Project Final Report

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1 Executive Summary

CFMATTERS offers a stratified next generation approach to antibacterial treatment in a disease model of chronic bacterial infection with super-imposed acute exacerbations. It is the first randomized, controlled trial comparing the use of microbiome-directed antibiotic treatment versus standard therapy. The analysis of the constituent microbiome and its genomes will pave the way for more effective therapeutic regimes and ultimately contribute to the development of personalized medicine and personalized CF treatment. CFMATTERS for the first time will offer patients a targeted treatment model personalized to the individual Microbiome.

CF is a unique disease model. In CF, acute polymicrobial bacterial infections overlap with chronic polymicrobial bacterial colonisation and thus findings from the CFMATTERS study have potential applications in other diseases involving Pseudomonas aeruginosa, and/or polymicrobial infection (both acute and chronic) such as wounds, burns, mechanical devices patients and ventilator associated pneumonias. CFMATTERS may potentially revolutionize the practice of antibiotic prescription in acute and chronic infections and limit the development of antimicrobial resistance globally by decreasing antibiotic consumption therefore decreasing the likelihood of bacterial resistance and side- effects from repeated non-personalized antibiotic courses. Not only will this potentially improve survival of the 36,000 EU patients with CF who die significantly younger from lung disease when compared to non-CF EU citizens, but will also have subsequent application of these principles to patients with other acute and chronic infections.

We believe that this approach will also have profound implications for the EU and global public policy makers. An estimated 25,000 patients die annually and approximately €0.9 billion is spent on additional health costs related to bacterial resistance. Furthermore, the consequent economic value of lost productivity at work resulting from illness and its treatment of patients is estimated to be at least €1.5 billion in Europe each year.

CFMATTERS may also lead to identification of within-host evolution of pathogens during infection, bacterial persistence mechanisms and identification of genetic resistance mechanisms. Metabolite profiling of sputum (pathogen) and/or interrogation of host defense proteins (host factors) may discover new biomarkers to supplement traditional clinical assessment of antibiotic responses of CF patients. Given the difficulties associated with assessment of response to antibiotic treatment the development of such a biomarker(s) would be a welcome advance for patients with acute and/or chronic microbial infection. The development of in-vivo models of co-infection will also offer unparalleled insight into the impact into bacterial cross talk, bacterial virulence, the efficacy of treatment regimens and interaction between the lung and GI tract in response to pathogenic bacteria in the lung.

Finally CFMATTERS is examining the consequences of antimicrobial therapy on the gut microflora. Little is known of the effect of antibiotic administration on gut microbiome composition and/or inflammatory implications. Understanding the consequence of antibiotic administration on the gut microbiome is crucial in addressing the associated clinical symptoms such as diarrhea, occurring in as many as 30% of patients receiving antibiotics. Interestingly CF patients are known to have high carrier rates for Clostridium difficile. Recent reports suggest an increase in incidence of C. difficile infection in Europe, with significant medical complications. Data has estimated C. difficile infections cost the EU €3 billion per annum. Using CF as a model to understand this problem has important potential benefits. In totality these studies will increase understanding of the gut and immunologic consequences of antibiotic treatment, which could have broad implications in many areas of medicine.

2 Summary of description of project context and objectives

Cystic Fibrosis Microbiome-determined Antimicrobial Therapy Trial in Exacerbations: Results Stratified (CFMATTERS) will provide a randomized multi-centre controlled trial of microbiome-derived antimicrobial treatments versus current empirical therapy for Cystic Fibrosis Patients. Simultaneously parallel human host-pathogen interaction studies in sputa, human gut faecal analysis and evaluation of in vivo co-infection exacerbation have been performed. Antimicrobial resistance is arguably the most significant challenge facing the EU health care system. The unnecessary use of antibiotics is a key driver in the development of antibiotic resistance. Cystic Fibrosis (CF) represents a unique disease model to study bacterial resistance and to explore therapeutic strategies for the same, as chronic lung infection overlaps with acute lung exacerbations caused by a multitude of organisms that traditionally evolve various mechanisms of resistance. With time, chronic polymicrobial infection develops, in the lung with the most dominant infecting organism being Pseudomonas aeruginosa, which is also important in other infectiong including wounds, burns and patients with medical devices, making it an important clinical target for the EU. In CF infections, empiric intravenous antibiotics are usually given for two weeks. Recurrent infections and treatments result in increasing antimicrobial resistance, and alterations in pathogen host interactions in the lung and gut flora.

Next- generation DNA sequencing technology now offers DNA-based personalised diagnostics and treatment strategies. Based on bacterial molecular profiling of sputum, the use of stratified targeted antibacterial therapy can be compared with standard empirical antibacterial therapy currently used. We believe this will reduce antibiotic usage, and optimize dosage and duration strategies, as the therapy will be tailored to the actual individual patient needs and consequently, decrease adverse effects as well as emergence of drug resistance. The throughput and quality of

next-generation DNA sequencing technology now brings DNA-based personalised diagnostics within immediate reach for routine application in medical diagnostics and treatment strategies.

CFMATTERS will undertake 4 key objectives:

1. Provide randomized, controlled clinical data regarding the treatment effectiveness of microbiome-derived, tailored antimicrobial treatments versus current empirical therapy.

2. Enhance the understanding of human host-pathogen interaction via molecular profiling of antimicrobial peptide synthesis and innate immune responses in responders to treatment versus non-responders.

3. Use in *vivo* models to determine the impact of the lung microbiome on host response to infection by:

    − investigating the role of host-pathogen interaction and pathogen-pathogen interaction in the development of acute and chronic infection

    − characterising the treatment effectiveness of tailored microbiome-derived antimicrobial therapy via measurement of the emergence of antibiotic resistance mechanisms

4. Examine the consequences of antimicrobial treatment of CF lung exacerbations on the gut microflora, its influence on disease progression, and determine the potential role of the GI tract as a reservoir for resistant bacteria.

2.1 Overall strategy of the work plan

CFMATTERS offers a stratified, next-generation approach to antibacterial treatment in a disease-model of chronic bacterial infection with super-imposed acute exacerbations. Based on bacterial molecular profiling of sputum, the use of stratified, targeted antibacterial therapy can be compared with standard empiric antibacterial therapy currently used. Parallel basic science studies will enhance our understanding of pathogen-host factors and interaction. CFMATTERS may revolutionize the practice of antibiotic prescription in acute and chronic infections and limit the development of antimicrobial resistance. It was divided into 10 work packages (WP).

2.1.1 List of participants:

Participant no.	Acronym	Participant organisation name	Country	Contact
01	UCC	University College Cork	Ireland	Barry Plant
02	QUB	Queens University Belfast	UK	Stuart Elborn
03	UPD	Université Paris Descartes	France	Pierre-Regis Burgel
04	UoD	University of Dundee	UK	Robert Ryan
05	UW	University of Washington	USA	Chris Goss
06	UKL-HD	University of Heidelberg	Germany	Marcus Mall
07	TEAGASC	Teagasc, Food Research Centre	Ireland	Catherine Stanton
08	CSA	Clininfo SA	France	Patrick Chevarier
09	GABO:mi	GABO:mi Gesellschaft für Ablauf-organisation:milliarium mbH & Co. KG Participation terminated on 30 June 2016	Germany	Birgit Fuchs
10	PAP	Papworth Hospital	UK	Charles Haworth
11	KU LEUVEN	University Hospital Leuven	Belgium	Lieven Dupont
12	APHP	Assistance Publique-Hôpitaux de Paris	France	Isabelle Fajac
13	HCL	Hospices Civils de Lyon Participation started on 1 April 2016	France	Isabelle Durieu
14	UHSM	University Hospital of South Manchester Participation started on 1 April 2016	United Kingdom	Andrew Jones
15	ART	ARTTIC SAS Participation started on 1 July 2016	France	Martin Dietz

2.1.2 WP 01 Clinical trial management and analysis

WP01 will establish reliable and effective management structures (including the Trial Management Committee [TMC] and the Trial Coordination Centre [TCC]) and ensure that the clinical trial (WP03) is conducted in compliance with the protocol and Good Clinical Practice, with an appropriate Statistical Analysis Plan.

2.1.3 WP 02 Data management

WP02 will establish the online data input interface (eCRF) and data coordination mechanisms to ensure reliable and efficient data capture and traceability of specimens for the clinical trial period.

2.1.4 WP 03 Conduction of clinical trial

WP03 will conduct a randomized, clinical trial comparing microbiome-derived therapy vs. empiric therapy in 252 patients with CF across 9 clinical sites. All trial sites will be large university medical centres (including 8 European sites and 1 US site- total patient numbers n=2442 with specialised units for the care of people with CF. The consortium partners have a proven track record of conducting clinical trials in people with CF. WP03 will involve the transport of clinical specimens from clinical sites to the Central Laboratory, determination of a microbiome-derived treatment algorithm and, ultimately, quantification of the benefits of the microbiome-derived treatment over standard empiric treatment inCF pulmonary exacerbations. WP03 will also involve completion of scientific reports and final manuscript preparation.

2.1.5 WP 04 Ethical, data and safety monitoring

WP04 will be responsible for RCT monitoring to appropriate standards. Independent Ethics, Data and Safety Review Group (EDSRG) and a Scientific Advisory Board (SAB) will be constituted and ensure same, to maximise patient safety.

2.1.6 WP 05 Microbial activities and functions

WP05 will use metatranscriptomics and metabolomics to assess bacterial gene expression and metabolite profiles of sputum samples from WP03 during exacerbation using both in vitro and in vivo models. This WP aims to investigate microbial factors to identify novel biomarkers for unresponsiveness to antibiotic therapy.

2.1.7 WP 06 Human host defence response

WP06 aims to investigate the in vitro treatment effectiveness of host-derived antimicrobial peptides (AMPs) and standard antibiotics, and to characterise host- factor protein profiles in the sputum of CF patients from both treatment arms (WP03). This WP will determine the association between host response, the lung microbiome, antibiotic administration and clinical outcome.

2.1.8 WP 07 Lung microbiome

WP07 will investigate novel co-infection models for host- pathogen interactions, and the role of the lung microbiome on host response to Pseudomonas aeruginosa during chronic airway infection using murine models. WP07 will also provide in vivo validation of antibiotic treatment strategies utilised in WP03, aimed at improving rational prescription regimens and reducing the development of antibiotic resistance.

2.1.9 WP 08 Gastrointestinal microbiota (Participants 01, 02, 07, 11)

WP08 will use next-generation pyrosequencing technology to profile the microbiome of the CF gut during clinical stability and exacerbation, and during acute and chronic antibiotic therapy

from a subgroup (n=120 from 3 clinical sites). WP08 will provide novel data regarding the influence of acute and chronic antibiotic therapy on the CF gut microflora and T cell pathways. WP 08 will also determine if the CF gut is a reservoir for antibiotic resistance genes and will evaluate strains of probiotics isolated from persons with CF in a murine model.

2.1.10 WP 09 Project management (Participants 01, 09)

The Coordinator assisted by ART will ensure the overall proper management of the project from the submission process to achieving the final objectives. ART is an experienced SME with longstanding, hands-on expertise in the management of EU Framework projects.

2.1.11 WP 10 Dissemination, Outreach and Training (All participants)

WP10 aims to promote an understanding of CFMATTERS research, and to disseminate the results generated in CFMATTERS to relevant professional sectors such as clinicians and scientists and also the general public, and patient support groups.

3 Description of the main S&T results / foregrounds

3.1 WP01: Clinical trial management and analysis

WP01 has established reliable and effective management structures and ensure that the trial is conduct rigorously, to a high standard and in compliance with the protocol and Good Clinical Practise.

- To develop efficient, high quality trial management and coordination structures
- To ensure the uniform, high-quality conduct of the trial and reliable capture of study data.
- To analyse trial data in accordance with approved Statistical Analysis Plan.
- Develop the Treatment Algorithm.

3.1.1 Task 1: Establish Trial Management Committee

3.1.1.1 Trial Management Committee

A Trial Management Committee has been established. The Trail Management Committee is chaired by the Project Coordinator Professor Barry Plant and member of the committee include the Principle Investigators at the clinical trial sites; Professor Joe Eustace (Director HRB-Clinical Research Facility, Cork (CRF-C)), Dr Charlie Haworth (Papworth, UK), Professor Chris Goss (Seattle, USA), Professor Lieven Dupont (Leuven, Belgium) , Professor Marcus Mall (Heidelberg, Germany), Professor Stuart Elborn (Belfast, Northern Ireland) and Professor Isabelle Fajac (Paris, France) along with the Project Manager Dr Evelyn Flanagan,the Research Nurse at the coordinating centre Mary Daly and the coordinating scientist Yvonne McCarthy. The role of the group is monitor the conduct and progress of the trial, ensure that the protocol is adhered to, take appropriate action to safeguard participants and ensure the quality of the trial data. The Trial Management Committee will also prepare and approve reports for the Ethical and Safety Committee and the Scientific Advisory Board of the CFMATTERS.

3.1.1.2 Trial Steering Committee (TSC)

A trial steering committee (TSC) has been also established. The TSC is made up of all work package leaders; Prof Barry Plant (UCC), Patrick Chevarier (Clininfo), Professor Chris Goss (UW), Prof Isabelle Fajac (AP-HP), Dr Robert Ryan (UoD), Dr Cliff Taggart (QUB), Dr Rebecca Ingram (QUB) and Professor Catherine Stanton (Teagasc) . The role of the TSC is to provide overall supervision of the trial and to ensure that the trial is conducted to rigorous standards.

3.1.1.3 Scientific Advisory Board (SAB)

The Scientific Advisory Board (SAB) has been appointed for the CFMATTERS study. The SAB is chaired by Pete Greenberg who is the Director of the Cystic Fibrosis Foundation-supported University of Washington CF Research and Development Center and Co-Director of the NIDDK-supported Cystic Fibrosis Core Center. The other panel members include Professor Kris De Boeck (UZ Leven, European CF President elect,), Dr Dominik Hartl (University of Tuebingen, Germany) and Professor Ute Römling (Karolinska Institutet, Sweden) who all have significant translational research experience in the area of CF. The SAB will be invited to attend General Assembly meetings and will consult the consortium and make recommendations regarding perspective data.
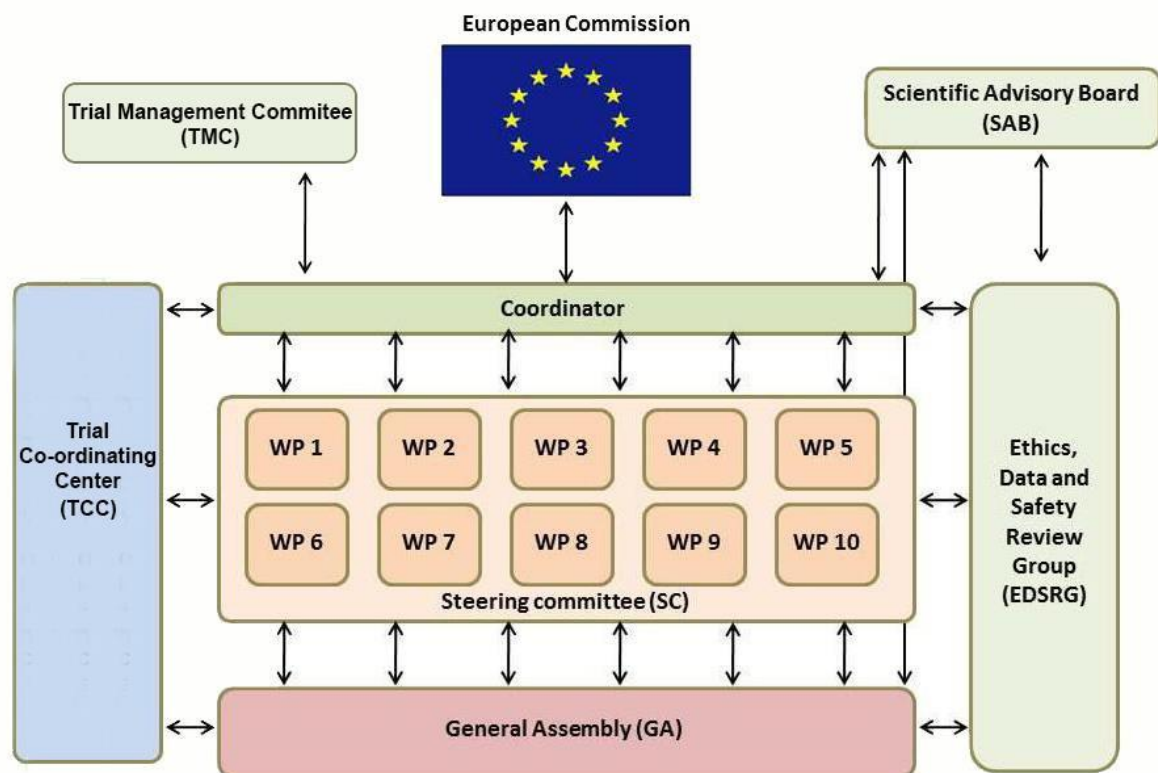
Figure 1: Governance structure in CFMATTERS

3.1.2 Task 2: Establish Trial Coordination Centre (TCC)

The Trial Coordinating Centre (TCC) has made a number of appointments to oversee the management of the CFMATTERS study at University College Cork. In addition to the co-ordinator Professor Barry Plant, an overview of the trial management staff is summarised below Table 1. Figure 2 shows TCC activity

Table 1: TCC Management Structure

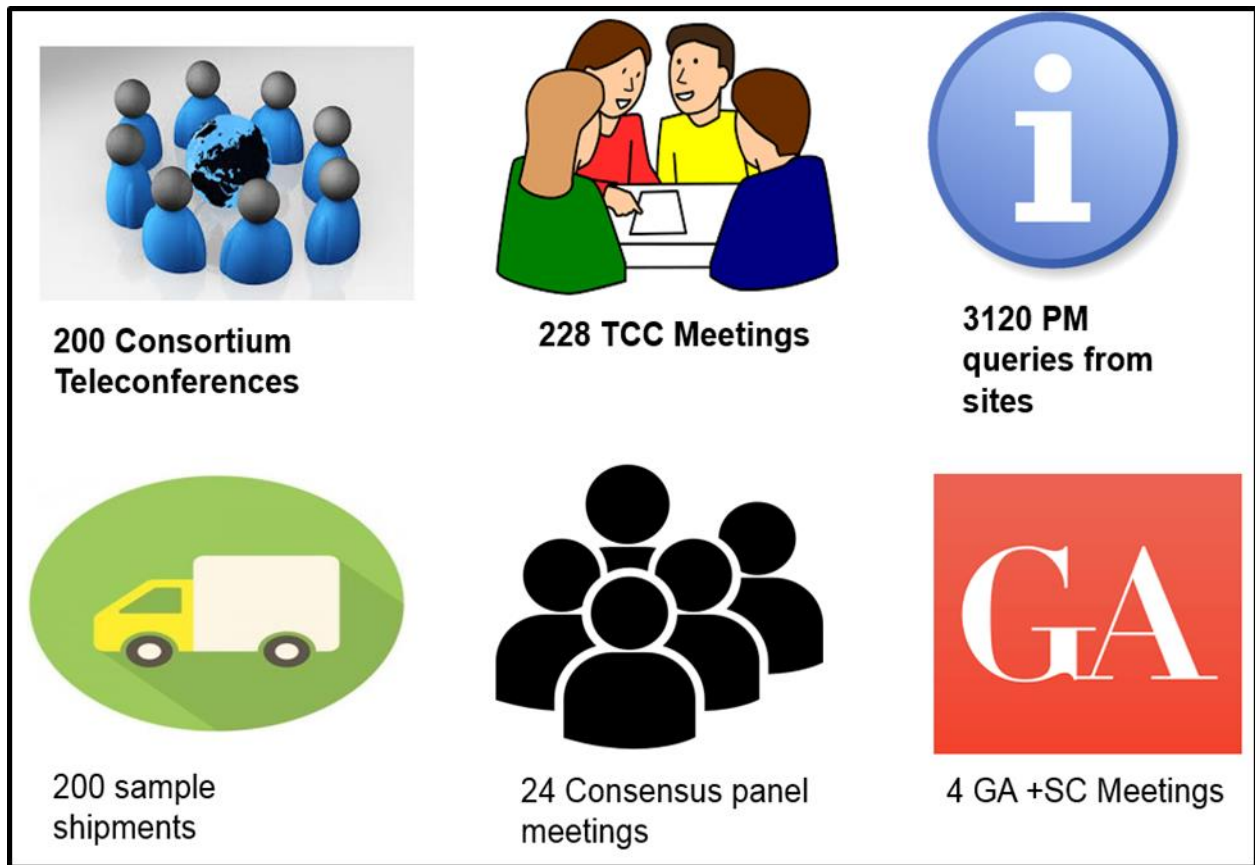| Title	Personnel
Project Manager	Dr Evelyn Flanagan
Research Nurse Manager	Mary Daly CNS
Clinical Fellow	Dr Nicola Ronan/ Dr Parniya Arooj
Data Manager	Dr Evelyn Flanagan
Quality Affairs Manager and Study Monitor	Dr Ruben Keane and Maire McCarthy
Clinical Research Facility Director	Professor Joe Eustace
Scientist	Yvonne McCarthy

Figure 2: TCC activity

3.1.3 Task 3: Develop Trial Initiation Plan and Oversee Site Training

The staff of the Trial Coordinating Centre oversaw the development and implementation of a study initiation plan and site training ensuring each site had the necessary resources, personnel and training necessary to carry out the study.

3.1.4 Task 4: Develop and Conduct Trial Monitoring Plan

Effective monitoring of clinical investigations is critical to the protection of human subjects and the conduct of high- quality studies. The CFMATTERS Study monitoring plan was developed by the CRF-Recent guidance documents from the U.S FDA and from the EMEA reflect a growing trend moving from the exclusive use of on-site monitoring towards using a variety of risk based monitoring strategies in clinical trials and studies. With these developments in mind the Trial Coordinating Centre approach to study monitoring of CFMATTERS consists of a combination of on-site and centralised data monitoring, with the centralised data monitoring concentrating on key risk indicators. In addition to a remote site initiation visit, each site will have a minimum of one onsite monitoring visit carried out by a member of the study co-ordinating team.

3.1.5 Task 5: Develop Treatment Algorithm

The CFMATTERS study is based upon the treatment of a CF patient exacerbation with either standard treatment or Microbiome determined treatment.

Standard therapy was determined by consensus as Ceftazidime in combination with Tobramycin at dosages as per table below:

Drug Name	Treatment dosage	Treatment Regimen	Treatment preferred route PO or IV	Treatment Duration
Ceftazidime	3g	Tds	IV	2 weeks
Tobramycin	5-10mg/kg	adjust according to site	IV	2 weeks

If a patient is intolerant or allergic to Ceftazidime,an alternate antibiotic Aztreonam (determined by consensus) in combination with tobramycin will be used at dosages as per table below:

Drug Name	Treatment dosage	Treatment Regimen	Treatment preferred route PO or IV	Treatment Duration
Aztreonam	2g	Tds	IV	2 weeks
Tobramycin	5-10mg/kg	adjust according to site	IV	2 weeks

Patients who are randomised to the Microbiome treatment arm of the study will receive the standard therapy as outlined above and an additional antibiotic based on the Microbiome analysis of their sputum sample.To select the third antibiotic the Consensus Expert Treatment Panel will review the Microbiome results and the subjects' reported allergies and other relevant clinical details. The panel considers the antibiotic which (i) optimally covers the 2nd, 3rd and 4th most abundant genera and (ii) which optimizes convenience of administration, cost and best combination (oral and/or intravenous) up to a maximum of 3 selected antibiotics.

In addition, to aid the selection of the Microbiome therapy, sample treatment vignettes (17 in total) and guiding principles for the selection of antibiotic based on the genus found in the sputum (below) were circulated to the CFMATTERS consortium.

