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Enhanced Correction of Skeletal Muscle and Brain Pathology in a Pompe Mouse Model Using Transferrin Receptor–Mediated Delivery of GAA

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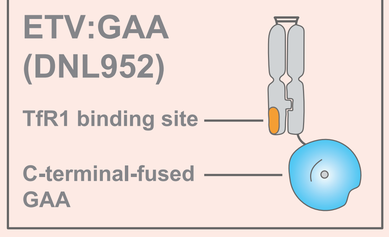
Introduction

- In Pompe disease, deficiency of GAA causes lysosomal glycogen accumulation in muscle and other tissues
- In muscle, accumulation of glycogen causes lysosomal vacuolization, autophagic buildup, and dysregulation of cellular pathways involved in energy metabolism, protein homeostasis, and muscle maintenance, ultimately resulting in progressive muscle weakness that remains an unmet need despite existing ERTs[1–4]
- In the nervous system, glycogen buildup causes severe neurological deficits in IOPD[5–7] and may contribute to muscle weakness in LOPD,[8–10] potentially through involvement of brainstem and spinal cord motor neurons. Therefore, innovative new approaches are needed to enhance ERT delivery to both muscle and the nervous system
- We have developed an ETV that binds human TfR to enhance delivery of biotherapeutics to TfR-expressing tissues through receptor-mediated cellular uptake and transcytosis
- Here, we report preclinical data on DNL952, an investigational ERT designed to treat Pompe disease that consists of the GAA enzyme fused to a TV to enhance its delivery to muscle and to the nervous system (**Figure 1**)

Figure 1. Structure of DNL952



Objective

- To characterize DNL952, a TfR-enabled ERT, by evaluating its mechanism of action and PK, and by assessing its PD efficacy, defined as glycogen clearance and correction of key pathological hallmarks of Pompe disease in a humanized Pompe mouse model

Methods

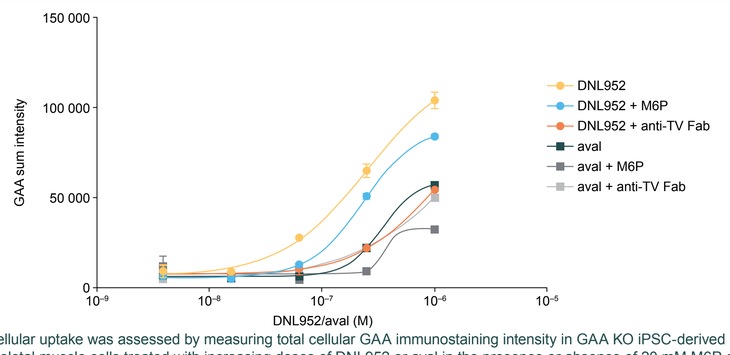
- Cellular uptake and lysosomal delivery of DNL952 or avalglucosidase alfa were evaluated using human GAA KO iPSC-derived skeletal muscle cells. Cellular internalization and trafficking were assessed by immunofluorescence microscopy. Lysosomal delivery of GAA protein was also measured by western blot analysis in lysosomes isolated from cell lysates using SPION-mediated fractionation
- A TfR$^{mu/hu}$ KI mouse model was generated that harbors the human TfR apical domain knocked into the murine TfR, rather than replacing the full-length receptor.[11] TfR$^{mu/hu}$ KI mice are healthy and have a normal phenotype (as assessed by clinical and anatomical pathology up to 12 months of age), and they express TfR1 levels and distribution in the brain and peripheral tissues that are comparable to those in WT (C57BL/6J) mice
- PK was characterized in TfR$^{mu/hu}$ KI and WT mice following a single IV dose of DNL952. Tissue concentrations were quantified using total human GAA enzyme immunoassays
- PD effects following single and multiple IV doses were evaluated in *Gaa* KO;TfR$^{mu/hu}$ KI mice, a Pompe disease model that is GAA-deficient[12] and expresses a chimeric TfR permitting DNL952 engagement
- PD endpoints included glycogen quantification using LC-MS/MS–based methods in quadriceps muscle and brain tissue, evaluation of lysosomal and autophagic dysfunction in muscle using an immunofluorescence method, and proteomics and lipidomics profiling using LC-MS/MS in quadriceps muscle to assess correction of biochemical and cellular pathways downstream of GAA activity restoration

Results

Cellular Uptake of DNL952 Occurs Through TfR- and M6PR-Mediated Processes

- DNL952 uptake in human GAA-KO iPSC-derived skeletal muscle cells was reduced by excess M6P, consistent with M6PR-mediated uptake, and by an anti-TV antibody that blocks the TfR-binding interface on DNL952 (**Figure 2**)
- Avalglucosidase alfa uptake was dependent on M6PR-mediated uptake, as expected

Figure 2. TfR- and M6PR-dependent receptor-mediated cellular uptake of DNL952

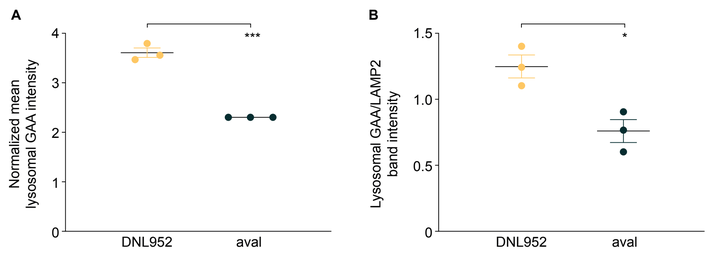


Cellular uptake was assessed by measuring total cellular GAA immunostaining intensity in GAA KO iPSC-derived skeletal muscle cells treated with increasing doses of DNL952 or aval in the presence or absence of 20 mM M6P or 10 mM anti-TV Fab (n = 3 experiments). Data are presented as mean ± SEM

