Ivacaftor is associated with reduced lung infection by key cystic fibrosis pathogens: A cohort study using national registry data

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Author contributions:
FF, DN and MJW contributed to the conception, design of the study and manuscript preparation. SC contributed to the design and statistical methodology. CW contributed to the interpretation of results and manuscript preparation. FF and SC performed the statistical analyses.

Funding: nil

Running head: Reduced prevalence of CF pathogens with ivacaftor

Subject category: 9.1 Adult Cystic Fibrosis

Word count: 2631

Key words:
Cystic fibrosis
Ivacaftor
Pseudomonas aeruginosa

Abbreviations:

AMR	Antimicrobial resistance
BCC	*Burkholderia cepacia* complex
BMI	Body mass index
CF	Cystic fibrosis
CFRD	Cystic fibrosis related diabetes
CFTR	Cystic fibrosis transmembrane conductance regulator
FEV1	Forced expiratory volume in one second
GOAL	G551D Observational Study
PR	Prevalence ratio

At a glance commentary:

Ivacaftor restores cystic fibrosis transmembrane conductance regulator (CFTR) function in people with cystic fibrosis and a gating mutation. Treatment is associated with improved lung function, increased weight and reduced exacerbation frequency. The question now arises as to whether some of the long-term treatments recommended to people with CF are needed in those who remain clinically stable whilst receiving ivacaftor. In-vitro data suggests ivacaftor may have bactericidal properties, however the long-term impact of CFTR restoration on chronic respiratory infection is unknown and greater understanding is vital to addressing the on-going need for chronic anti-infective therapies in this population.

This study includes the longest follow-up of respiratory infection in people receiving ivacaftor and provides evidence of sustained reductions in infection important CF pathogens. For the first time we have shown that lower post-ivacaftor *P. aeruginosa* prevalence appear to be driven by a combination of reduced acquisition and increased clearance of infection. These findings have important implications for antibiotic stewardship in people receiving ivacaftor.

Online Data Supplement: This article has an online data supplement, which is accessible from the issue's table of content online at www.atsjournals.org

Abstract

Rationale:

Ivacaftor can greatly improve clinical outcomes in people with cystic fibrosis (CF) and has been shown to have in-vitro antibacterial properties, yet the long-term microbiological outcomes of treatment are unknown.

Objectives:

To investigate changes in respiratory microbiology associated with long-term ivacaftor use.

Methods:

Retrospective cohort study utilising data from the United Kingdom CF Registry 2011-2016. Primary outcome was the annual prevalence ratios for key CF pathogens between ivacaftor users and their contemporaneous comparators. Multivariable log-binomial regression models were designed to adjust for confounders. Changes in *Pseudomonas aeruginosa* status were compared between groups using non-parametric maximum likelihood estimate for the purposes of Kaplan-Meier approximation.

Results

Ivacaftor use was associated with early and sustained reduction in *P. aeruginosa* rates (2016 Adjusted Prevalence Ratio [95% CI] 0.68 [0.58, 0.79], p<0.001) via a combination of increased clearance in those with infection (Ivacaftor: 33/87 [37.9%] vs. Non ivacaftor: 432/1872 [22.8%], p<0.001) and reduced acquisition in those without infection (49/134 [36.6%] vs. 1157/2382 [48.6%], p=0.01). The improved prevalence of *P. aeruginosa* infection was independent of reduced sampling in the ivacaftor cohort. Ivacaftor was also associated with reduced prevalence of *Staphylococcus aureus* and *Aspergillus* spp. but not *Burkholderia cepacia* complex.

Conclusion

In this study, long-term ivacaftor use was associated with reduced infection with important CF pathogens including *P. aeruginosa*. These findings have implications for antibiotic stewardship and the need for on-going chronic antimicrobial therapy in this cohort.

Introduction:

Cystic fibrosis (CF) is an ion-transport disease caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Dehydrated, viscid epithelial secretions result in a cycle of stasis, infection and inflammation in multiple organ systems but manifest most obviously in the lungs where chronic infection results in a progressive and irreversible decline in pulmonary function.

Recently, therapies aimed at correcting the underlying CFTR defect have become available and ivacaftor (Vertex Pharmaceuticals, USA), a CFTR potentiator, can restore CFTR function in people with a gating mutation such as G551D. Available in the UK since 2013, improved lung function, increased weight, reduced sweat chloride and improved exacerbation frequency have been demonstrated in short and long-term studies. (1, 2)

Chronic infection with classical CF pathogens such as *Pseudomonas aeruginosa* often requires long-term suppressive antibiotic therapy, which imparts a significant treatment burden on people with CF and also has implications for anti-microbial resistance (AMR). (3, 4) Ivacaftor may influence these respiratory pathogens and potentially reduce the need for such aggressive antibiotic therapy through two mechanisms. Firstly, the presence of a quinolone ring in its chemical structure may confer antibiotic properties and direct bactericidal activity has been confirmed in-vitro, where a potential synergism with colistimethate has been reported. (5-8) Secondly, correction of CFTR activity by ivacaftor may restore ion flux such that changes in the pulmonary microenvironment can influence

the ability of bacteria to survive in that niche. As some people with CF remain clinically stable having received over 5 years of ivacaftor therapy, understanding the long-term microbiological consequences becomes increasingly important for predicting future morbidity and also in implementing antibiotic stewardship.

