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EDITED TRANSCRIPT

ALNY - 2018 RNAi Roundtable: Platform Advances in RNAi Therapeutics

EVENT DATE/TIME: JUNE 26, 2018 / 2:30PM GMT

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PRESENTATION

Christine Regan Lindenboom *- Alnylam Pharmaceuticals, Inc. - VP of IR & Communications*

Good morning, everyone. Thanks for joining us for today's RNAi roundtable where we'll be discussing platform advances in RNAi therapeutics. I'm Christine Lindenboom, Vice President of Investor Relations and Corporate Communications at Alnylam. With me today are Martin Maier, Vice President of Research; Vasant Jadhav, Senior Director of Research; Mark Schlegel, Senior Scientist Research; and Kirk Brown, Associate Director of Bioanalytical Sciences.

Today's RNAi roundtable is the first in a series of roundtables that we are hosting this summer. Today's event is expected to run about 1 hour. Vasant will moderate a Q&A session at the conclusion of the presentation. If you'd like to submit a question, you could do so at any time during the event by typing your question in the Ask a Question field.

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

And with that, I will turn it over to Martin.

Martin Maier

Thank you, Christine, and welcome again, everyone, to our RNAi roundtable in which we will discuss some recent advances in our RNAi platform technology.

By way of introduction, RNAi therapeutics has emerged as a new class of clinically proven medicines with the potential to transform the treatment of a broad range of diseases. After many years of research and development, we are excited to see the translation of this Nobel Prize-winning science into a platform technology, which enables potent and durable silencing of disease-causing genes.

The progress on the platform technology also provides a product engine for sustainable pipeline of innovative new medicines, the first of which, patisiran, is about to enter commercial stage, which is very exciting to us.

So far, at Alnylam, we have developed 2 clinically validated and complementary platforms for the delivery of functionally intact active siRNAs to the liver as our target tissue. On the left, you see the schematic representation of our lipid nanoparticles, a multicomponent lipid formulation, which encapsulate the siRNA molecules, thereby protecting them for most of their journey from the site of administration to the site of action inside the target cells. The particles, which are administered intravenously, deliver the siRNA payload with high efficiency to liver hepatocytes using a targeting mechanism involving endogenous apoE. This delivery approach is used for patisiran.

And on the right-hand side, we have the schematic representation of our GalNAc-conjugate approach, in which a multivalent galNAc ligand is covalently attached to the siRNA. The ligand is designed to specifically recognize and bind to a cell surface susceptor, which is highly expressed on hepatocytes, thereby enabling targeted delivery of the siRNA conjugates to the liver. In this case, to ensure adequate protection throughout

the journey, the siRNA is extensively chemically modified. This delivery approach is utilized for all other development candidates currently in our pipeline, which brings us to the next slide summarizing Alnylam's current clinical development pipeline.

We have currently 6 active programs in the clinic across 2 therapeutic areas, genetic medicines and cardio-metabolic disorders. And of course, patisiran, which recently demonstrated fantastic results across all primary and secondary endpoints in the pivotal Phase III clinical study, is currently in registration for regulatory approval in the U.S. and in Europe.

As a new class of medicines, RNAi therapeutics represent a highly differentiated approach, which allows to address disease targets that are considered undruggable with small molecules and monoclonal antibodies. We can achieve highly efficacious, if needed, up to 99% knockdown of disease-causing proteins with a clamped pharmacodynamic effect compared to the saw-tooth pharmacology often seen with other drugs or other drug classes. And our advanced siRNA designs demonstrate a remarkable durability effect, which enables once-a-quarter and, in some cases, biannual or potentially even further expanded dosing regimens. As mentioned, GalNAc siRNA conjugates can be delivered by subcu injections, and the stability of our drugs allows us to avoid the many challenges of a cold chain. When these pieces combine, we believe they define a winning profile for our product even in competitive markets.

Alnylam's rich pipeline and corresponding clinical experience has allowed us to accumulate a significant safety database. With more than 10 programs in the clinic over the last few years, we have conducted more than 25 studies involving far -- over 1,200 patients or volunteers who have participated in the clinical studies. And looking, for instance, at the patisiran program, we have patients now with greater than 48 months of exposure.

Examining the platform-related findings that we've seen to date, from a safety perspective, we see a low incidence, about 24%, of generally mild and transient injection site reactions with our GalNAc conjugates. We've also seen a low incidence, about 2.9%, of mild asymptomatic and reversible LFT changes with transaminase elevations greater than threefold the upper limit of normal.

Now for the revusiran program, which was discontinued in October 2016, we observed an imbalance in mortality, which even after extensive evaluation could not be associated with a clear reason. However, it is important to note that we have not observed any safety signals similar to the revusiran program for any of our other programs.

Now to orient you for today's discussion, this slide shows the continued progress in our GalNAc conjugate platform, which was enabled through several major advancements in the clinical design. On the left, you see the Standard Template Chemistry with revusiran as the example, which is no longer in development. In the middle, we have the Enhanced Stabilization Chemistry conjugates, which constitute the bulk of our pipeline today, and the relevant programs shown in the box. And finally, on the far right, the ESC+ conjugate platform, which will serve as the go-to design for upcoming INDs from 2018 onwards.

