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Human Medicines Research and Development Support Division

Report on Enpr-EMA workshop on gastrointestinal (GI) outcome measures to evaluate CFTR modulators for the treatment of cystic fibrosis (CF)

Tuesday, 8 December 2015, at EMA

Background

Recently introduced new CFTR modulator treatments target the basic defect of cystic fibrosis. CFTR modulators offer the potential of a curative treatment. Some CFTR modulators have currently been registered for clinical use in CF patient with specific CFTR mutations; others are under development or tested in several clinical trials.

The currently accepted primary outcome measures for clinical trials in CF are sweat chloride, FEV1 and weight gain. In practice also pulmonary exacerbation rate (FDA) and fecal elastase (EMA) have currently been accepted as secondary outcomes for use in clinical trials.

In practice the currently used set of outcome measures show some drawbacks:

1. Usability in young children with still well preserved lung function and nutritional status.
2. Absent or weak mutual correlation between sweat chloride (representing CFTR function), FEV1 and weight gain.
3. The inability to perform dose finding studies.
4. No clear mechanistic explanation for the relation between CFTR correction and the measured outcome; e.g. treatment with Ivacaftor in CF patients with gating mutations resulted in significant weight gain. However, the mechanisms behind the observed weight gain are not fully understood.
5. For individual patients, outcome measures may vary in effect size or effect direction, indicating that a more personalized approach might be appropriate.
6. The potential presence or absence of additional treatment effects in other, by CF seriously affected organ systems are not evaluated (intestinal malabsorption and inflammation, gut microbiota, bile salt disturbed metabolism, exocrine pancreatic insufficiency and CF related diabetes).

Evaluating gastro intestinal (GI) outcome measures might help to elucidate some mechanisms and thus reduce the current knowledge gaps. (*Bodewes, Frank AJA, et al. "Cystic fibrosis and the role*

of gastrointestinal outcome measures in the new era of therapeutic CFTR modulation." Journal of Cystic Fibrosis 14.2 (2015): 169-177.)

Aim of the meeting:

To discuss with CF experts, regulators and industry representatives and to potentially reach agreement on gastrointestinal outcome measures to be included in clinical studies to evaluate efficacy (and safety) of CFTR modulators for regulatory submissions.

Agenda and summary of discussion

Co-chairs: Frank Bodewes (University Medical Center Groningen) and Elmer Schabel (Chair CHMP GI-Drafting Group)

Topic	Summary of discussion
Welcome and registration	
Brief overview of CFTR modifier pipeline	Presentation - Overview of CFTR Modifier Pipeline (Tim Lee)
Potential of gastrointestinal outcome measures in CFTR modulation. Is it possible to define a core set that should be evaluated in all studies?	Presentation - Potential of GI outcome measures in CFTR modulation (Frank Bodewes)
The role of intestinal organoids function for evaluation of CFTR modulators	Presentation - The role of intestinal organoid function for evaluation of CFTR modulators (Jeffrey Beekman)
Discussion of predefined questions (and answers received)	<p>Due to the very limited time available, the discussion focused on</p> <p>1) Organoids</p> <ul style="list-style-type: none">Available data indicate organoid function to be well correlated to exocrine pancreas function: The team of Jeffrey Beekman is currently analyzing CFTR biomarkers in newborns (sweat chloride concentrations, intestinal current measurement (ICM), organoids) vs clinical phenotype. Limited cohort of 17 individuals (non-F508del homozygous, 12 different genotypes) shows very good associations between high swelling in organoids and various classical CF parameters (IRT, fecal elastase, CT pragma score at 1 year) whereas high chloride sweat levels only correlated with fecal elastase and ICM showed no correlations. Further data will be presented at the ECFS meeting 2016. Preliminary data from adult CF with homozygous F508del (n=38): high organoid swelling group (top 19 vs bottom 19 swelling): significantly difference in BMI, borderline significance CT (p 0.054), FEV1 (p 0.08).

