Use of antisense oligonucleotides to correct DEB

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24-26 September 2017

EB2017, Salzburg, Austria
Declaration of interest

- Co-inventor on a patent of the UMCG on exon skipping.
- Signed a statement that I will not receive any share or royalties out of this patent.
- Collaboration with ProQR for *in vitro* experiments.
Puzzle like structure **COL7A1** gene

- 118 small exons
- 107 skippable exons (90%)
- Collagenous region encoding Gly-X-Y repeat (84 exons)
  - 67 exons dividable by 9 (80%)

Bornert et al., Mol Ther, 2016
Recessive dystrophic epidermolysis bullosa

Small amount of re-expression of type VII collagen could ameliorate the phenotype

Van den Akker et al., J Dermatol Sci, 2009
Antisense oligonucleotide-mediated exon skipping
# Exon skipping for epidermolysis bullosa

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Journal</th>
<th>Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>Goto et al.</td>
<td>J Invest Dermatol</td>
<td>70</td>
</tr>
<tr>
<td>2016</td>
<td>Bornert et al.</td>
<td>Mol Ther</td>
<td>13</td>
</tr>
<tr>
<td>2016</td>
<td>Bremer et al.</td>
<td>Mol Ther Nucleic Acids</td>
<td>105</td>
</tr>
<tr>
<td>2016</td>
<td>Turczynski et al.</td>
<td>J Invest Dermatol</td>
<td>73, 80</td>
</tr>
</tbody>
</table>
Patient homozygous for p.Arg2610Ter in exon 105

Bremer et al., Mol Ther Nucleic Acids, 2016
In vitro exon skipping of exon 105

Bremer et al., Mol Ther Nucleic Acids, 2016
In vitro exon skipping of exon 105

Bremer et al., Mol Ther Nucleic Acids, 2016
In vitro exon skipping of exon 105

Bremer et al., Mol Ther Nucleic Acids, 2016
In vitro restoration of type VII collagen production

Bremer et al., Mol Ther Nucleic Acids, 2016
Bremer et al., Mol Ther Nucleic Acids, 2016
In vivo exon skipping after systemic administration

- Five times a week 50 mg/kg of each AON via subcutaneous injections for 8 weeks at the tail base, i.e., approximately 7 cm distal from the skin grafts

Bremer et al., Mol Ther Nucleic Acids, 2016
In vivo restoration of type VII expression

Bremer et al., Mol Ther Nucleic Acids, 2016
In vitro exon skipping of exon 73 and 80

Exon 73

Exon 80

Turczynski et al., J Invest Dermatol, 2016
In vitro restoration of type VII collagen production

Turczynski et al., J Invest Dermatol, 2016
**In vivo restoration after local injection**

- 400 μg (20 mg/kg) was injected subcutaneously under the graft once or twice with a 7-day interval

Turczynski et al., J Invest Dermatol, 2016
Functionality of exon deleted variants

• Bornert et al. (2016) developed a screening tool for the functional analysis of exon-deletion in type VII collagen

• Deletion of exon 13 or exon 105 does not affect:
  - Conformation and thermostability of the triple helix
  - Binding to type IV collagen
  - \textit{In vitro} fibroblast adhesion and migration
  - \textit{In vivo} incorporation into the basement membrane zone

⇒ Altogether these results advocate AON-mediated exon skipping as therapeutic approach for DEB
What can we expect of exon skipping therapy?

- These data are still confidential and have therefore been removed. The manuscript has been submitted.

Bremer et al., submitted
Summary

• AON-mediated exon skipping was observed after local and systemic administration *in vivo*.

• Phenotypes caused by spontaneous dominant exon skipping could not be distinguished from phenotypes caused by other dominant DEB variants, whereas recessive exon skipping phenotypes were generally relatively mild in the spectrum of recessive DEB.

• It is anticipated that AON-mediated exon skipping for recessive DEB caused by bi-allelic null variants would lead to a clinically relevant improvement.
# Overview of FDA and/or EMA decisions on marketing authorisation of AONs

<table>
<thead>
<tr>
<th>Name</th>
<th>Disease</th>
<th>Mode of action</th>
<th>Chemistry</th>
<th>Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fomivirsen (Vitravene)</td>
<td>Cytomegalovirus retinitis</td>
<td>Inhibition of translation</td>
<td>DNA phosphorothioate</td>
<td>FDA: 1998 EMA: 1999</td>
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<tr>
<td>Pegaptanib (Macugen)</td>
<td>Neovascular age-related macular degeneration</td>
<td>Antagonistic binding to target protein</td>
<td>2’-O-methyl, 2’-fluorinated phosphorothioate</td>
<td>FDA: 2004 EMA: 2006</td>
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<tr>
<td>Mipomersen (Kynamro)</td>
<td>Familial hypercholesterolemia</td>
<td>RNase H induced RNA degradation</td>
<td>2’-O-methoxyethoxy phosphorothioate gapmer</td>
<td>FDA: 2013 EMA: Refusal 2013</td>
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<tr>
<td>Drisapersen (Kyndrisa)</td>
<td>Duchenne muscular dystrophy</td>
<td>Splice switching (exon skipping)</td>
<td>2’-O-methyl phosphorothioate</td>
<td>FDA: Declines approval 2016 EMA: Withdrawn by Applicant in 2016</td>
</tr>
<tr>
<td>Eteplirsen (EXONDYS 51)</td>
<td>Duchenne muscular dystrophy</td>
<td>Splice switching (exon skipping)</td>
<td>Phosphorodiamidate morpholino</td>
<td>FDA:2016 EMA: Under evaluation</td>
</tr>
<tr>
<td>Nusinersen (Spinraza)</td>
<td>Spinal muscular atrophy</td>
<td>Splice switching (exon inclusion)</td>
<td>2’-O-methoxyethoxy phosphorothioate</td>
<td>FDA: 2016 EMA: 2017</td>
</tr>
</tbody>
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Overview of FDA and/or EMA decisions on orphan designation of AONs for EB

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</tr>
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<tbody>
<tr>
<td>QR313</td>
<td>Epidermolysis Bullosa</td>
<td>Exon 73 skipping</td>
<td>--</td>
<td>FDA: 2017</td>
</tr>
</tbody>
</table>

- Topically applied, use of hydrogel
- Clinical trial to start in 2018

Challenges

• Personalised Medicine – mutations are scattered.

• High GC-percentage.

• Method of administration – for modified phosphorothioates after subcutaneous administration high percentage of skin reactions are observed in clinical trials – other method of administration (intravenous, topical).

• Optimising mucocutaneous delivery.
Acknowledgments

Department of Dermatology
UMCG, Groningen, the Netherlands
Marcel Jonkman
Jeroen Bremer
Daryll Eichhorn
Antoni Gostyński

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Hans Scheffer