Commentary | Open Access

Rare Disease and Orphan Drugs Journal

Argirò *et al. Rare Dis Orphan Drugs J.* 2026;5:30 **DOI:**10.20517/rdodj.2026.26

AAV9.LAMP2B gene therapy for Danon disease: from early clinical experience to evolving safety and trial design considerations

Alessia Argirò[1], Iacopo Olivotto[1,2]

Citation: Argirò A, Olivotto I. *AAV9.LAMP2B* gene therapy for Danon disease: from early clinical experience to evolving safety and trial design considerations. *Rare Dis Orphan Drugs J.* 2026;5:30. https://dx.doi.org/10.20517/rdodj.2026.26

Received: 24 Apr 2026
First Decision: 3 Jul 2026
Revised: 1 Sep 2026
Accepted: 10 Sep 2026
Published: 28 Sep 2026

Academic Editor: Daniel Scherman
Copy Editor: Ping Zhang
Production Editor: Ping Zhang

INTRODUCTION: DANON DISEASE AND THE RATIONALE FOR GENE REPLACEMENT

Danon disease is an X-linked cardiomyopathy caused by loss-of-function variants in the *LAMP2* gene (HGNC:6501; NCBI Gene ID: 3920). The estimated prevalence spans from 240 patients to 11807 persons with *LAMP2* loss-of-function variants, highlighting that this condition is likely underdiagnosed[1].

The *LAMP2* gene has 9 exons. Alternative splicing in exon 9 generates 3 isoforms: LAMP-2a, LAMP-2b, and LAMP-2c. LAMP-2b is mainly expressed in cardiac, skeletal muscle and in the central nervous system and its deficiency determines the disease development[2].

Over 160 separate *LAMP2* variants cause Danon Disease and genotype-phenotype correlation is yet to be clarified. However, exonic variants are usually associated with the absence of LAMP-2b, while intronic variants may still retain the ability to produce functional protein and be associated with milder phenotypes[3]. Furthermore, *de novo* variants are common, causing 40% of cases[4].

LAMP-2b is essential for autophagosome-lysosome fusion, a critical step in macroautophagy. Thus, LAMP-2b deficit leads to impaired autophagic flux, intracellular vacuolization, and progressive cellular dysfunction and death[5,6].

Furthermore, impaired mitophagy has a pivotal role in the pathophysiology of the disease.

In Danon human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), mitophagy is enhanced with persistence of an elevated number of damaged mitochondria, highlighting incomplete mitophagy. Abnormal mitochondria show impaired respiratory capacity and augmented oxidative stress, which can contribute

[1]Experimental and Clinical Medicine Department, University of Florence, Florence 50141, Italy.
[2]Cardiology Unit, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Meyer Children's Hospital, Florence 50139, Italy.

Correspondence to: Dr. Alessia Argirò, Experimental and Clinical Medicine Department, University of Florence, Florence 50141, Italy. E-mail: Alessia.argiro@unifi.it

to early cardiac dysfunction. Interestingly, mitophagy can be rescued providing functional LAMP-2b[7].

Clinically, Danon disease is characterized by hypertrophic cardiomyopathy, skeletal myopathy, and variable neurocognitive impairment. Cardiac disease is the dominant cause of morbidity and mortality, often progressing to advanced heart failure, malignant arrhythmias, and heart transplantation in young male patients and a subset of females[8].

Given the monogenic nature of the disease and the predominant cardiac involvement, Adeno-associated virus (AAV)-mediated gene replacement has emerged as a potential disease-modifying strategy.

Preclinical studies evaluating gene therapy in a *LAMP2*-knockout murine model demonstrated dose-dependent restoration of LAMP-2b expression, improvement in autophagic function, and normalization of cardiac histology, providing the foundation for clinical translation[9].

The following paragraphs will summarize and comment on the results of the Phase 1 Danon disease gene therapy study reported by Greenberg *et al.*[10], providing an update on the development of this therapeutic approach.

PHASE 1 CLINICAL EXPERIENCE: PROOF-OF-CONCEPT EFFICACY AND EARLY SAFETY SIGNALS

The first-in-human Phase 1 study evaluated *AAV9.LAMP2B* gene therapy in two pediatric and five adult male patients with symptomatic Danon disease (NCT03882437)[10]. The investigational product consisted of a recombinant AAV9 vector enclosing a full-length, wild-type version of the human *LAMP2B* transgene. The route of administration was intravenous and a low dose (6.7×10^{13} GC/kg) and a high dose (1.1×10^{14} GC/kg) were tested. The high dose was discontinued after one of the two patients treated at this dose developed thrombotic microangiopathy[11].

