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Original Article

Clinical effects of the three CFTR potentiator treatments curcumin, genistein and ivacaftor in patients with the CFTR-S1251N gating mutation

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ABSTRACT

Background: The natural food supplements curcumin and genistein, and the drug ivacaftor were found effective as CFTR potentiators in the organoids of individuals carrying a S1251N gating mutation, possibly in a synergistic fashion. Based on these *in vitro* findings, we evaluated the clinical efficacy of a treatment with curcumin, genistein and ivacaftor, in different combinations.

Methods: In three multi-center trials people with CF carrying the S1251N mutation were treated for 8 weeks with curcumin+genistein, ivacaftor and ivacaftor+genistein. We evaluated change in lung function, sweat chloride concentration, CFQ-r, BMI and fecal elastase to determine the clinical effect. We evaluated the pharmacokinetic properties of the compounds by evaluating the concentration in plasma collected after treatment and the effect of the same plasma on the intestinal organoids.

Results: A clear clinical effect of treatment with ivacaftor was observed, evidenced by a significant improvement in clinical parameters. In contrast we observed no clear clinical effect of curcumin and/or genistein, except for a small but significant reduction in sweat chloride and airway resistance. Plasma concentrations of the food supplements were low, as was the response of the organoids to this plasma.

Conclusions: We observed a clear clinical effect of treatment with ivacaftor, which is in line with the high responsiveness of the intestinal organoids to this drug. No clear clinical effect was observed of the treatment with curcumin and/or genistein, the low plasma concentration of these compounds emphasizes that pharmacokinetic properties of a compound have to be considered when *in vitro* experiments are performed.

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Abbreviations: ATP, adenosine triphosphate; AUC, area under the curve; BMI, body mass index; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CFQ-r, cystic fibrosis questionnaire-revised; DMSO, dimethylsulfoxide; FIS, forskolin induced swelling; ppFEV₁, percentage of predicted forced expiratory volume in one second; SCC, sweat chloride concentration.

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1. Introduction

Cystic Fibrosis (CF) is an autosomal recessive disease that affects over 70,000 patients worldwide [1]. In the last decade new treatments that enhance the function of the mutated cystic fibrosis transmembrane conductance regulator (CFTR) protein have been developed and introduced in the care for patients with CF. Currently, these treatments are available for only part of the patients with CF, depending on the combination of (the more than 2000 known) CFTR variants [2]. Clinical studies on the effect of these CFTR modulators show impressive improvement in clinical parameters, but indicate that CFTR function after treatment appears not yet on the level of healthy controls [3–9]. Another limitation of the currently available CFTR modulators is their lack of accessibility, which highlights the importance of exploring new therapies that can increase *in vivo* CFTR function, using interventions that are easily accessible to people.

In previous studies, we showed that we can generate intestinal organoids by isolating stem cells from rectal biopsies and expanding them in a 3D culture condition [10,11]. Adding the adenylyl cyclase activator forskolin to these cultures induces CFTR dependent swelling of organoids (forskolin induced swelling (FIS)) which can be used to study the residual CFTR function of an individual with CF, and the response to CFTR modulating drugs [11–14]. This makes this assay an ideal drug-screening platform to evaluate the effect of (combinations of) drugs on the function of the CFTR protein, also for individuals with specific mutations.

Using the intestinal organoid model to evaluate the *in vitro* effect of different compounds on CFTR function showed that the natural food supplements curcumin and genistein, and the potentiator ivacaftor (VX-770) were effective as CFTR potentiators in organoids of patients with a p.Ser1251Asn (S1251N) gating mutation [15]. These *in vitro* experiments also showed co-potentiator effects of these compounds, especially when genistein and ivacaftor were combined; the organoid response upon combined addition resulted in twice the organoid response compared to the sum of the responses upon addition of the single compounds. Earlier *in vitro* studies also showed that both curcumin and genistein can improve the gating of the CFTR protein through an ATP-independent and ATP-dependent mechanism, respectively [16–18]. The synergistic effect of curcumin and genistein on another CFTR gating mutant, G551D, has been previously described in two *in vitro* patch-clamp studies [19,20]. However whether the synergistic effect of these food supplements can also be observed in people with CF remains unknown [19–21]. In contrast, ivacaftor has not only shown positive effects on CFTR gating *in vitro* studies, but clinical trials have clearly confirmed its efficacy *in vivo*, mainly in patients with a CFTR gating mutation [3–5,22]. Worldwide S1251N is a relatively rare CFTR mutation, however, it is the most common gating mutation in The Netherlands. [23] Previous clinical trials showed that ivacaftor treatment of patients with the S1251N mutation resulted in a clear clinical effect, comparable to its effect in patients with the more common G551D gating mutation [3,4].