Topic	Summary of discussion
	<ul style="list-style-type: none"> • Organoids appear to be a good personalized predictor of response to CFTR modulator therapy; well suited to identify patients with rare mutations as potential treatment responders. Thresholds for responders have been established, but likely require further fine-tuning when more data is acquired. • Organoid function measurements more reproducible than rectal potential difference measurements with lower technical variability. • However, potential difference measurements i) may be better suited to evaluate in vivo treatment response ii) environmental influences can be captured in biopsies but not in organoids grown in vitro. • Rectal biopsies disliked by some older children and adolescents who (might) refuse to participate in clinical trials if biopsies are planned. • Dose finding with organoids possible by using serum of patients on treatment with CFTR modulators to determine impact of dose/ treatment on organoids swelling. • The Utrecht center is following a newborn cohort prospectively. <p>2) Defining core set of biomarkers</p> <ul style="list-style-type: none"> • Currently, FEV1 only biomarker accepted by regulatory bodies; however, a correlation between GI biomarkers and FEV1 may not necessarily be possible, as organs may respond differently; this raises the question how to validate individual biomarkers. EMA qualification advice should be considered. • It was proposed to add gut microbiota to list of biomarkers in the article in JCF 2015: microbiota is less difficult and cumbersome to evaluate than CFA; it was found to return to diversity under treatment with ivacaftor. However, it is influenced by frequent antibiotic treatment and thus difficult to interpret. • The following GI biomarkers were identified as most feasible and promising to be included in clinical trials for all ages subsets, including infants: <ul style="list-style-type: none"> – fecal elastase 1 – plasma FGF 19 – malabsorption blood tests

Topic	Summary of discussion
	<ul style="list-style-type: none"> • Adding fecal calprotectin and C4 to the above list was suggested. • Several biomarkers should be evaluated simultaneously in clinical trials to increase knowledge on <ul style="list-style-type: none"> – relationship of different biomarkers and CFTR function and genotype – their suitability for assessing treatment response <p>Postmeeting note:</p> <p>Prof. Stallings (Children's Hospital Philadelphia, US) commented on current evidence related to</p> <ul style="list-style-type: none"> • <u>Malabsorption blood tests (MBT)</u>, <p>which hold promise to provide a more accurate, specific and acceptable alternative to the 72-hour stool collection to quantify pancreatic-based fat malabsorption.</p> <p>This center has a new data set with subjects with CF gating mutation, pre and post-ivacaftor treatment to determine the mechanism of weight gain. The study is just completed, data are currently analyzed and expected available in a few months. The study will also provide data on resting energy expenditure, stool calprotectin, 72-hr fat collection for fat and calorie loss, fecal elastase, etc. The gating mutation study results will be the first indication of MBT utility to detect changes with CFTR modulator treatments.</p>
Wrap-up and conclusion/next steps	<ul style="list-style-type: none"> • Several promising biomarkers are currently available; however, at present, relationship between biomarker level and treatment response to CFTR modulators not yet established. • Many environmental factor's impact on treatment effect and may confound study results. • Organoid function very promising as PD biomarker; strongly recommended to obtain qualification advice from EMA • The identified three GI biomarkers listed above should be included in all clinical trials with CFTR modulators to define their sensitivity with regards to treatment response and to validate them.

Please also refer to the Annex 1 and 2 providing the compiled responses received to the questions circulated among experts in advance of the meeting.

Annex1: List of questions circulated to experts prior to the meeting

1. Is there a need to evaluate intestinal function in clinical studies with CFTR modifiers?
2. Can gastro intestinal outcome measures be useful in CFTR development?
3. Which of the following intestinal and pancreatic function tests might be related / suitable to reflect treatment effect:
 - a) Intestinal pH
 - b) Bile salts; intestinal re-uptake and hepatic synthesis assessment
 - c) Fatty acid stable isotope mucosal uptake
 - d) Fecal elastase
 - e) Coefficient of fat absorption, other methods
 - f) Malabsorption blood test
4. Can GI outcomes be related to improved metabolism and/or development of CF related diabetes
5. Can GI outcome be related to exocrine and/or endocrine pancreatic function
6. Can gastro intestinal outcome measures be related to body weight improvement with CFTR modifiers? (Increase of GI pH - publication by Rowe et al; Am J Respir Crit Care Med. 2014 Jul 15; 190(2): 175-84)
7. Which of the following might be related to improved body weight after CFTR modifier treatment
 - a) Fecal calprotectin
 - g) Gastro intestinal endoscopy
 - h) Videocapsule endoscopy
 - i) Other?
8. Can an intestinal or pancreatic endpoint when positioned versus FEV1/LCI serve as endpoint in clinical efficacy studies in 0-5 year old CF patients?
9. Is organoid function related to intestinal function parameter?
10. Is organoid function coupling to biopsy Ussing chamber measurement necessary
11. Should organoid function testing be mandatory prior to study inclusion? Can organoids predict change in intestinal function?
12. Could dose finding on intestinal, pancreatic endpoints result in different optimal dosing / contribute to dose adjustments of CFTR modifiers compared to FEV1 dose finding results?
13. Is increased risk for intestinal and pancreatic cancer decreased by CFTR modifiers?
14. Could/should the prevalence of intestinal malignancies been considered as a relevant long term treatment effect?