The inclusion criteria included: a pathogenic or likely pathogenic *LAMP2* variant, male sex, at least 15 years of age (for adults and adolescents) or at least 8 years of age (for the pediatric cohort), cardiac involvement [ECG, echocardiography or cardiac magnetic resonance imaging (MRI) abnormalities], and New York Heart Association (NYHA) class I-III symptoms (NYHA class II-III for pediatric patients).

The primary endpoints were the safety and toxic effects of the investigational product, myocardial LAMP-2b transduction and protein expression, and preliminary stabilization or improvement in heart failure symptoms and in cardiac structure and function.

During the study, the immunomodulatory regimen was progressively adapted: steroid doses were reduced to mitigate steroid-induced myopathy, tacrolimus was replaced with sirolimus to reduce the risk of renal toxicity, and rituximab was introduced. All patients exhibited increased left ventricular (LV) mass and elevated cardiac biomarkers, were in NYHA class II, and reported low quality of life.

An immunohistochemistry analysis to evaluate LAMP-2b expression was performed on endomyocardial biopsy samples, which were evaluated following semiquantitative grading by a blinded pathologist.

A blinded reviewer centrally evaluated LV mass obtained by cardiac MRI or echocardiography. All other measurements of cardiac structure and function were evaluated by echocardiography.

Since this was a single-arm study, safety and efficacy outcomes were described using observed values and absolute or relative changes from baseline at the individual-patient level. No formal statistical hypothesis testing was performed.

After 3-6 and up to 24-36 months, myocardial biopsies demonstrated increased LAMP-2b expression, reduced vacuolization, and normalization of cardiomyocyte morphology.

After 2-4 years, patients exhibited a reduction in LV mass, improvement in circulating cardiac biomarkers, and enhanced functional status and quality of life measures.

The only patient with baseline systolic dysfunction experienced progressive heart failure and ventricular arrhythmias requiring heart transplantation. The protocol was therefore refined excluding patients with reduced ejection fraction. Additional adverse events included thrombotic microangiopathy requiring transient dialysis and complement inhibition, reversible hepatocellular injury, and steroid-associated myopathy.

TRANSITION TO PHASE 2: THERAPEUTIC OPTIMIZATION AND EMERGING SAFETY CONCERNS

A Phase 2 program was initiated to evaluate a co-primary clinical endpoint, including increased LAMP-2b expression on immunohistochemistry and ≥ 10% reduction in LV mass (NCT06092034). Based on the initial efficacy observed in the low-dose cohort and to mitigate complement-mediated safety concerns observed in the high-dose cohort (related to thrombotic microangiopathy), the Phase 2 study was initiated at the low dose (6.7×10^{13} gc/kg)[12]. Importantly, the Phase 2 protocol incorporated a modified immunomodulatory regimen including a complement C3 inhibitor.

Early Phase 2 experience revealed unexpected and severe immune-mediated toxicity. In May 2025, two patients developed severe adverse events characterized by capillary leak syndrome leading to multi-organ dysfunction, with one fatal outcome following an intercurrent systemic infection. The sponsor suspended dosing in both the United States and European Union, and the U.S. Food and Drug Administration (FDA) placed the study on clinical hold[12].

REGULATORY REASSESSMENT AND PROTOCOL REVISION

Following detailed investigation, the clinical hold was lifted by the FDA in August 2025. The safety review concluded that the severe adverse events were likely the result of the combination of the complement C3 inhibitor introduced into the immunomodulation regimen and the investigational product. A potential mechanism has subsequently been proposed, whereby complement inhibition with the C3 inhibitor may have reduced AAV opsonization and increased vector uptake by endothelial cells, leading to vascular endothelial injury[13].

Following regulatory review, the Phase 2 protocol was substantially revised. The vector dose was reduced to 3.8×10^{13} GC/kg. The recalibrated dose was selected to achieve potency consistent with the dose range at which RP-A501 was associated with biological efficacy in Phase 1. This recalibration accounted for the higher proportion of full capsids in the current drug product and was developed in consultation with clinical experts and the FDA[14].

The revised protocol introduced sequential enrolment with safety review for initial participants, with a minimum four-week interval between administrations. This conservative approach was intended to allow early detection of dose-related toxicity signals.

In parallel, the immunomodulatory regimen was restructured. Prophylactic administration of the C3 complement inhibitor was discontinued, while maintaining immunosuppression with sirolimus, rituximab, and corticosteroids.

Given the recognized association between systemic AAV administration, complement activation, and thrombotic microangiopathy, a more reactive strategy was adopted for complement-mediated complications[15]. Accordingly, the protocol specified a lower predefined threshold for initiation of the C5 inhibitor eculizumab in the event of complement activation. The eligibility criteria remained unchanged.

