Project Proposal

A pilot study investigating the effects of a manuka honey sinus rinse on sino-nasal outcome test scores in cystic fibrosis patients

Sponsor	Swansea University, Swansea, SA2 8PP, UK
Study Coordination Centre	As above
Funder	The Hodge Foundation
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This protocol has been authorised by:

Chief Investigator Rowena Jenkins Signature Date

Principle Investigator Jamie Duckers Signature Date

This protocol has been authorised by:

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigator team, host organisation, and members of the research ethics committee, unless authorised to do so.

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1. Study Synopsis

Study Title	A pilot study investigating the effects of a manuka honey sinus rinse on sino-nasal outcome test scores in cystic fibrosis patients.			
Short Title	Manuka honey sinus rinse study – V0.1			
Study Design	Basic Science (feasibility / pilot study)			
Planned Sample Size	20-40 Participants			
Planned Study Period	18 Months			
Planed Study Period	**Start:**	01/10/2018	**Finish:**	31/03/2020
Primary Objective	Does the addition of manuka honey to the sinus rinse impact on the Sino Nasal Outcome Test (SNOT)-22 score of patients with cystic fibrosis?			
Secondary Objective(s)	**1.**	Does the addition of manuka honey to a sinus rinse alter the quality of life of patients with cystic fibrosis?		
	2.	Does the addition of manuka honey to a sinus rinse impact on the bacterial type/amount found in the nasal/paranasal cavities of patients with cystic fibrosis?		
	3.	Does the addition of manuka honey to a sinus rinse protocol change the structure of sputum mucus in patients with cystic fibrosis?		
Statistical Methodology & Analysis	Sino-nasal outcome tests and quality of life (questionnaire) scores will be compared before and after treatment with/without the manuka honey sinus rinse. Proportional change in bacteria seen within and between groups will be analysed using a contingency table and assessing co-variants. The molecular structural changes to sputum mucus will be corrected and vector normalized.			

2. Abbreviations

AE	**A**dverse **E**vents
AMR	**A**nti**m**icrobial **R**esistance
APR	**A**nnual **P**rogress **R**eport
AWACFC	**A**ll **W**ales **A**dult **C**ystic **F**ibrosis **C**entre
BMI	**B**ody **M**ass **I**ndex
CF	**C**ystic **F**ibrosis
CFQ-R	**C**ystic **F**ibrosis **Q**uestionnaire - **R**evised
CI	**C**hief **I**nvestigator
CRF	**C**ase **R**eport **F**orm
CT	**C**omputed **T**omography
ECFSPR	**E**uropean **C**ystic **F**ibrosis **S**ociety **P**atient **R**egistry
FEV1%	**F**orced **E**xpiratory **V**olume (in 1 second as percentage of predicted)
GCP	**G**ood **C**linical **P**ractice
GP	**G**eneral **P**ractitioner
ICH	**I**nternational **C**onference of **H**armonisation
ISF	**I**nvestigator **S**ite **F**ile
HCRW	**H**ealth and **C**are **R**esearch **W**ales
HRA	**H**ealth **R**esearch **A**uthority
HTA	**H**uman **T**issue **A**uthority
NICE	**N**ational **I**nstitute for Health and **C**are **E**xcellence
NHS	**N**ational **H**ealth **S**ervice
MHRA	**M**edicines and **H**ealthcare Products **R**egulatory **A**gency
MID	**M**icrobiology and **I**nfectious **D**isease
NRES	**N**ational **R**esearch **E**thics **S**ervice
PI	**P**rincipal **I**nvestigator
PIS	**P**articipant **I**nformation **S**heet
R&D	**R**esearch & **D**evelopment
REC	**R**esearch **E**thics **C**ommittee
SAE	**S**erious **A**dverse **E**vents
SDL	**S**tudy **D**elegation **L**og

SOPs	**S**tandard **O**perating **P**rocedure(s)
SNOT-22	**S**ino-**N**asal **O**utcome **T**est
TMF	**T**rial **M**aster **F**ile
UHB	**U**niversity **H**ealth **B**oard
UKCRN	**U**nited **K**ingdom **C**linical **R**esearch **N**etwork