DNL952 Exhibits Greater Cellular Uptake and Lysosomal Delivery in Muscle Cells

- In GAA KO iPSC-derived skeletal muscle cells, cellular enzyme uptake, as measured by GAA immunostaining intensity, was greater following treatment with DNL952 than with avalglucosidase alfa (**Figure S1**)
- Lysosomal delivery of the enzyme was greater following treatment with DNL952 than with avalglucosidase alfa (**Figure 3**)

Figure 3. (**A**) Lysosomal localization of DNL952 and avalglucosidase alfa in GAA KO iPSC-derived skeletal muscle cells. (**B**) Quantification of western blot bands of GAA/LAMP2 proteins from the isolated lysosomes using SPION-mediated fractionation



(A) Lysosomal localization was assessed by quantifying the mean GAA signal intensity co-localized with LAMP2-positive spots, normalized to untreated samples (n = 3). Data are presented as mean ± SEM
*P < 0.05; ***P < 0.001

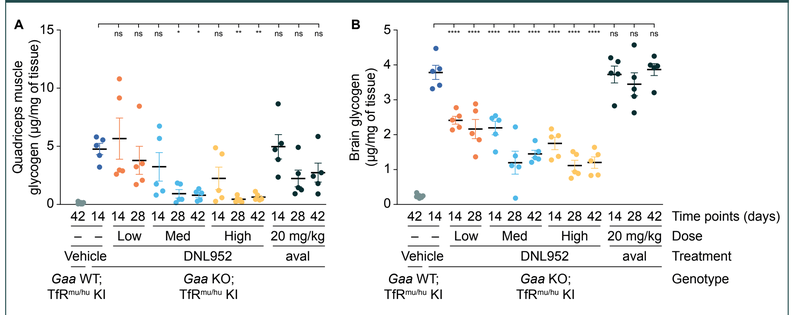
TfR Binding Enables Enhanced DNL952 Exposures in Quadriceps Muscle and Brain Tissue *In Vivo*

- DNL952 exposures in quadriceps muscle and brain tissue were compared between TfR$^{mu/hu}$ KI and WT mice that received a single IV dose of DNL952 at various dose levels to evaluate the TfR-dependence of tissue distribution
- Across all dose levels evaluated, DNL952 exposures were improved in both quadriceps muscle and brain tissue in TfR$^{mu/hu}$ KI mice compared to WT mice, indicating that the TfR enables enhanced DNL952 delivery to these tissues (**Figure S2**)

PD Response of DNL952 After a Single Dose Demonstrates Significant and Sustained Reduction of Muscle and Brain Glycogen

- *Gaa* KO;TfR$^{mu/hu}$ KI mice received a single IV dose of either vehicle, avalglucosidase alfa (20 mg/kg), or DNL952 at various dose levels
- DNL952 produced a dose-dependent and durable reduction in accumulated glycogen in both quadriceps muscle and brain, with greatest glycogen reduction at Day 28 after dosing across all doses that was sustained through at least Day 42 as evaluated in the medium- and high-dose groups (**Figure 4**)
- DNL952 demonstrated greater efficacy than avalglucosidase alfa in quadriceps muscle and brain after a single dose

Figure 4. Glycogen levels in (**A**) quadriceps muscle and (**B**) brain tissue at 14, 28, or 42 days after a single dose

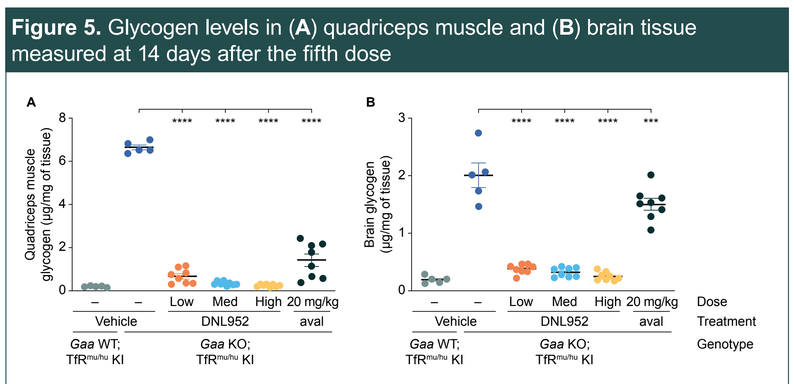


Gaa KO;TfR$^{mu/hu}$ KI animals (n = 5 per group) received a single IV dose of vehicle, DNL952 (at low, med, or high dose), or aval (20 mg/kg). Vehicle-treated *Gaa* WT;TfR$^{mu/hu}$ KI (n = 10) and *Gaa* KO;TfR$^{mu/hu}$ KI (n = 5) mice served as the nondisease and disease comparator groups, respectively. Levels were assessed using an LC-MS/MS–based method. Data are presented as mean ± SEM. *P < 0.05; **P < 0.01; ****P < 0.0001

PD Response of DNL952 After Multiple Doses Administered EOW Shows Near Normalization of Glycogen Levels in Muscle and Brain

- *Gaa* KO;TfR$^{mu/hu}$ KI mice received five IV doses administered EOW of either vehicle, avalglucosidase alfa (20 mg/kg), or DNL952 at various dose levels
- DNL952 treatment significantly reduced glycogen to near-normal levels in both quadriceps muscle and brain across various dose levels. DNL952 demonstrated greater efficacy than avalglucosidase alfa in quadriceps muscle and brain after multiple administration (**Figure 5**)

Figure 5. Glycogen levels in (**A**) quadriceps muscle and (**B**) brain tissue measured at 14 days after the fifth dose

