

Amlexanox: novel read-through approach for RDEB therapy

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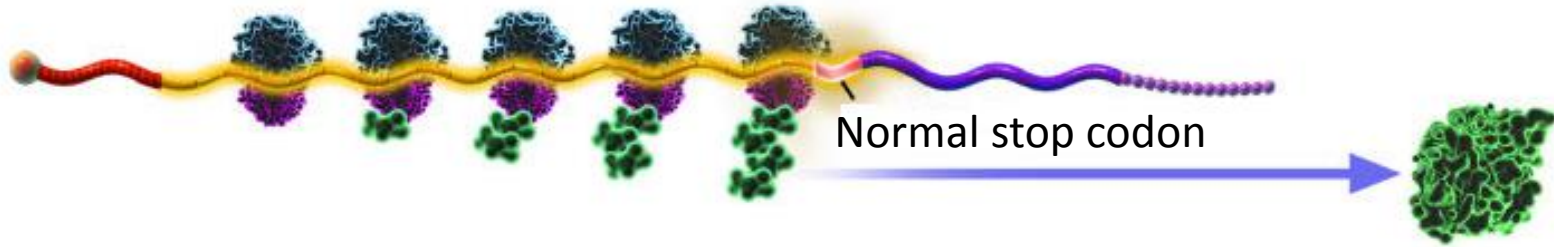
No conflict of interest to declare

About 46% of RDEB patients harbor at least one premature termination codon (PTC) mutation in *COL7A1*