3. Trial Background and Rationale

Cystic fibrosis (CF) is an inherited lifelong, life threatening disease which can affect multiple organs in the body. The inherited defect affects a cells chloride ion channels leading to thick and sticky secretions. This is highly problematic in the lung where there is an accumulation of mucus, which in turn blocks the airway and leads to increased breathing difficulties. In addition to this, the mucus acts as a rich nutrient source that promotes colonisation by a wide range of bacterial species, such as; Staphylococcus aureus, Pseudomonas aeruginosa and Burkholderia cepacia complex species. As infection and lung damage progress over the years, P. aeruginosa becomes the dominant infective species, accounting for 33% of all UK CF lung infections, with 55% of patients prescribed long term inhaled antibiotics to help try and prevent/remove these infections (ECFSPR Annual Data Report 2013). Long term exposure to antibiotics leads to the emergence of Antimicrobial Resistance (AMR) amongst bacterial cells, which is now recognised globally as one of the major threats to human health. Globally, 700,000 people die each year as a result of AMR, requiring the UK government to develop a 5 year plan in order to combat AMR (O'Neill 2014). The direct effects of AMR are far reaching, resulting in; an inability to control infections (which we once could with the same antimicrobial agents), increased morbidity, increased mortality, and decreased patient wellbeing, all of which lead to increased healthcare costs (Roberts 2009; Romling 2014). The upper respiratory tract (upper airways and sinuses) of CF patients are frequently infected and therefore act as reservoirs of infection for the lower respiratory tract (Johansen 2012). Therefore, we have an urgent need for new and sustainable antimicrobial treatments for those suffering with CF as over 80% of patients will require treatment as a result of chronic infection and subsequent inflammation which ultimately leads to lethal respiratory failure (Lyczak 2002).

To understand the impact (emotionally and socially) of CF and the treatments on everyday life, a specialised questionnaire way developed (CFQ-R) to aid healthcare professions in how they tackle patient health and treatment. In addition to this, patients undertake a Sino-Nasal Outcome Test (SNOT-22) questionnaire which determines the social and emotional consequences of the patient's nasal disorder. If scoring highly in this, patients are required to follow a nasal rinse procedure daily as part of standard clinical care to help alleviate some of the symptoms described above.

Manuka honey has a strong track record as an antimicrobial agent, inhibiting a wide range of opportunistic pathogens, many of which cause problematic chronic infections, and can colonise the

airway of CF patients (Cooper 2008; Jenkins 2015; Roberts 2012). It is thought that by combining manuka honey with the nasal rinse procedure, the patients emotional and social wellbeing (as determined by the two questionnaires) may improve and the amount/diversity of bacterial cells within the sino-nasal area might also be lowered and/or altered.

As part of routine care, patients with CF have an annual review meeting which involves attending the All Wales Adult Cystic Fibrosis (AWACF) Centre at the University Hospital Wales (UHW) Llandough. During this meeting nasal swabs, blood tests, sputum samples, chest X-rays and an assessment of sinus symptoms (via the SNOT-22 and CFQ-UK) is completed. They also attend other routine clinics during the year and can have stays as in patients. Over an 18-month period, some of these patients (which meet the set inclusion criteria) will be recruited to the study. Prior to the routine clinic meetings, patients are reminded (via) letter about their upcoming meeting. We intend to include information about this study and a patient information leaflet within this letter.

During any routine clinic meeting, patients scoring highly on the SNOT-22 questionnaire will be asked whether they wish to take part in the study. The healthcare professionals will ensure the patient has read the information leaflet, answering any questions they may have with regards to the study. Patients who wish to proceed will be asked to give informed consent and will become a participant of the study. Participants will be randomly assigned into two groups; Group one will follow the standard NHS nasal rinse protocol, and group two will follow a modified NHS nasal rinse protocol which requires the addition of manuka honey (supplied to the participants). Participants will then follow the nasal rinse procedure (with/without manuka honey) daily for 30 days.

As part of routine clinical care, patients return to the AWACF centre approximately one month after routine clinic meeting. During this meeting the same tests are completed and their results from the previous meeting are explained to them. A treatment plan is then agreed with the patient. Participants of the study will supply a nasal swab, sinus rinse sample, and sputum sample (all part of standard care) along with copies of their SNOT-22 and CFQ-UK questionnaires. Any effects manuka honey has on the questionnaire scores, the amount/diversity of bacteria within the sino-nasal area, and/or the structural composition of the sputum will be analysed and determined.

Conventional microbial culture analysis will be completed along with microbiota analysis using DNA extracted from bacteria within sputum, sinus rinse, and swab. This high resolution, cultivation independent method analyses all the bacteria present in the samples including those that are deemed "unculturable". Understanding the total microbiota and the effect of honey on these organisms will provide an all-encompassing picture of the therapeutic benefit the proposed sinus rinse protocol. Fourier transform infrared spectroscopy will be used to determine if the manuka honey rinse protocol causes any molecular structural changes to sputum mucus (secreted mucin glycoprotein) composition as mucin glycan composition maybe an indicator of disease state in cystic fibrosis (Schulz et al., 2007).

A clear picture that has emerged from the application of microbiome analysis to CF lung infections is that as patients age, accumulating multiple rounds of potent antibiotic therapy, the diversity of the bacteria in the lung reduces. Their infections become dominated with AMR infections such as Pseudomonas and Burkholderia. This low diversity state is commonly associated with severe lung disease and inflammatory damage (Li 2016).

The proposed sample size combines a power calculation and a consideration of logistical constraints. We expect to see 290 patients per year of which 50% should complete a SNOT-22 form. Of the 145 forms received around 72 (50%) should be above average. If we allow for a low recruitment rate (of 30-40%) we believe it is realistic to enrol at least 20-30 patients per year. Over the 18 months of the project we feel, therefore, a sample size of 30-40 can be realistically achieved. Savastano (2014) published summary details of SNOT-22 scores for CF patients (who should well approximate our sample group) reporting a mean (SD) scores of 30.5 (14.5). We draw our definition of a clinically significant change from Hopkins et al (2009), who propose 8.9 points. The study will be structured as a 2-sided equality trial, with before and after measurements for each participant. In order to obtain a power of 80% we would require a sample size of at least 21.