Here we asked whether the (combinations of) compounds that potentiated CFTR function in intestinal organoids would also be efficacious in people carrying the S1251N mutation. Therefore, we conducted three clinical trials in which we treated individuals with S1251N with (a combination of) curcumin, genistein and ivacaftor. As a secondary aim we investigated the plasma levels of curcumin, genistein and ivacaftor achieved in these patients and investigated the effect of plasma that was collected after treatment, in the organoid assay.

2. Methods

2.1. Design

We performed three multicenter clinical trials with different (combinations of) CFTR potentiators. Study 1 (NTR4585) was an eight-weeks open label intervention study with a combination of curcumin (AOV 811 curcuma longa) and genistein (AOV 805 genistein). Both compounds were produced by the company AOV (part of Atrium Innovations INC), information on the formulation specification and quality can be found in supplemental Table S1. Dosage schemes of curcumin and genistein were based on doses that were used in previous clinical trials described in literature. Curcumin dose was between 102.9 and 138.5 mg/Kg/day and genistein dose was between 3.3 and 5.0 mg/Kg/day in 3–4 doses (supplemental Fig. S1). Study 2, (NTR4873) was an eight-weeks observational study in patients that started with ivacaftor at the time that it was introduced in The Netherlands (supplemental Fig. S2). All patients received ivacaftor in a dose of 150 mg, twice daily. Study 3 (NTR6515) was a randomized double-blind placebo controlled cross-over study with eight weeks of genistein treatment (AOV 805 genistein, between 5.0 and 10.0 mg/kg/day) eight weeks of treatment with matched placebo and a four weeks washout period, in patients already receiving ivacaftor (supplemental Fig. S3). The placebo capsules were produced by the Erasmus MC hospital pharmacy (“Apotheek A15”) which is certified according to “good manufacturing practice” (GMP) standards.

2.2. Subjects

Included patients had at least one S1251N mutation, were at least 6 years old and already had a rectal biopsy to produce an organoid. Patients were not included in a study if they were already using another CFTR modulator (besides ivacaftor in study 3). In study 1 and 3, patients could not be included if there were any contra-indications for the use of curcumin and/or genistein or if they had an exacerbation needing intravenous therapy in the four weeks prior to the start of the study. The study protocols were reviewed and approved by the medical ethics committee of the University Medical Center Utrecht. All participants (and/or their legal representatives) provided written informed consent.

2.3. Clinical outcomes

In all 3 studies we evaluated the clinical effect of the treatment by measuring change in: percentage of predicted forced expiratory volume in one second (ppFEV₁, measured according to ATS-ERS standards and converted to percentage of predicted using reference values of Koopman et al. and Quanjer et al. [24–26]), airway resistance, sweat chloride concentration (SCC, sweat collected using a Macroduct® system and analysis performed according to the most recent version of the standard operating procedure of the European Cystic Fibrosis Society-Clinical Trial Network), quality of life examined by the cystic fibrosis questionnaire – revised (CFQ-r), fecal elastase concentration and body mass index (BMI). To monitor safety, patients were asked to keep a diary in which they had to report any possible side effects. Both the relation of these adverse events (AE's) to the study drug and the severity of the AE's (a 5 points scale from mild to fatal) was scored by a medical doctor at the site.