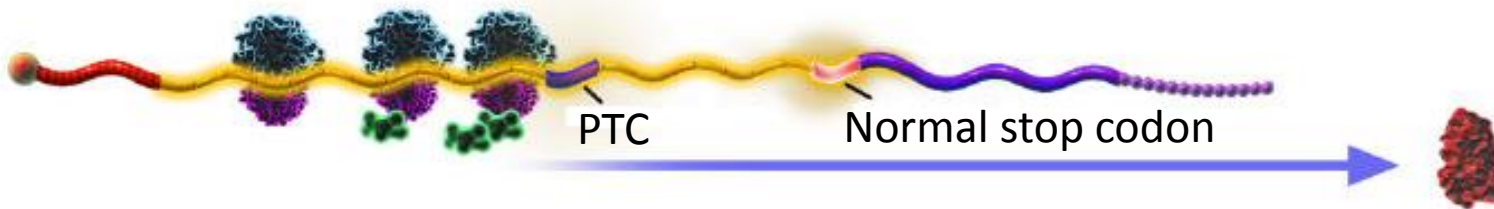
Mechanism of read-through PTCs

Normal translation



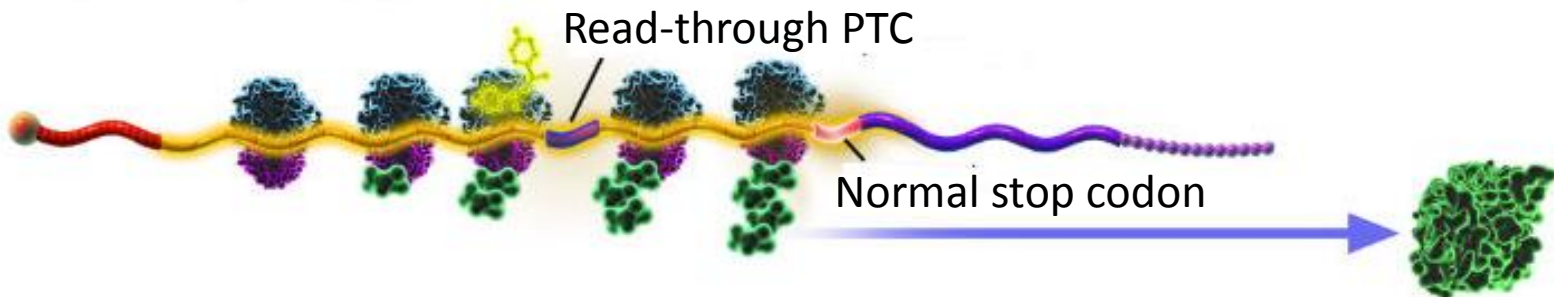
Functioning protein

Incomplete translation



Truncated protein

Read-through facilitated translation



Full-length protein

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

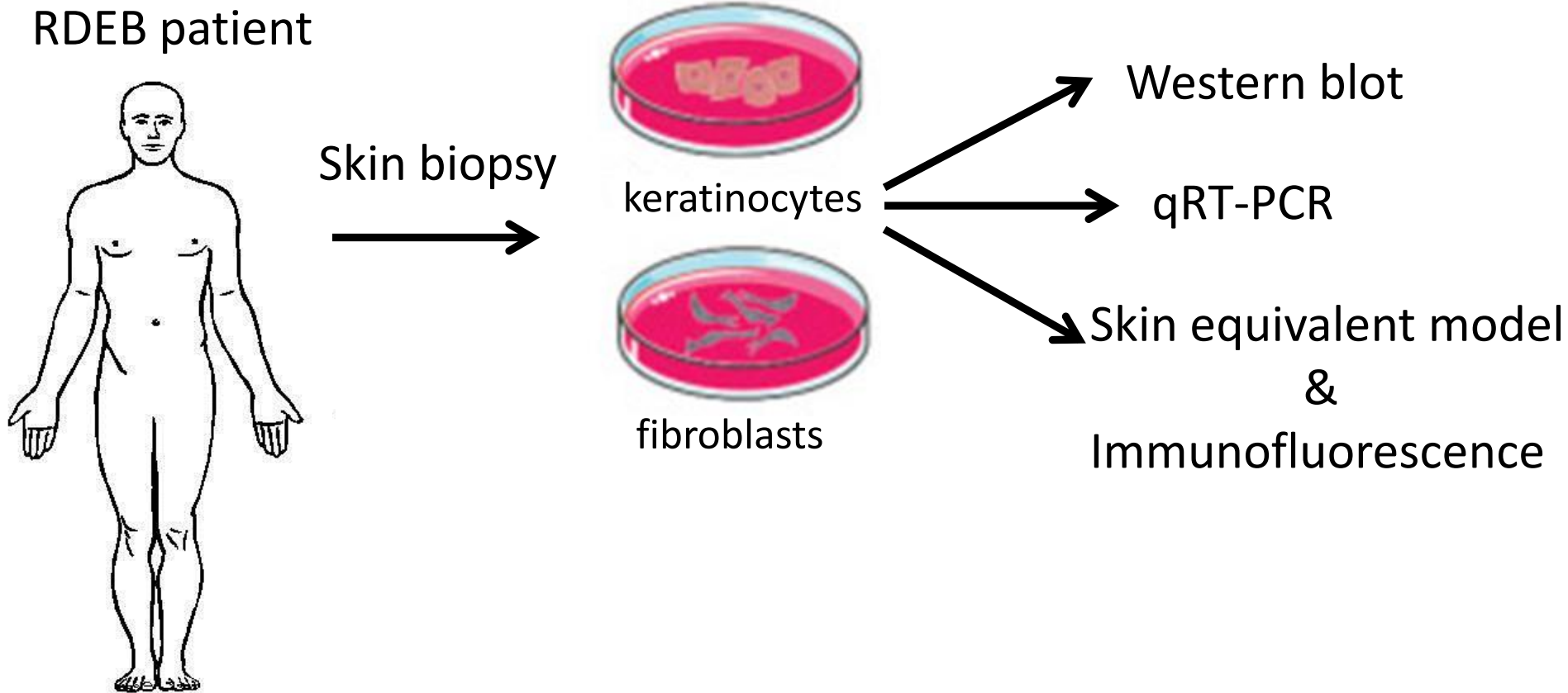
Gonzalez-Hilarion S et al., *Orphanet J Rare Dis*, 2012, Rescue of nonsense mutations by amlexanox in human cells.

Amlexanox for RDEB?

Our approach:

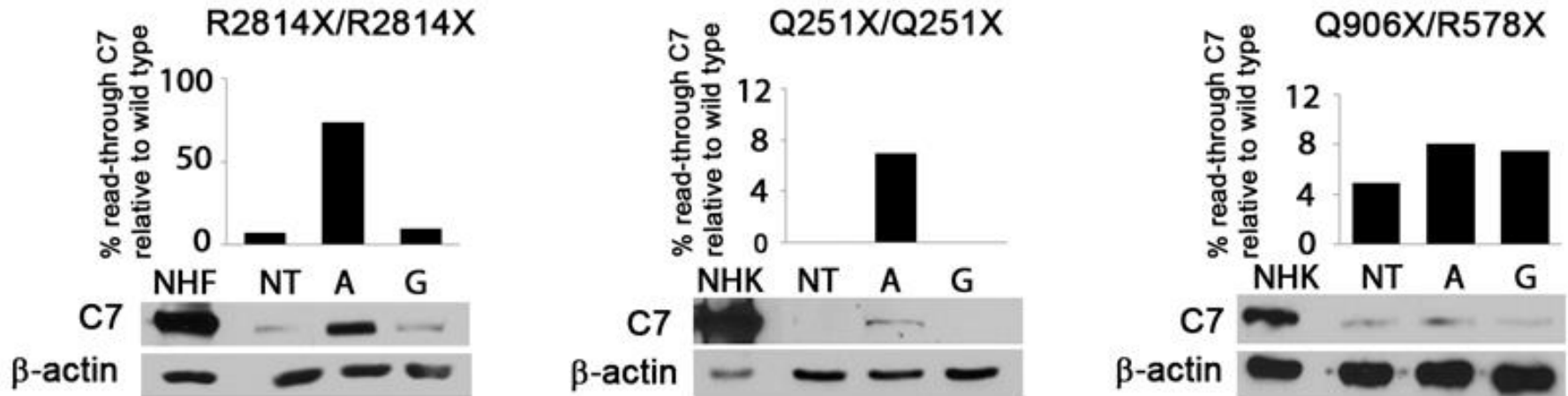
Gene correction by amlexanox enhanced
read-through of PTC

