A protocol for emulating a published randomised controlled trial using registry data: effects of azithromycin in young adults with cystic fibrosis

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Abstract

Introduction Target trial emulation can be used to evaluate the effects of treatments using observational data. The trial emulation approach involves specifying key elements of a protocol for a target trial (a randomised controlled trial designed to address the question of interest) and then describing how best to emulate the trial using observational data. Recent years have seen an uptake of target trial emulation in several disease areas, although there are limited examples in cystic fibrosis. This protocol describes a study which aims to assess the applicability of target trial emulation in cystic fibrosis (CF). We aim to emulate an existing trial in CF and assess to what extent the results from the trial can be replicated using registry data.

Methods and analysis We aim to emulate a published trial (i.e., the target trial) which found evidence for beneficial effects of azithromycin use on lung function in young adults with cystic fibrosis. Two emulated trials are planned: one using data from the UK CF Registry and one using data from the US CF Registry. The inclusion and exclusion criteria, treatment and outcome definitions, follow-up period, and estimand of interest are all designed to match the published trial as closely as possible. Inverse-probability-of-treatment weighting will be used in the emulated trials to account for confounding bias. Results obtained in the emulated trials using registry data will be compared to the results obtained in the published randomised controlled trial.

Ethics and dissemination Ethical approval has been granted by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref: 29609). This study has also been approved by the UK CF Registry Team and the North Star Review Board. The results of this study will be published in a peer-reviewed journal and presented at relevant scientific conferences.

Strengths and limitations of this study

- We use data from the UK and US CF Registries. These are the two largest national CF registries, and the UK CF Registry is cited as an exemplar patient registry in the NICE real-world evidence framework.
- We use the target trial emulation approach. This approach helps to clearly articulate the study design and to avoid certain biases. We provide an example of target trial emulation in a disease area where there are limited applications.
- The CF registries do not contain data on treatment doses or adherence, which limits our ability to match the treatment strategies in the target trial precisely.
- The CF registries do not contain data for all secondary outcomes used in the target trial.

Word count: 3926

1. Rationale and background

Randomised controlled trials (RCTs) are the gold standard approach for evaluating the effects of treatments. However, RCTs are expensive, and sufficiently large trials are not always feasible, particularly in patient populations with a rare disease, such as cystic fibrosis (CF). When an RCT is not feasible, an alternative is to use observational data to 'emulate' a trial [1]. The trial emulation approach involves specifying key elements of a protocol for a target trial (an RCT we would like to conduct, if it were feasible) and then describing how best to emulate the target trial using the observational data at hand. This approach combines the study design principles of RCTs, with an analysis appropriate for observational data.

Recent years have seen an uptake of target trial emulation in several disease areas [2-7]. There is also rising interest in emulating existing RCTs in an attempt to replicate the results from the existing RCTs using observational data. The RCT DUPLICATE initiative recently published the results of 10 trial emulations which used insurance claims data to replicate existing trials in cardiovascular disease [8]. They found that agreement in findings between RCTs and emulated trials varied depending on which agreement metric was used. In 9 of 10 cases, differences between effect estimates from the RCT and emulated trial were within expected random variation; however, results from emulated trials matched the direction and statistical significance of results from RCTs in only 6 of 10 cases. Matthews et al [9] used Swedish registry data to emulate the Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE) randomised trial [10]. Admon et al [11] used target trial emulation to predict results of the PreVent (Preventing Hypoxemia with Manual Ventilation during Endotracheal Intubation) Trial [12] before they were published.

Despite the widespread use of target trial emulation across other areas of medicine, there are limited applications within the CF literature [13]; thus, its applicability to CF remains unclear. We aim to assess the applicability of target trial emulation in CF using data from the UK and US CF Registries by emulating a published RCT within CF, and assessing the extent to which the RCT findings could be replicated. This protocol describes the proposed analysis plan for our trial emulation. We follow the reporting guidelines recommended in the HARmonised Protocol Template to Enhance Reproducibility (HARPER) [14].

2. Research question and objectives

The primary objective of this study is to emulate a published RCT in CF [15] and assess the extent to which the RCT results can be replicated using registry data. The RCT of Clement et al [15] provides the target trial that this study aims to emulate. Table 1 summarises the research question addressed in the target trial.

3. Data Sources

UK CF Registry

The UK CF Registry is a national database sponsored and managed by the Cystic Fibrosis Trust, with UK National Health Service research ethics approval. It records longitudinal data on approximately 99% of people with cystic fibrosis in the United Kingdom [16,17].

Data is collected on time-invariant variables, such as sex at birth, *CFTR* genotype, date of birth, diagnosis data, and longitudinal variables that change over time. Longitudinal data are collected at approximately annual review clinic visits and covers several domains including clinical measurements, hospital admissions, chronic medications, culture and microbiology, health complications, nutrition, physiotherapy, smoking, socioeconomic status, and outcomes (death and transplants).

US CF Registry

The US CF Registry began collecting data on CF patients in the United States in 1986 and is managed by the Cystic Fibrosis Foundation. It contains longitudinal information on approximately 80% of CF patients in the United States [18, 19].

Like the UK registry, data is collected on demographic characteristics and longitudinal variables that change over time. Data collection is organized around encounter visits at CF care centres, as well as annualized data collected in an interview format. Encounter visit data include relevant information regarding hospitalizations, clinical measurements, medication usage, culture and microbiology, and health complications. Annualized data capture relevant microbiology, nutritional and pulmonary outcomes, CF therapies, including CFTR modulator usage, complications, including Cystic Fibrosis-related diabetes (CFRD), transplantation outcomes, and survival. Since data is collected at both encounter visits and through an annual interview, where relevant, we will consider using yearly averages of longitudinal data to match the annual nature of the UK data collection process.

