

October 29, 2025

Emily Freilich, MD, Director
Center for Drug Evaluation and Research
Food and Drug Administration
Division of Neurology I
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: COMPLETE RESPONSE TO CLINICAL HOLD – IND 167326

In light of the additional information provided below, we are writing to respectfully request that the Agency lift the partial clinical hold placed on the clinical protocol entitled “An open-label, single ascending dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of intrathecally administered PrP siRNA in healthy adult patients at genetic risk for prion disease.”

This protocol concerns 2439-exNA, a prion protein (PrP)-lowering siRNA therapeutic developed for prion disease. As you know, prion disease is a rapidly and universally fatal neurodegenerative disease for which there is currently no standard of care. The drug showed a clean toxicology profile in GLP toxicology studies designed with Agency input and conducted in rats and dogs. The drug has been GMP manufactured, and the IND was filed in February 2025 with two clinical protocols. The first outlined a study in symptomatic prion disease patients, who typically die less six months from their first symptom, after experiencing a rapid neurological decline that culminating in akinetic mutism and death. The second outlined a study in asymptomatic carriers of pathogenic variants in the prion protein gene (*PRNP*) known to be likely to develop fatal prion disease (>90% lifetime risk for specific mutations). The symptomatic protocol was cleared in March 2025, while the presymptomatic protocol was placed on partial clinical hold in April 2025.

The stated rationale for the hold was that 2439-exNA is non-targeting (lacks pharmacological activity) in rats and dogs and therefore a toxicology study in the cynomolgus monkey would be required to address on-target risks of PrP-lowering. We fully agree that an understanding of the potential adverse consequences of on-target activity is important in supporting dosing and clinical monitoring of presymptomatic patients; however, as discussed below, the nature of this hazard has already been adequately characterized. Notably, a toxicology study in the NHP will not materially affect the risk assessment for on-target activity associated with 2439-exNA, and requiring such a study, in this context, is not consistent with the recent statements of Agency policy intending to limit the use of NHPs^{1,2}.

Many lines of evidence indicate that reducing PrP stands to be an effective way to treat prion disease. Prion disease is a gain-of-function disease in which a single protein, PrP, undergoes a conformational corruption into a self-templating conformer termed a “prion.” Prions then spread throughout the brain,

killing neurons. It is well established that either genetic or pharmacologic depletion of PrP dose-dependently protects against disease, as neither prion propagation nor neurotoxicity can occur in the absence of PrP. As the sole essential substrate for pathogen formation and root genetic cause of all cases of prion disease, PrP represents the clearest drug target in prion disease and our best hope for helping patients in our lifetimes.

Many lines of evidence likewise indicate that PrP reduction, up to and including complete ablation of PrP, is well tolerated. PrP knockout has been characterized in multiple mouse lines as well as in goats and cows. These models unanimously indicate that long-term reduction or lack of PrP is compatible with healthy life. Human genetic data further indicate that *PRNP* loss-of-function alleles are found in healthy older individuals, and indeed, occur in the general population at the frequency expected based on DNA mutation rates, indicating that such variants are not being removed from the human population by premature mortality or reduced fecundity. Of the PrP knockout phenotypes reported in the literature, one – an age-dependent demyelination of peripheral nerves – has been convincingly replicated by histology across animal models. However, any functional or behavioral impact of this histological phenotype is modest when seen and is not observed across all models. Further, the histological defect is seen only in complete knockouts, where it emerges only late in life, and is not seen in heterozygotes. Our protocol includes a full neurological exam at every visit via which, in human patients, any clinically meaningful peripheral neuropathy, such as change in reflexes or sensation, could be monitored. Dosing could be paused or discontinued, or dose levels reduced, if warranted.

In FDA's partial clinical hold letter, the Agency cited one review from the literature, Wulf 2017³ as the basis of their concern about potential deleterious effects of PrP reduction. The senior author of Wulf 2017 and the prion field's leading expert on PrP knockout, Dr. Adriano Aguzzi, has provided a letter (attached) in support of testing of proceeding with the 2439-exNA presymptomatic protocol based on the existing toxicology package. As he outlines in his letter, he does not believe that either any known risk of PrP reduction, nor the marginal knowledge that could be gained from an NHP study justify delaying a treatment trial in asymptomatic individuals.

The decline that PRNP mutation carriers experience once symptoms begin is precipitous and irrecoverable, and all available preclinical data strongly, across PrP-lowering modalities, strongly support the following hypothesis. 1) Preventive dosing offers a quantitatively greater degree of survival benefit than post-symptomatic treatment. 2) Preventive dosing offers a fundamentally different kind of benefit, as it extends healthy life rather than prolonging the symptomatic phase of disease. For these reasons, a preventive clinical study poses scientifically distinct questions from a symptomatic study; its value is not pre-empted by a symptomatic study. Further, its outcomes are not predicted by a symptomatic study, as the asymptomatic population offers the prospect of distinct learnings. The two most important endpoints from a first-in-human study of a PrP-lowering agent – safety and target engagement – are both more difficult to interpret in the symptomatic population; safety because of the complex and rapidly declining state of the patients, and target engagement because CSF levels of PrP may change as a function of

disease process. By contrast, asymptomatic PRNP mutation carriers are healthy and have stable intra-individual CSF PrP baseline levels over up to six years. These stable baselines provide our clearest opportunity to discern both the safety and pharmacodynamic properties of 2439-exNA.

