Conclusions: In addition to CFTR, other genetic and environmental factors strongly influence the severity of CF disease. For these two siblings, the severity of the disease correlates with IL-8 and NE levels in sputum samples. Validation of these protein biomarkers will help in routine disease monitoring and could potentially be used as outcome measures in clinical trials.

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27

MUTATIONS IN THE SECOND CYTOPLASMIC LOOP OF CFTR SUGGEST DISTINCT MODE OF ACTION BETWEEN POTENTIATORS VX-770 AND GLPG1837

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The current therapeutic strategy to repair cystic fibrosis-causing defects in the chloride channel CFTR is to develop novel and better correctors (to improve folding) and potentiators (to improve function). Galapagos-AbbVie identified a novel potentiator GLPG1837 by compound screening on mutant CFTR. YFP-halide efflux assays and single channel measurements showed ~2.5-fold improvement in channel activity by GLPG1837 compared to VX-770 (ivacaftor/Kalydeco) on G551D CFTR (1, 2). GLPG1837 successfully passed the Phase-2 clinical trials and proved to be the first potentiator after VX-770 to show competitive results on G551D patients. To identify potential differences in the mode of actions of these potentiators we studied their effects on CFTR folding and function.

Biochemical radiolabeling experiments showed that mutations in the intracellular loop 2 (ICL2) disrupt domain assembly between TMD1 and NBD2, a late folding event in CFTR, but in most cases do not impair CFTR trafficking towards the cell surface. Protease-susceptibility assays showed that VX-770 improved late TMD1 folding of many ICL2 mutations, but GLPG1837 did not.

YFP-halide efflux assays showed that these ICL2 mutants had varying effect on channel function, ranging from wild-type-like to function-defective mutants. GLPG1837 restored function of non-CF gating mutant E267K much better than VX-770. Residue E267 in ICL2 electrostatically interacts with K1060 in ICL4 to promote channel opening (3). This indicates that GLPG1837 is more efficient in compensating for this lost interaction.

Altogether, our biochemical and functional data suggests that potentiators VX-770 and GLPG1837 have a different mode of action.

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28

LAMPREY CFTR, AN EVOLUTIONARY ANCESTOR OF HUMAN CFTR, EXHIBITS NUMEROUS BIOPHYSICAL DISSIMILARITIES FROM HUMAN CFTR

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Lampreys, an extant representative of the jawless vertebrates, diverged from jawed vertebrates approximately 650 million years ago and possess a unique CFTR ortholog. Lamprey CFTR (Lp-CFTR) shares 46% sequence identity and 65% sequence similarity with human CFTR (hCFTR). The biophysical consequences of this dissimilarity remain unknown so we investigated the channel behavior and pharmacology of Lp-CFTR expressed in *Xenopus* oocytes. Like hCFTR, Lp-CFTR channel activity was PKA-dependent with whole cell currents stimulated by forskolin and IBMX, and excised macropatch currents activated by intracellular exposure to MgATP and PKA. Two blockers of hCFTR, NPPB and glibenclamide, blocked Lp-CFTR activity in a manner comparable to hCFTR. Surprisingly, GlyH-101 failed to block Lp-CFTR at Vm = -60 mV and $CFTR_{inh}172$ exhibited modest inhibition of Lp-CFTR in both the whole cell (TEVC) and inside-out macropatch configuration. The sole clinical hCFTR potentiator VX-770 (Kalydeco^{TM}) mildly potentiated hCFTR in the presence of MgATP + PKA, recorded with the inside-out macropatch technique. However, Lp-CFTR was significantly inhibited by VX-770 under the same experimental conditions. Furthermore, Lp-CFTR exhibited significant inward rectification, the most among all the CFTR species tested thus far, when it was recorded in symmetrical 150 mM Cl^- in inside-out macropatches. The single channel behavior of Lp-CFTR was also unique. Whereas open hCFTR channels occupy the full open state (f) over 98% of the time, Lp-CFTR randomly occupies the f, the subconductance 1 (s1), or subconductance 2 (s2) states. In summary, Lp-CFTR has unique pharmacological and biophysical characteristics compared to hCFTR. The differences may provide a tool for identifying the binding sites and working mechanism of VX-770 and may shed light on understanding the evolution of CFTR.

29

MODULATING READTHROUGH AND THE NONSENSE MEDIATED DECAY PATHWAY WITH ANTISENSE OLIGONUCLEOTIDES TO UPREGULATE THE EXPRESSION OF W1282X CFTR

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Introduction: Antisense oligonucleotides (ASOs) are an established platform for drug development. ASO treatments can downregulate their target mRNAs through an RNase H mechanism; alternatively, through steric blocking mechanisms, ASOs can upregulate target mRNAs leading to increased protein production. Nonsense mutations generate premature termination codons (PTCs) that can subject the CFTR transcript to degradation through the nonsense mediated decay (NMD) pathway. A significant challenge to drug development for nonsense mutated-CFTR is the lack of model systems in which to screen potential drug candidates. Likely because of this, there are no targeted therapies available for patients with nonsense mutated-CFTR. The W1282X mutated CFTR protein may be particularly primed for therapeutic restoration; in vitro models show that it retains minimal function and enhancing its expression can increase chloride channel function. W1282X is the second most common nonsense mutation that causes CF, with an allele frequency of 1.218% in CFTR2 www.cftr2.org). Therapeutics that generate relatively small increases in CFTR function could have a significant clinical impact.

Objective: To identify ASOs that upregulate CFTR with nonsense mutations by promoting PTC readthrough or through inhibition of the NMD pathway, with the eventual goal of developing this strategy for therapeutics.

Methods: We have employed three strategies to upregulate the expression of nonsense mutated-CFTR with ASOs: 1) block the deposition of exon junction complexes downstream of PTCs, which serve as a trigger for NMD; 2) block NMD-initiating mRNA cleavage by SMG6; and 3) downregulate NMD and translation termination factors through an RNase H mechanism. The ASOs for each strategy will be initially screened in cell culture models for their ability to upregulate CFTR before they are tested in more human disease-relevant models.

We have developed an in vitro cell line model of the human CFTR W1282X mutation. This model utilizes the TRex-FlpIn system to incorporate an expression mini gene for the W1282X mutation into HEK293 cells, which normally express very low levels of CFTR. This system has been validated to produce CFTR mRNA and protein in a dox-inducible manner, and, importantly, the W1282X transcript resulting from this expression mini gene is targeted for decay by the NMD pathway. Data generated thus far show promising levels of mRNA upregulation with the inhibition of multiple NMD pathway components using RNase H-activating ASOs. Experiments are underway to determine the protein-level consequences of W1282X mRNA upregulation. Additionally, screens are ongoing to determine the efficacy of our steric blocking ASOs.

Conclusions: ASO modulation of the NMD pathway can upregulate CFTR W1282X at the transcript level. Therapeutic strategies that promote PTC read-through or modulate the NMD pathway have the potential to generate a functional or partially functional CFTR protein, thereby providing benefit to CF patients.

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