PTC readthrough opportunities for RDEB therapy: novel candidate drugs

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350 million
People worldwide have one of over 7,000 rare diseases

- 80% are genetic in origin
- 50% are children
- 30% Die before 5th birthday
- 95% Have no treatment
- 11% Nonsense mutations
  Single base substitutions that introduce a premature termination codon (PTC)
- 10-20% for EB

Therapies directed at nonsense mutations could benefit many patients across many genetic disorders
Induction of full-length protein by premature termination codon (PTC) readthrough

WT
full-length protein
functional

PTC
no full length protein
low levels of mRNA (NMD)
and truncated protein

PTC readthrough
full-length protein
largely active
Part 1
A new look at gentamicin

Clinical trials for cystic fibrosis and Duchenne muscular dystrophy (ca 2000)
Improvements were observed but they were small and variable

“Different results may be attributable to different brands of gentamicin used in the mouse and human studies cited”


“The variable response found among different studies in CF patients, DMD mice models and DMD patients might be attributed to different brands, which might contain different relative concentration of each gentamicin component”


“The source of the antibiotic could have an influence on efficacy ... In commercial production, a particular mixture of these three isoforms (C1, C1a, C2) could be more or less effective in the laboratory or in the clinic. For clinical trials, it is important to use gentamicin from the same source”


“Given that gentamicin has variable effects and exhibits some toxicity, less toxic effective derivatives of this drug need to be developed for an effective DMD treatment”

Pichavant et al. Mol. Ther. 19:830 (2011)
Pharmaceutical gentamicin is not a pure compound
Model system: HDQ-P1 breast cancer cell line with homozygous $TP53$ nonsense mutation
Automated p53 western assay for readthrough
Gentamicin batches show variable readthrough activity

Major components (>97%)

Minor components (<3%)

R1
R2
C1
C1a
C2/C2a
C2b

HO
O
\(\text{CH}_3\)
\(\text{NHCH}_3\)
\(\text{NH}_2\)
\(\text{NH}_2\)

HO
O
\(\text{CH}_3\)
\(\text{NHCH}_3\)
\(\text{NH}_2\)

HO
O
\(\text{CH}_3\)
\(\text{NHCH}_3\)
\(\text{NH}_2\)

Minor components (<3%)

HO
O
\(\text{CH}_3\)
\(\text{NHCH}_3\)
\(\text{NH}_2\)

HO
O
\(\text{CH}_3\)
\(\text{NHCH}_3\)
\(\text{NH}_2\)

HO
O
\(\text{CH}_3\)
\(\text{NHCH}_3\)
\(\text{NH}_2\)
Major gentamicin components are inactive

Gentamicin B1 shows potent PTC readthrough activity
Activity in cells from rare genetic disease patients

**a**
- Gentamicin B1 (25 µg/ml)
- Gentamicin (100 µg/ml)

**b**
- Vinculin
- Pro-TPP1
- Mature TPP1

**c**
- Duchenne muscular dystrophy
- Schimke immuno-osseous dysplasia

**d**
- SMARCAL1

**e**
- Collagen VII

Neuronal ceroid lipofuscinosi
CLN2
Part 2
Enhancers of PTC readthrough by aminoglycosides

Small molecules that do not themselves induce readthrough but potentiate readthrough by aminoglycosides
Time course CDX5-1 + G418
Compounds also potentiate readthrough by gentamicin B1
Activity in cells from rare genetic disease patients

- Neuronal ceroid lipofuscinosis (lysosomal storage disease)
- Mutations in the CLN2 gene encoding tripeptidylpeptidase 1 (TPP1)
- Primary fibroblasts from a patient with nonsense mutations (R127X/R208X)
Summary

- Gentamicin B1: a minor gentamicin that potently induces PTC readthrough
- Four distinct structural classes of small molecules that potentiate PTC readthrough by aminoglycosides

Combination of B1 and a potentiatior may broadly suppress nonsense mutations in a variety of genetic diseases including EB
Summary

- Gentamicin B1: a minor gentamicin that potently induces PTC readthrough
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Combination of B1 and a potentiator may broadly suppress nonsense mutations in a variety of genetic diseases including EB

New collaborations with
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