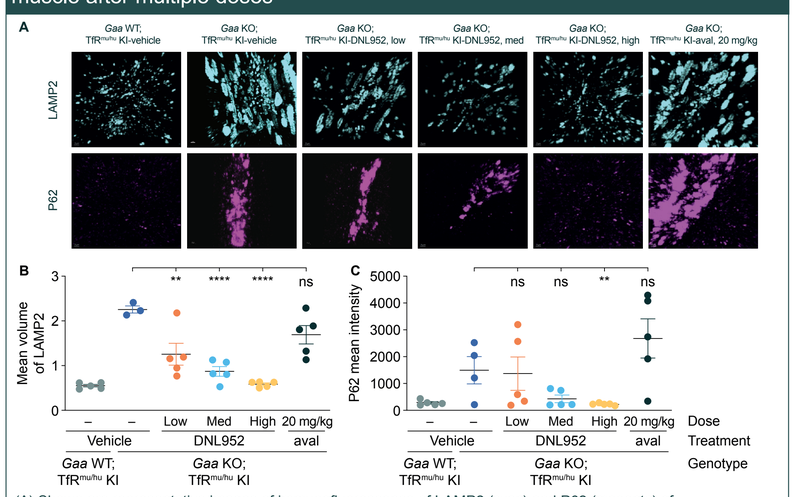


Gaa KO;TfR$^{mu/hu}$ KI animals (n = 8 per group) received five IV doses of vehicle, DNL952 (low, med, or high dose), or aval (20 mg/kg) administered EOW. Vehicle-treated *Gaa* WT;TfR$^{mu/hu}$ KI (n = 5) and *Gaa* KO;TfR$^{mu/hu}$ KI (n = 5) mice served as the nondisease and disease comparator groups, respectively. Levels were assessed using an LC-MS/MS–based method. Data are presented as mean ± SEM. ***P < 0.001; ****P < 0.0001

Multiple Doses of DNL952 Reduces Markers of Lysosomal and Autophagic Dysfunction

- Staining for LAMP2 and P62 revealed marked lysosomal vacuolization and autophagic buildup, respectively, in the quadriceps muscle of the disease model (vehicle-treated *Gaa* KO;TfR$^{mu/hu}$ KI mice) (**Figure 6A**)
- Treatment with five IV doses of DNL952, administered EOW, at a low, medium, or high dose resulted in a dose-dependent reduction of lysosomal volume and autophagic burden that was greater than that achieved with avalglucosidase alfa (**Figure 6B,C**)

Figure 6. Immunofluorescence staining with LAMP2 and P62 in quadriceps muscle after multiple doses



(A) Shown are representative images of immunofluorescence of LAMP2 (cyan) and P62 (magenta) of *Gaa* WT;TfR$^{mu/hu}$ KI (n = 5) and *Gaa* KO;TfR$^{mu/hu}$ KI mice treated with vehicle, DNL952 (low, med, or high dose), or aval (20 mg/kg) at 14 days after the final dose; n = 3–5 per group. Scale bar = 2 mm. (B,C) Data are mean ± SEM of quantified (B) LAMP2 and (C) P62 signal. **P < 0.01; ****P < 0.0001

Correction in Multiple Cellular Pathways After Multiple IV Doses of DNL952

- Integrated proteomic and lipidomic analyses revealed widespread dysregulation of metabolic and lysosomal pathways in quadriceps muscle of vehicle-treated *Gaa* KO;TfR$^{mu/hu}$ KI mice, consistent with impaired energy metabolism, lysosomal function, and lipid homeostasis characteristic of Pompe pathology (**Figure 7**)
- Treatment with DNL952 resulted in broad, dose-dependent correction of these pathways toward WT profiles. Pathway correction after treatment with avalglucosidase alfa was less extensive than that observed with DNL952 (**Figure 7**)

Figure 7. Heat maps of differentially regulated pathways identified by (**A**) proteomics and (**B**) lipidomics/metabolomics analyses after multiple doses

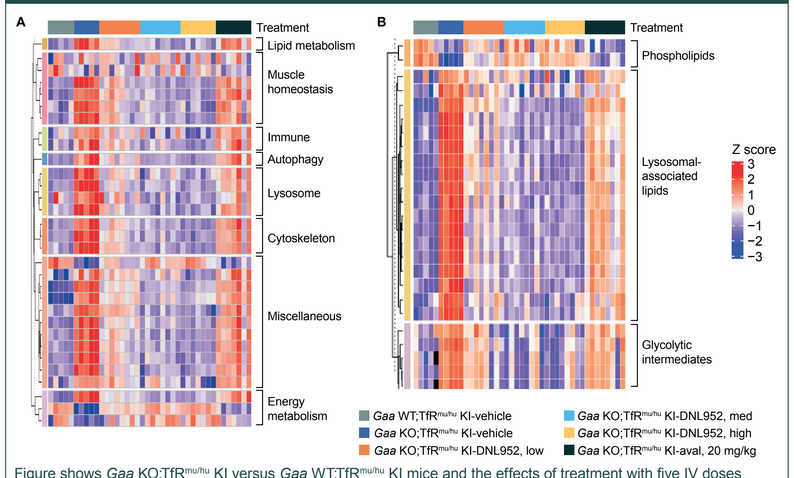


Figure shows *Gaa* KO;TfR$^{mu/hu}$ KI versus *Gaa* WT;TfR$^{mu/hu}$ KI mice and the effects of treatment with five IV doses EOW of DNL952 (low, med, or high dose) or aval (20 mg/kg) at 14 days after the final dose

