Journal of Cystic Fibrosis 23 (2024) 703–706

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Centralized intestinal organoid generation is a feasible and safe approach for personalized medicine as demonstrated in the HIT-CF Europe Organoid Study☆

Marlou C. Bierlaagh [a,b], Peter van Mourik [a,b], Annelotte M. Vonk [a,b], Johanna Pott [c], Danya Muilwijk [a,b], Gitte Berkers [a], Bente L. Aalbers [b], Frank P. Vleggaar [d], Sabine Michel [a], Sylvia F. Boj [c], Robert G.J. Vries [c], Jeffrey M. Beekman [a,b], Cornelis K. van der Ent [a,*], on behalf of the HIT-CF organoid study group

[a] Department of Pediatric Pulmonology, Wilhelmina Children's Hospital, UMCU, Utrecht, the Netherlands, member of ERN-LUNG
[b] Regenerative Medicine Center (UMCU), Utrecht University, Utrecht, the Netherlands
[c] HUB Organoids B.V. (HUB), Utrecht, the Netherlands
[d] Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht, the Netherlands

ARTICLE INFO

Keywords:
Intestinal organoids
Rectal biopsy
Suction biopsy
Forceps biopsy
Personalized medicine

ABSTRACT

Background: Patient-derived intestinal organoids (PDIOs) show great potential as in vitro drug testing platform for personalised medicine in Cystic Fibrosis and oncology. PDIOs can be generated by culturing adult stem cells obtained through rectal forceps biopsy or suction biopsy, but the safety of these procedures and the success rates of generating organoids after shipment to a centralized lab using these procedures has not been studied in this context. We here report the safety and success rates of both biopsy procedures and the subsequent generation of PDIOs in the international multicentre HIT-CF Organoid Study.

Methods: 502 biopsy procedures were conducted, on 489 adult people with Cystic Fibrosis from 33 different hospitals across 12 countries. Depending on the preference of the hospital, either rectal forceps biopsies or suction biopsies were obtained and internationally shipped to a central laboratory for organoid generation.

Results: No adverse events were reported for 280 forceps biopsy procedures, while 222 rectal suction biopsy procedures resulted in 2 adverse events, namely continued bleeding and a probably nonrelated gastroenteritis. The success rate of organoid generation from all biopsies was 95%, and the main reason for failure was insufficient sample viability (3.2%).

Conclusion: Our results indicate that both rectal suction biopsy and forceps biopsy procedures are safe procedures. The high success rates of PDIO generation from the obtained tissue samples demonstrate the feasibility of the organoid technology for personalised in vitro testing in an international setting.

1. Introduction

Patient-derived intestinal organoids (PDIOs) derived from adult stem cells are increasingly used to study organ development and diseases such as cancer, inflammatory conditions and inherited diseases [1–5]. PDIOs can be expanded almost indefinitely, and maintain functional characteristics of their parental organ [1,6,7]. The genetic stability and long-term expansion allow the generation of organoid collections in biobanks.

Initial studies in people with Cystic Fibrosis (pwCF) demonstrated the direct correlation of the in vitro response to drug treatment in organoids and the clinical response of the same patient [8]. Therefore, we have set up an international effort to determine the use of organoid based personalized in vitro drug testing in pwCF. In the HIT-CF Europe Organoid Study, rectal biopsies are collected from pwCF with ultra-rare genetic variants. The organoids are tested with a number of clinical phase compounds in central academic laboratories [9,10]. The tests will stratify patients for specific clinical drug trials based on the in vitro

☆ Trial identification number NTR7520, https://www.trialregister.nl/trial/7304

* Corresponding author at: Heidelberglaan 100, 3584, CX, Utrecht, the Netherlands.
E-mail address: K.vanderEnt@umcutrecht.nl (C.K. van der Ent).

https://doi.org/10.1016/j.jcf.2024.04.016
Received 12 January 2024; Received in revised form 12 April 2024; Accepted 28 April 2024
Available online 18 May 2024

response.

Intestinal adult stem cells can be harvested from the rectum through forceps or suction biopsy procedures, which are commonly used for other clinical indications and are generally considered safe [11,12]. Nevertheless, the risks of these biopsy procedures and the success rate for generating viable organoid cultures are unknown. In addition, we want to investigate other logistical and technical steps in the generation of PDIOs process that impact the success on a large scale. Previously, we identified a number of potential challenges such as the number of biopsies, the quality of the biopsies (i.e. viability and presence of stem cells in the obtained tissue), and the transportation time to the specialized organoid generation labs. These factors could impact the success rates of organoid establishment. We here report on the safety of both forceps- and rectal suction biopsies, the feasibility of international transport of biopsies and the success rate of organoid establishment in the HIT-CF Europe Organoid Study in adult pwCF [10].