Guiding principles for Microbiome Based Therapy Post Consensus review

	1st line Antibiotic	2nd Line Antibiotic	Rationale
Staphylococcus	Doxycycline	Linezolid	
Haemophilus influenzae:	Moxifloxacin	Doxycycline	Specificity
Pseudomonas aeruginosa	Ceftazadime /Aztreonam and Tobramycin.		This regimen will be initially considered as it has a focused spectrum of activity and will therefore have least impact on other members of the microbiome.
Streptococcus species	Moxifloxacin		
Stenotrophomonas maltophilia	Co-trimoxazole	Doxycycline	
Achromobacter xylosxidans	Tetracycline	Carbapenem. Co-trimoxazole	This organism is often pan-resistant and there is limited data on the best antibiotic for treatment. The most active agents are tetracycline, carbapenems, chloramphenicol and co-trimoxazole.
Burkholderia cepacia complex	Meropenem	Temocillin and Piperacillin/Tazobactam	This organism is also usually pan-resistant.
Prevotella and Veillonella spp.	Metronidazole	Meropenem, Co-Amoxiclav or Piperacillin/Tazobactam.	Primary data from QUB laboratory demonstrates both species in CF are generally sensitive to metronidazole.
Rothia.	Amoxicillin,	Doxycyline, Co-Amoxiclav	There is limited data available regarding the antimicrobial susceptibility of *Rothia* species. In general, they are susceptible to penicillin

Version 1: 09th March 2015

Figure 3: Guiding Principles for Microbiome Based Therapy Post Consensus review

3.1.5.1 Development of Statistical Analysis Plan

The statistical analysis plan of the CFMATTERS study is based on the final clinical trial protocol. The Statistical analysis plan was developed by Professor Chris Goss and Associate Professor Nicole Hamblett through an on-going collaboration of the Statistical Advisory Centre in Seattle, Washington (CF Therapeutics Development Coordinating Centre and the University of Washington, supported by the Trial Coordinating Centre, University College Cork and specifically the director of the HRB CRF-C Prof Joe Eustace. The SAP contains all modifications and updates to the planned analyses that were outlined in the original study protocol.

Final analysis was carried out on 106 patients randomized who had completed all visits outlined in the protocol. Table 1 shows a sample of the characteristics of the patient. The characteristics of the 106 randomised patients who had a 3-month visit are given in Table 3.

Table 2: Characteristics of randomised patients with a month 3 visit (n = 106).

	n	Control Arm Mean ± SD or n (%)	n	Active Arm Mean ± SD or n (%)	Test p-value
FEV % Predicted	61	48.8 ± 17.9	45	49.5 ± 16.8	0.73
Age	61	30.3 ± 9.2	45	31.7 ± 9.5	0.31
Sex	61		45		0.05
Males		36 (59%)		17 (37.8%)	
Females		25 (41%)		28 (62.2%)	
Height	61	61.6 ± 11.1	45	59.2 ± 12.1	0.27
Weight	61	169 ± 8.6	45	166.6 ± 10.4	0.1
BMI	61	21.5 ± 2.8	45	21.2 ± 3	0.33

Table 3: Characteristics of total number of patients randomised

	n	Control Arm Mean ± SD or n (%)	n	Active Arm Mean ± SD or n (%)	Test p-value
FEV % Predicted	110	52.1 ± 18.5	112	52 ± 18.9	0.94
Age	110	31.1 ± 9.1	112	31.4 ± 10.7	0.77
Sex	110		112		0.23
Males		60 (54.5%)		51 (45.5%)	
Females		50 (45.5%)		61 (54.5%)	
Height	110	61.4 ± 11.3	112	60.8 ± 12.6	0.55
Weight	110	168 ± 9.4	112	168.1 ± 9.4	0.74
BMI	110	21.6 ± 2.7	112	21.5 ± 3.7	0.17

3.1.5.2 Primary Outcome

The primary outcome of the clinical trial was the percentage change in recovery (post-exacerbation) FEV1 relative to the previous pre-exacerbation FEV1.There was no difference in the distribution of FEV1% predicted across study arms, at any time point. The FEV1 % predicted trajectory is given for each patient in figure 4. There is a clear dip at day 0, after which levels tend to return to those seen at baseline. Figure 5 shows the degree of overlap in the distributions of FEV1 % predicted at each time point, which is considerable. The mean FEV1 % predicted values are given for each arm, across all time points, in table 2 and figure 6. Table 3 also includes the estimated treatment effect of the active arm. These were derived from a linear regression of FEV1 % predicted on treatment arm, further adjusted for baseline FEV1 % predicted and centre. Log transformation of FEV1 % predicted did not appreciably alter model results (not shown).
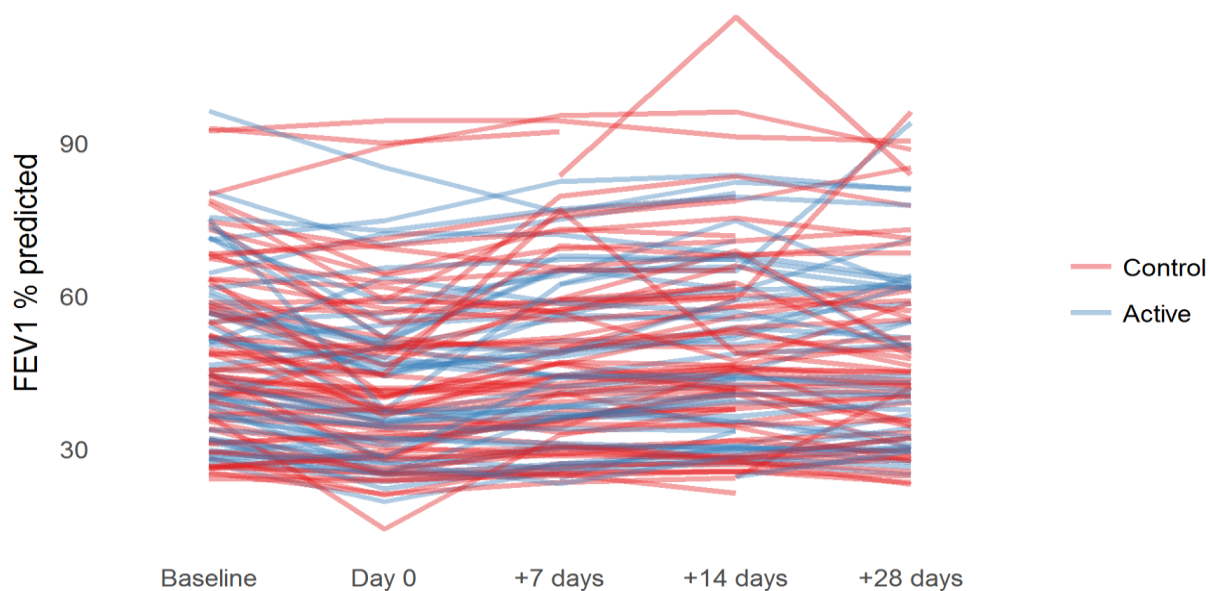
Figure 4: Distribution of FEV1% predicted in the control vs. active arm at each time point
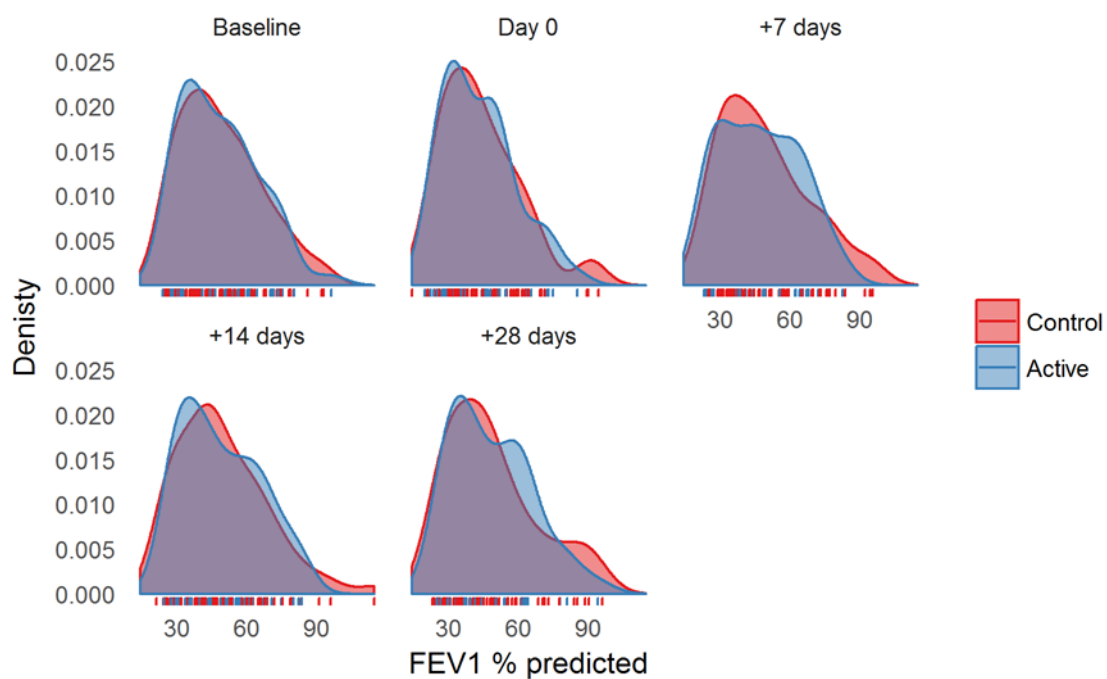

Figure 5: Distribution of FEV1% predicted in the control vs. active arm at each time point

Table 4: FEV1% predicted in the control vs. active arm at each time point

Visit	Arm	Mean (95% CI)	Effect (95%CI)*	P value*
Baseline	Control	48.89 (44.87 to 52.9)		
	Active	48.38 (44.15 to 52.61)		
Day 0	Control	43.81 (39.84 to 47.78)		
	Active	43.18 (39.18 to 47.19)		
+7 days	Control	49.09 (44.49 to 53.69)		
	Active	47.17 (42.58 to 51.76)	-2.43 (-5.46 to 0.61)	0.12
+14 days	Control	49.35 (44.85 to 53.85)		
	Active	48.95 (44.46 to 53.44)	-1.34 (-4.06 to 1.39)	0.34
+28 days	Control	48.69 (43.78 to 53.59)		
	Active	48.56 (43.79 to 53.32)	-0.36 (-4.25 to 3.54)	0.86
3 months	Control	47.44 (43.06 to 51.82)		
	Active	48.38 (43.22 to 53.55)	0.38 (-2.02 to 2.78)	0.76

* The effect of the active arm was estimated using linear regression, with adjustment for baseline FEV1% predicted and centre.
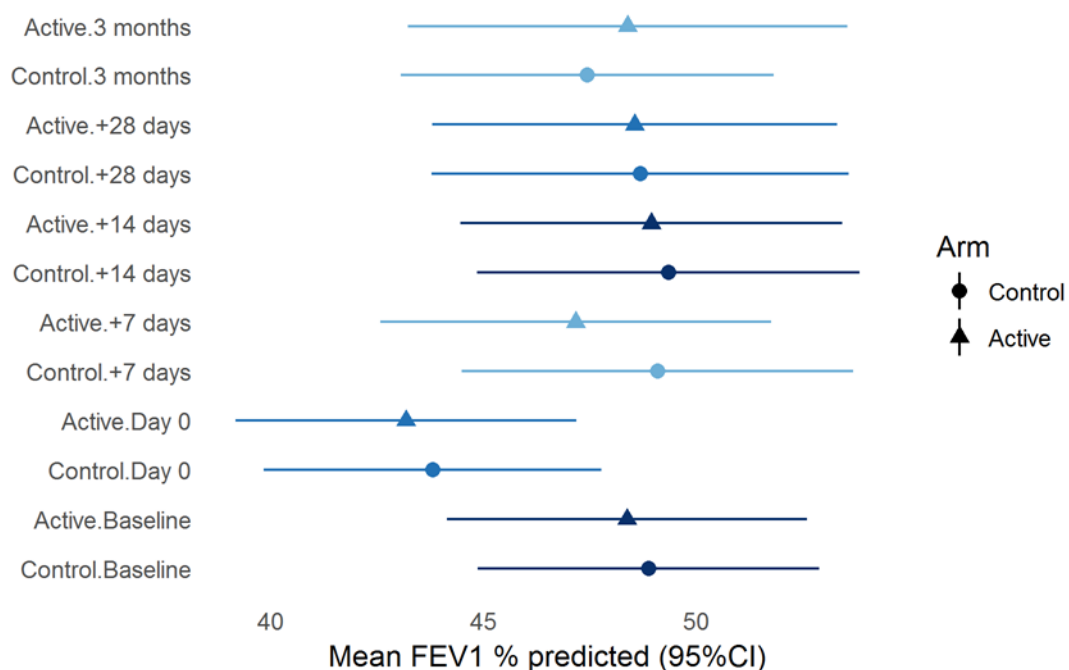

Figure 6: Mean FEV1% predicted in the control vs. active arm at each time point

CFMATTERS also looked at a number of secondary outcomes the results of which are examined below.

3.1.5.3   Time to second exacerbation

There was no appreciable difference between the control and active arms with respect to the time it took for patients to experience a second exacerbation, post treatment. The median times to second exacerbation were 29.1 weeks (95% CI 24.3 to 41.4) and 25.6 (19 to 35.4) respectively. The non-parametric survival curves for each arm are given in Figure 7. The effect of the active arm was estimated using the Cox proportional hazards survival model, stratified for centre. The resulting hazard ratio comparing active to control was 1.27 (95% CI 0.74 to 2.19). This means that the instantaneous risk of experiencing a second exacerbation at any given time point was slightly higher in the active arm, but this estimate was highly variable, and consistent with a null hypothesis of no difference between arms.
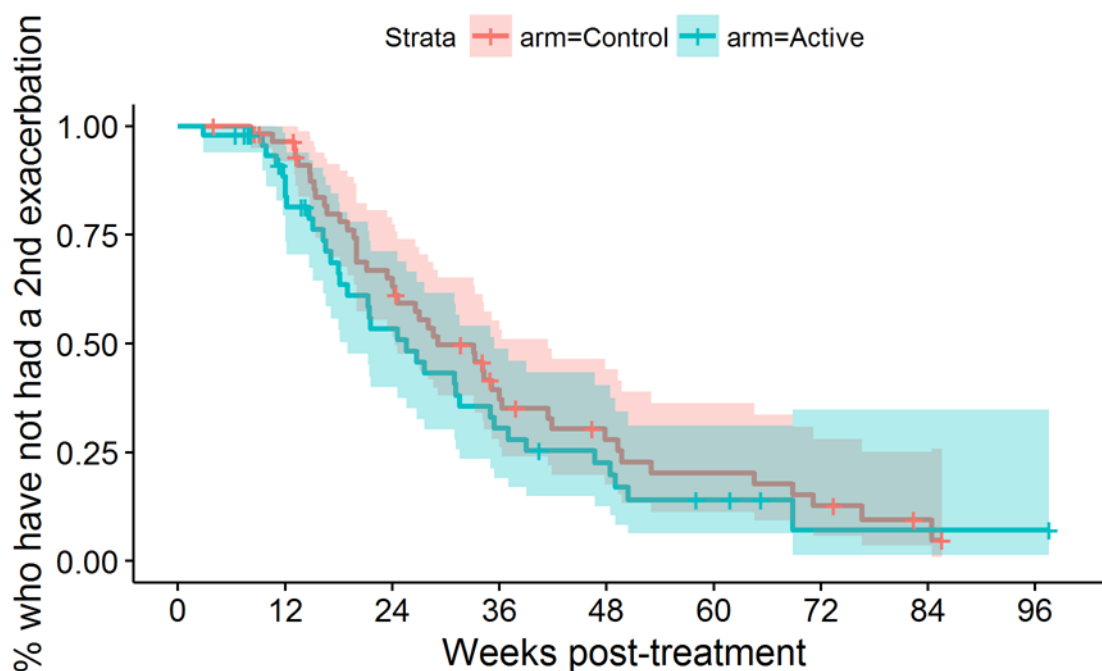

Figure 7: Control vs. active survival curves (and 95% CIs) for time to second exacerbation, post treatment.

3.1.5.4   Number of exacerbations

There was no difference between study arms in the number of post-treatment exacerbations, up to one year of follow-up. The median number of exacerbations was 3 in the active arm, vs. 2 in the control arm (the means were 3 and 2.4, respectively). The two-sided p-value from the Wilcox rank-sum test was 0.08, indicating that the overlap in distributions (Figure 8) was consistent with the null hypothesis of no difference.
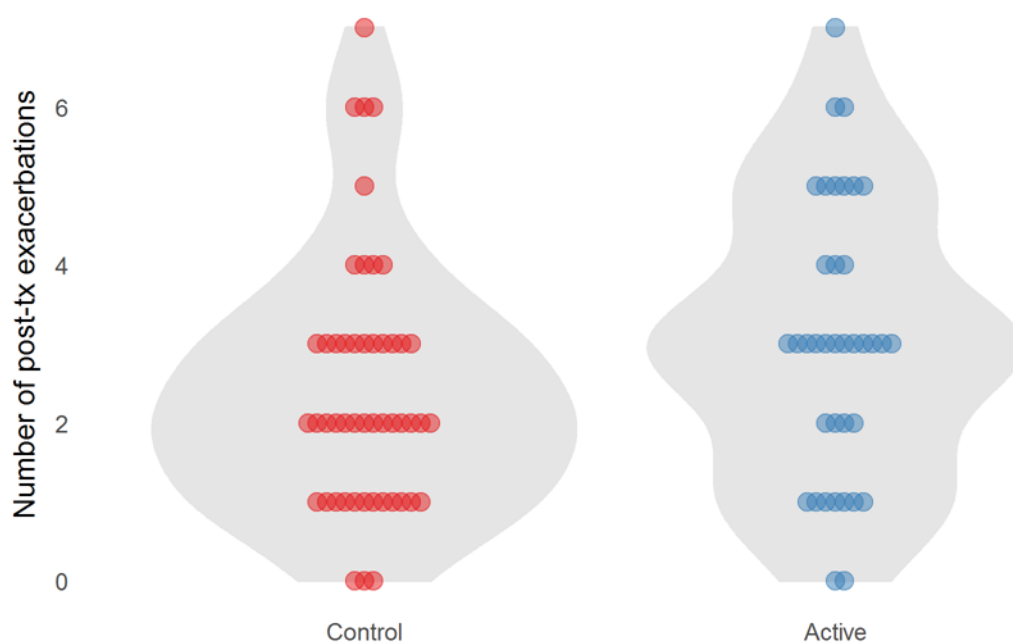
Figure 8: Number of post-treatment exacerbations

3.1.5.5 Number of IV days

Patients enrolled in the active arm tended to experience more days of IV treatment, up to one year of follow-up, than those in the control arm (Figure 9). The median number of IV days was 42 in the active arm, vs. 28 in the control arm (the means were 43.9 and 34.6, respectively). The two-sided p-value from the Wilcox rank-sum test was 0.05, indicating limited evidence that the data were inconsistent with a null hypothesis of no difference between arms.
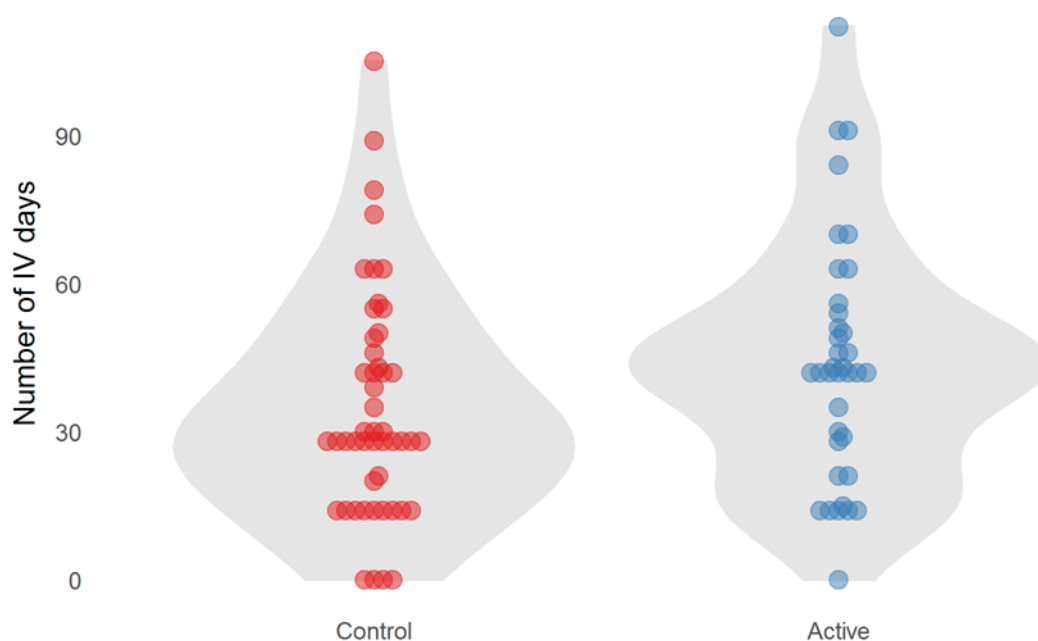

Figure 9: Number of post-treatment days of IV use

3.1.5.6 Quality of life

Differences between arms in CFQ-R component scores at +28 days were estimated using linear regression, with additional adjustment for centre and baseline FEV1 % predicted. The effect estimates for each model are given in figure 10. Among the 12 component items included in the CFQ-R, only the Health score was different between study arms, indicating that the mean score in the active arm was 10 units lower than in the control arm (95% CI -19.0 to -1.5; p = 0.02). However, it is worth noting that any reasonable adjustment for multiple comparisons would render the result non-significant. Individual level component scores and their distributions across all time points are given in Figure 11 and 12 and 14.
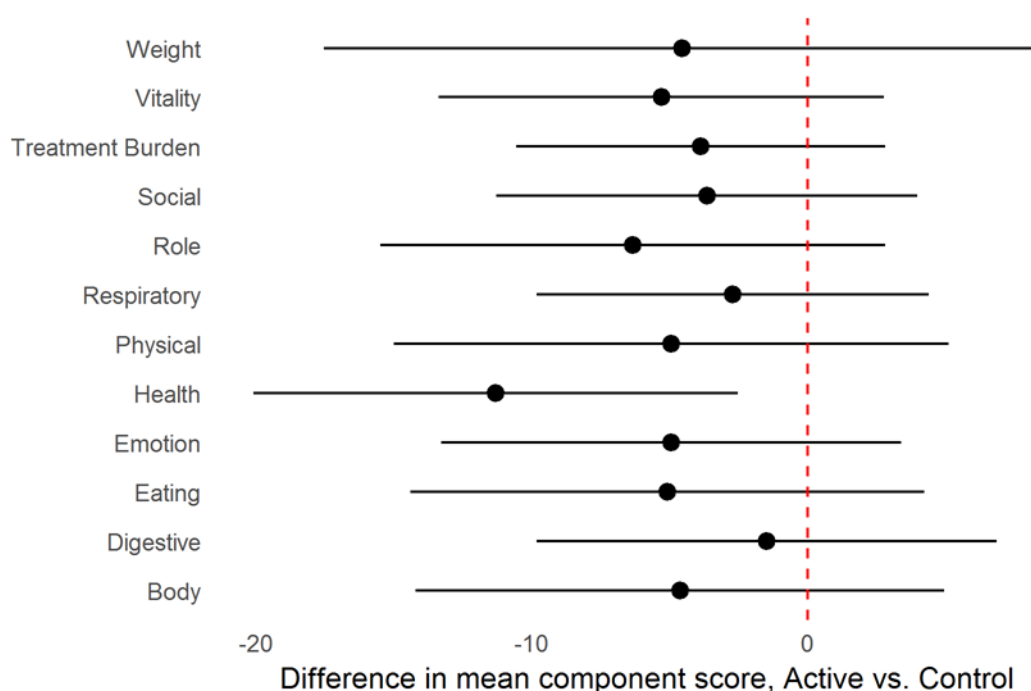