Methods used to evaluate Amlexanox read-through efficacy in RDEB cells



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

8/14 patient cells respond to amlexanox treatment

YES

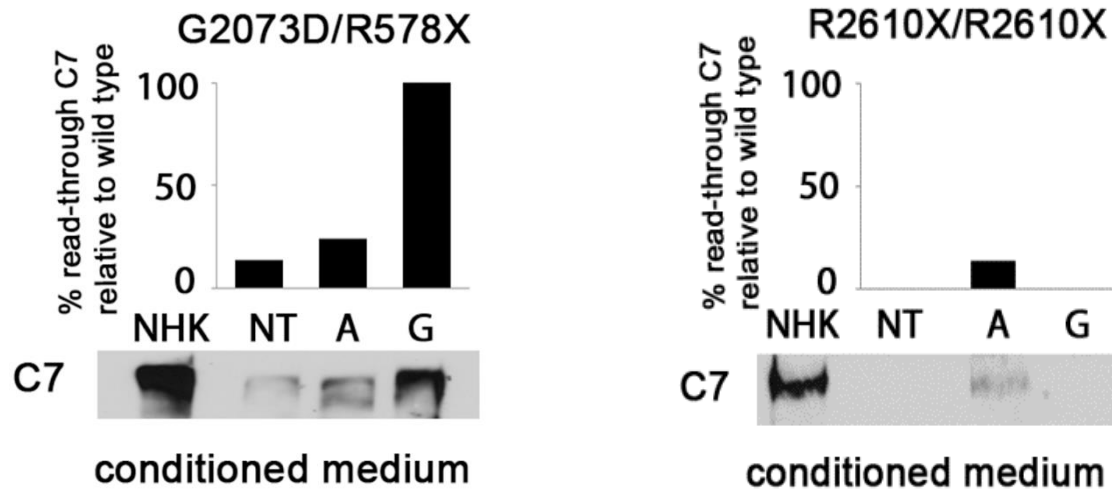
Cell line name	Mutation
RDEB14K	p.Q251X/p.Q251X
RDEB13K	p.G2073D/p.R578X
RDEB102K/F	p.R2814X/p.R2814X
RDEB5K	p.R578X/p.Q906X
RDEB111K	p.R2610X/p.R2610X
RDEB112K	p.R1343X/p.R2069C
RDEB113K	p.R1630X/p.R2069C
RDEB115K	c.4249delG/p.R1933X

NO

Cell line name	Mutation
RDEB53K	p.R669X/p.R669X
RDEB101K/F	p.R669X/p.R669X
RDEB110K	p.R2338X/p.R2338X
RDEB3K	p.R525X/-
RDEB107F	p.R578X/p.R2492X
RDEB116K	p.R1933X/p.R1933X

Is read-through type VII collagen functional?

