

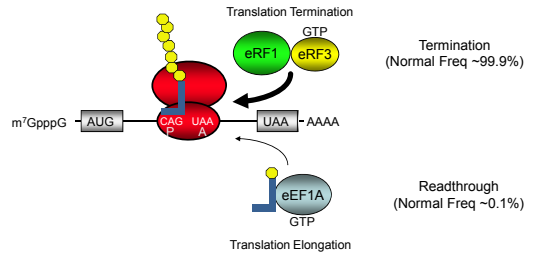
## Modulating Translation Termination To Treat Genetic Diseases

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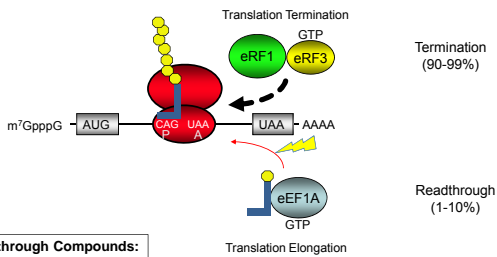
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### Basic Concept Behind Nonsense Suppression: Two Outcomes Are Possible When a Stop Codon Enters the Ribosomal A Site

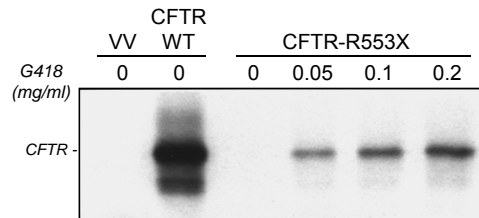


### Certain Drugs or Mutations Can Increase the Frequency of Stop Codon Suppression



- Readthrough Compounds:**
- Gentamicin
  - Amikacin
  - PTC124
  - Others

### The Aminoglycoside G418 Suppresses the CFTR-R553X Nonsense Mutation



Howard et al., Nature Medicine 2: 467-469 (1996)

Vaccinia-T7 expression system- HeLa cells

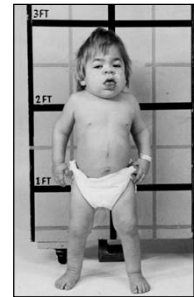
**Our Published CF Readthrough Studies Using the hCFTR-G542X Transgenic Mouse Model**

- ❖ The aminoglycosides gentamicin and amikacin suppress the hCFTR-G542X mutation in a transgenic mouse model.
  - ❖ References: Du et al., J. Mol. Med. 80: 595-604 (2002); Du et al., J. Mol. Med. 84: 573-582 (2006)
- ❖ The co-administration of gentamicin with the polyanion Poly-L-Aspartic Acid (PAA).
  - ❖ Greatly reduces the toxicity associated with gentamicin.
  - ❖ Enhances the level of readthrough obtained with gentamicin.
  - ❖ Prolongs the therapeutic effect after treatment is terminated.
  - ❖ Reference: Du et al., J. Biol. Chem. 284: 6885-6892 (2009)
- ❖ PTC124 suppresses the hCFTR-G542X mutation in a transgenic mouse model.
  - ❖ PTC124 is much less toxic than aminoglycosides and is orally bioavailable.
  - ❖ PTC124 is currently in Phase 3 clinical trials for cystic fibrosis and Phase 2 clinical trials for Duchenne muscular dystrophy and hemophilias A & B.
  - ❖ A recent publication in *The Lancet* showed phenotypic improvement in a significant fraction (~50%) of CF subjects treated with PTC124.
  - ❖ Reference: Du et al., Proc. Nat. Acad. Sci. USA 105: 2064-2069 (2008)

**The Lysosomal Storage Disease Mucopolysaccharidosis I-Hurler (MPS I-H)**

**Features of MPS I-H:**

- ❖ MPS I-H is an autosomal recessive lysosomal storage disease caused by mutations in the *IDUA* gene (encodes  $\alpha$ -L-iduronidase).
- ❖ The loss of this enzyme leads to an inability to break down glycosaminoglycans (GAGs) in lysosomes, leading to GAG accumulation throughout the body.
- ❖ Causes skeletal/joint abnormalities, abnormal facial features, mental retardation, heart, liver, and lung disease and death by  $\leq 10$  years of age.

