INTESTINAL ORGANOID MODELS AS A PATH FOR PERSONALIZED THERAPY DEVELOPMENT IN CYSTIC FIBROSIS

Annelotte Vonk[1], Jeffrey M. Beekman[1,2], Peter van Mourik[2], Cornelis K. van der Ent[2†], Adriana Villella[3], John Miller[3], Benito Munoz[3] , Marija Zecevic[3] and Geoffrey Gilmartin[3] on behalf of the HIT-CF Consortium

[1]Regenerative Medicine Center Utrecht, Utrecht, the Netherlands and [2]Department of Pediatric Pulmonology, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands; [3]Proteostasis Therapeutics, Inc., Boston, MA, United States. [†]Project Coordinator

INTRODUCTION

Cystic fibrosis (CF) is a rare genetic disorder with more than 90,000 patients worldwide. Mutation of both alleles of the cystic fibrosis transmembrane conductance regulator (CFTR) gene causes CF. Deficits in CFTR lead to imbalances in chloride and fluid exchange, causing mucus thickening and accumulation in multiple organs. Pathogenesis predominantly manifests in deteriorating lung function, digestion and fertility problems. Approved CFTR modulators are shown to slow, but not arrest disease progression.

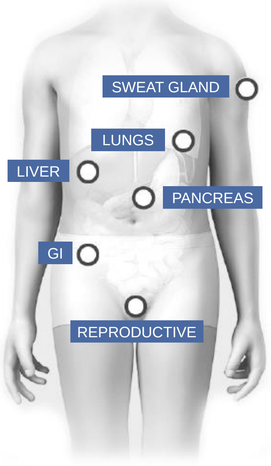
Approved CFTR modulators were developed using precision medicine with product labels restricted to specific CF genotypes. This limits accounting for response heterogeneity between patients with the same genotype, and prevents prediction of potential benefit for patients with genotypes not on the approved product labels. Several groups have demonstrated that patient-derived cells such as rectal organoids can accurately predict a donor's clinical benefit from approved CFTR modulators. This opens the possibility of personalizing therapies based on an individual's theratype instead of their genotype.

HIT-CF (www.hitcf.org) is a pioneering research project pursuing the transition from precision to personalized medicine for the treatment of CF. A consortium of academic, clinical and industry partners are collaborating to measure the in vitro response in patient-derived rectal organoids, and will use this data to select a subset of the patients for a confirmatory clinical trial. Up to 500 CF patients with ultra-rare genotypes will provide rectal biopsies for assaying as organoids for response to investigational CFTR modulators developed by Proteostasis Therapeutics, Inc., Eloxx Pharmaceuticals, Inc., and Flatley Discovery Lab, LLC. Selected high- and low-responders will be invited to participate in clinical trials initiating in 2020. Initial *ex vivo* data with CFTR modulators potentiator PTI-808 (dirocaftor) and corrector PTI-801 (posenacaftor) are described.

BACKGROUND

CF is a Systemic Disease and CFTR is Expressed in Multiple Tissues

- Although a systemic disease, morbidity and mortality is driven by progressive loss of lung function
- Other manifestations include digestive, metabolic, and reproductive disruptions
- CF is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene
- The CFTR gene encodes CFTR, a chloride channel that regulates ion flow across the apical membrane of epithelial cells (e.g. human bronchial epithelial cells)
- More than 2,000 CFTR mutations have been identified
- The most common CF-causing mutation, *F508del*, is present in at least one copy for up to 90% of the CF population
- More than 2,300 adult people in Europe with ultra-rare CFTR genotype (e.g. not F508del)

