N-LOREM SAFETY UPDATE

PS 2'MOE ASO ASSOCIATED NORMAL PRESSURE HYDROCEPHALUS (NPH)

PURPOSE: To provide information concerning possible PS 2′MOE ASO associated NPH and to provide guidance to minimize the incidence and severity of this potential adverse event.

DEFINITION: Normal pressure hydrocephalus (NPH) is defined as "radiographic evidence of ventriculomegaly with correlating symptoms of the syndrome"[1]

EXECUTIVE SUMMARY

Normal pressure hydrocephalus (NPH) has recently been observed to be a rare adverse event associated with long term intrathecal (IT) administration of PS 2′MOE ASOs. To minimize the risk of this adverse event, n-Lorem recommends:

Administer only PS 2′MOE ASOs that are products of a rigorous ASO discovery and preclinical development process.

If necessary induction of treatment consisting of three monthly IT injections, followed by the first IT injection of chronic treratment at the fourth month appears to be tolerated.

Unless the clinical situation demands and the tolerability of previous doses is acceptable, treat chronically no more frequently than every three months.

Limit doses to 100mg or less.

Perform routine monitoring of CSF cell count and protein levels.

Consider more frequent monitoring of patients with clinical manifestations that are of concern.

For a patient with symptomatic confirmed NPH, consider appropriate intervention, discontinue administration of the ASO and report the event immediately to the FDA and to n-Lorem.

SUMMARY OF CLINICAL OBSERVATIONS WITH PS 2′MOE ASOs

Nusinersen

To date, there have been no reports of NPH associated with IT administered PS 2′MOE ASOs in controlled clinical trials of nusinersen[2-4]. However, long term commercial treatment of patients with SMA with nusinersen has very rarely been associated with NPH. Further, in one patient with an in-dwelling catheter, infectious meningitis during nusinersen treatment was reported[5]. Long-term administration of nusinersen has also been associated with modest, but statistically significant asymptomatic increases in CSF protein concentrations. In a study in which repetitive analyses of CSF were performed in 56 SMA patients, CSF protein concentrations increased over the first 10 months of treatment, then were stable during the

remainder of the treatment. CSF cell count was variable, but may have increased slightly, but changes were statistically significant at only two time points[6-9].

Tofersen

A total of 36 and 72 patients with SOD1-caused ALS were treated for 28-weeks with placebo or 100mg on day 1, 15 and 28, then monthly during the controlled portion of the clinical trial and in the open label extension study[10,11]. During the 28-week controlled portion of the study and during the open label extension study, no incidences of NPH were observed. However, greater increases in CSF leukocyte counts and protein levels were observed in more patients treated with tofersen than the placebo group during the controlled portion of the trial. In the control group, 25% of the sham treated patients experienced an increase in CSF leukocytes while 78% of the tofersen treated patients experienced an increase. Similarly, 30% of placebo and 67% of tofersen treated patients experienced an increase in CSF protein levels. At the time of analysis of the open label extension study, both parameters showed numerical increases compared to 28 weeks (88 and 79% leukocyte and protein concentration respectively).

Tominersen

Long term treatment of patients with symptomatic Huntington's disease with tominersen has been associated with NPH in 0.6% of patients[12]. One patient who received 120mg monthly developed hydrocephalus in the open label extension study following the phase 1/2 study of tominersen [13, 14]. The most informative experience to date is in the Phase 3 study in patients with symptomatic Huntington's disease. In this clinical trial, approximately 781 patients were enrolled in either the control arm or 120mg tominersen/8 weeks or 120mg /16 week arms.

No patient in either the control or every 16-week arms experienced NPH. In contrast, 3 patients in the every 8 week arm experienced NPH. One patient who was treated with an every 4-week regimen that was evaluated in a previous study also experienced NPH, resulting in an overall incidence of 0.6%[12, 13,14]. There were also tominersen-associated increases in cerebroventricular volume (11.4%, 17.4% and 24.3% in the control arm, the every 16 week and the every 8-week arm, respectively, at 69 weeks). Further, there was a dose frequency-related increase in the number of patients who experienced an increase in CSF leukocyte counts (13.0%, 21.9% and 27.1% in placebo, every 16 week and every 8-week arms respectively. The fraction of patients who experienced an increase in CSF protein was numerically greater in the every 8 weeks treatment arm compared to control, while the percent patients with an increase on CSF protein in the every 16 week arm was numerically lower than the control arm (10.5%, 20.4 % and 7.8 % in the control, 120mg/8week and 120mg/ 16 arms respectively). The every 8 week regimen was also associated with a greater incidence of adverse events[12,13].