2. Methods

Adult pwCF were included in 33 hospitals in 12 different countries for the HIT-CF Organoid Study (NTR7520). All participating clinical sites were members of the clinical trial network of the European Cystic Fibrosis Society (ECFS-CTN) and the study was conducted in the following countries: Belgium, the Czech Republic, Denmark, France, Israel, Italy, the Netherlands, Poland, Portugal, Spain, Sweden and the United Kingdom. This study and the accompanying informed consent form were approved by independent ethics committees at each participating site. Written informed consent was obtained from each participant and/or the participant's legal guardian.

2.1. Biopsy collection

Forceps biopsies and rectal suction biopsies were obtained according to detailed working instructions based on a previously published protocol [13]. In short, participants were first treated with a (sodium phosphate) enema to cleans the colon/rectum. Forceps biopsies were obtained with a flexible endoscope. The endoscope was introduced rectally, and biopsies were obtained on sight, thereby avoiding any arteries or veins. For rectal suction biopsies, the device was introduced rectally, and the biopsy opening was positioned dorsolaterally and ~5 cm from the anal verge. To ensure enough material for stem cell isolation, two forceps biopsies or four rectal suction biopsies were obtained from each patient. If the biopsies were deemed to be of insufficient quality, the investigator could decide to obtain more biopsies.

2.2. Storage and transport of biopsies

Biopsies were stored in Ad-DF+++ with 0.1% primocin directly after the biopsy procedure and kept at 4 °C. Detailed information on the composition and the production of this media has been previously published [13]. Within 24 h of biopsy collection, biopsies were shipped to the central laboratory at Hubrecht Organoid Technology (HUB) in Utrecht, the Netherlands, using a courier service. Biopsies were shipped at 4 °C. target delivery time of samples was within 48 h after biopsy collection.

2.3. Crypt isolation and organoid generation

Crypts were isolated according to previously published methods [6,13,14]. In case organoid isolation failed, this was reported to the site and the investigator had the opportunity to re-biopsy a subject. Successful organoid establishment was defined as successful crypt isolation, organoid culturing and subsequent freezing of samples for future use in the study.

2.4. Data analyses

All data analyses were performed with SPSS Statistics version 29.0.1 (SPSS Inc., Chicago, IL, USA).

3. Results

Results are summarized in Table 1. A total of 502 biopsy samples were collected from 489 individual pwCF, with 13 individuals being successfully rebiopsied. Forceps biopsies were conducted at 28 sites, resulting in 280 samples, while rectal suction biopsies were performed at 9 sites, yielding 222 samples. Four sites performed both procedures.

Two adverse events were reported for the rectal suction biopsy procedures, which were considered serious adverse events. One biopsy procedure led to rectal bleeding which had to be surgically resolved and another associated with *Campylobacter* gastroenteritis, which was probably unrelated to the procedure. No other (serious) adverse events were reported.

Overall, 477 organoid cultures were successfully isolated from 502 biopsy procedure (success rate of 95%). One biopsy sample was lost during shipment due to incorrect storage temperature. A number of samples failed to generate organoids for different reasons. First, several samples were of insufficient viability (16 samples, 3.2%). Secondly, a number of samples were contaminated during the biopsy procedure (7, 1.4%) or in the organoid laboratory (1, 0.2%). The success rate of forceps biopsy procedures was 93.6%, in comparison to 96.4% for rectal suction biopsies (Table 1). The majority of samples were processed in the laboratory within 48 h after the biopsy procedure, while 17 of the 502 samples were processed between 48 and 72 h after collection, of which 1 failed due to insufficient viability.

4. Discussion

In our study, which included 489 subjects from 33 different hospitals in 12 countries, we achieved low biopsy complication rates and high success rates of organoid generation. These results underscore the feasibility of international studies using patient-derived intestinal organoids.

Organoid studies have demonstrated great potential to change the way drugs are developed and clinicals trials can be performed. It has opened up new avenues for personalized medicine and subject

Table 1
Subject characteristics and outcomes.