Figure 10: Active vs control differences in mean CFQ-R component scores at +28 days, adjusted for centre and baseline FEV1 % predicted
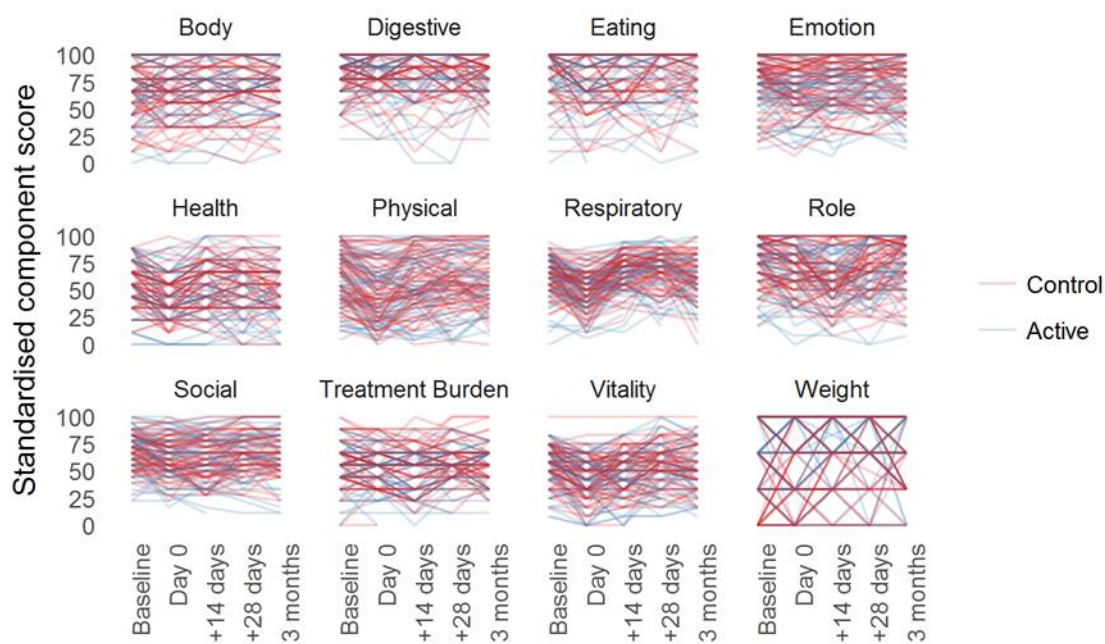
Figure 11: Individual level CFQ-R component scores across all time points
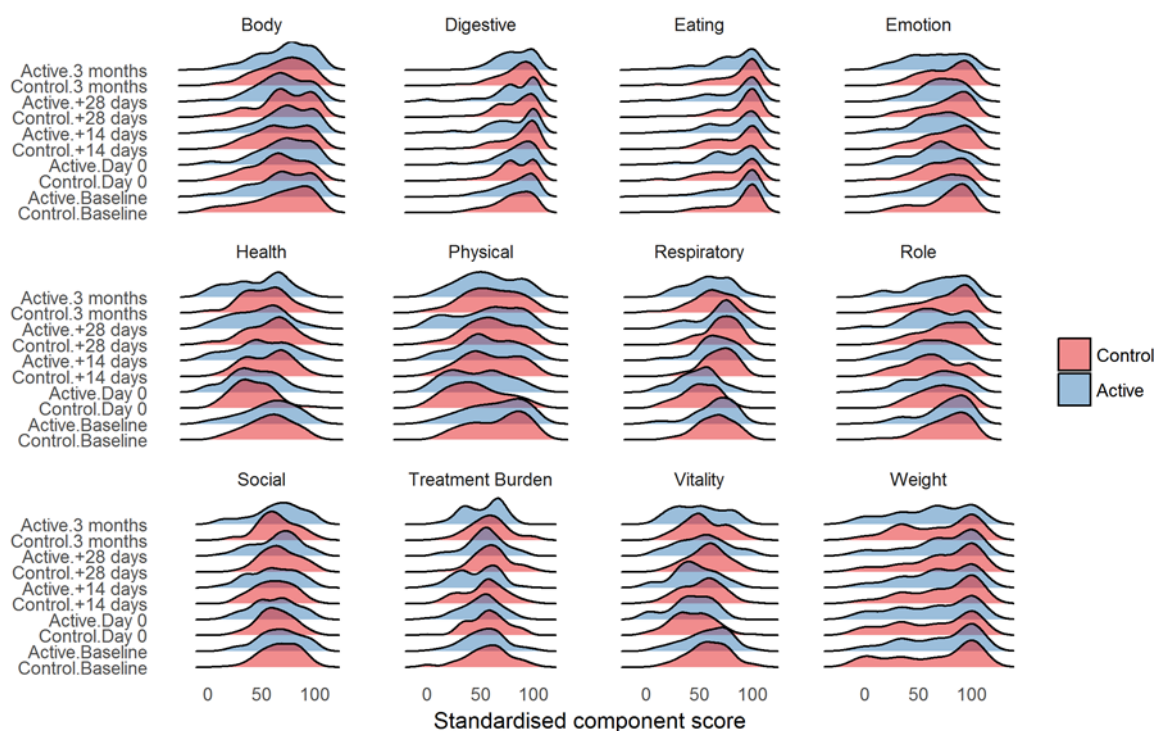

Figure 12: Distributions of CFQ-R component scores across all time points
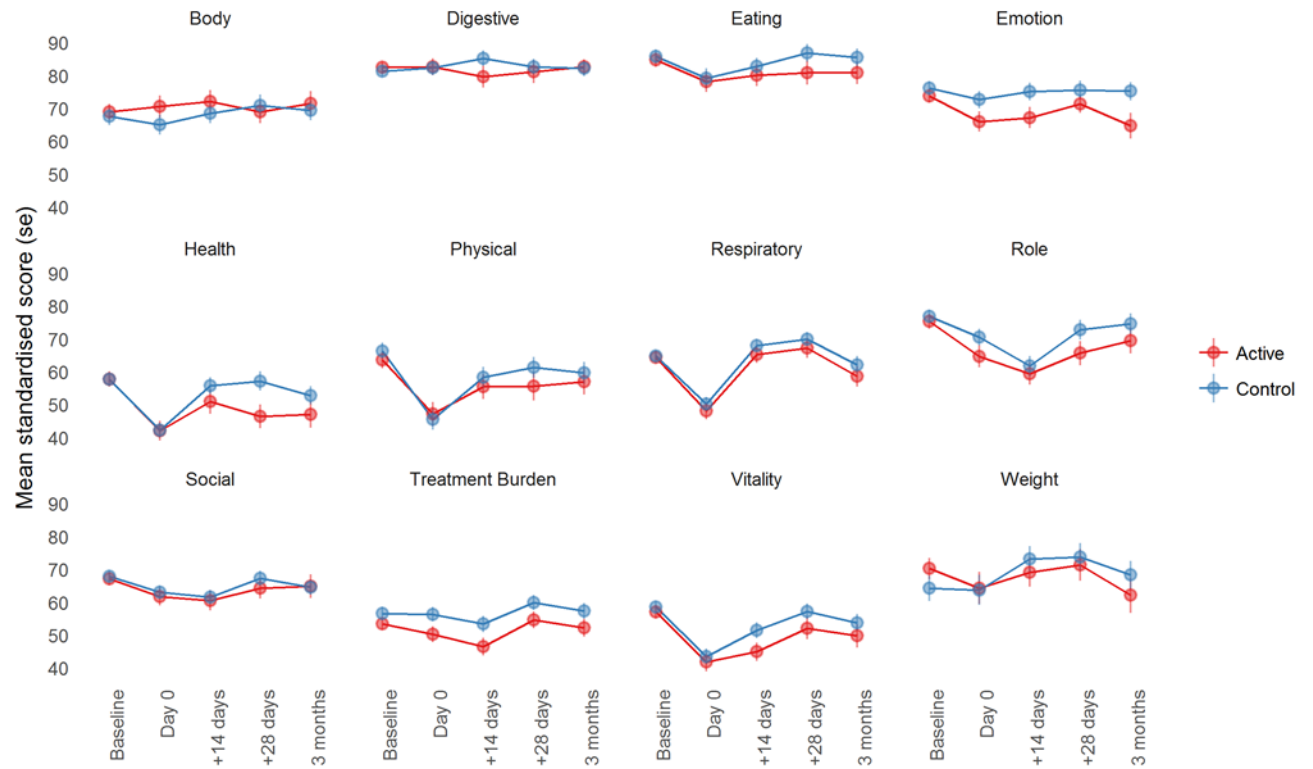
Figure 13: Distributions of CFQ-R component scores across all time points control vs active

3.2 WP02: Data Management

The main aim of WP2 is to establish the data input interface and data coordination mechanisms

- To develop validated (GAMP5) internet based online dedicated portal for trial data entry and randomisation procedures (eCRF).
- To ensure the traceability, safety and quality of all data entered.
- To monitor data quality throughout the entire project.
- To ensure specimen shipping tracking and centralisation.

3.2.1 Task 1: eCRF specification and data-management plan writing and acknowledgement

The electronic case report from (eCRF) has been specified in accordance with the current protocol. Specifications were used as input for the validation process

3.2.2 Task 2: To set up and develop a secure internet based online data management system

The eCRF is completed and live. The software has been validated using Gamp5 referential. It is compliant with FDA 21 CFR Part 11 and European directives 65/65EEC, 75/318/EEC and 2001/20/CE.

Several modules manage the data and the website:

- A dynamic electronic case report form (eCRF) application, for online controlled data entry by physician. This module includes various access rights management: read-only (including individual data); restricted to report and aggregated data; create or modify and monitor.
- A Data Manager level (residual error, access rights management, quality reports)
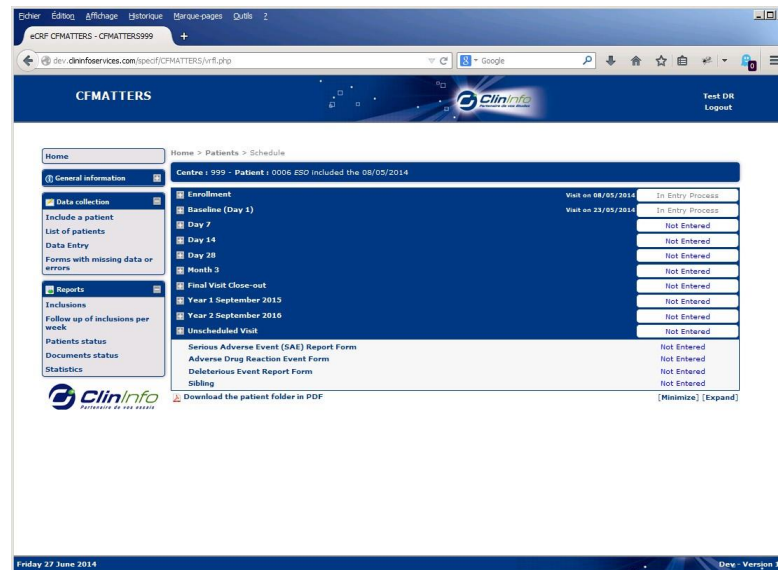- A Flexible Export Application

Figure 14: Patient menu for investigator

3.2.3   Task 3: User acceptance test of the eCRF

User acceptance testing was carried out by the UCC CRF-C data management department and the TCC. A test site was also made available for all sites to review.

3.2.4   Task 4: Randomization integrated in the eCRF

Randomization has been integrated into the ClinInfo web portal in accordance with the minimization algorithm outlined in the protocol. The randomization algorithm was generated by UW, integrated into the eCRF by CSA ClinInfo and reviewed and tested by the UCC CRF-C data management department.

3.2.5   Task 5: Data Blind review

The Trial Coordinating Centre are examining the blinded deleterious events, overall blinded event rate and blinded pulmonary exacerbation rate in real time for the CFMATTERS study.

3.2.6   Task 6: Run Requested data reports

A reporting platform has been developed by Clininfo and validated by the UCC- CRF-C data management department. The platform allows data to be extracted in SAS and CSV format. The platform also allows for field/site specific extraction. This allows for custom reporting to be carried out.

3.2.7   Task 7: Data management

A number of data management tasks are being carried out by the UCC CRF-C data management department and ClinInfo for the life cycle of the study.

A web based data management system, eCRF platform and the associated tools have been delivered. All user acceptance testing have been carried out by UCC. Patient data is being entered into the system. A comprehensive data management plan is also in place. The system will be maintained for the CFMATTERS rollover study.

3.3 WP03: Conduction of Clinical Trial

The main objective of WP03 was to conduct a randomized trial quantifying the benefits of treating pulmonary exacerbations in CF with stratified Therapy as compared to Empiric Therapy.

- Perform a randomized trial according to agreed protocol, GCP and highest ethical standards.
- Consent, enrol and randomize 252 eligible stable CF patients and follow them for up to 2 years or until three months before closeout of study.
- Obtain and transport serial sputum specimens to Central Laboratory for microbiome analysis
- Determine an antibiotic treatment regimen based on microbiome analysis in Tailored Therapy Arm
- Rigorously quantify the benefits of microbiome-guided Tailored Therapy versus routine Empiric Treatment in the treatment of routine pulmonary exacerbations.
- Reconcile all data queries from Data Management.
- Complete scientific reports and manuscript preparation.

3.3.1 Task 1: Potential subject identification, enrolment and randomization

The CFMATTERS study involved enrolment of 227 consenting subjects, who meet entry criteria in accordance with the protocol (including age >16 years, known CF with chronic pulmonary Pseudomonas colonization, chronic sputum production). Recruitment into the study ended the 31st of May 2017. Computer based randomization was performed (stratified by site and by FEV1) with a variable block size. Potential CFMATTERS study subjects are screened by their supervising clinician and their permission sought for initiating the consent process. Subjects are randomized on a 1:1 ratio to have their next subsequent eligible Pulmonary exacerbation treated by either empiric or Microbiome-determined therapy as per study protocol. Figure 15 shows current recruitment and Figure 16 shows the number of patients who have had an eligible exacerbation. Figure 17 shows the amount of amount of clinical data and samples collected.
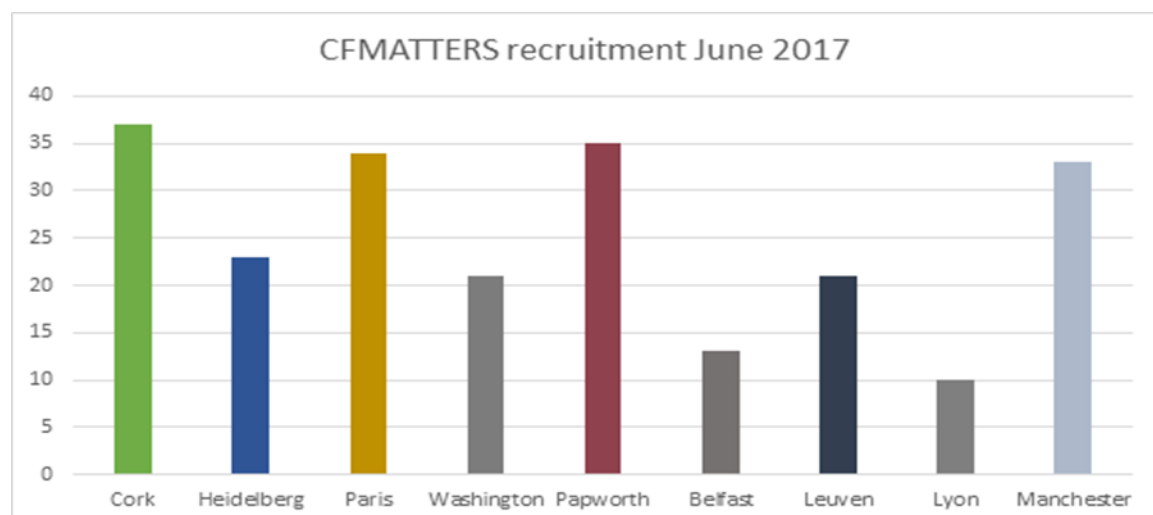

Figure 15: Current CFMATTERS trial recruitment according to clinical site
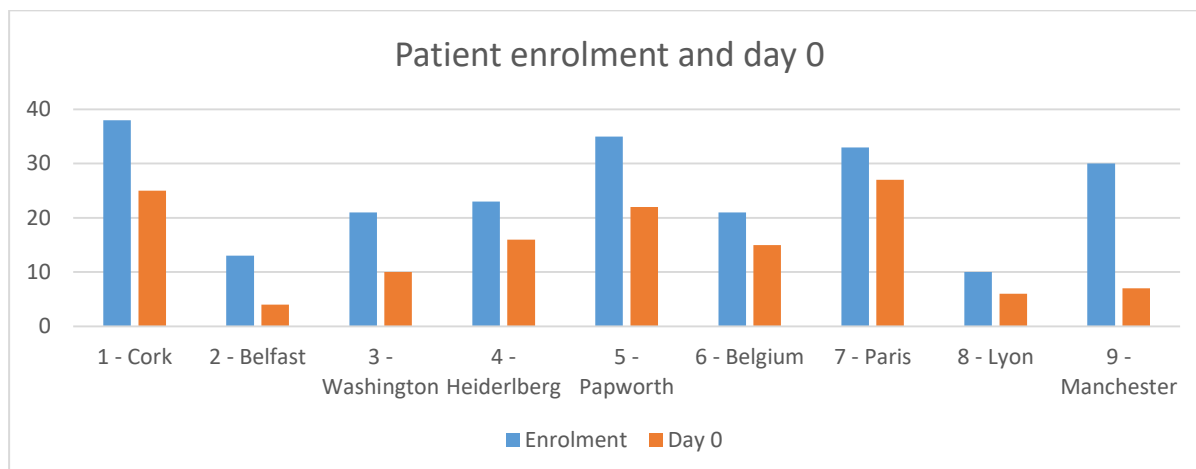
Figure 16: Number of patients who have had an eligible exacerbation (day 0) in each site
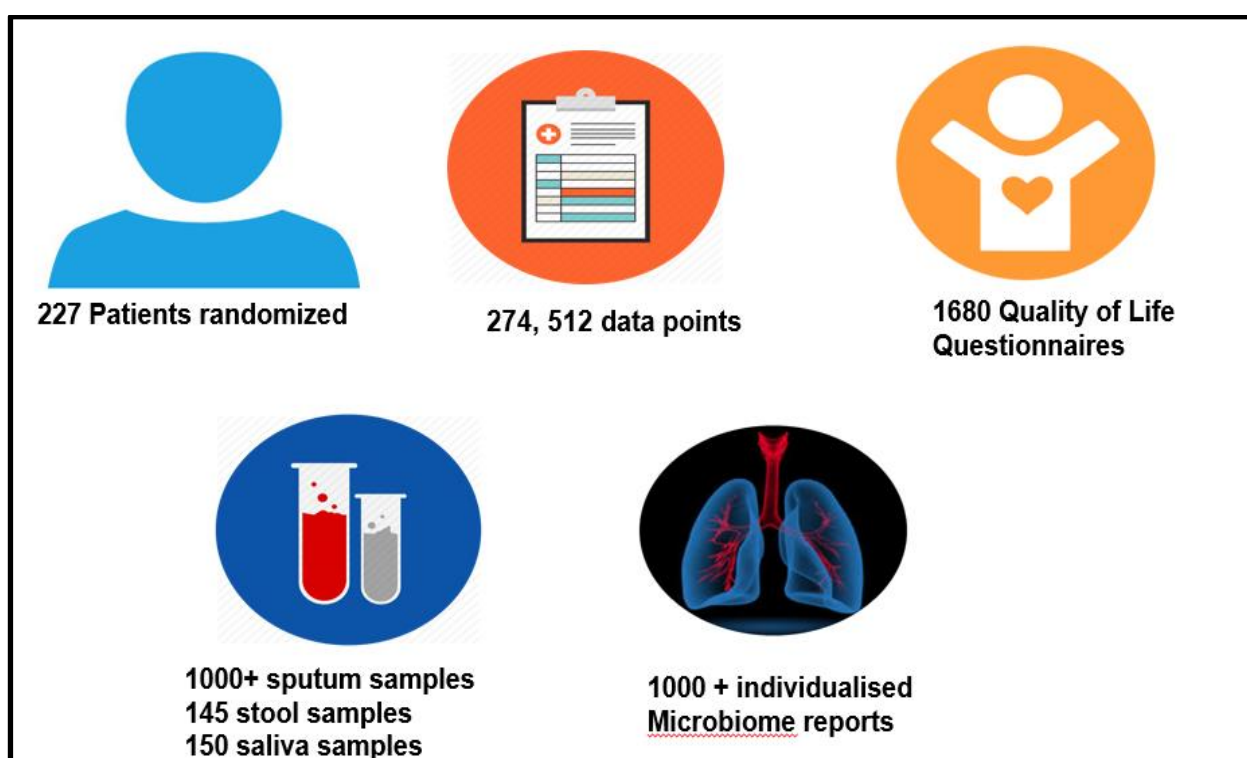

Figure 17: the amount of amount of clinical data and samples collected

3.3.2   Task 2: Participant Assessments

Once enrolled in the CFMATTERS study patients undergo an enrolment visit and are reviewed quarterly thereafter until an eligible exacerbation. During this visit clinical parameters are recorded as per the protocol (including standard spirometry, medical history, vital signs, symptom assessment using the novel Cystic Fibrosis Respiratory Symptom Diary (CFRSD)) and a sputum sample is collected. At four selected centres UCC, QUB, PAP and KU Leuven, serial blood, saliva and stool samples are being collected in accordance with the protocol. Sputum specimens are snap frozen and shipped to the CFMATTERS partner site at QUB fortnightly where the Microbiome analysis is carried out. Stool and blood samples are also snap frozen and sent to the TCC site at UCC and Teagasc for analysis in accordance with Work

package 8. CFMATTERS study participants are actively followed until 3 months after initiation of trial directed antibiotics (or 1 month if treatment initiated in month 33) and passively for up to 2 years post exacerbation by yearly medical note review. Figure 18 shows overview of study design and visits. Table 5 shows patients progress to date.
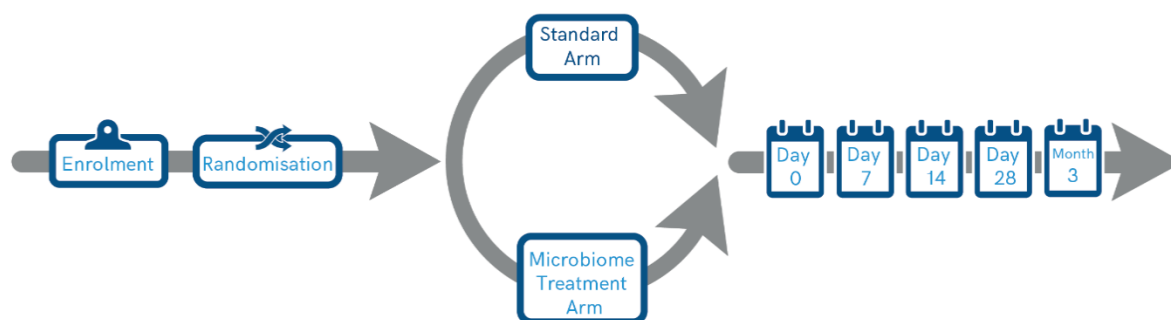

Figure 18: Schematic showing an overview of the study design and study visits.

Table 5: Patient progress June 2017

Patient Progress Update						
Sites	**Enrolment**	**Day 0**	**Day 7**	**Day 14**	**Day 28**	**Month 3**
Cork	37	25	23	24	23	24
Belfast	13	4	3	3	2	3
Washington	21	8	8	7	7	6
Heidelberg	23	16	16	16	16	16
Papworth	35	22	22	21	20	22
Belgium	21	15	5	13	10	10
Paris	34	27	27	26	27	26
Lyon	10	4	4	2	2	0
Manchester	33	3	1	0	0	0

3.3.3   Task 3: Review of Microbiome Sampling Frequency

The preferred sputum sampling frequency (currently 3 monthly) was assessed based on serial sputa samples in patients with quarterly sputa or sputum samples collected from patients post an non-eligible exacerbation (where repeat sampling is performed one month post this non-eligible exacerbation) in accordance with the protocol. It was decided to continue this frequency based on microbiome analysis.

