

Figure 1 (facing page). Trial Design for Cohort 2, DENV-3 RNA Viral Loads, and Mosnodenvir Plasma Concentrations.

Panel A shows the trial design for Cohort 2. Twice-daily loading doses (LDs) were administered for 2 days, starting on day -2, and the maintenance doses (MDs) were administered for 21 days, starting on day 1. The participants also received a subcutaneous injection of the recombinant dengue virus serotype 3 (DENV-3) strain rDEN3Δ30 on day 1. Panel B shows the DENV-3 viral load, assessed as the log₁₀ area under the concentration–time curve (AUC) from day 1 through day 29. The circles represent the values for the individual participants, and the circles on the dashed censoring line (target not detected) represent participants with undetectable DENV-3 RNA through day 29. In the box plots, the bottom of the box indicates the 25th percentile, the dark gray line the 50th percentile, and the top of the box the 75th percentile. Panels C and D show the mosnodenvir plasma concentrations over time (through day 85 in Panel C and through day 29 in Panel D) in the pharmacokinetic analysis. Mosnodenvir plasma concentrations below the lower limit of quantification (5.00 ng per milliliter [dashed horizontal line]) are not shown. The dashed vertical line indicates the start of the maintenance-dose phase and the inoculation with rDEN3Δ30 on day 1.

These data improve our understanding of dose-dependent antiviral activity of mosnodenvir in a human infection model and expand the evidence to weekly administration. The reduction in the loading period from 5 to 2 days and the transition from once-daily to once-weekly dosing are improved pharmacokinetic features that can facilitate use if this drug is developed further.

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Integration-Associated Paraspinal Neoplasm after AAV Gene Therapy

TO THE EDITOR: Krabbe disease is a rare autosomal recessive disorder caused by mutations in the *GALC* gene. Deficiency in galactosylceramidase (GALC) enzyme leads to the accumulation of the lipid psychosine, which causes widespread demyelination and neurodegeneration in both the central and peripheral nervous systems. Adeno-associated virus (AAV)-mediated gene therapy has emerged as a promising treatment strategy

for Krabbe disease. However, the safety profile of AAV-based therapy warrants careful consideration. Here, we report a case of paraspinal neoplasm in a 3-year-old boy with infantile-onset Krabbe disease (with biallelic *GALC* variants p.Arg220Ter and p.Tyr319Cys) after AAV gene therapy.

The patient had undergone two sequential transplantations of umbilical-cord blood, at 5 and 9 months of age, each after myeloablative,

alkylator-based conditioning. On day 29 after the second transplantation, he received gene therapy with intravenous FBX-101 at a dose of 3×10^{13} vector genomes per kilogram of body weight as part of the RESKUE trial.¹ FBX-101 is an AAVrh.10-capsid vector with AAV2 inverted terminal repeats that carries *GALC* under the control of a CAG promoter (consisting of a cytomegalovirus enhancer coupled with a chicken β -actin promoter). After treatment with FBX-101, the patient continued to have poor immune reconstitution. He received a donor lymphocyte infusion and underwent immunosuppression for management of graft-versus-host disease (Fig. 1A).

Surveillance magnetic resonance imaging 26 months later showed an incidental L2–L3 paraspinal mass. Biopsy showed a small, round, blue-cell neoplasm with a focal, epithelioid, nested appearance. The biopsy specimen was positive for CD56 and S100 and strongly positive for *GLI1* as assessed by in situ hybridization, with a Ki-67 index below 10%; the diagnosis was a *GLI1*-altered neoplasm.² The level of *GLI1* messenger RNA (mRNA) ranked in the 100th percentile among the 5143 samples from our pan-cancer cohort (Fig. 1B), with concordant *PTCH1* overexpression, and targeted sequencing showed a stable diploid genome without oncogenic mutations or fusions.