(Maisonneuve, Patrick, et al. "Cancer risk in cystic fibrosis: a 20-year nationwide study from the United States." Journal of the National Cancer Institute (2012): djs481.)

Is the relation to intestinal inflammation?

Is the relation to secretory and or absorptive function?

Is this relation to bile salt malabsorption?

Annex 2: Responses to list of questions

Received by Tim Lee, Anne Munck, Frank Bodewes, Jan Taminiau, Jeff Beekman, expert from French Competent Authority ANSM

1. Is there a need to evaluate intestinal function in clinical studies with CFTR modifiers?

- Yes
- YES
- Yes, CFTR modulators taken orally are circulating in the body, thus they can have an impact on CFTR function in organs where CFTR is located (i.e. pancreas, intestine, biliary tree. In CF, the three main GI functions are impaired: digestion, absorption, and motility resulting in significant nutritional status impairment and in GI complications. It would be of interest to have tests evaluating the CFTR modulation on parameters involved in digestion, absorption and motility.
- Yes, identify reasons for weight improvement, possible endpoint from age 0, allowing dose finding.
- Yes, gastro intestinal obstruction are an important clinical phenotype;
- Yes, they may serve as patient-specific biomarkers that may predict (organ-specific) long term treatment outcomes; stool samples or rectal biopsies are easily obtained (also from very young children) but their role as biomarkers needs to be evaluated against known biomarkers (e.g. SCC).
- Yes, the gastro intestinal tract displays several of the mayor phenotypical features of including fat and bile salt malabsorption and exocrine pancreatic insufficiency (EPI). Some of these traits are intrinsically related to the genotype and CFTR function (eg intestinal pH). Others are more related to CFTR related patho-physiologic disease development (eg pancreas fibrosis resulting in EPI and eventually CF related diabetes (CFRD)). Many features of the intestinal phenotype in CF already are preexisting or develop in early life and childhood. Additionally, some therapeutic effects of the CFTR modifier therapies currently have no clear mechanistic support. In particular, the reported improvement of BMI currently lacks a conclusive physiological explanation. Evaluation of intestinal functions during clinical studies can give direction to a support the point of action of CFTR modifiers in the complex clinical effects

My personal short list to discuss as additional gastro-intestinal endpoints

1. Fecal elastase-1 (already supported) all ages including newborns screening
2. Fasted serum FGF19 and C4 measurements in infants over 6 months of age
3. Coefficient of fat absorption (CFA) in particular for young children and infants
4. Fecal calprotectin in children over 3 years of age
5. Intestinal organoids

2. Can gastro intestinal outcome measures be useful in CFTR modifier development?

- If this question refers to CFTR modulator therapy development, then yes
- yes but probably in a specialized setting
- YES

- Yes, CFTR modulators development offer the potential of a curative treatment. Apart from the lung which is “the main target”, pancreas, intestine and biliary tree are other organs that we aim to target.
- Yes, The gastrointestinal tract presents ample opportunities to evaluate specific clinical symptoms of CF and of CFTR function (e.g. organoids). Clinical symptoms in the gastro-intestinal tract can be either intrinsic properties of the CF phenotype (e.g. bile salt metabolism) or subject to disease development (eg exocrine pancreatic insufficiency or intestinal inflammation).
- Symptomatology of CF in the gastro-intestinal tract frequently already present at birth or at a young age. These properties provide for opportunities the evaluate disease development in an early phase of the disease.

3. Which of the following intestinal and pancreatic function tests might be related / suitable to reflect treatment effect:

a) Intestinal pH

- This is likely to be difficult to measure in clinical trial setting, and is dependent on co-medication. However normalization of intestinal pH has been shown in patients taking Ivacaftor (Rowe et al. 2014). The measurement devices are currently too large for infants and small children (Bodowes et al 2015).
- In particular mucosal pH, bile pH, pancreatic juice pH
- Yes, proximal intestinal wireless motility “pH pill” has the potential to show a positive effect of potentiators (GOAL study). It is not yet applicable to young children because of the pill’s size. Nevertheless, we can contest the assumption that improved buffering of the duodenal juice by increasing the pancreatic enzyme activity is the unique mechanism of weight gain.
- interesting, but difficult in clinical practice (general anesthesia requested for the youngest children, thus only possible if centers with recognized repeated practice)

b) Bile salts; intestinal re-uptake and hepatic synthesis assessment:

