

CORRESPONDENCE



Dystrophin Immunity after Gene Therapy for Duchenne's Muscular Dystrophy

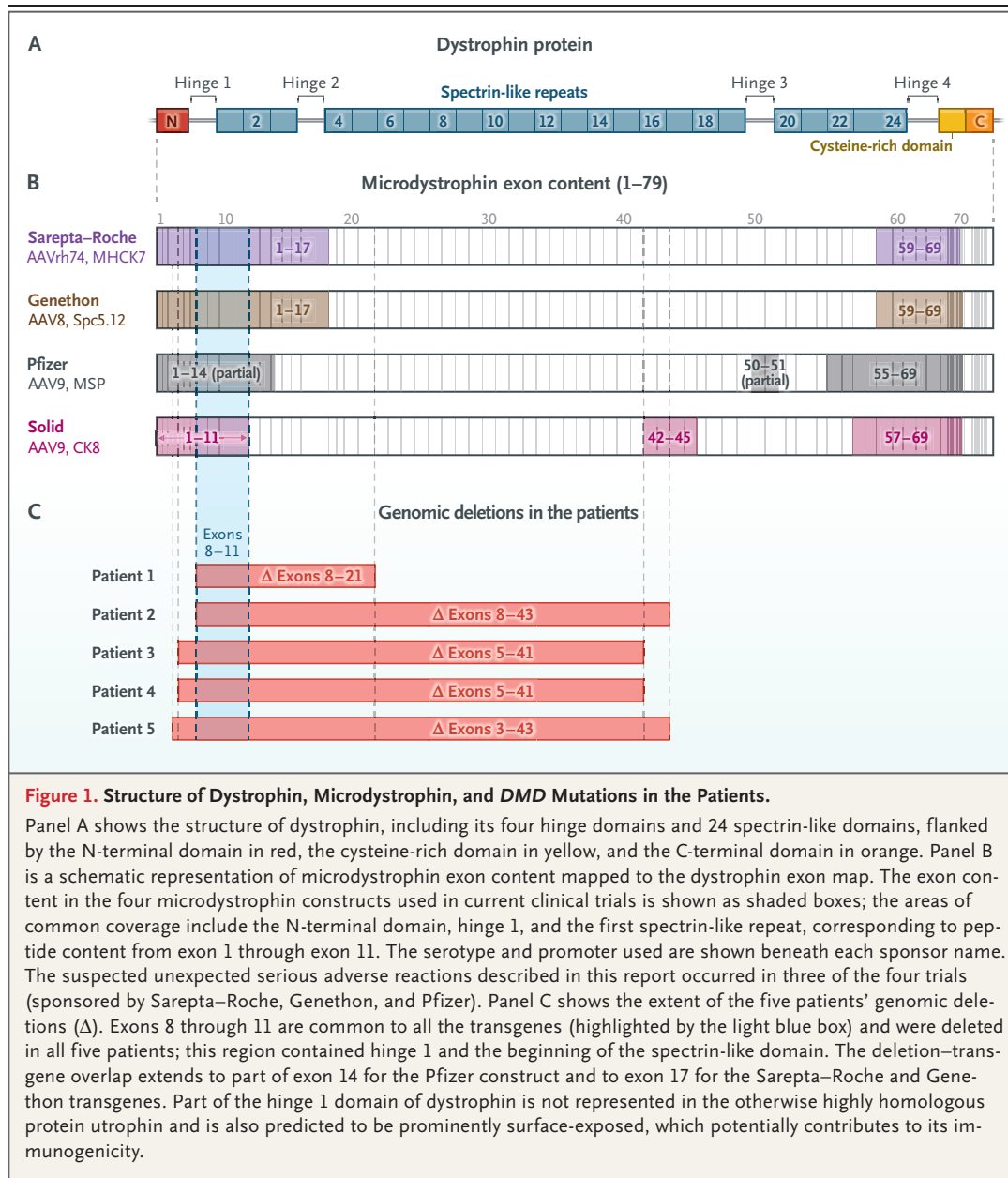
TO THE EDITOR: Duchenne's muscular dystrophy (DMD) is caused by loss-of-function mutations — often deletions — in *DMD* that lead to muscle dystrophin protein deficiency. Adeno-associated virus (AAV) gene therapy to deliver a shortened yet functional microdystrophin transgene that fits within the size constraints of AAV is under investigation in several studies. Mendell and colleagues¹ reported a strong T-cell immune response against epitopes encoded by an AAV-delivered microdystrophin after intramuscular delivery. The method of administration and the ubiquitous cytomegalovirus promoter that was used could have contributed to those findings.^{2,3}

