SAFETY BULLETIN

Novel adverse event of fever following PS 2'MOE ASO administration in a patient with KAND .

PURPOSE: to provide information concerning a new adverse event likely related to PS 2'MOE ASO and provide guidance to track this potential adverse event while monitoring safety of patients.

EXECUTIVE SUMMARY

A patient with KIF1A-associated neurological disorder (KAND) treated intrathecally with a phosphorothioate 2'-O-methoxyethyl (2'-MOE) antisense oligonucleotide for three years experienced transient, post-dose fevers beginning after the sixth dose, an event not previously observed with intrathecal ASOs. Extensive evaluation, including cerebrospinal fluid (CSF) analyses for cytokines, complement split products, and Factor H, revealed no evidence of alternative pathway activation. The etiology of this event remains uncertain. While inflammatory markers are elevated, it is unclear whether this is a manifestation of disease-associated chronic systemic inflammation or potentially ASO-related. Though some complement split products are elevated, the pattern is not consistent with the known mechanism by which some PS ASOs activate the alternate pathway. Alternatively, the ASO-associated fevers may reflect a drug-disease interaction as about 50% of KIF1A patients are known to have thermoregulatory issues. We believe that this may be a novel ASO-KIF1A disease interaction. It is important to note that this patient continues to show significant clinical benefits. A second patient treated with the same ASO has not experienced similar events, which supports continuation of dosing and initiating dosing in new patients.

To ensure patient safety, a comprehensive monitoring plan that has been endorsed by the Data Safety Monitoring Board (DSMB) and the U.S. Food and Drug Administration (FDA) has been implemented and includes

- Measuring CSF opening pressure prior to each dose
- Adjusting the dose of ASO if deemed necessary
- Enhancing clinical monitoring by continuing CSF cytokine and complement activation analyses
- Informing new KAND patients about this new adverse event

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SUMMARY OF CLINCIAL OBSERVATION AND ASSESSMENTS PURSUED TO UNDERSTAND ADVERSE EVENT

We have been treating a patient with Kinesin family member 1A (KIF1A) associated neurological disorder (KAND). *KIF1A*-related disorder (KAND) is a group of progressive genetic disorders caused by genetic variants in *KIF1A* due to autosomal dominant as well as recessive inheritance and associated with both a loss of function as well as gain of function molecular phenotype (Boyle et al., 2021; Pennings et al., 2020). Symptoms can range from mild to severe and can be life-threatening. The most common signs and symptoms are seizures, spasticity, cerebellar and cerebral atrophy, peripheral neuropathy, optic nerve atrophy leading to visual impairment, and intellectual disability. KAND is a progressive disease that is lethal.

Clinical presentation of the patient

The current patient has been treated for almost 3 years with an allele-selective ASO of the PS 2'MOE chemistry, has experienced repeated fevers and vomiting starting with dose 6 (at ~10 months on treatment); the patient has now received up to 14 doses. From dose 6 to dose 10 the fevers started faster and lasted longer, until pretreatment with dexamethasone was introduced at dose 10. This has alleviated some of the symptoms and has led to shorter bouts of fever resolving within 24 hours. Since starting treatment, the patient has shown important clinical improvements, such as fewer and shorter epileptic episodes, significant reduction in neuropathic pain, reduction in tremors, improved communication and awareness, and increased stamina and ability to partake in school and family activities (Ziegler et al., 2024). These changes have allowed her to put on her own shoes, feed herself, go to summer camp, move around her city in her wheelchair, and enjoy more quality time with family and friends. Normally, children with KAND lose skills over time, but in her case, the disease has not only stabilized—it has improved in several areas.

Etiology of the fevers and information on PS-ASO

It has been shown that 46% of patients with KAND experience difficulties with temperature regulation, including sporadic fevers unrelated to illness (Boyle et al., 2021). Although the fevers exhibited by this patient were not sporadic, and occurred consistently after each ASO injection, an underlying thermoregulation impairment associated with KAND may have exacerbated the fevers seen in this patient.

To understand the etiology of the fevers, CSF samples were analyzed for cytokines as well as complement split products and Factor H levels. It has been shown that phosphorothioate (PS) ASOs when administered systemically can activate the complement system, specifically through the alternative pathway, with no evidence of activation of the classical or lectin pathways (Shen et al., 2014). In non-human primates, administration of 2'-O-methoxyethyl (2'-MOE) PS ASOs triggered transient alternative pathway activation, whereas in humans, no activation was observed even at exposures comparable to those producing effects in monkeys. Mechanistic studies run by Ionis Pharmaceuticals have suggested that these effects are secondary to interactions with complement Factor H, a key regulator of the alternative pathway. Under normal conditions, Factor H works with Factor I to inactivate the alternative pathway C3 convertase, which is constitutively active at low levels. When Factor H function is impaired, C3 convertase becomes unregulated, driving alternative pathway activation. Evidence supporting this mechanism comes from several key observations. The addition of human Factor H to monkey serum can inhibit alternative pathway activation, confirming the central role of Factor H in regulating this response. Species differences in Factor H may also contribute to the observed effects, including potential variations in protein concentration across species. Furthermore, cynomolgus monkeys carry a sequence variant in Factor H that reduces protein function, analogous to a known human polymorphism associated with impaired complement regulation. In vitro assays and review of clinical safety data from 767 human subjects confirmed that PS ASOs have the potential to activate complement, but the magnitude of this effect depends on species-specific complement regulation. Overall, the greater sensitivity of monkeys to PS ASO-mediated complement activation is explained by weaker Factor H function, underscoring the importance of understanding complement biology when translating preclinical findings to humans (Shen et al., 2014).

The results of the longitudinal analyses of the CSF samples from this patient are difficult to interpret as there are no published references ranges for these analytes in pediatric population, and the results are magnitudes of levels lower than what is observed in adult patients. Nonetheless, the CSF analyses demonstrate elevations in C3a and C4a. Importantly, there is no evidence of alternative pathway activation, as neither C5a nor Bb were elevated, and there was no increase in terminal complement complex (MAC, sC5b9). The observed rise in C4a would implicate classical pathway activity, a mechanism that has

not previously been associated with ASO therapy. Therefore to date, the etiology of the post-dose fevers remains undetermined.

A comprehensive monitoring plan has been established and approved by the Data Safety Monitoring Board (DSMB) and the U.S. Food and Drug Administration (FDA). Key components include:

- CSF opening pressure assessment prior to each dosing occasion
- Continued monitoring of CSF cytokines and complement factors
- Serial CSF analyses in all patients treated with the investigational ASO
- MRI if deemed necessary by the investigator

A second patient has been treated with multiple doses of this ASO without evidence of post-dose clinical symptoms, supporting the risk–benefit assessment to proceed with treatment in the current and additional patients. Informed consents will be updated to reflect the occurrence of the fevers seen in the first patient.

KEY TAKEAWAYS

While the precise mechanism of the fevers remains unresolved, the events are mild, transient, and manageable. The patient has experienced substantial clinical benefits, including improvements across multiple neurologic and functional domains that would be lost if treatment were discontinued. Continued dosing with enhanced monitoring therefore remains the most appropriate course to safeguard both patient safety and therapeutic benefit.

References

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