3.3.4 Task 4: Assessment of Frozen Specimen Stability

This specifically relates to sputum samples in this study. This was carried out by WP08. Repeat microbiome analysis of stored sputum samples will allow assessment of stability of sputum microbiome overtime following storage at -80°C. Complete paper recently published with results. See WP08.

3.3.5 Task 5: Microbiome Analysis

All CFMATTERS trial participants randomised to the tailored therapy arm and 5% of those in empiric therapy arm will have the Microbiome of their sputum analysed following shipping to the Central Laboratory at the CFMATTERS partner site at QUB. The CFMATTERS Consensus Expert Treatment Panel is made up of three independent panels which rotate every six weeks and consist of a minimum of two clinical PIs from across the nine CFMATTERS clinical study sites at each meeting. This panel is supported at all meetings by the Trial Coordinator Professor Barry Plant (Clinical Physician), a Trial coordinating centre representative, and a representative from the laboratory in QUB performing the Microbiome analysis. To avoid bias where possible every attempt is made to ensure that the clinicians who participate in the consensus call are not discussing samples from their own clinical site. The consensus panel have reached agreement on all Microbiome determined antibiotic recommendation on all samples to date. The CFMATTERS clinical PI's decide to recommend a Microbiome determined treatment of an optimal antibiotic combination based on the ammonized Microbiome results, the subject's allergies and other relevant clinical details, selecting an antibiotic combination that treats the 2nd, 3rd and 4th most abundant Microbiome isolates, consideration of any additional organisms present >1% abundance, and also with respect to the antibiotic regimen convenience in terms of administration and cost. The guiding principles for the recommendations of the antibiotics and the reporting mechanism are reported in WP01.

3.3.6 Task 6: Identification and treatment of eligible pulmonary exacerbation

At all clinic or hospital contacts the participants will be assessed for a pulmonary exacerbation in accordance with the CFMATTERS study protocol. If identified, the patient's eligibility for study based assignment of therapy will be assessed and will be deemed ineligible if the attending physician believes subjects is too ill or if the pulmonary exacerbation criteria as per the protocol are not met. If deemed eligible, the patients recommended therapy is obtained from the eCRF and cross checked against subjects' known allergies. THE CFMATTERS study treatment arms are described in WP01. Both empiric and Microbiome determined Tailored Therapy recommendations may be modified at any time in accordance with clinical judgement of the treating physician. All other adjuvant therapies and duration of hospital stays will be as per local routine practise as agreed by the Trial Management Committee. Prospective sampling is summarised in Table 6 below:

*Table 6: Schedule of Study Visits (*Patient Stool will be collected in Cork, Leuven, Cambridge and Belfast. Sibling Stool will be collected in Cork, Leuven, Cambridge and Belfast at one study time point. Blood will be collected from Belfast and Cork and saliva will be collected in Cork only*

	Enrolment	Day 0	Day 7	Day 14 (+/-3 days)	Day 28 (+/- 7 days)	3 Months (+/- 14 days)
Informed Consent	X					
Vital Signs	X	X	X	X	X	X
Randomization of Treatment	X					
Review of Medication	X	X	X	X	X	X
Allergy History	X					
PE Worksheet		X				
CFQ-R	X	X		X	X	X
CFRSD	X	X	X	X	X	X
CFRSD (In-patient only)		Every day, Day 0 - Day 14				
Sputum Collection	X	X		X	X	X
Blood Collection*	X	X				X
Stool Collection *	X	X				X
Sibling Stool Collection*	X	X				
Saliva Collection *	X	X				X
Adverse Reaction Assessment			X	X	X	X
Standard Spirometry	X	X	X	X	X	X
Abbreviated Physical Exam		X	X	X	X	X

Ineligible pulmonary exacerbations will be treated in accordance with routine clinical practise, and re-assessed one month after completing of IV antibiotics in keeping with the CFMATTERS protocol.

3.3.7   Task 7: Acceptability of algorithm guided therapy

CFMATTERS has shown that it is feasible to develop an algorithm to guide therapy in CF based on consensus and vignettes. All patients enrolled now have a microbiome report and have been through a consensus panel. Figure 19 shows that more than 90% of the recommendations were adhered to across the nine clinical sites.
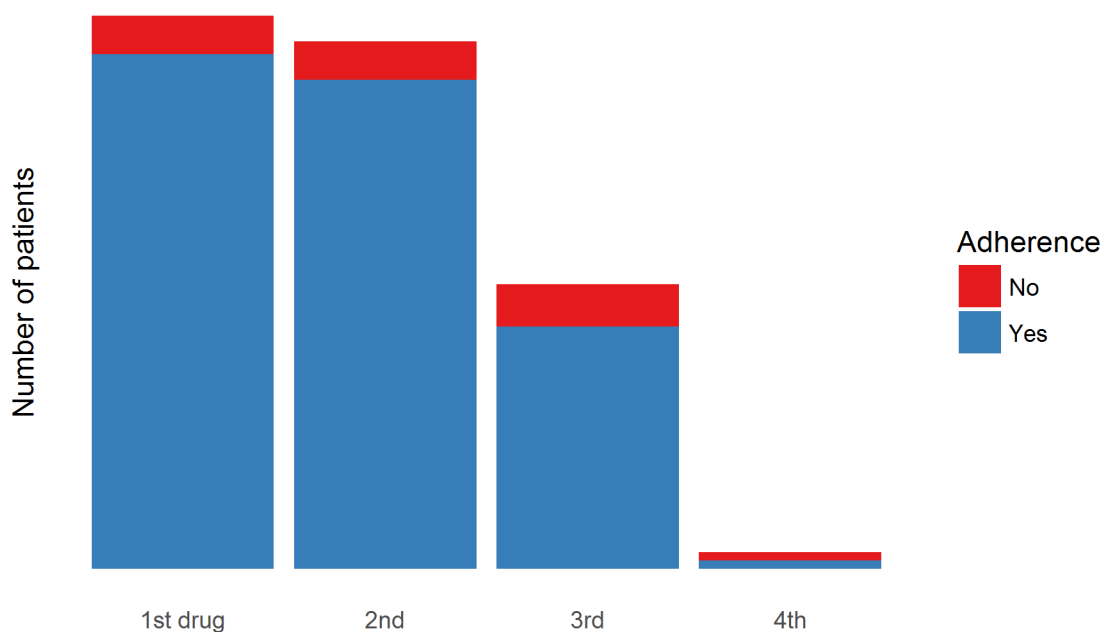
Figure 19: More than 90% of recommendations were adhered to.

3.3.8   Task 8: Data Cleaning Data clarification forms

The data query process is carried out via the eCRF. All queries are generated, tracked and resolved through the eCRF (data management /monitoring platform). Data cleaning was completed before final analysis.

3.4 WP 04: Ethical, data and safety monitoring

The overall aim of WP04 is to ensure the protection of participants rights and welfare
- To set up an Ethics, Data and Safety Review Group (EDSRG).
- Ensure that the study is performed according to highest ethical standards and to secure patients safety in accordance with national and international guidelines and regulations as outlined in Chapter 4 of Annex 1.
- Approve informed consent/assent procedures
- Approve study monitoring plan, review all monitoring reports
- Forward any actionable items to Trial Coordinating Centre, SAB and if necessary regulatory authorities
- Examine for any unexpected negative effect of study participation on subject outcomes

3.4.1 Task 1: Establish the Ethics, Data and Safety Review Group (EDSRG)

The Ethics, Data, Safety Review Group (EDSRG) for the CFMATTERS study was established in consultation with the Steering Committee. EDSRG Board members are Ulrike Pypops (CF patient and Attorney at law, Belgium), Dr Ed Mc Kone (CF Clinician and Researcher with Clinical trial experience, Ireland) and Professor Scott Bell (CF Clinician and Researcher with Clinical trial experience, Australia). As agreed by the Project Coordinator Professor Barry Plant and in agreement with the Steering Committee, Professor Scott Bell was appointed the Chair of the EDSRG Committee. All EDSRG members were sent a charter governing their membership of the EDSRG board

3.4.2 Task 2: Approval of Informed Consent/Assent Procedures and of initial ethics submission

All CFMATTERS sites received ethical approval before recruitment began. All ethical submissions were reviewed locally by the Ethical Committee as per local practice. Subsequent amendments were also approved by the local ethics committees.

3.4.3 Task 3: Review of Trial Monitoring

A Trial monitoring plan has also been created by the Quality and regulatory affairs manager (Dr Ruben Keane) and approved by the Trial Coordinating Centre. All site initiations and on site monitoring has been carried out across all nine clinical sites. Monitoring reports have been sent to the EDSRG and site sponsor.

3.4.4 Task 4: Review of Participant Outcomes

Each week at the CFMATTERS study meeting, all central data monitoring metrics are reviewed. Weekly metrics reports are sent to the consortium updating them on patient recruitment and patient progress. These reports also form the basis for the reports generated for the EDSRG review of the study.

3.4.5 Task 5 Review of Adverse Events

For the CFMATTERS Study a standard operating procedure for the reporting of a deleterious event, adverse event and serious adverse event has been generated and approved by the Trial Management Committee and circulated to the CFMATTERS consortium. All adverse event reporting is captured in the eCRF. All events are reviewed and the relationship between the

study participation/procedures and the adverse event assessed at the Trial Management weekly meeting. For all deleterious events and adverse events, an event report form is completed and if necessary the Trial Coordinating Centre can follow-up with the site and may recommend additional training or organise a monitoring visit.

All serious adverse events will be captured in the eCRF and an automatic email is sent to TCC staff including the Principal Investigator, an Independent clinician, Data Manager, Study Monitor, Project Manager and Project Coordinator. The serious adverse event is also reported to the CFMATTERS Consortium, the Ethical Safety Monitoring Committee, the local Ethics Committee and to the funding agency through the European Commissioner assigned to the CFMATTERS study. All CFMATTERS Partners will be advised in writing to contact their respective Ethical Review Boards. A written report on the serious adverse event will follow including a full description of the event and any sequelae.

3.5 WP05: Microbial activities and functions

Understanding the activities of microbial communities associated with CF patients with severe exacerbations may offer insights into the molecular basis of chronic infection and identify possible novel targets for therapeutic strategies to improve clinical outcome. The work described in this work-package aims to:

- Elucidate the transcriptional activities of microbial communities associated with patients with severe exacerbations who do or do not respond to empiric intravenous antibiotic treatment
- Identify the subset of functions that are involved in enhanced antibiotic tolerance by functional genomics
- Assess if metabolite profiling of sputum samples offers a biomarker for unresponsiveness to antibiotic therapy.

Overall, the identification of associations between microbial activities and patient treatment/outcome will provide a first step towards developing novel rapid diagnostic tools for the onset of exacerbation and/or informing treatment regimens for those sufferers with unresponsive infections.

3.5.1 Task 1: To determine if specific microbial determinants are expressed during periods of exacerbation that are unresponsive to antibiotic therapy using meta-transcriptomics

Not reported

3.5.2 Task 2: To examine if specific determinants of antibiotic tolerance are key conserved traits amongst P. aeruginosa isolates associated with chronic pulmonary infection

To examine phenotypic differences between P. aeruginosa isolates associated with chronic pulmonary infection, we subjected 12,000 isolates collected from the upper, middle, and lower lobes of ten lung pairs to tests measuring heritable phenotypes, including the production of the virulence factor rhamnolipid, swimming motility, amino acid auxotrophy, and resistance to antibiotics.

The proportion of isolates expressing most phenotypes differed regionally within each lung (Figure 20). For instance, ~ 40% of isolates from the right upper lobe of patient 1 were ciprofloxacin resistant, whereas all studied right lower lobe isolates were susceptible.

Likewise, the left lower lobe of patient 1 harbored mainly non-motile isolates, whereas nearly all clonally related siblings in the left upper lobe were motile. Regional differences were also seen in other traits in all ten lung pairs (Figure 20). Thus, regional populations likely differ markedly in virulence potential and treatment responses.

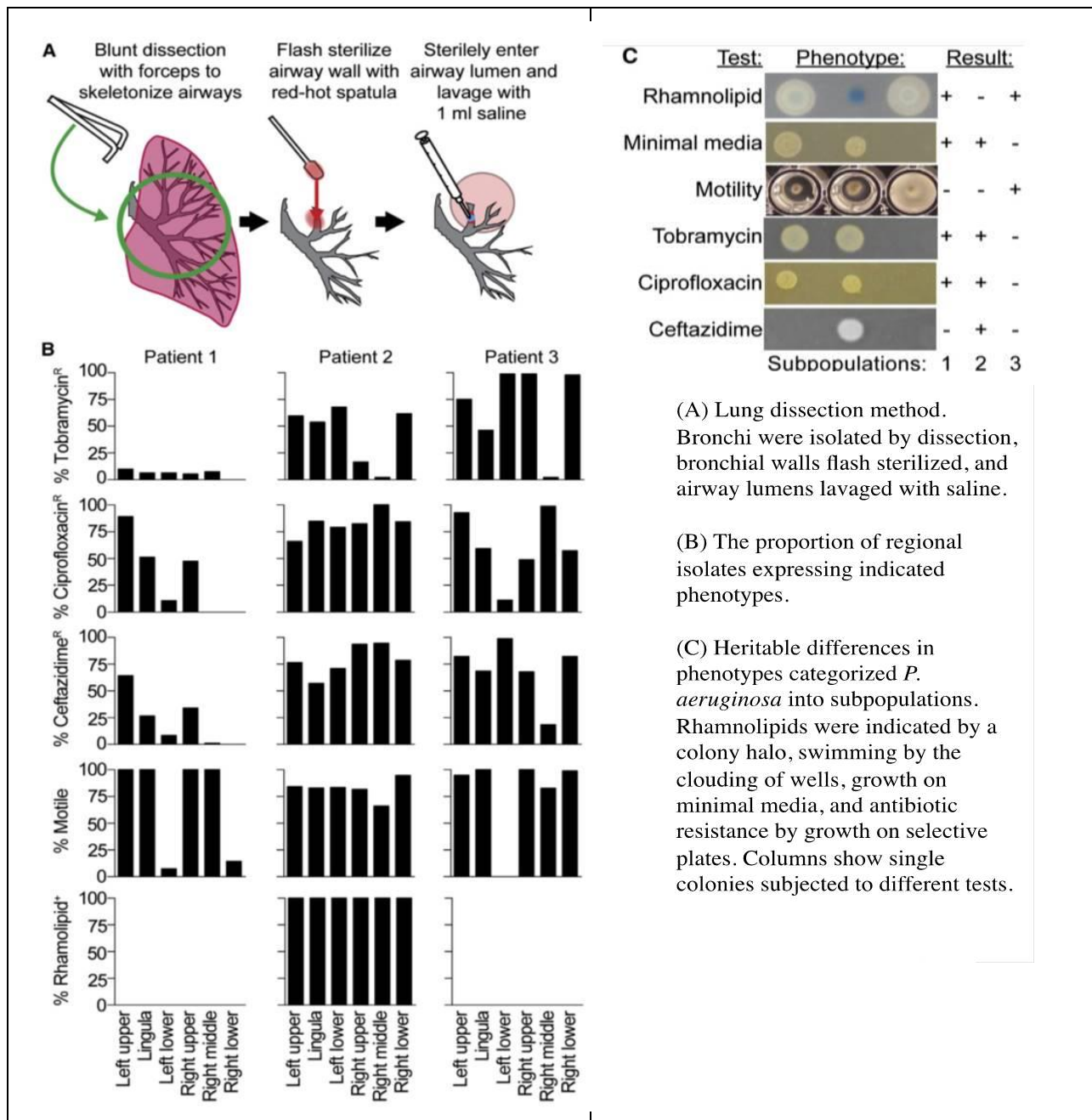The work has been published (Cell Host & Microbe 18, 307–319, September 9, 2015)

(A) Lung dissection method. Bronchi were isolated by dissection, bronchial walls flash sterilized, and airway lumens lavaged with saline.

(B) The proportion of regional isolates expressing indicated phenotypes.

(C) Heritable differences in phenotypes categorized *P. aeruginosa* into subpopulations. Rhamnolipids were indicated by a colony halo, swimming by the clouding of wells, growth on minimal media, and antibiotic resistance by growth on selective plates. Columns show single colonies subjected to different tests.

Figure 20: Regional Populations Vary in Infection Phenotypes

3.5.3 Task 3: To assess if metabolite profiling can be used to identify biomarkers for exacerbation and unresponsiveness to antibiotic therapy during these periods

Not reported

3.5.4 Task 4: To elucidate the contribution of specific microbial determinants to antibiotic tolerance in Pseudomonas aeruginosa

One approach to understanding the specific determinants of antibiotic tolerance of pathogen like P. aeruginosa is to identify processes required for its growth and survival in the in vivo environment. Such processes depend on the organ- ism's essential gene set. Both general and growth condition- specific essential genes can be distinguished. General essential genes are required under virtually all growth conditions, whereas condition-specific essential genes are required under a subset of conditions (like sputum from subjects with CF, studied here). We sought to identify both general and growth medium-specific essential genes of P. aeruginosa. To do this, we generated 13 fully independent, saturation-level transposon mutant pools on six different media and characterized them using Tn-seq. We analyzed three media in depth: LB nutrient agar, MOPS- pyruvate agar, and media made from cystic fibrosis sputa. Sputum medium was used because it corresponds to the bacterial growth medium in vivo in cystic fibrosis infections, in which bacteria reach high cell densities.

We found that 352 genes were required on all three media, and refer to these as "general" essential genes (Figure 21). An additional 199 genes were essential under a subset of the conditions and are called "condition-specific" essentials. Depending on the medium, 11–23% of essential genes were condition-specific. Nearly all of the general and condition-specific essential genes (99% and 90%, respectively) belong to the P. aeruginosa core genome.

Several functions associated with outer-membrane integrity and synthesis was specifically essential for sputum growth. These functions included the abundant outer-membrane OprI lipoprotein covalently attached to murein that helps stabilize the cell envelope and the function attaching it to murein (PA2854). An ortholog of outer-membrane chaperone protein Skp (PA3647) was also specifically essential on sputum medium. The global translational regulator RsmA was required for growth on sputum and LB, but not on minimal medium.
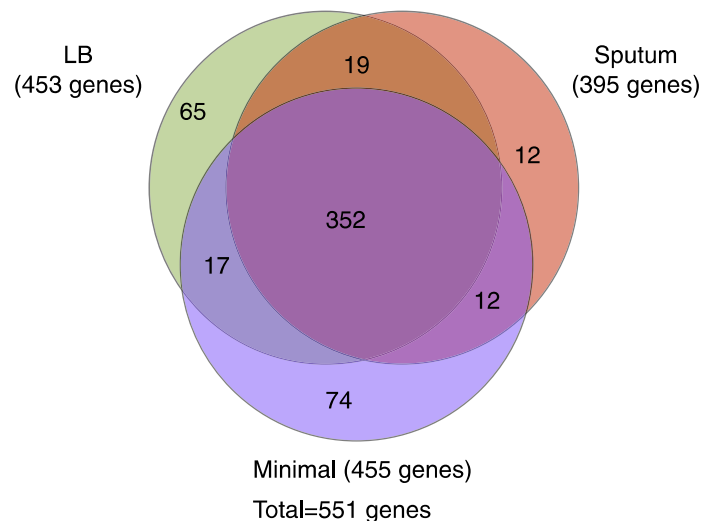

Figure 21: Overlap among genes essential under different growth conditions. The distribution of 551 genes found to be essential under at least one of the three growth conditions

Conversely, most genes for amino acid and nucleotide bio-synthesis were not essential on sputum. The result fits well with studies showing that cystic fibrosis patient sputum is rich in free amino acids. Genes needed for unsaturated fatty acid bio-synthesis (fabA, fabB, PA5174, and fabV/PA2950) were essential on minimal and LB but not sputum medium, suggesting that sputum contains unsaturated fatty acids. Unsaturated fatty acid synthesis has been proposed

as an antimicrobial drug target, but this finding suggests that its inhibition in P. aeruginosa may not be effective for treating cystic fibrosis pulmonary infections.

The work has been published (Proc Natl Acad Sci USA. 2015 Apr 21;112(16): 5189-94).

3.5.4.1 Studying P. aeruginosa gene variants that could cause exacerbations

We performed studies investigating associations between evolved Pa genetic variants and CF lung disease. We dissected lungs from CF patients immediately after removal for transplantation (as described above) and sequenced 100 clonally-related Pa from the upper, middle and lower lobe bronchi. We focused on a lung in which the left upper lobe was far more injured than other areas (Figure 22A), and searched Pa sequence data for explanations. We found that gene variants in a negative regulator of type 3 secretion (T188P in the exsD gene) were abundant in the destroyed upper lobe and rare in regions with milder disease (Figure 22B). Engineering wild-type and T188P exsD in the subject's clinical Pa isolate showed that T188P exsD caused major increases in macrophage and epithelial cytotoxicity and lethal virulence in murine lung infections (Figure 22 C-D).
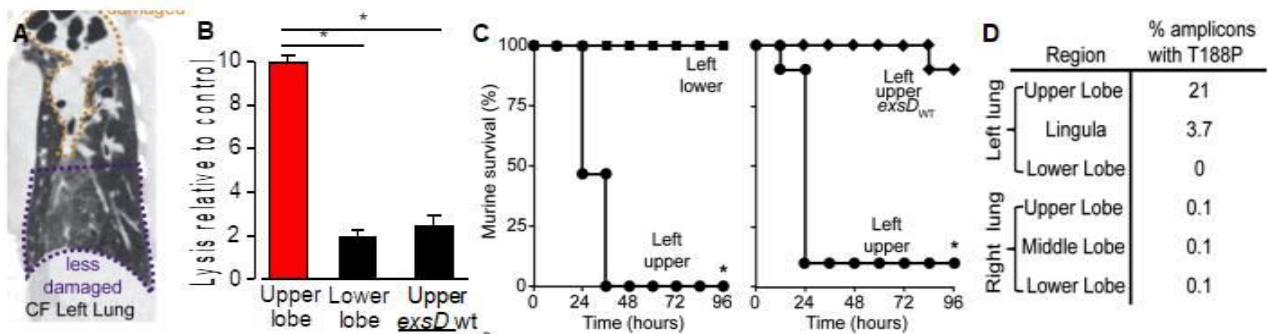

Figure 22: A. CT scan from case study 1#, subject showing severe upper and mild lower disease. B-C. An upper lobe isolate is compared to the lower lobe isolate, or the upper lobe isolate in which wild-type exsD was restored. B. Cytotoxcity to A549 epithelia, LDH

The findings above prompted us look for exsD gene variants in a different CF patient who suffered a severe, unexplained FEV1 decline (Figure 23) and had serial sputum samples banked. We PCR-amplified the exsD gene from DNA from whole sputum, and Illumina-sequenced exsD amplicons. No nonsynonymous esxD variants were detected at age 6 months and 7.5 years or at ages 12-14.5 (Figure 23). However, S164P exsD variants arose at the time of the marked FEV1 decline. Note this is a different esxD variant, and a different patient who is infected with a different Pa strain than described above. However, the S164P exsD variant from this patient also produced hyper-active type 3 secretion and increased cytotoxicity (Figure 24 + Figure 25).
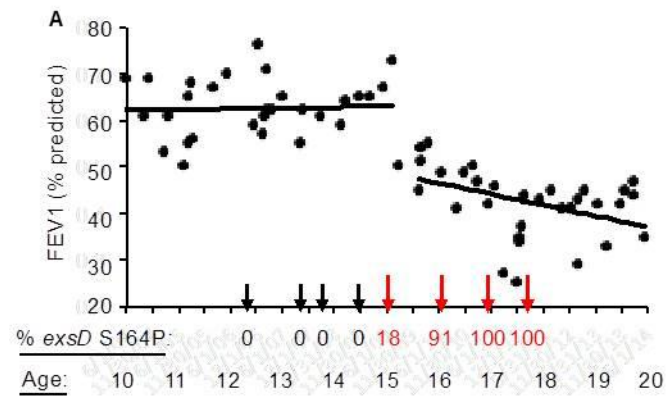
Figure 23: Lung function (FEV1) of subject described in case study 2; arrows indicate sputum samples which did not (black) and did (red) contain exsD S164P variants, and the proportion of S164P alleles is indicated.
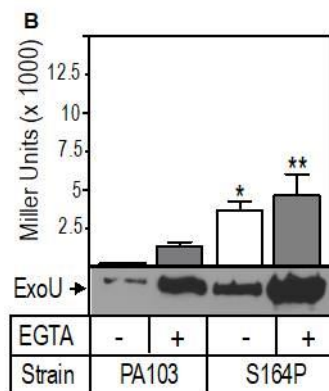