On the basis of recent investigations (performed with the assistance of clinical and other collaborators; see the Supplementary Appendix, available with the full text of this letter at NEJM.org), we now describe five boys with DMD, 7 to 9 years of age, who were enrolled in three sepa-

rate trials evaluating investigational gene therapies (ClinicalTrials.gov numbers, NCT04281485 and NCT04626674, and Eudra-CT number, 2020-002093-27) and in whom strikingly similar suspected unexpected serious adverse reactions (i.e., unexpected adverse reactions that are considered likely to be related to the treatment) occurred. The three AAV products used in the three trials in which these adverse reactions occurred were different microdystrophin transgenes under different muscle-specific promoters, packaged in different AAV serotypes (AAV9, AAV8, and AAVrh74) and delivered intravenously at doses between 1×10^{13} and 2×10^{14} vector genomes per kilogram of body weight. Symptom onset occurred 3 to 6 weeks after administration: all five patients had severe weakness of the proximal and distal limb muscles that led to loss of ambulation, as well as weakness of the bulbar and respiratory muscles, which led to receipt of transient ventilatory support in three of the patients (two with noninvasive ventilation and one with endotracheal intubation). The presence of myositis in the five patients was supported by an increase in the creatine kinase level relative to the baseline level (with myoglobinuria present in two patients), evidence of muscle edema on magnetic resonance imaging (MRI; performed in three patients), and T-cell infiltration on muscle biopsy (performed in two patients). Three patients had signs of myocarditis: elevated troponin levels, decreased ejection fraction, and focal wall-motion abnormalities; cardiac MRI performed in two of the patients showed increased T2-weighted signal intensity. Various immunomodulatory treatments were used among the trials (pulse-dose glucocorticoids, intravenous immune globulin, plasmapheresis, and tacrolimus), and

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symptoms resolved within 3 months. During follow-up, the adverse reactions were judged as resolved in three patients and as resolved with sequelae in two patients.

The timing of these adverse reactions was consistent with transgene expression, and laboratory findings suggested a cytotoxic T-cell immune response against dystrophin. All five patients had similar DMD mutations (Fig. 1): large overlapping deletions in the region from exon 8 to exon 21. When we mapped this deletion range

back to all four of the microdystrophin constructs that were currently being used in clinical trials, we found that epitopes encoded by exons 8 through 11, containing hinge 1 and the beginning of the spectrin-like repeat domains, were present in all the constructs but were missing in the patients and therefore represented nonself epitopes. Consistent with this constellation of findings, data from preliminary enzyme-linked immunospot testing (fully available for two of the patients) and antibody epitope mapping re-

vealed reactivity to peptide pools contained within exons 8 through 11, which suggested the presence of an immune response to this nonself epitope (Fig. 1). These findings resemble those in one of the patients in the study by Mendell et al. (deletion of exons 3 through 17).¹

These suspected unexpected serious adverse reactions highlight the complex and evolving immune biology related to gene-transfer therapies. To address these cases and their safety implications rapidly, the four sponsors currently running gene-therapy trials in DMD agreed to precompetitively collaborate, formed a working group chaired by academic investigators, and engaged experts from diverse backgrounds. Relevant information regarding the adverse reactions was shared, resulting in critical insights into the underlying mechanism and minimizing risks to future trial participants. All four of these current clinical trials have been adjusted so that patients are excluded from participation if they have genomic deletions that substantially overlap transgene sequences similar to those considered to pose a risk. Further epitope and HLA fine-mapping is in progress, with the aim of establishing a hierarchy of immunologic risk for patients with genomic deletions in this region, so that properly adjusted immunomodulation protocols can be developed to allow them to participate in clinical trials.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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Treatment Strategy for Rifampin-Susceptible Tuberculosis

TO THE EDITOR: With regard to the Two-Month Regimens Using Novel Combinations to Augment Treatment Effectiveness for Drug-Sensitive Tuberculosis (TRUNCATE-TB) trial conducted by Paton et al. (March 9 issue),¹ we disagree that there were “no evident safety concerns.” During the trial, the rifampin dose used in two trial groups was decreased from 35 to 20 mg per kilogram of body weight. According to the Supplementary Appendix of their article (available at NEJM.org), this dose change was made in response to a fatal case of drug-induced liver injury (a footnote in Table 3 of their article notes the fatality but provides no details). It is notable that the incidence of grade 3 or 4 hepatobiliary events decreased from 6.8% to 2.1% after the rifampin

dose was reduced (Table S15 in the Supplementary Appendix of their article).

A lack of significant differences in comparisons with wide confidence intervals should not be interpreted as equivalence.² For example, two pretomanid-based regimens were judged to have a safety profile similar to that of the standard regimen in a phase 2 trial,³ only to have a follow-up trial halted after three deaths from drug-induced liver injury.⁴ Given the substantial interest in high-dose rifampin, the adverse events that occurred in the TRUNCATE-TB trial should be highlighted and not relegated to the Supplementary Appendix. Finally, a primary goal of the tuberculosis research community should be to find regimens with less toxicity than the