Read-through type VII collagen forms stable triple helix



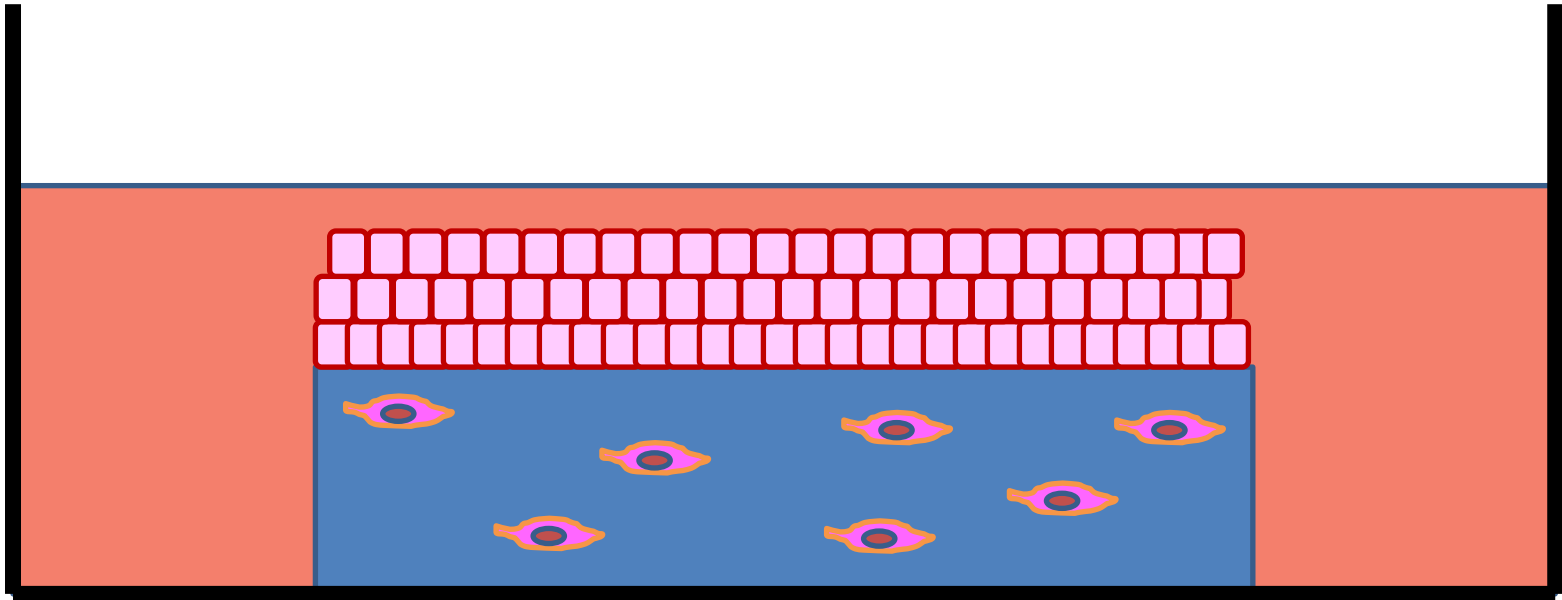
NHK- normal human keratinocytes

NT- non treated control

A- amlexanox

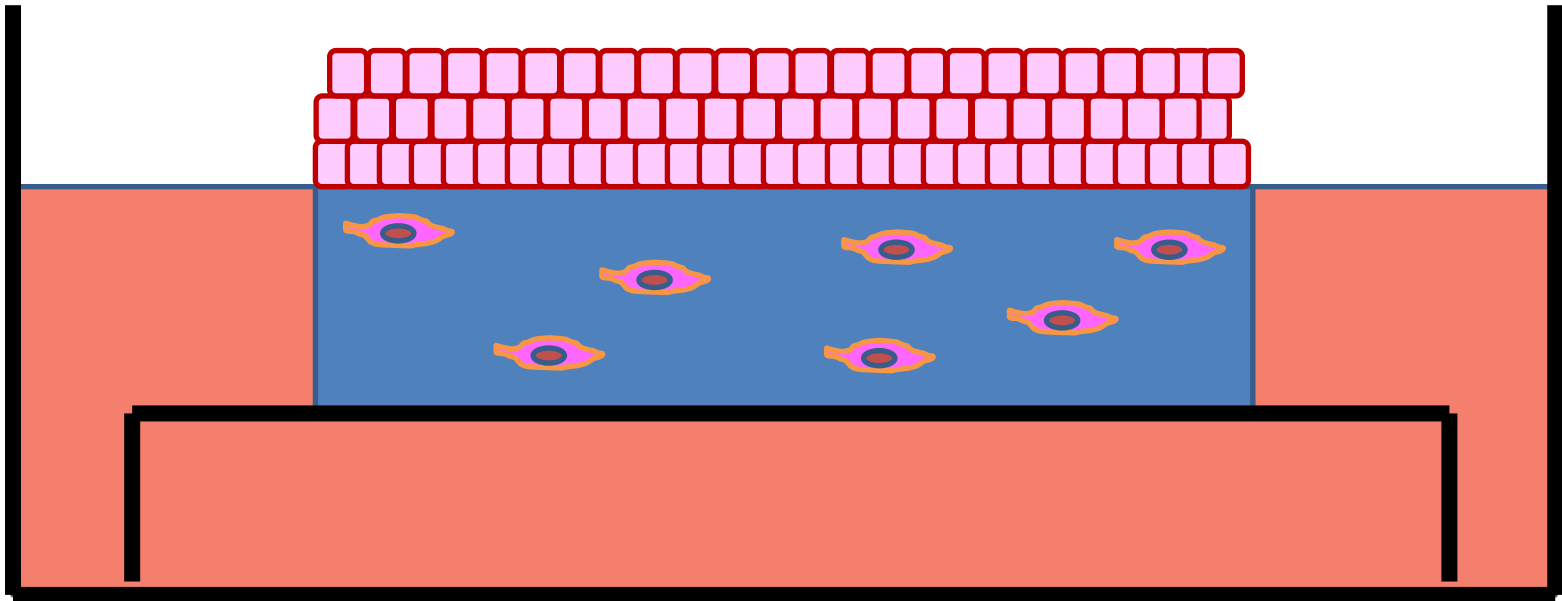
G- gentamicin

Organotypic Assay