Conclusions

- DNL952 uptake occurs via both M6PR- and TfR-mediated pathways. Notably, DNL952 achieved greater total cellular uptake and lysosomal delivery than avalglucosidase alfa, supporting enhanced delivery efficiency in human muscle cells
- A single dose of DNL952 produced rapid, dose-dependent, and durable glycogen reduction in muscle and brain
- Repeated dosing achieved near-complete glycogen normalization, reduced lysosomal vacuolization and autophagic buildup, and restored cellular metabolic homeostasis, demonstrating greater improvement than avalglucosidase alfa in correcting secondary disease mechanisms beyond substrate reduction
- Together, the *in vitro* and *in vivo* data highlight the differentiated mechanism of action of DNL952 and the speed, depth, and breadth of PD response of DNL952 in muscle and the nervous system, supporting the potential of DNL952 as an innovative new ERT for Pompe disease

ABBREVIATIONS

aval, avalglucosidase alfa; EOW, every other week; ERT, enzyme replacement therapy; ETV, Enzyme TransportVehicle™; GAA, acid α-glucosidase; *Gaa*, mouse acid α-glucosidase gene; Fab, antigen-binding fragment; IOPD, infantile-onset Pompe disease; iPSC, induced pluripotent stem cell; IV, intravenous; KI, knock-in; KO, knockout; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LAMP2, lysosomal-associated membrane protein 2; LOPD, late-onset Pompe disease; M6P, mannose-6-phosphate; M6PR, mannose-6-phosphate receptor; med, medium; ns, not significant; PD, pharmacodynamic; PK, pharmacokinetics; SEM, standard error of the mean; SPION, superparamagnetic iron oxide nanoparticles; TfR, transferrin receptor; TfR$^{mu/hu}$, a chimeric mouse–human transferrin receptor; TV, TransportVehicle™; WT, wild type.

REFERENCES

1. Ripolone M *et al. Neuropathol Appl Neurobiol* 2018;44:449–62.
2. Prater SN *et al. Orphanet J Rare Dis* 2013;8:90.
3. Kulessa M *et al. Neuropathol Appl Neurobiol* 2020;46:359–74.
4. Raben N *et al. Mol Genet Metab* 2003;80(1–2):159–69.
5. Kenney-Jung D *et al. Mol Genet Metab* 2024;141:108119.
6. van den Dorpel JJA *et al. J Inherit Metab Dis* 2024;47(4):716–30.
7. Tsai L-K. *Neuromuscul Disord* 2019;29:903–6.
8. Fuller DD *et al. J Neurophysiol* 2021;126:351–60.
9. Korlimarla A *et al. Ann Transl Med* 2019;7(13):289.
10. De Vito E. *Neuromuscul Dis* 2019;29:444–7.
11. Kariolis MS *et al. Sci Transl Med* 2020;12(545):eaay1359.
12. Raben N *et al. J Biol Chem* 1998;273(30):19086–92.

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DISCLOSURES

This poster was sponsored by Denali Therapeutics Inc. **RP**, **CVP**, **HN**, **IB**, **MM**, **RG**, **CT**, **JA**, **SD**, **AR**, **RM**, **TY**, **JHS**, **MF**, **NG**, **AH**, **SH-R**, **SC**, **AA**, and **CM** are employees of Denali Therapeutics Inc., which has filed patent applications related to the subject matter.

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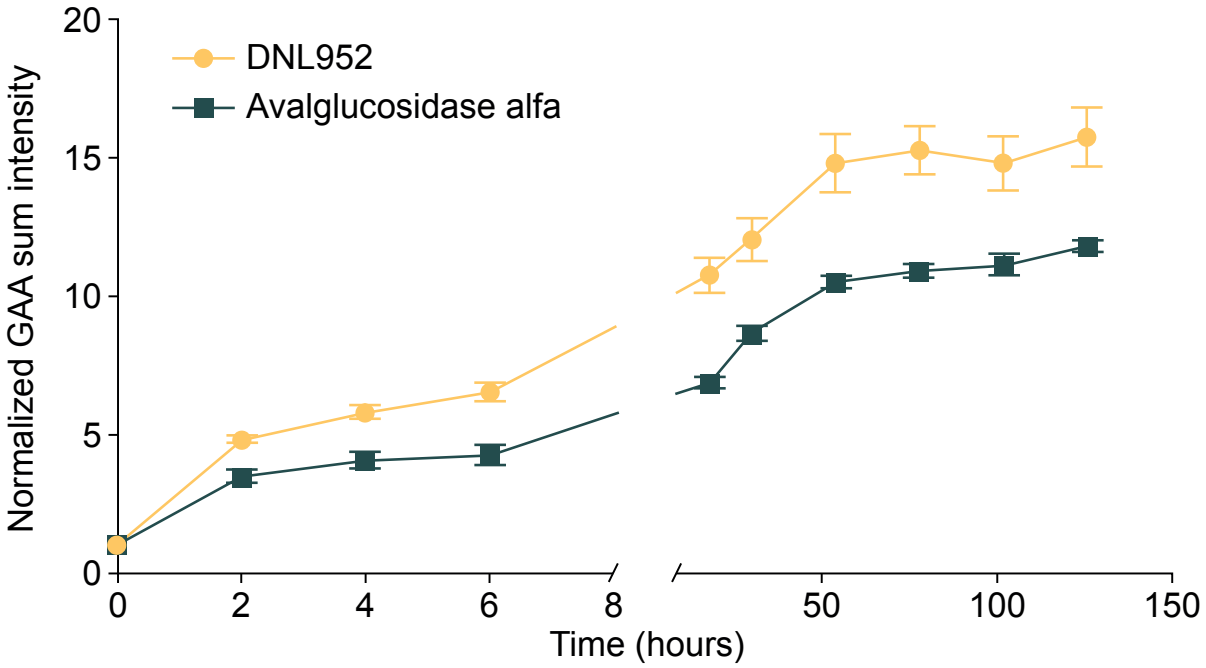
Presented at WORLD*Symposium*™, February 2–6, 2026, San Diego, CA, USA

