Molecular Therapy
Methods & Clinical Development
Brief report

Adeno-associated virus serotype 9 antibodies in neonates and young children: Seroprevalence and kinetics

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Gene therapies such as onasemnogene abeparvovec for spinal muscular atrophy (SMA) utilize adeno-associated virus 9 (AAV9) for targeted gene delivery, which requires an AAV9 antibody (AAV9-Ab) immunoglobulin G (IgG) ≤1:50 titer threshold. This retrospective cohort study evaluated age-related AAV9-Ab IgG seroprevalence for patients with SMA (part 1) and AAV9-Ab IgG kinetics and time to 1:50 titer threshold in newborns with elevated AAV9-Ab IgG titers (≥1:100) (part 2). A semi-quantitative ELISA assay was used in part 1 (*N* = 1,323 patients). For patients aged <12 months, 3.9% (*n*/*N* = 31/795) had elevated AAV9-Ab IgG titers (≥1:100); prevalence declined with age. In part 2, a new quantitative ELISA (linear mixed effects model) described continuous AAV9-Ab IgG concentrations for patients with initial titers ≥1:100. AAV9-Ab IgG concentrations waned according to first-order kinetics (58 samples; *N* = 18 patients). The model-based estimation of the AAV9-Ab IgG average half-life was 41 (95% CI, 38–44) days. Based on visualization, 200 ELISA units/mL was a reasonable approximate for the 1:50 titer threshold. In conclusion, initially elevated titers ≥1:100 in newborn patients declined with age. The new quantitative ELISA may allow for quantification of time to threshold for AAV9-Ab IgG retesting for onasemnogene abeparvovec treatment, leading to treatment as early as possible for patients with SMA.

INTRODUCTION

Adeno-associated virus (AAV) vectors are the leading platform for targeted disease treatment via gene delivery.[1] AAV serotype 9 (AAV9) vectors have been used in gene therapy to cross the blood-brain barrier and efficiently transduce cells.[2] Spinal muscular atrophy (SMA) is a primary candidate disease for gene therapy because of its genetic underpinnings. SMA is an autosomal recessive degenerative neuromuscular disease caused by biallelic deletion or mutation in the *survival motor neuron 1* (*SMN1*) gene, leading to irreversible loss of motor neurons.[3] SMA is characterized by progressive hypotonia, muscle atrophy, paralysis, and, in severe cases, swallowing and breathing impairment, permanent ventilatory support requirements, or infantile death.[4] Patients with SMA may benefit from gene therapies that replace the mutated or deleted *SMN1* gene.

The best outcomes with SMA treatment stem from early treatment of presymptomatic infants identified by newborn screening.[5–7] Passage of maternal antibodies before birth protects neonates and infants who are vulnerable to infections prior to immune system development and vaccination.[8] Both immunoglobulin G (IgG) (e.g., AAV9 antibodies [AAV9-Ab]) and immunoglobulin A (IgA) mediate protective functions; however, only IgG received via transplacental transfer may interfere with parenterally administered gene therapy due to its bloodstream circulation versus IgA, which is received via breastfeeding and remains in the gastrointestinal tract.[9] Circulating maternal AAV9-Ab IgG may bind and neutralize gene therapy vectors and may prevent transduction of gene therapy.[1,10,11] To this end, AAV9-Ab IgG titers are tested in patients to assess AAV9-based gene therapy eligibility.[2,12] AAV9-Ab IgG concentrations are typically elevated after birth, then decline before elevating again later in childhood due to wild-type AAV9 exposure; however, waning time course is unclear.[10,12,13] There is a specific window of eligibility for AAV9-based gene therapy in which patients are more likely to have AAV9-Ab levels below threshold.

