Development of AL00804, a novel brain penetrant glucosylceramide synthase inhibitor, to treat Gaucher disease and other neuronopathic glycosphingolipid storage diseases

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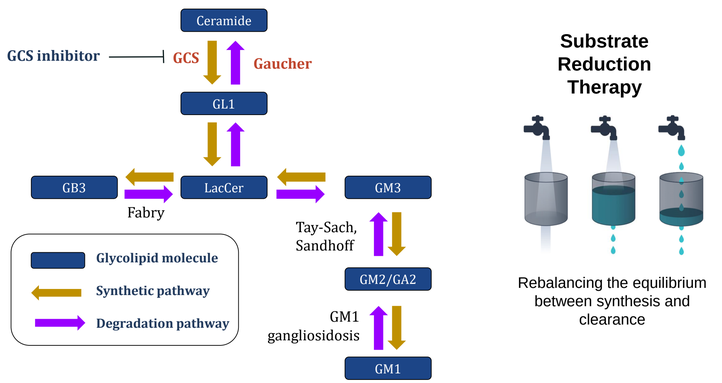
Gaucher disease (GD), a lysosomal storage disorder caused by glucocerebrosidase (GCase) deficiency, is characterized by accumulation of glucosylceramide (GL1) and its deacylated derivative lyso-GL1, which negatively impact the spleen, liver, bone marrow, and central nervous system (CNS). GD has also been associated with increased risk of developing Parkinson's Disease. Enzyme Replacement Therapy, the standard of care for GD, reduces GL1 and improves many visceral disease symptoms but does not cross the blood brain barrier to treat the CNS symptoms. Glucosylceramide synthase inhibitors (GCSi) have been shown to reduce GL1 synthesis, reduce GL1 and lyso-GL1 accumulation, and improve GD disease symptoms. However, the currently approved GCSi also does not efficiently penetrate into the brain to treat the CNS symptoms. We are developing AL00804, a GCSi with excellent drug-like properties including superior potency and brain penetration for the treatment of GD and other neuronopathic glycosphingolipid (GSL) storage disorders. The IC50 of AL00804 against GCS was determined to be approximately 10 nM. Pharmacokinetics studies were conducted in mice, rats, and dogs and support once daily, oral administration. Pharmacodynamic studies conducted in a pharmacologically induced mouse model (CBE) of neuronopathic GD show that AL00804 efficiently reduces brain GL1 and lyso-GL1 accumulation. Therapeutic efficacy evaluation in mouse models of neuronopathic GD, including induced (CBE) and genetic (4L;C*) models, demonstrated treatment with AL00804 reduced CNS disease symptoms, significantly delayed motor function decline and extended survival. The superior potency and brain penetration make AL00804 a potentially safer and more efficacious molecule for treating GD patients and other neuronopathic lysosomal storage disorders with GSL metabolism deficit.

GCS inhibition: substrate reduction therapy for glycosphingolipid storage disorders



Rebalancing the equilibrium between synthesis and clearance

Potency and Pharmacokinetic Properties: AL00804 is more potent and has greater brain penetration than Venglustat

A Potency

GCS enzyme assay (MDCK lysate)

AL00804 IC_{50} (nM) = 11.7 nM

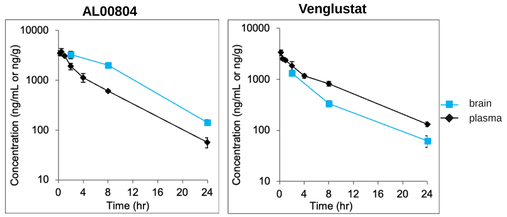
Venglustat IC_{50} (nM) = 76.5 nM

GCS cellular assay (K562 cells)