Figure 24: Miller assay measuring type 3 secretions gene expression $P_{exsA\text{-}lacZ}$ reporter) , Western blots show ExoU, a type 3 secretion cytotoxic

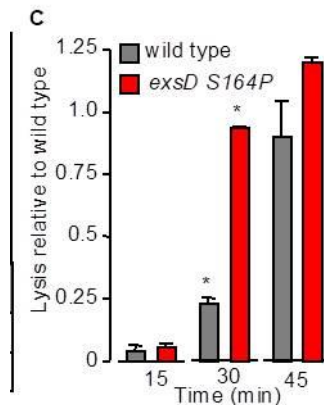*Figure 25: LDH release induced by strains after incubation with airway normalised (grey bars = Pa with wild exsD; red bars = isogenic Pa strains with S164PexsD*

3.5.4.2 Development of new methods to measure Pa gene variants in sputum.

UW think it is important that we measure Pa gene variants in sputum rather than cultured isolates, as CF Pa extensively diversifies during infection, and thus individual cultured isolates are not representative (Figure 22). We tested direct sequencing of CF sputum DNA to measure Pa gene variants. However, most DNA in sputum is human, and only 2-5% of reads aligned to the Pa genome (Figure 26). We developed the "target-capture" approach (Fig 6) based on a human exome sequencing methods that simultaneously target ~19,000 genes (a 39 Mb target region) using only 500 ng of DNA (the Pa genome is 6 Mb). This methods generates sequencing libraries from whole sputum DNA, and captures DNA fragments containing Pa gene targets with oligonucleotide probes. Probe-bound DNA fragments are purified using magnetic beads, Illumina sequenced, and gene variant abundance measured using our custom pipeline (BWA-MEM for alignment and VarScan2 for variant detection).
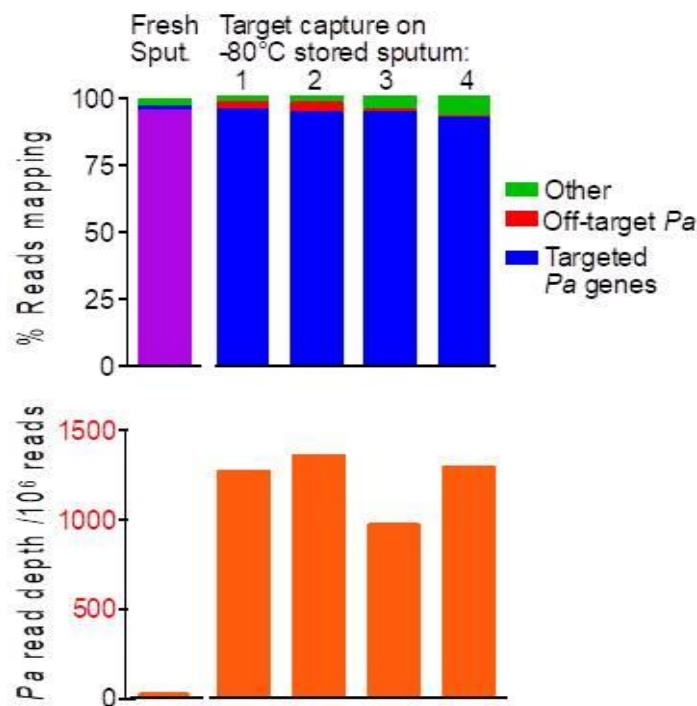

Figure 26: Sputum samples stored at -80□C

Whole sputum (Sput) was shot gun sequenced, and 4 sputum samples stored at -80°C were sequenced using target capture method. (Top) shows the percentage of reads aligning tp targets Pa genes, other Pa genes (Offf target) or other genomes (Other); (bottom) shows average read coverage Pa genes obtained. Note the marked improvement with target capture.

We tested the method using 30 Pa genes probes, and CF sputum that had been frozen for several years and found >90% of reads aligned to target Pa genes (Figure 26), yielding deep sequencing coverage. Note that each Pa chromosome fragments randomly and our analysis pipeline eliminates reads with identical start and stop positions. Thus sequence depth indicates the number of unique Pa chromosomes interrogated. The one gene (fliD) not captured in the experiment shown (Figure 28) has two divergent alleles types, and probes were designed against the type not present in this sputum. For genes with divergent alleles, we will design probes for the patient-specific allele using PCR amplicon sequencing as described above.

We tested target-capture sensitivity by spiking two sequenced Pa strains in CF sputum not infected with Pa. In addition to varying the ratio of the two strains, we varied the relative abundance of Pa to sputum DNA, wherein Pa DNA ranged from 2% to only 0.2 % of total DNA (i.e. host DNA comprised 98 to 99.8% of the total); Pa-infected CF sputum typically contains ~2-5% Pa DNA. The relative abundance of variants detected closely matched their expected frequencies, even when Pa DNA only comprised 0.2% of total DNA (Figure 29).
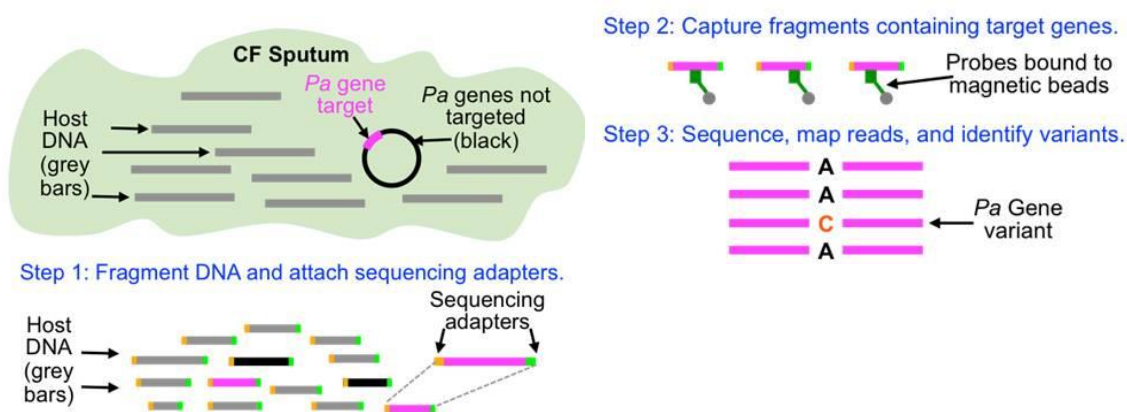

Figure 27: Target Capture Method

Step 1: Sequencing libraries generated from total sputum DNA
Step 2: DNA Fragments containing targeted Pa genes hybridise to probes and are pulled down by magnetic beads
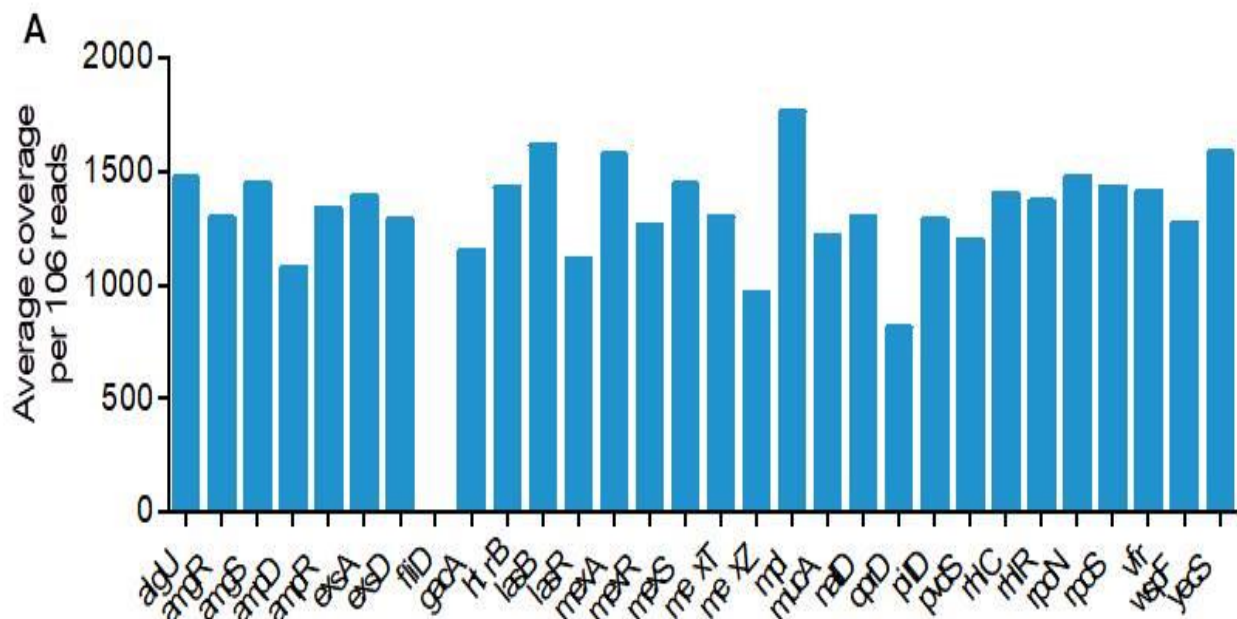**Step 3:** fragments are sequenced, aligned to the Pa genome, and variants are measured.

Figure 28: Unique coverage of individual Pa genes following target capture from frozen CF sputum samples per 1 million reads: representative of 4 experiments
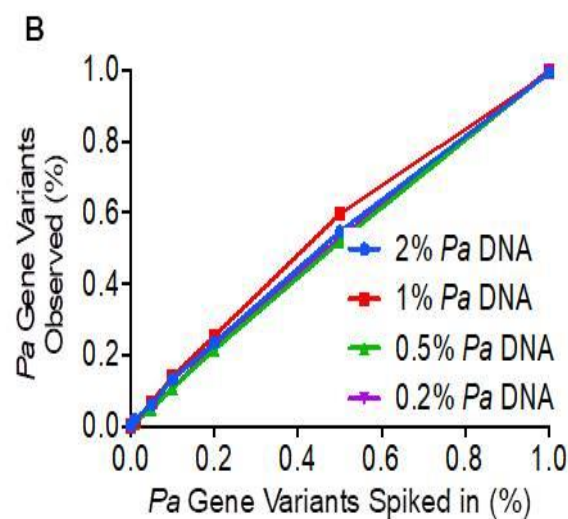

Figure 29: Pa Gene Variants

PA01 and Pa clinical isolate were added (at the "spike-in" ratio indicated on X axis) to CF sputum from non Pa-infected patient. The amount of Pa DNA relative to human sputum DNA was also varied (indicated by coloured lines). The mean variant abundance detected by target capture is plotted against expected values (based on spike in).

3.6 WP06: Human Host Defense Response

Host factors play an important role in determining and indicating polymicrobial colonisation in the CF lung and antibiotic treatment effectiveness. WP06 will investigate the association between key proteins of the innate host response, the lung microbiome, antibiotic administration and clinical outcome. WP 06 aimed to:

- Investigate the in vitro treatment effectiveness of host derived antimicrobial proteins/peptides (AMPs) and standard antibacterials (singly and in combination) on the growth inhibition of CF microbiome bacteria
- Characterise host factor protein signatures in sputum of CF patients undergoing standard and microbiome-directed antibiotic treatment regimens
- Correlate host factor profiles in sputum with clinical outcome, treatment success/failure (responder/non-responder) and gender.

3.6.1 Task 1: Susceptibility testing of specific members of the CF lung microbiome to growth inhibition by AMPs and antibiotics

The antibiotic susceptibilities varied with and across the genera. Antibiotic susceptibilities for a panel of antibiotics against all isolates tested. Chloramphenicol was the most effective antibiotic followed by ceftazidime, doxycyline and tobramycin.

Following evaluation of a number of AMPs against a range of bacteria, human beta defensin 3 and LL-37 were the most effective AMPs against most of the species and isolates tested. We have shown from Task 1 that the antimicrobial peptides, LL-37 and HBD-3, are the most effective innate antimicrobial peptides. We have also shown that the antibiotics, chloramphenicol, ceftazidime, doxycyline and tobramycin, were the most effective antibiotics against strains of tested bacteria.

3.6.2 Task 2: Quantification of host factor protein profiles in sputum of CF patients undergoing standard and microbiome-directed antibiotic treatment regimens and Task 3: Statistical analyses and interpretation of host factor profiles in CF sputum

Analysis of the sputum HDP levels has been carried out. Differences in HDP levels in microbiome versus empirical antibiotic treated patients have been determined and correlations of HDP levels against various clinical parameters have also been evaluated.

3.6.2.1 Results of Task 2 and 3

A number of HDPs have been analysed for individual protein levels or activity. A total of 738 sputum samples have been processed. We have determined the levels/activities of cathepsin S, cathepsin B, neutrophil elastase, SLPI, cleaved SLPI, lactoferrin, LL-37, IP-10 and total protein in these samples.

HDP levels in Microbiome versus Empirical Antibiotic Treated Sputum
CF patients undergoing an infective exacerbation were selected for antibiotic treatment using either microbiome-based therapy or empiric-based therapy. HDP levels in the sputum of both treatment groups was analysed using either ELISA or activity based assays. Some of the more significant data are presented below in Figure 30.
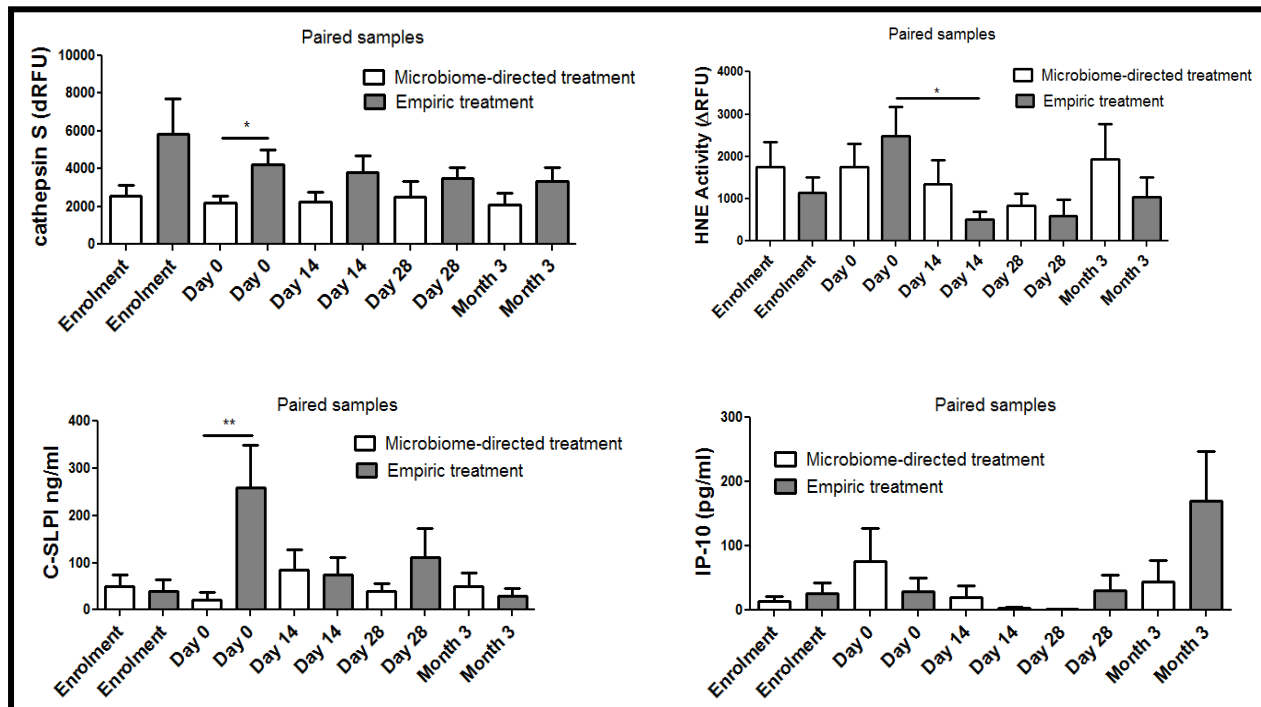
Figure 30: Levels/activities of cathepsin S, neutrophil elastase (HNE), cleaved SLPI (C-SLPI) and IP-10 were assessed in CF sputum samples

Neutrophil elastase (HNE) activity was shown to significantly decrease with empirical antibiotic treatment between days 0 and 14 compared to patients receiving microbiome treatment (Figure 30). There were also significant increases in cathepsin S and C-SLPI in the empirical treatment group although this occurred on Day 0 – the first day of exacerbation – so the significance of this finding is unclear. There also appeared to be some changes in IP-10 between groups although this did not quite reach significance (Figure 31).

Variations in HDP levels/activities over time
Levels and activities of the HDPs were assessed in sputum samples from patients before, during and post-exacerbation Figure 31.
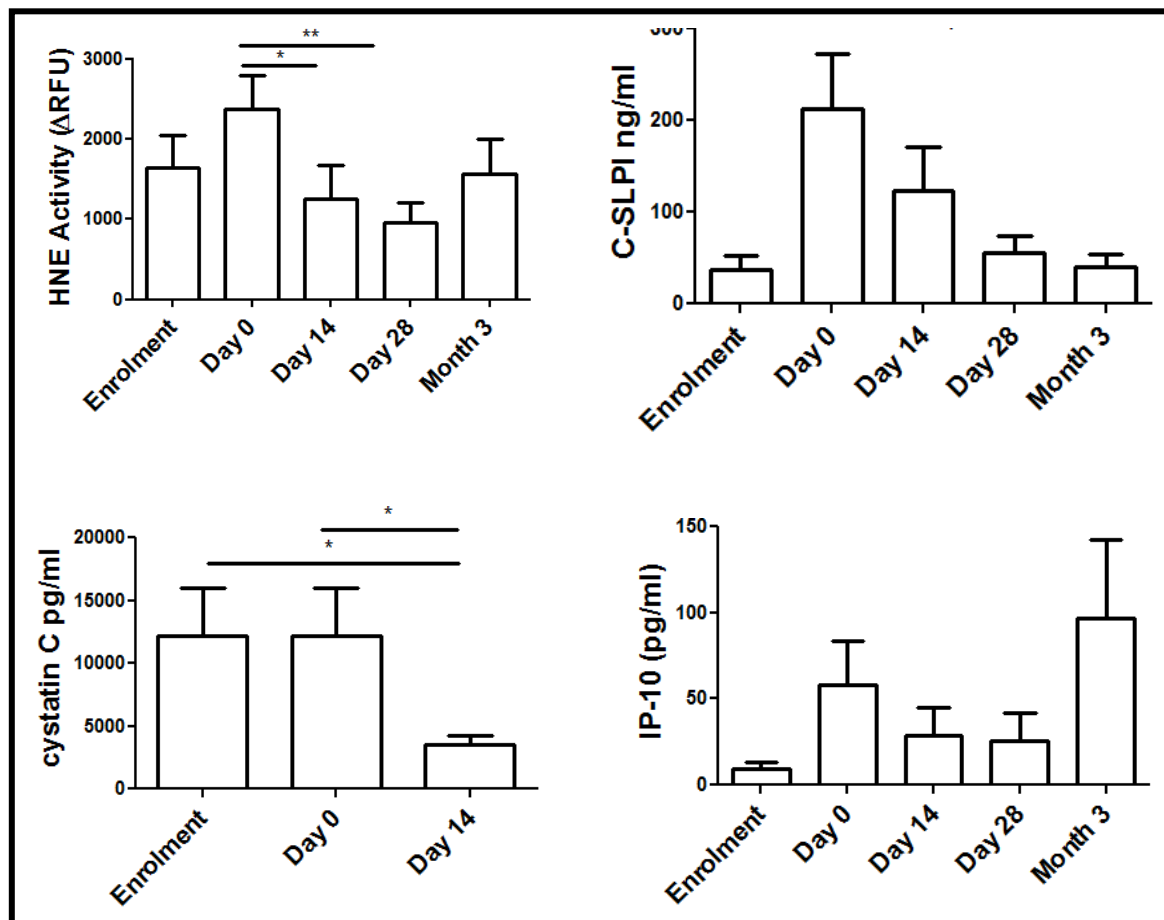
Figure 31: Levels/activities of cathepsin S, neutrophil elastase (HNE), cleaved SLPI (C-SLPI) and IP-10 were assessed in CF sputum samples pre, during and post-exacerbation.

HNE activity was shown to decrease significantly post exacerbation although interestingly, HNE activity did not quite reach significance between registration and Day 0 of the exacerbation (Figure 31). C-SLPI, a direct product of HNE activity, also increased at exacerbation and decreased post-exacerbation although these data did not quite reach significance (Figure 31). Interestingly, cystatin C levels were found to decrease following exacerbation (day 14) without actually increasing between registration and Day 0 (Figure 31). It is unclear why this should happen but it may be interesting to follow up this data in future studies to see if cystatin C may be utilised as a predictive marker of exacerbation. The cytokine IP-10, which is known to be involved in lymphocyte and neutrophil chemoattraction, also increased at Day 0 and decreased post-exacerbation although once again these data did not reach significance.

Correlations of HDP levels/activities to clinical and bacterial parameters
There were some instances of negative correlation between HNE activity and FEV1 (r = -0.1609) and positive correlation of C-SLPI with FEV1 (r = 0.09778) which was anticipated. HNE and C-SLPI also correlated in the same way to FVC. NE activity was found to positively correlate with Pseudomonas (r = 0.211) as expected and negatively correlate with Streptococcus (r = -0.3155) which was not anticipated.

3.7 WP 07: Lung microbiome

The main aim of WP07 is to assess the role of the lung microbiome on the establishment of chronic P. aeruginosa infection. In order to examine this, the following series of specific aims have been defined.

- To develop novel co-infection models for the assessment of host-pathogen interactions in vivo
- To investigate the role of the lung microbiome on host response to P. aeruginosa during chronic airway infection in vivo
- In vivo validation of antibiotic treatment strategies utilised in WP03, aimed at improving rational prescription regimens and reducing the development of antibiotic resistance

3.7.1 Task 1: Establish novel in vivo co-infection models

The work package as a whole aims to elucidate the complex interactions that occur within the CF lung between the host, the microbiome of the lung and the pathogenic organisms Pseudomonas aeruginosa. This fundamental scientific research complements the clinical component of the project assessing therapeutic treatment which targets the patient's specific microbiome compared to the current standard of care where therapy would be solely directed towards P. aeruginosa.

Thus, the aim of task 1 was to establish novel in vivo co-infection models, which can be used to elucidate the relationship between the lung microbiome constituents and P. aeruginosa. This model development included optimisation of the bacterial dose, route of infection (intranasal/ intra-tracheal) and timing of infection (i.e. sequential or simultaneous infection). These parameters have all now been established and the novel model developed provides us with a unique tool which facilitates assessment of the acquisition of P. aeruginosa infection, its dissemination from the upper respiratory tract into the lung and its influence on the establishment of chronic infection.

3.7.2 Task 2: Phenotyping of host response to P. aeruginosa in context of co-infection with different lung microbiome constituents.

We have demonstrated that the CF lung microbiome constituents are capable of altering the outcome of infection. An incongruity has been observed between the results observed in a larval model with a primitive innate immune system and mice. This indicates that the mammalian host response in intricate interactions moderating the response to respiratory pathogens. We have observed lung microbiome constituent alterations in the levels of inflammatory cytokines during P. aeruginosa infection. The spontaneous infection model developed within this project provides a unique tool to identify the host and microbiome factors responsible for alterations in the response to P. aeruginosa. Work carried out within this task has been published in the American Journal of Respiratory and Critical Care Medicine.

