QRX-421
Antisense oligonucleotide targeting Exon 13 mutations in the USH2A gene for treatment of non-syndromic RP and RP in Usher syndrome type II
RP associated with Usher Syndrome

Genetic cause of combined deafness and blindness

Symptoms: Pale optic nerve, thin vessels

Degeneration of Outer Nuclear Layer (ONL)

From Sandberg et al. 2008
Targeting Strategy

*In frame removal of Ex13 (642nt)*

Many pathogenic mutations in exon 13, including the two most common variants:
- G2299del (frameshift), causing Usher
- G2276T (Cys759Phe), causing RP

mRNA remains in frame (removal of 642 nt) Removes 4 laminin-EGF repeat domains (214 aa)

Strict requirement to show truncated (exon-13 deleted) mRNA leads to functional protein
QRX-421 for RP in Usher Syndrome

**USH2A exon 13 splice correction**

In wild-type cells, Ush2A protein enables protein transport through the connecting cilium.

In cells with the mutation, Ush2A protein is not active, hampering protein transport through the cilium.

Exclusion of mutated exon leads to restoration of functionality of Ush2a.
**QRX-421 for RP in Usher Syndrome**

**USH2A exon 13 splice correction**

**QRX-421:**
- Single stranded 21-mer RNA oligonucleotide
- P=S and 2’O-Me chemically modified for stability and uptake
- Designed to target USH2A exon 13 mutations
QRX-421 mediates exon 13 skip in vitro

Double exon-skipping in Ush2A is also present in wild-type cells

Wild type retinoblastoma cells treated with various AON’s
Patient-derived iPSC optic cups

Optic cup is an organoid model containing differentiated photoreceptor cells

Recoverin cone-arrestin

Details

Optic cups

Parfitt et al., 2016
QRX-421 mediated exon 13 skip in vitro and in optic-cups

Retinoblastoma

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<th>QRX-421</th>
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Exon 12 | Exon 13 | Exon 14

Exon 11 | Exon 14

In-frame

Out of-frame

2 months 3 months

Healthy Control

No AON  No AON

Ex13 mutation patient

No AON  No AON

QRX-421

QRX-421

2µM  10µM

Ex13 mutation patient

Ex12  Exon 13  Exon 14

Ex11  Exon 14

Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands
AON targeting Ex13 skip modifies mRNA and restores protein localization in Zebrafish retina

Restoration of Ush2a localization in zebrafish eyes

RT-PCR: Ush2a Ex13m +/-

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<thead>
<tr>
<th>Exon 12</th>
<th>Exon 13</th>
<th>Exon 14</th>
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<tr>
<td>Untreated</td>
<td>Treated</td>
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Ush2a antibody in fish retina showing localization at connecting cilia

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<tr>
<th>Wildtype</th>
<th>Ex13 mut</th>
<th>Ex13 mut + AONs</th>
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Co-staining with anti-centrin Ab showed Usherin localized at the connecting cilium

Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands
Loss of ERG in Exon 13 mutant Zebrafish

Reduced b-wave ERG amplitude in Exon 13 mutant fish

Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands
Restoration of b-wave ERG to wild-type level following Exon-13 deletion

Exon 13 deleted mutant zebrafish

Bands have been Sanger sequenced and confirmed to be Ex13-skipped

Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands
Efficient delivery of QRX-421 all retinal layers

Efficient delivery of QRX-421 to outer nuclear layer (photoreceptor cells)

- Immediate post IVT dose
- 7 days POST IVT dose

**Dose**

1 µl 25 µg/µl in C57/Bl6 mice
Summary: QRX-421 for USH2A Exon 13

mRNA profile restoration
- mRNA profile with exon 13 skip

Local (intravitreal) delivery to the eye
- Eye well validated target for oligo’s
- Efficient delivery to outer nuclear layer in the retina

mRNA profile restoration in eye-cups
- mRNA profile shows Ex13 Skip in patient-derived eye-cups

Restoration ush2a protein levels
- Significant increase in Ush2a protein levels

Functional restoration in Fish model
- protein and ERG restoration established

Clinical candidate selected
- QRX-421 selected as clinical candidate
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