Onasemnogene abeparvovec is a one-time AAV9 vector-based gene replacement therapy for patients with SMA that restores production of functional SMN protein, leading to improved motor outcomes and survival.[14–20] Safety and efficacy of onasemnogene abeparvovec for patients with elevated AAV9-Ab IgG titers (≥1:100) are not well established; therefore, onasemnogene abeparvovec treatment requires titers to be ≤ 1:50.[2,21] Repeated testing for AAV9-Ab is recommended for patients aged <6 months with elevated antibody titers to follow AAV9-Ab seroreversion.[22,23] However, timing for repeated

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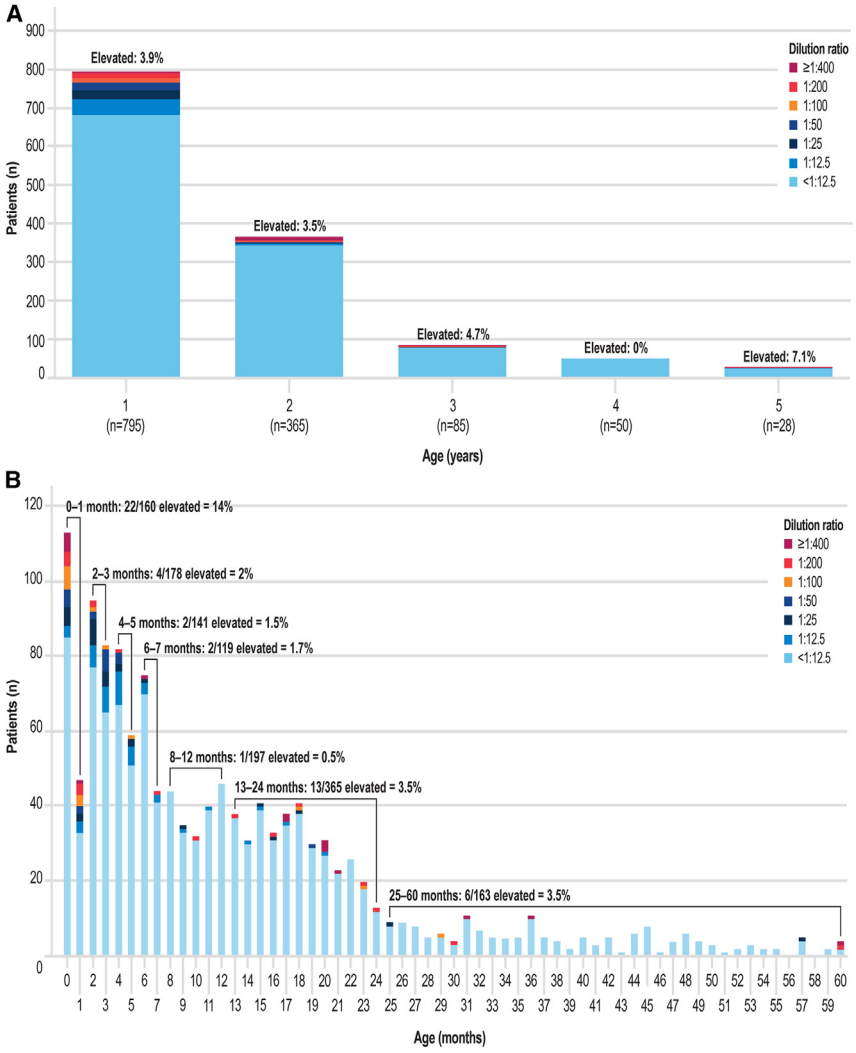

Figure 1. AAV9-Ab IgG titers in the first test for patients with SMA

(A) Panel of patients (N=1,323) by age in years.
(B) Panel of patients (N=1323) by age in months.
AAV9-Ab, anti-adeno-associated virus vector serotype 9 antibodies; IgG, immunoglobulin G; SMA, spinal muscular atrophy. Elevated titers in (A) and (B) refer to patients with AAV9-Ab IgG titers ≥1:100.

testing is unclear, and often varies according to each individual clinician's discretion.

A better understanding of seroreversion related to clearance kinetics for maternally derived antibodies will inform optimal antibody testing/retesting times to prevent delays in treatment.[12,24] The aims of this study were to: (1) establish age-related seroprevalence of AAV9-Ab IgG in neonates and young children with SMA, (2) determine AAV9-Ab IgG kinetics and estimate half-life of maternal antibodies in neonates with SMA, and (3) predict the time to threshold (AAV9-Ab IgG titers ≤1:50) in neonates with elevated AAV9-Ab IgG titers (≥1:100).