Now as we find from left to right, the first generation has provided the first human proof of concept for this delivery platform. Now for the second generation ESC conjugates, we now have human proof of concept across many of the programs within the clinical, and importantly, the benefits of the enhanced potency and durability has translated well into humans with significantly lower exposures compared to the first generation. And with the ESC+ conjugates, as we will discuss today, we anticipate that this next-generation conjugate will show further improvements both in specificity and therapeutic index.

Further, we are excited to share with you a recent initiative aimed at unlocking the potential of our technology for diseases of the central nervous system, thereby really expanding our platform, our pipeline of potentially transformative medicines into this new therapeutic area, adopting a strategy, which has been successfully applied for building our current pipeline. We will target disease-causing genes in the CNS, which are genetically validated, support early clinical-stage POC for a biomarker readout and the definable path to approval and patient access.

And with this, I will turn it over to my colleague, Mark Schlegel, who will discuss some of the recent advances in the ESC+ design.

Mark Schlegel

Thank you, Martin. To ground everybody, we're currently on Slide 14. So I would first like to briefly cover our Enhanced Stabilization Chemistry, or ESC, siRNA conjugate platform.

This platform utilizes an siRNA, which is chemically modified, in order to introduce metabolic stability, critical for achieving in vivo delivery as a conjugate. Such an siRNA can be conjugated to a trivalent N-Acetylgalactosamine, or GalNAc ligand, which is recognized, bound and internalized by the highly expressed asialoglycoprotein receptor, or ASGPR, on hepatocytes. Since the expression of the ASGPR receptor is highly hepatocyte specific, uptake and delivery of the siRNA conjugates is highly efficient and specific to the liver.

We have extensive experience so far with our ESC conjugate platform, both in preclinical species as well as in humans. The top of the slide outlines the process that we take from target identification to developmental candidate selection. During this lead finding process, there's a certain subset of conjugates, which show hepatotoxicity and LFC elevations in rats, which we call bad actors. While these candidates are never chosen to move forward as a developmental candidate, these examples may provide an opportunity to further understand some of the observations from the clinical studies, as Martin pointed out earlier.

So what are some potential drivers of rodent hepatotoxicity? We recently published a large body of work in Nature Communications, which I will summarize here. The first potential driver that we identified were non-rNAi effects, things such as the impact of chemically modified nucleotides, metabolism, protein binding and/or drug accumulation. We found, however, that the hepatotoxic effect appears to be sequence dependent, is not dependent on chemistry and that blocking RISC loading mitigates toxicity, all suggesting that non-rNAi effects are not a major driver.

Second, we thought that competition for the RISC machinery may play a role in the hepatotoxicity that we observed in rats. However, we found little evidence suggesting that to be the case, and that, in fact, different sequences, whether good or bad actors, show similar RISC loading across different studies and that the introduction of a short oligonucleotide designed to aggregate the activity of RISC-loaded siRNA, a so-called Reversir molecule, can also mitigate toxicity.

Finally, we have gathered evidence to suggest that off-target activity may be the major driver and that an enrichment of seed-mediated down regulation of off-target is driving hepatotoxicity.

So we're currently on Slide 17. In order to minimize off-target activity, we have developed a new approach, which we are calling our ESC+ conjugate platform. We believe that this approach 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 a microRNA-like mechanism. An approach that has been previously reported in the literature for the in vitro suppression of off-targets invokes the use of an additional chemically modified oligonucleotide in the antisense seed region to selectively destabilize off-target binding and, therefore, diminish the undesired seed-driven off-target activity.

Here, 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 are able to maintain on-target potency in vivo. Since these conjugates require exquisite metabolic stability for in vivo activity, it will be very important to ensure that additional modification has a minimal impact on stability. And second, we want to ensure that our ESC+ designs minimize off-target activity arising from seed matches to the 3'-UTR.

A potential solution for the reduction of seed-mediated off-target effects is a use of a thermally destabilizing modification like glycol nucleic acid, or GNA, within the seed region of siRNA. GNA is an acyclic, 3-carbon nucleotide analog, with a single stereo center that is thermally destabilizing in the context of RNA. In work that we published last year, we have shown that GNA can be well-tolerated in the siRNA conjugates, suggesting that this modification may be well suited for seed-pairing destabilization despite the shorter phosphate-to-phosphate and base-to-backbone distance in an RNA duplex.

In order to determine how the position of GNA impacts off-target activity, we utilize in vitro RNAseq to evaluate the walk of a single GNA nucleotide across the seed region of a model siRNA. In the MA plot on the top left, you can see that after dosing the parent ESC conjugate, that there are a lot

of -- there are a large number of differentially expressed genes shown in red or blue. Those are genes which are up or down regulated with statistical significance compared to the mock control. The introduction of GNA across positions 3 through 8 shows a position-dependent reduction of off-target knockdown while maintaining on-target potency.

