Amlexanox: novel read-through approach for RDEB therapy

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No conflict of interest to declare
About 46% of RDEB patients harbor at least one premature termination codon (PTC) mutation in *COL7A1*
Mechanism of read-through PTCs

Normal translation

Incomplete translation

Read-through facilitated translation

Normal stop codon

Normal stop codon

Normal stop codon

Functioning protein

Functioning protein

Full-length protein

Truncated protein

Read-through PTC

PTC

Amlexanox rescues nonsense mutations in human cells

- Amlexanox is an FDA Drug approved for mouth ulcers (topical)
- Currently in Phase II clinical trial for other disorders (oral)
- Well tolerated by humans when taken orally

Gonzalez-Hilarion S et al., *Orphanet J Rare Dis*, 2012, Rescue of nonsense mutations by amlexanox in human cells.

Amlexanox for RDEB?
Our approach:

Gene correction by amlexanox enhanced read-through of PTC
Methods used to evaluate Amlexanox read-through efficacy in RDEB cells

RDEB patient

Skin biopsy

Western blot

qRT-PCR

Skin equivalent model & Immunofluorescence

keratinocytes

fibroblasts

https://clipartfest.com/download/3d8169275c3b5f22a9d486d8858a722736fb6863.html
Can amlexanox read-through PTC mutations in *COL7A1* and result in full-length protein?
Amlexanox induces full-length type VII collagen synthesis in RDEB cells

NHK- normal human keratinocytes
NHF- normal human fibroblasts
NT- non treated control
A- amlexanox
G- gentamicin

Atanasova et al., J Invest Dermatol, 2017
8/14 patient cells respond to amlexanox treatment

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<th>Mutation</th>
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<td>p.Q251X/p.Q251X</td>
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<td>RDEB5K</td>
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<td>RDEB115K</td>
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Is read-through type VII collagen functional?
Read-through type VII collagen forms stable triple helix

NHK - normal human keratinocytes
NT - non treated control
A - amlexanox
G - gentamicin

Atanasova et al., J Invest Dermatol, 2017
Organotypic Assay

- Prepare matrix from fibrinogen and embed fibroblasts
- Plate keratinocytes on top
• Prepare matrix from fibrinogen and embed fibroblasts
• Plate keratinocytes on top
• Raise above liquid interface
- Prepare matrix from fibrinogen and embed fibroblasts
- Plate keratinocytes on top
- Raise above liquid interface
- Treat with amlexanox and fix sample after 1 and 2 weeks
- Evaluate skin integrity and stain for type VII collagen
Type VII collagen localizes to the dermal epidermal junction (DEJ) in skin equivalents.

- **DAPI**
- **Type VII collagen**

**wild type control**

**H&E**
Read-through type VII collagen localizes to the DEJ in skin equivalents

Q251X/Q251X

non-treated

amllexanox
How does amlexanox increase synthesis of type VII collagen?

PTC mutations are associated with mRNA instability.

Effect on nonsense mediated mRNA decay?
Amlexanox significantly increases $COL7A1$ transcript in RDEB cells

Atanasova et al., *J Invest Dermatol*, 2017
Nonsense mediated mRNA decay is dependent on UPF1 phosphorylation
Read-through synthesis of type VII collagen correlates with increase in UPF1 phosphorylation.
Conclusions

• Amlexanox enhances full-length type VII protein synthesis, which localizes to the DEJ in an organotypic skin equivalent

• Amlexanox increases \textit{COL7A1} transcript in RDEB cells

• Read-through efficiency correlates with increase in UPF1 phosphorylation
Acknowledgement