Methods:

Study Centers:

The clinical microbiology laboratory at the U. of North Carolina was the central laboratory processing all cultures per CFF protocol {Gilligan, Miller}.

Treatment Regimen:

In cases of severe study drug related gastro-intestinal side effects discontinuation of rifampin was allowed. Similarly, if a rash suspected to be related to TMP-SMX emerged on therapy, drug could be changed to minocycline if > 8 years of age, younger participants with a drug related rash would discontinue all oral antibiotics but continue with topical antibiotics and environmental decontamination. Compliance with study protocol was measured by self-reported study log and count of returned medications. At the end of the treatment participants completed a global assessment of protocol feasibility/burden.

Statistical Analysis:

Safety outcomes were monitored throughout the trial by a Data Monitoring Committee (DMC) appointed by the CF Foundation Safety Monitoring Board. One interim analysis with early stopping for futility was scheduled to take place after approximately half of the participants had been randomized into the study and had completed the Day 28 visit. Because of slow accrual, an early interim review and futility analysis of the primary endpoint was initiated when 24 participants completed the first 28 days of the study. Upon review of the interim results, the DMC recommended continuance of the study with a second interim analysis for early efficacy. The study team remained blinded to detailed findings presented to the DMC. Statistical ramifications of this unplanned efficacy analysis are addressed in the Online Supplement. The second interim review took place when 39 participants completed Day 28 visit. Guided by a formal stopping rule based on the primary endpoint, the DMC recommended that the enrollment of new participants be stopped because early efficacy of the primary endpoint had been met. The full details are highlighted below and contains the details of the interim analyses and statistical monitoring guidelines.

Results:

Table S1. Sensitivity Analyses for the Primary Endpoint

This table summarizes multiple sensitivity analyses for the primary endpoint used to evaluate the robustness of primary study result, i.e., proportion of participants with a negative culture for MRSA at Day 28. In particular, the primary efficacy analyses are conducted for the PPE population. In addition, the robustness of study results is assessed in relation to participants in the ITT-E population requiring treatment for MRSA prior to Day 28 in the observational control arm. In this scenario, participants are treated as positive for MRSA regardless of their culture result at Day 28. Lastly, the impact of missing culture data at Day 28 is assessed by assuming the missing cultures are either negative or positive in the ITT population. For participants who have both an OP and expectorated sputum sample available at a given visit, a positive respiratory culture result is based on MRSA being present in either the OP or expectorated sputum sample; a negative result is based on MRSA being absent from both the OP and expectorated sputum samples. The primary endpoint was assessed at Day 28.

		Treatment (N = 24)	Observational Control (N = 21)	Difference (95% CI) [5]	p-value [6]
PPE Population	Number (N_{PPE})	15	18		
	Number with Respiratory Culture at Day 28	15	18		
	MRSA Negative at Day 28, n (%)	12 (80%)	4 (22%)	58% (24%, 76%)	0.0016
	Number Cultures MRSA Positive at Screening	7	14		
	Changed to MRSA Negative from Screening to Day 28 [1], n (%)	4 (57%)	1 (7%)	50% (10%, 78%)	0.0251
Positive Result Assumption at Day 28 for participants Treated with Anti-MRSA Antibiotics in the ITT-E Population [2]	N_{ITT-E}	22	19		
	Number with Respiratory Culture at Day 28	22	19		
	MRSA Negative at Day 28, n (%)	18 (82%)	2 (11%)	71% (42%, 85%)	<0.0001
	Number Cultures MRSA Positive at Screening	12	15		
	Changed to MRSA Negative from Screening to Day 28 [1], n (%)	8 (67%)	0 (0%)	67% (32%, 86%)	0.0002
Negative Result Imputation at Day 28 in the ITT Population [3]	N_{ITT}	24	21		
	Number with Respiratory Culture at Day 28	24	21		
	MRSA Negative at Day 28, n (%)	20 (83%)	7 (33%)	50% (21%, 69%)	0.0009
	Number Cultures MRSA Positive at Screening	14	17		
	Changed to MRSA Negative from Screening to Day 28 [1], n (%)	10 (71%)	4 (24%)	48% (13%, 70%)	0.0122

		Treatment (N = 24)	Observational Control (N = 21)	Difference (95% CI) [5]	p-value [6]
Positive Result Imputation at Day 28 in the ITT Population [4]	N_{ITT}	24	21		
	Number with Respiratory Culture at Day 28	24	21		
	MRSA Negative at Day 28, n (%)	18 (75%)	5 (24%)	51% (22%, 70%)	0.0009
	Number Cultures MRSA Positive at Screening	14	17		
	Changed to MRSA Negative from Screening to Day 28 [1], n (%)	8 (57%)	2 (12%)	45% (12%, 68%)	0.0181

[1] Percent value is based on the number of participants with a MRSA positive respiratory culture result available at Screening.
[2] Assumes a positive Day 28 MRSA culture result for participants who received any oral, inhaled, or IV antibiotics active against MRSA prior to Day 28 in the observational control arm.
[3] Assumes missing Day 28 MRSA culture results are negative.
[4] Assumes missing Day 28 MRSA culture results are positive.
[5] 95% confidence interval calculated using the Newcombe-Wilson method without continuity correction.
[6] The p-value is obtained from the Fisher's exact test.

Adherence to Treatment

Two patients were on oral minocycline. Nineteen participants (83%) took at least 80% doses of rifampin, and 15 participants (65%) at least 80% doses of TMP/SMX or minocycline. The majority of the participants were compliant with topical antibiotic usage and environmental decontamination.

Safety Laboratory Assessment

Post-baseline assessments of serum chemistry and hematology were performed on Day 15 for participants who were randomized to the treatment arm and were 12 years of age or older. A repeated assessment of serum chemistry and hematology was performed on Day 28 if results were abnormal and deemed clinically significant at Day 15. Eleven participants in the treatment arm were assessed at Day 15 and only one of these participants was re-assessed at Day 28 due to clinically significant results at Day 15. This participant's AST and ALT values were abnormally high at Day 15 and subsequent emergent low RBC, Hemoglobin, and Hematocrit values were noted at Day 28 (all not clinically significant). Another participant had incomplete laboratory measurements (chemistry only, missing hematology) at Day 15 and had their hematology collected at Day 28 (normal results at both visits). A third participant had no laboratory measurements done on Day 15 and had assessments done on Day 28 instead (emergent high AST, not clinically significant).

