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# EDITED TRANSCRIPT

ALNY - 2017 RNAi Roundtable: Platform advances in RNAi therapeutics

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**OVERVIEW:**

Co. provided an update on platform advances in RNAi therapeutics.

## CORPORATE PARTICIPANTS

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**Joshua Brodsky**

**Maja Janas**

**Mark Schlegel**

**Vasant Jadhav**

## PRESENTATION

**Operator**

Thank you, ladies and gentlemen, for joining today's RNAi Roundtable. (Operator Instructions)

I would now like to turn the call over to Josh Brodsky for opening remarks. Josh, you may proceed.

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**Joshua Brodsky**

Thank you. Good afternoon, everyone. Thanks for joining us for today's RNAi Roundtable where we'll be discussing platform advances in RNAi therapeutics. I'm Josh Brodsky, Associate Director of Investor Relations and Corporate Communications at Alnylam. With me today are Akshay Vaishnaw, Executive Vice President of R&D; Vasant Jadhav, Senior Director of Research; Maja Janas, Senior Scientist, Early Development; and Mark Schlegel, Senior Scientist, Research.

Today's RNAi Roundtable is part of a series of roundtables that we are hosting this summer and early fall. Today's event is expected to run between 60 and 75 minutes.

Akshay will moderate the Q&A session at the conclusion of the presentations. If you'd like to submit a question, you can do so at anytime during the event by clicking the ask a question button that is located above the slide window on the webcast player.

Finally, as a reminder, we will be making forward-looking statements, and we encourage you to read our most recent SEC filings for a more complete discussion of risk factors.

And so with that, I will turn it over to Akshay.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Great. Thanks Josh, and welcome again, everybody to our RNAi Roundtable. By way of introduction and as we look at the pipeline that we have developed at Alnylam, you can see that we have 8 programs active in the clinic currently across 3 therapeutic areas in terms of genetic medicines, cardio-metabolic disorders and hepatic infectious disease.

We're very excited about patisiran, our Phase III program in TTR neuropathy, and as all of you are aware, we intend to have the Phase III readout in the second half of September with a view to filing the NDA by the end of the year.

In addition, we have fitusiran, which has entered Phase III; and inclisiran; and givosiran, which will soon start in Phase III. With this rich pipeline, we've accumulated a significant safety database as you can see here.

With a double-digit number of programs in the clinic over the last few years, we've conducted over 20 studies, and over 1,000 patients or volunteers have been evaluated with our RNAi therapeutics. When we look at the patisiran program, we have patients that have gone out to 36 months or beyond exposure.

Examining the platform-related findings that we've seen to date from a safety perspective, we see a low incidence, about 15%, of generally mild and transient injection site reactions with our GalNAc conjugates. We've seen also a low incidence, about 2.2%, of mild asymptomatic and reversal LFT changes that's greater than threefold the upper limit of normal for transaminases. And no evidence of safety signals similar to the revusiran program even when we consider the other programs in the pipeline.

And finally, when we look at the emerging ESC-GalNAc platform, which constitutes the majority of our pipeline, and we compare it to competing oligo platforms, we've seen evidence of thrombocytopenia, renal toxicity or systemic inflammatory changes.

Now to orientate you for today's discussion, this slide shows the 3 generations of products we've evaluated when it comes to GalNAc conjugates. On the left, you see Standard Template Chemistry conjugate, of which the best example is revusiran, which is no longer in development. In the middle, Enhanced Stabilization Chemistry conjugates, which constitutes the bulk of our pipeline today and the relevant programs as shown in the box. And finally, on the far right, the ESC+ conjugate platform, which will serve upcoming INDs for 2018 onwards.

Now as we've gone from left to right, from the first generation to the second generation, with the ESC conjugates, you can see we have human POC across many of the programs that are in the clinic, and we've enjoyed the benefits of the enhanced potency and durability with significantly lower exposures with the ESC-GalNAc conjugates.

And as we'll discuss today, with the ESC+ conjugates, we anticipate that this next-generation conjugate will show further improvements both in specificity and therapeutic index. And today, my colleagues and I will be discussing the second-generation ESC and the next-generation ESC+ conjugates with you.

And so with that, I want to turn over to Vasant Jadhav to discuss the ESC advances we've made of late.

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**Vasant Jadhav**

Well thanks, Akshay. Before we go into the potency and specificity improvement, let's look at the basics of organic sRNA conjugate technology.

It's a simple and very elegant approach of using highly modified sRNA conjugated with small molecule ligand for hepatocyte delivery via asialoglycoprotein receptor. It took more than a decade of effort to arrive at the chemical modification pattern suitable for surviving in extracellular and more importantly, in intracellular components needed for sRNA to reach its site of action inside a cell.

Our optimally designed line allows for very efficient delivery. In fact, conjugate activity is retained in preclinical models of reduced receptor of tables of more than 50%. This work will soon appear in a paper -- in peer-reviewed publication.