RESULTS

Patient population

For the seroprevalence study, AAV9-Ab IgG titers were measured in 1,323 patients with SMA 0–5 years of age (795 children aged 0–12 months). Patients were from 59 countries in Asia (excluding Japan), Europe, Canada, and South America. For the exploratory kinetics study, there were 18 newborns (<1 month old) with sequential tests for up to 25 weeks.

Part 1: Seroprevalence study

For patients aged <12 months, 3.9% (n/N = 31/795) of patients had elevated AAV9-Ab IgG titers at first testing (≥1:100) (Figure 1A). AAV9-Ab IgG titers decreased with age. The prevalence of elevated antibody titers was greatest at 0 to 1 month of age, with 14% (n/N = 22/160) of patients having AAV9-Ab IgG ≥1:100. However, prevalence decreased with age such that 0.5% (n/N = 1/197) of patients between 8 and 12 months of age had elevated titers ≥1:100 (Figure 1B).

Part 2: Exploratory kinetics study

A total of 58 samples from 18 patients with initially elevated AAV9-Ab IgG titers (≥1:100) were collected. For all 18 patients who had multiple sequential AAV9-Ab IgG tests, AAV9-Ab IgG concentrations waned according to first-order kinetics (Figure 2). The dynamics of AAV9-Ab IgG decline was adequately described by a linear mixed effect model (on a log scale) with random effect on the intercept (baseline AAV9-Ab IgG). Based on the final model parameter estimates (Table 1), the model-based estimation for transplacental AAV9-Ab IgG half-life was an average of 41 (95% CI, 38–44) days. A concentration of 200 ELISA units (EU)/mL was used as a desired AAV9-Ab IgG threshold; this value was suggested to serve as a proxy for the titer threshold of 1:50 based on visual inspection of the titer versus concentration (Figure S1).

DISCUSSION

Elevated AAV9-Ab IgG seroprevalence (≥1:100) was relatively low for patients with SMA; elevated titers were most prevalent in neonates and decreased with age. Elevated AAV9-Ab IgG declined after initial testing by first-order kinetics, demonstrating that elevated titers are likely to be temporary. Thus, all newborn patients will eventually display reduced AAV9-Ab IgG below the threshold for eligibility for onasemnogene abeparvovec treatment. The model-based estimation for average half-life (i.e., time for concentration to reduce by half)

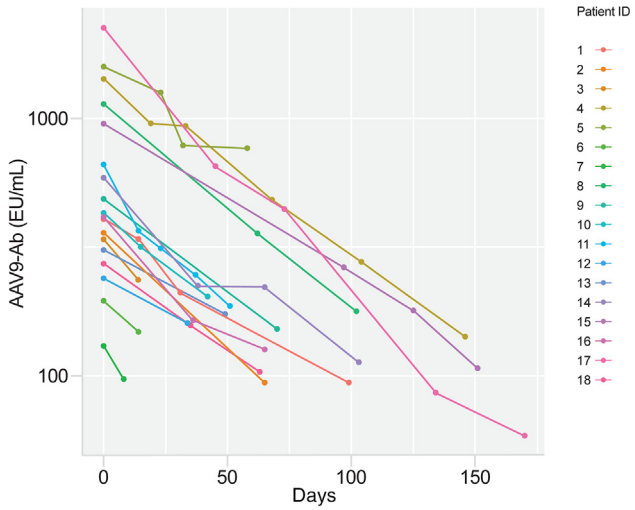

Figure 2. Waning of maternal AAV9-Ab IgG continuous concentration over time for patients with SMA with initial AAV9-Ab IgG titers ≥1:100

AAV9-Ab, adeno-associated virus vector serotype 9 antibodies; ELISA, enzyme-linked immunosorbent assay; EU, ELISA unit; IgG, immunoglobulin G; SMA, spinal muscular atrophy.

of AAV9-Ab IgG was 41 days, which is consistent with that of other transplacental antibodies.[25] Predictions for time to threshold were based on initial AAV9-Ab IgG concentrations: 200 EU/mL was predicted to be reached by 8 weeks and 13 weeks for those with initial samples of 500 and 1,000 EU/mL, respectively.

