Safety Evaluation of Chronic Antithrombin Silencing in Non-Human Primate and Expanded Therapeutic Index in a Hemophilia A Mouse Model

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Abstract

Background: ALN-AT3, a systemically administered investigational RNAi therapeutic targeting antithrombin (AT), is currently in clinical development (NCT02562198) for the treatment of hemophilia and other bleeding disorders. This preclinical work demonstrated that once weekly dosing of ALN-AT3 improves clotting factor levels in severe hemophilia A mice.

Aims: The objectives of these studies was to evaluate the safety and tolerability of ALN-AT3 when administered over a chronic dosing period to normal (non-disease) primate monkeys and hemophilia A mice at non-toxic dose levels.

Methods: ALN-AT3 was administered once weekly via subcutaneous injection to monkeys (6/sex/group) for 36 weeks of age (15, 45, 60, and 75 mg/kg, males only; 30 and 60 mg/kg, males and females combined). ALN-AT3 was administered weekly to HA mice (6/sex/group) for 26 weeks (0.15, 1, 2, 3, 5, 10, and 30 mg/kg). In both studies, potential test article-related effects were evaluated by clinical sign, body weight, clinical pathology, hematology, coagulation, serum chemistry, plasma AT levels, and histopathology via full tissue set.

Results: Chronic administration of 35 mg/kg ALN-AT3 was well-tolerated in monkeys, with no adverse changes to clinical condition or clinical or anatomic pathology parameters. There were no significant changes to coagulation parameters, fibrinogen levels, body weight, or food consumption observed in monkeys. No adverse effects were observed at dosing levels of 10 and 30 mg/kg in ALN-AT3-treated male HA mice. ALN-AT3 was administered weekly to female HA mice at 0.15 mg/kg (35%, 60%, and 70% mean steady state AT inhibition, respectively). ALN-AT3 was administered weekly to male HA mice (35/sex/group) at 0.15, 0.3, 1, 2, 3, 5, 10, and 30 mg/kg (30% and 60% mean steady state AT inhibition, respectively) for 104 days. In both studies, potential test article-related effects were evaluated by clinical sign, body weight, clinical pathology, hematology, coagulation, serum chemistry, plasma AT levels, microsomal protein levels, and histopathology via full tissue set.

Conclusions: The data demonstrate that chronic administration of ALN-AT3 was well-tolerated in monkeys, a relevant preclinical model. Further, chronic dosing at non-toxic dose levels in HA mice was also well-tolerated and resulted in a survival benefit relative to controls.

Background

The coagulation system has evolved to balance the need to control hemostasis with the need to prevent thrombosis. This balance is achieved through a complex interplay of clotting proteins in the extrinsic pathway of clotting (FVII, FIX, FX, thrombin, fibrin) and in the intrinsic pathway (AT, FIX, FVIII, FV, fibrinogen, factor XIII). The data demonstrate that chronic administration of ALN-AT3 was well-tolerated in monkeys, a relevant preclinical model. Further, chronic dosing at non-toxic dose levels in HA mice was also well-tolerated and resulted in a survival benefit relative to controls.

Figure 3. Summary of Findings – HA Mouse

Figure 4. Table of Pathology Findings – HA Mouse

Figure 5. A simplification of cytokine signaling in hemophilia and B lack of FVIII and FXVII, respectively, leads to a decrease in thrombin potential, resulting in a bleeding phenotype. This is a non-toxic dose level in hemophilia A mice for a given therapeutic index.

Table 1. A 9-Month Chronic Toxicity Study in cynomolgus Monkeys

Table 2. A 4-Month Chronic Toxicity Study in Hemophilia A Mice

Figure 1. Therapeutic Hypothesis: Rebalancing the Hemostatic System

Figure 2. Figure 2. A) Inhibition of plasma AT protein (ELISA) was evaluated at intermediate time points throughout the 9-month dosing period and 3-month recovery (sham-operated) period. Values for each animal (6/sex/group) were normalized to the individual animal pre-dose baseline (average of 100% pre-dose). B) AT protein levels (wt% remaining) in 30 mg/kg ALN-AT3 treated and saline controls (vehicle) in plasma from healthy Sprague-Dawley rats (n=3).

Figure 3. A) Inhibition of plasma AT protein (ELISA) was evaluated at the end of the dosing period (Day 184). Mean group values for ALN-AT3 treatment groups were normalized to the concurrent saline control group (individual animal pre-treatment values not technologically feasible). B) There were approximately 45% mortality in the control (saline) animals after the 12-week dosing period. Death or non-viability is secondary to hemorrhage. Lethal hemorrhages associated with the dosing procedure were described as firm/soft swelling at the injection site with rapid desiccation of the general condition. Hemorrhage noted or necropsy was consistent with the expected hemorrhagic disease condition in this strain of mice. In ALN-AT3 treated groups, mortality secondary to hemorrhage was greatly reduced, with 1 male and 1 female at 10 mg/kg and 1 male at 30 mg/kg (Figures 3A & 3B).

Table 3. Summary of Pathology Findings – HA Mouse

Conclusions

Chronic administration of 0.35 mg/kg ALN-AT3 was well-tolerated in monkeys with no adverse changes to clinical condition or clinical or anatomic pathology parameters, demonstrating the safety of mean 75% (45%–85%) target AT inhibition for 9 months in normal (non-disease) animals.

This is consistent with the hypothesis of improvement with pharmocodynamic- and pharmacokinetic-activity of AT.