- 48 hour faecal collections are unpleasant for clinical trial subjects and have poor feasibility, whether performed in ambulatory or hospital setting. Radioactive markers also generally unsuitable for this indication. The serum markers of increased bile acid synthesis (eg Plasma 4-cholesten-3-One (C4) (Axelson et al 1991) or Fibroblast Growth Factor 19 (Pattni et al. 2013) are of great interest and feasible in infants and young children, although not yet characterised in CF.
- Should improve bile acid pool, micellar concentration, decrease colonic bile salt exposure, improved digestion might accelerate transit and less exposure of esophagus to bile acids.
- Yes, there are biological tests validated in other diseases, but not in CF. If we plan to use them, preliminary validation work will be required.
- FGF19 and C4 measurement (frequently used end points to evaluate the enterohepatic cycling of bile salts. Representing respectively intestinal bile salt absorption and hepatic bile salt synthesis. Proven treatment effect on Ivacaftor (GOAL Study)
- interesting, but difficult in clinical practice (general anesthesia requested for the youngest children, thus only possible if centers with recognized repeated practice)

c) Fatty acid stable isotope mucosal uptake:

- This non-radioactive serum measurement of fat absorption following oral ingestion appears feasible and reliable in CF patients, is feasible in infants and young children, not yet assessed in clinical trials of CFTR modulator therapies.
- Probably rate limiting step in fat absorption, improve fat absorption.
- It has been studied in other disorders. It is not a routine exam and stable isotopes are not available in all countries.
- In principle effective test for post-lypilitic intestinal fat absorption, available for clinical trials. Potential to demonstrate treatment effect, high potential in subgroup analysis)

d) Fecal elastase:

- Stable, well characterized, used clinically, can be obtained from small samples of stool, particularly easy to collect from infants and small children.
- Improvement of pancreatic function, long term improvement.
- Yes, in toddlers receiving ivacaftor, an increase of fecal elastase¹ has been found in a third of the cohort. We don't know if it is a temporary or more permanent improvement. In the latter case, remaining pancreatic function may decrease the frequency of CF liver disease and diabetes mellitus. It is easy to collect, not expensive, and validated in CF.
- Potential of reflecting treatment effects in early phased of development as well as established (reported) exocrine pancreatic insufficiency)
- Yes, easy to undergo whatever the age (some variability but overall reliable), one sample is sufficient generally

e) Coefficient of fat absorption:

- Well characterized as an outcome measure in clinical trials. 72 hour record of dietary fat intake and faecal fat collection has difficulties and feasibility issues especially for patients. Can be difficult to do accurately in infants, toddlers, and young children.
- Stool fat dependent of fat intake, dietary intake is possible to assess.
- CFA is a burdensome evaluation for patients and even more constraining if they have to be inpatients for 3 days at least twice, as required in recent trials on PERT; thus hampering recruitment. Evaluation of fat malabsorption could be evaluated by stable isotopes with similar limitations as above (C).
- In particular in infants and toddlers CFA is good test for CFTR related intestinal fat absorption during short term interruption of pancreatic enzyme replacement therapy
- requesting 3 days of recollection, thus not easy in the adolescent population

f) Malabsorption blood test:

- Again, sounds feasible but not very well characterized and seems no data <12 years.
- Should be reviewed.
- Yes, there is some literature on this. Preliminary validation studies are needed. Many tests evaluating these parameters have been studied in other diseases in term of sensitivity/specificity but not in CF and also mainly in adults.

- In practice, only, exploration in coeliac disease (other nutrients are provided by very indirect measures)

- I have no experience with the malabsorption blood test; but add:

Fecal calprotectin (marker for inflammation. Often used as biomarker for intestinal inflammation. Well established in Clinical studies for IBD with high potential to reflect treatment effects)

4. Can GI outcomes be related to improved metabolism and/or development of CF related diabetes

- It is hoped that CFTR modulators will reduce incidence of CF related diabetes but this would need very long term follow-up to establish
- The inflammation and oxidative stress are related to glycemia and diabetes, also to pulmonary function. Glycemia balance can be of benefit on all functions, including digestive even if the direct influence is not demonstrated at this stage of the knowledge. It is supposed
- Yes, FGF19: There is extensive evidence that FGF19 is involved in glucose homeostasis:

"FGF19 levels are reduced in individuals with metabolic syndrome, non-alcoholic fatty liver disease and FGF19 levels are restored to normal values in obese patients who undergo Roux-en-Y gastric bypass bariatric surgery"

FGF 19 levels are also reduced in CF patients. Based on this observation it is probable that manipulation or correction of FGF19 in CF patients can potentially be related to glucose homeostasis or CFRD development or treatment

Stanley, Sarah, and Christoph Buettner. "FGF19: How gut talks to brain to keep your sugar down." *Molecular metabolism* 3.1 (2014): 3-4.