We have demonstrated that the CF lung microbiome is able to modulate P. aeruginosa infection, both positively and negatively. The microbiome constituents are able to moderate the production of inflammatory cytokines during subsequent P. aeruginosa. Conversely, we have also demonstrated that the host phenotype is able to alter the lung microbiome.

3.7.3 Task 3: Determine the biological relevance of low abundance pathogens using novel in vivo co-infection models.

These experiments utilised the spontaneous infection model developed as part of the CFMATTERS project. Outbred CD1 mice where inoculated intra-nasally with a titrated dose of

Haemophilus influenzae or a saline control. The animals were then exposed P. aeruginosa in their drinking water for 5 days, following a wash out period .the animals were culled and the levels of spontaneous P. aeruginosa in the upper and lower respiratory tract examined. A clear dose response between the inoculum of H. influenzae and the acquisition of P. aeruginosa was observed. Correlation analysis demonstrated that there was a significant relationship between the administered dose of H. influenzae and the CFU of P. aeruginosa detected in the respiratory tract ($p = 0.04$, $R2 = 0.91$) (Figure 32). It should be noted, however, that a large inoculum of H. influenzae had to be administered, below 107 no effect was observed (data not shown).

Animal are inoculated intra-nasally with 20 µl of a CF relevant microbiome component. The mice are then exposed to a clinical isolate of P. aeruginosa in their drinking water for a 5 day period. Freshly prepared water with P. aeruginosa were prepared daily. The mice were then provided fresh water for 3 days to ensure that any P. aeruginosa detected in the upper respiratory tract was due to colonisation rather than the animal recently having a drink. 8 days post exposure the animals were culled and the colony forming units (CFU) of P. aeruginosa in the upper respiratory tract (NALT) or lower respiratory tract (lung) determined by plating on selective media.
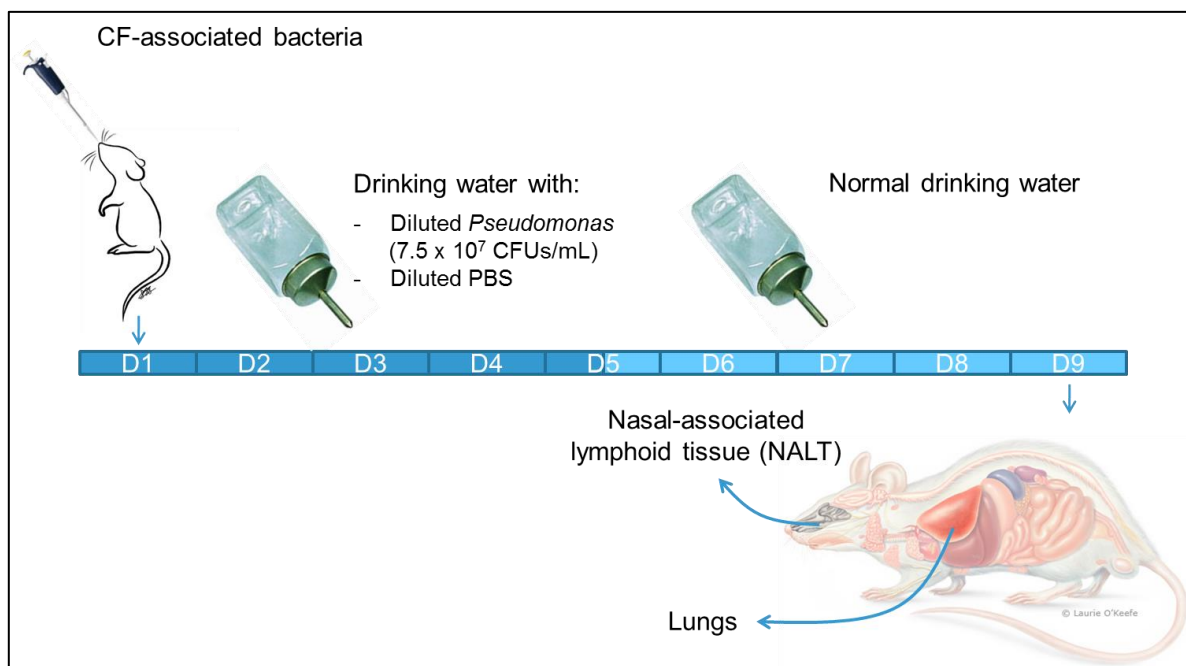
Figure 32: Schematic diagram depicting a spontaneous Pseudomonas aeruginosa infection model. Animal are inoculated intra-nasally with 20 µl of a CF relevant microbiome component.

There is a dose response between the amount of H. influenzae inoculated and the level of spontaneous acquisition of P. aeruginosa in the lungs of mice. Outbred CD1, sex matched, adult mice (n = 7 per group) were inoculated intra-nasally with varying doses of H. influenzae prior to exposure to P. aeruginosa in their drinking water (protocol outlined in Figure 32). On day 8, the mice were culled and the bacterial burden within the respiratory tract determined.
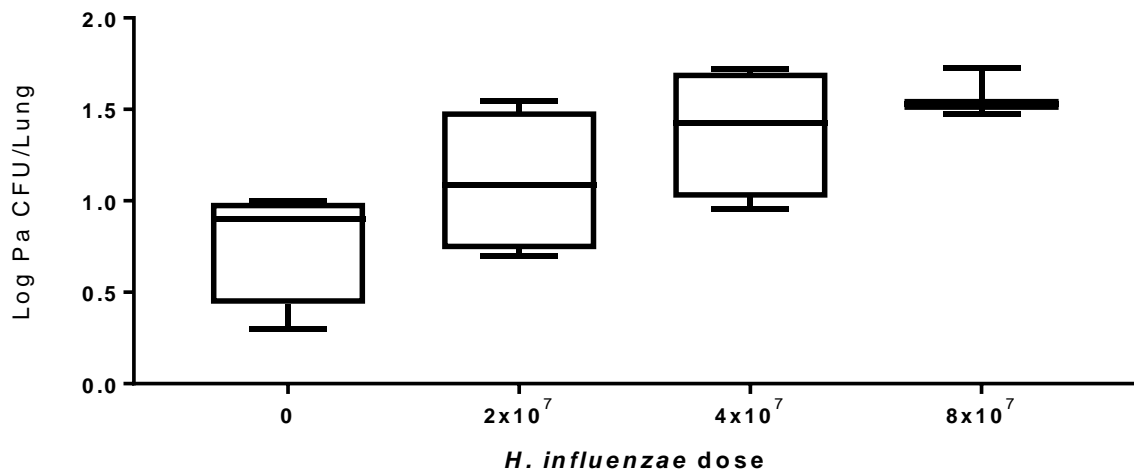
Figure 33: Dose response between the amount of H. influenzae inoculated and the level of spontaneous acquisition of P. aeruginosa in the lungs of mice
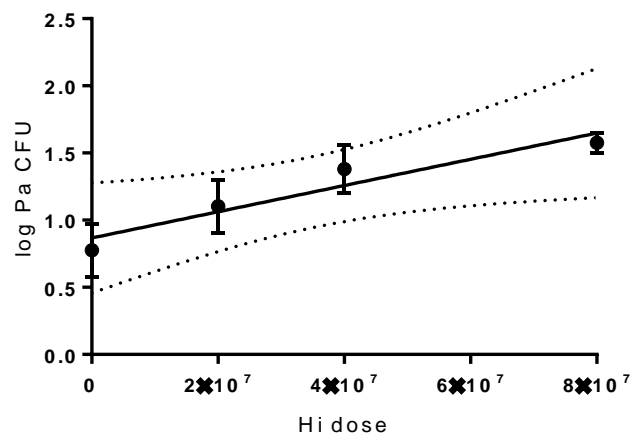

Figure 34: Correlation analysis verifies the relationship between the amounts of H. influenzae inoculated and the level of spontaneous acquisition of P. aeruginosa in the lungs of mice.

The results presented demonstrate that, in the case of H. influenza, the abundance of the organism clearly plays a role in its ability to exert an effect on the spontaneous acquisition of P. aeruginosa. It was, however, only when the initial inoculum and subsequent colonisation of the upper respiratory tract were at a high level this relationship was observed. This would suggest that antibiotic coverage for most organisms that are detected at low abundance during microbiome analysis is not required. This validates the approach within CFMATTERS of only considering the most abundant organism.

3.7.4 Task 4: Assessment of the antibiotic treatment strategies using murine models of co-infections

It was proposed that within this task we would use the novel murine co-infection models developed within the CFMATTERS project to assess the impact of co-infection on the efficacy of the antibiotics being used within the clinical trial. However, the work undertaken within WP7 demonstrated that individual CF lung microbiome constituents seem to exert little impact on the

outcome of P. aeruginosa infection.   Therefore, we have broadened the scope of the experiments within this deliverable and redesigned the experiments to allow us to ascertain if antibiotic induced alterations in the microbiome are capable of altering the outcome of P. aeruginosa infection.

In order to assess if antibiotic alteration in the microbiome of the mice resulted in any changes in P. aeruginosa infection outcome, we selected two of the antibiotics being utilised within the CFMATTERS study flucoxacillin and meropenem. The rational for this selection was that flucoxacllin, a narrow spectrum beta-lactam, will target gram positive bacteria, whilst in contrast meropenem is a broad spectrum antibiotic which will target a much wider range of organisms. Outbred CD1 mice were administered either flucoxacllin (25 mg/kg daily), meropenem (40 mg/kg daily) or a saline control intra-peritoneally for 5 days. Treatment was then withdrawn and they were given a 3 day 'washout' period to ensure no antibiotics remained within their system. The reported plasma half-life for flucoxacillin and meropenem in mice are 46 (Nauta & Mattie, 1975) and 90 minutes (Moon et al, 1997) respectively. The animals were then inoculated with P. aeruginosa intranasally, the lungs were harvested 48 hours post infection (Figure 33) and the number of bacteria within the lungs determined by plating of the lung homogenate on selective media. It can be seen (Figure 34) that pre-exposure to flucoxacillin resulted in a significant reduction (p = 0.04) in the bacterial burden P. aeruginosa within the lungs. This effect was not observed when the mice were pre-treated with meropenem. Given the rapid half-life of the antibiotics used within this study, the reduction in P. aeruginosa bacterial burden in is not due to a direct effect of the antibiotic. Therefore, pre-treatment with flucoxacillin has altered the murine host, presumably by changes to the microbiome, to make it less susceptible to P. aeruginosa.
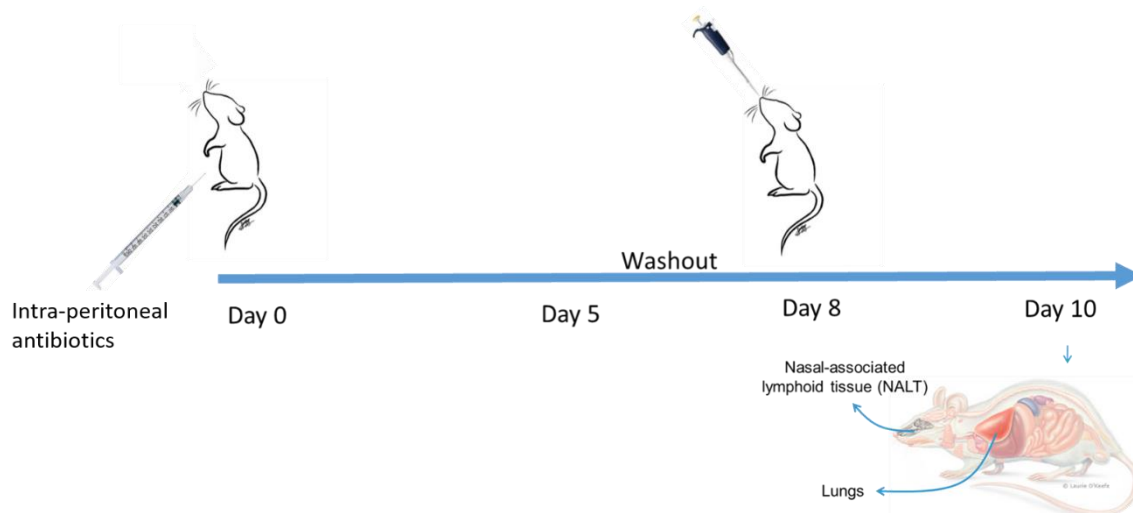

Figure 35: Schematic representation of the protocol utilised to examine the potential for antibiotic derived perturbation of the microbiome to alter the outcome of pathogenic infections within the respiratory tract.
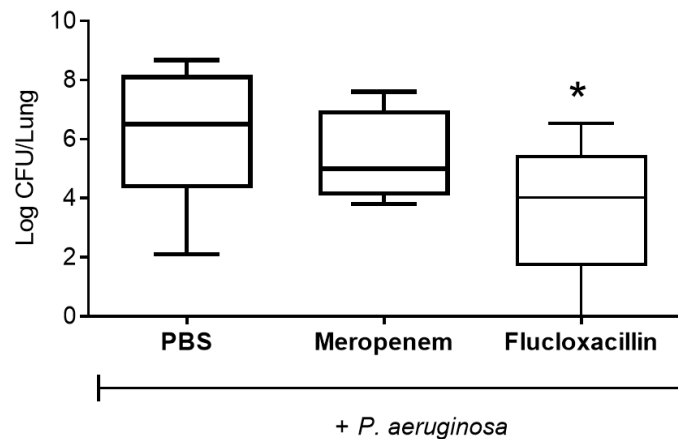
Figure 36: Antibiotic pre-treatment with flucloxacllin, but not meropenem, alteration in the microbiome has a protective effect against subsequent P. aeruginosa infection.

Given the rapid half-life of the antibiotics used within this study, the reduction in P. aeruginosa bacterial burden in is not due to a direct effect of the antibiotic. Therefore, pre-treatment with flucoxacillin has altered the murine host, presumably by changes to the microbiome, to make it less susceptible to P. aeruginosa. A small study examining patients admitted to hospital for surgical procedures has demonstrated that prophylactic flucoxacillin reduces the abundance of Staphylococus spp, although not the diversity, in the nasal microbiome (McMurray et al, 2016).

There is ongoing global debate regarding the role of anti-staphylococcal antibiotic prophylaxis in young children with CF, guidelines vary by country based on differing views of the risks versus benefits. Whilst it is clear that flucoxacillin prophylaxis does reduces S. aureus infections, there has been reports that it accelerates the development of chronic P. aeruginosa infection. However, the latest systematic review of clinical findings reports a trend towards a lower cumulative isolation rate of P. aeruginosa in the prophylaxis group at two and three years and towards a higher rate from four to six years (Smyth & Rosenfeld, 2017). It is therefore, essential to follow-up on the findings presented in this report. It is therefore essential to follow-up on the findings of this report to understand how flucoxacillin pre-treatment induced a protective effect against P. aeruginosa infection, this information may allow us target or supplement the microbiome of children with CF more effectively, allowing us to both prevent S. aureus infection but also limit P. aeruginosa colonisation.

3.8   WP 08: Gastrointestinal microbiota

The main aim of WP08 was to investigate the influence of the GI microbiota in the treatment of CF disease. This was planned to be achieved by the following:

- To determine the gastrointestinal microbiota composition of persons with CF during periods of stability, infective exacerbation and maintenance antibiotic therapy
- To investigate if antibiotic treatment alters CF gut microbiota composition and the development and function of T cell pathways
- To determine if the CF gut is a reservoir for antibiotic resistance genes
- To assess and evaluate strains of probiotics isolated from persons with CF in a murine model

3.8.1   Task 1: To determine the microbiota composition of the gut of CF patients during periods of stability, on maintenance antibiotic therapy-treatment Stool samples have been recruited from the CFMATTERS partners University College Cork, Papworth and Leuven. To date the following samples have been collected:

- 77 enrolment samples,
- 32 three month stable samples
- 12 six month stable samples,
- 8 nine month stable samples,
- 7 twelve month samples,
- fifteen month samples,
- eighteen month samples,
- twenty-one month samples,
- 1 twenty-four month sample,
- 1 twenty-seven month sample.

Additionally, we have received 26 exacerbation stool samples and 24 three month post-exacerbation stool samples, as well as having stool samples from 5 sibling controls and 12 non-sibling age-matched controls from the Cork site.

DNA was extracted from the faecal samples and sequenced using the Illumina MiSeq sequencing platform. The initial sequencing data has shown variation between samples from different individuals taken at the same time points, showing that each individual has a unique microbiota. Firmicutes are the most abundant phylum in CF faecal samples during stability (~60% of the phylum reads), with Bacteroidetes the second most abundant phylum. We have shown that in some patients, their gut microbiota shows significant fluctuations in bacterial profile during periods of pulmonary stability. This highlights the importance of studying longitudinal data in this cohort, to help determine the cause of such changes, independent of pulmonary exacerbation. Furthermore, when we compared the gut microbiota of the CF and control participants, they were very distinct. Alpha diversity indexes (which measure the diversity within samples) found the highest diversity in the controls (higher diversity is seen as a positive trait). Diversity decreased in the CF patients at exacerbation and recovered to near stable levels three months post exacerbation. Beta diversity (a measure of the diversity between groups) showed that CF and control samples cluster independently (Figure 37).

Additionally, we have also prepared the DNA from a small subset of CF patients for shotgun sequencing. This provided insights into both the composition and functionality of the CF gut

microbiota. The results showed that the gut microbiota of individuals with CF is functionally different to that of non-CF controls, with an increased capacity to metabolise xenobiotics, lipids and carbohydrates compared to the non-CF controls. Additionally, they have an altered metabolite profile.
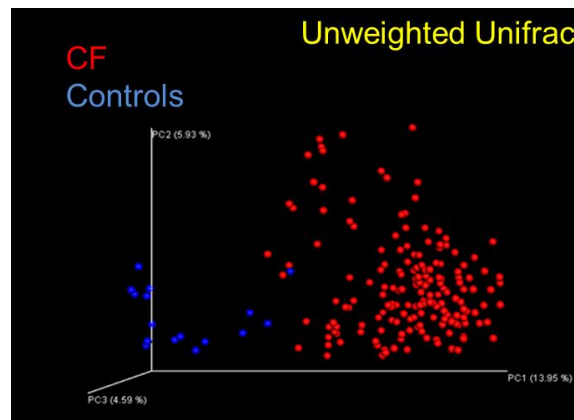

Figure 37: Unweighted Unifrac beta diversity measure of CF and controls, showing distinct separation of the two groups.

3.8.2 Task 2: To determine the impact of antibiotic therapy during infective exacerbation on the microbiota composition of the CF gut

To date there have been 26 exacerbation stool samples collected. At exacerbation, the alpha diversity decreases compared to stable samples. The gut microbiota at exacerbation also changes. We noted that patients could be separated into those with moderate changes at exacerbation, compared to those with large changes to their gut microbiota (Figure 38). Those with large changes to their gut microbiota during exacerbation, had large increases in *Enterococcus* levels compared to stable levels with some individuals becoming completely dominated by *Enterococcus* (>90% of genus in some patients). Additionally, a decrease in *Bifidobacterium* (up to 100 fold decrease) was seen in exacerbation samples compared to stable samples. Recovery of the levels of *Bifidobacterium* to enrolment levels was often incomplete even 3 months post-exacerbation.
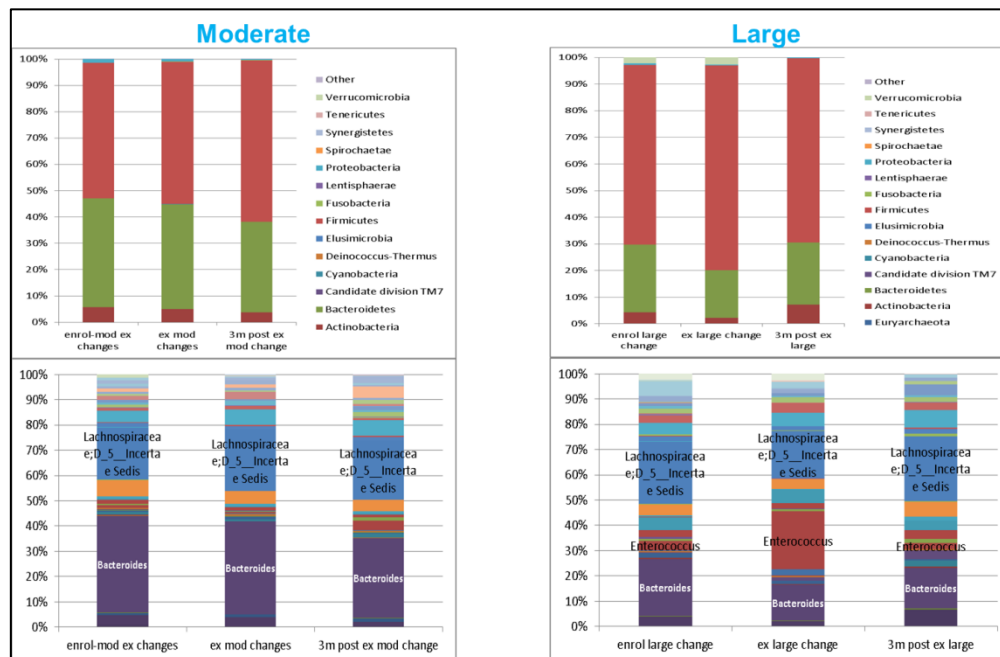
Figure 38: Gut microbiota composition at phylum and genus levels in CF patients who have moderate or large changes in their gut microbiota at pulmonary exacerbation

Correlation between the lung and gut microbiota

To identify if changes in the gut microbiota correlate to changes in the lung microbiota, we identified 7 patients for whom we have stable, exacerbation and 3 month post-exacerbation stool and sputum samples. We then conducted analysis to correlate the data between the lung and gut microbiota, with the aim of identifying how changes in one environment impact on the microbiota of the other environment. Initial analysis has demonstrated interesting correlations, similar to those observed by Hoen et al. 2015. This included a significant negative correlation between the Bifidobacterium and Bacteroides levels in the gut and the Pseudomonas levels in the lung (p= 0.02; r=-0.51 and p=0.05; r=-0.44 respectively). Further analysis on this work is on-going, to study a much larger data set.

C. difficile

Faecal samples (161) from 66 persons (46.97% male, median age 29.39+11.58 years) with CF attending three European CF centres were screened for C. difficile carriage using culture methods. In addition, C.difficile carriage rate in 14 age-matched healthy control subjects was also examined. Subjects were screened on enrolment, at 3, 6, 9, 12, 15 months, during pulmonary exacerbation and three months post exacerbation, where faecal samples were available. The prevalence of C. difficile carriage in CF persons at time of enrolment in the three centres was as follows: Cork 48.57% (17/35 subjects), Papworth 50% (6/12 subjects) and Leuven 47.37% (9/19 subjects). Carriage rate in the healthy control cohort was 7.14%. If we expand our examination of C. difficile carriage rate to include subjects who carried C. difficile at any sampling point, carriage rates are as follows: Cork-71.43%, Papworth- 66.67% and Leuven-63.16%. Most notably, no clear trend was seen in the prevalence of C. difficile between enrolment and exacerbation or 3m post exacerbation among the three sites investigated.

3.8.3 Task 3: To investigate if stratified treatment of CF patients alters gut microbiota and the development of and function of T-cell pathways

Peripheral blood was stained using the anti-human CD4/CD25 FITC/APC cocktail from eBiosciences.