Now let's look at the long journey of conjugate potency improvements. And to begin with, let's recap STC to ESC transition. The STC refers to Standard Template Chemistry, a first major breakthrough that allowed convincing POC in humans of GalNAc sRNA conjugate technology. However, this STC design required high doses for robust activity. And given the catalytic efficiency of RNAi pathway, we knew this can be improved. And the clue for this came from metabolic profiling of conjugates in liver, and that's shown here on this slide.

It became clear that there are regions of nuclease hotspots within the sRNA and they appear at the end, rather than in the middle, a significant one would expect from exonuclease activity. Now to improve the exonuclease protection, we turn to stabilization with few phosphorothioate linkages at the (inaudible). With these changes, we observed improved metabolic stability of conjugate, while retaining its inherent RNAi activity. This is the chemistry we refer to as Enhanced Stabilization Chemistry or ESC.

So how does it perform in vivo? Here is an example using our antithrombin sRNA conjugate in nonhuman primate. The STC version at 10 milligrams per kilogram is now done about 30% and recovered quickly within about 3 weeks of dosing. On the other hand, the ESC version, which is fitusiran, gave deeper knockdown and longer duration, even at tenfold lower dose. We are seeing similar profile for other sequences as well. The ESC designs are cornerstone of current pipeline that we have, and the latest DCs in our pipeline employ even more advanced ESC version, which we will talk about next.

So let's look at that. Once we identified the ESC design, we knew there must be room for further improvement. We are never satisfied with potency. The lower dose is always better. Our objective was to identify new, generalizable design with improved potency through optimal placement of 2 prime modifications, most specifically increasing the 2 prime all metal content as it further enhances metabolic stability while retaining the balance of inherent RNAi activity.

Our approach was to begin with motive identified by statistical analysis of large data sets of conjugate in vitro activity that we have assembled. Then apply the screening method using a set of sequences against multiple target and conditions for rapid and systematic evaluation of new designs. The screeding paradigm is shown here systematically. And through this approach, we identified advanced ESC design as shown in the next slide.

It is obvious that the advanced ESC design have a lot more 2 prime all metal content, which is expected to boost the metabolic stability.

So how does it perform? And here it is. We see about 3 full boost in potency as well as duration benefit. Note that advanced ESC was dosed at about threefold lower dose and still match the potency of ESC design. And so is this benefit coming from improved metabolic stability? Indeed, it is. In fact, on the right side, shows higher and sustained sRNA levels for the advanced ESC design.

How does that advanced ESC design now perform in NHP? Here, we compared the activity of revusiran in STC design with ALN-TTRsc02, the advanced ESC design. Revusiran needed high and multiple doses to see about 75% TTR knockdown in nonhuman primate. However, the advanced design TTRsc02 needed just single dose of 1 milligram per kilogram for more than 90% have done with much prolonged duration of effect.

Now adjusting for the dose difference, which is about 45-fold, and area under effect curve for TTR knockdown, about 1.95-fold, overall ALN-TTRsc02 shows 88-fold in vivo potency improvement over revusiran. That's incredible potency gain from STC to the advanced ESC design.

Well, the ultimate test for any of these designs is in humans. Earlier this month, we shared the preliminary study results from Phase I study of ALN-TTRsc02, which employs the advanced ESC design. We see dose-dependent and highly durable activity. Given the profile of our other ESC conjugate, this has now almost become an expectation.

To better appreciate the performance of advanced ESC design in humans, I would focus your attention, though, to the lowest dose we tested. This is shown as the black line in the middle of the graph. It's a dose of finite, a single dose, where we see about 50% TTR knockdown. Now for a 70-kilogram human, this would be about 0.07 milligram per kilogram dose giving ED50, the effective dose giving 50% knockdown. And this is about fourfold lower than what is needed in nonhuman primate for ED50, which is about 0.3 mgs per kg. Again, amazing translation from preclinical species to humans. In fact, we're seeing better activity in humans. And so we are looking forward to see how our additional advanced ESC designs in our pipeline perform.

Now if one looks at -- look back on more than a decade of this work and tries to measure the progress, how does it look? Well, it is shown here in this graph with time scale on X axis and ED50 in mind as indicator of potency on the Y axis, is truly a reflection of our commitment to platform research.

In early days of endo-light chemistry design, the ED50 was in the range of 100 milligrams per kilogram. The STC design was a major breakthrough. That's the design which provided human POC using conjugate technology. Over the years, potency has gone from 100 milligrams per kilogram to 0.1 milligrams per kilogram ED50 in mice. And given the catalytic nature of RNAi machinery, we hope to continue to improve the potency, but we'll have to optimize research beyond metabolic stability.

The chart here seems more like well-known Moore's law that predicts doubling of transistor capacity on integrator circuit every year or so. The Moore's law predict that this trend will continue into the foreseeable future. Well, our founder, Phil Sharp, who has nurtured Alnylam all these years and has witnessed closely our progress, urged us to draw the chart like this to show conjugate potency improvements over the years. And hence, currently, we call this as Sharp's law.

So this wraps up the story on conjugate potency improvements and the path we took from early designs to where we are now. In the next section, Maja will walk you through the path of selecting development candidates and the lessons learned from mechanistic work on hepatotoxicity.

