

I would like to thank the Usher Coalition for the invitation to present this USH Talk. My name is Hester van Diepen, and I'm the project lead of the Usher Development Program at ProQR. ProQR is a biotech company based in Leiden, in the Netherlands. And today I'm going to give you an update about our Usher Syndrome Type II Development Program.

As ProQR is a public company, we are required to show you these forward-looking statements. ProQR is a young biotech company that started about five years ago. I have been very happy working here, for about two years, and focus entirely on our programs on Usher syndrome. We also develop many other programs for other severe genetic diseases, including cystic fibrosis, a genetic lung disease, epidermolysis bullosa, which is a devastating skin-blistering disease, and a number of eye disorders, including Leber congenital amaurosis, and, of course, Usher syndrome.

Most genetic diseases are rare, and only less than 10% of them have a treatment. We aim to treat the genetic diseases using RNA therapies which are specifically designed for a certain mutation. So it's personalized for patient-specific genetic profile. With RNA therapies, we aim to treat the underlying cause of a disease. We focus on those genetic diseases where we believe we can have a big impact on people's lives.

This slide provides a general overview of drug development, which is the process of bringing a new medicine or therapy to the markets once a drug candidate has been identified as a lead compound in drug discovery. Our Usher program is currently in the preclinical development stage, which is a stage of research that begins before the clinical trial and testing in humans can actually start. During preclinical development, the safe dose for the first-in-man study is determined, and a product safety profile is assessed.

The different phases of the clinical research will determine if a new test or treatment works and if it's safe before it will be approved by regulatory authorities and made available for use by the physicians.

Building on our early collaboration with Erwin van Wijk, at Radboud UMC in Nijmegen, we are developing a novel RNA therapy. The lead compound is called QRX-421 and aims to treat retinitis pigmentosa-- or, in short, RP-- in Usher syndrome type II. It is being developed as an injection which will be administered directly into the eye of the patient, and it is not expected to have an effect on other parts of the body that may be affected by the disease. The therapy is therefore aimed to treat retinitis pigmentosa associated with Usher syndrome type II.

Usher syndrome type II is caused by a mutation, or a mistake, in the DNA, and DNA is inherited from both parents. QRX-421 is designed to target mutations in a specific part of the USH2A gene, and this part is called "exon 13." The most common known mutations in exon 13 of the USH2A gene are c.2299delG and c.2276G to T.

A list of known other mutations can be found in a specific database which is mentioned on this slide.

We also have another program targeting mutations within the USH2A gene causing Usher syndrome type II, which is called QRX-411. And this program was covered in the talk of Erwin van Wijk, which was also published in USH Talks on this website. So therefore, today I will only discuss QRX-421, which targets exon 13 mutations in the USH2A gene.

So our treatment strategy is to repair the genetic mistake at the level of the RNA. In people without Usher syndrome type II, DNA is transcribed into pre-messenger RNA, which is translated into messenger RNA. And the messenger RNA is the blueprint for making proteins.

More specifically, the USH2A pre-messenger RNA is the blueprint for the production of the usherin protein. An usherin protein is present in photosensitive cells, and these cells are called "photoreceptors" and are better known as "rods and cones." The usherin protein is important for maintenance of these photoreceptors.

In people with Usher syndrome type II and having a mutation in exon 13 of the USH2A gene, the mutation leads to breakdown of the RNA, and the breakdown of the RNA leads to the absence of the usherin protein in the photoreceptors. In the absence of the usherin protein, the photoreceptors cannot be well maintained, and this leads to degeneration of the photoreceptors and eventually to the start of vision loss associated with Usher syndrome type II.

Our compound QRX-421 is an antisense oligonucleotide that binds to the pre-messenger RNA to induce skipping of exon 13. Our hypothesis is that skipping of exon 13 from the pre-RNA leads to shortened mRNA and shortened-but-functional usherin protein expression. We expect that the restoration of usherin protein expression in the photoreceptors will lead to normal maintenance of the photoreceptors, with the aim to restore the vision loss associated with Usher syndrome type II. This hypothesis was tested in experiments in the laboratory.

So to test the ability of QRX-421 to skip exon 13 from the USH2A pre-mRNA, we conducted experiments in a so-called eyecup model. This eyecup model is a three-dimensional model and resembles the human retinal cell layer, including the presence of the photoreceptors. This model can be generated by isolating fibroblasts from the skin from a patient. And these fibroblasts are then reprogrammed into inducible pluripotent stem cells. These cells can be differentiated into a retinal cell layer in a dish containing the specific mutation as well as the photoreceptors.

This eyecup model can be used to test the effect of treatment with QRX-421. Eyecup data has been used, instead of animal efficacy data, in successful regulatory submission in both the US as well as the EU.