AL00804 IC_{50} (nM) = 9.7 nM

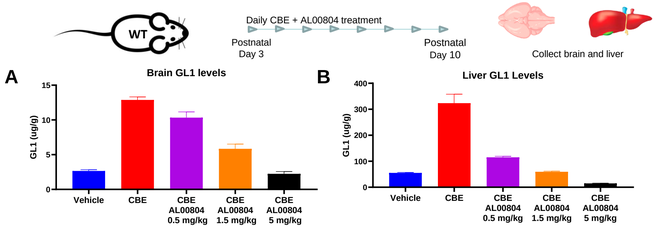
Venglustat IC_{50} (nM) = 165 nM

B Plasma and brain PK profiles



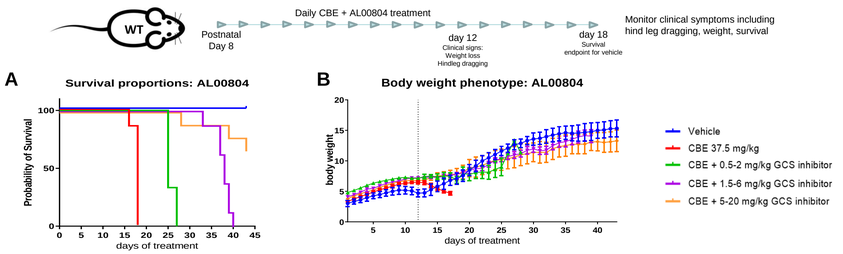
Potency and Pharmacokinetic profiles of AL00804 in mouse. GCS activity in both enzyme (cell free) and cellular assays was monitored using a labeled ceramide molecule as a substrate and then monitoring the glucosylated product by LCMS (A). Plasma PK and brain compound levels after a single intraperitoneal injection (IP) of AL00804 or Venglustat in mice (B). At 8 hrs, AL00804 has a brain to plasma (BP) ratio of approximately 3 and Venglustat has a BP ratio of 0.4.

AL00804 reduces glucosylceramide (GL1) in a pharmacological model of GD



Pharmacodynamic effects of AL00804 in a pharmacologically induced Gaucher disease model. Mice age postnatal day 3 were administered conduritol Beta epoxide (CBE) at 100 mg/kg/day (IP), a covalent inhibitor of the lysosomal enzyme GBA1, for 8 days. CBE injection resulted in accumulation of GL1 in brain (A) and liver (B) tissue. Coadministration of AL00804 dose dependently reduced GL1 levels in liver and plasma.

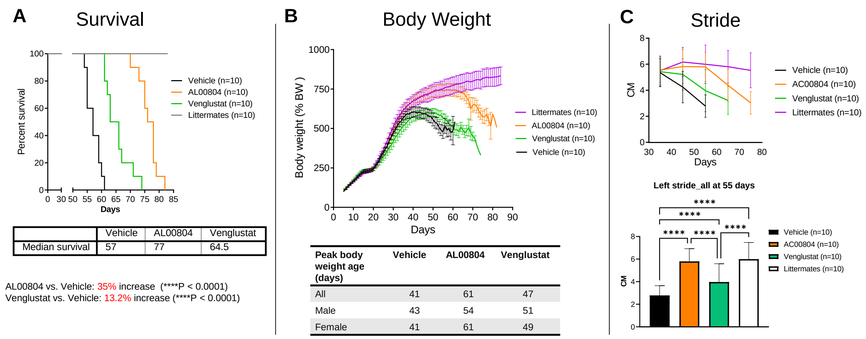
AL00804 extends survival in a pharmacologically induced model of neuropathic GD



Survival benefit of AL00804 in a pharmacological model of neuropathic GD. Mice age postnatal day 8 were administered CBE daily at 25 mg/kg/day (IP) until severe clinical signs were observed, and the survival endpoint was met. Coadministration of AL00804 dose dependently increased survival (A) and delayed clinical signs (B).

AL00804 improves survival and clinical signs in the 4L/C* mouse model of neuropathic GD

Animals (n=10)	Mice Enrolled (# female / # male)	Treatment (By IP)	Dose (mg/kg)
4L/WT	5/5	None	0
4L;C*	6/4	Vehicle	0
4L;C*	**4/6**	AL00804	2mg/kg @ P5-20 → 10mg/kg @ P21-EP
4L;C*	**4/6**	Venglustat	3mg/kg @ P5-20 → 15mg/kg @ P21-EP