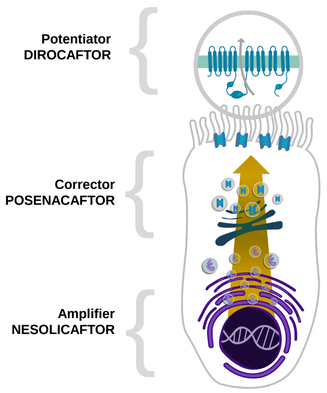


Disease Modifying Therapeutics Do Not Address All CF Genotypes

F508DEL HOMOZYGOTE | **F508DEL HOMO/HETEROZYGOTE** | **ULTRA RARE MUTATIONS**

- CFTR modulator therapeutics that target the underlying cause of the disease are available for limited set of genotypes:
  - Orkambi® for 1 homozygous genotype (F508del/F508del)
  - Kalydeco® for genotypes that include one of the 38 mutations
  - Symdeko®/Symkevi® for F508del homozygous genotype and 26 additional mutations
  - Trikafta® for genotypes that include at least one F508del
- These mutation/genotype-specific therapies leave at least 10% of people with CF without an approved modulator drug

Modulators Synergize to Maximize Mutated CFTR Protein Function



Potentiators such as dirocaftor* (DIR) enhance the ion transport activity of CFTR protein

Correctors such as posenacaftor* (POS) promote the maturation of CFTR protein to the cell surface

Amplifiers such as nesolicaftor* (NES) co-translationally increase the amount of CFTR protein

The results of a 28 day Phase 2 trial show the complementarity of the DIR/POS/NES triple combination versus the DIR/POS double combination with improvements in both lung function and sweat chloride, the latter being an established biomarker for CFTR function.

*dirocaftor, posenacaftor and nesolicaftor are investigational agents and are not approved by any regulatory agencies

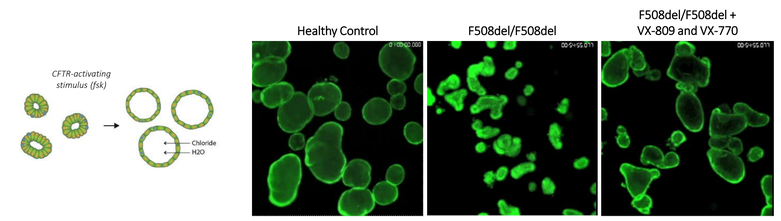
Positive Phase 2 Topline Results for F508del Homozygous Subjects (>18yo) with CFTR Modulator Combinations

	DIR/POS n=11	DIR/POS/NES n=11
Absolute improvement in percent predicted FEV_1 (compared to pooled placebo) (mean, 95%CI)	+3 (-2, 7) ns (n=10)[a]	+8 (3, 12) p ≤ 0.01 (n=11)[a]
Absolute improvement in Sweat chloride (compared to pooled placebo) (mean, 95%CI)	-15 mmol/L (-24,-6) P<0.01 (n=11)[a]	-29 mmol/L (-42,-16) P<0.0005 (n=11)[a]

[a]Total intent-to-treat population; number of subjects with non-missing measurements at week 4

HIT-CF APPROACH

Forskolin-Induced Swelling Assay Measures Individual CFTR Functional Rescue in Response to CFTR Modulator



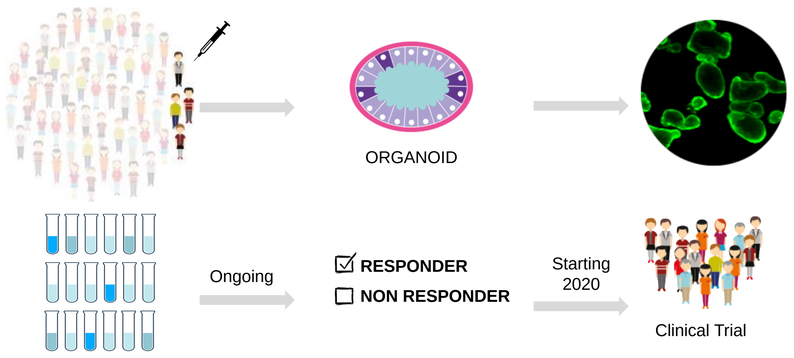
Beekman, J.M., Pediatric Pulmonology (2016)

Adapted from Dekkers et al., Nat Med, (2013)