We appreciate that the numbers of participants that will be recruited for this study, within the time frame (between 20-40 in 18 months), is not a true representation of the national population of CF patients. However, the results of this feasibility/pilot study will identify any trends in the effects of a nasal rinse supplemented with manuka honey on the bacterial amount/diversity within the infected sinuses. Antimicrobial interventions that do not rely on antibiotics to remove resistant strains from sinus infections have the potential to help alleviate symptoms in the sinus and upper airways, which may impact on the bacteria seen in the lower airways (Aanaes, 2013). Therefore, if a change in

bacterial diversity or a reduction in symptoms is observed, this study could inform a more in-depth study as manuka honey has already been demonstrated to eradicate both antibiotic sensitive and AMR bacteria in vitro and from chronic in vivo wounds.

Helping to manage upper respiratory infections without antibiotics could help to reduce an important driver for AMR, reduce the potential of cross contamination by AMR bacteria and help to reduce excessive healthcare costs. The research team have a wealth of experience in this area so are well placed to ensure a robust, well executed study that has the potential to inform and strongly influence treatment planning choices in the future, and ultimately improve the health outcome for patients. The information gained from this study would likely have impact on both clinical practitioners and patients in the long term, as demonstrating the presence of AMR could lead to a change in treatment regimens and help with reducing excessive healthcare costs.

4. Objectives and Outcome Measures/Endpoints

4.1 Primary Objectives

Objective	Outcome Measure / Endpoint
Does the addition of manuka honey to the sinus rinse impact on the SNOT-22 score of patients with cystic fibrosis?	SNOT-22 scores will be completed before and after the use of a sinus rinse with/without manuka honey.

4.2 Secondary Objectives

Objective	Outcome Measure / Endpoint
Does the addition of manuka honey to a sinus rinse alter the quality of life of patients with cystic fibrosis?	Quality of life questionnaires (CFQ-R) will be completed before and after the use of a sinus rinse with/without manuka honey.
Does the addition of manuka honey to a sinus rinse impact on the bacterial type/amount found in the nasal/paranasal cavities of patients with cystic fibrosis?	Swabs, a sinus rinse wash sample and a sputum sample for each participant will be collected before and after the use of a sinus rinse with/without manuka honey. Bacteria amount and types will be analysed in each sample.
Does the addition of manuka honey to a sinus rinse protocol change the structure of sputum mucus in patients with cystic fibrosis?	Sputum samples from each participant will be analysed before and after the use of a sinus rinse with/without manuka honey by fourier transform infrared spectroscopy.

5. Study Design

This is a single site study to be run at the AWACFC based in University Hospital Llandough (Cardiff and Vale Health Board) when patients attend any routine clinic meeting. The work on site will be carried out by the clinical research team as per the delegation of duties log.

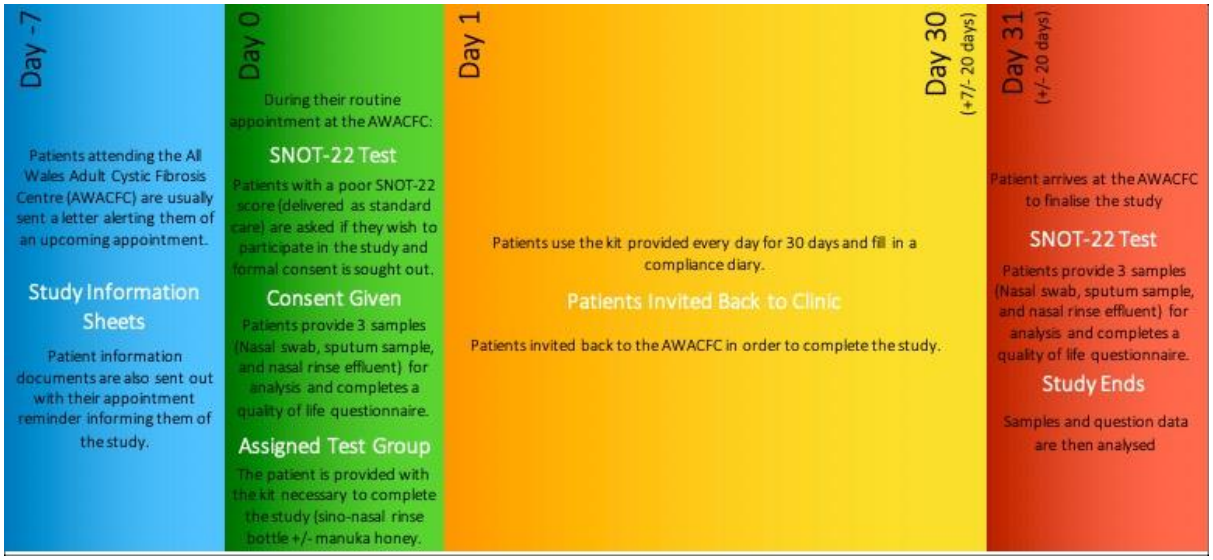
The AWACFC cares for 300 adults with CF from across Wales. Under standard clinical practise (NICE and CF Trust) all patients with CF receive an annual review meeting and routine clinic meetings each year as part of their routine clinical care. The AWACFC routinely write to patients who are due to attend the centre for their meetings with information explaining the meeting process. We intend to include information about this study, a patient information sheet and a patient consent form. During their meeting, participants who report significant nasal symptoms (measured using the SNOT-22 questionnaire) will be asked whether they wish to take part in this study. The clinical research team will check that patient has read the patient information sheet and answer any questions they may have. If patients want more time to think about the study, a separate clinic appointment can be arranged to accommodate this need. Patients who have given informed consent will become a participant of the study and be randomized to one of two groups; those who will use the standard sinus rinse (group one) and those who will have manuka honey added to the sinus rinse (group two). The participants will use their supplied nasal rinse protocol (with/without manuka honey) daily for 30 days while also filling in a participant compliance diary.