4. Research Methods

4.1 Study design

We will conduct two studies nested within existing longitudinal data sets (one using UK CF Registry data, one using US CF Registry data), designed using the target trial emulation framework. Table 2 summarises the key components of the target trial and emulated trials.

4.2 Study design diagram

Figure 1 describes the key design choices in our emulated trials. We make assumptions about the temporal ordering of variables measured at the same time-point and these assumptions are made explicit in the directed acyclic graph (Figure 2, Section 4.3.3).

4.3 Setting

4.3.1 Time-periods

The target trial was conducted from 2001 to 2003. We plan to consider three time periods for the emulated trials within the timeframe 2003-2018 (Table 3). Starting at 2003 allows a couple of years after publication of the early azithromycin trials [15,20] for the use of azithromycin to uptake in clinical practice. Ending the timeframe in 2018 means that we only use data from the time before CFTR modulators became widespread in clinical practice.

Table 3 defines the three time-periods considered in this study. For each time-period, the “recruitment-period” is the first two years within that timeframe, i.e., for 2003-2005, the “recruitment-period” is 2003-2004. This is defined as the period for which individuals are considered for inclusion in the study and is the same length of time as the recruitment period in the target trial. Individuals are included if they meet the inclusion and exclusion criteria in at least one of their annual reviews during the recruitment-period. Time 0 is defined as the date of annual review in which individuals meet the inclusion and exclusion criteria. For individuals who meet the criteria in both years during the recruitment period, time 0 is defined as the date of the first annual review in the recruitment period. Time 1 is defined as the date of the subsequent annual review and we assume that annual reviews are approximately 12 months apart.

4.3.2 Inclusion and exclusion criteria

The inclusion and exclusion criteria for the emulated trials are designed to match the criteria in the target trial as closely as possible. Table 4 summarises the inclusion and exclusion criteria for each emulated trial.

The data on liver function test results and serum creatinine levels (exclusion criteria 3 and 5) may have large amounts of missingness and therefore unusable. An alternative is to use indicator variables for any recorded non cystic fibrosis-related liver disease (for exclusion criteria 3), or chronic kidney disease (for exclusion criteria 5) at time 0.

Individuals who meet the inclusion criteria during the first year of the recruitment period, but then do not meet the exclusion criteria at that time will be reconsidered for inclusion in the second year of the recruitment period.

4.4 Variables

4.4.1 Treatment strategies

The active and comparator treatment strategies used in the target trial are provided in Table 2. We aim to match these strategies as closely as possible; however, the target trial specifies doses and frequency of treatment, and this information is not available in the UK or US CF registries.

For both the UK and US emulated trials, the active treatment is prescription of prophylactic oral or chronic oral azithromycin and the comparator is no prescription of prophylactic oral or chronic oral azithromycin.

Individuals who are recorded as taking the active treatment at time 1 are in the active treatment group, individuals who are not recorded as taking the active treatment at time 1 are in the control group.

4.4.2 Outcomes

Where possible, the emulated trials will replicate outcomes studied in the target trial; however, data are not available in the registries for all secondary outcomes. The primary outcome for both emulated trials is absolute FEV_1% at time 1. Secondary outcomes include:

- Prescription of IV antibiotics at time 2.
- FVC measured at time 1.
- BMI z-score measured at time 1.

FEV_1% is calculated using the Global Lung Initiative equations [21] and BMI z-scores are calculated using the WHO reference distribution [22].

4.4.3 Covariates

In the target trial, individuals were randomly allocated to the treatment or placebo strategy. In the emulated trials, randomisation is not possible. Instead, treatment decisions will be based on clinician and patient preference and therefore the association between prescription of azithromycin and FEV_1% is believed to be confounded by a number of factors. Based on clinical expertise, we developed a directed acyclic graph (DAG) to depict our assumed casual relationships between the variables in the UK and US CF registry data, and inform which factors need to be controlled for (Figure 2).

According to our DAG, the list of confounding variables that we should adjust for are as follows (we use data recorded at time 1 unless otherwise specified): age, number of days on intravenous antibiotics (IV days), non-IV hospital admissions, presence of *P. aeruginosa*, *Staphylococcus aureus* or Nontuberculous Mycobacteria, cystic fibrosis related diabetes (CFRD), use of hypertonic saline, inhaled antibiotics or DNase and rate of decline in FEV_1%, BMI z-score at time 0 and FEV_1% at time 0.

Age, FEV_1% and BMI z-score are continuous. Rate of decline in FEV_1% is calculated as difference between the absolute FEV_1% measured at time -1 and time 0. Data on treatment prescription, presence of infections, CFRD diagnosis, pancreatic insufficiency and non-IV hospital admissions will be binary indicators. Indicators for pancreatic insufficiency and non-IV hospital admissions are created using existing variables in the data (see Table A.1 in the appendix). Registry data provide dates for treatment with IV antibiotics (at home or hospital). These data will be used to create a variable indicating number of days on IV antibiotics since last annual review (including treatment administered at home and hospital). IV days will then be treated as a categorical variable with four categories: 0, 1-14, 15-28, 28+.

4.5 Data analysis

The following data analysis plan will be implemented in both the UK and US emulated trials.

4.5.1 Causal estimands of interest

The target trial reported the difference in mean changes (between month 0 and month 12) in FEV_1% between treatment groups in the total population, and subpopulations based on *P. aeruginosa* infection status. Let A denote an indicator variable for treatment strategy ($A = 0$ indicates no prescription of azithromycin and $A = 1$ indicates prescription of azithromycin).