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**Maja Janas**

Thanks, Vasant . Before Mark introduces our new ESC+ platform, I will first cover the clinical and nonclinical safety data of our ESC platform, focusing on mechanisms of hepatotoxicity we observed through the subsets of ESC siRNA at high doses in rat toxicity studies.

As Akshay described earlier, we have extensive human safety experience with encouraging results to date from 14 programs, more than 20 clinical studies, more than 1,000 patients or volunteers dosed for up to 3 years. As summarized in this table, in some of our programs, we see low incidence of about 2.2% of generally mild asymptomatic and reversible alanine aminotransferase, or ASC, increases that exceeded 3x the upper limit of normal.

This emerging profile is favorable for our ESC platform compared with other organ-nucleotide platform with no evidence of thrombocytopenia, renal toxicity or systemic inflammatory effects.

This favorable clinical safety profile is consistent with the wide therapeutic index from our nonclinical toxicity study. As you can see in the right-hand column of this table, we have large safety margins with our current ESC platform across our clinical programs, as calculated based on the no observed adverse effect level, or NOAEL, in monkey toxicity studies.

Below the table is a typical nonclinical safety profile of our development candidates. In the rats, we see nonadverse findings in the target organ, the liver; and our primary organ of elimination, the kidney. In the monkey, we see nonadverse findings in the liver and lymph nodes, all of which are manifestations of drug accumulation.

This is the selection process that allows us to identify development candidates with this favorable nonclinical safety profile. We start with in silico prediction and in vitro efficacy screening of a large number of siRNAs. We then screen most potent siRNAs for activity against a subset of predicted off-target mRNA with the highest sequence homology to the siRNA. siRNA does not affect this predicted off-target, are then screened for efficacy in rodent PD studies, and the subset was concerned in vivo activity is then screened for hepatotoxicity in rat toxicity study at doses that exceeds 100x the PD dose.

Those siRNAs that passed the rat toxicity screen are then tested in monkeys for efficacy, followed by DC declaration. At the rat toxicity screening stage of our selection process, about 60% of siRNAs are good actors and have the favorable safety profile I described on the previous slide. However, about 40% of siRNAs are bad actors that show adverse hepatotoxicity, typically manifested on hepatocellular degeneration and/or single-cell necrosis with liver function tests, or LFTs, that exceed 2x the upper limit of normal.

These adverse findings are only seen as high toxicological doses and are not observed at PD doses. Because most compound in the initial screen were in the ESC design, we consider these effects to be unlikely related to chemical modification. And because for most targets we could identify both bad and good actor siRNAs, on-target toxicity was unlikely.

We set out to systematically evaluate the underlying mechanism of bad actor hepatotoxicity. To meet the desired on-target activity, the antisense strand of the siRNA is loaded into the RISC complex and binds to the target mRNA with potent complementarity, resulting in potent catalytic mRNA cleavage.

The first potential cause of bad actor hepatotoxicity could be a non-RNAi dug effect such as protein binding, preservation of endo-lysosomal trafficking or metabolite generation.

The second potential cause could be competition for RISC loading with androgynous small RNA, resulting in the circulation of natural RNAi pathways.

The third potential cause could be undesired off-target activity, whereby, the RISC-loaded siRNA finds the partial sequence match to off-target mRNA, leading to translational repression and mRNA destabilization, analogous to microRNA. Because this off-target activity mimics microRNA-like activity, it will be most likely driven by the seed region, comprising the first 7 to 8 nucleotides of the antisense strand, and it would be much less potent by the catalytic on-target mRNA cleavage. Indeed, in vitro studies indicate that 50- to 100-fold higher sRNA concentrations are needed for eliciting off-target activity compared to on-target.

To begin today's [service] mechanism, we first block RISC loading of bad actor siRNA to focus on the contribution of non-RNAi drug effects. Through this end, we cupped the 5x ends of multiple bad actor siRNA to prevent 5x discoloration and therefore, incur RISC loading. These plus or minus cupped tests were tested in a series of rat toxicity studies of 30 mgs per kg, administered every other day over the course of 2 weeks.

In subsequent graphs, parent siRNAs will be shown in black, and the RISC-blocked siRNAs will be shown in gray. As expected, despite some blocks decreased RISC loading, but importantly, liver concentrations were equivalent, and therefore, any non-RNAi toxicities related to siRNA chemistry, trafficking or metabolism should be preserved.

We then observed hepatotoxicity by measuring serum transaminases elevations and evaluating microscopic changes in the liver. We observed that blocking sRNA RISC loading without altering the 2'-fluoro, 2'-O-methyl or phosphorothioate content, mitigated hepatotoxicity. Bad actor parent siRNAs in black showed the expected ALT elevations, while the same bad actors, in case of (inaudible) RISC loadings, have no liver enzyme elevation.

Consistent with the serum chemistry data, we observed microscopic evidence of hepatotoxicity with RISC-loaded bad actor sRNAs but not with siRNAs that were present in the liver in the same amount but were not loaded into it. Therefore, accumulation of chemically modified siRNAs in the liver is not sufficient for hepatotoxicity. RISC loading is likely required. To confirm this observation, we then tested the same bad actor sequence in the ESC chemistry or in the advanced ESC chemistry with overall higher 2 prime all metal content that Vasant described earlier.