While the introduction of GNA in positions 3 through 5 does not appear to have a significant effect, as seen on the MA plot, GNA placed at antisense positions 6 through 8 shows a strong reduction in off-targets and a generally much quieter profile in vitro.

So here now on Slide 20, let's take a look at the effects of GNA incorporation on inherent potency across a larger panel of sequences. On the top bar graph, we show the data from an in vitro screen across 47 sequences as ESC or ESC+ targeted against a single-target mRNA. This screen demonstrates that there are cases in which GNA is well tolerated when introduced into the seed region of the antisense strands, either maintaining or improving potency. And there are some cases in which it is not well tolerated.

Looking at the overall trends across a larger set of sequences, and target shows that there is a general shift towards lower potency after the incorporation of GNA. However, what we have learned is that we are able to find potent sequences for a given target and, if needed, individually optimize the design of a sequence of interest to fit our target product profile.

Next, we wanted to ensure that we could maintain on-target potency in vivo with our ESC+ conjugate. Here, these 2 sets of graphs show the pharmacodynamics of both ESC and ESC+ designs across a series of dose levels in mice and rats. As you can see, the ESC+ design maintains potency with only a slight difference in the effective dose required to provide 50% knockdown, or ED50, of the target gene.

A follow-up experiment compared the in vivo RNA seed profiles of an ESC versus an ESC+ conjugate in rats. This experiment, conducted across dose levels from 3 milligrams per kilogram up to 100 -- 120 milligrams per kilogram, demonstrates a much cleaner off-target signature as demonstrated by the MA plot on this slide. In fact, the ESC+ conjugate only starts to show differentially expressed genes at the highest dose of 120 milligrams per kilogram, whereas all other doses show a clean profile, resembling the controlled group of rats.

We additionally wanted to look at the impacts these ESC+ designs had on the therapeutic index of 2 sequences, both of which had shown hepatotoxicity as ESC conjugates in rats. 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 side of each of the graphs, you can see the pharmacodynamic profile of our conjugates. The blue dots indicate the level of silencing we observed at the specified dose for both ESC and ESC+ contributes. On the right side of each graph, the red dots indicate the toxicity grade associated with the specified dose for both ESC and ESC+ conjugates. While it is clear that the effective dose does not differ greatly between the ESC and ESC+ conjugates for these 2 sequences, the NOAEL has greatly shifted to a higher dose level for the ESC+ conjugate, and therefore, improved the therapeutic index in both cases. These improvements of over fivefold in the therapeutic index further improves our already wide dosing window where we can achieve effective silencing without causing toxicity.

Finally, an additional test for our ESC+ conjugates was to determine how well they translate into higher species. Here, we show that across 3 different sequences and several different GNA positions that our ESC+ conjugates maintain a pharmacodynamic profile in nonhuman primates that is very similar to our ESC conjugates, thereby providing us with confidence for their subsequent translation into humans for potency.

With this extremely encouraging data, we have transitioned into a new generation of conjugates, which, as mentioned before, are termed ESC+, as shown here on the right. These designs combine our Enhanced Stabilization Chemistry with seed-pairing destabilization to further improve the specificity and therapeutic index of our conjugates.

In conclusion, we have shown that RNAi mediated off-target effects and non-RISC competition or non-rNAi effects are important drivers of hepatotoxicity for a subset of conjugates in rodents. To mitigate these off-target effects, we have developed our next-generation ESC+ platform, which utilizes seed-pairing destabilization to further improve our already wide therapeutic index. This ESC+ platform is being applied to all future clinical candidates, and several programs are already advancing towards the clinic with the first trial set to begin in 2018.

With that, I'd like to turn it over to Kirk, who will provide you with an exciting update on CNS delivery of siRNA.

Kirk Brown

Thank you, Mark. I'm excited to provide an update today on the advances made in our platform to address CNS delivery. There are currently no therapies to prevent or reverse neurodegenerative diseases. And numerous dominantly inherited neurodegenerative diseases exist, including Alzheimer's, Parkinson's, Huntington's disease, ALS and spinocerebellar ataxias come to mind or many genetically validated targets are known but they lack a disease-modifying therapy. A therapeutic siRNA directed to a disease-causing CNS expressed gene represents a new frontier for RNAi. And we do anticipate superior potency, duration of activity as well as systemic safety versus antisense oligo.

Alnylam has made great advancements in conjugate-based delivery over the past nearly 15 years, as Mark and Martin have described. To build a potent and durable siRNA, one needs to combine an ability to select highly potent sequences; a stable optimized linker; and finally, a ligand with the capability to deliver to target cells of interest. These innovations are evident through publications back to our first gene silencing through systemic administration in 2004 to improve stabilization chemistry, to advance in vivo performance through hepatic targeting through GalNAc conjugation and, most recently, with improved specificity and reduced hepatotoxicity with our ESC+ technology.

These improvements are seen in the figure at the bottom right, revealing clear improvements in conjugate potency with each of these advancements.