2.4. Blood collection and storage

Before and after each treatment period a blood sample was collected which was used to measure the plasma concentration of

the compound and also to evaluate the effect of the plasma in the intestinal organoid model. At the end of each treatment period the blood collection was performed at the expected T-max of the treatment (3 h for curcumin + genistein, 4 h for ivacaftor and ivacaftor + genistein). Blood was collected in a Vacuette® heparin tube and centrifuged during 20 min at 450 g at 4°C to collect the plasma, which was immediately stored at –80 °C. Methods of measuring plasma concentrations of the three compounds as well as their metabolites can be found in supplemental Table S2.

2.5. Organoid measurements

Organoids were cultured according to previously described protocols [11]. Organoid swelling was measured as biological duplicates and FIS experiments were repeated at three independent culture time points. To evaluate FIS, calcein green labeled organoids were monitored by a Zeiss LSM800 live cell confocal microscope (37 °C, 5% CO₂) for 60 min with 10 min intervals. Zen Image analysis software (Zeiss) was used to quantify the total organoid area (µm²) per well for each time point after which relative swelling increase over time was normalized to time point zero ($t = 0$ min baseline of 100%). Response was defined by the area under the curve.

The response to the three potentiator treatments was evaluated in the intestinal organoids of patients included in the trials. The organoids were stimulated with a combination of 10 µM genistein (Sigma) plus 50 µM curcumin (Sigma), with 3 µM VX-770/ivacaftor (Selleck Chemicals LLC) and with a combination of 3 µM VX-770/ivacaftor (Selleck Chemicals LLC) plus 10 µM genistein (Sigma) together with 0.128 µM forskolin. The FIS observed after adding the three (combinations of) compounds was normalized to the mean FIS in response to dimethylsulfoxide (DMSO).

Organoid responses to plasma that was collected after treatment was evaluated together with forskolin (1.5% plasma and 0.128 µM forskolin for curcumin + genistein and ivacaftor treatment, 1% plasma and 0.320 µM forskolin for ivacaftor+genistein treatment) and normalized to the mean FIS in response to blank plasma under the same conditions.

2.6. Statistical analysis

Because of the limited number of patients with the S1251N mutation in the Netherlands, we were not able to power the study for small clinical effects. No formal power calculation was performed and a sample size of 15–20 patients per study was estimated based on the number of patients with the S1251N mutation numbers. Descriptive statistics were used to describe the study population included in the three trials. Depending on the nature and distribution of the characteristics we calculated proportions, means (including sd) and medians (including IQR). The effect of a treatment was evaluated by comparing the mean or median value of an outcome parameter in the study population before the start of a treatment with the mean or median value in the study population after eight weeks of treatment. For this comparison a paired samples *t*-test or Wilcoxon signed rank test was used, depending on the distribution of the values. A *p*-value < 0.05 was considered to indicate a statistically significant change. Analysis was performed using IBM SPSS Statistics version 22.

3. Results

3.1. Participants

A total of 13, 16 and 14 patients participated in study 1, 2 and 3, respectively. There were 13 patients who participated in both study 1 and 2, and 9 patients participated in all three studies. There were

no dropouts during the studies, consort diagrams for each study can be found in supplemental Fig. S4. The baseline characteristics of the included patients are shown in Table 1. This table not only shows that most patients carried the F508del mutation on the second allele, but also indicates that for most parameters the baseline values were already in the normal range at the start of study 3.

3.2. Clinical effect

Overall, treatment with ivacaftor resulted in a clear clinical effect showing significant improvement in virtually all clinical outcome parameters tested. For study 1 (curcumin + genistein) we observed no change in ppFEV₁ but in study 2 (ivacaftor) we observed a statistically significant increase of median ppFEV₁ of 9.5% compared to baseline. Treatment of patients with genistein on top of ivacaftor treatment (study 3) did not result in any further increase of median ppFEV₁. An overview of the changes in ppFEV₁ of individual patients is provided in supplemental Fig. S5. Changes in the other outcome parameters were in line with these findings in ppFEV₁, except for a small but statistically significant change of SCC and airway resistance upon curcumin + genistein treatment (Table 2). Analysis of the collected plasma showed that for the majority of patients (10 out of 13) curcumin was not detectable. Genistein showed median plasma concentrations of 3 and 14 µg/L (i.e. 0.01 and 0.05 µM) in study 1 and 3, respectively, and median ivacaftor concentrations were 1942 and 1160 µg/L (i.e. 4.95 and 2.96 µM) in study 2 and 3, respectively. Metabolites of curcumin and genistein after treatment in study 1 (shown in supplemental Table S3) were in the micromolar range.

