Amplifiers Enhance the Efficacy of Small Molecules to Promote the Translational Read-Through of CFTR Nonsense Mutations

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40th ECFS Conference - Seville, Spain
June 9th, 2017
Disclosures

• All authors are employees of Proteostasis Therapeutics, Inc.
• Proteostasis Therapeutics, Inc. has filed a provisional patent for the use of amplifier in phenotypic screening for CFTR modulators.
Amplifiers as a foundation for CF combination therapy development

**Potentiators**, such as PTI-808 and ivacaftor, act by increasing the opening time of the CFTR channel, resulting in higher ion flow.

**Correctors**, such as PTI-801 and lumacaftor/tezacaftor, are thought to facilitate the processing of mutated protein substrate, leading to improved delivery to the cell membrane.

**Amplifiers**, such as PTI-428, selectively increase the amount of immature CFTR protein in the cell, providing additional substrate for correctors and potentiators to act upon.

Amplifiers have been shown to enhance the efficacy of clinically active compounds and may serve as the lynchpin of CFTR modulator combination therapy approaches.
Amplifiers are designed to improve the efficiency of CFTR translation by enhancing successful signal-sequence targeting to the ER membrane.

- Amplifiers are designed to improve the efficiency of CFTR translation by enhancing successful signal-sequence targeting to the ER membrane.
- This slows CFTR mRNA degradation and tips the balance back in favor of CFTR protein biosynthesis.
- *In vitro* studies currently demonstrate that amplifiers increase the amount of unfolded CFTR protein.
Amplifier Enhances Aminoglycoside Read-Through of PTC Alleles in HBE Cells

• G542X/G542X HBE cells were treated with aminoglycoside in the presence or absence of amplifier.

• Amplifier and aminoglycoside activities are additive

**G542X/G542X HBE cells**

![](chart1.png)

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<th>Amplifier</th>
<th>G418</th>
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**mRNA**

![](chart2.png)

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**Activity (forskolin + ivacaftor)**

![](chart3.png)

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Amplifier Enhancement of Aminoglycoside PTC Read-Through is Conserved in FRT Cells

- Fischer Rat Thyroid (FRT) G542X-CFTR cells detect amplifier (PTI-CH) and aminoglycoside (G418) read-through activities (48 hr treatment)
- Amplifier (PTI-CH) and aminoglycoside (G418) activities are additive

**G542X mRNA**

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**G542X Protein**

- PTI-CH:
  - band C
  - band B
- G418:
  - 150 kDa

**G542X Activity (Conductance)**

- forskolin + ivacaftor
- CFTRinh-172

For more details, see the attached graph and images.
Amplifiers Enable Identification of Novel CFTR Modulators When Used in Combination Screening

Our strategy is to leverage the ability of amplifiers to increase the effectiveness of phenotypic screening to identify novel small molecules that enhance the translational read-through of CFTR PTC mutations.

~7-fold enrichment in true positives when screens were performed with amplifier
The CFTR-HRP Trafficking Assay as a PTC Read-Through HTS Assay platform

- HTS strategy is perform a phenotypic screen in combination with PTI amplifier in an assay using a full-length CFTR coding sequence

**FRT G542X-CFTR HRP cells**

- FRT Flp-In cells engineered to express G542X-CFTR with an HRP enzyme in extracellular loop 4 (ECL4)
- Full-length CFTR that traffics to the cell surface cleaves extracellular luminescent substrate
- Trafficking assay has been validated in a 384-well format (Liang et al., SLAS Technology 2017)
- Sensitive enough to detect G418 read-through activity

Zhang and Chen (2016) Cell
FRT G542X-HRP Trafficking Assay Detects Combination of Amplifier and Aminoglycoside PTC Read-Through Activities

- PTI-CH increased mRNA levels and enhanced G418-mediated read-through of the G542X-HRP
- Trafficking activity correlates with protein levels
- G418 and Amplifier (PTI-CH) activities are synergistic
- Amplifier (PTI-CH) combination screening 384-well format performance acceptable for HTS assay
  - >5-fold assay window between PTI-CH + G418 and PTI-CH
FRT G542X-HRP HTS in Combination with Amplifier

- HTS targeting G542X allele
  - Most prevalent allele, low basal read-through of PTC
  - No report of HTS screen with full-length G542X cDNA reported in the literature
- HRP trafficking assay was optimized to execute HTS in presence of amplifier (PTI-CH)
  - Further increased assay window to further sensitize assay to read-through agents (G418)
  - 384-well format; DMSO tolerability ≥ 1%: consistent Z’ scores
- Primary screen in combination with PTI-CH screen successfully executed

Hit cut-off: > 37% G418 activity
0.25% hit rate
Summary

- Amplifiers serve as a foundation for CF combination therapy and can enhance the efficacy of other CFTR modulators (correctors, potentiators).
- Amplifiers enhance the efficacy of read-through agents to promote the translational read-through of CFTR PTC mutations in primary patient cells and cell lines.
- Combination screening with amplifier was observed to enhance the “effectiveness” of phenotypic screens to identify novel, synergistic CFTR modulator compounds.
- The use of amplifier enabled the development of a sensitive PTC read-through assay using the G542X CFTR allele.
- Completed HTS in which compounds were screened in the FRT G542X-CFTR HRP trafficking assay in combination with amplifier.
- Currently characterizing HTS hits.
Acknowledgements

• Proteostasis Therapeutics, Inc
  • Hellen Kim
  • Danijela Dukovski
  • Daniel Qiu
  • Keith Chen
  • Josh Horan
  • Debora Melo
  • Lawrence Drew
  • Marija Zecevic
  • Po-Shun Lee
  • Ben Munoz

• The University of Alabama-Birmingham
  • Steve Rowe, MD
  • Venkateshwar Mutyam

• Emory University
  • Eric Sorscher, MD
  • Jeong Hong

• CFFT Lexington Lab
  • Martin Mense
  • Hermann Bihler
  • Feng Liang