This sRNA test was tested in a rat toxicity study at 100 mgs per kg administered weekly, 9x. First, we confirmed equivalent liver concentration and equivalent RISC loading for both chemical modification pattern. Consistent with our earlier observation that siRNA chemistry is not an important driver of bad actor hepatotoxicity, both the ESC and the advanced ESC design of this bad actor sequence showed serum LFT elevations and corresponding adverse microscopic changes in the liver.

Therefore, siRNA sequence, not the chemical modifications, is important for rat hepatotoxicity. These studies indicated that non-RNAi drug effects are not a major driver of bad actor rat hepatotoxicity. The distinguished competition for RISC loading from off-target effects, we then utilize an approach that allows RISC loading but blocks the activity of the RISC-loaded siRNA.

Through this end, we employed our unique Reversir technology to abrogate RNAi activity without changing siRNA chemistry or RISC loading. As we showed previously, the Reversirs are shored single-stranded GalNAc conjugated dose, that binds a synthetic target to the seed region of the antisense strand in functional RISC, thereby preventing hybridization to complementary mRNAs.

As shown in the graph below, administration of the Reversir molecule is a rapid and complete reversal of siRNA activity.

We employed the Reversir approach in 2 types of toxicity studies: prevention and treatment. In prevention-type studies, we first administered Reversir molecule at a high PD dose of 3 to 10 mgs per kg, followed by a toxic regimen of a bad actor siRNA, consisting of 3 weekly doses of 30 mgs per kg.

In treatment-type studies, we first elicited hepatotoxicity of a toxic regimen of a bad actor siRNA, followed by a single PD dose of Reversir. In subsequent graph, black bars represent the bad actor siRNA by itself, all with the controlled Reversir, while the gray bars represent the bad actor siRNA with a Reversir blocking the RISC-loaded antisense strand.

As expected, both liver concentration and RISC loading of bad actor siRNAs were unaffected by the (inaudible) Reversir. However, despite same liver siRNA concentration and same RISC loading, reversing the activity of the RISC-loaded siRNA did mitigate hepatotoxicity.

First, we evaluated serum transaminases. As expected, all 3 bad actors administered alone or with controlled Reversirs showed LFT elevation. However, either pretreatment with (inaudible) Reversir or post treatment with (inaudible) Reversir, eliminated these LFT changes. Consistent with mitigation of transaminase elevations, microscopic changes in the liver such as degeneration, single-cell necrosis and fibrosis were reduced with Reversir that blocked the activity of the RISC-loaded siRNA.

Therefore, competition for RISC loading with the androgynous microRNA is not a major contributor to rat hepatotoxicity. Seed-mediated hybridization of the RISC-loaded antisense strand appears to be important.

This series of investigative rat toxicity studies pointed to RNAi-mediated off-target effects as important drivers of rat hepatotoxicity. Indeed, we were able to capture these seed-mediated off-target effects by RNA's fixed global gene expression profiling at high doses in rat hepatocytes. The data is summarized in these thoughts.

Each point represents an RNA transfer, the X factor is abundance, and the Y axis is magnitude of change related to the control group. Blue horizontal lines indicate twofold change. RNA transcript in red are disregulated significantly with P value of less than 0.05.

Under the on-target mRNA, circled in blue, is typically the most highly down regulated mRNA, as expected. There are multiple off-target mRNAs, decreasing or increasing in response to the siRNA treatment, typically by less than twofold, which is consistent with the low potency of this microRNA light seed-mediated off-target activity.

Also consistent with this mechanism, seed match the antisense strand will significantly enrich only in the down regulated subset of the mRNAs. No seed match enrichment was observed for up regulated mRNAs all for the same strand.

Through this systematic approach via a number of studies, we concluded that for our ESC platform, seed-driven, RNAi-mediated hybridization based off-target effect, nonchemical modifications or competitions for RISC loading with endogenous small RNA appears to be important drivers for rat hepatotoxicity at exaggerated doses.

Now Mark will walk you through the -- our ESC+ strategy for mitigating these speed-driven off-target effects resulting in improved specificity and therapeutic index.

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**Mark Schlegel**

Thank you, Maja. Based on a very nice work that Maja has just shown you, which points towards seed-mediated off-target effects, we believe that it would be important to have a strategy to address this potential liability.

This new approach is what we are calling our ESC+ conjugate platform, and we believe that it will allow us to further improve our therapeutic index.

Let's take a step back and look again at the proposed mechanism of seed-mediated off-target binding via microRNA-like mechanism. An approach that has been previously reported in the literature invokes the use of an additional chemically modified nucleotide in the antisense seed region to selectively destabilize off-target binding and therefore diminish the undesired seed-driven off-target activity.

As shown on the top of this slide, we need to keep several key design features in mind when utilizing such an approach for our ESC+ conjugates. First, we must ensure that we're able to maintain on-target potency in vivo. Second, since we want to develop conjugates for subcu delivery using

this strategy of seed-pairing destabilization, it is very important that this additional modification has little to no impact on the metabolic stability of the conjugate.