Table S2. Most Frequently Occurring Serious and Non-serious Adverse Events (ITT Population)

System Organ Class Preferred Term	Treatment (N=24) n (%)	Observational Control (N=21) n (%)	Difference in Proportion (%)	(95% CI)[1]
Gastrointestinal disorders	11 (46)	5 (24)	22	(-33, 21)
Skin and subcutaneous tissue disorders	5 (21)	1 (5)	16	(-23, 10)
Injury, poisoning and procedural complications	3 (13)	0 (0)	13	(-24, 19)
Nervous system disorders	2 (8)	0 (0)	8	(-21, 18)
General disorders and administration site conditions	3 (13)	1 (5)	8	(-19, 16)
Renal and urinary disorders	1 (4)	0 (0)	4	(-19, 16)
Musculoskeletal and connective tissue disorders	1 (4)	0 (0)	4	(-18, 23)
Immune system disorders	1 (4)	0 (0)	4	(-15, 22)
Eye disorders	2 (8)	1 (5)	4	(-15, 22)
Ear and labyrinth disorders	2 (8)	1 (5)	4	(-12, 20)
Infections and infestations	3 (13)	2 (10)	3	(-12, 20)
Psychiatric disorders	1 (4)	1 (5)	-1	(-12, 20)
Blood and lymphatic system disorders	1 (4)	1 (5)	-1	(-12, 27)
Metabolism and nutrition disorders	2 (8)	2 (10)	-1	(-8, 26)
Investigations	3 (13)	3 (14)	-2	(-5, 31)
Congenital, familial and genetic disorders	0 (0)	1 (5)	-5	(-5, 36)

[1] 95% confidence interval calculated using the Newcombe-Wilson method without continuity correction.
[2] The p-value is obtained from Fisher's exact test.

Protocol Feasibility/Burden Assessment

Protocol feasibility/burden was assessed in 23 participants randomized to Rx, 87% reported they would be ‘Definitely’ or ‘Probably’ likely to take rifampin and 92% TMP/SMX or minocycline if prescribed again. Body wash, disinfectant wipes, and washing towels were reported by 87%, 87%, and 100% of participants, respectively, as ‘Definitely’ or ‘Probably’ likely. The lowest acceptance was noted for oral gurgle with 52% and 22% rating it as ‘Definitely’ or ‘Probably’ likely to do. Nasal ointment had intermediate acceptance (65% ‘Definitely’ and 13% ‘Probably’). The mean protocol burden measured on a scale from 0 (‘very easy’) to 10 (‘very inconvenient’) was 2.7 (SD=2.0).

Table S3.1 Changes in MRSA Status from Screening through Day 168 (ITT Population)

This table summarizes changes in MRSA culture status by visit from screening through day 168. For participants that have both an OP and expectorated sputum sample available at a given visit, a positive respiratory culture result is based on MRSA being present in either the OP or expectorated sputum sample; a negative result is based on MRSA being absent from both the OP and expectorated sputum samples. The primary endpoint was assessed at day 28.

		Treatment (N=24)	Observational Control (N=21)	Difference (95% CI) [4]	p-value [5]
Screening					
	Number with Respiratory Culture	24	21		
	MRSA Positive, n (%)	14 (58%)	17 (81%)	Difference = -23% (-45%, 4%)	0.1212
Day 28					
	Number with Respiratory Culture	22	19		
	MRSA Positive [1], n (%)	4 (18%)	14 (74%)	Difference = -56% (-74%, -25%)	0.0005
Change from Screening to Day 28					
	Number Cultures MRSA Positive at Screening [2]	12	15		
	Changed to MRSA Negative [3], n (%)	8 (67%)	2 (13%)	Difference = 53% (16%, 75%)	0.0069
Day 84					
	Number with Respiratory Culture	21	17		
	MRSA Positive [1], n (%)	8 (38%)	10 (59%)	Difference = -21% (-47%, 10%)	0.3275
Change from Screening to Day 84					
	Number Cultures MRSA Positive at Screening [2]	11	14		
	Changed to MRSA Negative [3], n (%)	4 (36%)	5 (36%)	Difference = 1% (-33%, 35%)	>0.9999
Day 168					
	Number with Respiratory Culture	21	15		
	MRSA Positive [1], n (%)	9 (43%)	7 (47%)	Difference = -4% (-33%, 26%)	>0.9999
Change from Screening to Day 168					
	Number Cultures MRSA Positive at Screening [2]	11	12		
	Changed to MRSA Negative [3], n (%)	4 (36%)	5 (42%)	Difference = -5% (-39%, 31%)	>0.9999
	Changed to MRSA Negative at Day 28 and Remained Negative through Day 168 [6], n (%)	2 (20%)	1 (8%)	Difference = 12% (-19%, 43%)	0.5714

	Treatment (N=24)	Observational Control (N=21)	Difference (95% CI) [4]	p-value [5]

[1] Percent value is based on the number of participants with both a non-missing MRSA culture result at screening and at the given visit.
[2] Number of participants with both a MRSA positive respiratory culture result available at screening and a non-missing MRSA culture result at the given visit.
[3] Percent value is based on the number of participants with both a MRSA positive respiratory culture result available at screening and a non-missing MRSA culture result at the given visit.
[4] 95% confidence interval calculated using the Newcombe-Wilson method without continuity correction.
[5] The p-value is obtained from Fisher's exact test.
[6] Percent value is based on the number of participants with a MRSA positive respiratory culture result available at screening and non-missing MRSA culture results at Day 28, Day 84, and Day 168.

Table S3.2 Changes from Screening to Day 28 and Day 168 in Secondary Clinical Endpoints (ITT Population)

This table summarizes changes in clinical measures from screening through Day 28 and Day 168.

		N	Treatment (N=24)	N	Observational Control (N=21)	Difference (95% CI) [1]	p-value[1]
FEV_1 (L)	Screening	20	2.46 (0.83)	17	2.28 (0.84)		
	28-Day absolute change	19	0.06 (0.13)	16	-0.05 (0.21)	0.11 (-0.01, 0.23)	0.0621
	168-Day absolute change	17	0.06 (0.18)	14	0.00 (0.16)	0.06 (-0.07, 0.19)	0.3383
	28-Day relative change	19	2.45 (6.50)	16	-2.44 (9.50)	4.89 (-0.63, 10.42)	0.0808
	168-Day relative change	17	3.35 (7.72)	14	0.27 (7.27)	3.08 (-2.47, 8.64)	0.2651
FEV_1 (% Predicted)	Screening	20	98.52 (21.62)	17	101.22 (11.80)		
	28-Day absolute change	19	0.74 (6.89)	16	-4.05 (9.72)	4.79 (-0.94, 10.52)	0.0983
	168-Day absolute change	17	-0.56 (6.45)	14	-5.24 (7.47)	4.69 (-0.43, 9.80)	0.0710
Weight (kg)	Screening	24	40.51 (16.96)	21	38.19 (19.78)		
	28-Day absolute change	23	0.44 (1.29)	20	0.37 (1.48)	0.07 (-0.78, 0.93)	0.8626
	168-Day absolute change	21	1.78 (1.76)	17	1.97 (2.75)	-0.19 (-1.68, 1.3)	0.7928
Weight (percentile)[2]	Screening	21	50.45 (27.40)	20	53.66 (23.91)		
	28-Day absolute change	20	0.75 (5.13)	19	1.45 (6.81)	-0.7 (-4.59, 3.2)	0.7191
	168-Day absolute change	17	2.77 (5.81)	17	0.04 (9.91)	2.72 (-2.95, 8.39)	0.3361
CFRSD-CRISS	Screening	24	17.79 (12.72)	21	12.24 (10.86)		
	28-Day absolute change	22	-3.77 (9.78)	19	2.95 (14.35)	-6.72 (-14.4, 0.95)	0.0841
	168-Day absolute change	21	-0.05 (11.07)	16	-5.19 (18.44)	5.14 (-4.76,15.04)	0.2989
CFQ-R RSS[3]	Screening	24	84.68 (11.37)	20	85.69 (9.33)		
	28-Day absolute change	22	-2.73 (16.29)	18	-2.47 (18.86)	-0.26 (-11.5,10.99)	0.9632
	168-Day absolute change	21	-5.50 (14.73)	16	-1.56 (15.91)	-3.94 (-14.2, 6.33)	0.4414

[1] Two-sample t-test is used to calculate the 95% confidence interval and corresponding p-value.
[2] Percentiles are derived using CDC standards in participants <= 20 years old.
[3] CFQ-R respiratory domain scale scores.