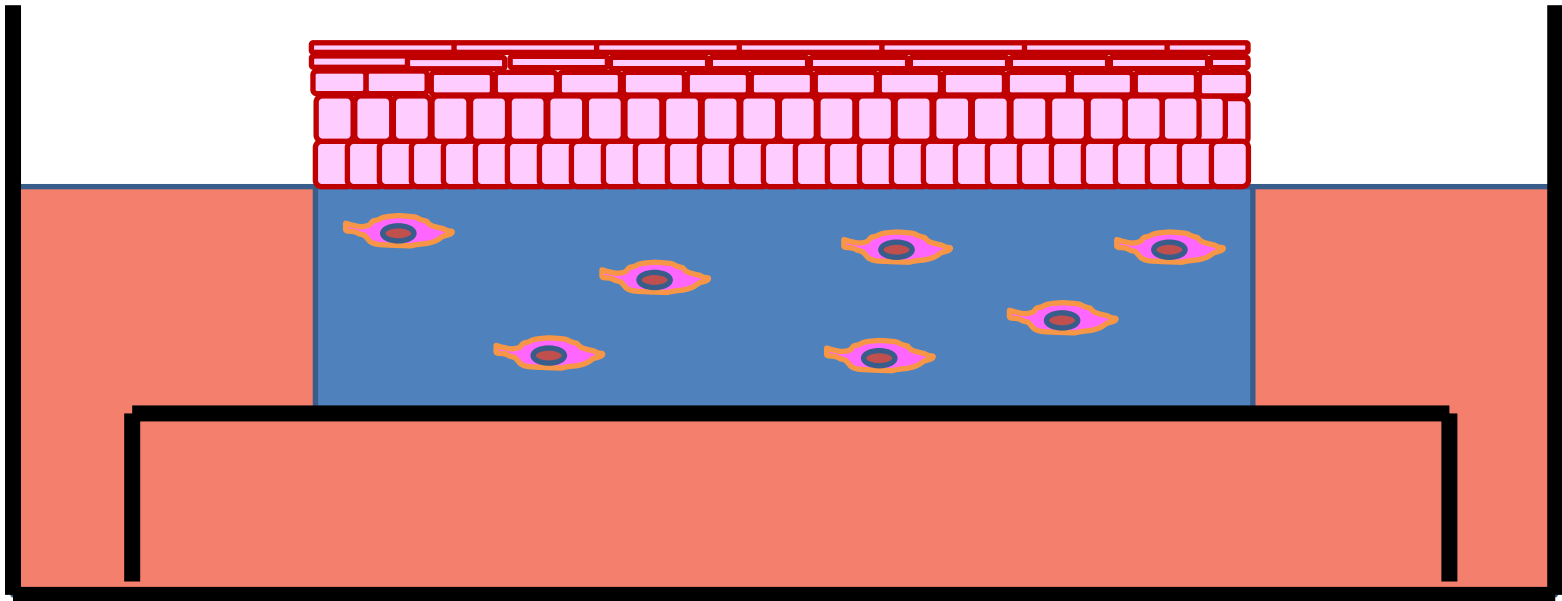
- Prepare matrix from fibrinogen and embed fibroblasts
- Plate keratinocytes on top

Organotypic Assay



- Prepare matrix from fibrinogen and embed fibroblasts
- Plate keratinocytes on top
- Raise above liquid interface