3.3. Organoid responses

To study the CFTR potentiating effect of the test compounds at plasma concentrations that were reached during treatment *in vivo*, we applied an organoid-based bioassay developed previously for measuring functional levels of the CFTR potentiator ivacaftor in human plasma and compared the organoid swelling responses to plasma collected after treatment with the responses to purified compounds (Fig. 1) [27]. In line with previous observations we found that micromolar concentrations of curcumin+genistein, ivacaftor and ivacaftor+genistein added *in vitro* to S1251N intestinal organoids were all capable of inducing forskolin-activated, CFTR-mediated organoid swelling [15]. In contrast, a clear gain in forskolin-induced organoid swelling was detected only with plasma samples collected after patient treatment with ivacaftor (study 2) but not when stimulated with plasma samples obtained after combined treatment with the food supplements genistein and curcumin or addition of genistein to ivacaftor treatment (study 1 and 3; Fig. 1).

3.4. Adverse events

The adverse events (AE's) that were reported by more than one patient are shown in Table 3. In all three studies the majority of patients reported at least one AE. The AE's that occurred during treatment with curcumin + genistein and were possibly or probably related to the study drug were headache and gastro-intestinal symptoms. During treatment with ivacaftor the reported AE's that were possibly or probably related to the study drug were coughing up more sputum, headache and gastro-intestinal symptoms. During study 1 and 3 there were no serious adverse events (SAE's), during study 2 there were 4 SAE's reported, of which none was possibly or probably related to the study drug.

Table 1
Baseline characteristics of patients.

	Curcumin + Genistein (n = 13)		Ivacaftor (n = 16)		Ivacaftor + Genistein [#] (n = 14)	
	n		n		n	
Male sex (n, %)	13	8 (61.5)	16	11 (68.8)	14	6 (42.9)
Age in years (median, IQR)	13	15.0 (10.0 – 33.0)	16	16.0 (9.8 – 34.3)	14	19.0 (14.0 – 30.5)
CFTR mutation on second allele (n, %)	13	F508del (12, 92.3) R117H (1, 7.7)	16	F508del (12, 75.0) R117H (2, 12.5) A455E (1, 6.3) 17171G > A (1, 6.3)	14	F508del (13, 92.9) UNK (1, 7.1)
ppFEV ₁ (median, IQR)	12	75.5 (64.0 – 93.8)	16	73.0 (65.3 – 93.0)	14	92.5 (61.5 – 108.0)
ppRawtot (median, IQR)	10	121.5 (103.8 – 156.8)	12	108.0 (88.3 – 133.5)	14	113.5 (91.5 – 162.8)
SCC in mmol/L (median, IQR)	13	80.0 (65.5 – 91.0)	16	74.0 (63.3 – 109.8)	13	25.5 (13.0 – 31.0)
CFQ-r respiratory (median, IQR)	13	66.7 (45.8 – 69.4)	16	83.3 (72.2 – 87.5)	14	86.1 (72.2 – 94.4)
CFQ-r digestion (median, IQR)	13	66.7 (61.1 – 77.8)	16	72.2 (66.7 – 100.0)	14	83.3 (66.7 – 100.0)
BMI (median, IQR)	13	20.3 (16.5 – 22.9)	16	19.7 (16.7 – 22.8)	14	20.8 (19.1 – 22.6)
Elastase (median, IQR)	11	<15 (<15.0 – 141.0)	16	<15 (<15.0 – 246.0)	13	79 (<15.0 – 367.5)

BMI, Body Mass Index; CFQ-r, CFTR, Cystic Fibrosis Transmembrane conductance Regulator; IQR, Inter Quartile Range; ppFEV₁, percentage of predicted Forced Expiratory Volume in one second; ppRawtot, percentage of predicted airway resistance; SCC, Sweat Chloride Concentration; UNK, unknown.