Table S4.1 Presence of MRSA at Other Body Sites: Axilla/Groin (ITT Population)

This table summarizes changes in MRSA culture status by visit from screening through day 168. All the participants in the treatment arm were treated through day 28 and the primary endpoint was assessed at day 28.

		Treatment (N=24)	Observational Control (N=21)
Screening			
	Number with Axilla/Groin Cultures	24	20
	MRSA Positive, n (%)	1 (4%)	0 (0%)
Day 28			
	Number with Axilla/Groin Cultures	22	19
	MRSA Positive [1], n (%)	0 (0%)	0 (0%)
Change from Screening to Day 28			
	Number Cultures MRSA Positive at Screening [2]	1	0
	Changed to MRSA Negative [3], n (%)	1 (100%)	0 (0%)
Day 84			
	Number with Axilla/Groin Cultures	21	17
	MRSA Positive [1], n (%)	0 (0%)	1 (6%)
Change from Screening to Day 84			
	Number Cultures MRSA Positive at Screening [2]	1	0
	Changed to MRSA Negative [3], n (%)	1 (100%)	0 (0%)
Day 168			
	Number with Axilla/Groin Cultures	21	15
	MRSA Positive [1], n (%)	0 (0%)	0 (0%)
Change from Screening to Day 168			
	Number Cultures MRSA Positive at Screening [2]	1	0
	Changed to MRSA Negative [3], n (%)	1 (100%)	0 (0%)

[1] Percent value is based on the number of participants with both a non-missing MRSA culture result at screening and at the given visit.
[2] Number of participants with both a MRSA positive axilla/groin culture result available at screening and a non-missing MRSA culture result at the given visit.
[3] Percent value is based on the number of participants with both a MRSA positive axilla/groin culture result available at screening and a non-missing MRSA culture result at the given visit.

Table S4.2 Presence of MRSA at Other Body Sites: Nares (Left/Right) (ITT Population)

This table summarizes by visit changes in MRSA status from screening and through day 168. All the participants in the treatment arm were treated through day 28 and the primary endpoint was assessed at day 28.

		Treatment (N=24)	Observational Control (N=21)	Difference (95% CI) [4]	p-value [5]
Screening					
	Number with Nares (Left/Right) Cultures	24	21		
	MRSA Positive, n (%)	6 (25%)	8 (38%)	Difference = -13% (-38%, 13%)	0.5197
Day 28					
	Number with Nares (Left/Right) Cultures	22	19		
	MRSA Positive [1], n (%)	2 (9%)	7 (37%)		
Change from Screening to Day 28					
	Number Cultures MRSA Positive at Screening [2]	4	8		
	Changed to MRSA Negative [3], n (%)	2 (50%)	2 (25%)	Difference = 25% (-24%, 64%)	0.5475
Day 84					
	Number with Nares (Left/Right) Cultures	21	17		
	MRSA Positive [1], n (%)	3 (14%)	6 (35%)		
Change from Screening to Day 84					
	Number Cultures MRSA Positive at Screening [2]	4	8		
	Changed to MRSA Negative [3], n (%)	2 (50%)	3 (38%)	Difference = 13% (-35%, 55%)	>0.9999
Day 168					
	Number with Nares (Left/Right) Cultures	21	15		
	MRSA Positive [1], n (%)	5 (24%)	4 (27%)		
Change from Screening to Day 168					
	Number Cultures MRSA Positive at Screening [2]	4	6		
	Changed to MRSA Negative [3], n (%)	1 (25%)	3 (50%)	Difference = -25% (-62%, 30%)	0.5714

[1] Percent value is based on the number of participants with both a non-missing MRSA culture result at screening and at the given visit.
[2] Number of participants with both a MRSA positive nare culture result available at screening and a non-missing MRSA culture result at the given visit.
[3] Percent value is based on the number of participants with both a MRSA positive nare culture result available at screening and a non-missing MRSA culture result at the given visit.
[4] 95% confidence interval calculated using the Newcombe-Wilson method without continuity correction.
[5] The p-value is obtained from Fisher's exact test.

Table S5. Changes in *P. aeruginosa* Status from Screening (ITT Population)

This table summarizes by visit changes in *P. aeruginosa* status from Screening and through Day 168. For participants that have both an OP and expectorated sputum sample available at a given visit, a positive respiratory culture result is based on *P. aeruginosa* being present in either the OP or expectorated sputum sample; a negative result is based on *P. aeruginosa* being absent from both the OP and expectorated sputum samples. All the participants in the treatment arm were treated through Day 28 and the primary endpoint was assessed at Day 28.

		Treatment (N=24)	Observational Control (N=21)	Difference (95% CI)[6]	p-value [7]
Screening					
	Number with Respiratory Culture	24	21		
	Pa Positive, n (%)	4 (17%)	4 (19%)	Difference = -2% (-26%, 20%)	>0.9999
Day 28					
	Number with Respiratory Culture	22	19		
	Pa Positive[1], n (%)	3 (14%)	4 (21%)		
Change from Screening to Day 28					
	Number Cultures *Pa* Positive at Screening [2]	4	4		
	Changed to *Pa* Negative[3], n (%)	1 (25%)	1 (25%)	Difference = 0% (-49%, 49%)	>0.9999
	Number Cultures *Pa* Negative at Screening[4]	18	15		
	Changed to *Pa* Positive[5], n (%)	0 (0%)	1 (7%)	Difference = -7% (-30%, 12%)	0.4545
Day 84					
	Number with Respiratory Culture	20	17		
	Pa Positive[1], n (%)	3 (15%)	4 (24%)		
Change from Screening to Day 84					
	Number Cultures *Pa* Positive at Screening [2]	4	4		
	Changed to *Pa* Negative[3], n (%)	1 (25%)	1 (25%)	Difference = 0% (-49%, 49%)	>0.9999
	Number Cultures *Pa* Negative at Screening[4]	16	13		
	Changed to *Pa* Positive[5], n (%)	0 (0%)	1 (8%)	Difference = -8% (-33%, 13%)	0.4483
Day 168					
	Number with Respiratory Culture	21	15		
	Pa Positive[1], n (%)	3 (14%)	3 (20%)		
Change from Screening to Day 168					
	Number Cultures *Pa* Positive at Screening [2]	3	3		
	Changed to *Pa* Negative[3], n (%)	0 (0%)	0 (0%)		
	Number Cultures *Pa* Negative at Screening[4]	18	12		
	Changed to *Pa* Positive[5], n (%)	0 (0%)	0 (0%)		

	Treatment (N=24)	Observational Control (N=21)	Difference (95% CI)[6]	p-value [7]

[1] Percent value is based on the number of participants with both, a non-missing *Pa* culture result at Screening and at a given visit.
[2] Number of participants with both, a *Pa* positive respiratory culture result available at Screening and a non-missing *Pa* culture result at a given visit.
[3] Percent value is based on the number of participants with both, a *Pa* positive respiratory culture result available at Screening and a non-missing *Pa* culture result at a given visit.
[4] Number of participants with both, a *Pa* negative respiratory culture result available at Screening and a non-missing *Pa* culture result at a given visit.
[5] Percent value is based on the number of participants with both, a *Pa* negative respiratory culture result available at Screening and a non-missing *Pa* culture result at a given visit.
[6] 95% confidence interval calculated using the Newcombe-Wilson method without continuity correction.
[7] The p-value is obtained from the Fisher's exact test.