Let $Y_t(0)$ denote the potential outcome under treatment $A = 0$ at time $t, t \in \{0,1\}$ where $t = 1$ is 12 months after $t = 0$. Similarly, let $Y_t(1)$ denote the potential outcome under treatment $A = 1$ at time t. Additionally, let PA_t denote *P. aeruginosa* infection status at time t. Then, the causal effects of interest in the target trial are defined as:

Difference in mean change in FEV_1% in total population:

$$E((Y_1(1) - Y_0(1))) - E((Y_1(0) - Y_0(0))) \quad (1)$$

In randomised controlled trials, this is equivalent to the difference in means at the end of follow-up (see Appendix):

$$E(Y_1(1)) - E(Y_1(0)) \quad (2)$$

In observational data, it is recommended against analysing change scores [23] and therefore we estimate the difference in means at the end of follow-up in the whole population, and subgroups defined by *P. aeruginosa* infection (Table 5).

4.5.2 Main analysis

We use inverse-probability-of-treatment weighting (IPTW) to account for confounding bias. This involves assigning weights to each individual to create a pseudo-population in which the distribution of baseline confounders is balanced between treatment groups. The weights are based on propensity scores, defined as the probability of treatment conditional on baseline characteristics:

$$PS = P(A = 1|\boldsymbol{C} = \boldsymbol{c})$$

where $\boldsymbol{C}$ is a vector of confounding variables (given in section 4.4.3). Propensity scores will be estimated using logistic regression with an indicator for azithromycin prescription as the outcome and confounders as the predictors. To allow flexibility, all interaction terms between treatment and confounders will be included in the propensity score model.

The weights ($IPT_{weights}$) are defined is the inverse probability of receiving the treatment received:

$$IPT_{weights} = \frac{A}{PS} + \frac{(1 - A)}{(1 - PS)}$$

The following models will then be fitted in the weighted population:

$$Y = \beta_0 + \beta_A A + \varepsilon \quad (3)$$

$$Y = \gamma_0 + \gamma_A A + \gamma_{PA} PA + \gamma_{A.PA} APA + \varepsilon \quad (4)$$

The estimated treatment effect in the whole population is given by $\hat{\beta}_A$. Estimated treatment effects in individuals infected with *P. aeruginosa* and individuals not infected with *P. aeruginosa* are given by $\hat{\gamma}_A + \hat{\gamma}_{A.PA}$ and $\hat{\gamma}_A$.

A robust standard error estimator will be used, which takes into account the fact that the propensity score is estimated [24].

Additional analysis in the UK Emulated Trial making use of data on treatment prescription dates

A limitation of this approach is that it assumes individuals with $A = 1$ at time 1 have been taking azithromycin for the past 12 months. Realistically, individuals may initiate treatment with azithromycin

at any time between time 0 and time 1. In the UK data, prescription dates data are available from 2016 onwards. Therefore, in the most recent time-period, we can conduct a second analysis using the UK data and making use of the dates data.

We define a new time variable, t^*, which measures time in months. For treated individuals, $t^* = 0$ on the first date they are prescribed azithromycin during times $t = 0$ and $t = 1$. For control individuals, $t^* = 0$ on the day of the annual review at time 0. We then fit the following models in the weighted data:

$$Y_{t^*=12} = \delta_0 + \delta_A A + \delta_{t^*} t^* + \delta_{At^*} A t^* + \varepsilon \quad (5)$$

$$Y_{t^*=12} = \alpha_0 + \alpha_A A + \alpha_{PA} PA + \alpha_{A.PA} APA + \alpha_{t^*} t^* + \alpha_{At^*} A t^* + \varepsilon \quad (6)$$

Where $Y_{t^*=12}$ is FEV_1% measured on the day of the annual review after $t^* = 0$ and closest in time to $t^* = 12$.

Model (5) will be used to estimate the treatment effect in the whole population and Model (6) will be used to estimate treatment effects conditional on *P. aeruginosa* infection.

Diagnostics

The distribution of weights will be assessed using boxplots. Methods such as stabilisation, trimming or truncating will be considered if there are extreme weights (which can lead to unstable effect estimates). Standardised mean differences will be used to compare the balance in the distribution of confounders between treatment and control groups in the original and weighted samples.

4.5.3 Sensitivity analyses

Sensitivity to the no unmeasured confounders assumption

Our analysis relies on the assumption that there are no unmeasured confounders. Unfortunately, there may exist some factors that are associated with both treatment prescription and outcome, which are not captured in the registries (denoted by U in Figure 1). Sensitivity to unmeasured confounders will be summarised using E-values [25].

Allowing individuals to enter the emulated trials more than once

For the main analysis, individuals will be included in the emulated trial once. Individuals "enter" the trial at time 0, which is defined as the earliest year within the recruitment period that they meet the inclusion and exclusion criteria. This approach restricts the analysis to using information from everyone at one time point only and may be inefficient. Alternatively, we can allow individuals to "enter" the trial twice if they meet the inclusion and exclusion criteria in both years during the recruitment period. Standard errors will need to take into account that individuals are included multiple times.

4.5.4 Missing data

The amount of missing data in each variable will be summarised in tables by treatment group.

Where there is missing data in binary time-varying variables that are usually static for long time periods, we will use a simple imputation approach. For missing visits where the prior visit and subsequent visit are equal, we will assume the missing value is also equal and impute accordingly. This approach will be used for the following variables: pancreatic insufficiency, *P. aeruginosa*, *Staphylococcus aureus*, NTM, CFRD, inhaled antibiotics, inhaled steroids, hypertonic saline and DNase.

The remaining missing data will be dealt with using multiple imputation by chained equations, which assumes that data are missing at random. The number of imputed datasets used will be determined using a two-stage procedure recommended by von Hippel [26].

4.5.5. Validation of results against the target trial

We will validate our results against the target trial by determining whether results from the emulated trials are compatible with the target trial. Three measures of success are used:

(1) do the emulated trials replicate the direction and statistical significance of the target trial?