Previous reports support our findings. AAV9-Ab IgG titers above 1:50 were detected in 13% (n/N = 115/882) of US infants with SMA and declined with age (1% had titers ≥1:50 at ≥21 months of age).[12] A UK study found that AAV seroprevalence was low in a population of pediatric patients (n = 260).[26] In another US study, neutralizing AAV2-Ab or AAV8-Ab titers ≥1:5 were detected in 36–59% (n/N = 353/751; n/N = 398/751) of neonates, respectively, and reached nadir at 7–11 months of age.[13]

Testing/retesting AAV9-Ab IgG, as needed, is recommended to identify patients who are eligible for onasemnogene abeparvovec treatment as early as possible for optimal outcomes. A case report described one patient with SMA with three *SMN2* copies who had AAV9-Ab IgG ≥ 1:50 at diagnosis at age 9 days. The patient was retested at 2-week intervals until reaching levels below the 1:50 threshold after 6 weeks. The patient was then treated with onasemnogene abeparvovec and subsequently achieved age-appropriate motor milestones and improved motor function scores by age 12 months.[27]

This is the first study to utilize the new quantitative ELISA to calculate the half-life of AAV9-Ab IgG, which indicates AAV9-Ab IgG waning speed. Future studies may utilize this quantitative ELISA to evaluate the time to threshold for each patient, which would impact recommendations for retest timing for newborn patients with initially elevated AAV9-Ab IgG. Understanding AAV9-Ab IgG waning and time to threshold would directly impact all stakeholders, allowing for realistic expectations to be set for onasemnogene abeparvovec treatment initiation. Although comparable US study data have been published, this is the first report from other countries. The worldwide expansion of newborn screening programs for SMA and increase in early SMA diagnosis further validates the need for elucidation of AAV9-Ab seroprevalence and kinetics.[28]

Table 1. Parameter estimates for the final model

Parameter estimation using Equation 1	Model 1 (final model)
Intercept fixed effect a_0 (SE)	6.198 (0.174)
Slope fixed effect b_0 (SE)	−0.119 (0.004)
Intercept random effect var_1 (SD)	0.521 (0.722)
Slope random effect var_2 (SD)	N/A
Random error var_3 (SD)	0.033 (0.180)

N/A, not applicable; SD, standard deviation; SE, standard error.

Limitations included the small sample size of the exploratory kinetics study, with a limited number of measurements for some patients. Samples were collected at the discretion of the physician during scheduled patient visits, which did not follow a standardized time sequence, highlighting the variability and inconsistency of testing in real-world practice. Despite these limitations, the model parameters were estimated with reasonable precision.

In conclusion, the likelihood of elevated AAV9-Ab IgG titers is more likely in newborns versus toddlers, although at a low rate (<15%). Elevations decline with age, allowing for eventual gene therapy treatment. Future considerations may utilize the new quantitative ELISA for screening for the most accurate and quantitative data predictive of the time needed to reach the 1:50 titer threshold. Future studies may investigate seroprevalence, clinical characteristics, and waning of AAV9-Ab IgG according to geographic region and also in correlation to the mother's AAV9-Ab IgG.

METHODS

See supplemental information, including Table S1, for detailed methods. In brief, parts 1 and 2 followed a retrospective cohort study design. Part 1 evaluated AAV9-Ab IgG seroprevalence. Part 2 (exploratory) evaluated AAV9-Ab IgG kinetics, half-life, and time to threshold in neonates with elevated AAV9-Ab IgG titers.

AAV9-Ab IgG testing

Part 1: Seroprevalence study

As described previously, initial AAV9-Ab IgG titers were measured in titrated samples with a standard screening semi-quantitative ELISA.[2] Results were expressed as dilution ratios, with titers as the inverse of the sample dilution based on predefined cutoffs.

Part 2: Exploratory kinetics study

Patients with initial titers $\geq$1:100 and at least two longitudinal samples were identified. Health care providers retested for antibodies that bind AAV9 at varying intervals according to their individual discretion. Both titer and continuous value (EU/mL) measurements were obtained for each AAV9-Ab IgG sample at a single dilution using a newly developed and optimized quantitative ELISA. Results were expressed as EU/mL.