	Total population Mean +/- SD (range) or n (%)	Rectal suction biopsy Mean +/- SD (range) or n (%)	Forceps biopsy Mean +/- SD (range) or n (%)
Subjects included	489	217 (44.4)	272 (55.6)
Age (years, mean)	32.4 +/- 11.4 (16 – 77)	31.9 +/- 10.7 (16 – 67)	32.7 +/- 12.0 (16 – 77)
Sex (female)	252 (51.5)	121 (55.8)	131 (48.2)
Number of biopsy samples	502	222 (44.2)	280 (55.8)
Biopsies per sample	3.6 +/- 1.3 (1 – 8)	3.6 +/- 1.4 (1 – 8)	3.7 +/- 1.2 (1 – 7)
Adverse events	2 (0.4)	2 (0.90)	0
Serious adverse events	2 (0.4)	2 (0.90)	0
Gastroenteritis	1	1 (0.45)	0
Hemorrhage	1	1 (0.45)	0
Successful organoid generation	477 (95.0)	214 (96.4)	262 (93.6)
Reasons for failure			
Insufficient sample viability	16 (3.2)	3 (1.4)	13 (4.6)
Contamination of sample	8 (1.6)	4 (1.8)	4 (1.4)
Shipment error	1 (0.2)	1 (0.5)	0

stratification in clinical trials [1,2,8]. The novelty of the technology and the requirement of specialized personnel have prompted us to investigate models of organoid application. Here we introduce an international collaboration using a centralized laboratory to implement personalized organoid cultures, which can be particularly relevant in rare diseases with large genetic variability such as Cystic Fibrosis. Our study shows that it is feasible to set up international studies and generate data on a large group of patients with a rare disease in centralized labs. The methods used in this study could be implemented for other diseases and efficient shipment of tissue material across countries demonstrates the feasibility of this personalized patient stratification approach for clinical trials, predictive diagnostic tests, and generation of biobanks comprised of internationally obtained samples.

Our study finds very low rates of serious adverse events (<1%) for both forceps biopsy and rectal suction biopsy, comparable to previous studies [11,12]. These data implicate that both rectal suction biopsies and forceps biopsies are effective tools for obtaining intestinal adult stem cells. Rectal suction biopsy is commonly used by pediatric gastroenterologists to diagnose Hirschsprung disease, and can be safely performed in newborns [12]. In contrast, rectal suction biopsies are rarely indicated in the adult population which limits the experience of adult gastroenterologists with this procedure. Ultimately, since both procedures were found to be safe, the choice of biopsy device should be based on the target population and the experience and preference of the operator.

Several examples of organoid biobanks exist, with success rates of organoid establishment ranging from ~60 – 90% [2,15,16]. However, these biobanks were generated from locally obtained samples. Shipment of live tissue samples over large distances requiring several days of transportation was considered to limit the organoid establishment rates. Here we show that standardized working instructions can lead to high success rates in a multicenter setting with extensive transport times of up to 72 h. The low failure rate of samples processed between 48 and 72 h after collection suggests that even longer transport times might be feasible. Whether these results can be extrapolated to other organoid models needs to be further investigated.

The largest proportion of failed samples was due to insufficient sample viability, 16 samples, with no difference observed between the suction and forceps groups. Sample viability depends on several factors, beginning with the quality of the biopsy immediately after it is taken. A good quality biopsy should have a pink color, be round in shape and, measure around 3 mm in width. Although not feasible at every facility, biopsies that are questionable can be examined under a light microscope where they should have a honeycomb structure. The second crucial step in preserving viability involves directly placing the biopsies at a temperature of 4 °Celsius, ice or fridge, and maintaining this temperature until processing [13]. Interestingly, only 8 samples failed due to sample contamination. There are several precautions taking up in the protocol used to avoid contamination [13]. Biopsies were transported in transport media containing antibiotics, and all laboratory procedures were performed in a laminar flow cabinet. Considering, the rectal biopsy procedure and transportation, this demonstrates that the combination of the standardized procedure, media conditions, and transportation allow for efficient collection of clean samples.

In this international, multicentre study, establishment of intestinal organoids from both rectal suction and forceps biopsies was highly successful. These data implicate that international biobanks can be effectively generated through shipment of fresh samples to a central laboratory and support the implementation of these procedures for research and future clinical indications.