- Forskolin (fsk) stimulation leads to opening of the CFTR ion channel and subsequent swelling due to ion and fluid transport into the lumen in a CFTR-dependent manner. Functional readout assesses the impact of both CFTR mutations and additional patient-specific genetic factors that act on CFTR function[1]
- Literature has shown that organoid responses to marketed CFTR modulators correlate with clinical response parameters in treated CF patients such as indicators of lung function improvement (change in ppFEV1 or percent predicted Forced Expiratory Volume in 1 second) and CFTR target engagement (SCC or changes in Sweat Chloride Concentration)
- In a study with 35 CF patients with multiple genotypes, the organoid assay was shown to have a Positive Predictive Value of 100% and a Negative Predictive Value of 80%[2] with the clinical response of treated patients

[1]Dekkers *et al.*, 2013 [2]Berkers *et al.*, 2019

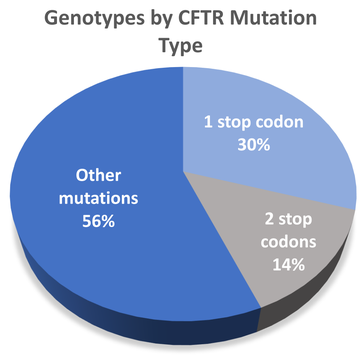
Organoid Based Personalized Medicine Strategy



- Ultra-rare donors provide biological samples (rectal suction biopsy) for organoid culturing and modulator response profiling
- The organoid assay identifies responders/non-responders for inclusion in a clinical trial

Patient Enrollment and Ex Vivo Study Status as of December 2019

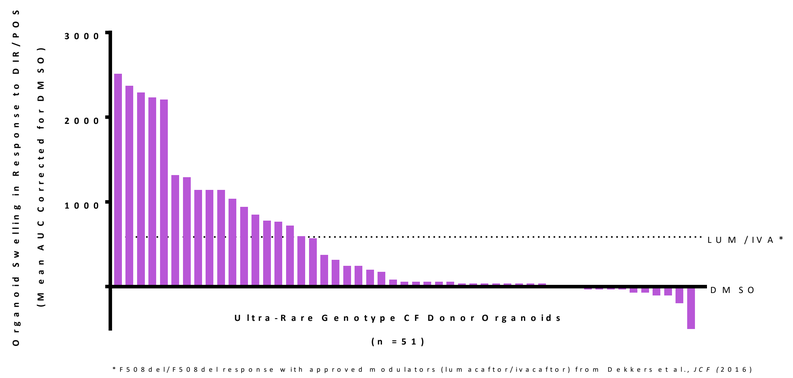
Country	# Patients
Israel	28
Czech Republic	10
The Netherlands and Germany	81
Belgium	30
Sweden	21
Italy	84
Poland	8
England	26
Spain	26
Denmark	4
France	11
Total included	**329**



- Patient samples from 329 patients have been collected through European CF Society's network of clinical trial sites. All patients meet the inclusion and exclusion criteria for the subsequent clinical trial (W.H.O. ID: NTR7520)
- Organoid culturing success rate is approximately 95%
- Standardized assay and protocol (manuscript submitted) is practiced by 3 independent laboratories currently performing *ex vivo* study with investigational drugs
- *Ex vivo* study with DIR, POS and NES to identify potential clinical responders will exclude genotypes that carry two stop codon mutations due to lack of CFTR protein synthesis

INITIAL EX VIVO STUDY RESULTS

Initial Identification of Potential Responders to DIR/POS Dual Modulator Combination



- Preliminary results suggest 75% (51/68) of donor organoids can be assessed for responsiveness
- 31% of assessable organoids show DIR/POS responsiveness superior to literature-reported LUM/IVA response in F508del/F508del organoids
- Secondary validation of responses, including expanded conditions and controls is underway
- DIR/POS/NES triple combination profiling is initiating

PROJECT STATUS & NEXT STEPS

- The HIT-CF project has developed the processes and infrastructure to recruit eligible CF patients, collect rectal biopsy samples, culture and biobank biological materials and perform *ex vivo* study with investigational agents through qualified laboratories
- Targeted collection of 500 donor samples is projected to complete in Q1 of 2020. *Ex vivo* study assessing responses to DIR, POS, and NES is projected to complete in the first half of 2020
- Organoid responsive patients will be invited to join a cross-over, placebo controlled clinical study with DIR, POS, NES in the second half of 2020

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The HIT-CF consortium is made up of the following partners:

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