And finally, our ESC+ design should minimize off-target activity and thereby increase our therapeutic index.

With those features in mind, let's take a look at the performance of our ESC+ conjugates in a rodent model system in vitro. For this initial proof of concept, we chose a sequence, which behaves as a classical bad actor in rats, that is a sequence which shows toxicity in a standard 2-week study, using weekly doses of 30 to 100 milligrams per kilogram. Here, you can see that the inherent on-target potency is maintained in the ESC+ conjugate, where we have incorporated a new modification into the seed region of the ESC sequence.

If we now look at the RNA seq analysis showing the off-target activity for the ESC conjugate on the top right of the slide, one can see that there are a significant number of gene transcripts, as highlighted by the red dots, which are differentially expressed. Of these differentially expressed genes, a large portion possesses the perfect seed match with the administered siRNA. Interestingly, we also see a number of genes that are up regulated in this RNA seq experiment.

The RNA seq analysis for the corresponding ESC+ conjugate shown on the bottom right indicates fewer differentially expressed genes and a quieter off-target profile. In fact, we observed similar profiles of several additional sequences, where most seed-mediated off-targets are abrogated in vitro.

Here, we also observed a reduction in the number of differentially expressed genes that are up regulated in this experiment. This suggests that most of these are likely downstream targets of those genes down regulated by the ESC conjugate. So far, this is looking pretty good.

Next, we wanted to ensure that we could maintain on-target activity in vivo with this ESC+ conjugate. Here, these 2 graphs show the pharmacodynamics of both ESC and ESC+ designs when subcutaneously administered across a series of dose levels in rats. As you can see, the ESC+ design maintains in vivo potency, with only a slight shift in the effect of dose to provide 50% knockdown.

With that information in hand, namely that our ESC+ conjugate designs could maintain in vivo potency while reducing off-target effect, we wanted investigate the impact of our ESC+ design had on hepatotoxicity in the rat. Indeed, the ESC conjugate that we chose as our model sequence is a classical bad actor, as pictured in the liver section on the left. A section from the liver of a rat treated with the improved ESC+ conjugate on the right points to a more normal liver architecture and an overall reduction or elimination of toxicity signals when compared to the corresponding ESC design. These encouraging data support the theory that seed-mediated off-target pairing is a potential driver a hepatotoxicity in rodents.

Finally, we wanted to look at the impact this ESC+ design had on the therapeutic index of this set of compounds. The therapeutic index is defined as the ratio between the effective dose and the dose level at which there are no observed adverse effects or NOAEL.

On the left, you can see the profile of our ESC conjugate. The blue dots indicate the level of silencing we observed at the specified dose, while the red dots indicates the toxicity grade associated with the same specified dose. The calculated therapeutic index, as indicated by the dotted line in this graph, is 66 for the ESC conjugate. This value was typical of our ESC conjugate and provides a significant dosing window where we can achieve effective silencing without causing toxicity. When we now take a look at our ESC conjugate design on the right, there's a clear and significant shift in NOAEL dose, with only a minimal impact on the effective dose. This results in a therapeutic index of greater than 400 for the ESC+ conjugate and, therefore, greater than 6-fold improvement over the corresponding ESC conjugate.

And here, we should note that we will present further details on our ESC+ conjugate platform at the upcoming OTS Meeting in September.

So far, what I have shown you is that we're able to improve our safety profile and therapeutic index using our ESC+ conjugate strategy in a rodent model. Now I would like to walk you through an example where we wanted to utilize observations from the clinic to improve our conjugate design.

Last year, we discontinued development of ALN-AAT after observing dose-dependent and transient transaminase elevations in the clinic, as pictured on this slide. Since we believe that this observation may have been driven by off-target effects, we chose to develop an ESC+ version of our ALN-AAT sequence.

Consistent with our previous example, our developed ESC+ conjugate targeting AAT or ALN-AAT02 possesses an identical sequence to ALN-AAT.

As shown here, the RNA seq analysis indicates that ALN-AAT02 shows a profile where most but not all off-targets are diminished in hep 3B cells, which is a human cell line. In addition, as indicated by the circle dots in the RNA seq analysis, ALN-AAT02 was able to maintain inherent potency in vitro.

There's one gene in particular that is down regulated by both siRNAs, which is not abrogated using our ESC+ strategy. We can say, however, that this regulation of this gene is not an on-target effect, but is specific to the hep 3B cell line and is currently under investigation.

Now that we have shown you that we can reduce off-target effects with ALN-AAT02, we wanted to ensure that we can maintain on-target activity in vivo. Since this AAT targeting sequence is not rodent cross-reactive, we chose to perform the ultimate test for our ESC+ conjugate and evaluate how well the ESC+ design for ALN-AAT02 translates into higher species.

Here, we show that ALN-AAT02 demonstrates a similar level of knockdown to ALN-AAT when dosed subcutaneously in nonhuman primates. Based on this extremely encouraging data, we plan to advance ALN-AAT02 into clinical development in 2018 to investigate our ESC+ conjugate strategy in humans.