Figure S1.1 Absolute Change in FEV_1 (Liters) from Screening through Day 168 (ITT Population)

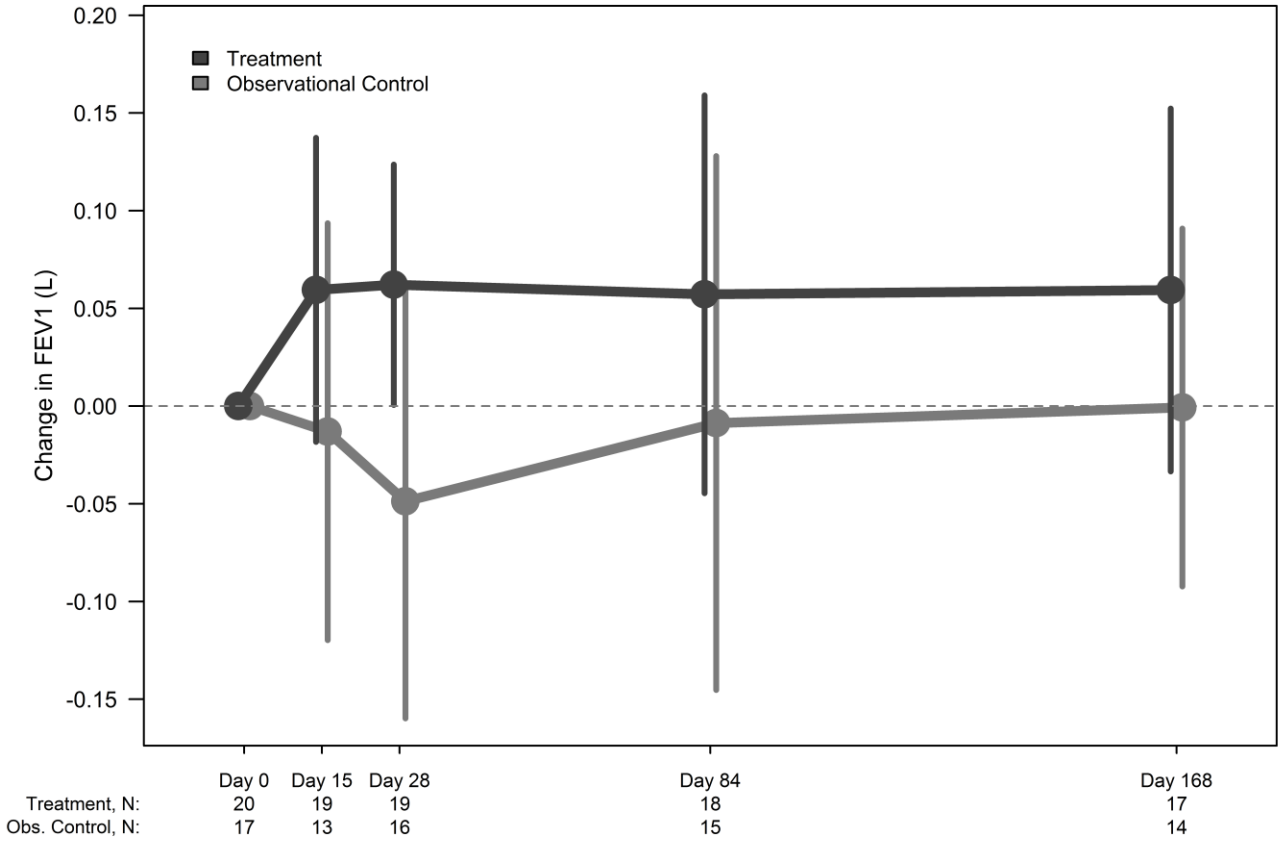


Figure S1.2 Absolute Change in FEV_1 (% Predicted) from Screening through Day 168 (ITT Population)

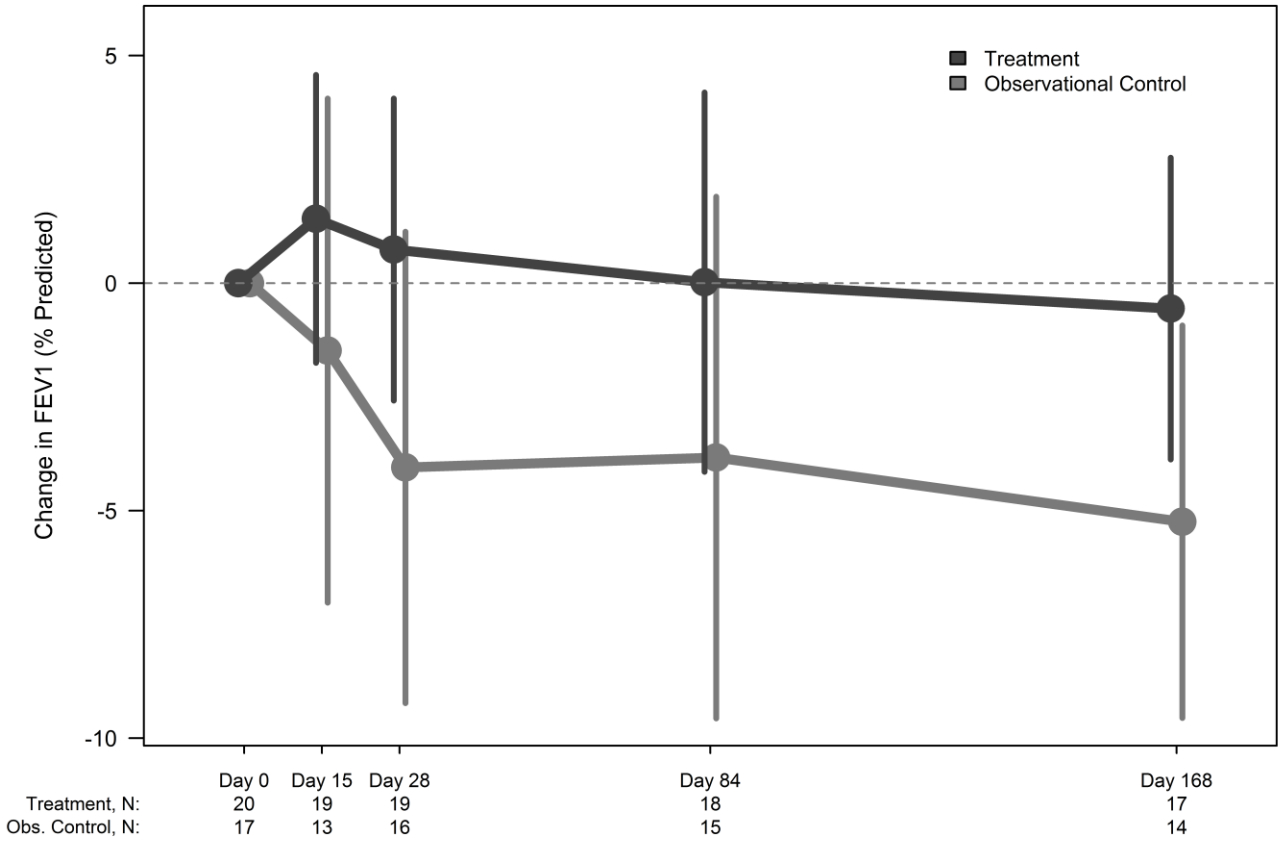


Figure S2.1 Change in Weight (kg) from Screening through Day 168 (ITT Population)