CReditT authorship contribution statement

Marlou C. Bierlaagh: Formal Analysis and writing – original draft. **Peter van Mourik:** Investigation, formal analysis and writing -original draft. **Annelotte M. Vonk:** investigation and writing – review & editing. **Johanna Pott:** Resources, investigation and writing – review & editing. **Danya Muilwijk:** Investigation and writing – review & editing. **Gitte Berkers:** Investigation and writing – review & editing. **Bente L. Aalbers:** Investigation and writing – review & editing. **Frank P. Vleggaar:** Resources and writing – review & editing. **Sabine Michel:** Investigation and writing – review & editing. **Sylvia F. Boj:** Conseptualization and writing – review & editing. **Robert G.J. Vries:** Conseptualization and writing – review & editing **Jeffrey M. Beekman:** Conseptualization, methodology and writing – review & editing. **Cornelis K. van der Ent:** Conseptualization, supervision, and writing – review & editing. The HIT-CF organoid study group: Investigation

Declaration of competing interest

No conflict.

Acknowledgements

Financial support: This study was funded by the European Union's Horizon 2020 research and innovation program under grant agreement No. 755021, and grants for patient travel from the CF Trust UK and Mukoviszidose e.V.

We would like to thank Rachelle Schwanen, Ka Wai Lai, Nilofar Ehsani, Ewout Spaan, Marvin Statia, Emily Cole, Suzanne Kroes and Begoña Aguilera from Hubrecht Organoid Technology for their excellent work in processing all the biopsies and generating organoids.

The HIT-CF Organoid Study group consists of the following people:

Prof. Dr. Helmut Ellemunter, Dr. Katharina Niedermayr (Medical University of Innsbruck, Cystic Fibrosis (CF) Center, Department of Child and Adolescent Health, Univ. Clinic for Paediatics III, 6020 Innsbruck, Austria), Dr. Anabela S. Ramalho, Dr. Francois Vermeulen (Katholieke Universiteit Leuven), Elke De Wachter MD, PhD, Eef Vanderhelst MD, PhD (CF Center, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium), Tereza Dousova, Lucie Borek-Dohalska (Department of Pediatrics, University Hospital Motol and Second Faculty of Medicine, Charles University, Prague, Czechia), Marianne Skov, Tacjana Pressler (CF Center Copenhagen, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark), Dr. Edward F Nash, Dr. Marcus Mottershead (University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK), Daniel Peckham (Leeds Teaching Hospitals NHS Trust), Dr. Mary Carroll (Adult Cystic Fibrosis Unit, University Hospital Southampton NHS FT, Southampton, UK), Janisha Patel (Department of Hepatology, University Hospital Southampton NHS FT, Southampton, UK), Dr. Helen Barker, Marlene Taveira (Royal Papworth Hospital NHS Foundation Trust), Dr. MD. FRCD Nicholas J. Simmonds (1.Adult Cystic Fibrosis Center, Royal Brompton Hospital, London, UK 2. National Heart and Lung Institute Imperial College, London, UK), Dr. MD MRCP Kinesh Patel (Imperial College, London, UK, Royal Brompton Hospital, London, UK), Dr. Helen L Barr, Mrs. Jessica Longmate (Wolfson Cystic Fibrosis Center, Department of Respiratory Medicine, Nottingham University Hospitals NHS Trust, Nottingham, UK), Dr. Michael D Waller (Adult Cystic Fibrosis and Respiratory Medicine, King's College Hospital NHS Foundation Trust, London, UK), M.D. Peter J. Barry (1. Manchester Adult Cystic Fibrosis Center, Wythenshawe Hospital, Manchester university NHS Foundation Trust, Southmoor Road, Wythenshawe, United Kingdom 2. Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, University of Manchester), Dr. Dipesh H Vasant (1. Gastroenterology, Wythenshawe Hospital, Manchester University NHS Foundation Trust, Southmoor Road, Wythenshawe, United Kingdom 2. Division of Diabetes, Endocrinology and Gastroenterology, University of Manchester, Manchester, United Kingdom), Pr Michael Fayon, Dr Julie Macey (University Hospital, Hôpital Pellegrin-Enfants, Pediatric Cystic Fibrosis Reference Center (CRCM), Center d'Investigation Clinique (CIC 1401), F-33000 Bordeaux, France), Dr. / M.D. Nadine Desmazes-Dufeu (Bordeaux University, Center de Recherche Cardio-Thoracique de

