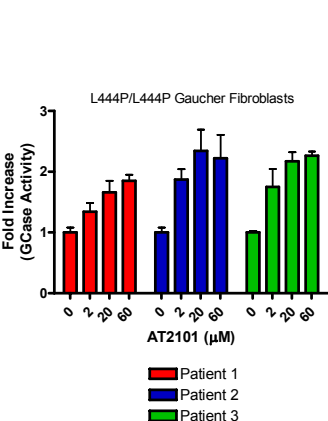
Top: AT2101 treatment of N370S/N370S Gaucher fibroblasts resulted in a 2- to 3-fold increase in GCase activity as measured by 4-MU- $\beta$ -Glc. Bottom: AT2101 treatment increased N370S GCase protein levels as shown by GCase immunoreactivity (green) and its colocalization with lysosomes (LAMP-1, red) in Gaucher (N370S) fibroblasts.



Lieberman et al. (2007) Nature Chem. Biol 3(2):101

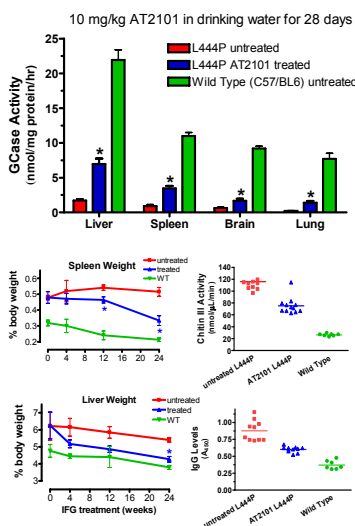
## 5. AT2101 Increases L444P GCase Levels in Patient-Derived Cells

A dose-dependent increase in GCase levels were observed with AT2101 treatment of L444P/L444P Gaucher patient fibroblasts. L444P GCase was immunoprecipitated using a polyclonal anti-GCase (G. Grabowski) prior to GCase activity assay.



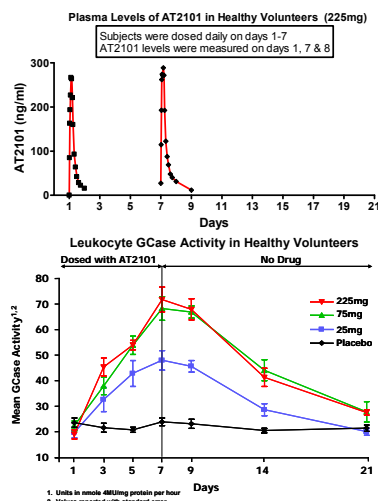
## 6. AT2101 Increases L444P GCase Levels in a Mouse Model

Oral treatment of L444P knock-in mice with AT2101 (10mg/kg isofagomine free base) increased GCase levels in liver, spleen, brain and lung (top) and significantly decreased liver and spleen weights and plasma chitin III and IgG levels (bottom).



## 7. AT2101 Increases WT GCase Levels in Humans

In a repeat-dose Phase 1 clinical trial for AT2101 (orally administered to healthy volunteers) was found to have a plasma elimination half-life of 14 hrs (top). AT2101 was well-tolerated and increased WT GCase levels in leukocytes (up to 3.5-fold) and GCase levels remained elevated for >1 week after dosing ended (bottom).



## Conclusions

- AT2101 binds to the active site of GCase
- AT2101 stabilizes GCase in the ER and promotes trafficking of N370S GCase to the lysosomes
- AT2101 has a lower affinity for GCase at lysosomal pH
- N370S GCase is stable in lysosomes even in the absence of AT2101
- AT2101 also rescues L444P GCase
- The plasma half-life of AT2101 is much shorter (14 hrs) than the half-life of N370S or wild type GCase (> 3 days)
- A dosing interval can be used to control AT2101 concentration peaks and troughs to maximize for "chaperoning" during peaks and substrate turnover during troughs
- AT2101 may be an effective treatment for majority of Gaucher patients