[#] Measurement at baseline of period in which patient received genistein, during baseline measurement all patients were using ivacaftor (2dd 150 mg).

Table 2
Effects of potentiator treatment.

	Curcumin + Genistein (n = 13)		P-value	Ivacaftor (n = 16)		P-value	Ivacaftor + Genistein [#] (n = 14)		P-value
	n			n			n		
Organoids									
Organoid response to drug at 0.128 μ M forskolin (median, IQR)*	13	1092 (688 – 1541)		15	2225 (1942 – 2626)		8	3240 (2807 – 3712)	
Clinical response, change in:									
ppFEV ₁ (median, IQR)	12	0.0 (–3.8 – 4.0)	0.799	16	9.5 (6.0 – 20.0)	0.001	14	0.0 (–2.0 – 3.5)	0.591
ppRawtot (median, IQR)	10	–15.5 (–23.0 – –2.0)	0.019	12	–7.0 (–14.8 – 7.5)	0.306	13	–1.0 (–30.0 – 28.0)	0.917
SCC in mmol/L (median, IQR)	11	–6.0 (–16.0 – –1.0)	0.047	15	–48.0 (–88.0 – –28.0)	0.001	13	2.0 (–2.5 – 3.3)	0.529
CFQ-r respiratory (median, IQR)	13	8.3 (–5.6 – 20.8)	0.099	16	9.7 (0.0 – 16.7)	0.018	14	0.0 (–5.6 – 8.3)	0.205
CFQ-r digestion (median, IQR)	13	11.1 (0.0 – 22.2)	0.310	16	5.6 (–8.3 – 30.6)	0.182	13	0.0 (0.0 – 22.2)	0.200
BMI (median, IQR)	13	0.2 (–0.2 – 0.7)	0.196	16	0.4 (0.0 – 1.0)	0.023	14	0.3 (–0.2 – 0.7)	0.331
Elastase (median, IQR)	10	0.0 (0.0 – 8.3)	0.285	13	3.0 (0.0 – 16.0)	0.018	11	0.0 (–24.0 – 0.0)	0.398
Plasma:									
Concentration of the drug in plasma after treatment in μ g/L (median, IQR)	13	Curcumin: ND Genistein: 3 (2 – 15)		16	Ivacaftor: 1942 (1028 – 2588)		13	Ivacaftor: 1160 (954 – 1636) Genistein: 14 (5 – 31)	

BMI, Body Mass Index; CFQ-r, Cystic Fibrosis Questionnaire revised; CFTR, Cystic Fibrosis Transmembrane conductance Regulator; IQR, Inter Quartile Range; ND, Not Detectable; ppFEV₁, percentage of predicted Forced Expiratory Volume in one second; ppRawtot, percentage of predicted airway resistance; SCC, Sweat Chloride Concentration.

* Response of organoids to drug minus response of organoids to DMSO; [#] Compared to the effect of ivacaftor alone; clinical response upon adding genistein treatment to ivacaftor (2dd 150 mg).

4. Discussion

In this study the effect of oral administration of three compounds with CFTR potentiator activity in intestinal organoids was evaluated in people carrying the S1251N CFTR gating mutation. The three clinical trials performed are among the first drug trials that were initiated on the basis of their CFTR stimulating effect in the intestinal organoid model. Although not all trials were performed in a double blinded setting, they all had a comparable design which included an eight-weeks treatment period and similar outcome parameters.

We observed a clear clinical effect of treatment with ivacaftor alone (study 2), with a significant improvement in ppFEV₁, SCC, CFQ-r and BMI values. The observed clinical effectiveness of ivacaftor therapy was comparable to the outcome of a previous trial

on the effect of ivacaftor in patients with a non-G551D mutation [4]. In contrast, combination therapy with the natural food supplements curcumin and genistein (study 1) or supplementation of ivacaftor with genistein (study 3) failed to show a statistically significant clinical effect. Despite the dosages of the supplements approached the upper limits used in previous clinical trials (supplemental Table S3), the plasma concentrations of the food supplements during treatment were in the submicromolar range, which is far below the concentration that was effective in the *in vitro* organoid swelling experiments (3–100 μ M genistein; 25–200 μ M curcumin) [15]. The plasma concentrations of ivacaftor were in line with the known pharmacokinetic properties of this compound and reached concentration (3–5 μ M) corresponding with maximal swelling of the S1251N organoids *in vitro* [15,28].

