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

are genetic in origin

are children

Die before 5th birthday

Have no treatment

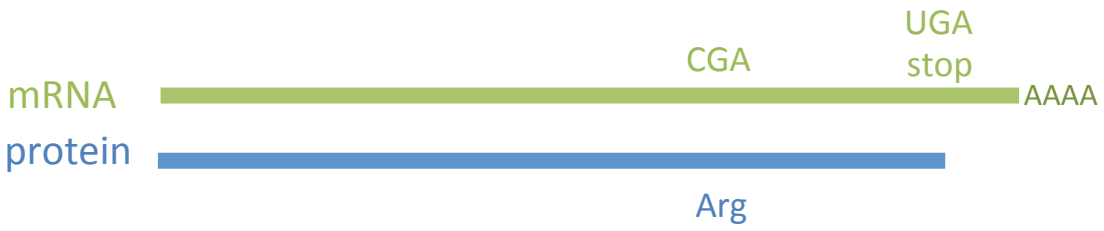
10-20% for EB

Nonsense mutations

Single base substitutions that introduce a premature termination codon (PTC)

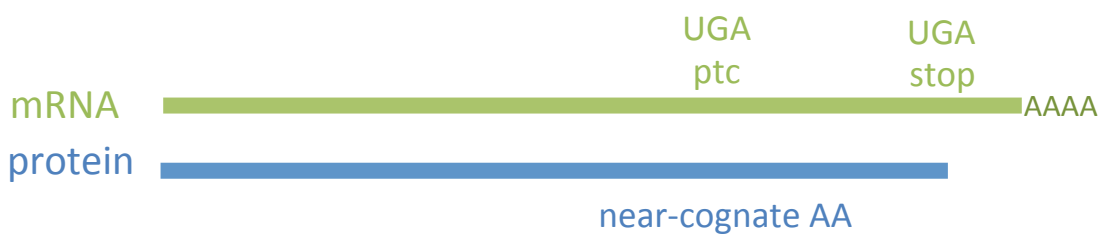
Therapies directed at nonsense mutations could benefit many patients across many genetic disorders

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"

Karpati and Lochmuller Ann. Neurol. 49:693 (2001)

"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"

Linde and Kerem Trends Genet. 24:552 (2008)

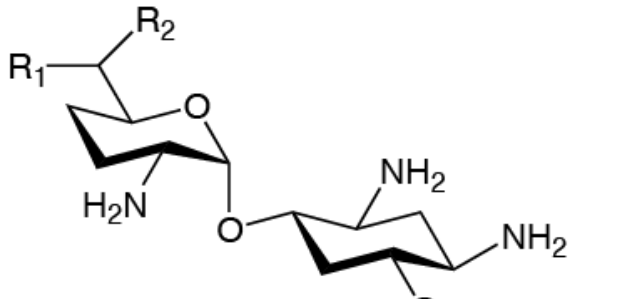
"The source of the antibiotic could have an influence on efficacy … In commercial production, a particular mixture of these three isoforms (C1, C1a, C2) could be more or less effective in the laboratory or in the clinic. For clinical trials, it is important to use gentamicin from the same source"

Malik et al. Ther. Adv. Neurol. Dis. 3:379 (2010)

"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)

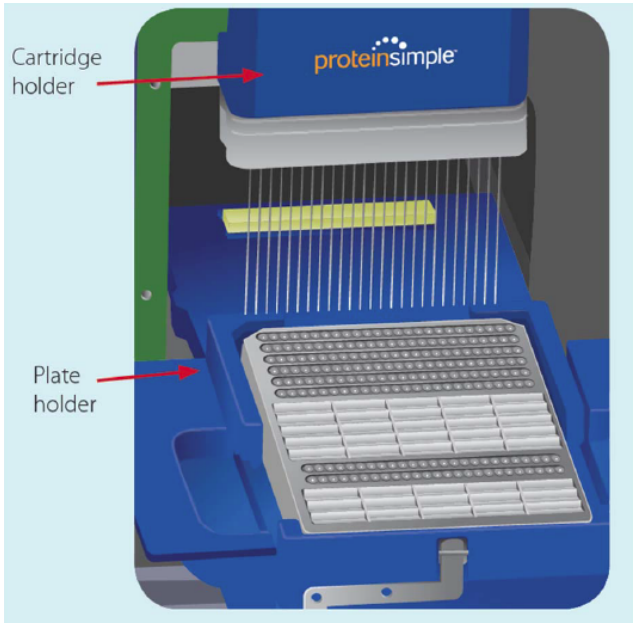
Pharmaceutical gentamicin is not a pure compound