Animal studies:

Perry, Rachel J., et al. "FGF1 and FGF19 reverse diabetes by suppression of the hypothalamic-pituitary-adrenal axis." *Nature communications* 6 (2015).

Kir, Serkan, et al. "FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis." *Science* 331.6024 (2011): 1621-1624.

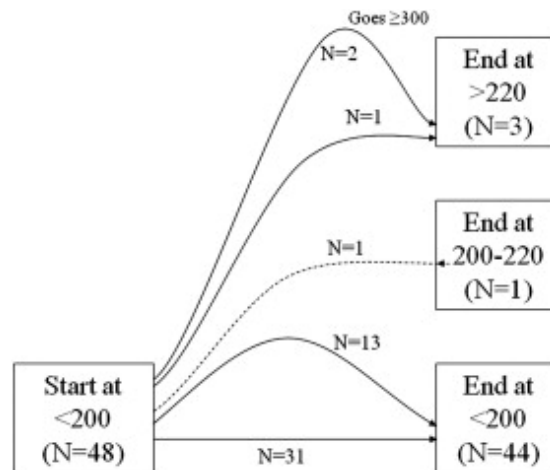
Vrieze, Anne, et al. "Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity." *Journal of hepatology* 60.4 (2014): 824-831.

5. Q: Can GI outcome be related to exocrine and/or endocrine pancreatic function

- YES
- Studies of Ivacaftor in young children have showed an increase in faecal elastase in a proportion of subjects but this needs further characterization
- There is a lack of knowledge
- Yes:

1. Fecal elastase - Established measure of EPI

O'Sullivan, Brian P., et al. "Evolution of pancreatic function during the first year in infants with cystic fibrosis." *The Journal of Pediatrics* 162.4 (2013): 808-812.



Abstract: McKay, K., et al. "231 The effect of ivacaftor on exocrine pancreatic function in patients with cystic fibrosis and the G551D CFTR mutation who are naïve for ivacaftor." Journal of Cystic Fibrosis 14 (2015): S117.: "In CF patients with at least one G551D mutation, ivacaftor substantially improves fat intake and decreased fat excretion in near 80% of patients (normalising in 3). These are major factors contributing to the improved growth seen in these patients."

This study was supported by Vertex Pharmaceuticals.

2. Fat malabsorption in CF is not only EPI dependent. CFA can be used to measure the combination of pre- and post lypolitic intestinal fat malabsorption.

Wouthuyzen-Bakker, Marjan, Frank AJA Bodewes, and Henkjan J. Verkade. "Persistent fat malabsorption in cystic fibrosis; lessons from patients and mice." Journal of Cystic Fibrosis 10.3 (2011): 150-158.

6. Q: Can gastro intestinal outcome measures be related to body weight improvement with CFTR modifiers? (Increase of GI pH - publication by Rowe et al; Am J Respir Crit Care Med. 2014 Jul 15;190(2):175-84)

- Unclear – body weight improvement likely to be multifactorial.
- There is a lack of knowledge
- Many factors might contribute, all should be tested in a coherent manor and protocol to find out which or which combinations are relevant.
- CFA; under the assumption that growth and weight gain are related to fat absorption CFA could be a indirect measure or indicator for body weight improvement. However it likely weight gain is multi factorial (physical activity, lung disease...)
- Intestinal pH can be related to 1) PERT activity, endocrine, non-pancreatic lipase activity (eg gastric lipase) and post lypolitic fat absorption (micelle form micelle formation and bile acid precipitation)
- Probably relationship, as sometimes a spectacular increase of weight is observed (e.g. +17 kg in two years for a young girl in ORKAMBI, etc). Probably multifactorial:
 - fluidification of secretions at all levels: intestinal (better absorption?), pancreatic (recovery of a residual function?), biliary?
 - duodenal Ph better acting as buffer therefore increasing enzyme absorption

- but also potential decrease of pulmonary inflammation, and functional improvement and pulmonaire et amélioration fonctionnelle and drainage = less secretions ingested, less bacteria ? (could be studied)
- all these factors contributing to increase the appetite, concomitantly to less expenditure of energy due to pulmonary infections and inflammation.