Fig. 1. Organoid response to pure drug and plasma collected after treatment with drug. Organoid swelling was measured as biological duplicates and FIS experiments were repeated at three independent culture time points. (Mean $\pm$ SD of three culture points). Organoid response to the pure drug (left): Area under the curve (AUC) of swelling of organoids from patients included in the trials after measuring for 60 min. Responses after adding a combination of 10 μ M genistein plus 50 μ M curcumin, 3 μ M VX-770/ivacaftor and a combination of 3 μ M VX-770/ivacaftor plus 10 μ M genistein all together with 0.128 μ M forskolin. Responses are normalized to the mean organoid response to DMSO with 0.128 μ M forskolin. Organoid responses to plasma collected after treatment (right): AUC of swelling of organoids with a F508del/S1251N mutation after measuring for 60 min. Responses after adding 1.5% plasma together with 0.128 μ M forskolin (for plasma collected after treatment with curcumin + genistein and ivacaftor) and 1% plasma together with 0.320 μ M forskolin (for plasma collected after treatment with ivacaftor + genistein). Responses are normalized to the mean organoid response to blank plasma in combination with 0.128 μ M forskolin (for curcumin + genistein and ivacaftor) and 0.320 μ M forskolin (for ivacaftor + genistein).

Table 3
Adverse events.

	Curcumin + Genistein (n = 13)	Ivacaftor (n = 16)	Ivacaftor + Genistein [#] (n = 14)
<i>Number of patients that reported:</i>			
Any adverse event (n, %)	12 (92)	15 (94)	7 (50)
Adverse event (possibly) related to study drug (n, %)	9 (69)	10 (63)	1 (7)
The following adverse events were reported* (n, %)			
Bloating	–	1 (6)	–
Common cold	–	1 (6)	–
Cough	1 (8)	–	–
Cough up more sputum	1 (8)	7 (44)	–
Decreased appetite	–	1 (6)	–
Diarrhea	1 (8)	1 (6)	1 (7)
Flatulence	3 (23)	–	–
Headache	4 (31)	5 (31)	1 (7)
Malaise	1 (8)	–	–
Nausea	1 (8)	3 (19)	–
Night sweats	1 (8)	–	–
Sinus complaints	–	1 (6)	–
Skin rash	–	–	1 (7)
Sore eyes	–	1 (6)	–
Sore nose	–	1 (6)	–
Sore throat	–	1 (6)	–
Sputum less viscous	–	1 (6)	–
Stomach ache	2 (15)	4 (25)	1 (7)
Throwing up	1 (8)	2 (13)	–
Vertigo	1 (8)	–	–
Yellow stool	4 (31)	–	–

*Only adverse events that were (possibly) related to the study drug and were reported by more than one patient are shown #Events upon adding genistein treatment to ivacaftor (2dd 150 mg).

In comparison with previous pharmacokinetic studies in which comparable doses of curcumin and genistein were taken orally (supplemental Table S3) the plasma concentrations of genistein and curcumin measured in our study are remarkably low. [32–39]. However, when we also take the concentration of metabolites and conjugates into account, both curcumin and genistein reached concentrations that were very similar to the values reported in the literature (405 and 955 μ g/L, respectively). This comparison suggests that the previous studies have included metabolites and conjugates of curcumin and genistein in their analysis as well. Importantly, the results of our clinical trials and the lack of organoid response to the plasma of treated patients suggest that these metabolites, un-

like the original compound, are unable to stimulate CFTR as we see no clear functional effect *in vivo* and *in vitro*, with the possible exception of the sweat glands. The modest but significant reduction in sweat chloride levels observed in study 1 (Table 2) suggests that curcumin and/or genistein may accumulate in the sweat duct over time and might reach high enough local concentrations to stimulate S1251N CFTR in a subset of patients.