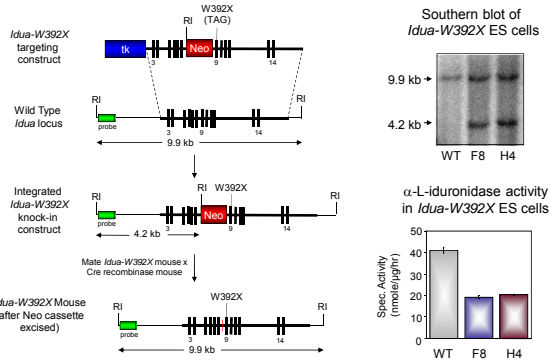


Neufeld and Muenzer, *The Mucopolysaccharidoses*, in *The Metabolic and Molecular Bases of Inherited Disease*, 2001.

**Why make a knock-in *Idua-W392X* Mouse as a model for PTC Suppression?**

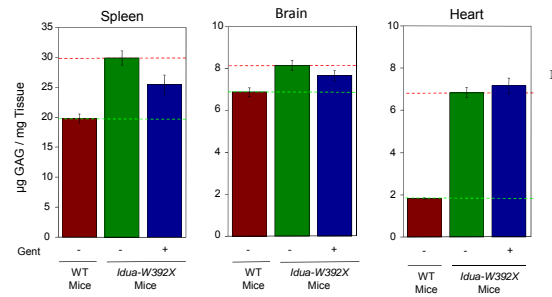
- ❖ Current treatments are not ideal:
  - ❖ Bone marrow transplant
  - ❖ Enzyme replacement therapy
- ❖ PTCs are found in 70% of MPS I-H patients of European descent.
- ❖ *IDUA* mRNA from MPS I-H patients with PTCs undergoes Nonsense-Mediated mRNA Decay (NMD).
- ❖ Provides an important *in vivo* test of the utility of PTC suppression to treat genetic diseases.

