Use of antisense oligonucleotides to correct DEB

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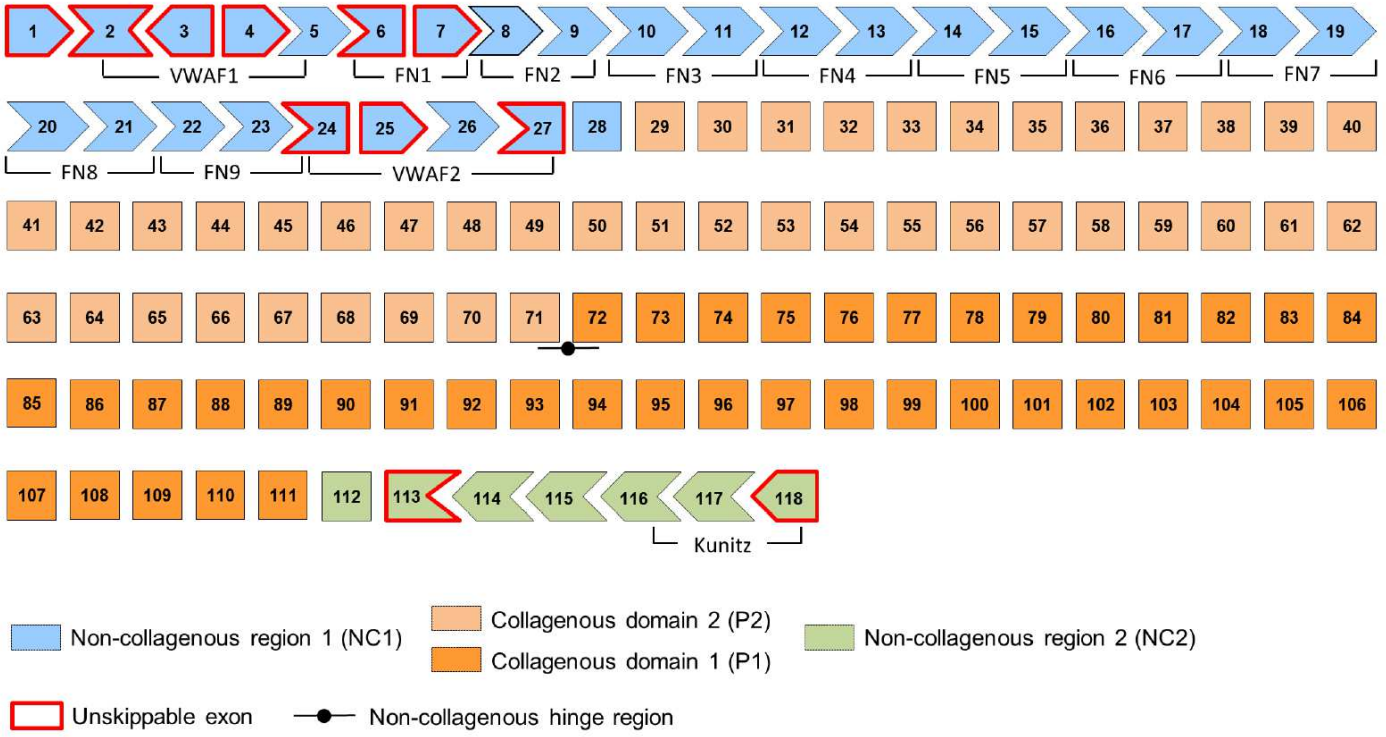
Declaration of interest