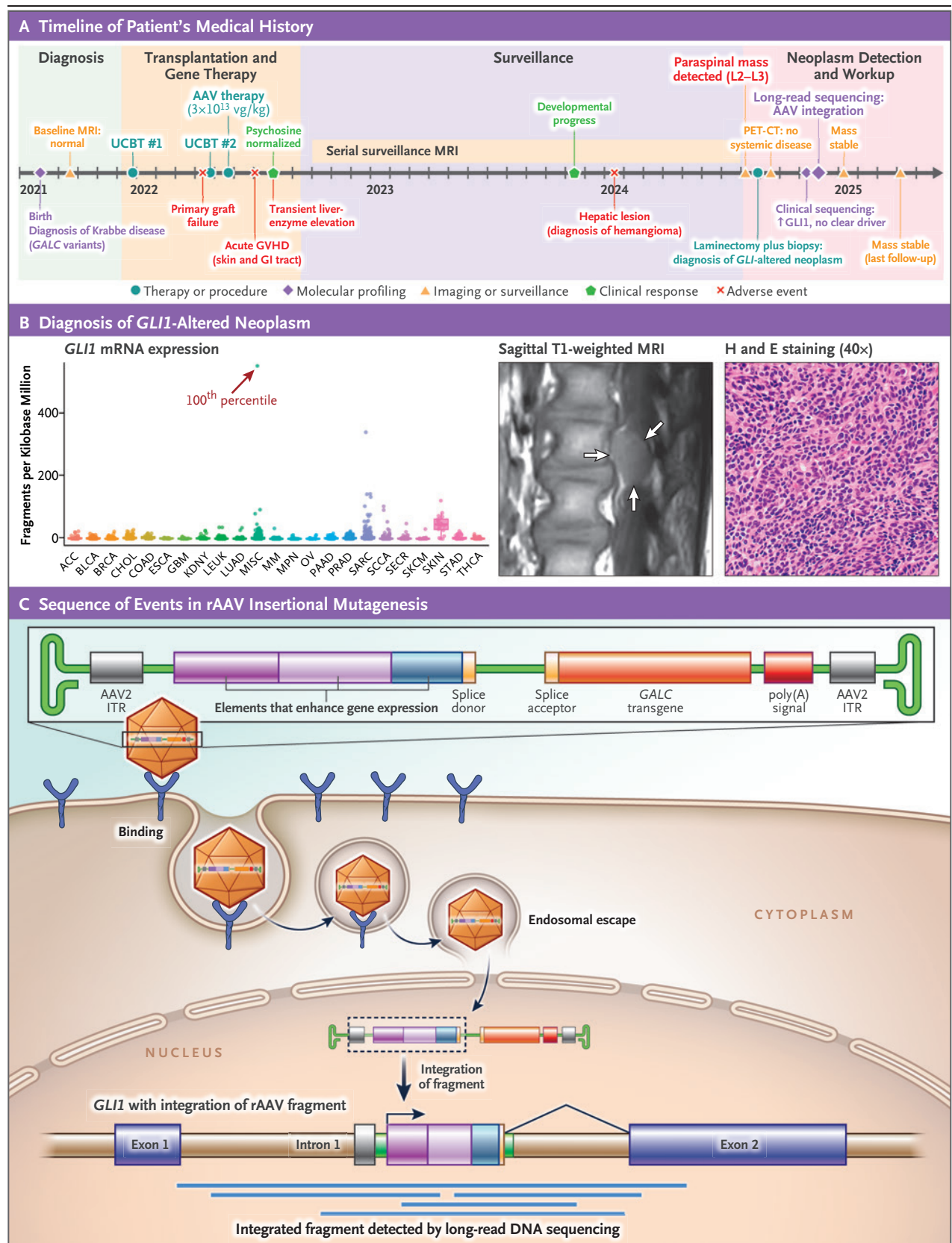
However, long-read sequencing with a nanopore sequencer (Oxford Nanopore) identified a clonal 1038-bp insertion (present in 44% of the reads) in *GLI1* intron 1; this insertion was flanked by 115-bp target-site duplications (Fig. 1C). The integrant was a subgenomic vector fragment that comprised a partial AAV2 inverted terminal repeat, the cytomegalovirus enhancer, the chicken β -actin promoter, exon 1, and a splice donor and lacked the *GALC* transgene and polyadenylation signal. The splice donor generated a chimeric transcript with chicken β -actin exon 1 joined to *GLI1*, which drove full-length *GLI1* expression, and allele-specific hypomethylation that was confined to the integrant allele established the integration as the proximate cause of *GLI1* activation (see the Supplementary Appendix, available with the full text of this letter at NEJM.org).

AAV vectors are predominantly nonintegrating and have an established safety record across thousands of recipients³; nonetheless, we report this case of AAV integration-driven oncogenesis shortly after the report by Ahrens-Nicklas et al.⁴

of a *PLAG1*-activated tumor. In the case described by Ahrens-Nicklas et al. and in the present case, the integrants were truncated, rearranged subgenomic structures that retained a CAG promoter–enhancer and a splice donor and activated a developmental transcription factor. Unlike the mucopolysaccharidosis type I integrant described previously, which retained fragments of the *IDUA* transgene, the integrant in this case had no transgene or polyadenylation sequence, a finding that warrants concern that a snap-back

Figure 1 (facing page). Gene Therapy–Associated Insertional Mutagenesis.