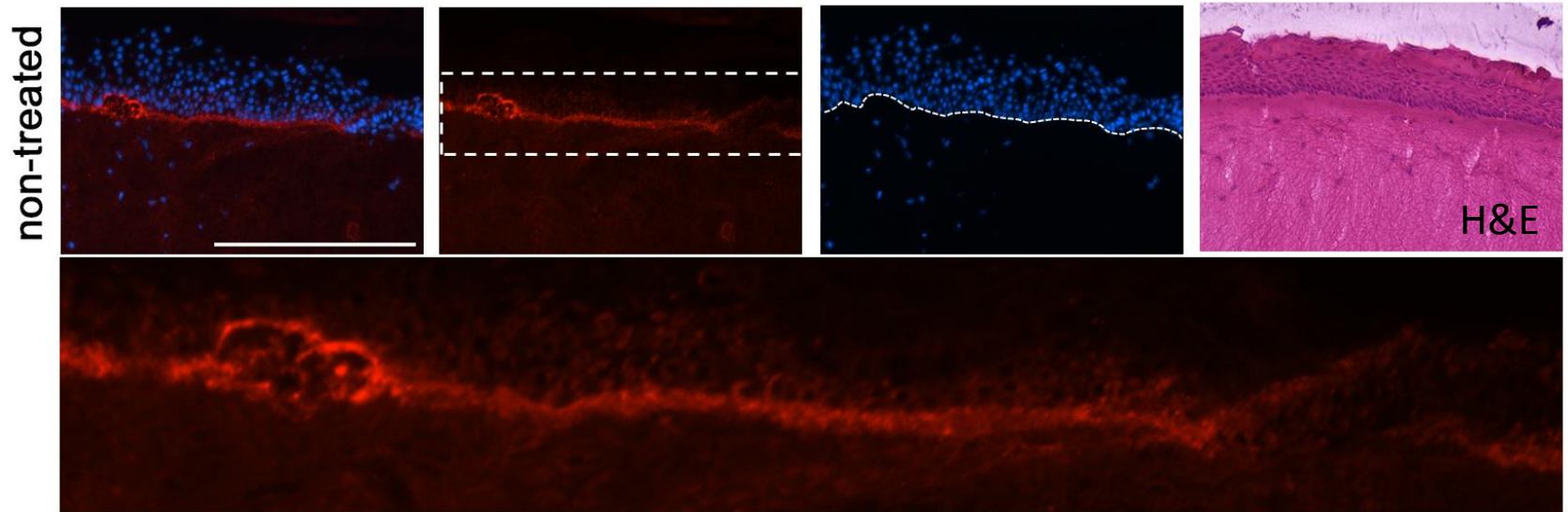
Organotypic Assay



- 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

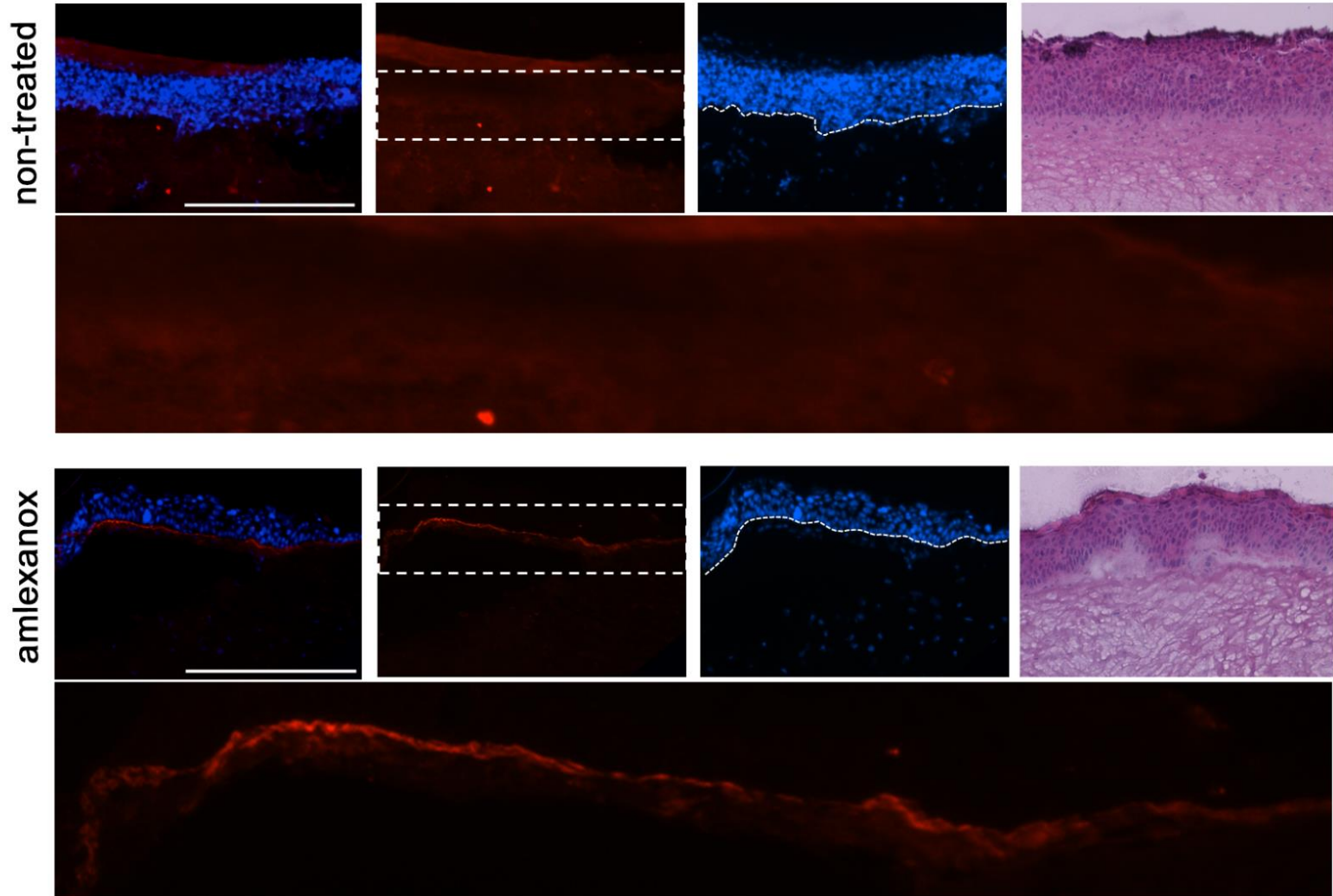
wild type control



- DAPI
- Type VII collagen

Read-through type VII collagen localizes to the DEJ in skin equivalents

Q251X/Q251X



How does amlexanox increase synthesis of type VII collagen?