The study will not involve additional clinic visits for patients, however a nasal swab and sinal rinse solution would be provided in addition to standard measurements.

6. Study Management

The trials management group (consisting of Jamie Duckers, Anna Sayers, Cendl Hawkins, Jess Williams, Rowena Jenkins, and Aled Roberts) will meet up every 3 months and will be in contact via e-mail and phone during the interim if any part of the study needs discussing. The full list of duties is outlined in the delegation of duties log. Standardized SOPS for each clinical procedure will be followed for each activity.

7. Study Flow Chart



8. Participant Identification

Between 20-40 patients will be recruited for this study over an 18-month period. Patients will be recruited opportunistically during their routine clinic meetings. Normally the AWACFC runs a clinic for CF patients everyday Monday to Friday. We would estimate recruiting an average of two patients per month, taking into account the inclusion/exclusion criteria (see below), and the potential for participants to drop out of the study. We would expect to recruit all patients within the 18-month period.

8.1 Inclusion Criteria

The following criteria will be used to determine the eligibility of a patient to participate in the study.

- The patient is willing and able to give informed consent.
- The patient must be ≥ 18 years.
- The patient must have an established diagnosis of CF (one or more of the following)
    - Sweat chloride >60mEq/L
    - Presence of two CF causing mutations.
- The patient must have chronic symptoms of rhinosinusitis according to the criteria of the European Position Paper on Rhinosinusitis (appendix A).
- The patient scores greater ≥ 7 on their SNOT-22 questionnaire.

8.2 Exclusion Criteria

The following criteria will be used to determine the ineligibility of a patient to participate in the study.

- The patient has ever tested positive for the bacteria Mycobacterium tuberculosis.
- The patient is currently using a nasal rinse protocol.
- The patient has undergone sinus surgery within 6 months.
- The patient suffers from nasal bleeding.
- The patient is currently undergoing systemic antibiotic therapy for infective exacerbation.
- The patient is using overnight oxygen via nasal cannula.
- The patient is participating in another clinical trial or has done so within the last 30 days.
- The patient has a known allergy to bee products.
- The patient has an objection to the use of bee products

9. Study Procedures

(See attached study procedures document)

9.1 Recruitment

A member of the clinical research team (as per the delegation of duties log) will identify potential participants when arranging their routine clinic meeting at the AWACFC. As part of the letter sent out informing the patient of their routine clinic meeting, the clinical research team will include a letter, patient information sheet, and patient consent form. This will allow the patient to read through the information prior to attending their routine clinic appointment. During this week period, they are able to call the clinical research team to ask any questions. At the meeting appointment, the patient will have another opportunity to ask questions about the study. These patients will be recruited opportunistically at their meeting if they meet the inclusion criteria without any excluding criteria.

All potential participants will be recruited from the AWACFC during routine clinical meeting. All data gathered during this process will only be seen by the clinical research team whom will also be delivering their standard care, so there should be no issues with confidentiality. This information will be passed onto the academic research team (as per the delegation of duties log) in an anonymised state so as to project the study participant.

Participants will provide answers to a SNOT-22 questionnaire as part of standard practice and if their score meets the inclusion criteria, the clinical research team will discuss the trial in detail with the patient. If the patient is happy to proceed, informed consent will be taken, and the patient will become a participant of the study immediately with all their data being randomised against a participant ID number. As per the patient consent form their GP will be subsequently notified of their involvement in the study. Participants will then be randomly separated into two groups:

- Group 1 - Those who will use the standard sinus rinse.
- Group 2 - Those who will use the standard sinus rinse with the addition of manuka honey.

During their routine clinic meeting (Visit 1), all participants of the study will provide the following data/samples:

- SNOT-22 questionnaire.