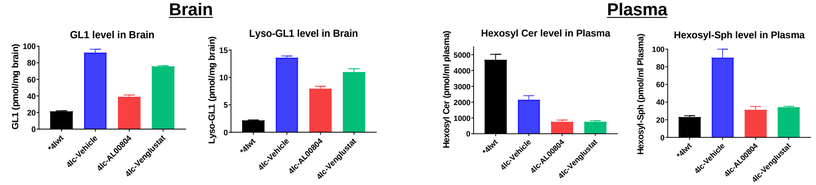
	Vehicle	AL00804	Venglustat
Median survival	57	77	64.5

AL00804 vs. Vehicle: 35% increase (****P < 0.0001)
Venglustat vs. Vehicle: 13.2% increase (****P < 0.0001)

Peak body weight age (days)	Vehicle	AL00804	Venglustat
All	41	61	47
Male	43	54	51
Female	41	61	49

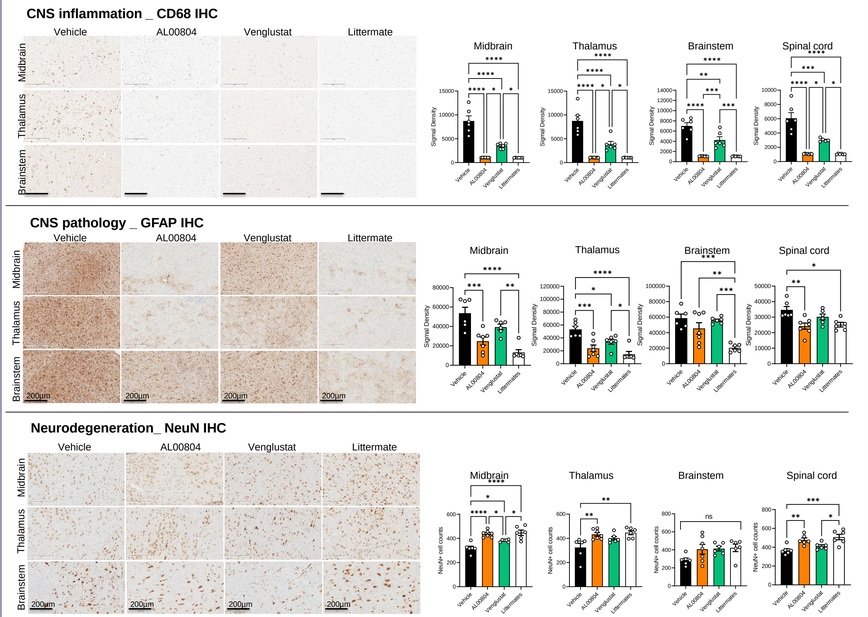
Survival and clinical sign benefit of AL00804 in the 4L/C* model of nGD. Mice age postnatal day 5 were administered AL00804 by IP injection until the survival endpoint was met (A). Body weight was monitored (B) and gait analysis (C) was performed throughout the study.

AL00804 produced greater brain reduction of GL1 and lyso-GL1 compared to Venglustat (when plasma GL1 reduction was equivalent for both compounds)



Pharmacodynamic effects of AL00804 in the nGD model. 4L/C* mice were treated with AL00804 by IP injection from postnatal day 5 to postnatal day 50 and brain and plasma were collected for GL1 measurement by LCMS.

AL00804 reduced histological markers of inflammation, gliosis, and neurodegeneration



Histopathological benefits of AL00804 in the 4L/C* nGD model: 4L/C* mice were treated with AL00804 by daily IP injection from postnatal day 5 to postnatal day 50. On day 50, brain was collected for histological evaluation of markers of CNS inflammation (CD68), gliosis (GFAP), and neurodegeneration (NeuN). Treatment with AL00804 reduced inflammation and gliosis markers and preserved neuron number.

Conclusions

Conclusion: AL00804 is a novel, orally available, potent, and selective GCSi that efficiently penetrates the brain to reduces GL1 synthesis. The increased potency and superior brain penetration compared to Venglustat make AL00804 a potentially safer and more efficacious molecule for treating neuropathic GSL storage disorders. When tested head-to-head in a neuropathic model of Gaucher disease, AL000804 performed better than Venglustat. At dose levels for these compounds that produced equal reduction of GL1 in the periphery, AL00804 produced greater GL1 reduction in the brain and this resulted in greater benefits to survival, clinical signs and histopathological markers.