Supplementary data

Supplementary methods

Calculation of AUC for the FIS assay (see supplementary Figure S2)

Two wells are used for each condition. Images of each well are taken at 10 minutes interval during the FIS assay, and typically contain 15 to 60 organoids. The total organoid area is calculated from each picture, and normalized to the area at t0 (considering area at t0 as 100%). This normalized area is plotted vs time, to calculate the AUC (area under the curve) for each of the wells.

For each experiment, the mean of the AUCs of the 2 wells is calculated for each of the forskolin concentrations and modulators used. The experiment is repeated on three different days, and the final AUC reported is the mean (and SEM) of the AUCs from the three experiments. The final graph summarizes the changes in AUC for each forskolin concentration and modulator (combination).

Supplementary discussion

Supplementary information about rare mutations analyzed in this study

The results on CFTR rescue in the organoids of CF patients with different rare mutations observed in this study correlates well with the position, structural and functional information previously reported about these mutations, as described below.

Mutation E92K [1], located in the first transmembrane (TM1) part of membrane spanning domain 1 (MSD) is thought to abrogate a salt bridge needed for correct protein folding [2]. CFTR function was rescued by lumacaftor in the organoids, suggesting E92K is a class II mutation, concurring with results in heterologous cells systems [2,3] showed to be a

processing/trafficking mutant as E60K [4]. E92K was also rescued by correctors GLP2222 [5], FLD304 and FLD160 [6] supporting the folding defect.

The L159S mutation has been reported in the CFTR1 database in a pancreatic sufficient patient diagnosed at 3 months of age. Both patients tested in this study are also pancreatic sufficient, and one has a sweat chloride in the intermediate range in line with a high residual function observed in the FIS assay. This mutation lies on the first intracellular loop of MSD1 within 5A of a putative docking site for corrector molecules [7]. The Q237E position, also in MSD1 (TM4), being in a transmembrane domain, may be involved in pore formation. Thus, there may be alterations in the gating properties of the CFTR channel when this position is mutated. The FIS assay of this mutant protein showed some residual function but mainly high rescue of CFTR function with ivacaftor, which may reflect the rescue of the putative gating defect.

We found residual CFTR function and rescue of function by ivacaftor and lumacaftor- ivacaftor in F508del/R334W organoids. The R334W mutation, located in TM6 of MSD1, reduced single-channel conductance by ~60% by impeding ion-ion interactions within the CFTR pore [8], thus suggesting disturbed gating or conductance. This is in agreement with the FIS results where most of the CFTR rescue came from ivacaftor. In contrast, in FRT cells, this mutation is reported as associated with normal CFTR expression, but very low function and no rescue of function by ivacaftor [9]. Hence assessment of benefit from modulators seems needed in subjects.

We noted moderate responses to ivacaftor in organoids with mutations A455E, D1152H, 3849+10Kb C>T and R117C. Also when expressed in FRT cells, these mutations have >10% improvement in CFTR function [9], findings that led to FDA approval for ivacaftor treatment for these mutations [10].

We observed considerable residual function for mutation E831X, however no rescue with the modulators, in accordance with E831X being a splicing mutation that induces exon 14b skipping in 76% of transcripts, a premature stop codon in 16% and lack of one amino acid in

8% [11]. The latter transcripts result in a functional protein, that may be responsible for this residual function observed in organoids homozygous for this mutation.

We found almost no response to modulators in I1234V/W1282X organoids although in CFTR2, I1234V is annotated as having 107% expression and 40% of wild type function in FRT cells. In fact, other studies already showed that this is not a missense mutation but a splicing mutation and the resulting truncated protein lacking 6 amino acid from the N-terminal portion of second nucleotide binding domain (NBD2) has very low function [12,13].

Organoid results for mutations R334W and E831X contradict findings in FRT cells. Discrepancies between findings in organoids and FRT cells have been reported before. Based on data in FRT cells, mutation G970R was included in the clinical trial assessing the effect of ivacaftor in patients with non G551D gating mutations. However, a benefit was seen in all subjects except those with the G970R mutation [14]. Subsequently rescue of CFTR function by ivacaftor was proven absent in organoids of patients with the G970R mutation and the mutation was proven to induce alternative splicing [15]. This demonstrates the superiority of using the patients' own tissue rather than heterologous expression of mutations in non-human cell lines.

We highlight 2 non-characterized non-responding/swelling mutations, 1648_1652dupATCAT and K464E. The duplication 1648_1652dupATCAT induces alterations in the reading frame leading to a premature stop codon and resulting in no production of normal CFTR protein. In K464 (lysine) localized in the walker A region of the first nucleotide binding domain is completely conserved in CFTR sequences across different species [16]. This amino acid is thought crucial for the ATP binding [17].

Lumacaftor and ivacaftor was the treatment used for all the analysis in the paper except for the patients that were treated clinically, for which the FIS assays were done posteriorly and at that time were already using tezacaftor (also 3μM) and ivacaftor. The number of assays done

with teza/iva combination was very reduce, not allowing similar correlation as the ones done for the luma/iva.

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