Supplemental File

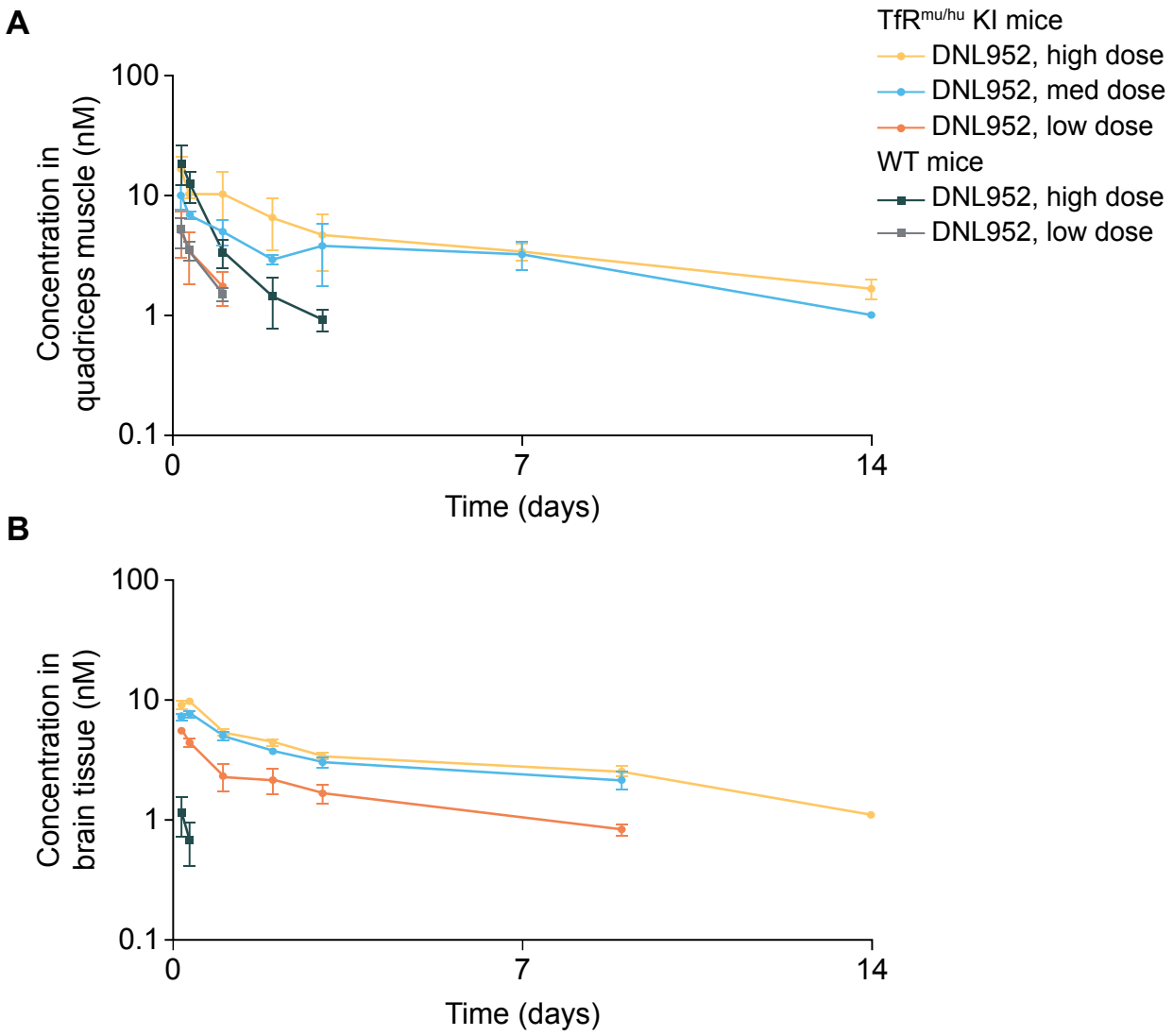
Enhanced Correction of Skeletal Muscle and Brain Pathology in a Pompe Mouse Model Using Transferrin Receptor–Mediated Delivery of GAA

Figure S1. Cellular uptake of GAA by DNL952 and avalglucosidase alfa



Cellular uptake was assessed by measuring total cellular GAA immunostaining intensity in GAA KO iPSC-derived skeletal muscle cells treated with DNL952 or avalglucosidase alfa at 1 mM for 2, 4, 6, 18, 30, 54, 78, 102, and 126 hours (n = 3). Data are presented as mean ± SEM

Figure S2. Total human GAA concentrations in (**A**) quadriceps muscle and (**B**) brain tissue of TfR$^{mu/hu}$ KI and WT C57BL/6J mice following a single IV bolus dose of DNL952



Data are presented as mean ± SD

ABBREVIATIONS

GAA, acid α-glucosidase; iPSC, induced pluripotent stem cell; IV, intravenous; KI, knock-in; KO, knockout; med, medium; SD, standard deviation; SEM, standard error of the mean; TfR$^{mu/hu}$, a chimeric mouse–human transferrin receptor; WT, wild type.