- Cystic Fibrosis Quality of life (CFQ-R) questionnaire.
- Clinical Record Form (CRF) data.
- Nasal swab.
- Sputum sample.
- Nasal rinse wash solution.

Participants will then be given a nasal rinse kit (with/without manuka honey depending on the group they have been assigned), a set of instructions on how to use the kit, and a compliance diary. Participants will use their respective kits as per the instructions daily and fill in the compliance diary, ensuring to make note of any observations in the compliance diary. Participants will then return to the AWACFC 31 days (+7/- 20 days) later for their standard follow up appointment (Visit 2). During this visit a second set of data/samples will be collected as per below:

- SNOT-22 questionnaire.
- Cystic Fibrosis Quality of life (CFQ-R) questionnaire.
- Clinical Record Form (CRF) data.
- Nasal swab.
- Sputum sample.
- Nasal rinse wash solution.

Following the collection of samples by the clinical research team during visit 1 and 2, they will be collected by the academic research team whom will process the nasal swab, sputum sample, and nasal rinse wash solution the same day, extracting bacteria and bacterial DNA from the samples. The structural composition of the sputum mucus will also be determined from the sputum sample. Other analysis of the samples we be completed at a later date where the amount/types of bacteria present will be determined.

9.2 Screening and Eligibility Assessment

Potential participants would be sent written information at least one week prior to their routine clinic meeting. At the routine clinic appointment an assessment of sinus symptoms is routinely performed by a respiratory physiotherapist. If patients describe sinus symptoms a SNOT -22 questionnaire is administered. The physiotherapist will inform the clinical research team who will then assess whether the patient meets the inclusion criteria for the trial and will take informed consent if the patient is keen to participate. In the study.

9.3 Informed Consent

Informed consent will be taken in written form after the participants have had time to consider the information and ask any questions they may have to the clinical research team. Once happy, patients will be asked to confirm the following statements, and sign the patient consent form, after which they will become a participant of the study:

- I confirm that I have read and understand the participant information sheet (version 5.0 27/02/2020) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
- I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.
- I agree to the use of anonymised data within publications and for medical notes and research project data to accessed by authorised individuals from Swansea University, regulatory authorities, or Cardiff and Vale University Health Board for monitoring and audit purposes.
- I agree to my clinician being informed of the results who, in turn will inform me.
- I agree to take part in the above study.
- I agree to my GP being informed of my participation in this study.

9.4 Randomisation, Blinding, and Unblinding

A blocked randomisation list will be generated prior to start of study using the electronic random number generator tool (see below). The participants of the study will not be blinded to the treatment they are receiving because the addition of manuka honey to the sinus rinse is obvious and there is no way to negate this. The academic research team will be blinded to which groups the participants (only identifiable by their participant ID number) are assigned. This information (the group allocations and treatment regimens) will only be made available to the academic research team once analysis of the data begins.

- GraphPad QuickCalcs
  - https://www.graphpad.com/quickcalcs/randomize1/

9.5 Baseline Assessments

General information will be collected from participants via the clinical record as will information pertaining to their antibiotic usage, their comorbidities, and more specific cystic fibrosis information as per below:

- Age.
- Sex.
- Height
- Weight.
- BMI.
- Smoking status.

- Antibiotic usage (last three months).
    - IV antibiotics.
    - Oral antibiotics.
    - Nebulised antibiotics.
    - Long term antibiotic usage.

- Co-morbidities.
- Cystic Fibrosis specific information.
    - SNOT-22 score
    - FEV1% score. .

- Is the participant undergoing sinus treatment.
- Is the participant used a nasal spray.
- Is the participant recovering from endoscopic surgery.

9.6 Study Visits and Follow Up

During their routine clinic meeting (Visit 1), all participants of the study will provide the following data/samples:

- SNOT-22 questionnaire.
- Cystic Fibrosis Quality of life (CFQ-R) questionnaire.
- Clinical Record Form (CRF) data.
- Nasal swab.
- Sputum sample.
- Nasal rinse wash solution.

Participants will then be given a nasal rinse kit (with/without manuka honey depending on the group they have been assigned), a set of instructions on how to use the kit, and a compliance diary. Participants will use their respective kits as per the instructions daily and fill in the compliance diary, ensuring to make note of any observations in the compliance diary. Participants will then return to the AWACFC 31 days (+/- 7 days) later for their standard follow up appointment (Visit 2). During this visit a second set of data/samples will be collected as per below:

- SNOT-22 questionnaire.
- Cystic Fibrosis Quality of life (CFQ-R) questionnaire.
- Clinical Record Form (CRF) data.
- Nasal swab.
- Sputum sample.
- Nasal rinse wash solution.

9.7 Sample Handling

Samples will be handled in accordance with HTA regulations and the safety operating procedures (SOPs) set out in NHS/university procedures. Participant samples will be processed and destroyed within 7 days according to local HTA regulations (bacterial cells and bacterial DNA extracted from participant samples will be kept as per the respective SOPs. Any material left over after processing will be disposed of via an approved method.