- 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

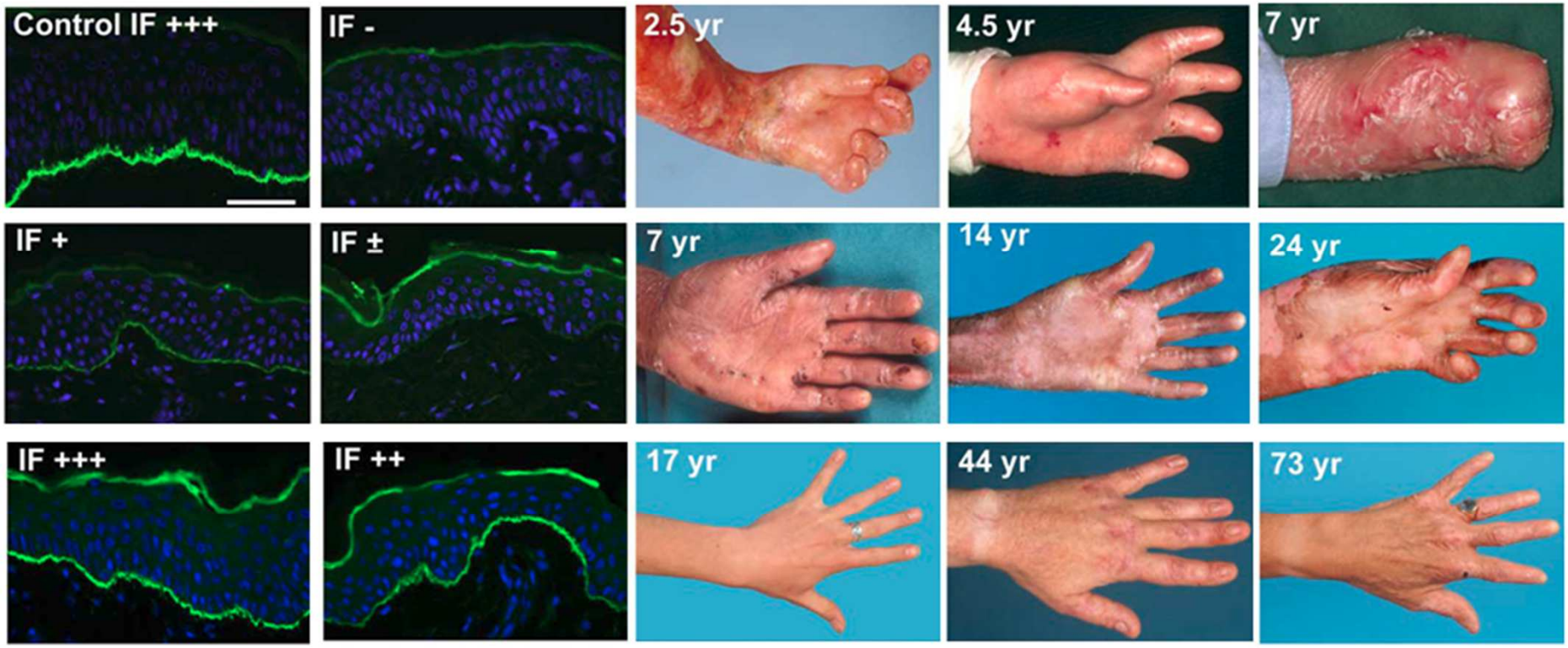


118 small exons

- 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

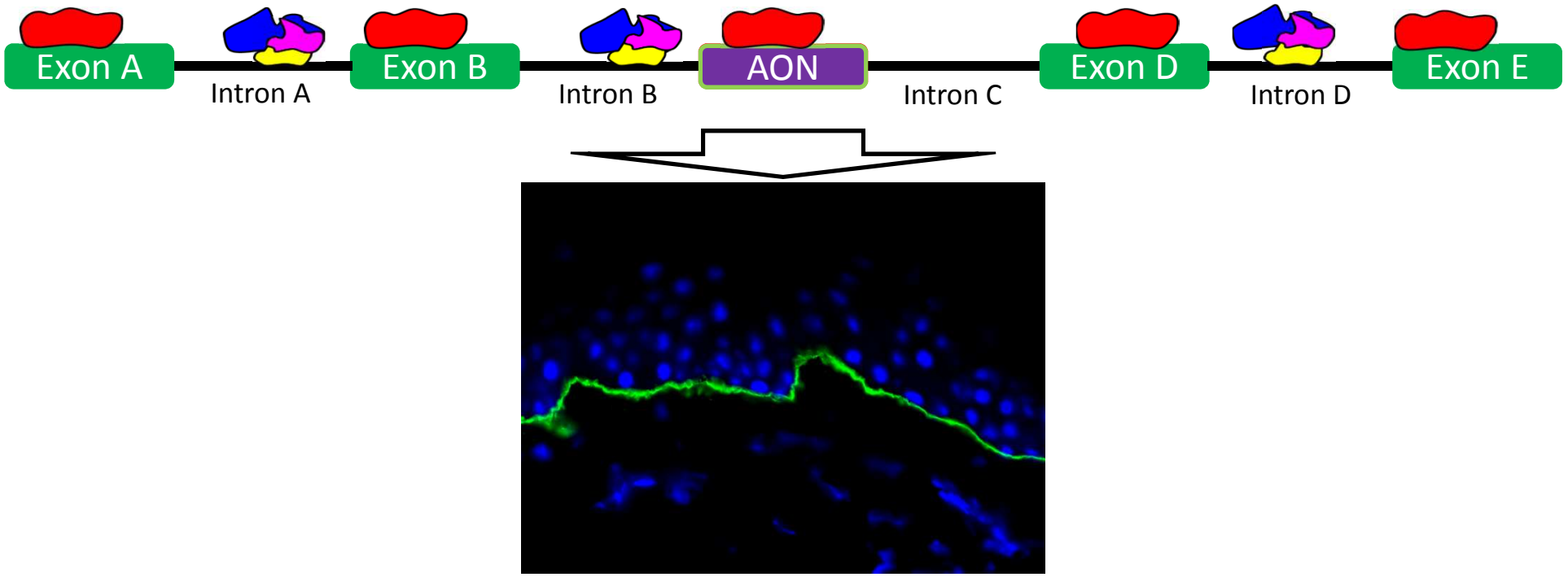