RBC lysis was performed, followed by intracellular staining using the Whole Blood Foxp3 Staining kit (eBiosciences).Cells were analysed on the Guava easyCyte HT system (Merck Millipore) using guavaSoft™ software version 2.7Cells in the lymphocyte gate were analyzed, with T-regs being identified as CD4+/CD25+ and Foxp3+, in the upper right-hand quadrant. For each patient samples were analyzed at enrolment, exacerbation and 3 months post exacerbation.
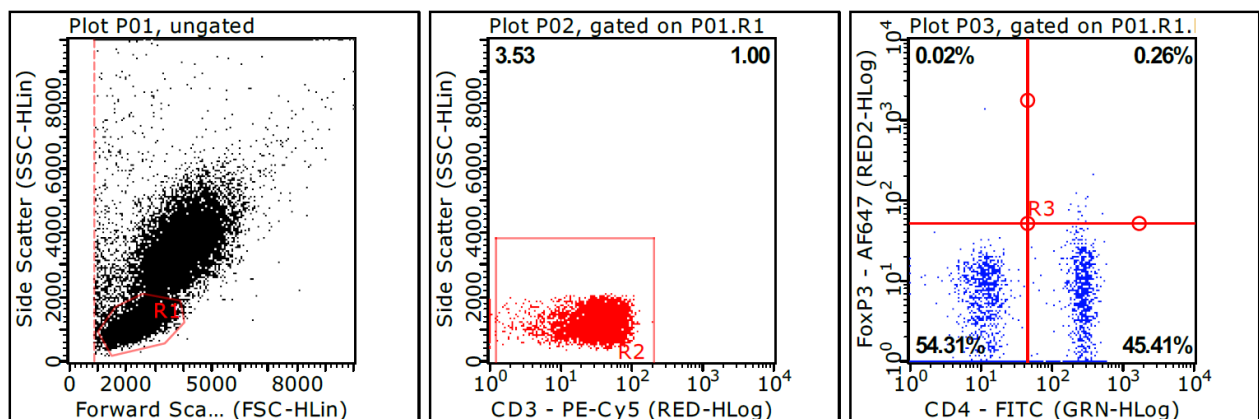

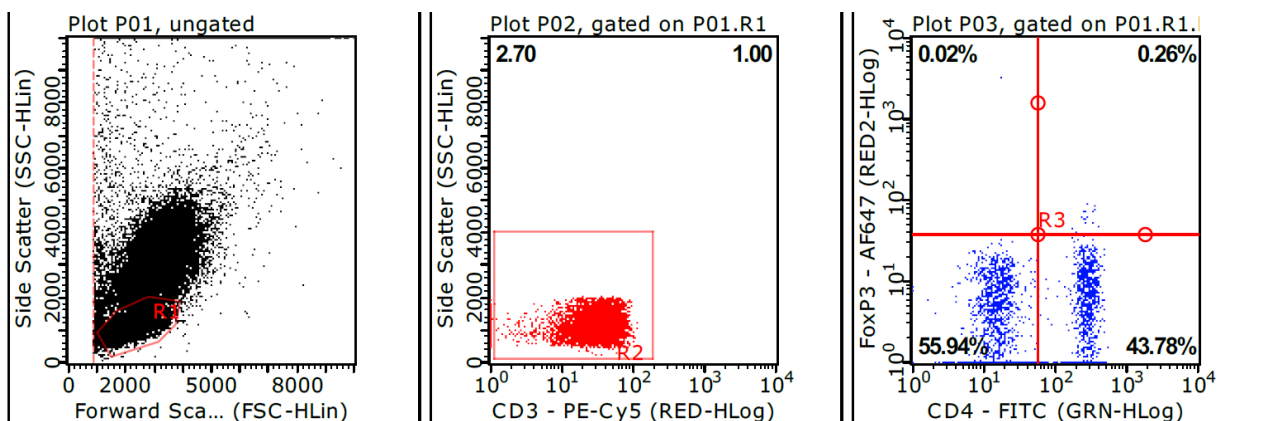*Figure 39: Example of flow cytometry at enrolment*

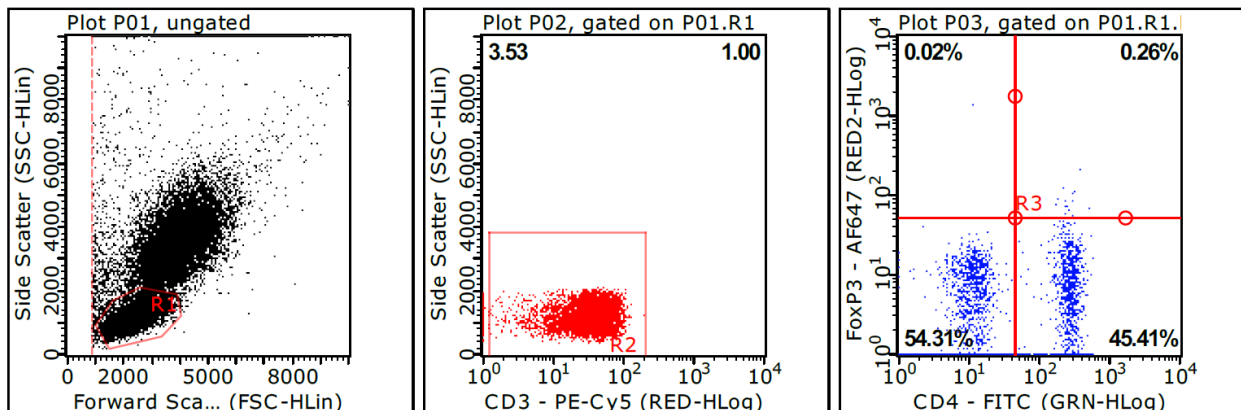*Figure 40: Example of flow cytometry at day 0*

Figure 41: Example of flow cytometry after treatment

There were no significant differences in T-regs at any time point between patient samples.

3.8.4 Task 4: To determine if the CF gut is a reservoir for antibiotic resistance genes

For each patient that has been recruited a portion (1g) of the fresh faecal sample has been collected and used for culture based analysis to determine the prevalence of antibiotic resistant isolates in CF samples. Each sample is serially diluted and plated on 1) Wilkens Chalgrens Agar (WCA; total bacteria counts), 2) WCA with vancomycin (40mg/L), 3) WCA with ciprofloxacin (40mg/L) and 4) WCA with metronidazole (40mg/L). Colonies are enumerated on antibiotic containing plates and compared to plates without antibiotics. In addition, gDNA is quantitatively assessed for antibiotic resistance genes associated with vancomycin, ciprofloxacin and metronidazole using quantitative PCR. At present the qPCR standards are being established for subsequent assays of gDNA. The culturing data suggests that the majority of total anaerobes cultured were resistant to vancomycin, metronidazole and ciprofloxacin. CFU/g levels were similar across time points.
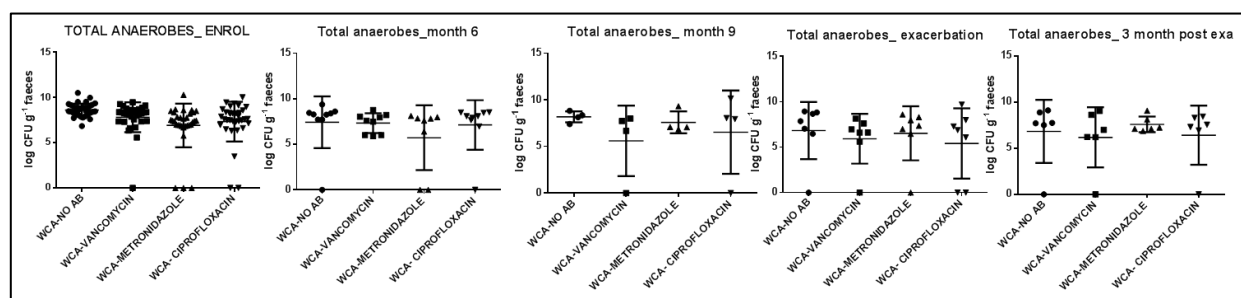
Figure 42: Log cfu/g faeces of total anaerobes and vancomycin, metronidazole and ciprofloxacin resistant anaerobes for enrolment, 6 month and 9 month stable samples and exacerbation and 3 months post exacerbation samples

Additionally, from our shotgun metagenomic sequencing study we demonstrated that there were higher abundances of antibiotic biosynthesis pathways in those with CF, compared with controls. These included gentamicin biosynthesis PWY-7025 (5066 vs. 4594 in CF vs. control gut microbiota), neomycin PWY 7016 (13.7 vs. 9.17 CF vs. control), paromycin biosynthesis PWY 7018 (1891 vs. 1575 CF vs. control), fosfomycin biosynthesis PWY 5757 (3.45 vs. 1.92 in

CF vs. control gut), kanamycin biosynthesis PWY 7000 (3.34 vs. 2.75 CF vs. control) and streptomycin biosynthesis PWY 5940 (79.0 vs. 68.5 CF vs. control gut).

3.8.5 Task 5: To assess strains of Lactobacillus and Bifidobacterium from the gut of stable CF subjects who are receiving chronic macrolide therapy with a view to determining their potential usefulness as probiotics

3.8.5.1 Culture Independent analysis

Alpha diversity metrics chao1, Simpson, Shannon, PD_whole tree, observed species show that in the absence of antibiotic combination treatment, the probiotic treated samples show reduced diversity. This reduced diversity of probiotic-only treated samples is reflected in the subsequent taxonomic analysis. At phylum, family and genus taxonomic levels there is a reduction in taxonomic diversity of probiotic-only treated (both LGG and L. plantarum) samples relative to probiotic-antibiotic treated samples. At phylum level this decrease in diversity is explained by an increase in the relative abundance of the *Firmicutes* taxa at phylum level, *Enterococcaceae* at family level and *Enterococcus* at genus level.

The results of this study demonstrated the ability of our *L. plantarum* strain to survive in the model of the gastrointestinal tract. However, the results of the sequencing analysis are unexpected, with a decrease in alpha diversity in the probiotic treated groups. This may reflect technical issues with this experiment, with issues with the initial inoculum.

3.8.6 Task 6: In vivo study to assess the therapeutic potential of probiotic administration with antibiotics for CF potential

The results of Task 5 demonstrated the ability of our L. plantarum strain to survive in the model of the gastrointestinal tract. However, the results of the sequencing analysis are unexpected, with a decrease in alpha diversity in the probiotic treated groups. This may reflect technical issues with this experiment, with issues with the initial inoculum. As a result, while Task 5 proves the ability of our probiotic to survive in the gastrointestinal tract (as tested in a model of the colon) we were unable to progress with testing this strain in a murine model. Before testing in a murine model we will need to repeat the in vitro testing to determine if the probiotic beneficially modulates the gut microbiota. We will then progress with testing this potential probiotic, compared to the commercially available LGG strain in a murine model.

3.9 WP 10: Dissemination

Our main objective is to disseminate the results generated in CFMATTERS to all target audiences including the general public and relevant professional sectors such as clinicians and scientists outside of the CFMATTERS consortium.

- To promote an understanding of, and interest in, CFMATTERS research and role of clinical science in society through communication with all the potential audiences, in particular patient organizations and public health authorities.

- To create a clear dissemination and knowledge exchange plan between all CFMATTERS participants, developing mutual specialisation, standard operational procedures, supporting management and training, thus strengthening the complementarity of the partners

- To regularly check the relevance of the CFMATTERS objectives and scope through periodic interaction between General Assembly, Steering Committee and the Advisory Board

3.9.1 Task 1: Design of a detailed dissemination plan

A dissemination plan and best practice guide for the CFMATTERS study was designed during the first six months of the award. This plan detailed internal and external dissemination activities relating to the study and was circulated to the CFMATTERS consortium. Internally, an electronic mailing list of all CFMATTERS staff was created (all@cfmatters.eu) to ensure effective communication between all study staff. Additionally, a weekly CFMATTERS teleconference is held each Monday to discuss study related information or queries, and minutes of this meeting are circulated shortly thereafter. The guidelines for the external dissemination of CFMATTERS activities are listed in the CFMATTERS final overall Dissemination Guide according to the rules agreed in the Consortium agreement. The dissemination plan details guidelines for the publications requirement including authorship plan and adherence to special clause 39, logo and website usage and grant acknowledgement.

3.9.2 Task 2: Develop a corporate project identity (Logo, Website, Smartphone application (APP), project flyer)

The CFMATTERS project logo is shown below:

The logo symbolizes the lungs which are made of tiny circular pills that symbolise the personalised antimicrobial treatment recommended by CFMATTERS. The corporate project identity is based on the colours of the project logo with the project colours of CFMATTERS being light blue and light grey.
The CFMATTERS website (www.cfmatters.eu) was designed based on the corporate identity of CFMATTERS and was launched on 31.03.2014.
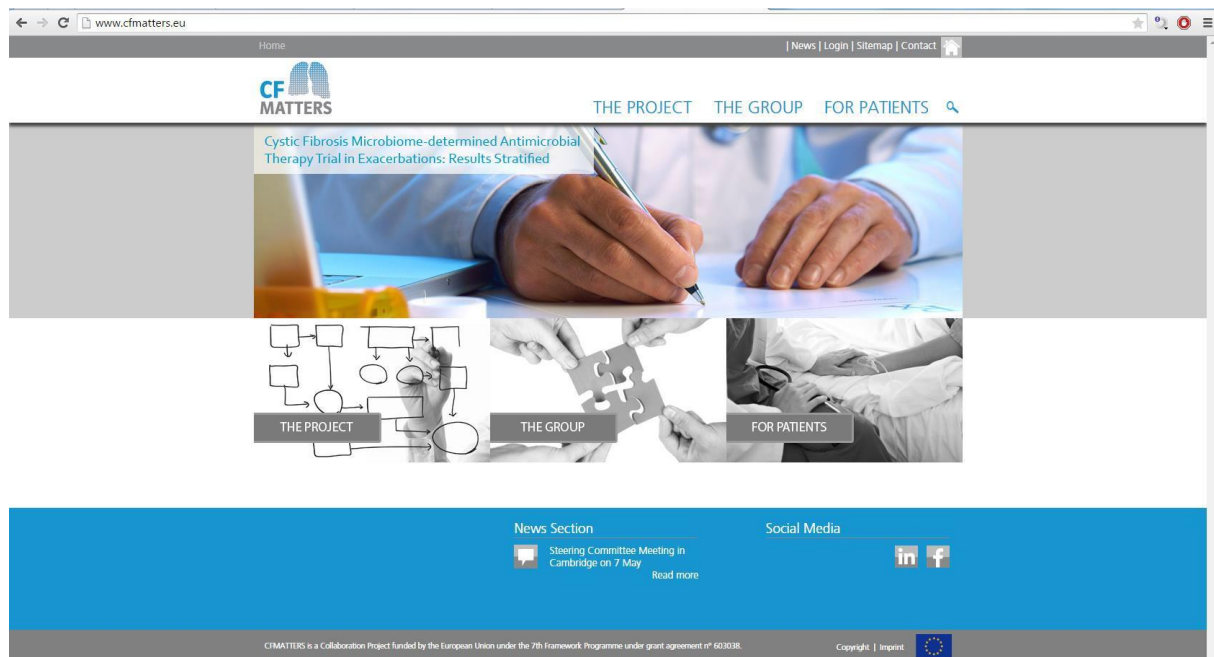
Figure 43: CFMATTERS Public Website

The website informs the public about the project and its objectives and partners. It also includes information about the project for patients with Cystic Fibrosis. Additionally, the website contains a password protected internal website which provides the CFMATTERS members with information on trainings, meetings, project management, WPs and EC documents. The internal section of the website is linked to the project management tool :milliarium. The website is updated regularly with news about CFMATTERS and the Consortium.

In total the website had 14,873 visits (79.47% new visitors).

3.9.3 Smartphone Application 1: CF Outpatient Data Analytics Smart Phone App

This game app for android Smartphone is based on the popular mobile game "Flappy Bird". It requires the patient to blow into the microphone in order to control the bird's movements (as seen in Figure 45). Since completing the development of the app a small stress test was performed with three adult CF patients from the Cork University Hospital Adult CF Day Ward. This stress test was done to evaluate the performance of the system and its desirability for the CF adult Cohort. These patients were given a smartphone with the app pre-installed and asked to play the game over two months. Custom alert criteria for these patients was not required. During these two months, no SMS alerts were sent to the patients. Although the game was not played every day, it was played frequently on days of game play (between 2-7 game plays). All three patients agreed that this game was non-intrusive to their daily lifestyle and could be implemented easily. Likewise, the patients commented favourably on the game and app design, as it did not appear to be *"just another medical app".* Due to this, patients felt comfortable playing the game in front of family and friends. All three patients agreed they would play the game again.
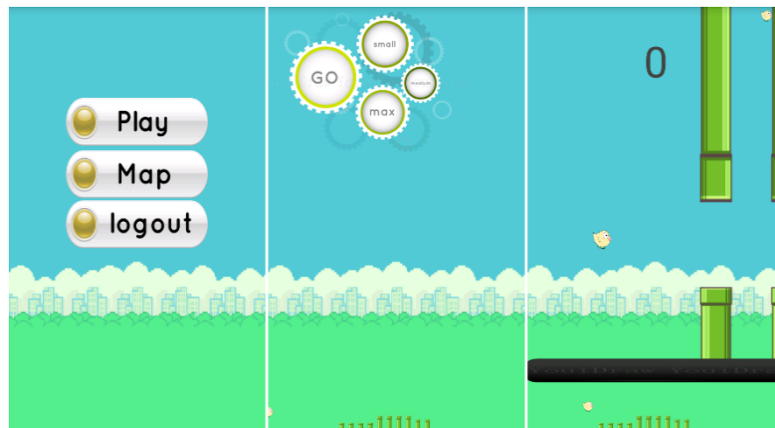
Figure 44: Game Main Menu, Custom Map Creator, and Game Screen.

3.9.4 Smartphone Application 2 CF Patient Passport App

Following on from the development of the CF game app, further research was conducted into other apps that could be of benefit to CF adult patients. A short survey evaluating mHealth app types and features was given to 49 CF adult patients in the Cork University Hospital. The results found that of the 49 patients, only two currently have CF apps installed on their devices. This appears to be mainly attributed to the lack of awareness of apps for CF. It was also found that these CF adults were most interested in CF news/research updates or a means in which to record their basic medical information. Recording medical information in this way is similar to that of a patient passport. Therefore, a CF patient passport app was developed (see Figure 46) using PhoneGap so that it can be deployed to Android, iOS, Blackberry, and Windows Phone. The app is currently being tested with five adults from the Cork University Hospital Adult CF Day Ward.
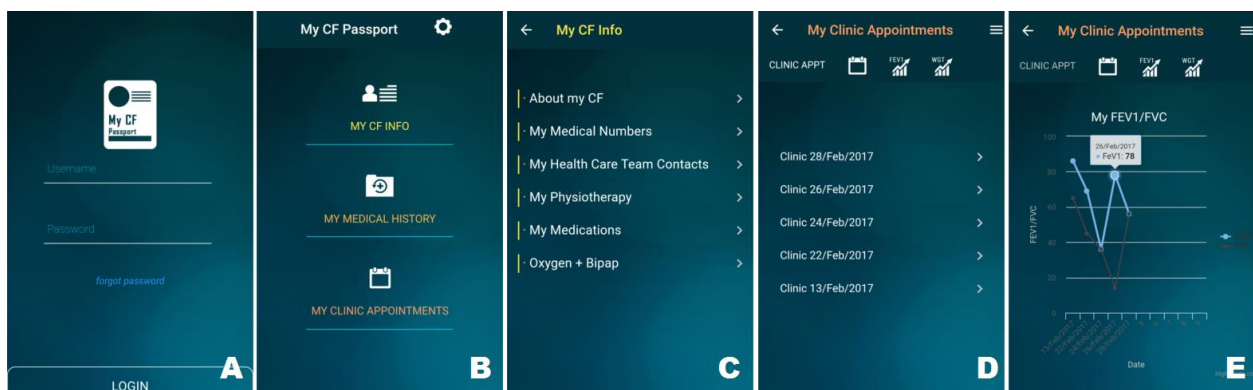
Figure 45: A) Login Screen. B) Main Menu. C) My CF Info Screen. D) My Clinic Appointments Screen. E) Graph with FEV1 % and FVC % data.

3.9.5 Task 3: To engage non-specialist audiences in CFMATTERS research

3.9.5.1 What is a Portacath, Gastrostomy Tube, Bronchoscopy?

Previously, one e-learning tool (portacath) was created for CF patients. Since then two additional tools focusing on Gastrostomy tubes and Bronchoscopies have been developed (see Figure 46). All three tools have been made available over the internet so that patients can

access them via personal devices. These tools are also directly accessible via bedside tablets in the Cork University Hospital. The tools incorporate 2D images, 2D animations, photos, and videos. All three tools collect data regarding device interaction. Over the last twelve months, it was found that these tools are more frequently accessed via personal devices. From the data collected thus far, it appears that patients prefer 2D images and photos while in a hospital setting. Likewise, that data shows tendencies for patients to access 2D animations and videos from personal machines/devices.

Figure 46:Screenshots of the three e-learning tools (Portacath, Gastrostomy, Bronchoscopy).

3.9.6 3D Visualisation Tool

3.9.6.1 The Lung Experience

The Virtual Reality Lung Experience tool was tested with a small group of students. Since then, the students feedback has been implemented. When the tool was first developed, consumer Virtual Reality (VR) headsets were not available. However, these headsets have now made their way to the market. Hence the VR tool was further enhanced using the HTC Vive. The HTC Vive allows for richer 3D visualisation, room scale VR, and the tracking and representation of input devices (see Figure 47). The VR tool was then subjected to a small pilot study with eight medical students (4th and 5th Year) who were on respiratory attachments. The users were given a pre-questionnaire regarding educational content in the tool. The users were then allowed to explore the VR tool to find and interact with this content. On completion, the users were given a post questionnaire based on the educational content as well as five usability questions. The results showed a 35% (on average) increase in knowledge across the two questionnaires. More importantly seven of the eight participants agreed that this tool was beneficial, they would use it again in future, and they would like more VR educational content to support their learning.
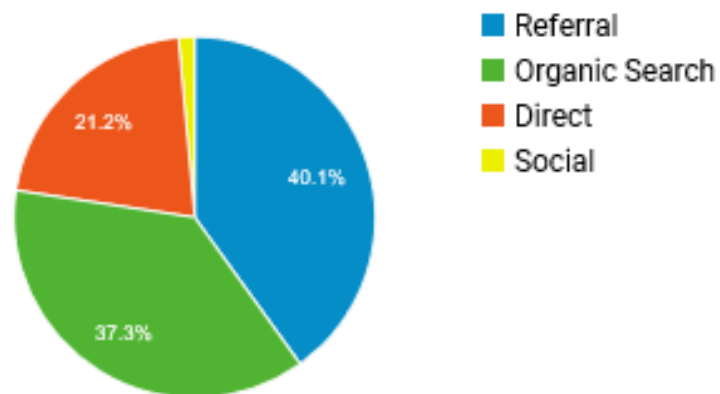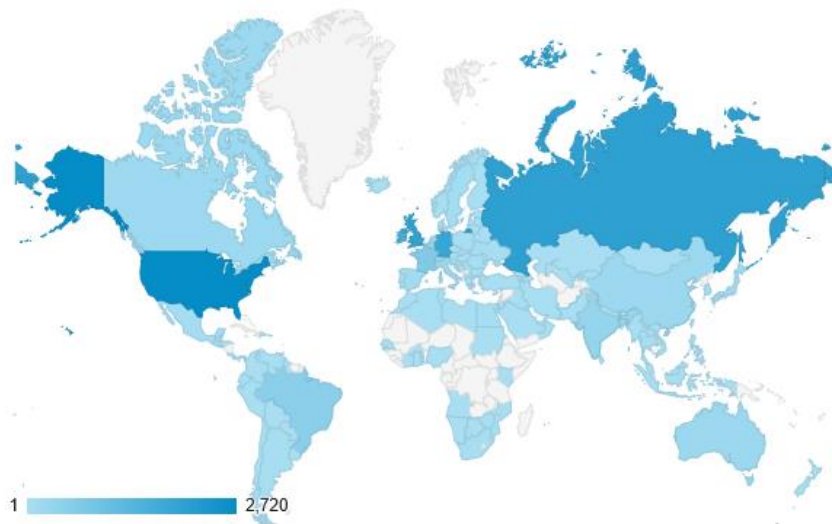

Figure 47:The HTC controllers and Vertex Painted Mucus.