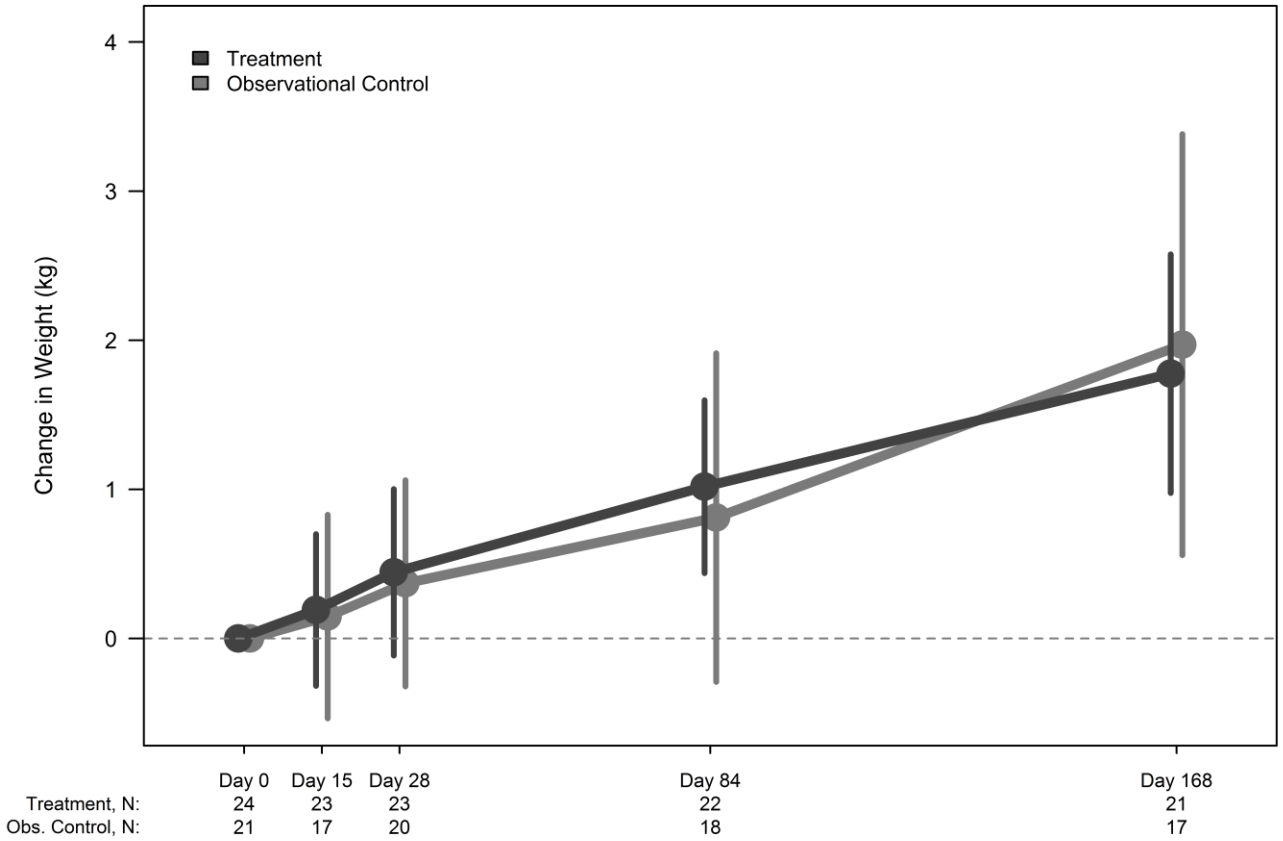


Figure S2.2 Change in Weight (Percentile) from Screening through Day 168 (ITT Population)

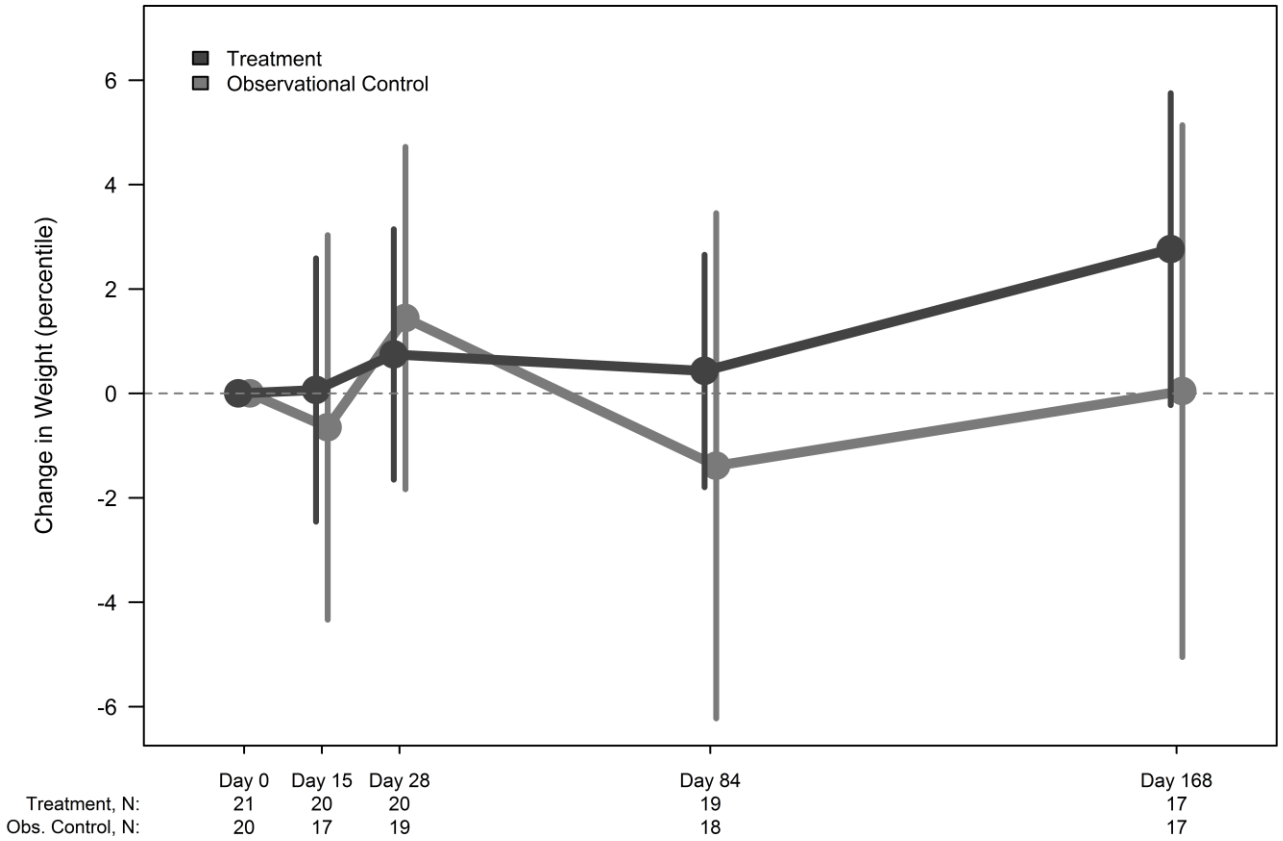


Figure S2.3 Change in BMI (Percentile) from Screening through Day 168 (ITT Population)