Panel A shows the clinical timeline from the diagnosis of Krabbe disease with *GALC* variants through two umbilical-cord-blood transplantations (UCBT 1 and 2), adeno-associated virus (AAV)–based gene therapy with FBX-101 (delivered at a dose of 3×10^{13} vector genomes [vg] per kilogram of body weight), and detection of the paraspinal neoplasm. Clinical sequencing was performed with the use of the MiOncoSeq assay at the Michigan Center for Translational Pathology. GI denotes gastrointestinal, GVHD graft-versus-host disease, and PET-CT positron-emission tomography–computed tomography. Panel B shows the level of *GLI1* expression (red arrow) in the tumor sample as compared with that in samples from a pan-cancer cohort, as well as sagittal T1-weighted magnetic resonance imaging (MRI) of the L2–L3 mass (arrows) and hematoxylin and eosin (H and E) staining of the tumor sample. The following cancers were represented in the pan-cancer cohort: adrenocortical carcinoma (ACC), bladder cancer (BLCA), breast cancer (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), kidney cancers (KDNY), leukemia (LEUK), lung adenocarcinoma (LUAD), miscellaneous or rare cancers (MISC), multiple myeloma (MM), myeloproliferative neoplasms (MPN), ovarian cancer (OV), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), sarcoma (SARC), squamous-cell carcinoma (SCCA), secretory cancers (SECR), cutaneous melanoma (SKCM), other skin cancers (SKIN), stomach cancer (STAD), and thyroid cancer (THCA). mRNA denotes messenger RNA. Panel C shows a subgenomic vector fragment (comprising a cytomegalovirus enhancer [dark purple], a chicken β -actin promoter [light purple], the first exon of the chicken β -actin gene [blue], and a splice donor and lacking the *GALC* transgene and the polyadenylation signal) integrated into the *GLI1* intron 1 and spliced to exon 2. The integrant drove an increase in *GLI1* expression and was detected as a 1038-bp insertion by long-read sequencing, with the insertion independently confirmed by polymerase-chain-reaction assay (see the Supplementary Appendix). Horizontal blue lines indicate individual long reads from nanopore sequencing. ITR denotes inverted terminal repeat, and rAAV recombinant AAV.



or promoter-only species that arose during vector manufacturing was the integrating substrate.⁵ A potential contribution of the context of transplantation in this case, which involved therapy with toxic effects on DNA, as well as immunosuppression, is discussed in the Supplementary Appendix.

The integration event was promptly reported to the treating physicians and the Food and Drug Administration. At the time of the last follow-up, the patient remained asymptomatic. These findings emphasize the need for cassette designs without splice donors, as well as attention to vector purity and extended genomic surveillance after AAV gene therapy.

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Extended DAPT for Multivessel Coronary Artery Disease

TO THE EDITOR: Tian and colleagues (July 16 issue)¹ assess whether patients with multivessel coronary artery disease who had been event-free while receiving dual antiplatelet therapy (DAPT) for 12 months after percutaneous coronary intervention (PCI) may benefit from extending DAPT. In the Dual Antiplatelet Therapy in Patients with Coronary Multivessel Disease (DAPT-MVD) trial, 8250 patients were randomly assigned to continue DAPT (clopidogrel plus aspirin) or receive aspirin monotherapy for 12 months. Extending DAPT led to a lower risk of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (the composite primary efficacy end point) without increasing the risk of major bleeding.

The percentage of patients with nonfatal myocardial infarction was lower with DAPT than

with aspirin monotherapy, with the largest between-group difference involving myocardial infarction attributable to nontarget vessels during the first 24 months. This finding suggests that the protective effect of DAPT includes segments other than the stented target segments. In addition to the mechanisms discussed by the authors, cardiovascular benefit may reflect a balance among plaque destabilization, thrombosis, and healing. Many plaque ruptures or erosions remain clinically silent.² Whether destabilization leads to myocardial infarction, stroke, or plaque healing may depend on prothrombotic, antithrombotic, and fibrinolytic activity. Occlusive thrombosis may therefore require a “double hit” of plaque destabilization in the presence of sufficient local and systemic thrombogenic stimuli or impaired healing.³⁻⁵ In this context, prolonged