The above scientific and disease-specific risk-benefit considerations form the basis of our request for reconsideration of our protocol and lifting of the clinical hold. Separately, it is worth noting that as academic investigators developing 2439-exNA without a pharmaceutical partner, we do not have the resources to perform a GLP toxicology study in NHP, nor do we have the prospect of raising these resources. The preclinical and IND-enabling studies performed to date were funded by NIH mechanisms (R61NS119717; U01NS132994), and discussions with NIH staff have made clear that these mechanisms cannot support the NHP study described in the preclinical hold letter. Therefore, the NHP study requirement functionally amounts to an indefinite hold.

In summary, we believe that the large existing body on PrP native function, the unique risk-benefit profile of prion disease, and the existing toxicology package for 2439-exNA combine to form a strong scientific rationale for proceeding with the protocol entitled, “An open-label, single ascending dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of intrathecally administered PrP siRNA in healthy adult patients at genetic risk for prion disease.”

Thank you for your consideration. We are available at your convenience to discuss this matter further, as it is our top priority.

Sincerely,

[REDACTED]

Sonia Vallabh

Director, Prion Therapeutic Science, Broad Institute of MIT and Harvard
Assistant Professor, Department of Neurology, Harvard Medical School

[REDACTED]

Eric Vallabh Minikel

Director, Prion Therapeutic Science, Broad Institute of MIT and Harvard
Assistant Professor, Department of Neurology, Harvard Medical School

[REDACTED]

cell: [REDACTED]

[REDACTED]

Cambridge, MA 02142

References

1. U.S. Food and Drug Administration. FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs [Internet]. 2025. Available from: <https://www.fda.gov/news-events/press-announcements/fda-announces-plan-phase-out-animal-testing-requirement-monoclonal-antibodies-and-other-drugs>
2. U.S. Food and Drug Administration. Roadmap to Reducing Animal Testing in Preclinical Safety Studies [Internet]. 2025. Available from: <https://www.fda.gov/media/186092/download?attachment>
3. Wulf MA, Signore A, Aguzzi A. The biological function of the cellular prion protein: an update. BMC Biol. 2017 02;15(1):34. PMID: PMC5412054



University of Zurich, Institute of Neuropathology
CH-8091 Zurich

Emily Freilich, MD

Director

Division of Neurology

Office of Neuroscience

Center for Drug Evaluation and Research

U.S. Food and Drug Administration

Prof. Dr. Adriano Aguzzi

Institute Director

Phone

September 8, 2025

Re: Support for clinical evaluation of the PrP-lowering siRNA 2439-exNA in asymptomatic PRNP variant carriers

Dear Dr. Freilich,

I am a Professor of Neuropathology specializing in prion diseases for over 30 years. I have authored more than 200 peer-reviewed papers on prions, collectively cited over 75,000 times; my H-index is 142 (i.e., at least 142 publications have each been cited ≥ 142 times).

I am writing to express my support for the clinical testing of the PrP-lowering siRNA therapeutic 2439-exNA in asymptomatic individuals at genetic risk for prion disease, based on the currently available nonclinical safety data. More broadly, I support the therapeutic hypothesis of lowering prion protein (PrP) in prion disease for both symptomatic patients and asymptomatic carriers of highly penetrant PRNP pathogenic variants. Prion disease—most commonly manifesting as Creutzfeldt-Jakob disease (CJD)—is rapidly progressive and uniformly fatal. The interval from first symptoms to death may be only months, leaving scant opportunity for meaningful post-symptomatic intervention. Preventive paradigms must therefore be contemplated for those at established genetic risk.

A protocol entitled “*An open-label, single ascending dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of intrathecally administered PrP siRNA in healthy adult patients at genetic risk for prion disease*” was recently submitted to enable dosing of 2439-exNA into asymptomatic PRNP mutation carriers. The protocol was placed on clinical hold for “*insufficient information to assess risks to human subjects*,” with the Agency noting that while the nonclinical data are adequate to support a study in symptomatic patients, they were viewed as not adequate for presymptomatic carriers. The rationale cited that PrP-siRNA was not pharmacologically active in rat or dog toxicity studies (precluding on-target assessment), that it is active in cynomolgus monkey, and that reducing PrP might adversely affect neurobehavioral function (Wulf et al., 2017). The Agency requested a GLP-compliant intrathecal cynomolgus monkey study of duration similar to or longer than proposed human exposure.