Supplement to poster presented at WORLD*Symposium*™, February 2–6, 2026, San Diego, CA, USA

034

A Phase 1, Multicenter, Open-Label Study Design to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of DNL952 in Adult Participants with Late-Onset Pompe Disease

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Introduction

- In Pompe disease, GAA deficiency causes glycogen accumulation in muscle, resulting in progressive motor deterioration and respiratory weakness despite available ERTs
- In addition, there is growing evidence that glycogen buildup in the nervous system causes severe neurological deficits (including seizures and encephalopathy) in IOPD,[1–3] and may contribute to weakness in LOPD[4–6]
- Therefore, innovative new approaches are needed that enhance ERT delivery to muscles and to the nervous system

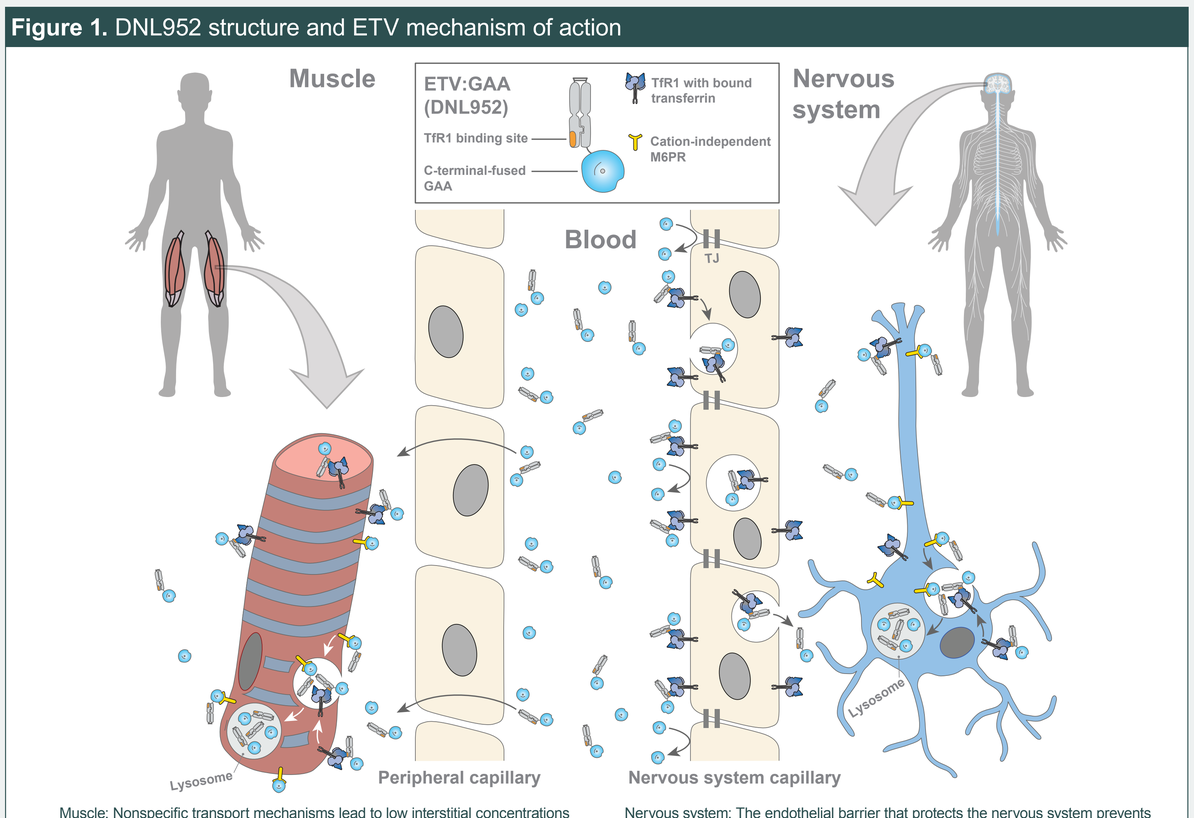
Conclusions

- DNL952 is an investigational, next-generation ERT for Pompe disease that leverages the TfR to improve enzyme delivery to muscles and to the nervous system
- Study DNLI-J-0001 is the first in-human study of DNL952
- Safety, PK, and PD data obtained in this study will support identification of a well-tolerated and potentially effective dose for future studies in Pompe disease
- For more information, please visit ClinicalTrials.gov (NCT07354724)

Background on DNL952

- DNL952 is a novel, investigational ERT for Pompe disease that has been designed to enhance GAA delivery to muscles and to the nervous system
- DNL952 (ETV:GAA) consists of recombinant GAA fused to an ETV – with an Fc region engineered to bind the TfR – which improves GAA distribution to TfR-expressing tissues (such as muscles and the nervous system) via receptor-mediated cellular uptake and transcytosis (**Figure 1**)
- The PD effects of DNL952 were evaluated in *Gaa* KO;TfR$^{mu/hu}$ KI mice, a Pompe disease model that is GAA-deficient and also expresses a chimeric TfR. This TfR binds the ETV with similar affinity to the human TfR while preserving the function and expression of the murine TfR, thereby enabling the evaluation of TfR-mediated pharmacology. *Gaa* KO;TfR$^{mu/hu}$ KI mice received five IV doses administered EOW of either vehicle, DNL952 at various dose levels, or avalglucosidase alfa 20 mg/kg
- Vehicle-treated *Gaa* KO;TfR$^{mu/hu}$ KI mice developed glycogen accumulation in quadriceps muscle and in brain tissue. In addition, staining for the lysosomal marker LAMP2 and autophagosomal marker P62 revealed marked lysosomal vacuolization and autophagic buildup in the quadriceps, consistent with impaired lysosomal function and autophagic flux, pathologic hallmarks of Pompe disease that are also observed in muscle biopsies from human patients[7–9]
- DNL952 treatment significantly reduced glycogen to near-normal levels in both quadriceps muscle and brain tissue across various dose levels. DNL952 demonstrated greater efficacy than avalglucosidase alfa in correcting glycogen accumulation in both of these tissues (**Figure 2**)
- Treatment with DNL952 at low, medium, and high doses resulted in dose-dependent reduction of lysosomal volume and autophagic burden that was greater than that achieved with avalglucosidase alfa (**Figure 3**)
- The differentiated mechanism of action of DNL952 and data from nonclinical studies support its potential as a next-generation therapy for Pompe disease (see Poster 290 [Priya R *et al.*] for additional nonclinical data)