(2) do the estimated effects from the emulated trials lie within the 95% confidence intervals reported in the target trial?

(3) is there a difference in the results from the emulated trials and the target trial?

These are the criteria that were used in the RCT DUPLICATE Project [8]. To assess criteria (3), we implement the approach used by Admon et al [11]. This involves calculating the difference in difference estimate (i.e., the difference between the target trial effect estimate and emulated trial effect estimate) and obtain 95% confidence intervals for the difference using bootstrap with 10,000 replicates. Emulation is considered a success if 0 lies within the bootstrap confidence interval for the difference in difference estimates.

5 Limitations

If there are differences in the results between the target trial and emulated trials, we will seek to explain the reasons for these differences. Here, we identify a number of potential reasons we may observe differences in the results, due to either limitations regarding data availability in the registries, differences in sample size, or differences in the study populations.

Data availability

The target trial specified a particular dose of azithromycin depending on an individual's weight. The trial also reported a high adherence, estimated at 95% for azithromycin and placebo. Neither the UK nor US Registry provide details on treatment doses or adherence and must rely on data for treatment prescription. It is possible that individuals in the registry will take different doses to those given in the target trial or did not take their prescribed treatment at all.

The emulated trials cannot replicate the exclusion criteria of the target trial precisely. For example, the target trial includes a criterion based on liver function tests: individuals are excluded if they have liver disease with liver function tests more than twice the laboratory upper limit. In the UK Registry, closest variable to this criterion is an indicator for acute liver failure with liver function tests greater than three times the laboratory upper limit. In the US Registry, data is not collected on liver function tests and instead, a binary indicator for liver disease will be used. Moreover, the target trial includes a criterion based on serum creatinine and creatine clearance levels. Although this is collected in the UK Registry, we expect large amounts of missingness. An indicator function for kidney disease will be used a proxy for this criterion, where data on serum creatinine levels are not usable.

The target trial calculated the outcome, FEV_1%, using the Knudson equations [27]; we plan to use the Global Lung Initiative (GLI) equations in the emulated trial [21]. However, previous research suggests that results will be minimally affected by choice of reference equations [28].

The main analyses in both the UK and US emulated trials will use data from consecutive annual review visits. We assume that the annual review visits are 12 months apart and that individuals in the treatment group were taking azithromycin for the 12 months in between visits. Realistically, the visits are only approximately one year apart, and individuals may begin treatment with azithromycin at any time during the time between visits. We address this limitation to some extent in an additional analysis for the UK Registry data, in which we incorporate prescription dates data.

Finally, our analysis relies on the assumption of no unmeasured confounders. It is possible that there are some factors associated with both azithromycin prescription and the outcome, that are not collected in the registry. If there are confounders that we have not controlled for in the analysis, our results would be biased. This is an important assumption which is untestable using the observed data

and so we plan a sensitivity analysis to assess how sensitive our results are to unmeasured confounders.

Sample size

The target trial included 82 individuals (40 in the treated group and 42 in the placebo group). The authors note in their discussion that it is possible the study was not adequately powered to detect significant differences in FEV_1%.

We have not performed sample size calculations for the emulated trials and there is some debate as to whether sample size calculations are needed in studies using observational data [29-31]. We plan to use all the available data in the UK CF Registry, or US CF Registry, and expect much larger sample sizes than were observed in the target trial.

Differences in the study populations

Ideally, we would conduct the emulated trials using data from a similar time-period as the target trial, to ensure homogeneity in the clinical settings. However, in 2007, the UK CF Registry introduced a new web-based data collection system which improved data collection and data quality. The target trial was conducted from 2001 to 2003 and so if we restricted the emulated trials to this period, we would not make use of the years with higher data quality. On the other hand, using later years could result in differences in the clinical setting between the emulated and target trials. For this reason, we have suggested multiple time periods for the emulated trials and will compare results between time periods.

The geographical locations are different between the target and emulated trials. The target trial was conducted on individuals with CF recruited in France, whereas the emulated trials will focus on individuals with CF from the UK or US. Moreover, there may be differences in distribution of patient characteristics, or severity of disease between the trials. In the target trial, on average, the individuals were young (mean age at baseline: 11 years) with mild lung disease (average FEV_1% at baseline: 85.5). Although we impose the same inclusion criteria regarding age and FEV_1% at baseline, the samples in the emulated trials may be on average, older, with worse lung disease at baseline.

6 Ethics and dissemination

UK Emulated Trial

This project will use anonymised data from the UK Cystic Fibrosis Registry, which has Research Ethics Approval (ref: 07/Q0104/2) and from the US Cystic Fibrosis Registry, which has IRB approval (Ref: XXXX). No additional data beyond that contained in the registries will be collected for the project. Ethical approval has been granted by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref: 29609). The study has also been approved by the UK CF Registry Team and the North Star Review Board.

We plan to publish the results of this study in a high-ranking peer-reviewed journal. Findings will also be presented at relevant scientific conferences such as the European Cystic Fibrosis Conference, the North American Cystic Fibrosis Conference, and the International Society for Clinical Biostatistics.

7 Conclusions

This work is being undertaken by the CF Trial Emulation Network, a new multidisciplinary international collaborative network. We plan to replicate multiple trials on the effects of the use of azithromycin on health outcomes for people with cystic fibrosis.

This work will contribute to the evidence base for the target trial emulation approach in CF. If the trial emulations are a success, we could extend the research to study questions beyond the trial. For example, the longer-term effects of azithromycin, effects of azithromycin use on other outcomes such as risk of NTM infection, or combination effects of multiple treatments. Such questions are often difficult to study in RCTs due to additional costs or lack of statistical power.

If we can consistently replicate the results of existing trials, then we can gain confidence in future studies using registry data to investigate questions that may not be answered in a RCT.