As the senior author of Wulf et al., 2017 and a member of the prion research community with long-standing experience on native PrP function, I respectfully disagree with the interpretation that the available evidence warrants blocking a presymptomatic study or that a new 9-month cynomolgus study is likely to materially change the on-target risk assessment. It is my professional opinion that:

1. The native-function literature does not provide adequate grounds to preclude a carefully monitored first-in-human study in asymptomatic carriers; and
2. While a 9-month cynomolgus study may add knowledge, the gain in knowledge may not be commensurate with the delay it would impose on patient access.

Scientific basis. As detailed in the IND and in Wulf et al., 2017, three decades of work across multiple species and PrP-knockout models converge on a consistent finding: **complete, lifelong germline ablation of PrP (from the zygote stage onward) yields a late-onset, peripheral demyelinating polyneuropathy (PMP)** with measurable histopathology and **modest functional impact** (e.g., slower locomotion in mice). This germline-null state simultaneously maximizes depth (~100% loss) and duration (lifelong) of target suppression—a worst-case perturbation relative to any feasible pharmacologic intervention, including 2439-exNA, which produces **partial and reversible** PrP lowering over **finite** periods and with **heterogeneous** tissue exposure.

Several observations collectively argue that the on-target neuropathy risk under clinical conditions is low and manageable:

- **Threshold-and-time logic.** In mice, PMP emerges only after ~60 weeks of **uninterrupted, complete** PrP absence. By contrast, non-human primate (NHP) toxicology achieves only **partial** lowering for **<60 weeks**; it thus fails at least one necessary condition (depth and/or duration) for PMP expression observed in the null.
- Importantly, heterozygously ablated mice (one genomic allele is missing while the other is still active) do **not** develop PMP, implying substantial functional reserve at ~50% gene dosage, which models the therapeutic situation more plausibly than a full biallelic ablation.
- **Histology vs. function.** Even under the extreme null, pathology has limited behavioral consequences. This decoupling suggests that adult-onset, **partial** lowering is less likely to produce clinically overt dysfunction, particularly in the short-to-intermediate term.
- **Human genetic context.** Available human genetic data indicate that **heterozygous loss-of-function** PRNP alleles can occur without a clear adverse phenotype signal at the population level, consistent with tolerance of partial PrP reduction.
- **Species/time scaling.** A 60-week latency in mice maps to late adulthood on a species-relative timescale; shorter-duration, partial suppression in NHPs

provides limited opportunity for a cumulative demyelinating process to manifest.

- **On-target vs. platform risk.** The knockout literature informs **on-target** risk from PrP reduction. Any **off-target** or platform-specific class effects are addressed by standard toxicology packages and controls in non-target species; they do not undermine the on-target safety inference.

To be clear, **the neuropathy signal is real and documented**. In germline-null models, peripheral myelin changes and slower movement are observed. At the same time, those data delineate an **upper bound** of risk under conditions far more extreme than any clinical use case of 2439-exNA. Given partial, reversible suppression and finite exposure, **clinically overt neuropathic symptoms—if they occur—are likely to be mild**. Because of the regeneration potential of Schwann cells, they would be amenable to management through dose interruption and monitoring (e.g. by simple nerve conductance velocity testing) and could conceivably even be reversible.

Risk management in the clinic. The proposed protocols already incorporate prudent safeguards—dose escalation, careful PK/PD characterization, and stopping rules. Additional targeted monitoring can directly test the low-risk prediction: periodic **nerve conduction studies**, **serum neurofilament light (NfL)** as a sensitive axonal/myelin injury biomarker, and **clinical neurologic examinations** focused on large-fiber function. Because 2439-exNA is an oligonucleotide, its pharmacology is **reversible**; dosing can be paused at the first signal of concern, allowing drug levels and pharmacodynamic effect to **wash out within weeks**.

Benefit–risk in a lethal disease. CJD and related genetic prion diseases are **invariably fatal** and **horrendous** neurodegenerative conditions with a very narrow therapeutic window once symptomatic. As in oncology—where nontrivial toxicities are accepted to prevent or delay lethal disease—the ethically appropriate comparison is not between “no risk” and “any risk,” but between a **modest, monitorable risk** of peripheral nerve effects and the **certainty of death** without preventive options. On this calculus, presymptomatic evaluation of 2439-exNA is justified, provided robust safeguards are in place.

Regulatory path. In light of the above, I respectfully support the request that the Agency **reconsider the presymptomatic hold** and allow initiation of the proposed study in asymptomatic PRNP variant carriers **without requiring a new GLP intrathecal cynomolgus study** prior to first-in-human dosing. This approach is also consistent with the Agency’s stated aim to minimize **unnecessary non-human primate studies** when human safety can be assured by other means. The totality of evidence—(1) the knockout and human genetic data defining the upper bound of on-target risk, and (2) the specific attributes of 2439-exNA (partial, reversible modulation) together with vigilant clinical monitoring—supports advancing 2439-exNA into both symptomatic patients and at-risk carriers **expeditiously**.



I would be pleased to discuss any aspect of this assessment with the review team.
Thank you for your consideration.

Sincerely,



Adriano Aguzzi, MD
Professor of Neuropathology and Director
Institute of Neuropathology, University Hospital Zurich