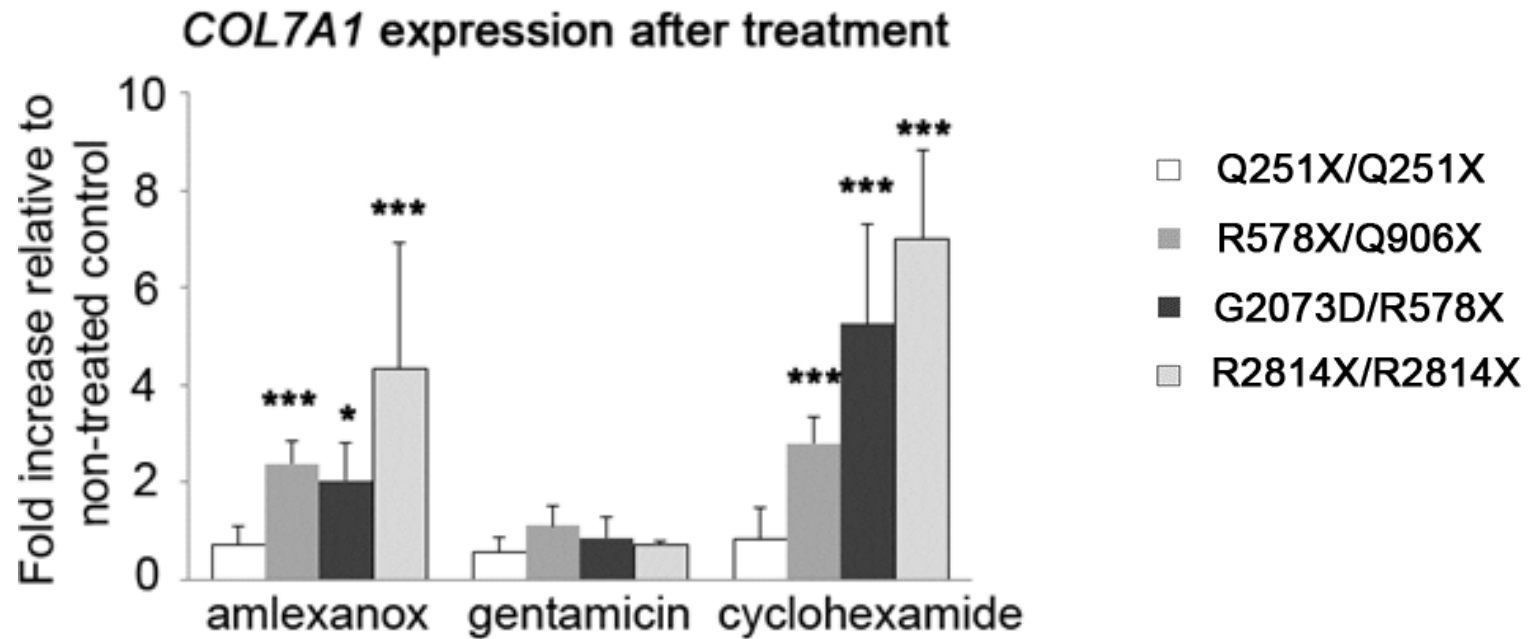


PTC mutations are associated with mRNA instability.