7. Which of the following might be related to improved body weight after CFTR modifier treatment

- The underlying mechanisms of weight gain are possibly a combination of factors.

Considering extradigestive factors: if CFTR modulators improve the pulmonary function and decrease the rate of pulmonary exacerbations, patients will present a reduced hypercatabolism status with decreased resting energy expenditure (weight gain). Their improved general condition will have a positive psychological impact that can increase their appetite (weight gain).

Considering pancreatic factors:

if CFTR modulators improve CFTR mediated bicarbonate secretion, it will increase PERT activity and improve digestion (weight gain) and decrease calorie fat stool losses (weight gain). Improved digestion may result in relief of abdominal discomfort, thus increasing appetite (weight gain);

if CFTR modulators reduce intestinal mucus production and normalize the mucus composition, it may improve intestinal absorption (weight gain) and improve intestinal comfort with increasing appetite (weight gain);

a direct impact of CFTR modulators on CFTR ions transfer may result in an improved hydration of the lumen content with a positive impact on constipation (40% of the patients) thus improving abdominal discomfort with increased appetite (weight gain);

CFTR modulators may also improve dysmotricity (lumen hydration, mucus content) and we can add other possible improvements on GI inflammation and microbiota diversity.

a) Fecal calprotectin

- Unlikely
- Yes, possibly
- Yes
- Inflammation might be related to anorexia. Easy to perform for general information.
- Yes, specific of intestinal inflammation and perhaps of other more global markers of the inflammation.

b) Gastro intestinal endoscopy

- Inflammation might be detected, also colonic inflammation due to bile salts, and improvement.
- Not very feasible as a clinical trial endpoint
- I am not sure that it can be relevant except if the data analysis is centralized. It is however invasive (general anesthesia for children)
 - Invasive, and of low contribution, with exception of the biopsies, bile salts measures?

c) Videocapsule endoscopy –

- To detect inflammation beyond endoscopic reach.
- Intestinal pH likely to be a factor in improving body weight and can be measured this way.
- Less invasive than endoscopy for macroscopic evaluation; no biopsies can be performed.
- For macroscopic aspects only, invasive, for ages >> 6 y. to avoid general anesthesia (capsule to be swallowed if possible)

d) Other?

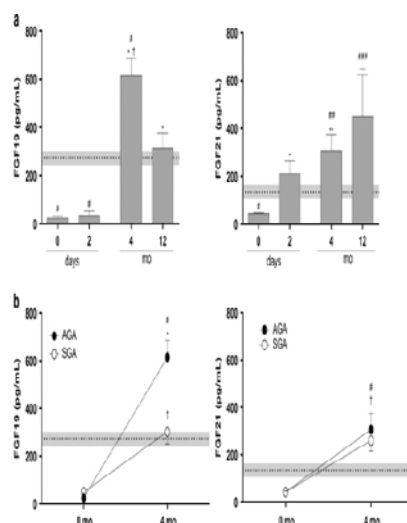
- Possibly improvements in faecal elastase may be a factor
- Surrogate of increased appetite: leptine ? Intestinal microbiote variation?
- FGF19 (Pournaras, Dimitri J., et al. "The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control." *Endocrinology* 153.8 (2012): 3613-3619.

8. Can an intestinal or pancreatic endpoint when positioned versus FEV1/LCI serve as endpoint in clinical efficacy studies in 0-5 years old CF patients?

- Yes. Sweat chloride response will remain important. A signal using FEV1 is not possible in 0-5 year old patients (both due to lack of feasibility but also where measurable FEV1 increasingly normal in this age group).
- LCI we wait to see if an efficacy signal is possible in this age group. However it is more likely that GI endpoints will be needed in this age group.
- Yes, it can be a secondary important objective (in neonates, some recovery of pancreatic function can be expected)
- It is too early to reply to this question, it might be possible.
- Yes; should only be comparable to FEV1/LCI in dose response range at older ages.
- Yes

1.1 FGF19 from 6-12 month

Sánchez-Infantes, D., et al. "Circulating FGF19 and FGF21 surge in early infancy from infra-to supra-adult concentrations." *International Journal of Obesity* (2015).



1.2 CFA from birth

1.3 Calprotectin years and older

"The suggested cutoff level for adults ($<50 \mu\text{g/g}$) can be used for children aged from 4 to 17 years regardless of sex".