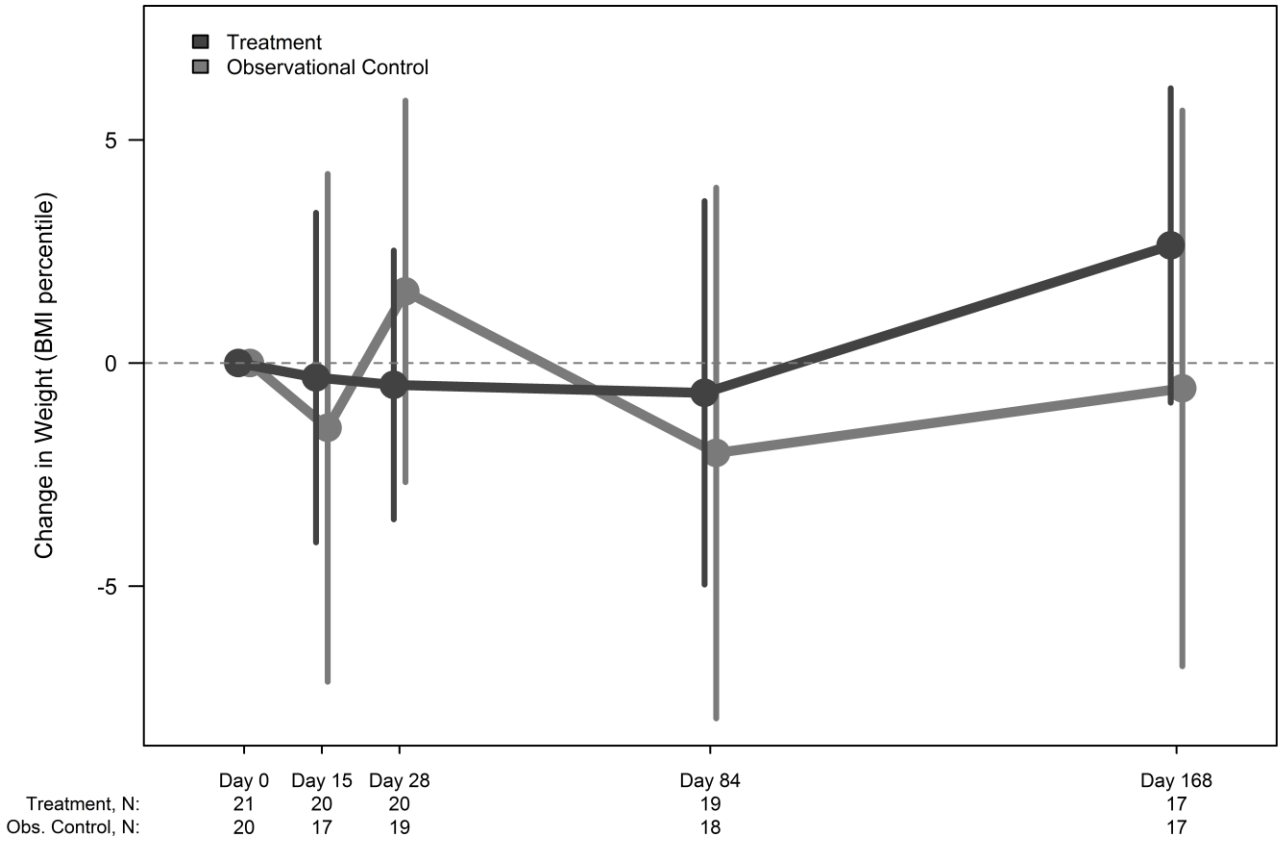


Figure S3.1 Change in CFRSD-CRISS from Screening through Day 168 (ITT Population)