Now to endeavor outside of the liver into extrahepatic tissues, we applied lessons learned from our past innovations to enable the delivery and subsequent durable knockdown within the CNS space. First, we need to select the CNS target of interest and identify a potent sequence. For our CNS proof of concept, we identified targets that are ubiquitously expressed in CNS, but importantly, also in the liver. This allowed us to perform lead selection in vitro and in vivo using our traditional GalNAc conjugate chemistry prior to moving into the CNS space.

In the figure, you can see the gene expression of this particular target in the CNS in yellow and also the expression identified there in the liver.

On the next slide, for the in vitro efficacy screen, 45 siRNAs were selected and tested in primary mouse hepatocytes at 2 different concentrations. At 24 hours, target knockdown was assessed using qPCR. Two sequences revealed extremely highly potent knockdown.

Next, we move in vivo into mice with the most potent sequences from the in vitro screen. Seven sequences were taken forward. Importantly, these used GalNAc chemistry for liver delivery to assess in vivo knockdown at post-dose days 7 and 21. siRNA, too, was selected to move forward with the CNS study because it showed the most potent and durable activity with sustained knockdown in the liver up through day 21.

On the next slide, I'm showing the intrathecal study design for our novel conjugates. This was a single-dose time course in rats. We selected 2 targets for the sake of sequence specificity. This was a 900-microgram single IT bolus injection. We sacrificed animals at post-dose days 2, 4, 7, 14 and day 28 and then evaluated drug levels as well as knockdowns. Specifically, we analyzed 3 regions of the spinal cord, lumbar, thoracic and cervical, the prefrontal cortex, cerebellum as well as the remaining brain. We also looked at drug levels in the CSF and plasma. We looked at histology on day 28. And lastly, in addition to mRNA knockdown and tissue distribution of our compounds, we confirmed RISC loading through Argonaute immuno-precipitation experiment.

On the next slide, we're looking at the amount of knockdown across 3 regions of the spinal cord. Starting with the lumbar cord on the top left, we're comparing 3 different conjugates against a single CNS target, which we're calling target 1 versus a controlled siRNA. What you see is there's rapid onset of RNAi activity within the first 48 hours, with maximum knockdown of greater than 80% target reduction by day 7 with conjugate 3. This observed knockdown is sustained through to the end of the study on day 28. We see somewhat comparable activity with all 3 tested conjugates in this tissue.

As we move to the thoracic and cervical spinal cord regions, we see that conjugate 2 and 3 show more robust activity. Conjugate 3 also reveals more rapid onset over the other 2, as we saw in the lumbar cord region.

On the next slide, we take a closer look at the regions more distal to the side of the delivery. On the top left, we observe knockdown in the cerebellum for all 3 conjugates. The timing of the onset of the knockdown differs for each conjugate tested. Conjugate 3 again reveals the most rapid onset

of activity with peak knockdown seen at day 7, reducing the message by about 65%. The knockdown is sustained through to the end of the study in the cerebellum. Conjugate 2 shows slower onset but also reaches a similar degree of knockdown as conjugate 3.

In the frontal cortex, we observed conjugate 3 producing 50% knockdown by day 7.

And lastly, in the bottom center, we observed 40% to 50% sustained knockdown in the remaining rat brain for conjugates 2 and 3.

On the next slide, a single conjugate targeting a second independent ubiquitously expressed gene is shown compared to its mass control. On the top left, we are showing all 3 regions of the spinal cord on a single figure. Again, we see rapid onset of activity and 50% knockdown within 48 hours, with sustained knockdown of 75% throughout the duration of the study in all 3 regions of the spinal cord.

Moving to the brain on the top right. Here, showing 3 regions of the brain on a single figure, we observed 65% knockdown, which is maintained throughout -- to day 28.

On the next slide, Slide 37, we take a closer look at the benefit of the conjugate over an unconjugated control siRNA. In the top figure, we compare the clearance of an unconjugated siRNA in gray with that of a conjugated siRNA in blue. Both show rapid clearance from the CSF space.

A comparison of drug levels is shown in the middle figure. What is clear is that the conjugated siRNA reveals much greater uptake and stability in the brain over the unconjugated siRNA tested. siRNA concentrations in the mid-single-digit microgram per gram range were observed.

Moving to the bottom figure. Increased drug levels of the conjugated siRNA in the brains resulted in more potent knockdown on the target gene.

On the next slide, we take a closer look at the tissues themselves. On the top figure, we used an siRNA antibody to detect compound in various regions of the brain. Here we're showing strong uptake in Purkinje cells and neurons.

At the bottom of the page, looking at a cross-section of the spinal cord, we stained for target protein in control-treated and conjugated siRNA-treated animals. On the left, there is dark staining of the target protein from the control. On the right, following treatment with a conjugate against target 1, we observed 65% reduction in protein levels by IHC, confirming the mRNA analysis shared previously. And you can see that the knockdown is pretty evenly distributed across the white and gray matter of the spinal cord.

In conclusion on the next slide. We see rapid clearance from the CSF following IT bolus injection. Tissue uptake was observed across all CNS tissues examined, with drug levels in the low to mid-single-digit microgram per gram range. Robust siRNA endurable silencing of the target mRNA was observed across all the CNS regions following a single IT administration. And importantly, the silencing extended out through the end of the study on day 28.