Bordeaux, U1045, F-33000 Bordeaux, France), Dr. / M.D. Marlène Murris, Dr. / M.D. Marion Dupuis (Service de Pneumologie-Allergologie CRC Mucoviscidose Adulte Center de Transplantations pulmonaire, Hôpital Larrey, Toulouse, France), Raphaël Chiron Alexandre Coudrat (Cystic Fibrosis Center, Hôpital Arnaud de Villeneuve, Center Hospitalier Universitaire de Montpellier, Univ Montpellier, France), Isabelle Durieu, Quitterie Reynaud (Adult Cystic Fibrosis Care Center, Hospices Civils de Lyon, EA HESPER 7425, Université de Lyon, France), Silke van Koningsbruggen, Jan-Christoph Thomassen (CF Center and Experimental pulmonology, Children's Hospital, Faculty of Medicine, University of Cologne, Germany), Susanne Naehrig (Medizinische Klinik V (Pneumology), Cystic Fibrosis Center for Adults, LMU University of Munich, Munich, Germany), Dr. MD Felix C. Ringshausen, Dr. Annette Sauer-Heilborn (Department of Respiratory Medicine, Hannover Medical School (MHH) and Biomedical Research in End-stage and Obstructive Lung Disease Hannover (BREATH), German Center for Lung Research (DZL), Hannover, Germany), Simon Y. Graeber, Marcus A. Mall (1. Department of Pediatric Pulmonology, Immunology and Critical Care Medicine and Cystic Fibrosis Center, Charité-Universitätsmedizin Berlin, Berlin, Germany, 2. Berlin Institute of Health (BIH), Berlin, Germany, 3. German Center for Lung Research (DZL), Berlin, Germany), Dr. Sivagurunathan Sutharsan, Dr. Matthias Welsner (Division of Cystic Fibrosis, Department of Pulmonary Medicine, University Medicine Essen – Ruhrlandklinik, Essen), MD Michael Lorenz (Cystic Fibrosis Center / Ped. Pneumology, University of Jena, Jena, Germany), Sabine Wege (Department of Pulmonology and Critical Care Medicine, Thoraxklinik at the University of Heidelberg, Heidelberg, Germany), Prof. Michael Wilschanski, Malena Cohen-Cymberknoh (Center for Cystic Fibrosis, Hadassah Hebrew University Medical Center, Jerusalem, Israel), Dario Prais MD, MD Meir Mei-Zahav MD (Pulmonary Institute, Schneider Children's Medical Center of Israel, Petah Tikva, Israel; Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel), Dr. MD, head physician of the CF center Vincenzina Lucidi, Dr. PhD, researcher of the CF center Fabiana Ciciriello (Pediatric Hospital Bambino Gesù, Rome, Italy), Dr. Vito Terlizzi, Dr. Giovanni Taccetti (Cystic Fibrosis Regional Center of Tuscany, Anna Meyer Children's University Hospital, Firenze, Italy), Cresta Federico, Carlo Castellani (Cystic Fibrosis Center, IRCCS Istituto Giannina Gaslini, Genoa, Italy), M.D. Giovanna Pizzamiglio, PhD Leonardo Terranova (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Internal Medicine Department, Respiratory Unit and Adult Cystic Fibrosis Center, Milan, Italy), M.D. Dr. Carla Colombo, Arianna Bisogno (Cystic Fibrosis Center Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy), PhD MD Paola Melotti, Davide Treggiari, (Azienda Ospedaliera Universitaria Integrata Verona, Italy) Prof. Claudio Sorio (University Dept. of Medicine, General Pathology Division, Univ. of Verona), Barbara Messore, Sara Demichelis (Azienda Ospedaliero Universitaria San Luigi Gonzaga, Adult Cystic Fibrosis Center, Pulmonology Div., Turin, Italy), Dorota Sands, Lukasz Wozniacki (Institute of Mother and Child, Cystic Fibrosis Department, ul., Warsaw, Poland), Celeste Barreto, Carolina Gonçalves (Departamento de Pediatria, Hospital de Santa Maria (CHULN), Centro Académico de Medicina de Lisboa, Lisboa, Portugal), Silvia Gartner (Pediatric Pulmonology and Cystic Fibrosis Unit, Hospital Universitari Vall dHebron, Barcelona, Spain), Antonio Alvarez Fernandez (Adults Cystic Fibrosis Unit. Hospital Universitari Vall dHebron, Vall dHebron Insitut de Recerca (VHIR) Barcelona, Spain), prof MD PhD, Lena Hjelte, MD PhD Isabelle de Monestrol (Stockholm CF Center, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden), Marita Gilljam MD, PhD (Department of Respiratory Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden), Per Hedenström (Unit of GI Endoscopy, Sahlgrenska University Hospital, Gothenburg, Sweden), Prof. Dr. Alexander Möller (University of Zurich), Dr. F. Singer (Division of Respiratory Medicine, Dept of Pediatrics, Inselspital, University of Bern, Bern, Switzerland), Dr. Marleen Bakker (Department of Pulmonology, Erasmus MC, University Medical Center, 3015 GD Rotterdam, the Netherlands), Dr. H.M. Janssens (Division of Respiratory Medicine and Allergology, Dept of Pediatrics, University Medical Center Rotterdam, Erasmus MC-Sophia, Rotterdam, The Netherlands), Dr. J. Altenburg (Department of Respiratory Medicine, Amsterdam University Medical Centres, University of Amsterdam, Amsterdam, Netherlands), Dr. S.W.J. Terheggen-Lagro (Department of Pediatric Pulmonology, University Medical Center Utrecht, The Netherlands).