9.8 Discontinuation/Withdrawal of Participants

All participants have the right to withdraw from the study at any time. In addition to this, the chief investigator (CI) may discontinue a participant from the study at any time if the they consider it necessary. The reason for withdrawal will be recorded on the CRF. The participant will be replaced if possible within the time frame using the same recruitment procedure as was used for the original participant. If you wish to withdraw from the study, all identifiable samples will be destroyed, however all the data generated up to the date of your withdrawal will remain for analytical purposes. Withdrawing from the study will in no way affect your current or future care. If in the future, you were no longer able to give consent to partake in the study, we would follow the procedure as if you had withdrawn from the study

9.9 Study Amendments

The CI will seek approval for any substantial amendments to the protocol or other study documents from the Sponsor (Swansea University), Research Ethics Committee (REC) and NHS Research & Development (R&D) Office(s) where appropriate. Amendments to the protocol or other study documents will not be implemented prior to these approvals being granted. Non-substantial amendments will be notified to the REC for information and may also need to be reviewed and accepted by R&D departments, or through other research governance mechanisms, before they can be implemented in practice. It is the sponsor's responsibility to classify amendments as being non-substantial or substantial and the chief investigator will seek advice from the sponsor when necessary.

9.10 Definition of End of Study

The end of the study will be the date of the last visit of the last participant undergoing the trial.

10. Products, Devices, Techniques, and Tools.

Below is a brief (layman) explanation of some of the devices, techniques and tools being employed in this study. Each element has been expertly selected using good clinical and academic judgement to ensure the primary and secondary objectives can be answered in the most efficient way possible.

10.1 Devices

Medihoney.

Medihoney (a sterile medical grade honey) is manufactured by Integra Lifesciences and is CE marked. The honey will be dissolved into the standard sinus rinse (which forms part of standard clinical care for the treatment of CF sinus infections) at a final concentration of 40 %.

10.2 Techniques and Tools

SNOT-22 Questionnaire.

The SNOT-22 questionnaire is a validated method used to determine the severity of the social/emotional consequences of a patient's nasal disorder. This questionnaire has no right or wrong answers and should reflect the feelings of the patient to their nasal disorder (ranging from "no problem" through to a "moderate problem", with "problem as bad as it can be" having the highest rating). Some of the areas surveyed in this questionnaire relate to; sneezing, runny nose, dizziness, nasal discharge, ability to sleep, fatigue etc…

CFQ-R Questionnaire.

The CFQ-R questionnaire is used to understand the impact of CF and the associated treatments on the patients everyday life, allowing adjustments to be made in treatments to benefit the patient. The CFQ-R is a quality of life questionnaire that has been specifically designed for CF patients. Some of the areas surveyed in this questionnaire relate to; demographics, quality of life, school/work and daily life activities, and symptom difficulties.

CRF.

The CRF is used to record various data pertaining to the patient's demographics, antibiotic usage, comorbidities, and specific information relating to their condition (CF) and their treatment. This form is based on facts rather than the previous two questionnaires which focus more on the patient's feelings.

Microbial Diversity Assessment (Basic Microbiology).

Basic microbiological techniques (culturing bacterial species on selective bacterial growth media) will be used to determine the total number of bacteria in each biological sample. The selective growth media will provide some indication as to the bacterial species present in the samples and the ratio of them.

Microbial Diversity Assessment (Bioinformatics).

Advanced bioinformatics techniques (sequencing of bacterial cells) will be used to determine the community composition and the species of bacteria present within biological samples. This particular method will allow non-culturable bacterial cells to also be identified (which wouldn't be picked up through basic microbiology methods).

Sputum Mucus Structure.

Bruker Tensor high throughput Fourier transform infrared spectrometers will be used to determine the molecular structure of mucus sputum.

11. Safety Reporting

11.1 Adverse Events

An Adverse Event (AE) is any untoward medical occurrence in a trial participant which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and/or unintended sign (including abnormal laboratory finding), symptom, or disease. We will therefore record signs of an allergic reaction such as;

- Rashes.
- Hives.
- Itching.
- Red and/or swollen skin.
- Blistered and/or peeling skin
- Wheezing.
- Tightness in the chest or throat.
- Trouble breathing, swallowing, and/or talking
- Unusual hoarseness
- Swelling of the mouth, face, lips, tongue, and/or throat
- Nasal irritation.

The following (see below) may be expected with routine use of a sinus rinse protocol and will therefore be recorded but not reported. May can often be avoided by following the administration instructions in the patient information booklet.

- Temporary increase in nasal congestion (often improves with continued use).
- Ear discomfort whilst rinsing.
- Drainage of leftover solution from the nose after rinsing.
- Accidental ingestion of solution (not recommended but deemed not harmful).

11.2 Serious Adverse Events

A Serious Adverse Event (SAE) is any adverse event that meets one or more of the criteria listed below:

- Results in death
- Is life-threatening
- Requires hospitalisation or prolongs existing hospitalisation
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- Other medically important condition

11.3 Reporting Serious Adverse Events

In the event of a SAE, the study coordination centre will be contacted by phone and then sent a completed SAE form within 24 hours as above. The research team will report all related SAEs as required by their REC and/or R&D Office.