CURRENT STATUS AND EARLY PHASE 2 EXPERIENCE

Six patients were treated in the Phase 2 study at the higher dose (6.7×10^{13} GC/kg) prior to the temporary clinical hold[16]. After the protocol amendment, three patients have been treated and no thrombotic microangiopathy, capillary leak syndrome, or other significant safety concerns were observed[17].

Although data remain preliminary, this cohort represents an important opportunity to evaluate whether dose reduction and modified immune modulation can preserve efficacy while reducing toxicity.

BROADER IMPLICATIONS FOR CARDIAC GENE THERAPY

The clinical development of *AAV9.LAMP2B* gene therapy provides several broader insights relevant to cardiovascular gene therapy programs.

The findings observed in the Phase 1 study provided preliminary evidence that partial restoration of LAMP-2b expression may be associated with measurable improvements in cardiac structure and function.

The emergence of complement-mediated toxicity underscores the importance of innate immune pathways in systemic AAV administration. Indeed, complement activation may represent a central determinant of both acute toxicity and organ-specific injury[18].

This experience highlights the critical role of dose optimization. AAV-related toxicity is dose-dependent, with higher doses increasing the risk of systemic inflammatory complications.

In a systematic review and meta-analysis of 801 studies including 1,972 treated patients, approximately 30% of AAV recipients experienced an immune-mediated adverse event, although the severity varied substantially by event type. Thrombotic microangiopathy, observed in 4.7% of patients, was associated with substantial morbidity and showed a characteristic early onset, clustering predominantly during the first week after vector administration. Notably, higher vector doses were associated with increased risk of immunotoxicity, whereas multimodal immunosuppression was associated with a lower risk[19].

The restriction of eligibility to patients with preserved ejection fraction reflects an evolving recognition that advanced myocardial remodeling may be less amenable to reversal.

Finally, the inability to re-dose due to neutralizing antibody formation remains a major limitation of current AAV platforms, emphasizing the importance of first-dose optimization and long-term durability assessment.

LIMITATIONS OF CURRENT EVIDENCE

Despite encouraging signals, several limitations remain. The small sample sizes and early-phase design preclude definitive conclusions regarding clinical efficacy or long-term safety.

A subset of female patients with Danon disease may present with a severe clinical course comparable to males, characterized by life-threatening arrhythmias and advanced heart failure[20]. Thus, eligibility to females could be potentially extended to patients with severe pediatric- or adolescent-onset disease.

Longer-term follow-up is required to assess durability of gene expression, late toxicity, and clinical outcomes, including survival and survival free from heart transplantation.

Comprehensive mechanistic studies evaluating complement activation, cytokine profiles, and vector-host immune interactions remain limited but are essential for future optimization.

Finally, gene therapy administration may be associated with prolonged hospitalization and significant adverse events. Evaluating the experiences of patients and their caregivers, through patient-reported outcomes and qualitative interviews, could help identify areas for intervention to reduce treatment burden and improve overall care.

CONCLUSION

The clinical development of *AAV9.LAMP2B* gene therapy for Danon disease represents an important early clinical experience with cardiac-directed gene replacement therapy. Phase 1 studies demonstrated biological activity and provided preliminary evidence of clinical benefit, whereas subsequent Phase 2 experience revealed important safety challenges in the context of a modified immunomodulatory regimen.

These findings refine the path forward by highlighting critical determinants of safety, including immune modulation strategy, dose definition, and patient selection. Continued iterative optimization will be essential to fully realize the potential of gene therapy as a disease-modifying approach in Danon disease and other inherited cardiomyopathies.

DECLARATIONS

Authors' contributions

Writing - original draft preparation: Argirò A
Writing - review & editing: Olivotto I

Availability of data and materials

Not applicable.

AI and AI-assisted tools statement

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Dr. Argiro A has no conflicts of interest to declare. Prof. Olivotto I is supported by the European Union Grant Agreement no. 101137115 (SMASH-HCM [Stratification, Management, and Guidance of Hypertrophic Cardiomyopathy Patients using Hybrid Digital Twin Solutions]; call: HORIZON-HLTH-2023-TOOL05); has received research grants from BMS, Cytokinetics, Amicus, Chiesi, Tenaya, Boston Scientific, and Medtronic, and has received advisory board or speaking fees from BMS, Cytokinetics, Amicus, Chiesi, Bayer, Edgewise, Boston Scientific, Tenaya, Rocket Pharma, Lexeo, Imbria, and Novo Nordisk.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