Recessive dystrophic epidermolysis bullosa



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

Van den Akker et al., J Dermatol Sci, 2009

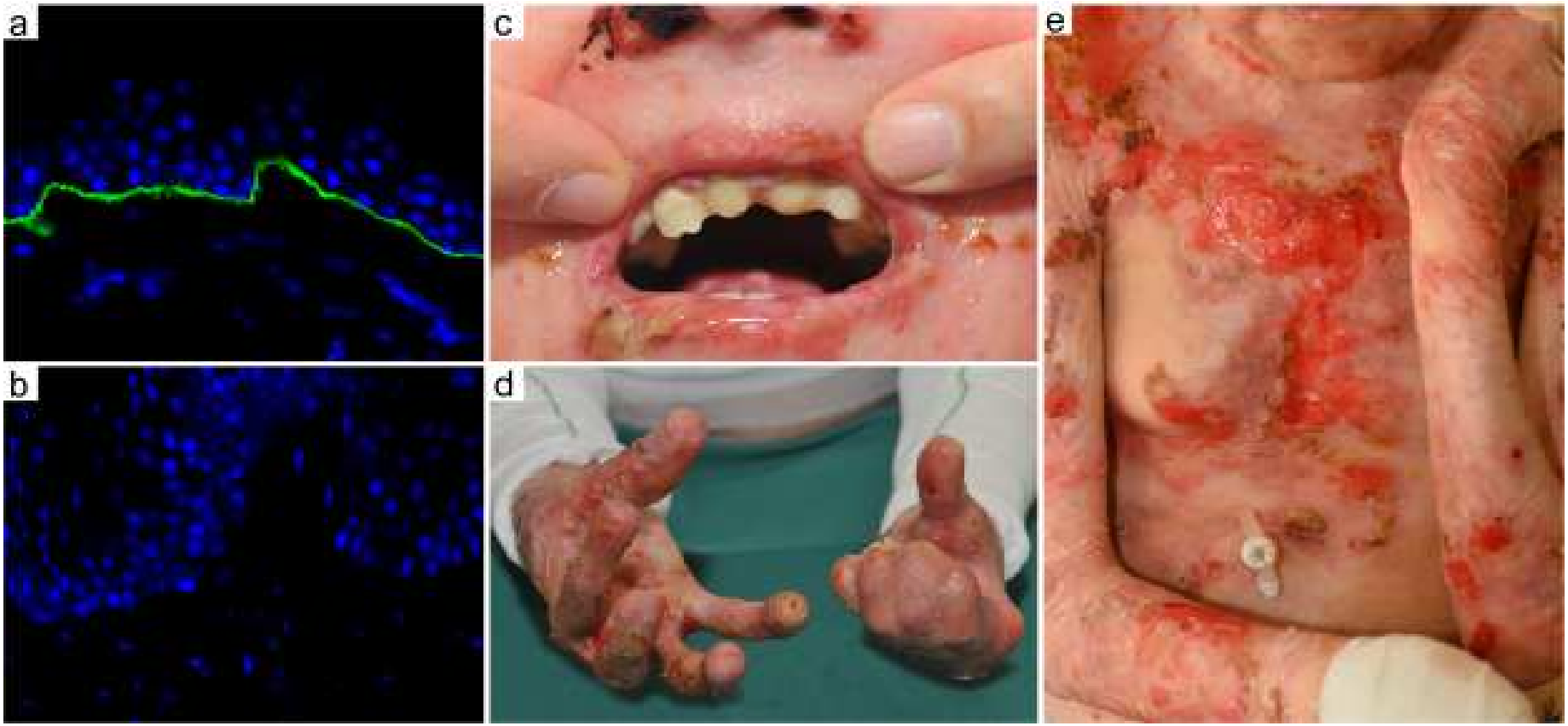
Antisense oligonucleotide-mediated exon skipping