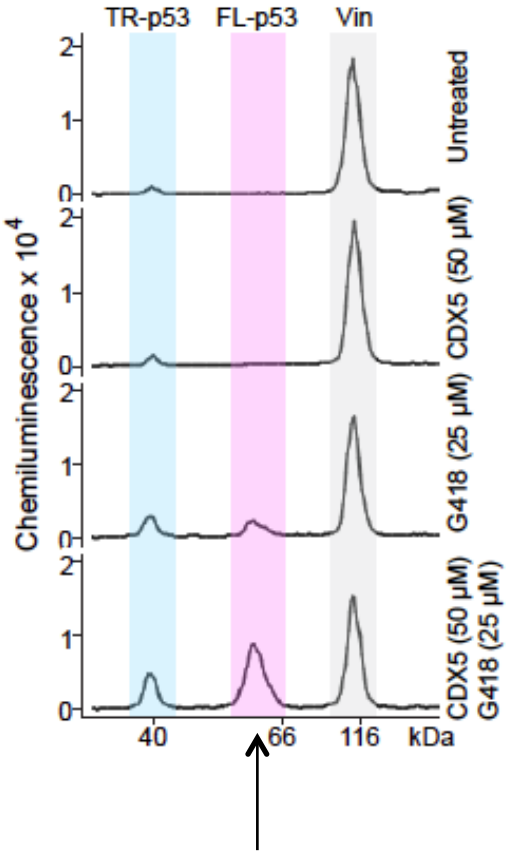


USP		R_1	R_2
25-50%	C1	CH_3	$NHCH_3$
10-35%	C1a	H	NH_2
25-55%	C2/C2a§	CH_3	NH_2
	C2b	H	$NHCH_3$

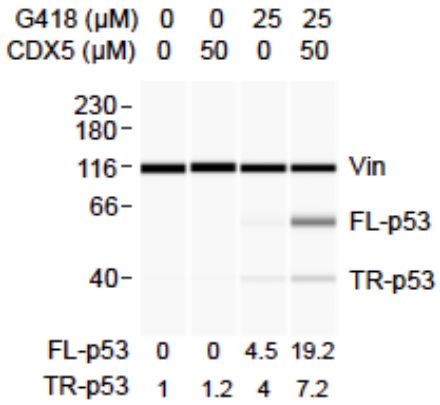
Model system: HDQ-P1 breast cancer cell line with homozygous *TP53* nonsense mutation