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Availability of data and materials

UK Registry Data are available following application to the UK CF Registry Research Committee. https://www.cysticfibrosis.org.uk/the-work-we-do/uk-cf-registry/apply-for-data-from-the-uk-cf-registry.

Authors contributions

All authors contributed to the study question and design. EG wrote the first draft of the protocol manuscript. All authors contributed to further drafts and approved the final version.

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Competing Interests
GD reports speaker honoraria from Chiesi Ltd and Vertex Pharmaceuticals. RHK reports a speaker honorarium from Vertex Pharmaceuticals.

Appendix

Proof that the estimands defined in equations (1) and (2) are equal in RCTs

Note that causal inference analyses in RCTs assume exchangeability (i.e. $\left(Y_t(1), Y_t(0)\right) \perp A$) and consistency (i.e. $A = a \Rightarrow Y_1 = Y_1(a)$). Furthermore, randomisation ensures that the observed outcome at time 0 is independent of treatment (i.e., $Y_0 \perp A$). Given these assumptions:

$$
\begin{aligned}
E[Y_0(0)] &= E[Y_0(0)|A=1] && \text{By exchangeability} \\
&= E[Y_0|A=1] && \text{By consistency} \\
&= E[Y_0] && \text{Since } Y_0 \perp A
\end{aligned}
$$

Therefore:

$$
\begin{aligned}
E[Y_1(1) - Y_0(1)] - E[Y_1(0) - Y_0(0)] &= E[Y_1(1)] - E[Y_0(1)] - E[Y_1(0)] + E[Y_0(0)] \\
&= E[Y_1(1)] - E[Y_0] - E[Y_1(0)] + E[Y_0] \\
&= E[Y_1(1)] - E[Y_1(0)]
\end{aligned}
$$

This result is not true in non-randomised studies, it is not true that the outcome at time 0 is independent of treatment.

Table A.1: Details on the variables used in the UK CF Registry

Variable	Code Name	Description	Further details
Inclusion Criteria			
Age	age	age (in years) at annual review	
FEV_1%	gli_percpredfev	FEV_1% taken on the day of the annual review	Calculated using the Global Lung Initiative Equations
Exclusion Criteria			
Intolerance to macrolides [criteria 1]	s04drugintolerancemacrolideantib	Indicator variable for drug intolerance to macrolide antibiotics	NA
Prescription of macrolides [criteria 2]	tr_macrolides_prophylacticoral trtype_chronoralantibio_macrolid	Indicator for prescription of macrolides since last annual review	New indicator variable for prescription of macrolides (M) will be created. $M = 1$ if either of the listed variables are equal to 1. $M = 0$ if both of the listed variables are equal to 0.
Acute liver failure [criteria 3]	s06cmpshepatobilaryacuteliver	Indicator for Acute liver failure (no underlying liver disease, ALT >3x ULN, INR > 2, not responsive to vitamin K)	An indicator for any liver disease (s06cmpsliverdisease) will be used if missingness is too great in this variable.
Cirrhosis with portal hypertension [criteria 4]	s06cmpscirrhosis	Indicator for cirrhosis with portal hypertension	NA
Serum creatinine [criteria 5]	s03serumcreatinine	Serum creatinine	If the data quality in this variable is poor, an indicator for chronic kidney disease will be used instead (s06cmpsrenalfailure)
DNase [criteria 6a]	tr_dnase	Prescription of DNase since last annual review	NA
Inhaled tobramycin [criteria 6b]	tr_tobramycindrypowderinhaled tr_tobramycinsolutioninhaled	Prescription of tobramycin since last annual review	New indicator variable for prescription of macrolides (T) will be created. $T = 1$ if either of the listed

			variables are equal to 1. $T = 0$ if both of the listed variables are equal to 0.
Inhaled steroids [criteria 6c]	trtype_corticost_inha trtype_corticost_inhacombobronch	Prescription of any inhaled corticosteroids since last annual review Prescription of any inhaled corticosteroids combined with bronchodilators since last annual review	New indicator variable for prescription of inhaled steriods (IS) will be created. $IS = 1$ if either of the listed variables are equal to 1. $IS = 0$ if both of the listed variables are equal to 0.
Treatment			
Prescription of azithromycin	tr_azithromycin_prophylacticoral tr_azithromycin_chronicoral	Prescription of azithromycin since last annual review	New indicator variable for prescription of macrolides (A) will be created. $A = 1$ if either of the listed variables are equal to 1. $A = 0$ if both of the listed variables are equal to 0.
Outcomes*			
FEV_1%	gli_percpredfev	FEV_1% measured on the day of the annual review	Calculated using the Global Lung Initiative equations
FVC			
BMI z-score	bmi age dmg_sex	BMI taken on the day of review Age on the day of the review Sex	Variables used to calculate BMI z-scores using the WHO reference distribution
IV days	s02homeivoveralltotaldays s02hospivoveralltotaldays	Number of days on IV treatment at home since last visit Number of days on IV treatment in the hospital since last visit	Listed variables are used to calculate total number of days on IV antibiotics since last annual review (home or hospital). New categorical variable is created (categories defined in section ??)
Confounders			
FEV1%	gli_percpredfev	FEV_1% measured on the day of the annual review	Calculated using the Global Lung Initiative equations
BMI z-score	bmi age dmg_sex	BMI taken on the day of review Age on the day of the review Sex	Variables used to calculate BMI z-scores using the WHO reference distribution