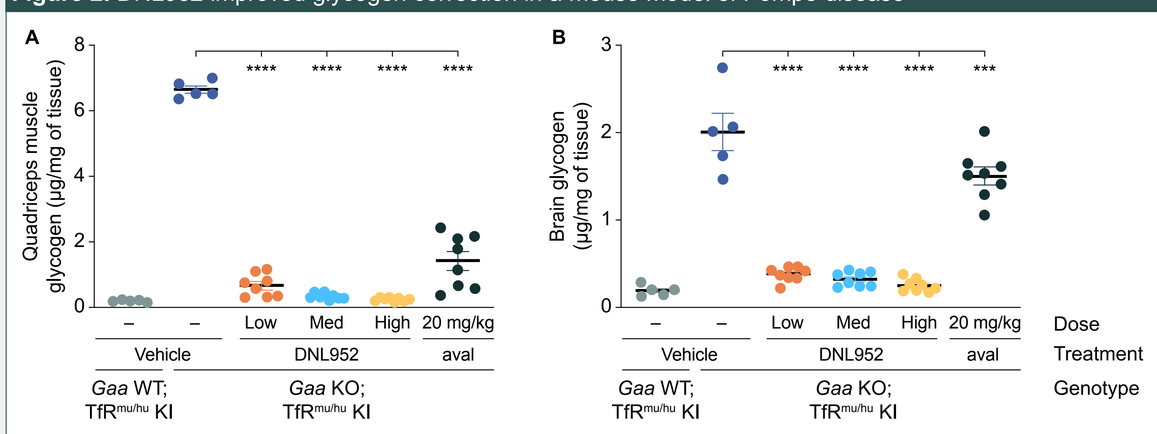
Figure 1. DNL952 structure and ETV mechanism of action



Muscle: Nonspecific transport mechanisms lead to low interstitial concentrations of GAA ERTs. The ability of DNL952 to engage the TfR, in addition to the M6PR, enables improved cellular uptake and lysosomal delivery

Nervous system: The endothelial barrier that protects the nervous system prevents efficient delivery of GAA. TfR engagement by DNL952 enables receptor-mediated transcytosis across the endothelium as well as cellular uptake and lysosomal delivery

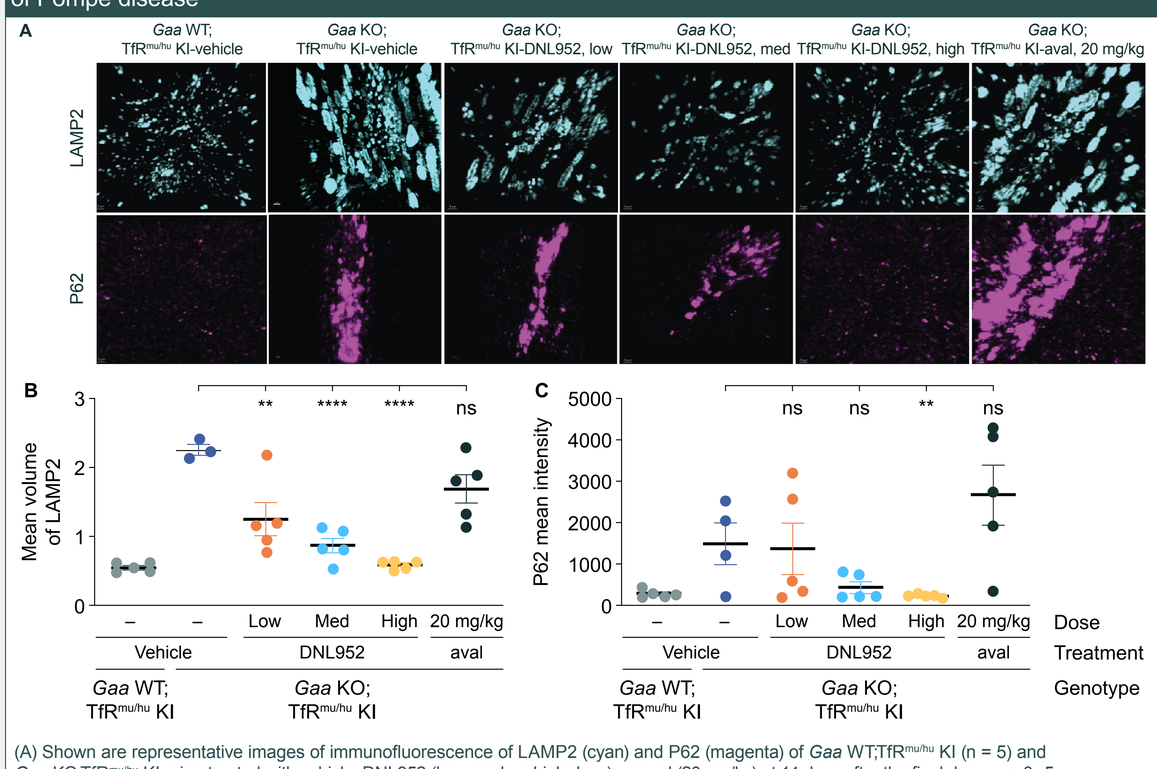
Figure 2. DNL952 improved glycogen correction in a mouse model of Pompe disease



Gaa KO;TfR$^{mu/hu}$ KI animals (n = 8 per group) received five IV dosings of DNL952 (low, med, or high dose) or aval (20 mg/kg) administered EOW. Vehicle-treated *Gaa* WT;TfR$^{mu/hu}$ KI (n = 5) and *Gaa* KO;TfR$^{mu/hu}$ KI (n = 5) mice served as the nondisease and disease comparator groups, respectively. Levels were assessed using an LC-MS/MS–based method. Data are presented as mean ± SEM

$^{***}P < 0.001$; $^{****}P < 0.0001$

Figure 3. DNL952 improved correction of markers of lysosomal and autophagic dysfunction in a mouse model of Pompe disease



