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DEVELOPMENT OF NEW NANOLUC LUCIFERASE-BASED DUAL READTHROUGH/NONSENSE-MEDIATED MRNA DECAY (DUAL RT/NMD) REPORTERS TO IDENTIFY NEW COMPOUNDS TO TREAT CYSTIC FIBROSIS CAUSED BY NONSENSE MUTATIONS

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In-frame premature termination codons (PTCs) comprise 11% of all disease-associated mutations. Translation of PTC-harboring mRNAs generates truncated proteins that are unstable and/or lack normal function. Drugs have been identified that suppress translation termination at a PTC to restore full-length protein (also referred to as readthrough of the PTC). A subset of aminoglycosides has been shown to induce PTC readthrough, but the amount of full-length protein generated by these drugs has not been sufficient to correct the phenotype of many diseases, including cystic fibrosis (CF). Furthermore, aminoglycoside toxicity limits their long-term clinical use. In addition, PTCs also frequently elicit nonsense-mediated mRNA decay (NMD). NMD is a conserved mRNA surveillance pathway that preferentially degrades PTC-containing mRNAs, thereby diminishing the pool of mRNAs available for translation and PTC readthrough. Most current compounds induce PTC suppression or mediate NMD inhibition alone, although a few have the capacity to mediate both responses to some extent. The goal of this study is to develop a reporter system that will allow us to systematically identify new compounds with the ability to simultaneously induce PTC suppression and reduce NMD, which would provide the optimal therapeutic potential for CF patients.

Here, we describe the development and characterization of NanoLuc luciferase-based dual RT/NMD reporters as well as NMD-only and RT-only control reporters in Fischer rat thyroid (FRT) cells. The response of these reporters to PTC suppression and NMD inhibition was tested by the treatment of cells with a variety of PTC suppressors and NMD inhibitors both individually and together. Our results show that NMD inhibition alone increased the NanoLuc luciferase activity and the mRNA abundance expressed from the dual RT/NMD reporter by 1.5-4 fold. Treatment with a variety of readthrough drugs increased NanoLuc activity 2-50 fold. Importantly, the combination of NMD inhibitors and readthrough drugs increased NanoLuc activity by as much as 700-fold (depending on the PTC context and the cell line). The synergistic effect of combining NMD inhibition with readthrough observed with the dual RT/NMD reporter was not seen in the NMD-only and RT-only controls. These results demonstrate that this new NanoLuc luciferase-based RT/NMD reporter system can be used to simultaneously monitor both NMD inhibition and PTC suppression. We anticipate that the use of this system will allow the identification of new, more effective compounds for the treatment of CF in patients that harbor PTCs.

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LUMACAFTOR/IVACAFTOR IN CF PATIENTS WITH HOMOZYGOUS P.PHE508DEL MUTATIONS: IMPROVING PERSONALIZED MEDICINE UTILIZING INTESTINAL ORGANOIDS

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Introduction: Lumacaftor and ivacaftor combination therapy is expected to debut in the Netherlands in 2016 as standard care for patients above 12 years of age who are homozygous for the p.Phe508del mutation. It is known that drug responses to this therapy may vary significantly and it is not clear yet which patients will benefit most. Recent developments enable us to assess the effects of CFTR-modifying drugs in vitro with a functional CFTR assay using patient-derived intestinal organoids (Dekkers JF, et al. Nat Med. 2013;19:939-45). This assay may facilitate future personalized medicine approaches, by predicting clinical responses based on the effects on the function of CFTR p.Phe508del mutant proteins in organoids. The objective of this project is to evaluate the clinical response to treatment with lumacaftor/ivacaftor in patients with homozygous p.Phe508del mutations and to assess the in vitro-in vivo correspondence of this response. This project is part of the HIT-CF program.

Methods: We aim to measure treatment effects of two times daily 200/125 mg lumacaftor/ivacaftor therapy after six months of treatment in one hundred and sixty p.Phe508del patients. To evaluate the clinical response, baseline measurements are performed, including quality of life (CFQ-R), lung function (FEV_1, expressed as % predicted), body mass index (BMI), sweat chloride concentration (SCC) and rate of pulmonary exacerbations during the preceding period of six months. Concurrently intestinal stem cell cultures will be derived from rectal biopsies, and tested for therapy-induced activity of the CFTR protein. Subsequently in vitro-in vivo correspondence of treatment will be evaluated.

Results: Currently baseline measurements have been performed in 158 patients. The median age is 23 years (IQR 16-29) and 52% of the patients are male. The measurements show a large variability in disease severity. The median score of the CFQ-R respiratory domain is 72.2 (IQR 55.6-83.3), FEV_1 71% predicted (IQR 55-86), BMI 20.3 kg/m^2 (IQR 18.6-22.1) and SCC 95 mmol/L (IQR 89-101). Preliminary data of in vitro organoid CFTR responses to lumacaftor/ivacaftor show quite variable responses in individual patients. Updated results will be presented at the conference.

Conclusion: The results of this project will facilitate personalized medicine approaches for lumacaftor/ivacaftor combination therapy in homozygous p.Phe508del patients with CF, by validating the predictive capacity of drug testing in intestinal organoids in vitro.

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INHIBITION OF INTERLEUKIN-8 SIGNALING BY ALPHA-1 ANTITRYPSIN: SUPPORT FOR THE USE OF AEROSOLIZED ALPHA-1 ANTITRYPSIN THERAPY IN CYSTIC FIBROSIS

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Introduction: Cystic fibrosis (CF) is characterised by neutrophil-dominated airway inflammation, in part attributable to the potent chemotactic agent interleukin-8 (IL-8). High levels of both neutrophil elastase (NE) and IL-8 have been found in the bronchoalveolar lavage fluid (BALF) of patients with CF. Alpha-1 antitrypsin (AAT) is an acute phase protein that possesses both immune regulatory and anti-inflammatory properties. This glycosylated protein is posttranslationally modified via the addition of N–glycosidically linked oligosaccharides such as sialic acid, giving rise to different glycoforms. The aim of this study was to investigate the ability of AAT versus AAT with increased sialylation (sAAT) to inhibit IL-8 signaling. The biological consequence of the AAT-induced inhibition was investigated at the level of neutrophil chemotaxis.

Methods: BALF was obtained from patients with CF (n=16) or healthy controls (n=10). The level of IL-8 in BALF was quantified via ELISA. The active level of NE in CF BALF was quantified by a specific fluorescence resonance energy transfer substrate (FRET). AAT was purified from human plasma using Alpha-1-Select Resin. AAT glycoforms were determined by isoelectrical focusing. The ability of AAT or sAAT to inhibit NE (500nM) was determined via FRET. The ability of sAAT or AAT (5μg/mL) to bind IL-8 (10ng/mL) was determined by slot blot. Neutrophil chemotaxis in response to IL-8 (10ng/mL) in the presence of AAT or sAAT (1mg/mL) was measured in a Boyden chamber. Statistical significance was obtained by Student's-*t* test, Mann-Whitney test, or one-way ANOVA followed by a post hoc Bonferroni test. Ethical approval was granted from Beaumont Ethics committee.

Results: Ex vivo analysis demonstrated significantly higher levels of IL-8 in BALF of patients with CF compared to healthy controls (n=10, p=0.04). The active level of NE found in CF BALF ranged from 2.03 - 18.17nM (n=6). Results of FRET analysis demonstrated no significant difference in the anti-NE capacity of AAT compared to sAAT (n=3, ns). Slot blot analysis revealed that sAAT bound higher levels of IL-8 compared to AAT (n=6, p=0.002). The neutrophil chemotactic index in response to IL-8