Contact details for reporting Serious Adverse Events (SAEs)

ATTENTION FOR DR JAMIE DUCKERS

Please send SAE forms to:

All Wales Adult Cystic Fibrosis Center, University Hospital Llandough,

Penlan Road, CF62 2XX

Phone: 02920 716488 Fax: 02920 7152947 (Mon to Fri 09:00 – 17:00)

All Serious adverse event in studies sponsored by Swansea University must be reported to the sponsor immediately and within 24 hours of the research team becoming aware of the event. Swansea University Research Governance Office should be informed at resgov@swansea.ac.uk"

A serious adverse event (SAE) occurring to a participant will be reported to the REC that gave a favourable opinion of the study by the Principal Investigator (PI; Jamie Duckers). Reports of related and unexpected SAEs will be submitted to the REC within 15 working days of the PI becoming aware of the event, using the National Research Ethics Service (NRES) report of serious adverse event form (see IRAS/NRES website).

Unrelated and Expected SAEs do not require reporting but a copy of the SAE report should be retained in the study master file for audit purposes by research governance, unless alternative arrangements have been agreed during study set up. The Chief Investigator (CI; Rowena Jenkins) will send the Annual Progress Report (APR) to the main REC and Sponsor using the NRES template.

11.4 Urgent Safety Measures and Serious Breaches of GCP

The CI and PIs may take immediate safety measures to protect research participants against any hazard to their health or safety without prior authorisation from the REC or sponsor, however they must alert the sponsor and CI as soon as possible. The CI will notify the REC of the presenting

issue within 3 days of the urgent measure setting out the reasons for the urgent measure and the plan for further action. If a site PI identifies the presenting issue, he or she should also inform their local R&D department.

In the event that a serious breach of GCP is suspected, this will be reported to the Sponsor and REC immediately and will be investigated by the sponsor. Any corrective action required will be undertaken by the CI and REC informed. If necessary, a protocol amendment will be submitted for review.

12. Statistics and Analysis

12.1 Description of Statistical Methods

Qualitative analysis will include the difference between the SNOT-22 and quality of life questionnaire (CFQ-R) scores being assessed before and after treatment within and between both patient groups (with/without manuka honey). To determine if manuka honey caused a change in the amount/type(s) of bacteria present compared to the control group, a contingency table with odds ratio will be prepared taking into account co variants. Infrared spectra will be baseline corrected and vector normalised. Absorbance from specific infrared wavenumbers representative of mucin glycan molecular structures will be compared against in-house sputum spectral libraries to determine increase or decrease in glycosylation patterns as well as structural changes post-treatment. Comparisons in absorbance at key wavenumbers between groups will be analysed using ANOVA.

12.2 Number of Participants

The proposed sample size combines a power calculation and a consideration of logistical constraints. We expect to see 290 patients per year of which 50% should complete a SNOT-22 form. Of the 145 forms received around 72 (50%) should be above average. If we allow for a low recruitment rate (of 30-40%) we believe it is realistic to enrol at least 20-30 patients per year. Over the 18 months of the project we feel, therefore, a sample size of 30-40 can be realistically achieved.

Savastano (2014) published summary details of SNOT-22 scores for CF patients (who should well approximate our sample group) reporting a mean (SD) scores of 30.5(14.5). We draw our definition of a clinically significant change from Hopkins et al (2009), who propose 8.9 points. The study will be structured as a 2-sided equality trial, with before and after measurements for each participant. In order to obtain a power of 80% we would require a sample size of at least 21. We therefore propose that a target sample size of 30, since this will be both achievable and will deliver sufficient power, even allowing for drop outs.

12.3 Analysis of Outcome Measures/Endpoints

All participant data will be analysed by the academic research team after the end point of the study. The data will be sorted into the two treatment groups before analysis is carried out. The

primary outcome measures will be to determine whether the SNOT-22 score of the manuka honey treatment group has improved compared to the standard treatment. The secondary outcomes will be to see if; the lifestyle questionnaire scores have improved, the amount/type(s) of bacteria has changed, and whether the structure/composition of sputum mucus has changed in the same two groups. If any participants withdrew before the second visit/second set of samples were taken then that data will be excluded from analysis.

13. Data Management

13.1 Analysis of Outcome Measures/Endpoints

Direct access to data will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations.

13.2 Data Recording and Record keeping

Clinical data collected in both visit one and visit two will be recorded on the CRF. The clinical data which is routinely collected as part of standard clinical practice will be entered onto the CF registry and is kept indefinitely as part of standard clinical practice. The data from the SNOT-22 score, CFQ-R and CRF will be kept on a local clinical database (UHB) and anonymised data will be kept for 10 years after the completion or discontinuation of the trial.