Automated p53 western assay for readthrough

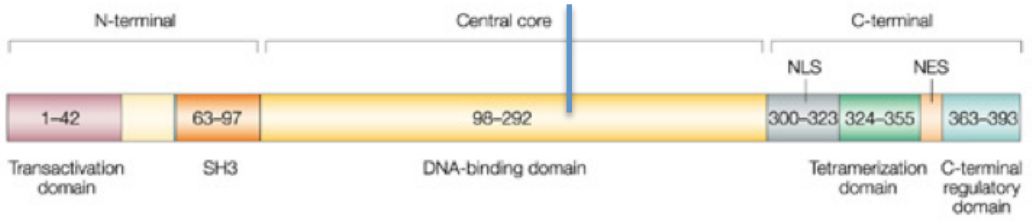




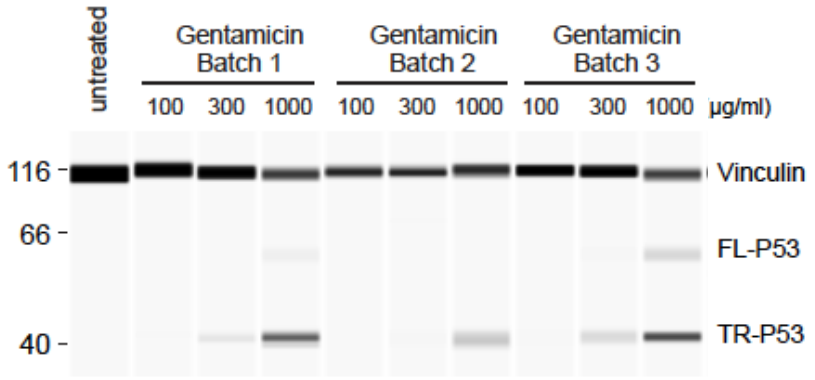
Pseudoblot representation



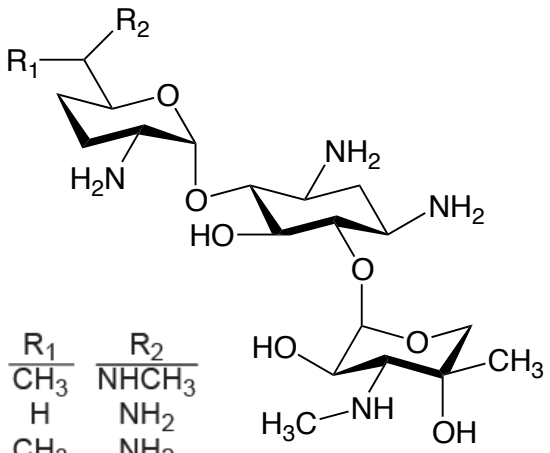
R213X



Gentamicin batches show variable readthrough activity

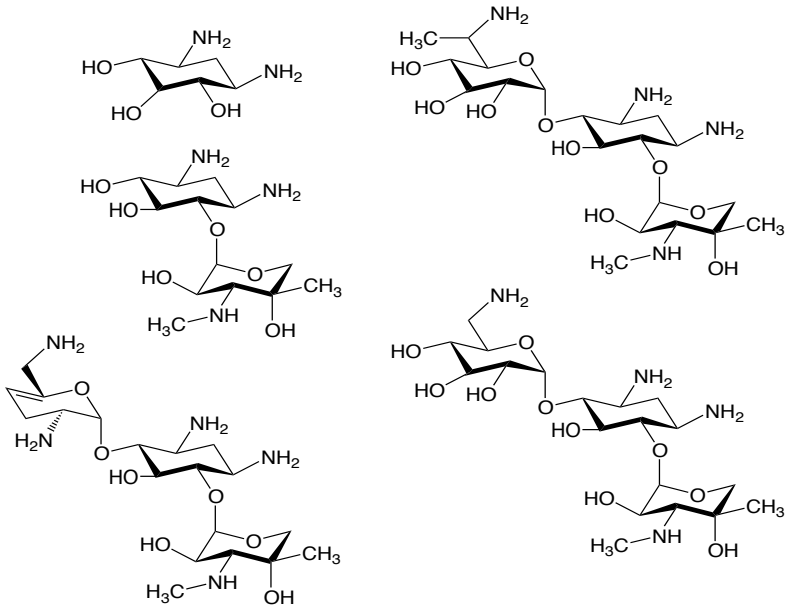


Major components (>97%)