(A) Shown are representative images of immunofluorescence of LAMP2 (cyan) and P62 (magenta) of *Gaa* WT;TfR$^{mu/hu}$ KI (n = 5) and *Gaa* KO;TfR$^{mu/hu}$ KI mice treated with vehicle, DNL952 (low, med, or high dose), or aval (20 mg/kg) at 14 days after the final dose; n = 3–5 per group. Scale bar = 2 mm. (B,C) Data are mean ± SEM of quantified (B) LAMP2 and (C) P62 signal. $^{**}P < 0.01$; $^{****}P < 0.0001$

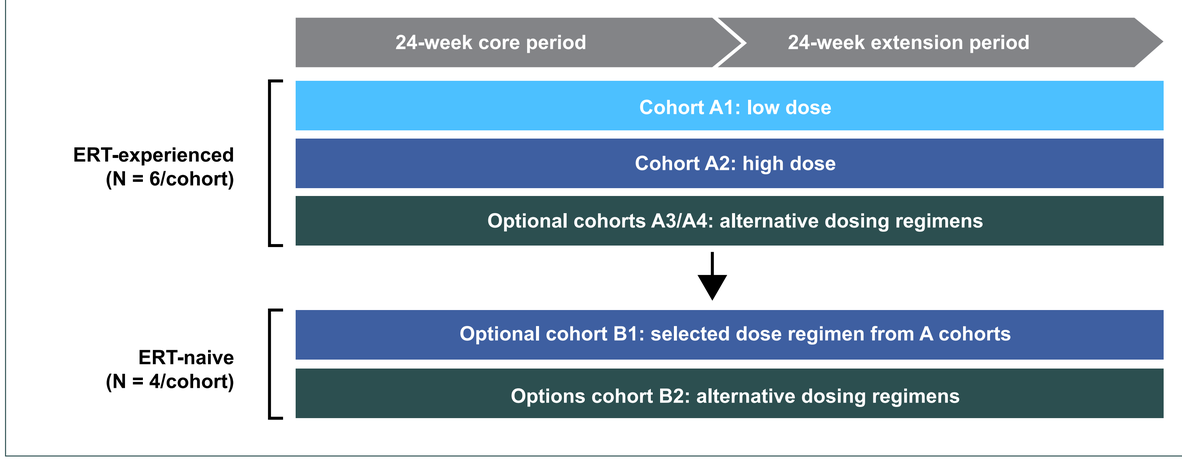
Study Design

- Study DNLI-J-0001 is a Phase 1, multicenter, open-label study to evaluate the safety, tolerability, PK, and PD of DNL952 in adult participants with LOPD (**Table 1**; **Figure 4**)

Table 1. Overview of the Phase 1 study design

		Study overview
Key eligibility	All cohorts	• Age ≥ 18 and ≤ 75 years • Confirmed diagnosis of LOPD • Upright FVC ≥ 30% of predicted normal value • Able to ambulate ≥ 40 m (use of assistive devices is acceptable)
	A cohorts: ERT-experienced	• Have received avalglucosidase alfa or cipaglucosidase alfa at a dose of 20 mg/kg every 2 weeks for at least 12 months
	Optional B cohorts: ERT-naive	• Have not received any ERT for at least 12 months and have received no more than four total doses at any time
Sample size		• Up to 32 participants
Key endpoints	Primary	• Safety and tolerability
	Secondary	• PK • Immunogenicity
	Exploratory	• PD: urine Glc4, serum CK, and exploratory biomarkers • Efficacy: motor and respiratory strength and function and patient-reported outcomes

Figure 4. Phase 1 study schema



- Two planned dose-exploration cohorts will enroll ERT-experienced participants
- Additional optional cohorts may be included to explore alternative doses or dosing frequencies, or to evaluate DNL952 in ERT-naive participants

ABBREVIATIONS

aval, avalglucosidase alfa; CK, creatine kinase; EOW, every other week; ERT, enzyme replacement therapy; ETV, Enzyme TransportVehicle™; Fc, fragment crystallizable; FVC, forced vital capacity; GAA, acid α-glucosidase; *Gaa*, mouse acid α-glucosidase gene; Glc4, glucose tetrasaccharide; IOPD, infantile-onset Pompe disease; IV, intravenous; KI, knock-in; KO, knockout; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LAMP2, lysosomal-associated membrane protein 2; LOPD, late-onset Pompe disease; M6PR, mannose-6-phosphate receptor; med, medium; ns, not significant; PD, pharmacodynamics; PK, pharmacokinetics; SEM, standard error of the mean; TfR, transferrin receptor; TfR$^{mu/hu}$, a chimeric mouse–human transferrin receptor; TJ, tight junction; WT, wild type.

REFERENCES

1. Kenney-Jung D *et al. Mol Genet Metab* 2024;141:108119.
2. van den Dorpel JJA *et al. J Inherit Metab Dis* 2024;47:716–30.
3. Tsai L-K *et al. Neuromuscul Disord* 2019;29:903–6.
4. Fuller DD *et al. J Neurophysiol* 2021;126:351–60.
5. Korlimarla A *et al. Ann Transl Med* 2019;7:289.
6. De Vito E *et al. Neuromuscul Disord* 2019;29:444–7.
7. Ripolone M *et al. Neuropathol Appl Neurobiol* 2018;44:449–62.
8. Prater SN *et al. Orphanet J Rare Dis* 2013;8:90.
9. Kulessa M *et al. Neuropathol Appl Neurobiol* 2020;46;359–74.

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DISCLOSURES

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ACB, **IVC**, **MG**, **SH-R**, **SD**, and **DJ** are employees of Denali Therapeutics Inc., which has filed patent applications related to the subject matter. **MDT** and **CH** were employees of Denali Therapeutics Inc. at the time the study was designed.

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