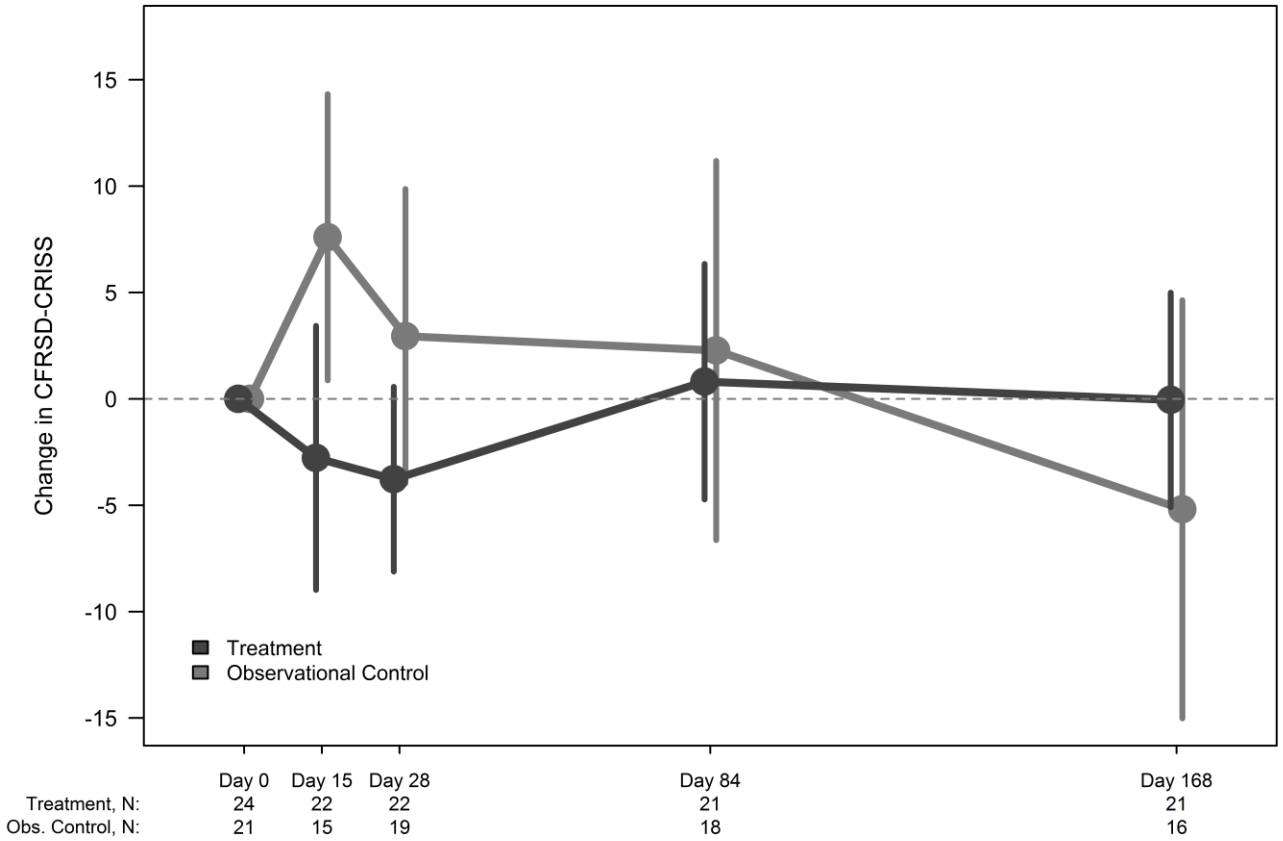
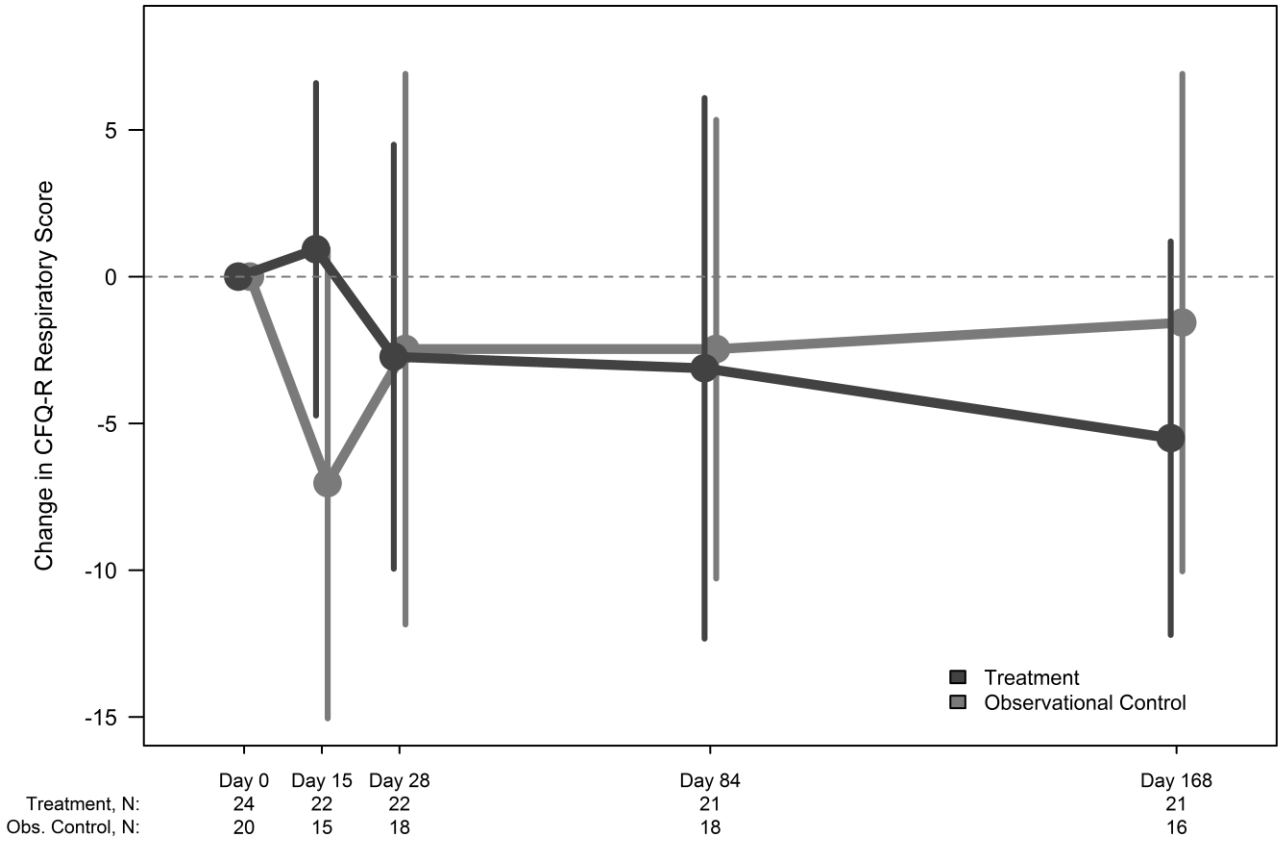


Figure S3.2 Change in CFQ-R Respiratory Symptom Scale from Screening through Day 168 (ITT Population)



Statistical Monitoring Guidelines and Interim Primary Endpoint Analyses

Summary of the Primary Endpoint and Original Sample Size Calculations

The primary endpoint for the study is the proportion of respiratory cultures negative for MRSA at Day 28. With an anticipated dropout rate of approximately 10%, the proposed sample size for the study of 90 participants was established to ensure that at least 80 subjects would be available in the efficacy population for analysis. In prior CF studies of 6 month duration and including regular microbiologic cultures, an average of 94% of the randomized subjects had microbiology results available at any one visit whereby missing results were attributable to both early withdrawals from the study and missed study visits. A sample size of 80 subjects, 40 randomized to the treatment arm and 40 randomized to the control arm will provide 80% power to detect a difference in the proportion negative for MRSA at Day 28 of 30% or greater using a two sided 0.05 level chi square test. This assumes that approximately 80% of the subjects in the treatment arm are negative for MRSA at day 28 and 50% of the subjects in the control arm are negative for MRSA at day 28 which accounted for potential transient infection among the controls.

Interim Data Monitoring Committee Reviews

Oversight for this trial was provided by a Data Monitoring Committee (DMC) from the Cystic Fibrosis Foundation Data Safety Monitoring Board (DSMB). A single interim analysis was scheduled to take place in the Spring of 2013, after a projected 40 out of 90 participants had completed their Day 28 study visit. Given the slower than expected enrollment (approximately 1 participant per month), only 27 participants had completed their Day 28 study visit as of Spring 2013. As the study has been ongoing for over a year, it was decided to continue with the scheduled interim analysis in Spring 2013 to enable a detailed assessment of safety and feasibility. In addition, an interim futility analysis took place based on the primary endpoint, the difference between treatment groups in MRSA negativity at Day 28. Upon review of the interim report, the DMC recommended continuance of the study as well as the addition of a second interim analysis, to be carried out when 55 participants have completed Day 28, or Fall 2014, whichever occurred first. The DMC requested a formal efficacy analysis be conducted at the second interim. Because the interim efficacy analysis was not planed *a priori,* the sensitivity of the results to different stopping boundaries was assessed.

In September 2014, guided by a formal stopping rule based on the primary endpoint (the difference between treatment groups in MRSA negativity at Day 28), the DMC recommended that the enrollment of new participants be stopped because the primary endpoint has been met. The data collection continued for participants already enrolled in the study.

The confidential closed interim safety reports and listings were available only to members of the DMC and select members in the TDN Biostatistics and Clinical Data Management Unit (BCDM) who prepared the report. An open version of the interim safety report was presented to the principal investigators which contained limited sections related to enrollment and demographics.