Data analysis

The study was descriptive; no hypothesis was tested. Data were analyzed by R software (43.1, R Project, Boston, MA). Part 1 results were reported as percentages of patients with elevated AAV9-Ab IgG. Part 2 results for model-based predictions according to model parameter estimates were reported as concentrations (EU/mL) over time and mean (95% CI).

DATA AND CODE AVAILABILITY

All data generated or analyzed during this study are included in this published article and are available from the authors under reasonable request.

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AUTHOR CONTRIBUTIONS

This project was conceptualized by R.W.v.O., N.M., C.L.B., and K.W.D. Authors contributed in part to the following: data curation, R.W.v.O., C.L.B., and K.W.D.; formal analysis, R.W.v.O., S.X., C.L.B., K.W.D., and M.S.; investigation, R.W.v.O., N.M., C.L.B., K.W.D., and M.S.; methodology, R.W.v.O., S.X., C.L.B., K.W.D., A.T., C.v.B., and M.S.; supervision, R.W.v.O., N.M., C.L.B., K.W.D., and A.T.; validation, R.W.v.O., C.L.B., and K.W.D.; visualization, R.W.v.O., S.X., K.W.D., and M.S. All authors contributed to the writing and review of the manuscript, with R.W.v.O., N.M., S.X., C.L.B., K.W.D., and M.S. as the main contributors for draft 1. R.W.v.O., N.M., and C.L.B. contributed to funding acquisition, project administration, and resources.

DECLARATION OF INTERESTS

R.W.v.O., C.L.B., K.W.D., M.S., S.X., and N.M. are employees of Novartis AG and own Novartis stock or other equities. H.S. was an employee of Novartis AG at the time of the study. A.T. and C.v.B. are employees of Cerba Research and contracted consultants for Novartis AG. This study was funded by Novartis AG.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtm.2024.101344.

REFERENCES

1. Wang, D., Tai, P.W.L., and Gao, G. (2019). Adeno-associated virus vector as a platform for gene therapy delivery. Nat. Rev. Drug Discov. *18*, 358–378. https://doi.org/10.1038/s41573-019-0012-9.

2. Day, J.W., Finkel, R.S., Mercuri, E., Swoboda, K.J., Menier, M., van Olden, R., Tauscher-Wisniewski, S., and Mendell, J.R. (2021). Adeno-associated virus serotype 9 antibodies in patients screened for treatment with onasemnogene abeparvovec. Mol. Ther. Methods Clin. Dev. *21*, 76–82. https://doi.org/10.1016/j.omtm.2021.02.014.

3. Wirth, B., Karakaya, M., Kye, M.J., and Mendoza-Ferreira, N. (2020). Twenty-five years of spinal muscular atrophy research: from phenotype to genotype to therapy, and what comes next. Annu. Rev. Genom. Hum. Genet. *21*, 231–261. https://doi.org/10.1146/annurev-genom-102319-103602.

4. Prior, T.W., Leach, M.E., and Finanger, E. (2000). In Spinal Muscular Atrophy, GeneReviews®., M.P. Adam, J. Feldman, and G.M. Mirzaa, et al., eds. (Seattle, WA: University of Washington), https://www.ncbi.nlm.nih.gov/books/NBK1352/.

5. Strauss, K.A., Farrar, M.A., Muntoni, F., Saito, K., Mendell, J.R., Servais, L., McMillan, H.J., Finkel, R.S., Swoboda, K.J., Kwon, J.M., et al. (2022). Onasemnogene abeparvovec for presymptomatic infants with three copies of SMN2 at risk for spinal muscular atrophy: the Phase III SPR1NT trial. Nat. Med. *28*, 1390–1397. https://doi.org/10.1038/s41591-022-01867-3.

6. Strauss, K.A., Farrar, M.A., Muntoni, F., Saito, K., Mendell, J.R., Servais, L., McMillan, H.J., Finkel, R.S., Swoboda, K.J., Kwon, J.M., et al. (2022). Onasemnogene abeparvovec for presymptomatic infants with two copies of SMN2 at risk for spinal muscular atrophy type 1: the Phase III SPR1NT trial. Nat. Med. *28*, 1381–1389. https://doi.org/10.1038/s41591-022-01866-4.