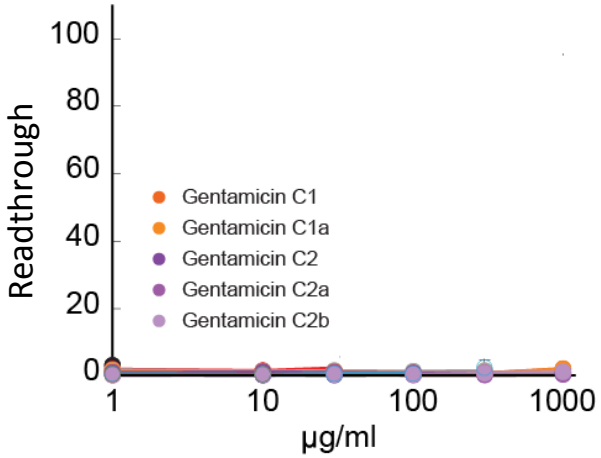


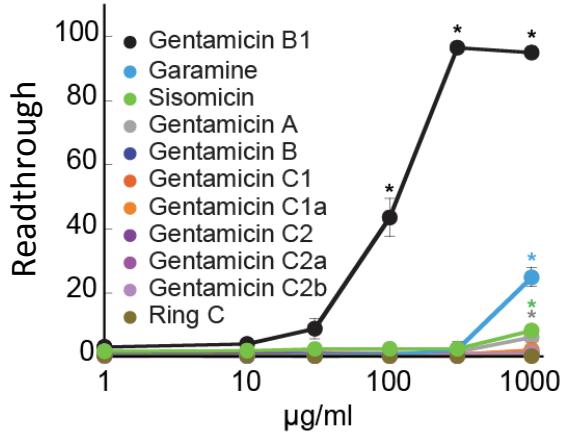
	R_1	R_2
C1	CH_3	$NHCH_3$
C1a	H	NH_2
C2/C2a§	CH_3	NH_2
C2b	H	$NHCH_3$

Minor components (<3%)

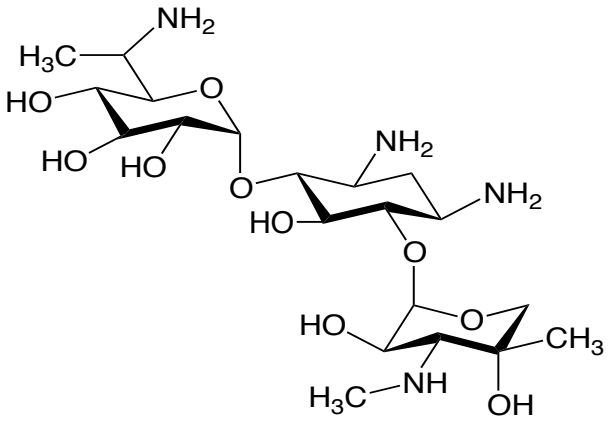


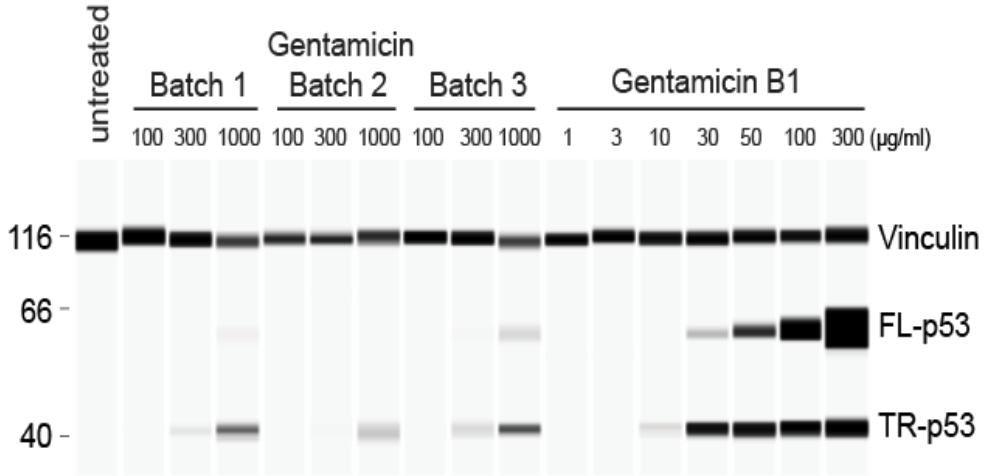
Major gentamicin components are inactive