Exon skipping for epidermolysis bullosa

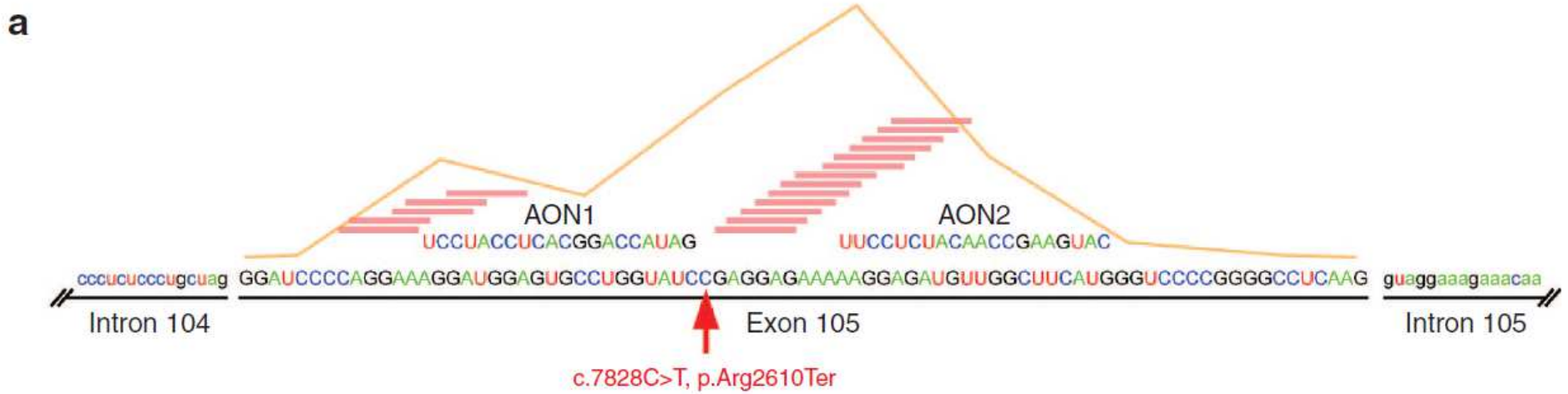
Year	Authors	Journal	Exons
2006	Goto et al.	J Invest Dermatol	70
2016	Bornert et al.	Mol Ther	13
2016	Bremer et al.	Mol Ther Nucleic Acids	105
2016	Turczynski et al.	J Invest Dermatol	73, 80

Patient homozygous for p.Arg2610Ter in exon 105



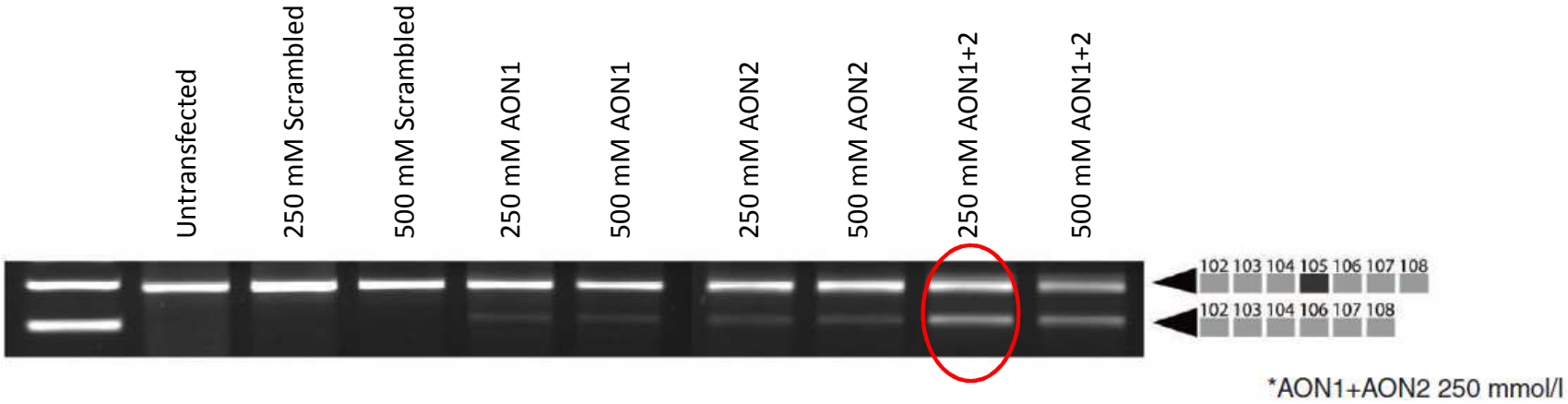
Bremer et al., Mol Ther Nucleic Acids, 2016