Age	Age	age (in years) at annual review	
Pancreatic insufficiency	tr_creon1000 tr_creon2500 tr_creon40000 tr_creon5000 tr_creon8000 s07lipase trtype_pancreaticenzymetherapy	Each variable is an indicator for whether the treatment listed in variable name was prescribed since last annual review.	New indicator variable for prescription of macrolides (P) will be created. $P = 1$ if any of the listed variables are equal to 1. $P = 0$ if all of the listed variables are equal to 0.
IV days	s02homeivoveralltotaldays s02hospivoveralltotaldays	Number of days on IV treatment at home since last visit Number of days on IV treatment in the hospital since last visit	Listed variables are used to calculate total number of days on IV antibiotics since last annual review (home or hospital). New categorical variable is created (categories defined in section ??)
Non-IV hospital admissions	s02hospnonivoveralltotaldays	Total number of admitted in hospital (non-IV) since last visit	New indicator variable for non-IV hospital admissions will be created (V). $V = 1$ if the total number of non-IV hospital days is greater than 1. $V = 0$ if the total number of non-IV hospital days is equal to 0.
P. aeruginosa	s05culturespeciespseudoaeruginos	Culture growth (pseudomonas aeruginosa) since last visit	NA
Staphylococcus aureus	s05culturespeciesstaph	Culture growth (staphylococcus aureus) since last visit	NA
NTM	s05ntmpulmonarydisease s05ntmhaspositive is_ptcurrentpostivesample is_pttreatmentlastannual cmpl_ntm_atypmyco	Has the patient been on treatment for NTM pulmonary disease at any time since last annual review? Has the patient had NTM positive samples since last annual review?	New indicator variable for prescription of macrolides (N) will be created. $N = 1$ if any of the listed variables are equal to 1. $N = 0$ if all of the listed variables are equal to 0.
CFRD	S06CMPsCFRDDiabDiagnosis	CFRD status will the following options: WHypGly = CFRD with fasting hyperglycaemia; WOHypGly = CFRD without fasting	New indicator variable for CFRD will be created (D). $D = 1$ if s06cmpscfrddiabdiagnosis is equal to “CFRD”, “WHypGly”,

		hyperglycaemia; CFRD = CFRD (fasting hyperglycaemia status unknown); IGT = Impaired glucose tolerance; Indet = Indeterminate; N = No CFRD	"WOHypGLy" or "Y". $D = 0$ otherwise.
Hypertonic saline	tr_hypertonicsalinesolution	Indicator for prescription of hypertonic saline since last visit	NA
Inhaled antibiotics	trtype_inhaledantibiotics	Indicator for prescription of inhaled antibiotics since last visit	NA
DNase	tr_dnase	Indicator for prescription of DNase since last visit	NA
Inhaled steriods			

Table A.2: Details on the variables used in the US CF Registry

Variable	Code Name	Description	Further details
Inclusion Criteria			
Age	Age_YrEnd	Age on 12/31 of review year	
FEV_1%	GLI_FEV1_pct_predicted	FEV_1% on encounter date of visit	Calculated using the Global Lung Initiative Equations
Exclusion Criteria			
Intolerance to macrolides [criteria 1]	A_drugallergies4	Indicator variable for drug intolerance to macrolide antibiotics	
Prescription of macrolides [criteria 2]	chronic_macrolide_type1	Indicator for prescription of azithromycin at encounter visit	
Acute liver failure [criteria 3]	hepatobiliary2_4	Indicator for Acute liver failure (no underlying liver disease)	An indicator for any liver disease (hepatobiliary2_1) will be used if missingness is too great in this variable.
Cirrhosis with portal hypertension [criteria 4]	hepatobiliary1_3 cirrhosiscomplications1 (esophogeal varices) cirrhosiscomplications2 (gastric varices) cirrhosiscomplications3 (GI bleed related to varices) cirrhosiscomplications4 (splenomegaly) cirrhosiscomplications5 (hypersplenism) cirrhosiscomplications6 (ascites) cirrhosiscomplications7 (encephalopathy)	Indicator for cirrhosis with portal hypertension	Requires hepatobiliary1_3 (liver disease, cirrhosis) = 1 AND any one of the cirrhosiscomplications variables = 1
Serum creatinine [criteria 5]	serum_creatinine	Serum creatinine	
DNase [criteria 6a]	dornasealfa	Prescription of DNase at encounter visit	

Inhaled tobramycin [criteria 6b]	tobi	Prescription of tobramycin at encounter visit	
Inhaled steroids [criteria 6c]	corticosteroids2 (Inhaled) corticosteroids3 (Inhaled in combination with bronchodilator)	Prescription of inhaled steroid	New indicator variable for prescription of inhaled steroids (A) will be created. $A = 1$ if either of the listed variables are equal to 1. $A = 0$ if both of the listed variables are equal to 0.
Treatment			
Prescription of azithromycin	chronic_macrolide_type1	Prescription of azithromycin at encounter visit	
Outcomes*			
FEV_1%	GLI_FEV1_pct_predicted	FEV1% on encounter date of visit	Calculated using the Global Lung Initiative Equations
FVC	FVC_pct_predicted	FVC% on encounter date of visit	
BMI z-score	bmi_zscore encounterage gender	BMI taken on the encounter date Age on the encounter date Patient's gender	Variables used to calculate BMI z-scores using the WHO reference distribution
IV days	NumPulmHomeIVNights	Number of nights on home IV for Pulmonary Exacerbations	
Confounders			
FEV1%	GLI_FEV1_pct_predicted	FEV1% on encounter date of visit	Calculated using the Global Lung Initiative Equations
BMI z-score	bmi_zscore encounterage gender	BMI taken on the encounter date Age on the encounter date Patient's gender	Variables used to calculate BMI z-scores using the WHO reference distribution
Age	encounterage	Age on the encounter date	
Pancreatic insufficiency	Creon1203 Creon1206 Creon1212 Creon1224 Creon1236	Each variable is an indicator for whether the treatment listed in variable name was prescribed since last annual review.	New indicator variable for prescription of macrolides (P) will be created. $P = 1$ if any of the listed variables are equal to 1. $P = 0$ if all of the listed variables are equal to 0.
IV days	NumPulmHomeIVNights	Number of nights on home IV for Pulmonary Exacerbations	