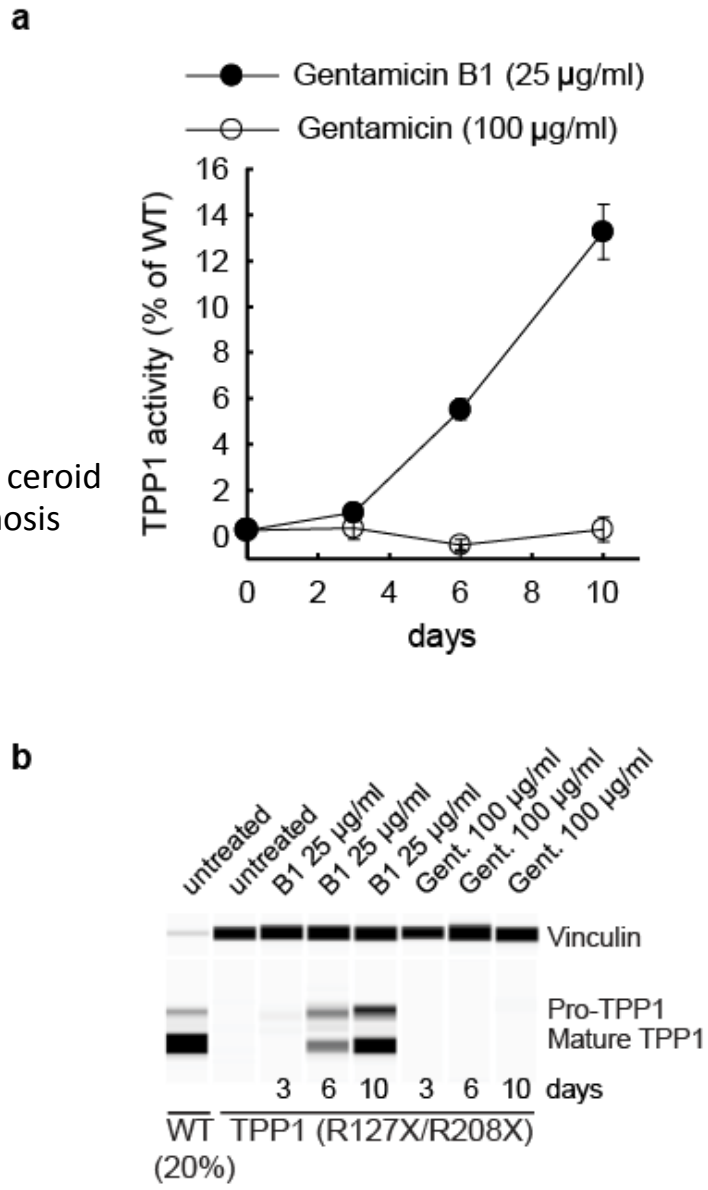
Gentamicin B1 shows potent PTC readthrough activity