In vitro exon skipping of exon 105



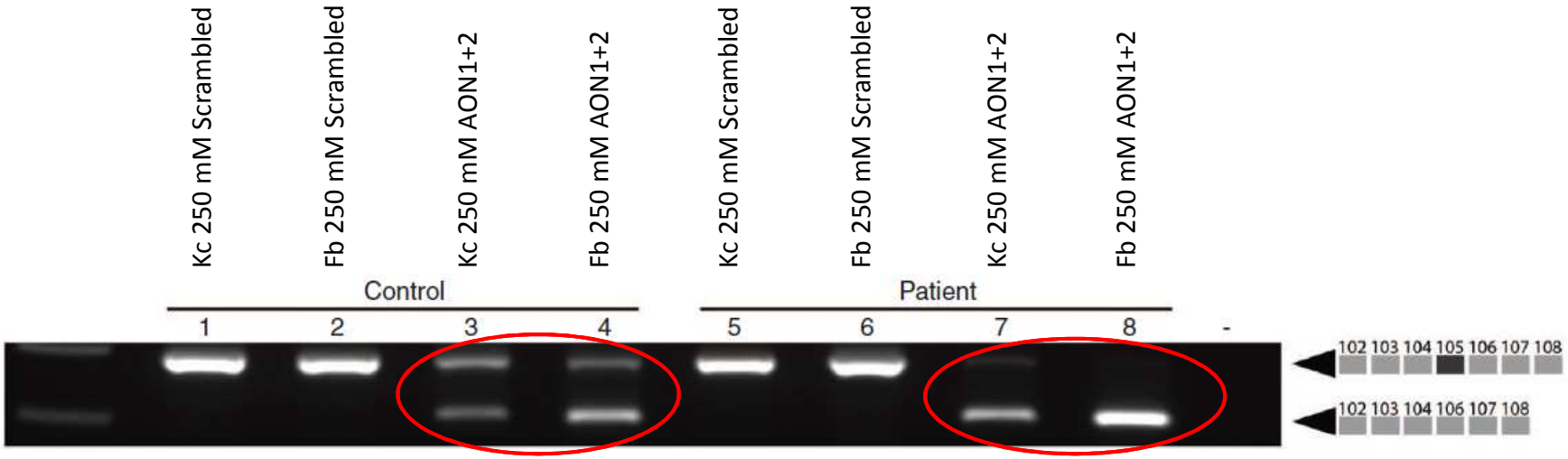
Bremer et al., Mol Ther Nucleic Acids, 2016

In vitro exon skipping of exon 105



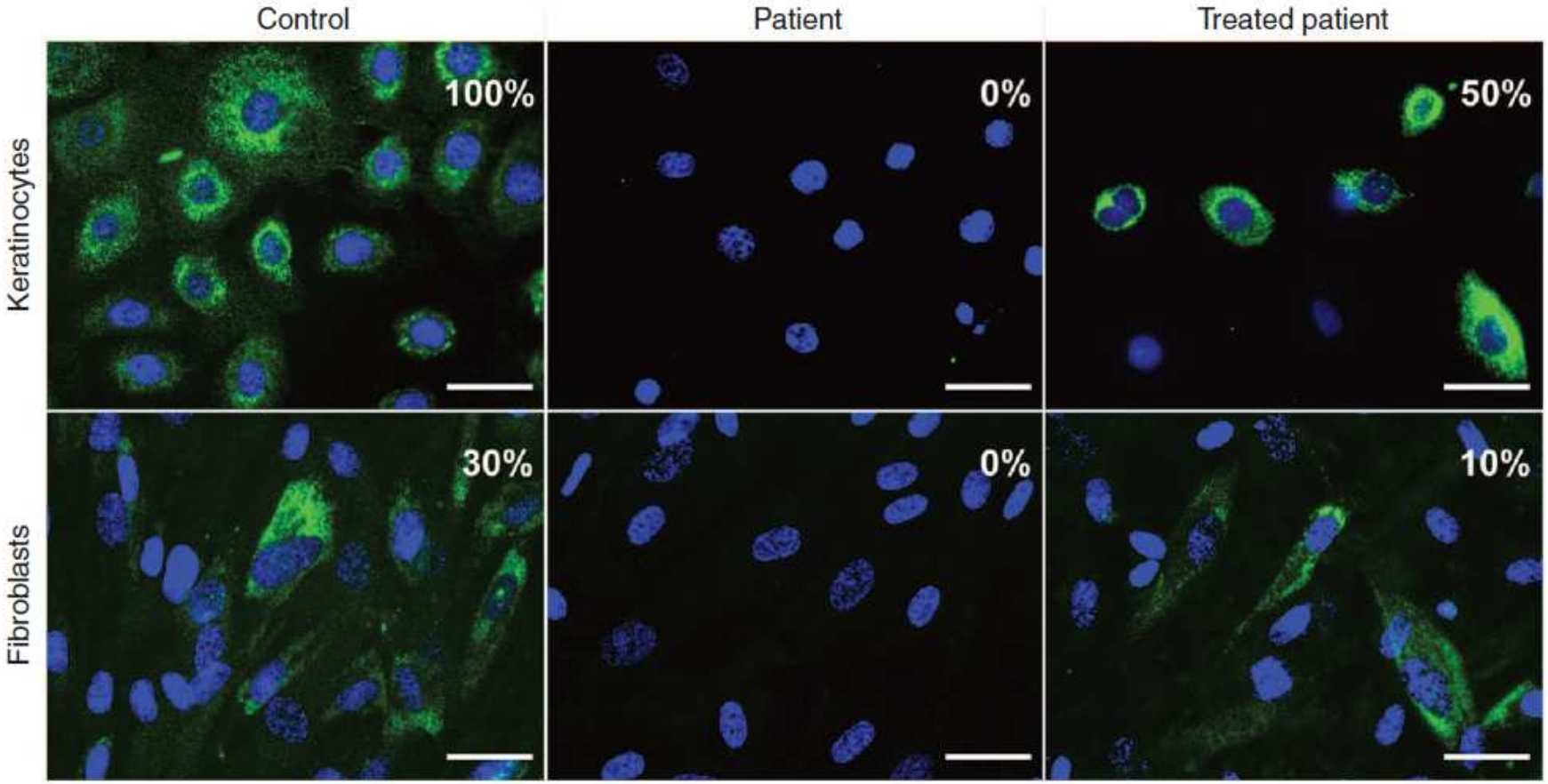
Bremer et al., Mol Ther Nucleic Acids, 2016