The enhanced tissue uptake in activity for siRNA conjugates compared to the unconjugated siRNA was quite clear. And importantly, conjugates administered by IT dosing was extremely well tolerated.

On the next slide, we share the Alnylam CNS pipeline strategy, which, as I laid out at the start, will focus on genetically validated, CNS-expressed target genes, ideally with a biomarker that's detectable within the CSF and trackable. And from there, we plan to identify a candidate by the end of this year moving into our first IND in late '19 to early '20, with multiple INDs per year starting in 2020.

Christine Regan Lindenboom - *Alnylam Pharmaceuticals, Inc. - VP of IR & Communications*

Thanks, Kirk. We will now open it up for Q&A. (Operator Instructions)

QUESTIONS AND ANSWERS

Vasant Jadhav

All right. Well, thank you, everyone, for joining us for this RNAi roundtable, the first one for this year. Just as a reminder, we are going to focus today on -- more on the platform research than on clinical part of this work. And questions related to the clinical programs will be answered in the subsequent sessions. So we encourage you to submit the questions, as Christine mentioned. There are a couple that have come in, and they are related to AAT02 and, in general, about where are we progressing with ESC+ conjugates in clinic, and is there any delay with these ones.

Mark Schlegel

Yes. Vasant, I think that's a good question. So regarding AAT02, we have reported clinical data -- or preclinical data, I'm sorry, on AAT02 last year at our roundtable series. So I would encourage you to look at that. In terms of new data, I think, as it's coming along, we would potentially also report that. And in terms of the IND for AAT02, we plan to file that this year and start that trial this year.

Vasant Jadhav

Thanks. Thanks, Mark. And just to confirm that, there is no delay with the ESC+ programs. We are moving full force ahead. As we guided earlier, it will be later this year for the AAT02. And again, we're moving full force ahead in this one.

All right. So one other, let's see what's the second question here. "Are patients being dosed with the ESC+ technology yet?"

We've sort of discussed that just now. And as we said, the AAT02 will be starting later this year, so not yet right now.

All right. So another one. Based on the data that we have shown so far with the CNS delivery and maybe, Martin, you can answer this one, "So what benefit do you expect for CNS delivery or other oligo technologies? Because we know there are other oligo technologies, which are also in the CNS space. You're now entering in this space. How do you think we'll be differentiating with the other technologies?"

Martin Maier

Yes. So first of all, let me say it's really wonderful to see the life-changing impact that the ASO drug, Spinraza, has had on patients with spinal muscular atrophy, and it's really encouraging for the whole field. I think for targets requiring modulation of the pre-mRNA through changing the splicing pattern, for instance, technologies such as the antisense are well suited. Now we believe that as far as silencing of disease-causing genes is concerned, we believe our technology is actually quite well suited. And based on the advances we have made in the conjugate design, we expect superior potency and duration and systemic safety profile for our siRNAs for CNS applications. I think also maybe worth mentioning is that these drugs -- considering that these drugs will be administered via the intrathecal route, the -- we believe the duration of effect will be actually very critical here for the drugs to be viable.

Vasant Jadhav

Yes. Thanks, Martin. So another question has come, and this is related to CNS. "Wondering how you would prioritize your choices of pipeline disease candidates. What factors will lead to choosing one versus another? Any color would be useful here." Maybe, Kirk, if you could address this one.

Kirk Brown

Certainly. I mean, we're going to focus, as I said in the presentation, on genetically validated targets in the -- of CNS expressed genes, preferentially with a known and detectable biomarker. Now that could include things such as Huntington, obviously, because it's a target with a biomarker and potentially some derisking has occurred. But there are also numerous other genetically defined neurodegenerative diseases that also come to

mind, which I outlined in the first slide of the presentation. A great deal of work is going on at the moment to select our first target, and we do intend to select that target by the end of this year, and we'll be able to present a little bit more information on that later part of this year. But at the moment, we're not disclosing anything about which targets we're focusing on at this time.

Vasant Jadhav

Thanks, Kirk. So another question has come up, and it's on the ESC+ approach. So Mark, based on the presentation and the data that we have, as you know, we shared the data last year, and now we have this new update. So just to summarize, what are the key things have we learned about the ESC+ technology since last year?

Mark Schlegel

Sure. I think one of the biggest things that we've learned about ESC+ is essentially on the -- along the lines of generalizability. So we've shown that these ESC+ designs are both potent and durable just like their ESC counterparts. We've shown this generalizability across multiple targets. And also importantly, we've shown it in nonhuman primates, so now across species as well. Second is that we've also done a lot of work to further improve our ability to assess off-target potential of our siRNAs, and we feel very confident about the translation of this ESC+ technology to humans and also the improved specificity in humans as well.

Vasant Jadhav

Yes. Well, thanks, Mark, for that update. Again, just going back to the CNS question. And this one is on how deeply does the CNS delivery can penetrate into deeper brain structures such as the basal ganglia. Kirk?