Activity in cells from rare genetic disease patients

Neuronal ceroid lipofuscinosis CLN2

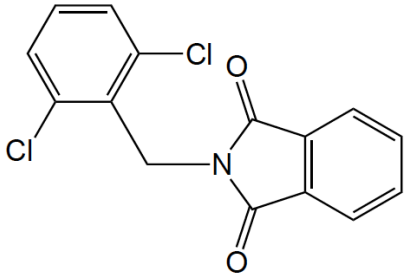


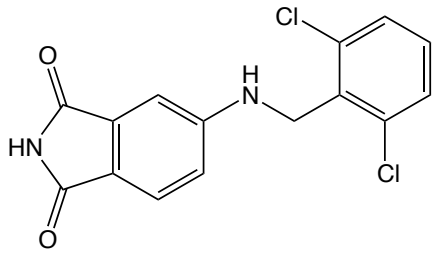
Duchenne muscular dystrophy

Schimke immuno-osseous dysplasia

Recessive dystrophic epidermolysis bullosa

Part 2
Enhancers of PTC readthrough by aminoglycosides

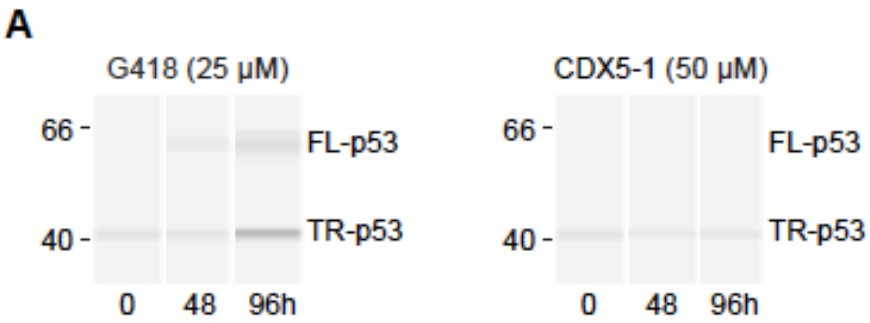




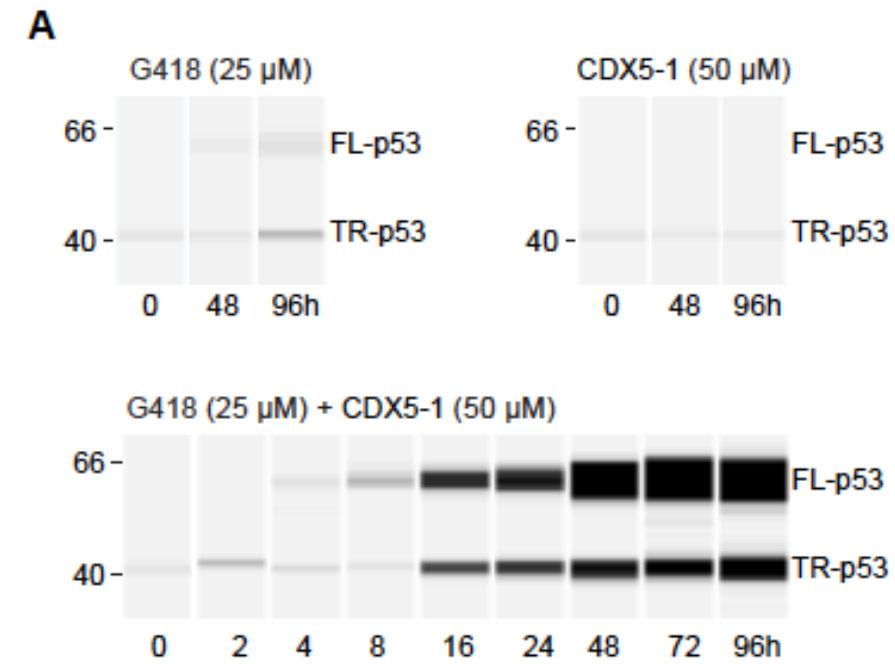
CDX5 ---------> 200 analogs --------> CDX5-1

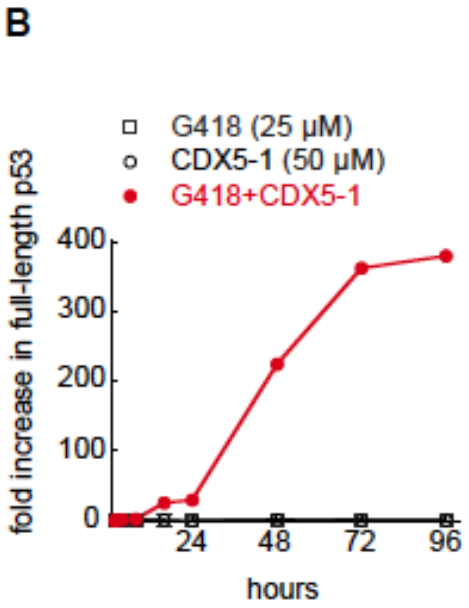
Small molecules that do not themselves induce readthrough
but potentiate readthrough by aminoglycosides