**Construction of an *Idua-W392X* Knock-In Mouse**

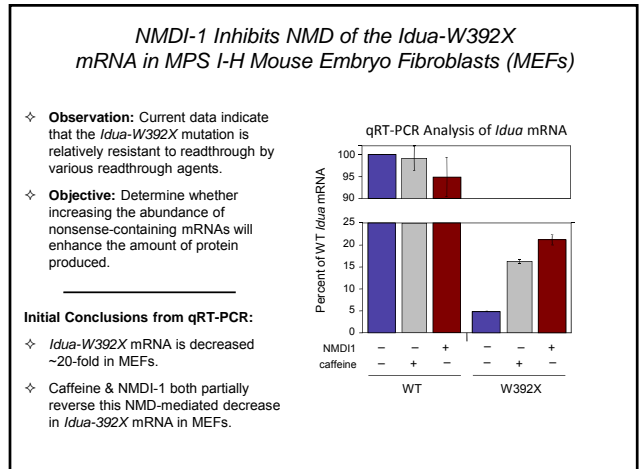
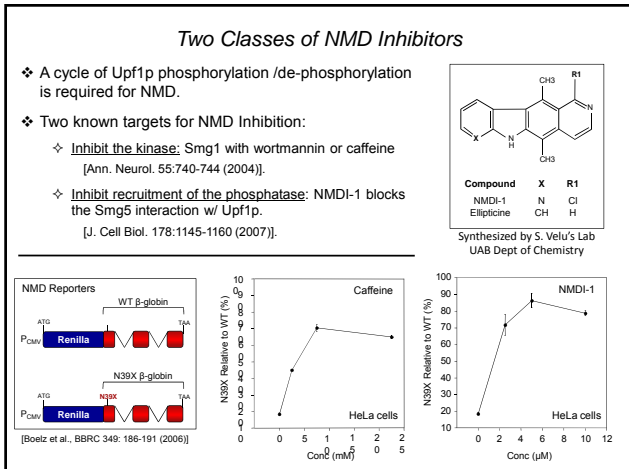
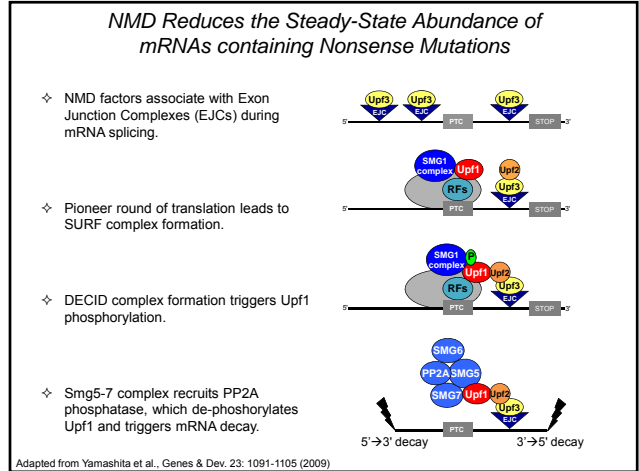
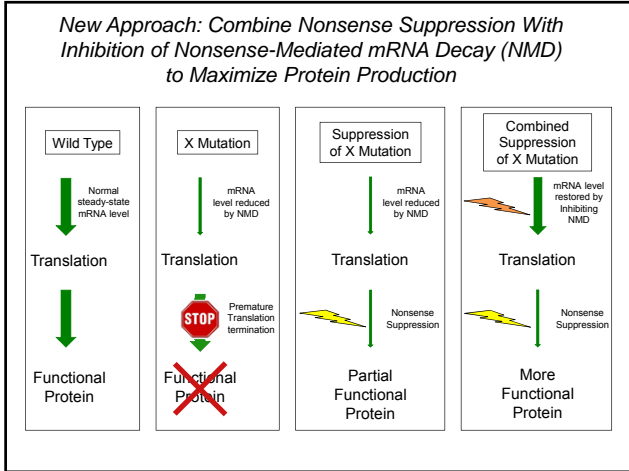


Wang et al., Mol. Genet. & Metab. 99: 62-71 (2010)

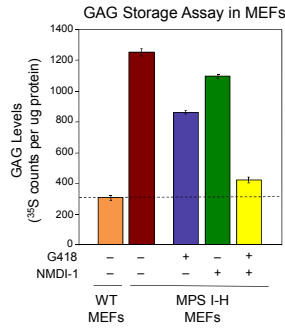
**PTC Suppression by Gentamicin Promotes Only Modest GAG Reduction in *Idua-W392X* Mice**



❖ 11 week old mice injected subcutaneously twice daily with 30 mg/kg gentamicin for 10 days.

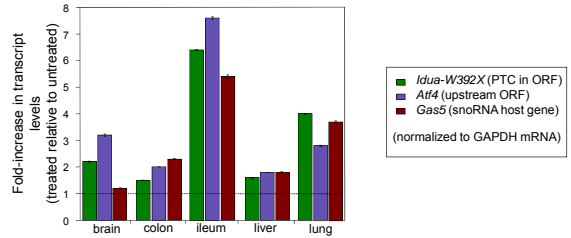


**Combination Therapy in MPS I-H Mouse Embryo Fibroblasts (MEFs) Reduces GAGs by Enhancing Readthrough**



❖ **Conclusion:** Combining nonsense suppression with NMD inhibition provides a much greater decrease in GAGs than either treatment alone.