Non-IV hospital admissions	NumPulmHospNights	Total number of admitted in hospital (non-IV) since last visit	
P. aeruginosa	pseudomonasaeruginosa	Culture growth (pseudomonas aeruginosa) at visit	NA
Staphylococcus aureus	staphylococcus_aureus	Culture growth (staphylococcus aureus) at visit	NA
NTM	ntm_treatment	Was the patient started on treatment for NTM	
CFRD	cfrd_status		
Hypertonic saline	hypertonicsaline	Indicator for prescription of hypertonic saline at visit	NA
Inhaled antibiotics	medscurrentepisode4	Indicator for prescription of inhaled antibiotics at visit	NA
DNase	dornasealfa	Indicator for prescription of DNase at visit	NA
Inhaled steroids	corticosteroids2 (Inhaled) corticosteroids3 (Inhaled in combination with bronchodilator)	Prescription of inhaled steroid	New indicator variable for prescription of inhaled steroids (A) will be created. $A = 1$ if either of the listed variables are equal to 1. $A = 0$ if both of the listed variables are equal to 0.

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Table 1: Description of the primary research question addressed in the target trial (Clement et al, Thorax 2006;61:895-902)

Objective:	Investigate whether long-term use of azithromycin is associated with respiratory benefits in young patients with CF
Population:	CF patients aged older than 6 years and forced expiratory volume in 1 second (FEV_1%) of 40% or more.
Exposure:	Oral azithromycin
Comparator:	Placebo pills
Primary Outcome:	Change in FEV_1%
Time:	12 months
Setting:	Patients recruited from 18 CF accredited care centres in France
Main measure of effect:	Difference in change in FEV_1% from baseline between treatment groups

Table 2: Key components of the target trial and emulated trials

Protocol Component	Target Trial	Emulation of the target trial using UK/US CF Registry data
Eligibility Criteria	Include: French individuals diagnosed with cystic fibrosis (sweat chloride >60mmol/l or a genotype known to cause the disease), aged 6-21 years, with the ability to perform pulmonary function tests with FEV_1%>40, and the ability to swallow tablets. Individuals were excluded if they had the following: 1. Allergy to macrolide antibiotics 2. Long-term (>3 months) with macrolides during the 12-month period before study entry 3. Liver disease with liver function tests >2 times the laboratory upper limit 4. History of portal hypertension 5. Kidney disease with serum creatinine > 150 µmol/l and/or creatinine clearance <50ml/min 6. Use of any of the following in the 3 months before study entry: DNase, inhaled tobramycin, inhaled steroids	Include: Individuals who have an observation date in the registry within the study recruitment period (defined in section 4.3.1), aged 6-21 years, with FEV_1%>40. The ability to swallow tablets is assumed. Individuals will be excluded if they have the following: 1. Intolerance to macrolide antibiotics 2. Prescribed oral macrolides 3. Liver disease 4. Cirrhosis 5. Kidney disease 6. Prescribed DNase, inhaled tobramycin or inhaled steroids. Further details on inclusion and exclusion criteria are provided in section 4.3.2.
Treatment strategies	The active intervention was azithromycin supplied as 250mg tablets and the comparator was placebo pills. Individuals weighing less than 40kg took one tablet 3 days per week and individuals weighing more than 40kg took two tablets 3 days per week.	The active intervention is prescription of oral azithromycin and the comparator is no prescription of oral azithromycin. Further details on the treatment strategies are provided in section 4.4.1

Assignment procedures	Individuals were randomised to treatment strategy. Randomisation was stratified according to centre and *P. aeruginosa* infection status. The patients and all study investigators remained blinded to the treatment assignment until study completion.	In the emulated trials individuals are not randomly assigned to the treatment strategy. This is accounted for in the analysis.
Follow-up period	12 months	As in the target trial
Outcome	Primary outcome: mean change in FEV_1% between month 0 and month 12. Secondary outcomes included: evaluation of the number of pulmonary exacerbations, the use of antibiotics, modifications of microbiological analysis of sputum or throat cultures, changes in FVC, nutritional status with measurement of body mass index (BMI), and quality of life.	Primary outcome: absolute FEV1% at the end of follow-up. Secondary outcomes include: prescription of IV antibiotics, FVC, BMI z-score. Further details on the outcomes are provided in section 4.4.2.
Causal contrasts of interest	Intention-to-treat	Per-protocol
Analysis plan	For continuous outcomes, mean differences between treatment groups were estimated using mixed models; for binary outcomes, logistic regression was used; for count outcomes, Poisson regression was used. Models were adjusted for interactions taking into account effects due to centre and baseline characteristics (including *P. aeruginosa* infection status)	A direct acyclic graph is used to inform which variables need to be controlled for (see section 4.4.3). Confounding by measured variables will be accounted for using inverse-probability-of-treatment weighting. Further details on the analysis plan are provided in section 4.5.