7. Baranello, G., Gorni, K., Daigl, M., Kotzeva, A., Evans, R., Hawkins, N., Scott, D.A., Mahajan, A., Muntoni, F., and Servais, L. (2021). Prognostic factors and treatment-effect modifiers in spinal muscular atrophy. Clin. Pharmacol. Ther. *110*, 1435–1454. https://doi.org/10.1002/cpt.2247.

8. Albrecht, M., and Arck, P.C. (2020). Vertically transferred immunity in neonates: mothers, mechanisms and mediators. Front. Immunol. *11*, 555. https://doi.org/10.3389/fimmu.2020.00555.

9. Niewiesk, S. (2014). Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. Front. Immunol. *5*, 446. https://doi.org/10.3389/fimmu.2014.00446.

10. Colella, P., Ronzitti, G., and Mingozzi, F. (2018). Emerging issues in AAV-mediated in vivo gene therapy. Mol. Ther. Methods Clin. Dev. *8*, 87–104. https://doi.org/10.1016/j.omtm.2017.11.007.

11. Gray, S.J., Matagne, V., Bachaboina, L., Yadav, S., Ojeda, S.R., and Samulski, R.J. (2011). Preclinical differences of intravascular AAV9 delivery to neurons and glia: a comparative study of adult mice and nonhuman primates. Mol. Ther. *19*, 1058–1069. https://doi.org/10.1038/mt.2011.72.

12. Day, J.W., Mendell, J.R., Burghes, A.H.M., van Olden, R.W., Adhikary, R.R., and Dilly, K.W. (2023). Adeno-associated virus serotype 9 antibody seroprevalence for patients in the United States with spinal muscular atrophy. Mol. Ther. Methods Clin. Dev. *31*, 101117. https://doi.org/10.1016/j.omtm.2023.101117.

13. Calcedo, R., Morizono, H., Wang, L., McCarter, R., He, J., Jones, D., Batshaw, M.L., and Wilson, J.M. (2011). Adeno-associated virus antibody profiles in newborns, children, and adolescents. Clin. Vaccine Immunol. *18*, 1586–1588. https://doi.org/10.1128/CVI.05107-11.

14. Al-Zaidy, S.A., and Mendell, J.R. (2019). From clinical trials to clinical practice: practical considerations for gene replacement therapy in SMA type 1. Pediatr. Neurol. *100*, 3–11. https://doi.org/10.1016/j.pediatrneurol.2019.06.007.

15. Mendell, J.R., Al-Zaidy, S., Shell, R., Arnold, W.D., Rodino-Klapac, L.R., Prior, T.W., Lowes, L., Alfano, L., Berry, K., Church, K., et al. (2017). Single-dose gene-replacement therapy for spinal muscular atrophy. N. Engl. J. Med. *377*, 1713–1722. https://doi.org/10.1056/NEJMoa1706198.

16. Duque, S.I., Arnold, W.D., Odermatt, P., Li, X., Porensky, P.N., Schmelzer, L., Meyer, K., Kolb, S.J., Schümperli, D., Kaspar, B.K., and Burghes, A.H.M. (2015). A large animal model of spinal muscular atrophy and correction of phenotype. Ann. Neurol. *77*, 399–414. https://doi.org/10.1002/ana.24332.

17. Thomsen, G., Burghes, A.H.M., Hsieh, C., Do, J., Chu, B.T.T., Perry, S., Barkho, B., Kaufmann, P., Sproule, D.M., Feltner, D.E., et al. (2021). Biodistribution of onasemnogene abeparvovec DNA, mRNA and SMN protein in human tissue. Nat. Med. *27*, 1701–1711. https://doi.org/10.1038/s41591-021-01483-7.

18. Day, J.W., Finkel, R.S., Chiriboga, C.A., Connolly, A.M., Crawford, T.O., Darras, B.T., Iannaccone, S.T., Kuntz, N.L., Peña, L.D.M., Shieh, P.B., et al. (2021). Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of SMN2 (STR1VE): an open-label, single-arm, multicentre, phase 3 trial. Lancet Neurol. *20*, 284–293. https://doi.org/10.1016/S1474-4422(21)00001-6.