3.9.7 Task 4: Ensure that the output of the project is disseminated in an efficient and timely fashion

3.9.7.1 CFMATTERS Public Website

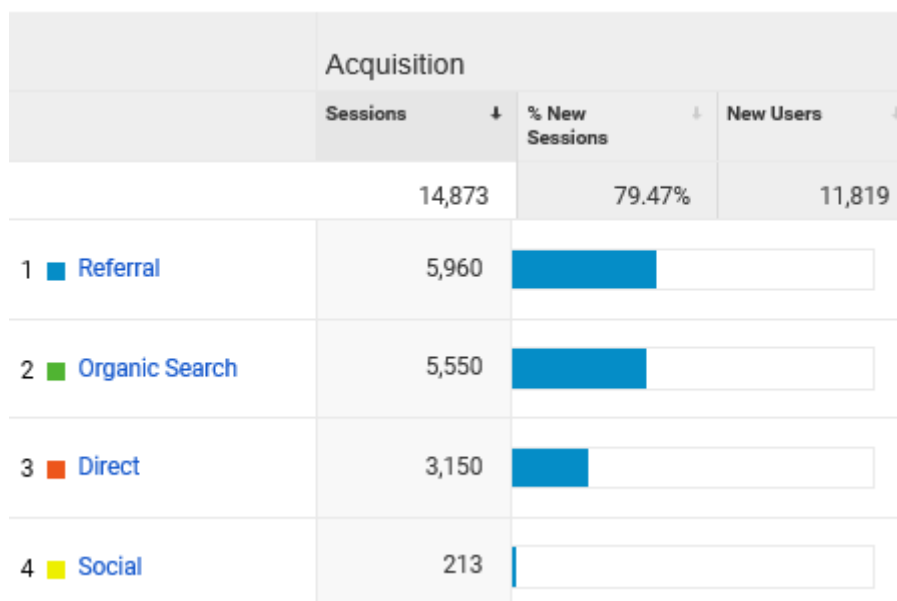Throughout the project lifecycle the project office has maintained and updated the CFMATTERS public website.

Figure 48:CFMATTERS User Acquisition M1 - M45

3.9.7.2 CFMATTERS Dissemination Video

The final dissemination video for the CFMATTERS project can be found at the following link: https://youtu.be/483IA14GxrA. This has also been impeded into the CFMATTERS public website: https://www.cfmatters.eu/the-project/ as to maximise the audience demographic and accessibility of the video.

The video has been produced by ARTTIC, with the support of UCC and project Principle Investigators. The focus of the video is to provide an oversight of the project, the key project objectives, and how we as research community can move forward in the development of Cystic fibrosis treatment, utilising the microbiome, utilising the knowledge generated within CFMATTERS.

3.9.8 Task 5: To train and develop all CFMATTERS research staff through organisation meetings and joint workshops with specific emphasis on harmonisation of procedures and documentation

CFMATTERS places a strong emphasis on the development and training of scientists, clinicians and clinical research staff of the future with a focus on translational bedside to bench research. To date over 18 research staff comprising both basic and clinical research staff have be directly funded through this proposal. A clinical research nurse is located at each of clinical trial partner sites and MD, PhD and Postdoctoral students are located in at both the trial coordinating centre, Teagasc, Queens University Belfast and the University of Dundee. The CFMATTERS study training day took place on September 3rd 2014 and focused on all aspects of the clinical study and was attended by all CFMATTERS study sites. Further training of staff is supported by the Trial Coordinating Centre as required. Additional training and updates on the trial is provided to all consortium members that attend the general assembly meetings. All sample collection, sample shipment, safety and site file processes have been standardized across all sites. Bioinformatics is an important component to the CFMATTERS study and there have been training between, the site biostatistician, QUB and the TCC to ensure the data is processed, analsysed and stored utilising FAIR principles. Researchers from Teagasc spent a number of

weeks training on bioinformatics and sequencing techniques to standardise the processes between the two groups to enhance the lung-gut axis research output.

4 Potential impact, main dissemination activities and exploitation of results

4.1 Potential Impact

4.1.1 WP01: Clinical trial management and analysis

CFMATTERS is the first randomised control trial investigating the effectiveness and safety of microbiome-directed, tailored antimicrobial treatments versus current empirical therapy in the treatment of pulmonary exacerbations in CF patients positive for Pseudomonas aeruginosa. It is also the largest EU/US cystic fibrosis collaborative academic study to date. The trial has also given us the first longitudinal microbiome dataset (before/after antibiotic treatment) providing a unique opportunity to characterize the clinical characteristics and microbiome changes for patients before, during and after pulmonary exacerbations and treatment.

CFMATTERS has shown that you can successfully employ a microbiome derived antimicrobial therapeutic strategy in a clinical cohort with active bacterial infection. The study successfully enrolled 227 patients within a limited period of time across multiple different jurisdictions with varied healthcare modalities. The inclusion/exclusion criteria allowed for a well matched heterogeneous cohort of adult patients. This resulted in 106 patients across the nine clinical sites completing the trial to date. An important observation from the CFMATTERS trial was the willingness of clinicians across multiple centres with multiple treatment modality options to embrace this new technology either as part of the multidisciplinary consensus panel reaching an agreement on all patient treatments or as part of the clinical team on site. The study offered clinicians across all sites an alternative treatment option using this microbiome approach. The decision to adopt this approach was left to the clinician on site. Our data shows that 90% of the consensus panel recommendations were adhered to by clinicians on site.

CFMATTERS has demonstrated that adopting a next generation approach to antibacterial treatment is clinically safe. Based on analysis of the 106 patients that have completed the study the clinical outcome measures (FEV1, QoL,) in the treatment arm did not negatively impact on patients and was at worse equivalent to standard care. To date people have postulated that the microbiome maybe equivalent/ better with no robust data to support these claims. At this point in the study CFMATTERS can confirm that there is no treatment disadvantage. Moving forward the CFMATTERS rollover study will collect all the data on the remaining cohort of patients over the next 12 months which will definitively demonstrate if there is a treatment advantage in adopting the current protocol in a clinical setting.

At the inception of CFMATTERS five years ago using next generation microbiome derived treatment was a time consuming/ costly/ labour intensive approach. In the intervening years since the inception of the study, rapid diagnostic microbiome same day technology has become available. Utilising the microbiome data and clinical results from CFMATTERS will inform the development of same day rapid diagnostic microbiome reports to allow an even greater individualised/ real time approach in the treatment of bacterial infection. CFMATTERS utilises a model of acute/chronic infection in the lung and gut. However, it is important to recognise that our findings have applications beyond cystic fibrosis .Our data has implications not only in the treatment of other acute/chronic bacterial infections but also in minimising antibiotics side effects with the potential development of probiotics strains. Additional studies maybe necessary to quantify the success or not of this strategy.

Finally it must be noted that at this point in the study, CFMATTERS data does suggest that this technology is a treatment advantage for patients and therefore in the clinical arena adopting a labour intensive/costly approach to treating acute infection is not immediately necessary. Standard culture based technology at this point remains the appropriate approach for clinical services. A number of exciting new technologies have emerged in recent years with the promise to have greater accuracy and provide faster times to result in hopes of improving the provision of care and patient outcomes. However, the challenge in evaluating new methods lies not in the technical performance of tests but in defining the specific advantages of new methods over the present gold standards in a practicable way and understanding how advanced technologies will prompt changes in medical and public health decisions. With rising costs to deliver care, enthusiasm for innovative technologies should be balanced with a comprehensive understanding of clinical and laboratory ecosystems and how such factors influence the success or failure of test implementation. Often, enthusiasm to adopt new test methods outpaces evidence to support their routine use. The CFMATTERS rollover study will build on the data obtained during the CFMATTERS trial and help to provide robust data to help make the decision of whether microbiome derived therapy should be the new gold standard for treatment of patients with bacterial infections.

Education is another important impact of the study. It has allowed MDs, PhDs, and postdocs to acquire the core knowledge, skills and attitudes to establish them as new researchers in cystic fibrosis care with the subsequent development of dedicated research groups across Europe and the US.

The impact on European and US research is outlined below in accordance to the individual work packages.

4.1.2 WP05: Microbial activities and functions

Impact and future directions
The impact of the work performed is revealed from the publications that have resulted from funding. We have performed studied that have significantly advanced understanding of CF infections. For example, our work indicates that regional isolation drives bacterial diversification within CF lungs. While it was is known that bacterial lineages that chronically infect cystic fibrosis (CF) patients genetically diversify during infection, the mechanisms driving diversification were previously unknown. Our studies also improved understanding general and condition-specific essential functions of P. aeruginosa. The essential functions of a bacterial pathogen reflect the most basic processes required for its viability and growth, and represent potential therapeutic targets. The profile of essential genes revealed that P. aeruginosa is highly vulnerable to mutations disrupting central carbon-energy metabolism and reactive oxygen defenses. The essential function profile thus fundamental insights into P. aeruginosa physiology as well as identifying candidate targets for new antibacterial agents. Finally our preliminary studies suggest novel mechanisms that could cause CF exacerbations involving blooms of highly virulent P. aeruginosa variants. We have also developed new methods to test this idea, using the novel target capture sequencing approach.

Understanding the mechanisms responsible for stable or accelerated clinical decline could suggest novel approaches to slow disease. This goal is particularly important in chronic infections like those in CF and others, which cannot generally be eradicated. We plan future work to exploit the finding and methods developed here to understand mechanisms of disease flares in CF and understand the remarkable persistence of chronic infections despite vigorous immune defenses and antibiotics.

4.1.3 WP06: Human host defence response

Impact and future directions

There is an urgent need to develop new effective antimicrobials as the pipeline of antibiotic development has ceased. In addition, all of the most recently developed antibiotics are associated with antimicrobial resistance. This is important in a wide range of infections and particularly relevant in CF where the use of antibiotics is a constant feature. In this study, we evaluated a number of endogenous human antimicrobial peptides (AMPs) for their potential use as both stand-alone antibiotics and in combination with established therapies including tobramycin and ivacaftor. We found that the AMP, LL-37, had utility alone and in combination with tobramycin against CF relevant bacteria including Streptococcus and S. aureus. Other combinations of tobramycin and ivacaftor were also effective in reducing the growth of Streptococcus and S. aureus species. As mentioned above, the need for new antimicrobials is urgent and although some of the AMPs evaluated in this study, including LL-37, have therapeutic potential there are drawbacks associated with the use of the AMPs as they may be expensive to produce and cause systemic toxicity problems. Nevertheless, there is the potential for direct delivery of AMPs to the lung to avoid systemic toxicity and the demand for new AMPs may drive down the production costs of some of these peptides. In addition, the use of endogenous human AMPs may reduce immune-based or off-target effects of these peptides in the treatment of infection.

During infection, one of the body's strategies for dealing with bacteria is to produce innate host defence proteins (HDPs) many of which play a direct or indirect role in eradicating pathogens in the lung. As part of the clinical trial in CF MATTERS, in which CF patients undergoing exacerbation are treated with two different antibiotic regimens, we analysed the levels and/or activities of some key HDPs in sputum samples obtained from both patient cohorts before, during and following exacerbation. Although we are waiting for the trial to end, we did identify some potentially interesting HDP signals that may be of significance. One of these HDPs, cleaved SLPI (C-SLPI), was shown to increase during exacerbation in a similar way to human neutrophil elastase (HNE), which is known to increase during infection in CF. This finding indicates that C-SLPI could be evaluated further as a potential biomarker of exacerbation in CF and requires further investigation. We also observed an interesting feature with the protein cystatin C which decreases post-exacerbation to levels significantly below that observed before exacerbation and at the start of exacerbation. Therefore, cystatin C needs to be evaluated further for its value as a marker, or predictor, of exacerbation. Predictors of exacerbation have major utility in the treatment of patients prior to exacerbation. However, there is still a significant amount of work to be carried out to determine the value of any of these HDPs as biomarkers or predictors of exacerbation.

4.1.4 WP07: Lung microbiome

Impact and future directions

This project has brought together 3 European laboratories which utilise in vivo models to understand infection in the context of CF. This collaboration has resulted in a sharing of expertise and an enhancement in the research models used in each laboratory. In addition, we have developed a new model which more closely mimics infection in CF patients. This will strengthen ongoing CF research in Europe.

This research also represents the first comprehensive in vivo study on the impact of the lung microbiome on the outcome of P. aeruginosa. It is essential that this research is carried out in parallel to the clinical studies as it has provided insight into interactions between bacterial species that may not have been discernible purely from the human samples. These experiments may help to shape post-hoc analysis of the clinical studies. Additionally, the insight of protective communities within the lung may help facilitate future lung probiotic studies.

There is currently controversy around the use of anti-Staphylococcal prophylaxis in children with cystic fibrosis due to reports of earlier acquisition of chronic P. aeruginosa infection. These findings substantiate the most recent Cochrane systematic review which reports that at early ages P. aeruginosa is reduced in treated children. However, by 6 years old these findings are reverse. Extension of these findings may help us to understand the mechanism involved in these divergent outcomes and develop more effective therapies to limit P. aeruginosa infection.

The in vivo models utilised within this project have been significantly enhanced by the collaboration, however, since submission of this project, there have significant advancements in murine models and these have yet to be explored in the context of CF. Examples include; using mice with a humanised immune system, if the animals were reconstituted with CF patient PBMCs the impact of the skewed immunity seen in CF on the microbiome and the outcome of infection could be assessed. The animals used within these studies had an endogenous murine microbiome, the use of gnotobiotic mice may provide a 'cleaner' system in which to study the interaction between CF relevant lung microbiome constituents and pathogenic organisms. Furthermore, it may be possible to reconstitute both the GI tract and lungs of gnotobiotic mice with CF or healthy control human microbiota. A model combining all these elements would represent a step-change in preclinical models for CF. The work conducted within this project has demonstrated that under the right circumstances, the lung microbiome can reduce susceptibility to P. aeruginosa. This opens up the possibility of future investigates exploring the potential of lung probiotics to maintain lung health and limit chronic infection for CF patients.

4.1.5 WP08: Gastrointestinal microbiota

Impact and future directions
We have provided the largest longitudinal investigation into the gut microbiota of adult CF patients. We have demonstrated the impact that pulmonary exacerbation and its associated treatment has on the CF gut microbiota. Our findings show that during exacerbation, there is a decrease in diversity and in beneficial bacterial populations. This presents the opportunity for therapeutic intervention e.g. through probiotic therapy to help minimise changes to the gut microbiota, with the aim of maximising health outcomes. As the largest longitudinal gut microbiota study in an adult CF cohort, we provide invaluable information to help advance our understanding of how the CF gut is altered during different stages of pulmonary disease and this will help us to design targeted interventions to help minimise the impact of such therapies on beneficial gut bacteria.
Furthermore, we can use such information to advance our understanding of the lung-gut axis and investigate how changes in the gut microbiota impact on the lung microbiota. This could have significant impact for the clinical community, if populations change in the CF gut presage changes in the lung microbiota, which are intrinsically linked to the health of the CF patient, the gut then becomes a viable target for manipulation to benefit the lung microbiota. Our data has shown correlations between the microbiota of these two environments, implying a role of gut microbiota in lung health. As pulmonary disease is the leading cause of death in CF patients, understanding all factors contributing to pulmonary health and infections will advance our ability to improve clinical care and patient outcomes.
Furthermore, we have significantly advanced our understanding of the carriage rate of C. difficile within the CF community, which will enable us to further investigate why this cohort has such high asymptomatic carriage rates. Our study is the largest multi-centre, longitudinal study of C. difficile carriage in CF adults. We have demonstrated an even higher rate of C. difficile carriage is recorded when you consider carriage as any positive C. difficile culture at any visit timepoint. Comparing this data to existing data that relied on carriage rates measured at just one timepoint, demonstrates the need for longitudinal studies to ensure accurate capturing of carriage rate data. We have also demonstrated the diversity of ribotypes present in CF patients and how such ribotypes change longitudinally. This presents us with the opportunity to further

investigate such ribotypes to determine their genetic similarity and may enable greater understanding of the transmission of different ribotypes between patients.

Future work will include extensive interrogation of the gut data and the C. difficile data to fully understand the changes seen to the gut in CF, the mechanisms involved in asymptomatic carriage of C. difficile in CF patients and work towards developing beneficial interventions to maximise health outcomes.

4.1.6   WP10: Dissemination, Outreach and Training

A plan for the future dissemination and exploitation is in place for CFMATTERS.

Smartphone Application 1: CF Outpatient Data Analytics Smart Phone App
Impact: The impact of this smartphone game is its ability to engage and motivate the user. In addition to this it collects data that has the potential to impact the health care team.

Future Works: The data recorded through this stress test found a possible correlation between gameplay ability and self-reported therapy compliance. However, further testing and interpretation is needed for this exploratory data. Future works for this would include more testing with a larger group. Investigating the use of a smartphone spirometer to be amalgamated into the system. Lastly, the development of more games for CF adults based on popular games found on the app stores.

Dissemination, Outreach and Training: CF Patient Passport App
Impact: To date, a paper or digital CF passport is not publicly available for patients. By creating a digital CF passport, patients can have access to their basic medical information. This will allow patients to receive care when travelling abroad and between CF centres. It may also encourage these CF adults to become active participants in their own healthcare. It is also anticipated that this app will improve patient compliance as it will allow patients to become more aware of their own symptoms.

The development of this and the previous smartphone app has also lead to the publication "A General mHealth Design Pipeline". It is anticipated that this publication will aid mHealth designers and developers in future mHealth projects.

Future Works: Future works include the completion of the app beta test. Once this is completed, patient feedback captured via a questionnaire will be incorporated into the app. The app will then undergo a review/certification process before being deployed to app stores.

What is a Portacath, Gastrostomy Tube, Bronchoscopy?
Impact: Three educational resources which can be accessed by CF patients anywhere with an internet connection. All content was validated by the CF multidisciplinary team.

Future Works: include further collection of data pertaining to device interactions. Further e-Learning tools can also be created using the same framework.

3D Visualisation Tool: The Lung Experience
Impact: 4th and 5th year medical students on respiratory attachments are given free or "non-contact" hours in order to study. Hence the Lung Experience can be of benefit to these students as it is an active/practical learning tool that can complement the students practical learning during respiratory attachments. Moreover, seven of the eight participants agreed they would like more VR to support their learning.

Future Works: Although the VR industry is proliferating fast, there are still limitations towards hardware cost and reliance. Hence future works would include the porting of the current VR tool to become compatible with mobile VR. By doing so medical students can explore the Lung Experience via their smartphone and mobile VR headsets. Such headsets include the Google Carboard which costs less than EUR3.00.

The Lung Experience can also be used in an outreach or patient context. It will also be integrated into the European Cystic Fibrosis online learning platform. The technology will also be used at the Irish Thoracic Society for patients and family members to use. Future works would include evaluating this system in such a context.

Virtual reality (VR) is considered to be the "next big thing" in technology and was estimated to be worth $2.7 billion in its first consumer year (2016). In addition to this, VR devices are predicted to be as commonly owned as smartphones in the coming years.

Virtual reality educational applications and software have proven effective for teaching and reifying complex or abstract concepts. A recent project by INVIVO Communications shows that exposure to computer generated 3D medical models in a virtual environment with 360 degree of freedom using the Oculus Rift is engaging among medical professionals and consultants. Other medical VR examples include the collaborative project between Rémi Rousseau and the MOVEO Foundation, which utilise head mounted displays (HMD) and 360 degree video recordings. This project allows students to watch surgical procedures from the surgeon's first person perspective. As these video recordings are 360 degrees, the student also has the freedom to look around the theater to observe the activities of the entire surgical team. Another use for VR in medical education can be seen in the "World of Comenius" project, where the user wears a HMD and interacts with a 3D anatomical educational model through touching and grabbing individual components. The user can also teleport within a 3D animation of a blood vessel or brain tissue, depending on the anatomical areas uncovered.

To date VR has been used in therapy simulation, surgery simulation, DNA visualisation, and many more medical spaces. VR offers medical professionals and patients:

- Total Immersion and a feeling of presence or "being there"
- Visually rich environments
- Natural and interesting interactions
- Opportunity for practical learning to support instruction

Specifically for patients, VR has been used for:
- Pain and stress relief
- Self-Management
- Cognitive rehab for stroke victims
- Social skill for autism
- Anxiety relief
- Paralytics muscle control
- Visualising health

Smart phone App
Impact: The impact of this smartphone game is its ability to engage and motivate the user. In addition to this it collects data that has the potential to impact the health care team.

Future Works: The data recorded through this stress test found a possible correlation between gameplay ability and self-reported therapy compliance. However, further testing and interpretation is needed for this exploratory data. Future works for this would include more testing with a larger group. Investigating the use of a smartphone spirometer to be amalgamated into the system. Lastly, the development of more games for CF adults based on popular games found on the app store.

4.2 Main dissemination activities

During the project lifetime CFMATTERS conducted various dissemination activities aimed at promoting its research to the widest and varied audience possible. The Dissemination Plan (DP) was released developed and signed off by all consortium members.. The DP provided guidelines for dissemination activities by project partners by presenting information on the dissemination strategy of the project, the aim of the dissemination actions, the communication and dissemination tools to be used, and the activities and mechanisms for information exchange with various stakeholders.

Key dissemination tools and activities conducted by the project are described below. Details can be found in WP10 above.

- **Project website**: The website, www.cfmatters.eu, It has been used as an important dissemination channel, describing project activities and outcomes such as latest news, articles, presentations, internal and external documents.

- **TCC Helpdesk services:** Helpdesk services were provided to the project community, they have been implemented through the use of:
    - The TCC contact email
    - The TCC contact number
    - A contact email specific for the Final Report
- **Logo, PPT and deliverable templates**: A logo was created to establish an identity for the project. Templates for PowerPoint presentations and deliverables were designed to ensure congruent presentation to external audiences., permitting unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

- **Dissemination database**: Project contacts were consolidated into a mailing list (all@cfmatters.eu) these were used for
    - Project communication
    - Meeting arrangements
    - Experts identified during the preparation of the project
    - Partners' contacts

- **eNewsletter:** e-Newsletters were disseminated during the project period, highlighting project activities, outputs, upcoming events and other information of interest. e-Newsletters

- **Press releases**: Press releases were published during the project period to world news media, various cystic fibrosis and research groups related mailing lists and the project dissemination database.

- **Project documentation**: Dissemination material produced by the project include:
    - Posters (information and research results for conference presentations)
    - Leaflet summarising the concept of the project
    - Brochure presenting the project key outcomes

- Publications/Presentations. Please see dissemination activity list provided in TEMPLATE A2 - LIST OF DISSEMINATION ACTIVITIES.

- **Social Media:** The social media accounts of the project (i.e. LinkedIn and Facebook) were created .They are accessible from the project website.

- **Calendar:** A Google Calendar was created to monitor the contribution of partners in dissemination activities and ensure their timely delivery. The different project deliverables, milestones and prospective events were added to this calendar to give a clearer vision of project activities and expected outputs. A calendar file was then shared with all partners to be integrated into their own calendar. They would then receive reminder emails to (i) manage their deliverables/milestones and to (ii) publish news on project tasks, pilot's achievements, etc.

- **Project trainings:** Training was provided to all study staff throughout the study. Refer to WP01.

4.3   Exploitation of results

The CFMATTERS consortium will continue to advertise its outcomes beyond the project duration, promoting the original objectives of the trial. CFMATTERS has developed new research techniques which will impact future cystic fibrosis research within the consortium and the wider scientific community. CFMATTERS results will be presented at a dedicated symposium at the 2018 European Cystic Fibrosis Society conference as well as the upcoming North American Cystic Fibrosis Conference in November 2017.

The activities organised by the project and the outcomes obtained have led to new ideas for research proposals and plans to better integrate existing projects. As a result of this, contacts with potential funding organisations is being carried out to provide longer-term funding opportunities.

4.4   CFMATTERS rollover study

227 patients were enrolled for CFMATTERS, the final results were carried out on 107 patients (completed month 3). The consortium at the final General Assembly agreed to continue to collect data on all patients that have not completed the trial to date. This will allow us to have a complete dataset, with samples collected at all time points for all patients. This additional data will further elucidate our results to date. Ethical approval has been received from sites to continue with the rollover study

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