Time course CDX5-1 + G418



Time course CDX5-1 + G418

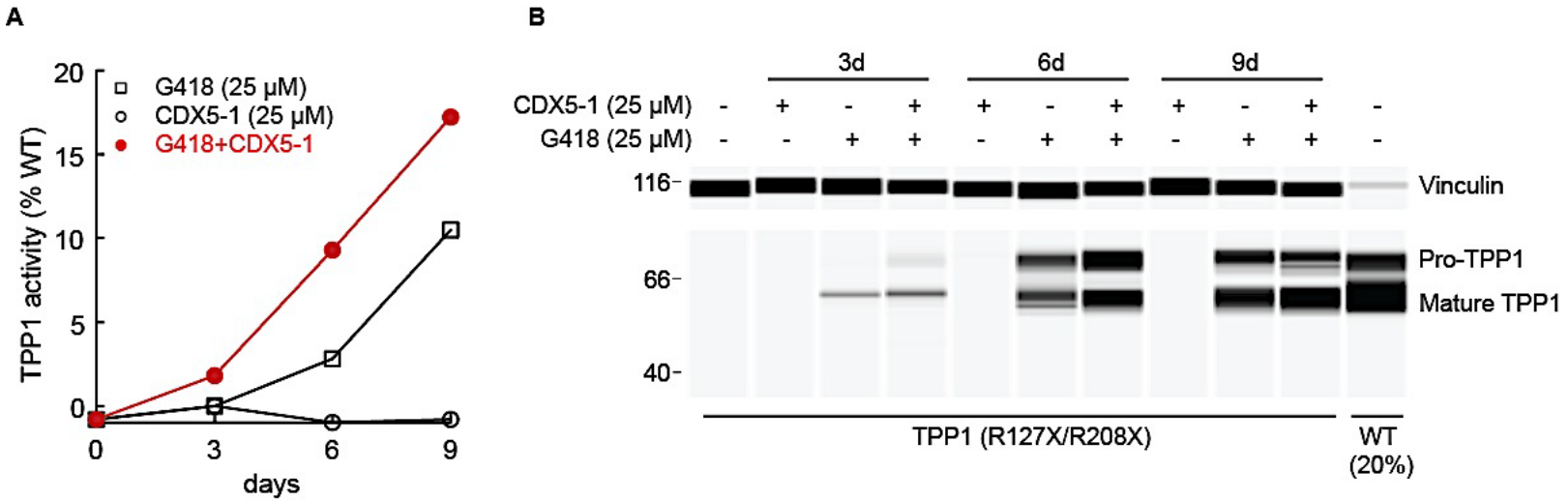


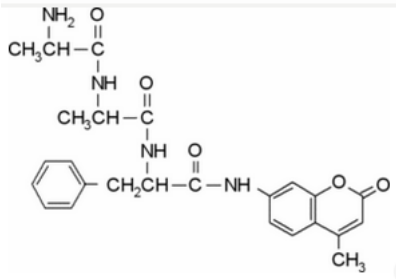


Compounds also potentiate readthrough by gentamicin B1

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)





Ala-Ala-Phe-7-amido-4-methylcoumarin

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 potentiator may broadly suppress nonsense mutations in a variety of genetic diseases including EB

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 potentiator may broadly suppress nonsense mutations in a variety of genetic diseases including EB

New collaborations with
Cristina Has, Fernando Larcher and Andrew South
First EB grant, starting Sept 1 2017

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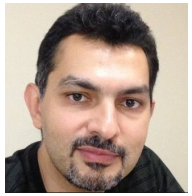
Aruna Balgi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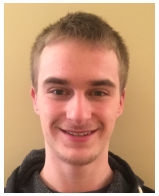
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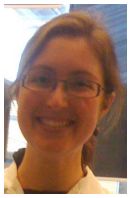
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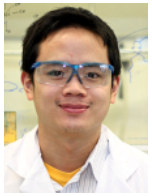
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