Most plausibly, the low plasma concentrations of genistein and curcumin reached in the circulation, and the apparent lack of CFTR potentiation by their metabolites underlie the lack of clinical efficacy observed. Moreover, in case of study 3, all clinical parameters were already drastically improved by the ivacaftor treatment and

were for some already within the normal range (Tables 1 and 2). This makes it difficult to detect a potential further clinical improvement by the additional administration of genistein.

Poor absorption of the food supplements from the intestinal tract, in combination with their fast metabolism in the liver could well account for the low plasma levels found in our clinical trials. Here it should be noted that, among the various CFTR-expressing epithelia, intestinal epithelial cells are exposed at their apical border to much higher concentrations of the food supplements passing the gut and therefore would have a better chance of functional repair by these CFTR potentiators. Unfortunately the most direct test to verify this assumption, *i.e.* CFTR intestinal current measurements (ICM) in rectal biopsies before and after treatment, was not included in our studies [29]. However our studies did allow an indirect assessment of CF-related gastro intestinal dysfunction in the S1251N patients on the basis of CFQ-r, BMI and fecal elastase tests. The outcome of these tests did not reveal signs of a significant improvement following an eight weeks treatment with the food supplements.

Recent attempts in healthy volunteers to improve oral bioavailability of curcumin by its dispersion with colloidal nanoparticles (named theracurmin) resulted in a ~27-fold higher area under the blood concentration-time curve (area under the curve, AUC) in comparison with curcumin powder [30]. However the serum levels reached remained within the submicromolar range, *i.e.* below the level needed for effective CFTR potentiation [31]. Intravenous administration of food supplements could in principle bypass this hurdle, but chronic treatment would be strenuous for the patient and would no longer meet the “Generally recognized as safe” (GRAS) FDA designation of the food supplements.

Because there are a limited number of patients with a S1251N mutation in the Netherlands, we could not include more patients in the trials. As a result the trials were underpowered to detect small changes in clinical parameters, nevertheless the results of treatment with ivacaftor show that a clear clinical effect could be identified.

4.1. Conclusion

In conclusion, we evaluated the effect of three CFTR modulator treatments, which were found effective in intestinal organoids of patients with a S1251N mutation. We observed a clear improvement in multiple clinical parameters upon treatment with ivacaftor, which is in line with the clear functional response of intestinal organoids to this drug. This further supports that intestinal organoids can be used as a valuable tool for *in vitro* drug screening and selection of therapies. The lack of a clear and statistically significant improvement in the clinical parameters upon treatment with the natural food supplements curcumin and genistein most plausibly results from the low plasma concentration of these compounds. This highlights the importance of implementation of the pharmacokinetic properties of a compound when performing *in vitro* experiments. We can account for these pharmacokinetic properties by measuring the swelling response of organoids generated from rectal biopsies to plasma samples of the patient who is using the drug.

Contributors

G.B. designed and coordinated the clinical trials, provided clinical data, analyzed and interpreted results and generated article text and figures. R.v.d.M. provided patient material and clinical data and revised the manuscript. P.v.M provided clinical data, interpreted results and revised the manuscript. A.M.V, E.K. and S.S. were responsible for organoid cultures, performed the FIS experiments and revised the manuscript. H.G.M.H, C.M, G.H.K, J.R, H.M.J,

Y.B.d.R and M.M.K. provided patient material and/or clinical data and revised the manuscript. J.M.B designed the organoid experiments, interpreted results and generated article text. C.K.v.d.E designed and coordinated the clinical trials, provided patient material and clinical data, interpreted results and generated article text. H.R.d.J. designed and coordinated the clinical trials and revised the manuscript.

Declaration of Competing Interest

J.M.B. and C.K.v.d.E are inventors on a patent application related to these findings. GHK reports research funding from the Lung Foundation of the Netherlands, GSK, Ubbo Emmius Foundation, Vertex, TEVA the Netherlands, TETRI Foundation, outside the submitted work and participation in advisory boards from GSK and PureIMS, outside the submitted work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jcf.2020.04.014](https://doi.org/10.1016/j.jcf.2020.04.014).

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