Improved Specificity and Therapeutic Index with ESC+ siRNA Conjugates Utilizing Seed-Pairing Destabilization via Novel Chemical Modifications

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Abstract

• In the process of lead finding for developmental candidates, a subset of GalNAc-siRNA conjugates showed rat hepatotoxicity at toxicological doses and therefore fell out of contention due to our new ESC+ conjugate platform was investigated across multiple siRNAs with regards to the impact on potency, off-target mRNA dysregulation, and therapeutic index.

Background

Subset of Conjugates Shows Rat Hepatotoxicity at Toxicological Doses and Drop Out of Development Candidate Selection Process

Mitigation of RNAsi-mediated Off-Target Activity Through Seed-Pairing Thermal Destabilization

Design Considerations for ESC+ Conjugates to Improve Specificity

ESC+ Maintains On-Target Activity while Reducing Off-Target Effects in vitro

Therapeutic Index Improved >6-fold in Rodents Using ESC+

In Vivo Activity of ESC+ and ESC Comparable in Rodents

In vitro and in vivo potency while largely reducing off-target effects as demonstrated through in vitro RNAseq across multiple sequences.

ESC+ design demonstrated an improved safety profile and greater than 6-fold improvement in therapeutic index over the corresponding ESC design.

ESC+ has been advanced into clinical development in 2018.

Therapeutic Index Determined on the basis of therapeutic index of ESC+ conjugates. Toxicology studies were performed using a weekly dose of siRNA for a course of two weeks (3 doses). Animals were sacriﬁced 24 hours after the last dose and both oral and subcutaneous blood samples were assayed. The drug or compound determined to cause a change in liver at least twice the upper limit of normal was deﬁned as a ‘bad actor’ and all others were deﬁned as “good actors.”

Figure 7. Determination of the therapeutic index of ESC+ conjugates. Toxicology studies were performed using a weekly dose of siRNA for a course of two weeks (3 doses). Animals were sacriﬁced 24 hours after the last dose. Error bars represent the SD from each cohort (n=3).

Summary

• Developed an ESC+ GalNAc-siRNA conjugate design which utilizes a GNA modification in the antisense seed region to destabilize pairing with seed-driven off-targets.

• GNA substitution can have a minimal impact on in vitro and in vivo potency while largely reducing off-target effects as demonstrated through in vitro RNAseq across multiple sequences.

• A bad actor sequence which was converted to an ESC+ design demonstrated an improved safety proﬁle and greater than 6-fold improvement in therapeutic index over the corresponding ESC design.

• The ESC+ design has been applied to additional pre-clinical programs with successful translation from rodents into non-human primates.

• Plan to advance first ESC+ conjugate, ALN-AAT02, into clinical development in 2018.

• All future candidates planned to employ ESC+ designs.

References


9. Previous work has demonstrated that the use of chemically modified nucleotides aimed towards seed-pairing destabilization can reduce microRNA-like off-targets while maintaining on-target activity in vitro.

10. We investigated the impact of galactosamine nucleic acid substitution in the seed region on the mitigation of off-target effects via seed-pairing destabilization. This new ESC+ conjugate platform was investigated across multiple siRNAs with regards to the impact on potency, off-target mRNA dysregulation, and therapeutic index.

11. In the process of lead finding for developmental candidates, a subset of GalNAc-siRNA conjugates showed rat hepatotoxicity at toxicological doses and therefore fell out of contention due to our new ESC+ conjugate platform was investigated across multiple siRNAs with regards to the impact on potency, off-target mRNA dysregulation, and therapeutic index.

12. The ESC+ design has been applied to additional pre-clinical programs with successful translation from rodents into non-human primates.

13. Plan to advance first ESC+ conjugate, ALN-AAT02, into clinical development in 2018.

14. All future candidates planned to employ ESC+ designs.