In vitro exon skipping of exon 105

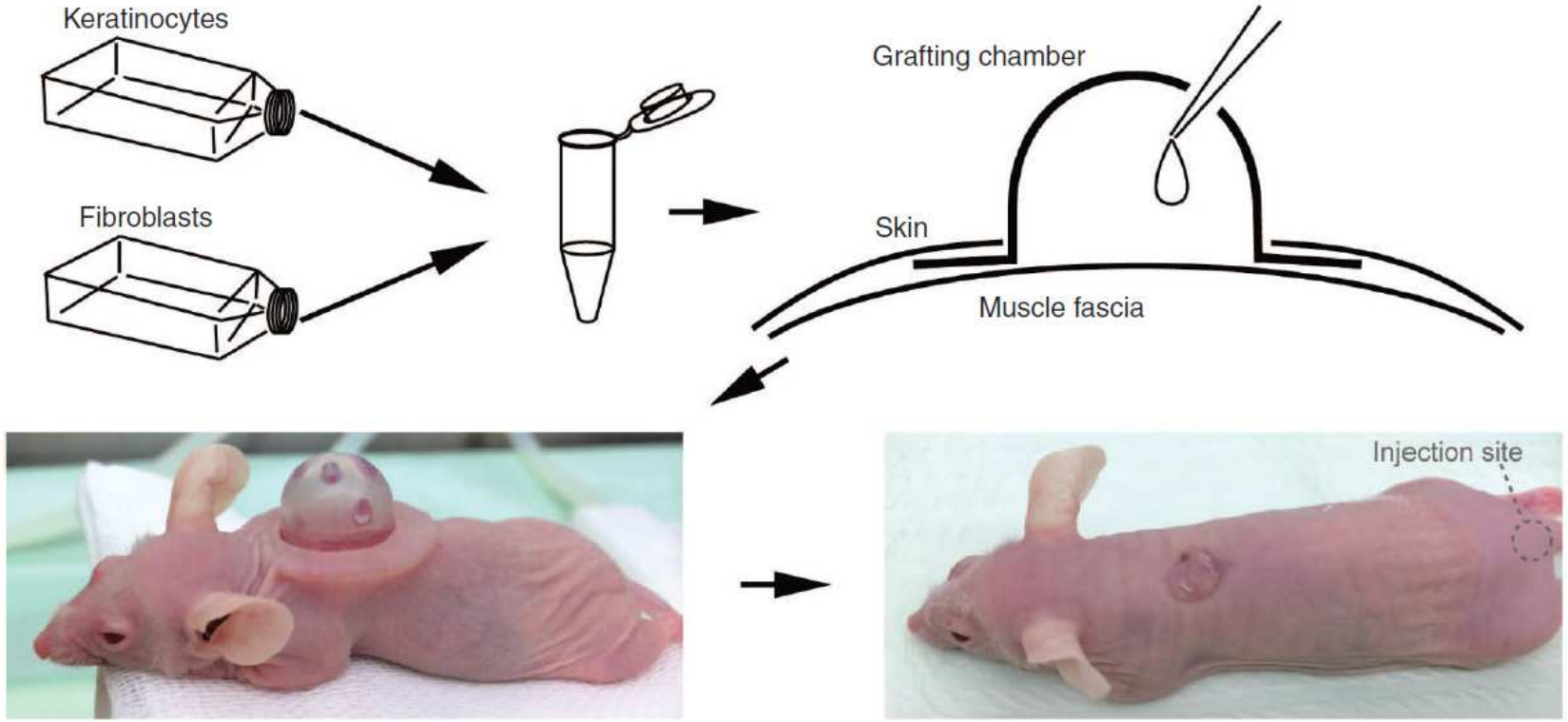


Bremer et al., Mol Ther Nucleic Acids, 2016

In vitro restoration of type VII collagen production

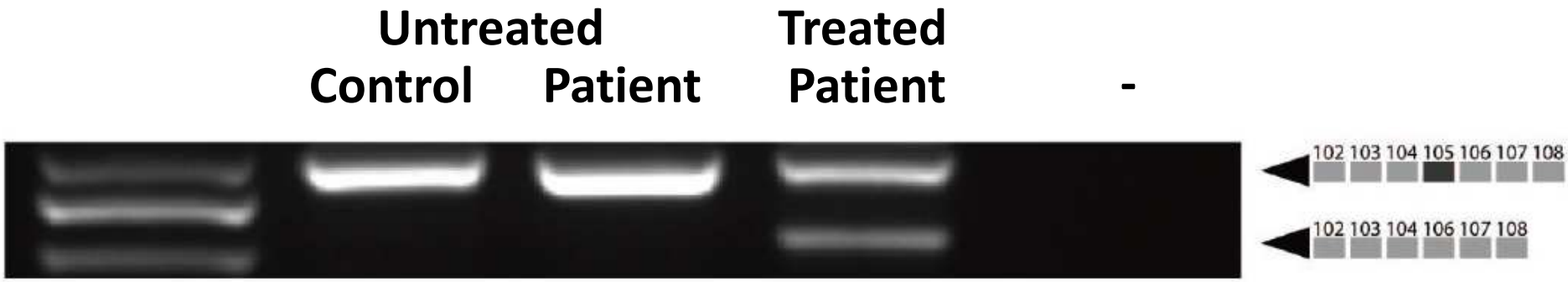


Bremer et al., Mol Ther Nucleic Acids, 2016



Bremer et al., Mol Ther Nucleic Acids, 2016

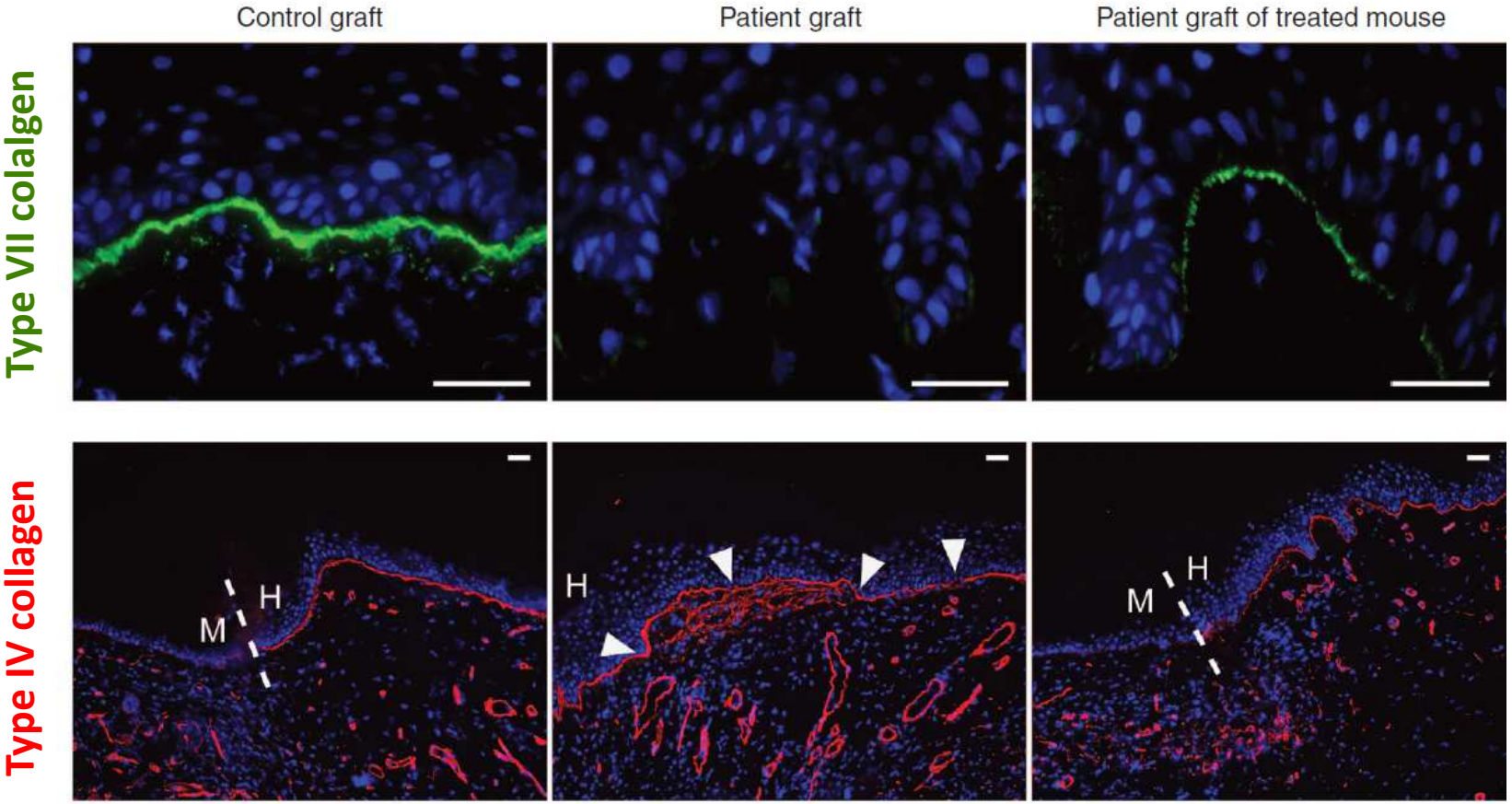
In vivo exon skipping after systemic administration