Fagerberg, Ulrika Lorentzon, et al. "Fecal calprotectin levels in healthy children studied with an improved assay." *Journal of pediatric gastroenterology and nutrition* 37.4 (2003): 468-472.

9. Q: Is organoid function related to intestinal function parameters ?

- In terms of the cell function yes
- Yes (fecal elastase linked to deficiency of the pancreas, if that is the question ?)
- Yes, in general CFTR2 database know how on disease severity is recapitulated in organoid measurements; we are currently defining these relations
- For compound heterozygotes, Class 2 defects, degree of resting CFTR function and improvement should be tested in each individual patient.

10. Is organoid function coupling to biopsy Ussing chamber measurement necessary ?

- Can only be done in specialized centers, reflecting only the CFTR in digestive mucosa
- Organoid function might not be identical to enterocytes in line as a membrane, when function restoration might be better or worse.
- Depends on what you want to achieve. Are we aiming to include or exclude people from treatment?

To repurpose/extend existing therapies to rare mutations: yes! Especially organoids are suited; our experience with cftr modifier in Ussing chamber on rectal biopsies indicates that many biopsies do not respond suggesting the drugs do not penetrate the tissue.

To extent 809/770 treatment to F508del single allele expressors: yes, this may work by selecting the top responders.

For difficult treatments with limited overall treatment efficacy: e.g. 809/770 in F508del/F508del: Do we want to generate exclusion criteria? I think yes when we will validate specificity and sensitivity but these drugs take a long time before their true effects can be measured (e.g. hospitalization, rate of FEV1 decline).

11. Should organoid function testing be mandatory prior to study inclusion? Can organoids predict change in intestinal function?

- It is an important and very interesting question; I don't believe that we have the answer.
- Yes, secretory activity should be related to tested functions.
- Organoids appear a very good personalized predictor of response to CFTR modulator therapies. For people with CF who have rare mutations then n of 1 studies or other very small number studies using organoid results as screening criteria seems a very appropriate and feasible way forward. Clinical Trial Networks, Pharma, Regulators, and Healthcare providers have a responsibility to consider carefully how people with rare mutations are not excluded from eventual access to better treatments for CF.

- If studies are intended to be performed in the youngest population, fecal elastase is the most simple, non invasive and easy to perform measure, no dependent of the consumption of food, enzyme supplementation. Steatorrhea (over 3 days) eventually, but more variable.
- First question: I don't think so, but may depend on the context. It will certainly help to generate a better insight on one's individual cftr function and potential response to treatment, which is highly usefull for rare mutations; It may be a cost effective tool to select treatments for rare mutations and part of a n=1 treatment scheme.

Question two: we need to learn whether within genotype differences in organoids have a meaning in the clinic (sensitivity/specificity) for response to treatment and disease progression (in 2016 we aim to generate the first data sets). Response to cftr modulator treatment in published literature (FEV1) is positively associated with response in organoids, as well as pulmonary function.

In terms of individual prediction: I think the CFTR dependent clinical response can to a great extent be predicted providing that PK is ok, but many clinical phenotypes are not reversible or fully CFTR dependent (e.g. FEV1, pancreatic function).

12. Could dose finding on intestinal, pancreatic endpoints result in different optimal dosing / contribute to dose adjustments of CFTR modifiers compared to FEV1 dose finding results?

- I am not sure that it can be predictable by using intestinal/pancreatic endpoints. In current daily practice there is even no relationship between level of fecal elastase and the optimal pancreatic enzyme replacement therapy dosage. We still need to learn more on these aspects.
- Possible- but will require a lot of work as unclear if levels required for correction will be the same in lungs as in GI tract.
- We stimulate organoids with plasma before and after treatment to indicate the functional activity of modulators in blood (to help conventional PK studies); this may help dose finding studies and we are currently setting this up in studies with Vx
- Possible
- Yes. This answer involves:

1) Organ specific therapeutic effects in general

2) Variance in organ specific therapeutic effects between patients.

In the GOAL and other studies all end points reported (sweat chloride, FEV1 and weight gain), group wise, display a positive treatment effect of Ivacaftor. However there is no or weak correlations between the individual endpoints used e.g. (Cl- vs. weight gain, Cl- vs. FEV1, weight gain vs. FEV1). This implies that there is a strong intra-individual and/or organ specific variance in the direction and magnitudes that patient do respond to the treatment.

We find the same results for markers of intestinal bile salt reabsorption (FGF19/C4), indicating that therapeutic effect in the intestine may differ from effect in eg lung or sweat gland.