REFERENCES

1. Boslough M, Massera D, Taylor M. How ultra-rare is Danon disease? An estimate of prevalence based on multiple data sources. *Eur J Heart Fail.* 2026;28:xuag193.282. DOI
2. Yu K, Peng B, Zhao D, Wang Y. Danon disease: two case reports and literature review. *Medicine.* 2026;105:e46993. DOI
3. Cetin H, Wöhrer A, Rittelmeyer I, et al. The c.65-2A>G splice site mutation is associated with a mild phenotype in Danon disease due to the transcription of normal LAMP2 mRNA. *Clin Genet.* 2016;90:366-71. DOI
4. Sugie K, Komaki H, Eura N, et al. A nationwide survey on Danon disease in Japan. *Int J Mol Sci.* 2018;19:3507. DOI
5. Del Favero G, Bonifacio A, Rowland TJ, et al. Danon disease-associated LAMP-2 deficiency drives metabolic signature indicative of mitochondrial aging and fibrosis in cardiac tissue and hiPSC-derived cardiomyocytes. *J Clin Med.* 2020;9:2457. DOI
6. Nishino I, Fu J, Tanji K, et al. Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature.* 2000;406:906-10. DOI
7. Hashem SI, Murphy AN, Divakaruni AS, et al. Impaired mitophagy facilitates mitochondrial damage in Danon disease. *J Mol Cell Cardiol.* 2017;108:86-94. DOI
8. Hong KN, Eshraghian EA, Arad M, et al. International consensus on differential diagnosis and management of patients with Danon disease: JACC state-of-the-art review. *J Am Coll Cardiol.* 2023;82:1628-47. DOI
9. Manso AM, Hashem SI, Nelson BC, et al. Systemic AAV9.LAMP2B injection reverses metabolic and physiologic multiorgan dysfunction in a murine model of Danon disease. *Sci Transl Med.* 2020;12:eaax1744. DOI
10. Greenberg B, Taylor M, Adler E, et al. Phase 1 study of AAV9.LAMP2B gene therapy in danon disease. *N Engl J Med.* 2025;392:972-83. DOI
11. Johnson V. Danon disease gene therapy yields sustained clinical benefit. CGTlive. Available from: https://www.cgtlive.com/view/danon-disease-gene-therapy-yields-sustained-clinical-benefit [Last accessed on 22 Sep 2026].
12. Rocket pharmaceuticals provides update on phase 2 clinical trial of RP-A501 for Danon disease. Rocket Pharmaceuticals, Inc. Available from: https://ir.rocketpharma.com/news-releases/news-release-details/rocket-pharmaceuticals-provides-update-phase-2-clinical-trial-rp/ [Last accessed on 22 Sep 2026].
13. Parajuli S, Gallagher T, Flotte TR. Immune Toxicities in AAV Gene Therapy: Overview for Clinicians. *Int J Mol Sci.* 2026;27:3196. DOI
14. Rocket pharmaceuticals announces positive clinical safety update from initial three patients treated with RP-A501 Under modified phase 2 protocol for Danon disease. Available from: https://ir.rocketpharma.com/news-releases/news-release-details/rocket-pharmaceuticals-announces-positive-clinical-safety-update [Last accessed on 22 Sep 2026].
15. Salabarria SM, Corti M, Coleman KE, et al. Thrombotic microangiopathy following systemic AAV administration is dependent on anti-capsid antibodies. *J Clin Invest.* 2024:134. DOI
16. Rocket pharmaceuticals announces FDA has lifted the clinical hold on the pivotal phase 2 trial of RP-A501 for the treatment of danon disease. Rocket Pharmaceuticals, Inc. Available from: https://ir.rocketpharma.com/news-releases/news-release-details/rocket-pharmaceuticals-announces-fda-has-lifted-clinical-hold/ [Last accessed on 22 Sep 2026].
17. Rocket pharmaceuticals reports second quarter 2026 financial results and highlights recent progress. Rocket Pharmaceuticals, Inc. Available from: https://ir.rocketpharma.com/news-releases/news-release-details/rocket-pharmaceuticals-reports-second-quarter-2026-financial/ [Last accessed on 22 Sep 2026].
18. Kropf E, Markusic DM, Majowicz A, Mingozzi F, Kuranda K. Complement system response to adeno-associated virus vector gene therapy. *Hum Gene Ther.* 2024;35:425-38. DOI
19. Maurizi N, Ammirati E, Silver E, et al. Incidence, timing, and clinical significance of adverse immune events after gene replacement therapy: a systematic review and meta-analysis. *Mol Ther.* 2026;34:1340-51. DOI
20. Gandaeva L, Sonicheva-Paterson N, McKenna WJ, et al. Clinical features of pediatric Danon disease and the importance of early diagnosis. *Int J Cardiol.* 2023;389:131189. DOI