Of note, in the phase 1/2 study of tominersen in patients with symptomatic HTT, tominersen was administered at doses of 10-120mg monthly IT for a total of four doses and a dose and time dependent increase in cerebroventricular volume was observed, but the incidence of NPH was zero[14].

BACKGROUND PREVALENCE OF NPH

Interpreting the clinical observations in PS 2′MOE ASO treated patients is confounded by a significant background incidence of NPH in patients with no CNS disease and that increase is exaggerated in patients with a variety of neurological diseases. NPH displays a U-shaped relationship with age. In children, NPH has been observed in 85/100,000. In adults, 17/100,00 and the elderly 175/100,000[15]. The number of

patients experiencing NPH is also increased in patients with neurodegerative diseases and schizophrenia (For example[7,15-17]).

BACKGROUND PREVALENCE OF INCREASED INTRAVENTRICULAR SPACE

Similarly, the interpretation of potential PS 2′MOE ASO (or other drug) related increases in cerebroventricular volume is also confounded by an age-related variation in the prevalence of ICV increases. In adults, an age-related decrease in brain volume and comparable increase in ICV volume was observed from the thirties to 60s and in the elderly, the changes in volumes appeared to be accelerated[18]. As an element of the AGES-Reykjavik study, MRIs were conducted on a population of patients over 65 years of age and this showed a steady decline in total brain tissue and a comparable increase in ICV volume[19]. Further, numerous neurodegenerative diseases and schizophrenia are associated with an increase in intraventricular space[20,21].

CONCLUSIONS

NPH is a potential, rare adverse event associated with chronic administration of PS 2′MOE ASOs intrathecally.

- In the very limited experience to date, patients with NPH appeared to also display increases in CSF cell count and CSF protein levels. Increases in putative markers of inflammation were reported in some patients as well.
- NPH appears to be dose and schedule dependent.
- With tominersen, chronic dosing of 120mg monthly or bimonthly is associated with a less attractive safety profile and a higher incidence of NPH than less frequent doses of 120mg.
- The mechanism resulting in NPH is unknown and being investigated.
- Interpretation of possible PS 2′MOE ASO induced NPH is complicated by a U-shaped relationship of NPH with age and the fact that numerous diseases are also associated with an increase in NPH.

Asymptomatic increases in ICV volume may be caused by aging and numerous diseases and should not be equated with NPH.

GUIDANCE TO MINIMIZE THE RISK OF NPH

Demand a PS 2′MOE ASO that has been selected after a thorough rigorous discovery and development process.

- Sophisticated experience-based A.I.-informed design algorithm designed.
- Initial screen of at least approximately 500 ASOs directed to different sites in the target RNA.
- Off-target analyses.
- Specialized *in-vitro* screens to avoid pre-inflammatory ASOs.

8 week rodent tolerability screens that include both in-life and histological assessment of safety of at least 20 potent ASOs.

Thorough multi-dose level 3 month GLP toxicity studies in rodents.

Rigid selection criteria throughout the entire process.

CLINICAL MANAGEMENT GUIDANCE

Unless clinically required and supported by prior tolerability,treat chronically at a dose frequency no greater than every three months. (Induction with two to three monthly doses appears to be tolerated).

Limit the maximum dose used to less than or equal to 100mg.

Monitor CSF protein level and cell count routinely and should clinical observations warrant, increase clinical and potentially CSF monitoring frequency.

Patients should be monitored for any change in gait or mental status and any significant change in headaches.

In patients with clinical manifestations that may be of concern or significant increases in CSF cellularity and protein levels, consider more frequent monitoring.

In the event of symptomatic, confirmed NPH:

Consider an appropriate intervention.

Discontinue ASO administration.

Report the event as an expedited SAE to the FDA.

Report the event to n-Lorem immediately.