19. Mendell, J.R., Al-Zaidy, S.A., Lehman, K.J., McColly, M., Lowes, L.P., Alfano, L.N., Reash, N.F., Iammarino, M.A., Church, K.R., Kleyn, A., et al. (2021). Five-year extension results of the phase 1 START trial of onasemnogene abeparvovec in spinal

muscular atrophy. JAMA Neurol. *78*, 834–841. https://doi.org/10.1001/jamaneurol.2021.1272.

20. Mercuri, E., Muntoni, F., Baranello, G., Masson, R., Boespflug-Tanguy, O., Bruno, C., Corti, S., Daron, A., Deconinck, N., Servais, L., et al. (2021). Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy type 1 (STR1VE-EU): an open-label, single-arm, multicentre, phase 3 trial. Lancet Neurol. *20*, 832–841. https://doi.org/10.1016/S1474-4422(21)00251-9.

21. ZOLGENSMA® (onasemnogene abeparvovec-xioi) (2023). Prescribing Information (Novartis Gene Therapies, Inc). https://www.novartis.com/us-en/sites/novartis_us/files/zolgensma.pdf.

22. Aharoni, S., Bistritzer, J., Levine, H., Sagi, L., Fattal-Valevski, A., Ginzberg, M., Noyman, I., Cohen, R., and Nevo, Y. (2023). Adeno-associated virus serotype 9 antibody titers in patients with SMA pre-screening for treatment with onasemnogene abeparvovec – routine care evidence. Gene Ther. *30*, 101–106. https://doi.org/10.1038/s41434-022-00339-0.

23. Kichula, E.A., Proud, C.M., Farrar, M.A., Kwon, J.M., Saito, K., Desguerre, I., and McMillan, H.J. (2021). Expert recommendations and clinical considerations in the use of onasemnogene abeparvovec gene therapy for spinal muscular atrophy. Muscle Nerve *64*, 413–427. https://doi.org/10.1002/mus.27363.

24. Albrecht, M., Pagenkemper, M., Wiessner, C., Spohn, M., Lütgehetmann, M., Jacobsen, H., Gabriel, G., Zazara, D.E., Haertel, C., Hecher, K., et al. (2022). Infant immunity against viral infections is advanced by the placenta-dependent vertical transfer of maternal antibodies. Vaccine *40*, 1563–1571. https://doi.org/10.1016/j.vaccine.2020.12.049.

25. Oguti, B., Ali, A., Andrews, N., Barug, D., Anh Dang, D., Halperin, S.A., Thu Hoang, H.T., Holder, B., Kampmann, B., Kazi, A.M., et al. (2022). The half-life of maternal transplacental antibodies against diphtheria, tetanus, and pertussis in infants: an individual participant data meta-analysis. Vaccine *40*, 450–458. https://doi.org/10.1016/j.vaccine.2021.12.007.

26. Perocheau, D.P., Cunningham, S., Lee, J., Antinao Diaz, J., Waddington, S.N., Gilmour, K., Eaglestone, S., Lisowski, L., Thrasher, A.J., Alexander, I.E., et al. (2019). Age-related seroprevalence of antibodies against AAV-LK03 in a UK population cohort. Hum. Gene Ther. *30*, 79–87. https://doi.org/10.1089/hum.2018.098.

27. Eisenkölbl, A., and Pühringer, M. (2024). Repeated AAV9 titer determination in a presymptomatic SMA patient with three SMN2 gene copies - a case report. J. Neuromuscul. Dis. *11*, 493–498. https://doi.org/10.3233/JND-221659.

28. Therrell, B.L., Padilla, C.D., Borrajo, G.J.C., Khneisser, I., Schielen, P.C.J.I., Knight-Madden, J., Malherbe, H.L., and Kase, M. (2024). Current status of newborn bloodspot screening worldwide 2024: a comprehensive review of recent activities (2020–2023). Int. J. Neonatal Screen. *10*, 38. https://doi.org/10.3390/ijns10020038.