And in conclusion, what we've shown you today is that our platform advances continue to drive the potency of our GalNAc-siRNA conjugate, allowing potentially quarterly to even biannually dosing at low doses in humans. We've demonstrated that RNAi-mediated off-target effects are an important driver of hepatotoxicity for a subset of our ESC conjugate and rodent toxicity studies, with no evidence for chemical -- for an impact for chemical modifications on the observed toxicity.

We've also developed an ESC+ design to mitigate these seed-mediated off-target effects, which improves the specificity and further expands our therapeutic index for our conjugate. We plan to advance our first ESC+ conjugate, ALN-AAT02, into clinical development in 2018, and finally, all future candidates at Alnylam will employ the ESC+ design.

And with this, I would like to turn it back over to Akshay.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Great. Thanks very much, Mark. And with that, I'd like to open the session for Q&A. You can send the questions in per the usual mechanism, as Josh described. We've already had a number of questions, let me begin with the following question.

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## QUESTIONS AND ANSWERS

**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

How would you describe the difference between antisense versus siRNA when both use GalNAc technology? Can you tell us a little bit about the safety and efficacy profile of each using GalNAc? Vasant, do you want to start with that?

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**Vasant Jadhav**

All right. Thanks, Akshay. Yes, it's a great question. Let's begin with, first, the efficacy or the potency of these molecules. So if one looks at just the inherent potency of siRNA and antisense oligonucleotide, there's clearly a big difference here with (inaudible) in multiple studies, in head-to-head comparison, siRNA have much better inherent activity for gene silence compared to antisense molecules. Now currently, the GalNac has boosted antisense oligo potency in vivo has improved quite a bit, but we still believe that the data that we have seen with other conjugate with GalNAc siRNA conjugates, we are seeing better potency and much better duration compared to the antisense oligonucleotide, even at GalNAc conjugate.

In terms of the safety of these molecules, so far, we have shown, as Akshay went for the data, with all the programs that we have, our profile looks very, very encouraging. We have no signs of thrombocytopenia or any other things that are associated with the safety profile of our molecule. So we remain very encouraged with our molecules. And yes, I mean, there are other things with antisense technology that have been reported and it will -- we'll see, as more data comes from the GalNAc, ASOs in clinic, how the profile emerges for these molecules.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Yes, and I would just add to that, Vasant, that for antisense oligonucleotides, even at lower exposures, which I think, they anticipate by using GalNAc technology, some of the class effects that have been seen to date, which you alluded to, thrombocytopenia, kidney effect, may persist. And interestingly enough, in that regard, the recently approved drug has been rather for SMA, which is administered intrathecally at very low exposure systemically, therefore, still seems to be associated with some of those issues. But as you say, in the fullness of time, we'll see with the data (inaudible)

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**Vasant Jadhav**

Yes, actually, just to add just a little bit on that one, the liver-to-kidney ratio for our GalNAc-siRNA conjugate is, at the PD dose, about 34 or more. And even with the GalNAc conjugation or antisense oligo, that ratio has not improved a whole lot, you still have the ASOs going into the kidney.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Okay. Thank you. Next question, does the ESC+ version of our platform have more (inaudible) modifications relative to the original ESC out of the platform. What are the other major differences between ESC+ versus ESC. So Vasant, do you want to start with that?

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**Vasant Jadhav**

Yes. So as we discussed in the presentation, the ESC design had a specific pattern of 2 prime modifications and 6 phosphorothioate at the end of the molecule. In the advanced ESC design, our purpose was to reduce -- or in fact, to increase the own -- 2 prime (inaudible) of content, and the reason for that one is the (inaudible) content increasing in that improves the metabolic stability. But improving the metabolic stability, we hope to improve the potency of our conjugate. And that's exactly what we're seeing with the ESC design, that increased 2 prime (inaudible) content has improved the potency and the duration. And just by the numbers, I think...

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Approximately.

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**Vasant Jadhav**

Approximately, there are 20 to 22 final methods in the earlier design, ESC design, and they're about 8 to 10 in advanced ESC+ design.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Right. Thank you. Just going to the next question now. What is the typical level of protein binding seen for siRNAs compared to antisense oligonucleotide? Maja, do you want to handle that?

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**Maja Janas**

So protein binding assays, we typically see about 50% protein binding across our platform compared to, my belief, more than 90% for ASOs. So we do see much lower plasma protein binding for our conjugates compared to ASOs.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

And in fact, my understanding from the literature has always been that a high degree of protein binding was engineered into antisense oligonucleotides by virtue of the phosphorothioate modifications, so those carriage on the albumen to potentiate delivery to organ failure.

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**Maja Janas**

And with our GalNAc technology, we don't really need such high protein bindings and we did efficient delivery.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Right. Good. So I think, a lot of questions are interested in the comparison to antisense oligonucleotides. Here's another question, do LycA ASOs have the same durability as GalNAc-siRNA? I think, you touched on that a little bit, Vasant, maybe you want to go at that again.