REFERENCES

1 Isaacs, A. M., Williams, M. A. & Hamilton, M. G. Current Update on Treatment Strategies for Idiopathic Normal Pressure Hydrocephalus. *Current Treatment Options in Neurology* **21**, 65, doi:10.1007/s11940-019-0604-z (2019).

2 Darras, B. T. *et al.* An Integrated Safety Analysis of Infants and Children with Symptomatic Spinal Muscular Atrophy (SMA) Treated with Nusinersen in Seven Clinical Trials. *CNS drugs* **33**, 919-932, doi:10.1007/s40263-019-00656-w (2019).

3 Finkel, R. S. *et al.* Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet (London, England)* **388**, 3017-3026, doi:10.1016/s0140-6736(16)31408-8 (2016).

4 Finkel, R. S. *et al.* Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. **377**, 1723-1732, doi:10.1056/NEJMoa1702752 (2017).

5 Özütemiz, C., Karachunski, P. & Nascene, D. R. Nusinersen injections in adults and children with spinal muscular atrophy: a single-center experience. *Diagnostic and interventional radiology (Ankara, Turkey)* **26**, 596-602, doi:10.5152/dir.2020.19607 (2020).

6 Freigang, M. *et al.* Increased chitotriosidase 1 concentration following nusinersen treatment in spinal muscular atrophy. *Orphanet Journal of Rare Diseases* **16**, 330, doi:10.1186/s13023-021-01961-8 (2021).

7 Viscidi, E. *et al.* The incidence of hydrocephalus among patients with and without spinal muscular atrophy (SMA): Results from a US electronic health records study. *Orphanet Journal of Rare Diseases* **16**, 207, doi:10.1186/s13023-021-01822-4 (2021).

8 Müschen, L. H. *et al.* Cerebrospinal Fluid Parameters in Antisense Oligonucleotide-Treated Adult 5q-Spinal Muscular Atrophy Patients. *Brain sciences* **11**, doi:10.3390/brainsci11030296 (2021).

9 Wurster, C. D. *et al.* Routine Cerebrospinal Fluid (CSF) Parameters in Patients With Spinal Muscular Atrophy (SMA) Treated With Nusinersen. **10**, doi:10.3389/fneur.2019.01179 (2019).

10 Miller T, AAN meeting (2021).

11 Miller, T. *et al.* Phase 1–2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. **383**, 109-119, doi:10.1056/NEJMoa2003715 (2020).

12 Boak, L., McColgan, P., CHDI meeting, *Basel, Switzerland* (2022).

13 Schobel, S., CHDI meeting, *Basel, Switzerland* (2021).

14 Tabrizi, S. J. *et al.* Targeting Huntingtin Expression in Patients with Huntington's Disease. *The New England journal of medicine* **380**, 2307-2316, doi:10.1056/NEJMoa1900907 (2019).

15 Isaacs, A. M. *et al.* Age-specific global epidemiology of hydrocephalus: Systematic review, metanalysis and global birth surveillance. *PloS one* **13**, e0204926, doi:10.1371/journal.pone.0204926 (2018).

16 Jaraj, D. *et al.* Prevalence of idiopathic normal-pressure hydrocephalus. *Neurology* **82**, 1449-1454, doi:10.1212/wnl.0000000000000342 (2014).

17 Eleftheriou, A., Nilsson, M. & Lundin, F. A patient with Idiopathic Normal Pressure Hydrocephalus and Amyotrophic Lateral Sclerosis. Coincidence or a genetic link between the diseases? **4**, 93-96, doi:10.24911/ejmcr/173-1570436579 (2020).

18 Scahill, R. I. *et al.* A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Archives of neurology* **60**, 989-994, doi:10.1001/archneur.60.7.989 (2003).
19 Sigurdsson, S. *et al.* Brain tissue volumes in the general population of the elderly: the AGES-Reykjavik study. *NeuroImage* **59**, 3862-3870, doi:10.1016/j.neuroimage.2011.11.024 (2012).
20 Vanhala, V. *et al.* Prevalence of Schizophrenia in Idiopathic Normal Pressure Hydrocephalus. *Neurosurgery* **84**, 883-889, doi:10.1093/neuros/nyy147 (2019).
21 DeLisi, L. E. Regional brain volume change over the life-time course of schizophrenia. *Journal of psychiatric research* **33**, 535-541, doi:10.1016/s0022-3956(99)00028-x (1999).