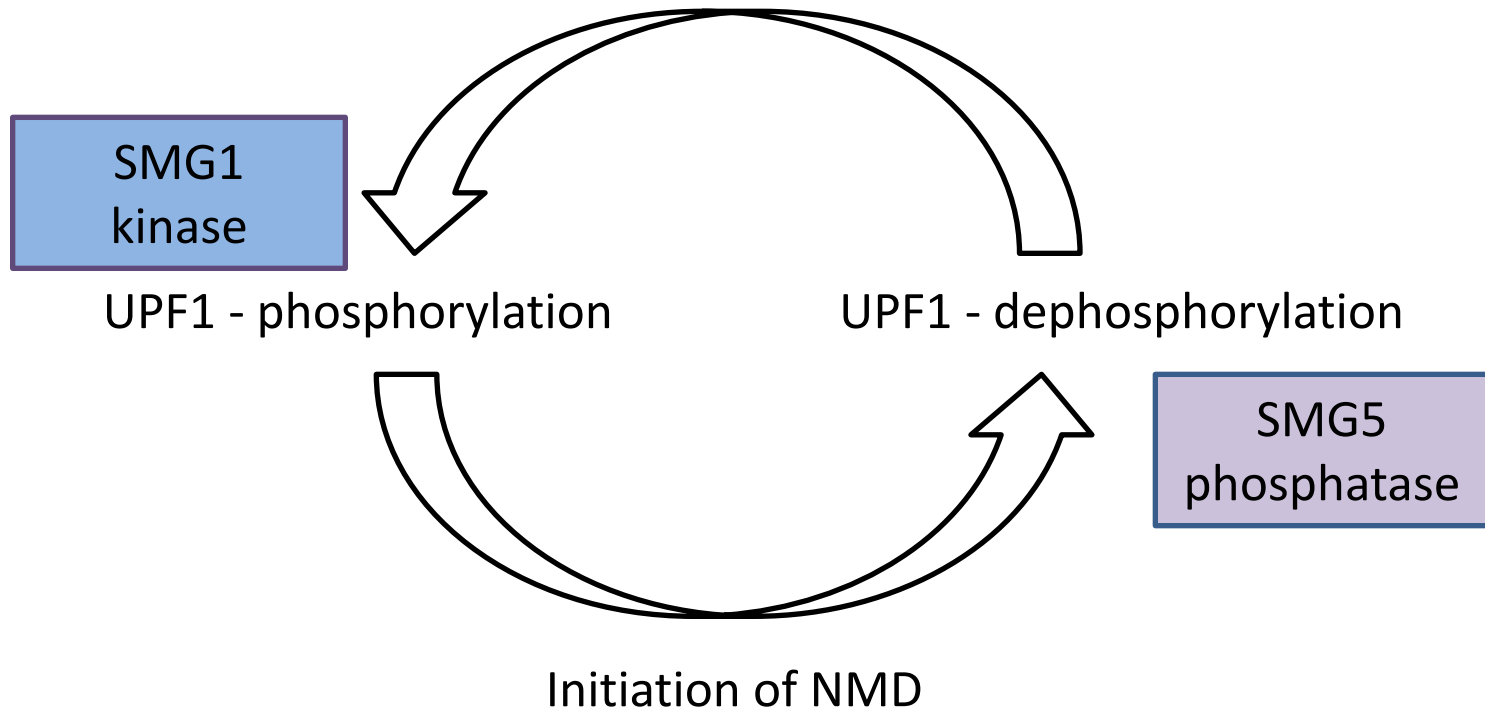


Effect on nonsense mediated mRNA decay?

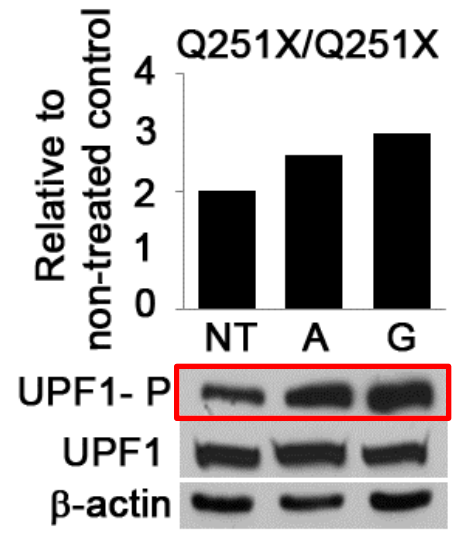
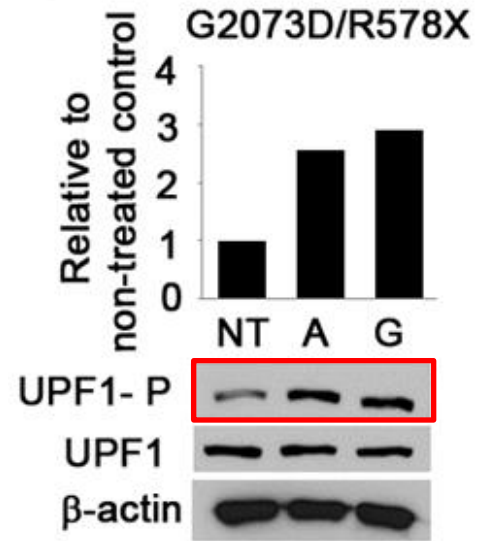
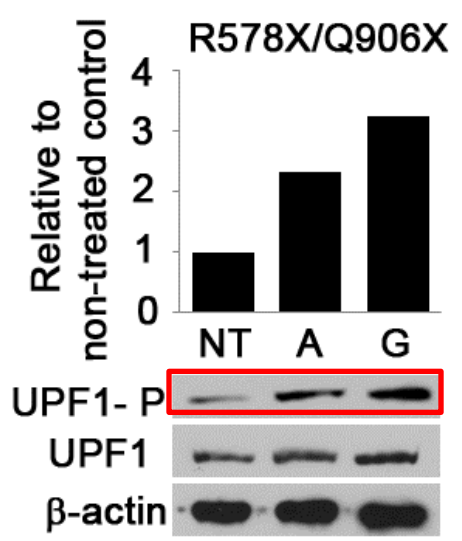
Amlexanox significantly increases *COL7A1* transcript in RDEB cells



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 *COL7A1* transcript in RDEB cells
- Read-through efficiency correlates with increase in UPF1 phosphorylation

Acknowledgement