This finding can have 2 potential consequences:

- Potential therapeutic effects of drugs are under estimated
- Some patient may benefit from therapeutic effects currently not tested in clinical trials

Concerning dose finding

It is not to be excluded that gastro-intestinal endpoints could provide be more sensitive or responsive treatment responses than eg FEV1 and weight gain making them more suitable for dose response evaluation studies.

13. Is increased risk for intestinal and pancreatic cancer decreased by CFTR modifiers?

- Unclear – would need long term follow-up
- Should be tested. Colon might be best target and esophagus
- I don't know
- Perhaps no, excepting a decrease of intestinal inflammation (mutagen decrease?)
- It potentially is. Several registries and patient cohort studies have recently reported a higher prevalence of intestinal malignancies in cystic fibrosis patients compared to the general population. Additionally a high incidence of malignancies is reported in CF patients after lung transplantation. This observation suggests that there is either a direct or indirect link to the development of malignancies and CFTR function in CF disease.

In the studies published is obvious that in particular intestinal and biliary malignancies are more prevalent among CF patients. This observation suggests that the intestinal tract is potential more at risk for the development of a malignancy.

In general it is suggested that colon cancer development is related to 1) obesity, 2) sedentary lifestyle, 3) carbohydrate- and fat rich diets 4) intestinal inflammation (IBD) and 5) elevated fecal excretion of secondary bile acids.

Billings, Joanne L., et al. "Early colon screening of adult patients with cystic fibrosis reveals high incidence of adenomatous colon polyps." *Journal of clinical gastroenterology* 48.9 (2014): e85-e88.

Mehta, Anil. "Cystic fibrosis as a bowel cancer syndrome and the potential role of CK2." *Molecular and cellular biochemistry* 316.1-2 (2008): 169-175.

Alexander, C. L., et al. "The risk of gastrointestinal malignancies in cystic fibrosis: case report of a patient with a near obstructing villous adenoma found on colon cancer screening and Barrett's esophagus." *Journal of Cystic Fibrosis* 7.1 (2008): 1-6.

Hernandez-Jimenez, Isaac, Daniel Fischman, and Pramil Cheriya. "Colon Cancer in Cystic Fibrosis patients: Is this a growing problem?." *Journal of Cystic Fibrosis* 7.5 (2008): 343-346.

Meyer, Keith C., et al. "Colon cancer in lung transplant recipients with CF: increased risk and results of screening." *Journal of Cystic Fibrosis* 10.5 (2011): 366-369.

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14. Could/should the prevalence of intestinal malignancies been considered as a relevant long term treatment effect?

(Maisonneuve, Patrick, et al. "Cancer risk in cystic fibrosis: a 20-year nationwide study from the United States." Journal of the National Cancer Institute (2012): djs481.)

- CF patients are screened by colonoscopy from age 40 years for polyp development in Minnesota.
- Yes as described above
- Yes, and could be collected through existing CF Registries
- I guess so, but relations below will be very hard to establish

Is the relation to intestinal inflammation?

- Unclear
- Probably yes, but not proved with induction of hypermutable cells?
- Yes as clearly proven by the increased malignancy risk in IBD; see reference Ullman et al and Jess et al. In addition CFTR related changes in microflora could be involved in both inflammation and bile salt metabolism
- IBD occurs in CF more frequently, is in itself related to increased cancer risk.

Is the relation to secretory and or absorptive function?

- Unclear
- Bile acids are implicated for colonic cancer and esophageal cancer.
- Potentially because of relation between intestinal cancer and high fat diet and obesity
- Is more the malnutrition a cancer risk, indirect consequence of malabsorption?

Is this relation to bile salt malabsorption?

- Unclear
- Yes - Bile acids are intestinal luminal aggressors. However no intestinal disease has been described were bile acids seem to be the primary aggressor. In vitro the toxic effects of bile acids on gastric mucosa and the development of peptic ulcers has been described. However the physiological concentration in vivo bile acids do not seem to be related to direct toxicity.
- Bile acids have the potential to be toxic in vivo but a second hit in conjunction with gut bacteria, in a mucosa that is already under stress or inflamed (review Pavlidis 2015)
- Raufman et al 2015 recently for the first time describe an in vivo mouse model (Asbt ko mice) in which endogenous bile acids can promote colon neoplasia. They address that it is not directly clear if this involves a direct BA effect or a secondary effect on eg flora.
- Bacterial flora composition has been associated with development of colon neoplasia.
- Irritation/inflammation factor?

Is this relation to diet?

- Unclear