The information required by the academic research team will be anonymised by the clinical research team using random study numbers generated by GraphPad Quick Calcs when the participants are allocated into groups. Only the PI will hold the link code and all the information on local databases will be password protected. The PI will be responsible for data entry and quality; data entry will be checked by two members of the clinical research team to ensure the data is entered correctly. The academic research team will be responsible for the analysis of the data both qualitative and quantitative. The data will be backed up on an encrypted USB and also transferred to the academic team in this way.

The original hard copy CRFs will be kept in secure fireproof locked cabinet until the analysis is complete. If there is a data loss/electronic failure, then the data can be recovered from the original hard copies.

13.3 Participant Confidentiality and Data Protection

The PI will maintain the data under a password protected database and only the usual clinical care team will see secure maintenance of the data and the linking code in separate locations using encrypted digital files within password protected folders and storage media. Data transferred to the

academic research team will already be anonymized and they will not have access to patient linked information at any point.

13.4 Record Storage and Retention

The Trial Master File (TMF) and Investigator Site File (ISF) containing essential documents will be kept for a minimum of 10 years after completion of study. Documents (paper and electronic) will be retained in a secure location during and after the study has finished. A label stating the required retention time should be placed on the inside front cover of the medical records for study participants. Essential documents pertaining to the study shall not be destroyed without permission from the Sponsor.

14. Quality Assurance Procedures

The study may be subject to inspection and audit by Swansea University under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK policy framework for health and social care research Nov 2017.

15. Ethical and Regulatory Considerations

The study will be conducted in compliance with the principles of the Declaration of Helsinki (2013) and the principles of Good Clinical Practice and in accordance with all applicable regulatory guidance, including but not limited to the Research Governance Framework for Health and Social Care in Wales (2nd ed, 2009) or applicable frameworks in the other UK countries. This protocol and related documents (and any subsequent amendments) will be submitted for review to the relevant parties. Annual progress and safety reports and a final report at the conclusion of the study will be submitted to the REC within the timelines defined.

15.1 Ethical Approval

Before the start of the study, approval will be sought from the REC. Any substantial amendments that require review by REC will not be implemented until the REC grants a favourable opinion for the study. All correspondence with the REC will be retained in the Trial Master File/Investigator Site File. An Annual Progress Report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. It will be the CI's responsibility to produce the annual reports as required. The CI will notify the REC of the end of the study. If the study is ended prematurely, the CI will notify the REC, including the reasons for the premature termination. Within one year after the end of the study, the CI will submit a final report with the results, including any publications/abstracts, to the REC.

15.2 Peer Review

The Hodge Foundation reviewed the project before awarding the money to the CI. It was also reviewed internally before being submitted to the funders.

15.3 Governance Review

The study will be reviewed for both study-wide and local governance compliance.

15.4 Reporting

The CI shall submit once a year throughout the study or on request, an Annual Progress Report (APR) to the REC, host organisation and Sponsor. In addition, an end of study notification and final report will be submitted to the same parties.

15.5 Expenses and benefits

No expenses will be paid as both study visits are part of normal standard care for the participants.

16. Finance and Insurance

16.1 Funding

The study is funded by The Hodge Foundation. They have contributed £302,000 to cover the costs of the trial and associated research.

Staff Costs	
Jamie Duckers (1.0 session for 18 months)	£18,720
Cendl Hawkins (0.5 session for 13 months)	£2,445
Aled Roberts (100% for 36 months)	£143,196
Rowena Jenkins (10% for 36 months)	£13,996
Study Consumable Costs	
Printing and stationary	£100
General laboratory consumables	£26,331
Bioinformatics and sequencing	£13,000
Honey	£2,000
Non-Study (but related project) Costs	
Microscopy and sample imaging	£7,682
Multi-species bacterial work	£12,500
Immunology experiments	£14,291
Lung modelling	£31,710
(Other) bioinformatics, sequencing, and computer	£5000
Conference and project dissemination	£12,000
TOTAL	£302,971

16.2 Insurance

For Swansea University employees/students/honorary staff members, Swansea University insurance applies. Where NHS staff are named on the protocol and/or research projects 'NHS indemnity and Swansea University insurance applies.

16.3 Finance and Other Competing Interests

The academic and clinical research team have no competing interests to declare, nothing being used in this study is owned by or related to any of the people involved in the study.

17. Publication and Registration Policy

Ownership of the data arising from this study resides with the study team and their respective employers. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared. Authors will acknowledge that the study was funded by The Hodge Foundation.

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study. Summaries of results will also be made available to Investigators for dissemination within their clinical areas (where appropriate and according to their discretion). The outcomes of the study will also be displayed at the AWACFC so participants and attendees of the clinic can see the results of the study. The research will be registered on the public UKCRN database and also the CF trust trials tracker.

18. References

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19. Appendix A

Clinical definition of rhinosinusitis in adults Rhinosinusitis in adults is defined as: inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip): - ± facial pain/pressure - ± reduction or loss of smell and either endoscopic signs of: - nasal polyps, and/or - mucopurulent discharge primarily from middle meatus and/or - oedema/mucosal obstruction primarily in middle meatus.