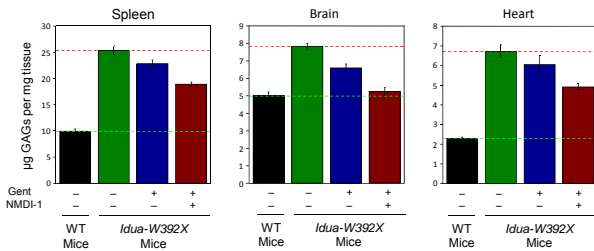
**NMDI-1 Inhibits NMD of Different Classes of NMD Substrates in *Idua-W392X* Mice**



❖ NMDI-1 (5 mg/kg) was administered subcutaneously (once daily) for 3 days to 4-week old *Idua-W392X* mice.

❖ **Conclusion:** NMDI-1 inhibits NMD of different classes of NMD substrate in organs of the MPS I-H mouse.

**PTC Suppression Combined With NMD Inhibition Enhances GAG Reduction in *Idua-W392X* Mice**



❖ 11 week old mice were injected with gentamicin (30 mg/kg) subcutaneously once daily for 14 days.  
 ❖ NMDI-1 (5 mg/kg) was administered by subcutaneous injection during the final 3 days of gentamicin treatment.

❖ **Conclusion:** NMD suppression enhances protein production resulting from PTC suppression.

**Overall Summary**

- ❖ Our studies suggest that nonsense suppression is a promising treatment for cystic fibrosis and MPS I-H patients that carry a nonsense mutation.
- ❖ This therapeutic approach could also be applicable to patients with many other genetic diseases that carry nonsense mutations.
  - ❖ According to the National Organization for Rare Disorders (NORD), there are ~6000 rare genetic diseases.
  - ❖ Roughly 25 million people in the U.S. have a rare genetic disease.
  - ❖ 10-12% of all mutant alleles are predicted to be nonsense mutations.
  - ❖ ~2.5 million people in the U.S. are predicted to have a genetic disease caused by a nonsense mutation.
- ❖ For any genetic disease, a unique minimum threshold of protein function is required to provide a phenotypic improvement.
- ❖ For some diseases, nonsense suppression alone may restore a sufficient level of protein production to surpass that threshold for some diseases.
- ❖ For other diseases whose response to nonsense suppression does not surpass that minimal threshold, a combination of approaches may provide additional protein function and phenotypic improvement. Possibilities include:
  - ❖ nonsense suppression by aminoglycosides plus PAA co-administration
  - ❖ nonsense suppression plus NMD inhibition
  - ❖ nonsense suppression plus Enzyme Replacement Therapy (ERT)

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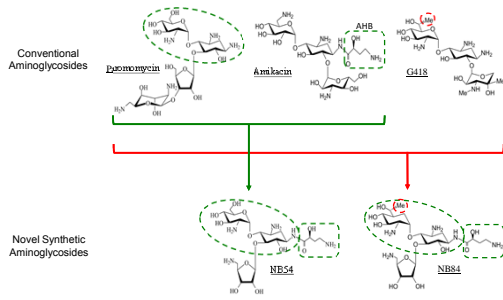
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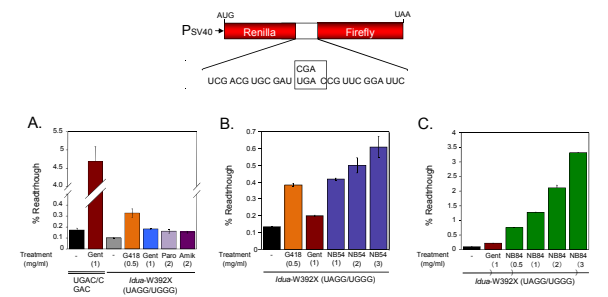
- ✦ Erik Schweibert, PhD

*Novel Synthetic Aminoglycosides Designed to Suppress Nonsense Mutations With Less Toxicity*



NB54 & NB84 designed and synthesized by Timor Baasov's Lab, Technion, Israel

*Synthetic Aminoglycosides Suppress Nonsense Mutations More Efficiently While Exhibiting Less Toxicity In Vitro*



NB54 & NB84 designed and synthesized by Timor Baasov's Lab, Technion, Israel

Assays in HEK293T cells