Results: For this study, 90 intestinal organoids derived from PwCF have been selected from our organoid biobank, 45 of which harbor F508Del/F508Del or F508Del/minimal function mutations, whereas the other 45 harbor rare mutations. Measurements for this screen are ongoing.
Conclusions: This project is in progress, but preliminary data show promising results for the FT triple-combination treatment.

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Delivery of gp64-pseudotyped lentivirus carrying codon-optimized cystic fibrosis transmembrane conductance regulator provides better functional restoration in human cystic fibrosis airway epithelial cultures

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Background: Cystic fibrosis (CF) is caused by mutations in the gene encoding CF transmembrane conductance regulator (CFTR) protein, resulting in impaired chloride conductance across epithelia lining the respiratory airways. Gene therapy using lentiviral vectors (LVs) offers a promising approach for treating CF through stable genome integration for long-term CFTR transgene expression. Pseudotyping lentiviruses with specific glycoproteins increases their infectivity and modifies tropism. LVs pseudotyped with gp64, a baculovirus envelope protein, have been shown to transduce airway epithelia to deliver the CFTR gene in a pig model of CF [1]. We have developed gp64-pseudotyped LVs carrying the coding sequence for human (h)CFTR (gp64-LV-hCFTR), insulator elements for greater safety and expression, and a promoter that constitutively drives expression of the gene for human CFTR. Marquez Loza et al [2] used codon-optimized CFTR constructs and demonstrated a significant increase in CFTR function. Following that precedence, we designed various codon-optimized versions of gp64-LV-hCFTR to minimize long-term gene silencing and establish codon-specific molecular assays for hCFTR detection, quantification, and localization of messenger ribonucleic acid transcripts.
Methods: Codon-optimized versions of gp64-LV-hCFTR were designed, and the LV constructs were evaluated in Fischer rat thyroid (FRT) cells and primary CF human airway epithelia (HAE). CF-HAE or FRT cells were transduced with gp64-LVs at the time of seeding at various multiplicities of infection (MOIs), and cells were allowed to differentiate at the air-liquid interface (ALI). CFTR-specific chloride conductance was measured by Ussing chamber assay. Vector copy number (VCN) was determined by droplet digital polymerase chain reaction assay.
Results: Twenty-one days after transduction, gp64-LV-hCFTR restored CFTR-mediated chloride conductance across polarized HAE layers in a MOI dose-dependent manner. Correction of CF varied depending on the lentiviral purification process and correlated with increasing VCN, with full functional restoration achieved at 5 VCNs or fewer per cell. Using a gp64-LV reporter vector containing identical regulatory elements, we were also able to demonstrate reporter gene expression after more than 9 months in transduced CF HAE cultures, indicating durability of transgene expression. Assessment of codon-optimized CFTR plasmids in FRT cells showed even greater CFTR correction than with wild-type (WT) hCFTR. The codon-optimized CFTR LV constructs also showed successful transduction and improved CFTR functional activity by 2 to 3 times while maintaining a VCN similar to that of WT CFTR in four primary CF-HAE donors tested.
Conclusions: These data suggest the potential for greater therapeutic efficacy of gp64-LV-hCFTR with a codon-optimized hCFTR transgene. This approach not only results in significanly greater CFTR correction than with WT CFTR, but also allows design of specific, sensitive molecular assays for detection, quantification, and localization of the vector and gene expression.

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Identification of organoid responders to cystic fibrosis (CF) transmembrane conductance regulator modulators in the Human Individualized Treatment for Cystic Fibrosis Europe project—Underlining the need for new treatment strategies for people with ultra-rare mutations

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Background: People with cystic fibrosis (PwCF) who carry ultra-rare CF transmembrane conductance regulator (CFTR) mutations are often not included in drug development pipelines of pharmaceutical companies. Currently, based on their genotype, no efficient modulator combination is approved for 15% to 20% of PwCF, although they could potentially benefit. These numbers are even higher outside Europe. The forskolin-induced swelling (FIS) assay in intestinal organoids has been found to be highly predictive of a donor's clinical response [1]. The Human Individualized Treatment for CF project (HIT-CF; www.hitcf.org) was designed to bring drugs to PwCF with ultra-rare mutations. Intestinal organoids of PwCF with ultra-rare mutations were screened with compounds that had already passed phase I and II clinical trials (dirocaftor (DIR)/posenacaftor (POS)/nesolicaftor (NES) and ELX-02). DIR is a novel potentiator, and POS is a novel corrector. NES, a first-in-class CFTR amplifier, selectively increases the amount of immature CFTR protein. ELX-02 is a readthrough compound designed for PwCF carrying nonsense mutations.
Methods: Founr hundred eighty-nine 489 patient-derived organoids (PDOs) were collected from PwCF from 18 European countries and Israel. All PDOs with at least one nonsense mutation were screened with ELX-02, and those with no more than one nonsense mutation with DIR/POS. The FIS assay was conducted as previously published [1].
Results: Screening was technically successful in 471 PDOs with ELX-02 (n = 221) or DIR/POS (n = 380), with 130 PDOs included in both screens. In the ELX-02 screen, PDOs were incubated with the drug for 48 hours before FIS, and a PDO sample from a G542X/G542X donor was used as a positive control. In the DIR/POS/NES screen, PDOs were incubated for 24 hours with DIR/POS using a PDO from a F508del/F508del donor incubated for 24 hours with tezacaftor/ivacaftor as the positive control. NES was screened in only a subselection. FIS responses in the PDOs were categorized into three classes: (1) higher response than in the positive control, (2) no organoid swelling, and (3) organoid swelling but not classified as 1 or 2. This classification allows estimation of how many PwCF who carry ultra-rare mutations could benefit from current and upcoming CFTR modulating therapies. The minimum swelling associated with clinical response is not yet known. The largest group (55% of PDOs) was classified as class 3, followed by class 1 (23%) and class 2 (22%). These results are preliminary; a more detailed analysis will be presented at the conference.
Conclusions: Based on organoid responsiveness to ELX-02 and DIR/POS, up to 78% of PwCF that carry ultra-rare mutations could benefit from upcoming CFTR modulating therapies. Although we have taken a great step forward in personalized medicine for PwCF, there is still a large unmet need for those who have *CFTR* class VII (unrescuable) mutations or variants that cannot be restored by the tested CFTR modulating therapies.
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