Statistical Monitoring Guidelines and Stopping Rules for the Planned Futility and Efficacy Monitoring

We begin by presenting the stopping boundaries as if they were in place as part of the original study design. With a planned total sample size of approximately 40 per group with available Day 28 microbiology results, we assumed in the original planning of the trial that half (50%) of the control group subjects would be negative at Day 28 and under these assumptions have 80% power to detect a 30% increase in the proportion negative at Day 28 in the treated group. For the purposes of calculating formal stopping boundaries for futility and efficacy, we used the hypothesized treatment effect of 30% or more for which the study has reasonable power. Assuming an Emerson-Fleming bound (1989)(1) with boundary parameter P=0.4 in calculation of a stopping rule for futility (which provides a less conservative bound and greater chance to stop for futility in this study design due to anticipated concerns for slow enrollment prior to starting the study), and a conservative O'Brien-Fleming (1979) (2) boundary (P=1) to allow the study

to stop for overwhelming evidence of efficacy (a much more conservative bound which provides less chance of stopping early for efficacy unless the evidence is very strong), the two-sided stopping rule for futility would suggest that the trial be stopped early at the second interim analysis (when data through Day 28 are available for approximately 53 participants) if the observed difference between treatment groups (early intervention minus observational arm) is less than 16.3%. Likewise, the stopping rule for efficacy would suggest that the trial be stopped early at the second interim analysis if the treatment effect is greater than 31.4%. If the trial is to continue until completion, an observed difference of 20.9% or greater would be judged statistically significant with revised power of 73% at a one-sided experiment level of significance of 0.025.

The actual stopping boundaries were computed at the time of the interim analyses and were revised according to the actual sample size at the time of the interim. Also, adjustments to these stopping boundaries were made after accounting for the first interim review. Thus, the actual stopping boundaries differed slightly from those provided above. Splus SeqTrial and R RTCdesign software was used to calculate the formal stopping boundary and to adjust for the interim analyses in the final analysis of the primary endpoint.

Summary of the Primary Endpoint Data at the Time of the Interim Analyses

As of August 20 2014, a total of 42 participants (22 in the treatment arm, 20 in the observational control arm) have completed Day 28 with a total of 39 participants (21 in treatment arm, 18 in observational control arm) with respiratory culture results available at Day 28. Table 1 describes the MRSA results at Day 28.

Table 1. MRSA Results from Respiratory Cultures at Day 28 (Observed Data)

	Treatment	Observational Control	Difference (95% Confidence Interval)
Number with Respiratory Cultures	21	18	
Number MRSA Negative	17 (81%)	4 (22%)	59% (28%, 76%)

Note: Among a subset of participants who were positive for MRSA at screening (n=27), the difference in the proportion negative for MRSA at Day 28 is 53% (16%, 75%).

Using the pre-defined stopping boundaries under the monitoring plan which have been adjusted for the first interim review and interim sample size, we would have exceed the efficacy bound if the observed difference between treatment groups was 35.3% or greater. The observed difference between groups adjusted for the stopping rule was 55.3%, 95% CI (25.7%, 83.9%), p=0.0002, which exceeded the stopping boundary for efficacy. Thus, there was overwhelming evidence demonstrating that the early MRSA eradication treatment is more efficacious than symptomatic management with respect to MRSA negativity at day 28 and it was recommended to stop the trial early for efficacy based on these stopping boundaries.

Sensitivity Analyses

It is recognized that the decision to conduct an efficacy analysis at the second interim review was unplanned. Therefore, an additional analysis was performed to evaluate the sensitivity of the inference based on the primary endpoint to variation in stopping rules. Sensitivity analyses of this sort are described in Emerson and Banks (1994)(3) and Emerson (1995)(4). Table 2 displays estimates of the p-values, 95% confidence intervals, and treatment differences for several group sequential designs for evaluating early efficacy; all inference estimates presented in this table are adjusted for the corresponding group sequential stopping rule. The group sequential designs differ from each other with respect to both the shape of the efficacy

boundary and the size of the group sequential test corresponding to the stopping boundary. The boundary shape is determined by the parameter P, which is a measure of conservatism at the earlier analyses. Higher values of P cause the group sequential test to be more conservative at the earlier analyses (i.e. harder to stop). A Pocock (1977)(5) boundary corresponds to a boundary shape parameter of P=0.5 while an O'Brien-Fleming (1979)(2) boundary corresponds to P=1.0, indicating the increased conservatism when using an O'Brien-Fleming boundary. Similarly, the design becomes more conservative by assuming smaller type 1 error as also evaluated in the table.

Group sequential designs for these two most commonly used boundary shapes are presented in Table 2. Because the stopping rule for efficacy at this second interim analysis was not specified in advance, this sensitivity analysis allowed us to assess how sensitive the inference (related to difference in the proportion MRSA negative between treatment groups) was to a myriad of stopping rules that the DMC could have considered. As previously noted, the estimates of treatment differences, 95% confidence intervals, and p-values shown in this table are adjusted for the corresponding group sequential stopping rule that would have been used.

Table 2 Sensitivity Analyses for Evaluating Efficacy

	Interim Cohort w/ Culture Results at Baseline and Day 28 (N=39)		
Size of Test (one-sided)	**Pocock (1977) Boundary (P=0.5)**	**O'Brien-Fleming (1979) Boundary (P=1.0)**	**Alternative conservative boundary (P=1.2)**
α=0.005			
p-value	0.0002	0.000005	0.000003
95% Confidence Interval	26%,91%	33%, 84%	33%, 81%
Bias-adjusted mean	58%	58%	57%
α=0.010			
p-value	0.0002	0.00002	0.000003
95% Confidence Interval	26%,91%	31%, 84%	33%, 84%
Bias-adjusted mean	58%	58%	58%
α=0.025			
p-value	0.0002	0.0002	0.00001
95% Confidence Interval	26%,91%	26%, 84%	32%, 84%
Bias-adjusted mean	59%	55%	58%

There is no or little difference in the p-values and 95% confidence intervals between the different stopping rules, and the treatment effect estimates vary by no greater than 4%. Under all bounds evaluated that could have been chosen *a priori,* the decision would be to stop early for efficacy at this interim review.

Summary

In summary, we present a thorough evaluation of the placement of a post hoc interim efficacy bound on the primary endpoint. Given the difficulties with study enrollment, it was deemed necessary for the DMC to weigh the efficacy evidence at this interim review while taking into consideration the overall study objectives and potential benefits of accumulating further data.

References

1. Emerson SS, Fleming TR. Symmetric group sequential test designs. Biometrics. 1989;45(3):905-23.
2. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. Biometrics. 1979;35(3):549-56.
3. Emerson SS, Banks PLC. Interpretation of a leukemia trial stopped early. In: Lange N, Ryan L, Billiard L, Brillinger D, Conquest L, Greenhouse J, editors. Case Studies in Biometry. New York City: John Wiley & Sons; 1994.
4. Emerson SS. Stopping a clinical trial very early based on unplanned interim analyses: a group sequential approach. Biometrics. 1995;51(3):1152-62.
5. Pocock SJ. Group sequential methods in the design and analysis of clinical trials. Biometrika. 1977;64:191-9.