Kirk Brown

So we were able to see uptake in the basal ganglia as well as very deep regions within the hippocampus. All 3 regions of the hippocampus were covered with siRNA. We have a very sensitive means to identify our compound within target tissues. And at the moment, all regions of the brain have shown uptake of our siRNA, and that includes both of the conjugate-targeting sequences presented today.

Vasant Jadhav

Thanks, Kirk. Another question that has come up is, "Do we expect improved duration of activity with the ESC+ molecules?"

And maybe I will take that one. So the pharmacodynamic profile looks very similar between the ESC and the ESC+ molecules, as Mark showed earlier. The key improvement, though, with the ESC+ chemistry is, what we expect is the improved safety profile. We already have a wide therapeutic index with ESC+, which should be even better. And we expect this to happen because of substantially reduced off-target effects that will come with the ESC+ designs. So based on our experience with other molecules at higher doses, our expected increases in therapeutic index with ESC+, so take these 2 together, we think that we have the potential to further extend the duration for programs where it is needed with the ESC+ design. So in principle, it is visible to increase the duration with the ESC+ candidates and potentially have a dosing once every year, and that would be fully very exciting.

All right. Let's go back and see what other questions that we have received. And there is one here, which is, "Do we expect AAT02 potency similar to TTRsc02?"

So this is very similar to what I talked about before. Now TTRsc02 is an ESC candidate. AAT02 will be an ESC+ version. We do see differences in the potency and the duration of the molecules depending on the target, but we are very optimistic that AAT02 will have sufficient potency and duration to have very attractive profile.

All right. So moving on. So Kirk, you explained and sort of introduced the CNS work that we are now -- Alnylam is moving into. And as we look back and say, Alnylam has been very successful in focusing on the liver delivery, and we have this great pipeline with 6 active programs, waiting on the patisiran, all these are liver-based programs. So how is the CNS work going to affect the liver pipeline moving forward? Maybe it'd be good to get that understanding from you.

Kirk Brown

Sure. So we continue to have many hepatic target opportunities, which means there will be a balance of CNS and hepatic target programs advancing into the clinic in 2020 and beyond. Prior to our focus on solving liver delivery, we did do extensive work in the CNS area. But unfortunately, at that time, the siRNA design was not as advanced as it is today. And same for the liver, we believe it plays a critical role in enabling our safe and efficacious target silencing in the CNS. So potentially, we're going to be having a balanced approach moving forward looking at both hepatic and extrahepatic targeting.

Vasant Jadhav

Thanks, Kirk. Yes, indeed. There are many, many opportunities that we have in liver. We'll continue to look at them. CNS is just another area where we can find interesting opportunities for the future pipeline beyond 2020. And we'll make a call based on the profile of these molecules, what's the best one to move forward.

All right. Just reading through the questions, and there's one here on CNS. It's about the proprietary nature of the conjugate for the CNS. "When will this be published?" Kirk, any insight on that one? I sort of know the answer, but what are your thoughts, Kirk?

Kirk Brown

Right. So the conjugates we use for the CNS application obviously incorporate chemistry advances that we've developed so far for the liver. But at this time, the conjugates that we use, we are not going to be disclosing them at this time. They're obviously not GalNAc. But the nature of them, we're not going to disclose at this time. How soon we'll publish that, I think, is still a topic of great internal debate.

Vasant Jadhav

Yes. Well, again, thanks, Kirk.

Just looking at the questions. There's one more here. The question is -- I'm just reading it out. "So I'm interested in improving the binding properties of GNA through chemical modifications. I'm wondering in general how chemical modifications on GNA monomers will reduce or increase binding affinity towards targets?" So Mark, will you please take this one?

Mark Schlegel

Yes. So as I had outlined, one of the key features of GNA is that it's actually a thermally destabilizing modification, especially in the context of an RNA duplex. So in terms of improving the binding properties of GNA, that's probably not something necessarily that we're looking at, at the moment.

Vasant Jadhav

Okay. GNA is a great example, though. And one of the questions that has come up is, "What kind of chemical modifications we are exploring and continue to explore."Any insight on that one would be great." So Martin, would you like to comment on that one?

Martin Maier

Sure. We obviously have a very extensive chemistry toolbox, which -- of nucleic acid modifications, and we use them judiciously at the sites under the points where we want to address specific issues, so let's say, for instance, to further enhance the in vivo performance, the stability of our conjugates. And the GNA is a good example here, which we have employed, specifically here to address the specificity questions and -- at a single incorporation into our siRNAs. So we continue really to expand our chemistry toolbox and tool set, which we use, of course, only after really extensive derisking in preclinical studies.

Vasant Jadhav

All right. Thanks, Martin. Another question here is on, "Could you tell us more about the conjugation chemistry for CNS targeting? How does it differ from ESC+? "

And maybe I will chime on this one because I think we addressed the first part that, at this point, as Kirk mentioned, we are not going into the details over the conjugation chemistry of the CNS conjugate. But just -- we're just confirming that the conjugation chemistry used for CNS, that is a different thing than the ESC+ design itself. ESC+ design means the use of seed destabilization modification like GNA to reduce the off-target effects. So that's the basic design -- the siRNA design that we have. In terms of the conjugates, the ligands, which take the siRNA for particular tissues, those are going to be different depending on the tissues. For example, in liver or in hepatocytes, it's GalNAc. For CNS, it will be a different one. At this point, we're just not revealing it.