References

[1] Clevers H. Modeling Development and Disease with Organoids. Cell 2016;165(7):1586–97. https://doi.org/10.1016/j.cell.2016.05.082. Jun.

[2] Sachs N, et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. Cell 2018;172(1–2):373–86. https://doi.org/10.1016/j.cell.2017.11.010. Jan.

[3] Bar-Ephraim YE, Kretzschmar K, Clevers H. Organoids in immunological research. Nature Reviews Immunology 2019. https://doi.org/10.1038/s41577-019-0248-y. Dec.

[4] Sato T, et al. Single Lgr5 stem cells build crypt – villus structures in vitro without a mesenchymal niche. Nature 2009;459(7244):262–5. https://doi.org/10.1038/nature07935.

[5] Dekkers JF, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med 2013;19(7):939–45. https://doi.org/10.1038/nm.3201. Jul.

[6] Sato T, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 2011;141(5):1762–72. https://doi.org/10.1053/j.gastro.2011.07.050. Nov.

[7] Sachs N, et al. Long-term expanding human airway organoids for disease modeling. EMBO J 2019;38(4):e100300. https://doi.org/10.15252/embj.2018100300. Feb.

[8] Berkers G, et al. Rectal Organoids Enable Personalized Treatment of Cystic Fibrosis. Cell Rep 2019;26(7):1701–1708.e3. https://doi.org/10.1016/j.celrep.2019.01.068.

[9] Beekman JM, et al. Biobanking: towards increased access of biomaterials in cystic fibrosis. Report on the pre-conference meeting to the 13th ECFS Basic Science Conference, Pisa, 30 March-2 April 2016. Journal of Cystic Fibrosis 2017;16(5):616–21. https://doi.org/10.1016/j.jcf.2017.04.009. Sep.

[10] P. van Mourik, S. Michel, J.M. Beekman, and C.K. van der Ent, "Rationale and design of the HIT-CF Organoid Study: stratifying Cystic Fibrosis Patients Based on Intestinal Organoid Response To Different CFTR-modulators," *Transl Med Commun*.

[11] Servidoni MF, et al. Rectal forceps biopsy procedure in cystic fibrosis: technical aspects and patients perspective for clinical trials feasibility. BMC Gastroenterol 2013;13:91. https://doi.org/10.1186/1471-230X-13-91. May.

[12] Friedmacher F, Puri P. Rectal suction biopsy for the diagnosis of Hirschsprung's disease: a systematic review of diagnostic accuracy and complications. Pediatr Surg Int 2015;31(9):821–30. https://doi.org/10.1007/s00383-015-3742-8. Sep.

[13] Vonk AM, et al. Protocol for Application, Standardization and Validation of the Forskolin-Induced Swelling Assay in Cystic Fibrosis Human Colon Organoids Protocol for Application, Standardization and Validation of the Forskolin-Induced Swelling Assay in Cystic Fibrosis. STAR Protoc 2020:100019. https://doi.org/10.1016/j.xpro.2020.100019.

[14] Boj SF, et al. Forskolin-induced Swelling in Intestinal Organoids: an In Vitro Assay for Assessing Drug Response in Cystic Fibrosis Patients. Journal of Visualized Experiments 2017;(120):1–12. https://doi.org/10.3791/55159.

[15] Yan HHN, et al. A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. Cell Stem Cell 2018;23(6):882–897.e11. https://doi.org/10.1016/j.stem.2018.09.016. Dec.

[16] Konstan MW, et al. Efficacy response in CF patients treated with ivacaftor: post-hoc analysis. Pediatr Pulmonol 2015;50(5):447–55.