Table 3: Description of the time-periods considered in the UK and US registry data

Time period	Justification for time-period	UK data	US data
2003-2005	Closest time-period to the target trial, allowing a couple of years for use of azithromycin to uptake in clinical practice. This will only be conducted using UK data as the US registry did not collect data on azithromycin use during this time.	✓	×
2006-2008	US registry started collecting data on azithromycin in 2006. Therefore, this time-period is as close to the time-period used in the target trial as is possible for the US registry.	✓	✓
2016-2018	UK registry started collecting data on treatment dates in 2016. Therefore, this time-period is a more recent period that pre-dates widespread use of CFTR modulators, but also allows use of treatment dates data.	✓	✓

Table 4: Details of inclusion and exclusion criteria for the UK and US Emulated Trials

	UK Emulated Trial	US Emulated Trial
Inclusion Criteria	Individuals will be considered for inclusion if they have a clinically confirmed diagnosis of cystic fibrosis (i.e. are present in the UK CF Registry) and have an observation date within the recruitment-periods defined in section 4.3.1, aged between 6 and 21 years, and obtained FEV_1%>40 on their pulmonary function test (taken on the day of the annual review). It is assumed that all individuals have the ability to swallow tablets.	Individuals will be considered for inclusion if they have a clinically confirmed diagnosis of cystic fibrosis (i.e. are present in the US CF Registry) and have an observation or encounter date within the time-periods defined in section 4.3.1, aged between 6 and 21 years, and obtained FEV_1%>40 on their pulmonary function test (taken from the annual averaged value). We excluded patients with any FEV1%<40 in the encounter level data. It is assumed that all individuals have the ability to swallow tablets.
Exclusion Criteria	Exclusion criteria are as follows: 1. Intolerance to macrolide antibiotics recorded at any time during study period. 2. Prescription of chronic oral or prophylactic oral macrolides (including azithromycin) recorded at time 0. 3. Acute liver failure with > 3 x the upper laboratory limit, INR>2, or not responsive to vitamin K at time 0 4. Recorded cirrhosis with portal hypertension at time 0. 5. Serum creatinine levels > 150 µmol/l at time 0. 6. Prescription of DNase, inhaled tobramycin or inhaled corticosteroids recorded at time 0. 7. No follow-up visit for time 1.	Exclusion criteria are as follows: 1. As in UK Emulated Trial 2. As in UK Emulated Trial 3. Non-cystic fibrosis related liver disease recorded at time 0. Laboratory results from liver tests are not available in the US registry. 4. Recorded cirrhosis at time 0. Portal hypertension is not available in the US registry. 5. As in the UK Emulated Trial 6. As in the UK Emulated Trial 7. As in UK Emulated Trial

Table 5: Causal estimands of interest in the emulated trials and how to interpret them

Estimand	Interpretation
$E(Y_1(1)) - E(Y_1(0))$	Expected difference in FEV_1% at month 12 if everyone had taken azithromycin for 12 months, compared to a scenario where no-one took azithromycin for 12 months.
$E(Y_1(1)\|PA_1 = 1) - E(Y_1(0)\|PA_1 = 1)$	Expected difference in FEV_1% at month 12 if everyone who was infected with *P. aeruginosa* had taken azithromycin for 12 months, compared to a scenario where everyone who was infected with *P. aeruginosa* did not take azithromycin for 12 months.
$E(Y_1(1)\|PA_1 = 0) - E(Y_1(0)\|PA_1 = 0)$	Expected difference in FEV_1% at month 12 if everyone who was not infected with *P. aeruginosa* had taken azithromycin for 12 months, compared to a scenario

where everyone who was not infected with P. *eruginosa* did not take azithromycin for 12 months.

Figure Captions

Figure 1: Directed Acyclic Graph depicting assumed confounding relationships for the association between azithromycin and FEV_1%. U represents any unmeasured confounders. Subscripts denote time.

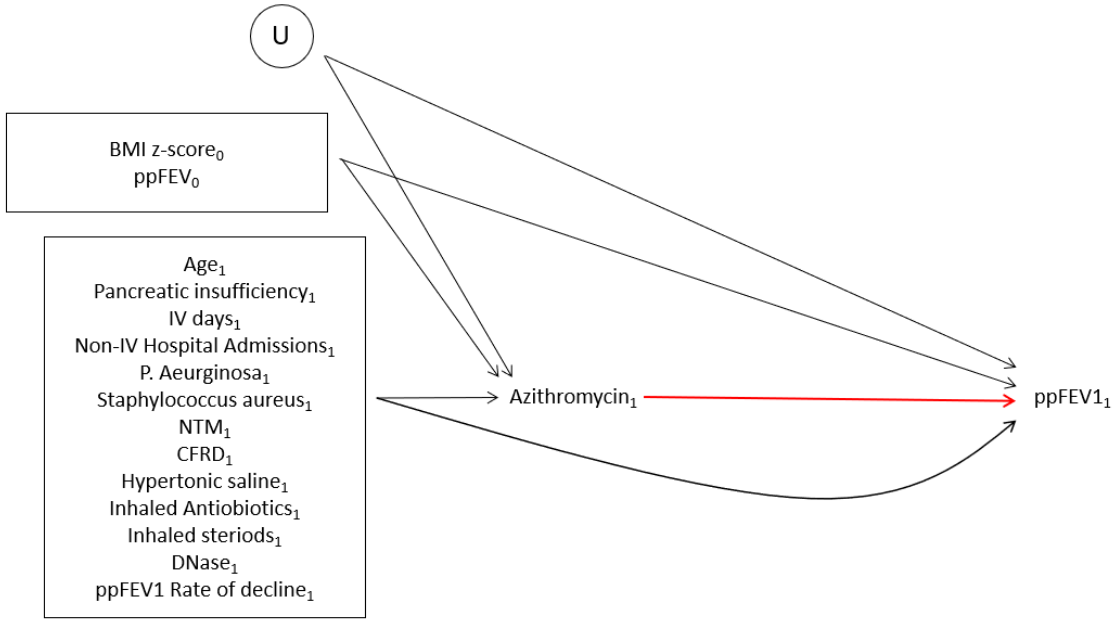

Figure 2: Study Design Diagram illustrating key design choices and their temporal ordering. Time 0 is the time of meeting inclusion criteria. Time -1 is 12 months prior to meeting inclusion criteria and time 1 is 12 months after meeting inclusion criteria