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**Vasant Jadhav**

Yes, definitely. I think, what we're seeing with our GalNAc-siRNA conjugate is not only incredible potency but very long duration, the duration that is going, just if we take the example of the data that we looked at, ALN-TTRsc02, is almost upward here, where we are seeing steady reduction in the TTR protein. The data that we have seen so far with the GalNAc ASOs, we don't think that the duration is like what we see with our conjugate (inaudible)

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Indeed. Turning to some of the off-target effects, here's a questioner who's asking, and Maja, I think, you might be able to help here. What sequencing technologies are we using to ensure that we have a good categorization of the off-target effect?

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**Maja Janas**

So we use the most advanced technology that is available, which is RNA sequencing, or RNA seq, to capture both mRNAs and also (inaudible) RNAs. So we have a very comprehensive coverage of the transcriptome.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Good. Thank you. The next question is why is the therapeutic margin of fitusiran so much smaller? I think, here, the question is referring to a safety table you showed, Maja, earlier on, where we compared the NOAEL in the rat and the monkey for our various programs. Recalling the data, I think, the fitusiran NOAELs where in this low single-digit, and they contrast to the generally triple digit NOAELs in terms of mg per kg for the other programs. And the answer is relatively straightforward that, for fitusiran, with the on-target effects against antithrombin, we tend to see on-target toxicity resulting in thromboembolic problems in the rat and the monkey, and that leads to the lowered NOAEL. Now of course, we've done fitusiran study in hemophilia mice, and there, mice tolerate intensive dosing weekly at over 100 milligram per kilogram, with no evidence of thromboembolic, is sort of similar to what we've seen in the clinic today. So that's the response there.

Turning to other questions, here's one. Why doesn't your in silico screening algorithm help you avoid the kinds of off-target effects that we discussed today. Maja?

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**Maja Janas**

So basically, there are 2 types of off-target, so those that have very high complementarity to our siRNA, and those are relatively easy to predict and relatively easy to screen against because there's a very low number of them. However, the more important ones are the ones that only have the seed match, which is basically only a [7-degree] that match. And when we just take a randomly chosen (inaudible) seed, it will appear in an average of 1,500 [indiscernible UTR, so you can imagine, it's a very daunting task to look at all of them and assess their contribution -- any potential contribution to hepatotoxicity. So we thought the better strategy would be to mitigate this off-target instead of having to predict them and evaluate them. And that's the approach we're taking.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Good. A couple of questions for you here, Mark, I think. When will our first ESC+ program be in the clinic? And do we have freedom to operate for base pairing destabilization modifications?

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**Mark Schlegel**

Thanks, Akshay. So our first ESC+ conjugate program in the clinic will be ALN-AAT02. As I had I mentioned, this will go in 2018, so that is next year. And I should also mention that all of our future DCs will employ this strategy. In terms of freedom to operate, what I can tell you is that we're using proprietary modifications at the moment. These are distinct from what you would find in the literature. And you should look out for further details on these at the OTS meeting coming up in September.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Great. The next questioner has the question, what's our best guess for the mechanism of LFT changes that we've seen in humans? Maja?

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**Maja Janas**

So I guess, at this point, we can only speculate, but our best guess based on indirect evidence is RNAi-mediated off-target effects for the following reasons. So we see LFT elevations in the clinic but only sporadically and not across all of our programs. And also, the kinetics of these LFT elevations are most consistent with RNAi-mediated off-target effects. And also, as we've just showed you, based on our nonclinical studies, off-target effects are the most likely mechanisms, based on the studies I just described, including the RISC loading study, the Reversir study and the fact that we cannot mitigate toxicity by changing chemical modification pattern of our siRNA. But I mean, the ultimate proof will be taking ESC+ to the clinic.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Good. To the next question, here we are. Back to siRNA versus antisense oligo, this questioner asked, the LFT effects that have been reported for antisense oligos, are those occurring via the same mechanism that you've discussed today?

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**Maja Janas**

So we don't believe that they are occurring via the same mechanism. As we just showed you, in our case, RNAi-mediated off-target effects appear to be an important driver. And this mechanism is not really consistent with the mechanism of action ASOs, which bind to the RNA page and not

to the RISC complex. So in the case of ASOs, there could be other hybridization-based effect that could be driving these LFT changes, or it could be due to their chemical modification patterns, such as higher phosphorothioate compared to siRNA.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

The next questioner wants to discuss the cost of goods for ESC+ conjugates (inaudible) or our ESC or siRNA platforms. And my anticipation is that we wouldn't see any significant changes. But Mark, you'd probably know more detail.

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**Mark Schlegel**

Yes, that's correct, Akshay. We don't anticipate any change in the cost of goods for our ESC+ conjugates, as these new modifications have a similar cost to the current modifications that we're using for our ESC conjugates.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Just looking at additional questions here that are coming in. Are we considering follow-on ESC+ versions for our current DCs like fitusiran and givosiran?

Of course, fitusiran and givosiran, they're active programs in the clinic. We've shared the data from them, and the data currently look very encouraging, both from a safety and efficacy perspective. Similarly, to other sponsors with their other programs, as these drugs proceed and they go to approval, I'm sure that we look forward to them being successful drugs. But we will continue to make enhancements to our platform. And as appropriate, we will bring in follow-on programs to the clinic subsequent to the initial approval.