Analysis of RNA of control eyecups, from a person without Usher syndrome type II, with no mutation, revealed

clear fragments containing exon 13 in the mRNA. In eyecups derived from Usher patients with the USH2A exon 13 mutation, similar RNA fragments were present containing the exon 13 mutations. These eyecups containing the mutation were then treated by adding a solution of QRX-421 to the eyecups, which was refreshed every other day for four weeks.

When we analyzed these eyecups, after treatment, we determined that, in addition to the exon 13 RNA fragment, another, slightly shorter fragment was present, without exon 13. And this data showed that QRX-421 is effective in skipping exon 13 at the level of the RNA in a laboratory setting.

To measure the effect of skipping exon 13 at the mRNA level on usherin protein expression, we conducted experiments in a zebrafish model. And this zebrafish model does have the exon 13 mutation in the USH2A gene, in a similar way compared to Usher syndrome type II exon 13 patients. In the zebrafish with exon 13 mutation--

Oh. I will repeat this slide. To measure the effect on skipping of exon 13 in the mRNA on usherin protein expression, we made use of a zebrafish model containing the exon 13 USH2A mutation in a similar way compared to exon 13 USH2A mutation which is present in patients. We treated these zebrafish with the zebrafish-specific antisense oligonucleotides and determined exon 13 skipped at the level of the mRNA. We determined a similar size of fragments compared to the experiments we conducted in eyecups and confirmed the exon 13 skip in the mRNA.

As a next step, we determined the effect of skipping exon 13 on usherin protein expression. So, in the control zebrafish, without the mutation present in the genome, we determined normal usherin protein expression in the retina. In the zebrafish having the exon 13 USH2A mutation, we did not detect any usherin protein expression in the retina. We treated these fish with zebrafish-specific antisense oligonucleotides and determined restoration of usherin protein--

I will repeat the slide again. To measure the effect on skipping of exon 13 in the mRNA on usherin protein expression, we performed experiments in a zebrafish model containing the exon 13 mutation in the USH2A gene. We treated these fish with zebrafish-specific antisense oligonucleotides and measured the effect--

Excuse me. I will start again. To measure the effect on exon 13 skipping of exon-- To measure the effect of skipping of exon 13 in the mRNA on usherin protein expression, we performed experiments in a zebrafish model containing a mutation in exon 13 of the USH2A gene. We treated these zebrafish with zebrafish-specific antisense oligonucleotides and determined an exon 13 skip at the level of the mRNA.

We determined a similar size of fragments compared to the experiments we performed in eyecups and confirmed the exon 13 skip. Next we determined the effect of skipping on exon 13 and usherin protein expression in the

retina of these zebrafish. In the control zebrafish, without the mutation, we determined normal usherin protein expression in the retina. In the zebrafish with an exon 13 mutation in the USH2A gene, we did not detect any usherin protein expression in the retina of these zebrafish. After treating these zebrafish with antisense oligonucleotides, usherin protein was restored in the retina.

The question remains, if the restored usherin protein in the retina of these zebrafish, of the exon 13 mutant zebrafish, is functional, and therefore we performed functional experiments in these fish. A functional measurement of the retina is recording of electroactivity in the eye of the zebrafish, with specific electrodes. And this is called an "electroretinogram recording," also known as an "ERG recording." Electrical activity is important for photoreceptor cells, in order to be functional. And the size of the amplitude of the ERG signal on the y-axis is a measurement of functionality of the photoreceptors.

The amplitude of the ERG signal in the retina of exon 13 mutant zebrafish is reduced, while the amplitudes of the ERG signal after treatment with zebrafish-specific antisense oligonucleotides was restored to normal levels. And this data indicates that the usherin protein is functional after exon 13 skip in a zebrafish model.

To date, much work has been done in the laboratory and in the zebrafish model to test the hypothesis that skipping of exon 13 in the USH2A pre-mRNA leads to shortened but functional usherin protein expression. By restoring functional usherin protein expression, QRX-421 aims to treat the underlying cause of retinitis pigmentosa associated with Usher syndrome type II. QRX-421 is intended to be administered by injections into the eye of patients.

While QRX-421 has shown promising results in the laboratory, it has not yet been tested in humans. There is still much we need to do and to learn about QRX-421 and its effects in the retina. ProQR hopes to move this program towards the clinic, but at this time it's too early to talk about expected timelines. But we are very happy, and we look forward to keeping the Usher community updated on our progress.

So I would like to thank the Usher community. I would like to thank Erwin van Wijk and colleagues at Radboud UMC, in Nijmegen. I would like to thank the regulators. And I would like to mention that, for more information, you can visit our website and stay updated on our progress on [www.proqr.com](http://www.proqr.com). Thank you.