All right. We'll keep moving on. Since there has been -- again, Martin, this might be coming your way. "Since there are examples of antisense oligonucleotides working well in the CNS space and they still have extensive PS linkages, which are known to cause significant toxicities for the ASOs, so why don't you think that similar things could happen with the PS linkages that you have in the siRNAs?"

Martin Maier

All right. Yes. So maybe to -- if we -- as we compare across those 2 different therapeutic platforms, it's important to highlight the structural differences maybe first. So antisense oligonucleotides typically contain a full phosphorothioate backbone. In our case, the siRNAs have -- only the phosphorothioates had very selective -- selected positions within the siRNA, namely here, in our case, at the (inaudible) of the 2 strands and only add up to maximally 6 phosphorothioates [and not in a switch]. So we believe some of the toxicity findings from ASOs are actually related to the presence of this phosphorothioate backbone in the protein binding, which comes along with this chemical modification. It's also important that if we think about these physical -- chemical features that single-stranded antisense oligonucleotides will have a different interaction -- different possibilities of interaction with proteins than, say, a double-stranded siRNAs with a much more defined secondary threshold -- structure. So overall, we believe that we will have -- we won't see -- with our designs and with our siRNAs, we won't see and we haven't seen any signs of thrombocytopenia, prone inflammatory effects or renal toxicities in none of the programs, which we have moved forward so far. So we believe that, actually, our profile is quite safe and maybe superior to a profile where you do have these highly modified phosphorothioate backbones.

Vasant Jadhav

Great, great. So again, coming back to the CNS. There's one question on why does knockdown with the CNS-targeted agent went faster in the frontal cortex than the other brain structures? So Kirk, do you think this is sort of an experimental variation? Or do we really see the difference between the duration of effect here between the different brain regions?

Kirk Brown

Right. It's a great question. So in the first test that I shared with the multiple different conjugates targeting the first target, the first message, we see a subtle waning in the frontal cortex compared to cerebellum and the remaindering brain. However, in the second independent targeting

siRNAs, that molecule had slightly different chemical modification. And we believe that, that subtle difference allowed us to see much more robust and consistent knockdown across all regions of the brain, which was shown in Slide 36. So the second independent targeting siRNA, we see extremely comparable knockdown in cerebellum cortex and the rest of brain in this particular study, and that compound is most likely the chemical composition and conjugate that we would move forward with.

Vasant Jadhav

Yes. All right. I think another question. It's pretty clear that CNS is on people's minds. More and more questions are coming on that one. I believe we've shown the data for the next question that has come up, but maybe Kirk can elaborate that on further. So what's our sense for how -- in particularly those CNS siRNAs distribute through the brain? For example, is it moving through the CSF and along the perivascular spaces? Is it diffusing through the brain parenchyma? Is it taken up by cells and distributed (inaudible)? So Kirk, I know we don't have a whole lot of data on this one, but maybe you can elaborate a bit more what you've seen so far.

Kirk Brown

Certainly. Looking closely at the levels of drug in the various spinal cord regions, we do see a subtle gradients of uptake, so closer, more proximal to the side of dosing. In the lumbar region, we saw greater levels of drug taken up there. And as we move up the spinal cord, we saw slightly less and less and then less so also in the brain, but still clearly enough to provide us with robust and stable knockdown. We do believe there's likely a diffusion-based event occurring. However, follow-up image analysis in tracing of our siRNAs are gearing up. So we'll have more data this year on that as we investigate this further.

Vasant Jadhav

All right. Thanks, Kirk. Another is sort of a broad question. "With the seemingly near-term breakthrough with enhanced delivery, where do you see RNAi in 3 to 5 years?"

and I will -- I'll take this on. Maybe Martin can chime on this one. And this is really the question which when we sort of look back and say -- see where we have come from, it's been a long journey. We just recently celebrated the 16th birthday of Alnylam. Kudos. And it took a long time. It took a long time to achieve -- to be at this place where, after 16 years of effort, we are now awaiting the approval of first candidate. So even when you have the breakthroughs, it takes time for these ones to translate. Having said that, we are super excited that the progress that we have done, the advances that we have done over the years, including for liver delivery, that will benefit us for anything else we do now in other tissues like in CNS. So what will happen in 3 to 5 years is not something that I would answer, but I would say for myself and maybe for the team that we are thrilled and just say the opportunities are huge for RNAi. It's finally arriving after all these Nobel prize-winning science, all the promises, we are on the verge on delivering on the promise of RNAi therapeutics and taking the lessons from antibody field where the monoclonal antibodies, after the discovery, took long time to come up with the first drug. But now they are the major class of drugs. We hope RNAi becomes the same way as the monoclonal antibodies in the future. Martin, if you want to add something.