Okay. The question -- the next questioner wants to go over the (inaudible) utilization in ESC+ versus ESC approximately.

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**Vasant Jadhav**

I think, what we talked about with the ESC design, and we should be -- just to clarify here, the ESC design doesn't mean there is that fixed pattern. We have the recipe of how to design this molecule. And over the time, we have changed the content of the (inaudible) in our molecules, but in general, the ESC would say that it is (inaudible) content to (inaudible) content is similar, maybe around 40%, 50% of each in that range. For advanced ESC, definitely, the (inaudible) content has increased. And this content is in the range of about 30, 35 (inaudible) in the advanced ESC molecules.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Okay. So going to the AAT01 versus AAT02 comparison, we showed in vitro results. This questioner asked for AAT01 versus 02, with the former showing greater off-target effects. Was AAT01 hepatotoxic in the rat similar to some of the other examples? And why do we take it forward only to find LFT changes. Maja?

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**Maja Janas**

No. In fact, ALN-AAT had a very similar favorable safety profile in our nonclinical studies. Does not differentiate from our other development candidates. But now, in light of the still dependent, transient, LFT elevation in the clinic, we started with the right thing to do, develop ESC+ follow-on molecule. And this example just highlights the inherent difficulty in predicting off-target across (inaudible) of the transcriptome are not (inaudible). So again, ESC+ platform, we're hoping to mitigate off-target across species without worrying about conservation of off-target effects.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Yes. And I think, the destabilization modification, which appears to be a general solution for off-target effect, should carryover across species and across transcripts. Good.

The next question, the question is asking how far do we think we can extend (inaudible). Or how much more potent can we make conjugates as we advance towards 2020? Vasant?

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**Vasant Jadhav**

Wow. Yes, I mean, would love to extend the (inaudible) as long as we can. Again, we have to look at the potency or the catalytic efficiency of RNAi missionary, which is incredible. So we do hope to keep improving the potency of our molecules. But as mentioned during the presentation, that the potency gains that we have so far are largely to enhancing the metabolic stability of our molecules. But to go beyond this one will have to work on the future of the siRNAs that only -- that not just only improve the metabolic stability but interaction with the risk and RNAi pathway (inaudible). So we hope to continue the path of improving the potency and go further and further.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Good. The next questioner asks, maybe, Maja, you can take this, will AAT02 have the same sequence as AAT01?

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**Maja Janas**

Yes, as Mark mentioned, the sequence is exactly the same.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Yes, indeed. And we're very excited about that because, I think, that will help us evaluate the impact of ESC+ approach on the LFT changes, so we have very interesting translational experiment to compare those data.

The next questioner asked, the advanced ESC or ESC+ has very few (inaudible), is that out of some concern for (inaudible) or are you moving towards conjugates with fewer or less (inaudible)? Vasant?

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**Vasant Jadhav**

Yes, it's a great question. Truly, as we mentioned about going towards advanced ESC design, increasing the (inaudible) content was not really for the reason of producing the (inaudible), it was really for improving the metabolic stability of our molecules. And we showed that data that advanced ESC design with higher (inaudible) content have increased metabolic stability and that includes potency. We should also note that with this improved metabolic stability, the potential for monomer formation with our molecules becomes less and less. So I would, again, just say the same thing, it was not driven by the desire of having lower (inaudible) content, it was really to improve the potency of our molecules through metabolic stability, and by doing that, we also get the benefit of reduced monomer release.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Good. I think, we have time for one last question. And the questioner is asking, what do you anticipate will be the dosing regimen for ALN-TTRsc02 in the clinic? Mark?

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**Mark Schlegel**

For ALN-TTRsc02 in the clinic, I'm not sure I can...

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

You showed those data, and I would suggest that per the data we discussed, we're very encouraged about the potency and durability of lower exposures for TTRsc02. And I think, we can anticipate that the dosing will be once every 3 or 6 months. So with that, I would hand it over to you, Josh.

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**Joshua Brodsky**

Okay. Excellent. Thank you, Akshay. And thank you to the rest of our speakers as well. This concludes our RNAi Roundtable for today. The replay and slides will be posted on the Alnylam website today at alnylam.com/capella, with the transcript to follow shortly thereafter.

We have 2 remaining RNAi Roundtable in the series and we hope you can join us on Thursday, September 7, at 10:30 a.m. Eastern time, as we discuss givosiran in development for the treatment of acute hepatic porphyrias. And then, our final roundtable of the 2017 series will be on Tuesday, September 12, at 10:30 a.m. Eastern time, and that will focus on fitusiran in development for the treatment of hemophilia and rare bleeding disorders. For more details for these events, please visit www.alnylam.com/capella. Thanks, everybody. Have a great afternoon.

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**Akshay K. Vaishnaw** - *Alnylam Pharmaceuticals, Inc. - EVP of Research & Development*

Goodbye.

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**Mark Schlegel**

Bye.

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**Operator**

Ladies and gentlemen, thank you for your participation in today's conference. This does conclude the program and you may now log off. Everyone, have a great day.

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