- 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

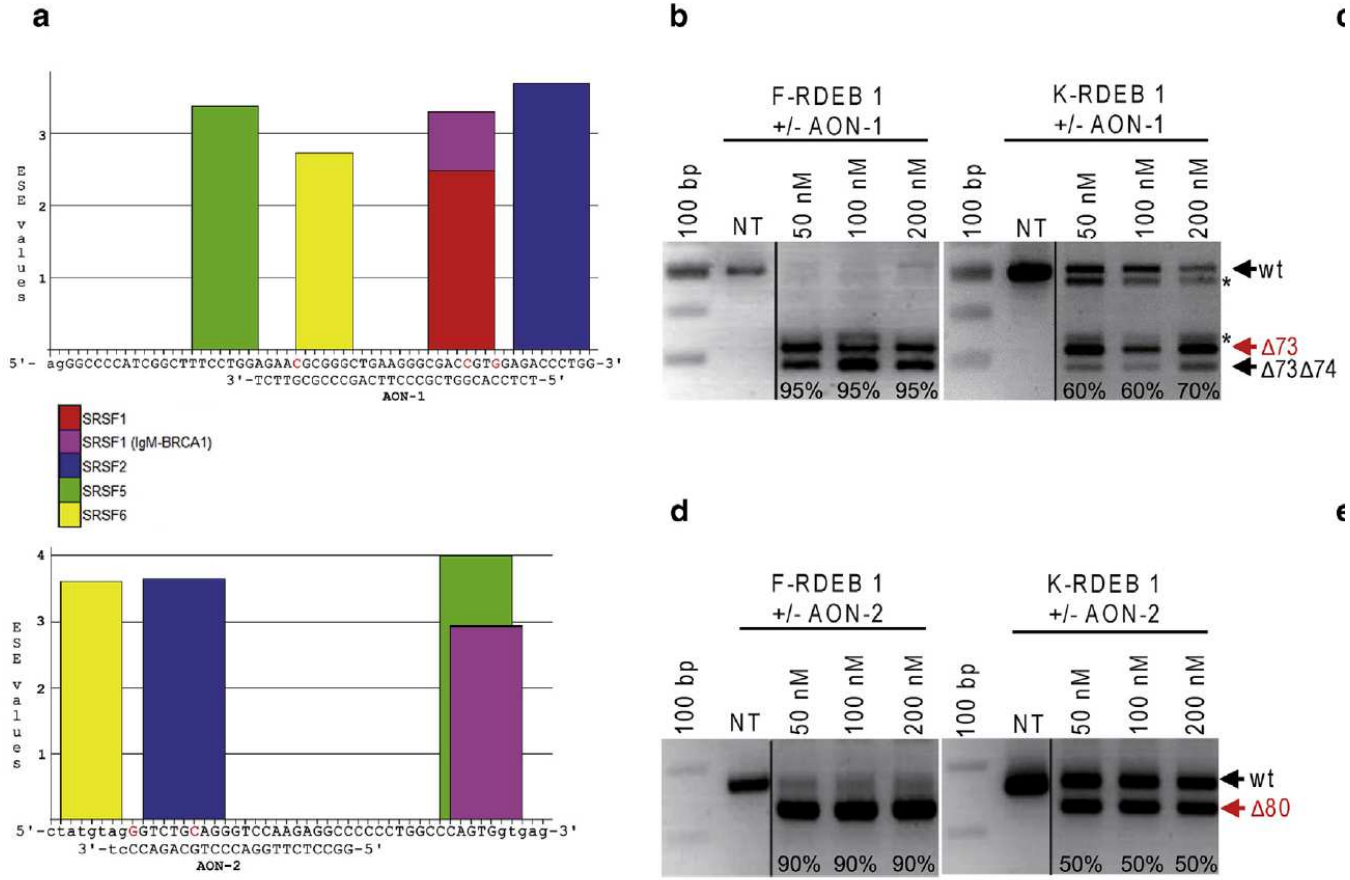


Bremer et al., Mol Ther Nucleic Acids, 2016

In vitro exon skipping of exon 73 and 80

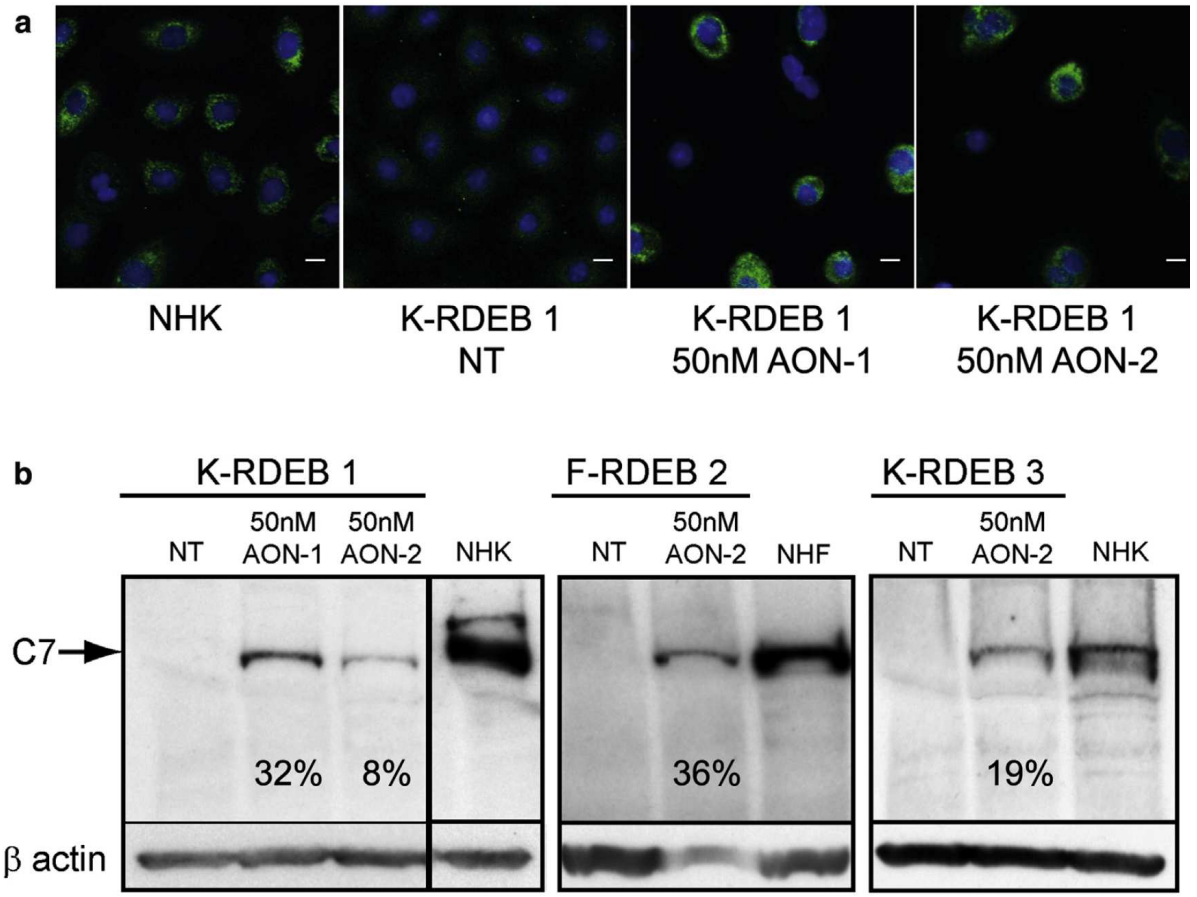
Exon 73

Exon 80



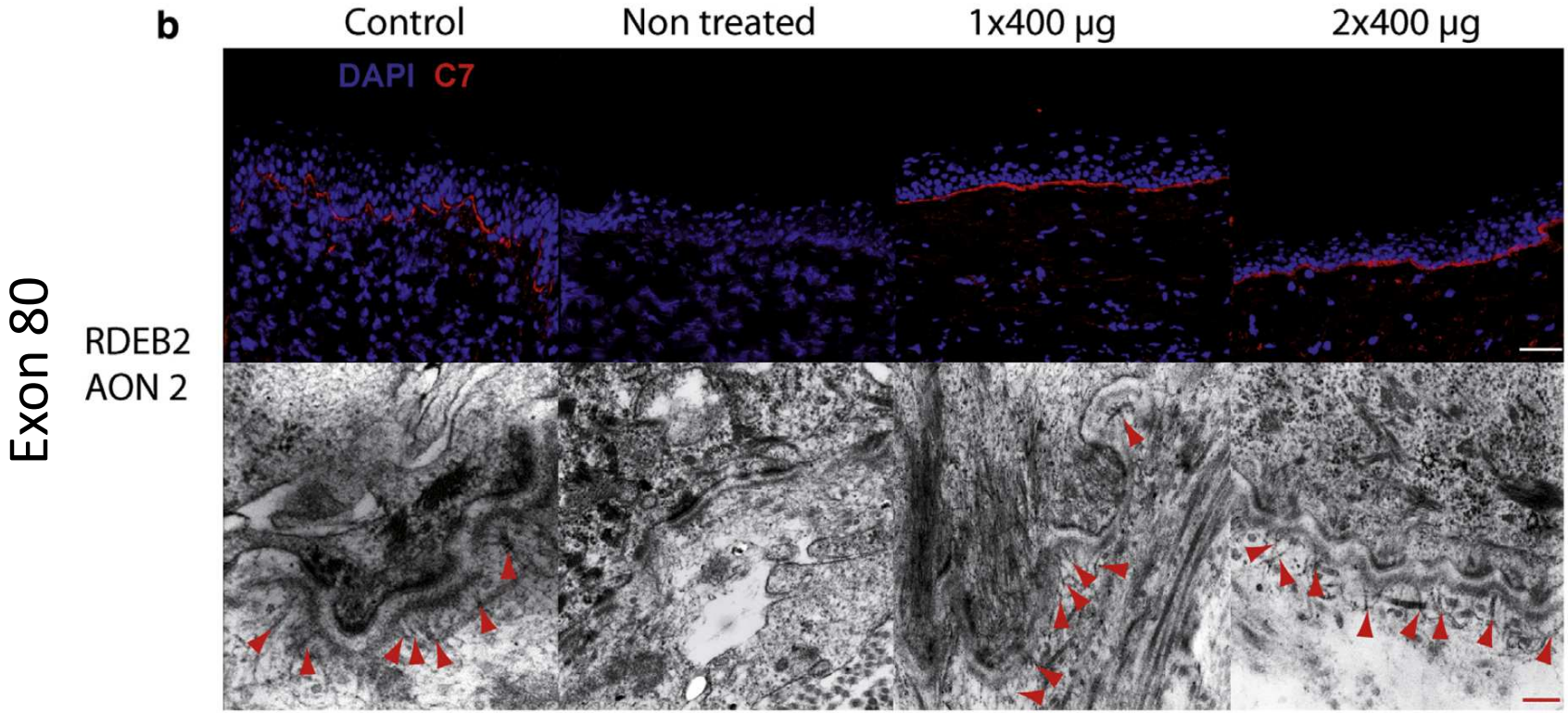
Turczynski et al., J Invest Dermatol, 2016

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
  In vitro fibroblast adhesion and migration
  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

Name	Disease	Mode of action	Chemistry	Approval
Fomivirsen (Vitravene)	Cytomegalovirus retinitis	Inhibition of translation	DNA phosphorothioate	FDA: 1998 EMA: 1999
Pegaptanib (Macugen)	Neovascular age-related macular degeneration	Antagonistic binding to target protein	2′-O-methyl, 2'-fluorinated phosphorothioate	FDA: 2004 EMA: 2006
Mipomersen (Kynamro)	Familial hypercholesterolemia	RNase H induced RNA degradation	2'-O-methoxyethoxy phosphorothioate gapmer	FDA: 2013 EMA: Refusal 2013
Defibrotide (Defitelio)	Severe hepatic veno-occlusive disease in haematopoietic stem-cell transplantation therapy	Non-specific protein interactions / unknown	Polydisperse mixture of phosphodiester nucleotides	FDA: 2016 EMA: 2013
Drisapersen (Kyndrisa)	Duchenne muscular dystrophy	Splice switching (exon skipping)	2'-O-methyl phosphorothioate	FDA: Declines approval 2016 EMA: Withdrawn by Applicant in 2016
Eteplirsen (EXONDYS 51)	Duchenne muscular dystrophy	Splice switching (exon skipping)	Phosphorodiamidate morpholino	FDA:2016 EMA: Under evaluation
Nusinersen (Spinraza)	Spinal muscular atrophy	Splice switching (exon inclusion)	2′-O-methoxyethoxy phosphorothioate	FDA: 2016 EMA: 2017

Overview of FDA and/or EMA decisions on orphan designation of AONs for EB

Name	Disease	Mode of action	Chemistry	Approval
QR313	Epidermolysis Bullosa	Exon 73 skipping	--	FDA: 2017

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

http://www.proqr.com/wp-content/uploads/2017/07/ProQR_RD-Day-June-15-2017.pdf

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

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