Martin Maier

Yes. Thanks. I mean, I totally agree, Vasant. I think from a -- if you think about where we started, and as you said, the evolution of the clinical design has really enabled us to make great progress not only in our preclinical mouse, but now translating actually to the humans. And we -- what we have -- from a chemistry -- if you ask me from a chemistry perspective, I would say we can always further optimize all designs and we will. We will try to make them safer. We will try to make them more potent. I think there is -- a lot has been done to date, and we are very happy with what we see, especially with the ESC+ conjugates. And we are very excited to see the translation of this new chemistry in humans soon. As we mentioned, it's going to hopefully go into patients by the end of this year. So we will get some readout on ESC+ chemistry by early or mid-'19. And in terms of when you think a little bit further across our pipeline, we will -- we expect to have 3 marketed products, which is very exciting, by 2020. So this is

only 2 years away. And that will be very exciting, I think, for the field and obviously for us as we see the -- our drugs now reaching patients and reaching the market.

Vasant Jadhav

Exactly. Yes, I will concur. I think there was also this report of recognizing 2017 as the year of advanced medicines, and we believe, with RNAi, we lead that kind of phenomenon. It's absolutely amazing to see the progress in other fields as well. It's sort of all coming together. So 3 to 5 or 10 years from now, advanced medicines will be the thing of the day. And we're looking forward to that, and we are super excited to be part of it.

All right. So just going back to the questions. So this is another one. Maybe, Kirk, going to the CNS. We sort of touch based on this one. "Do we expect to be able to achieve targeted uptake and illustrate knockdown in specific subset of cells in the brain?"

And we haven't really shown much data on this one, but our history of doing the targeted delivery in the liver, in hepatocyte specific gives us the confidence, right, that something like this is feasible. But at this point, we are not getting into the details of this one. Anything else you want to add?

Kirk Brown

Yes. I mean, right now, we're looking at a lot of colocalization work to try to evaluate all the different cell subtypes that we're overlapping with siRNA uptake. And again, we'll be able to share more of that information most likely later on in this year.

Vasant Jadhav

Yes, yes. So there's another question. "Would we be looking or would Alnylam be looking into the delivery in CNS using AAV vectors that have tropism to CNS?"

I think, just head on, right, that we are -- our CNS delivery data is using the conjugates. So it's not a remediated delivery at all. These are defined molecules for CNS delivery, and that's what we are focusing on. Are there going to be any other forms of the delivery other than intrathecal? Well, this is the first kind of delivery that we have done with the intrathecal delivery. But as the field grows, as our experience -- we gain the experience in this one, we'll explore other means. But intrathecal delivery is the one that we are going right now.

Okay. All right. Just going through more of the questions. Let me -- okay. So this one that we see on the screen, just reading it first for myself before I read it out to everyone. All right. I'll -- it's a long question. I'll just read it out. Maybe, Kirk, you and others can comment on that one. So the question is, "We have heard previously that single-stranded antisense, if it is better through the brain than double-stranded siRNA. Is this the case?" Kirk?

Kirk Brown

I think, previously, the people have -- were under the impression that single-stranded ASOs do penetrate deeper into brain regions. And some groups have shown that with -- certainly, with Spinraza, it's got very good deep brain penetration. In our case, I mean, there are elements of stability and chemistry as well as the conjugate itself that allow us better tissue distribution than, I think, had been previously assessed with natural siRNA. So my thought is earlier studies probably did not use the chemistry and the conjugates that we're using here to see the uptake that we're observing. Yes. But we have not done a head-to-head comparison at this time, so I wouldn't want to say one versus the other.

Vasant Jadhav

Yes, exactly. We have not done the head-to-head comparisons. So there is no reason to believe that our siRNA conjugates will have any inferior distribution than with the other oligo modalities. Martin, if you want to add something.

Martin Maier

Just to add, one of the features of our siRNA without conjugation actually is that they can be modulated fairly well by adding a conjugation ligand to them, like we did very successfully in the liver with GalNAc because, by themselves, they really don't have any nonspecific delivery uptake. They're, in a way, neutral molecules, which require some sort of ligand for them to enter the cells, which allows you a little bit more flexibility, I think, in terms of if you are after, say, specific cell types. As we have shown in the hepatocytes of the liver, you may be able to modulate this delivery with a single ligand better than if you had sort of a mixed mode of delivery.

Vasant Jadhav

All right. Well, thank you all for very lively questions. We have just about come to the end of our presentation. And Christine, if you want to close it off.

Christine Regan Lindenboom - *Alnylam Pharmaceuticals, Inc. - VP of IR & Communications*

Sure. Thanks, Vasant, and thanks to the rest of the speakers as well and to you for your questions. This concludes our RNAi roundtable for today. The replay and slides will be posted on the Capella section of the Alnylam website later today and at alnylam.com/capella with a transcript to follow shortly thereafter. We hope you can join us for our next RNAi roundtable on Tuesday, July 24, at 10 a.m. Eastern, as we discuss givosiran in development for the treatment of acute hepatic porphyrias as well as for other events in the series as shown here on Slide 42. For more details, please visit alnylam.com/capella.

Thank you, everyone. Have a great day, and goodbye.