The objective of this study was therefore to investigate the long-term microbiological outcomes associated with the use of ivacaftor.

Methods:

Study design

We undertook a retrospective cohort study utilising data from the UK CF Registry for the period 2011-2016. The UK CF Registry Steering Committee approved the study. The UK CF Registry is a Research Ethics Committee approved (Huntingdon Research Ethics Committee 07/Q0104/2) database holding demographic, clinical care, medication and health outcome data with excellent coverage of the CF population. The UK CF Registry data includes the annual presence or absence of key CF pathogens in respiratory cultures for each subject. For 2016, additional data including the number of respiratory cultures and number positive for *P. aeruginosa* were available.

Population

People with CF aged 6 and under were excluded from the study due to lack of consistent lung function and microbiology data. All individuals with at least one documented G551D mutation, who started ivacaftor treatment in 2013, were still receiving ivacaftor in 2016 and had complete microbiology data (a recorded status for each pathogen of interest for each year), formed the treatment cohort. The rest of the CF population formed the non-ivacaftor

comparator group. Subjects were excluded from the comparator cohort if they had received ivacaftor at any point since 2013 or if they had incomplete microbiology data for the study period.

Outcomes

The outcomes of primary interest were the annual prevalence ratios for each CF pathogen. Secondary outcomes included time to *P. aeruginosa* infection in those previously uninfected, and vice versa: time to *P. aeruginosa* clearance in those previously infected.

Primary outcome analysis and adjustment

Prevalence of a positive respiratory culture for *P. aeruginosa, Staphylococcus aureus, Aspergillus* spp. and the *Burkholderia cepacia* complex (BCC) were calculated annually 2011-2016. The two years preceding ivacaftor initiation in 2013 were included to allow more robust comparison of pre-ivacaftor microbiological trajectories. Annual prevalence ratios were calculated using two approaches. Firstly, the unadjusted ratio between the annual prevalence of each pathogen in the treatment and comparator cohorts. Secondly, a log-binomial regression model adjusted for known confounders identified from a review of the literature, see Supplement. Visual representation of identified potential associations in a direct acyclic graph was used to identify variables that were confounders, but not mediators or colliders, for inclusion in the model. Results from both unadjusted and adjusted analyses are presented as prevalence ratios with 95% confidence intervals.

Acquisition and clearance analyses

To assess whether changes in *P. aeruginosa* prevalence in the ivacaftor cohort were driven predominantly by changes in clearance, acquisition or a combination of the two, we classified any individual with positive respiratory cultures in each of the two years prior to ivacaftor initiation in 2013 as “infected” and any subject with no positive respiratory

cultures in that period as “uninfected”. Acquisition was defined as any member of the “uninfected” group with a subsequent positive respiratory culture. Clearance was defined as any member of the “infected” group without a recorded positive respiratory culture in any subsequent year. Given the precise timepoints for clearance and acquisition were known only within a defined 12-month period, we treated events as interval-censored data and calculated the non-parametric maximum likelihood estimate for the purposes of Kaplan-Meier approximation. (9)

Sensitivity analyses

To address the robustness of our primary analysis we performed a number of further analyses. Quantitative sample data, i.e. number of respiratory samples per year, was only available for the year 2016 and consequently could not be included in the analysis throughout. To test the impact of this variable on effect estimates we performed two sensitivity analyses by firstly including total sputum samples in a regression model for 2016 data and secondly, a restricted analysis, where all subjects providing < 3 sputum samples in 2016 were excluded. Given an in-vitro synergism between colistimethate and ivacaftor has been reported, (8) we performed a separate second restriction analysis excluding those receiving inhaled colistimethate preparations at any stage during the study period.

Statistical analyses

All analyses were performed in R (v3.3.3, R Foundation for Statistical Computing, 2018). Summary statistics including counts and percentages for categorical variables and means and standard deviations for continuous variables were used to describe the study cohorts. Between-group comparisons were made using t-tests or Mann-Whitney test for parametric and non-parametric continuous data respectively, with Chi-square test used for categorical

data. Unadjusted *p*-values are presented throughout with statistical significance considered <0.05.

Results:

Study cohort

In 2013, 276 people with CF and at least one G551D mutation met the inclusion criteria for the ivacaftor group and 5296 were included in the non-ivacaftor comparator group, see Supplement: Figure S1). Baseline characteristics of both groups for the year 2012 are presented in Table 1.

Reduced prevalence of CF pathogens following ivacaftor initiation

In 2012 the ivacaftor cohort had similar *P. aeruginosa* prevalence as their matched comparators (128/276, 46.4% vs. 2536/5296, 47.9%), see Table 2, however following ivacaftor initiation in 2013 the annual prevalence of *P. aeruginosa* fell in the ivacaftor cohort (48.9%, 40.6%, 37.0% and 35.9% for 2013-2016 respectively), see Figure 1. Smaller reductions were observed for *S. aureus* (prevalence 33.3%, 30.1%, 25.7%, and 30.1% for 2013-2016 respectively). From 2013 to 2016, *Aspergillus* spp. prevalence in the ivacaftor cohort fell from 12.0% to 4.7%, but for the same time-period there was <1% change in BCC prevalence in both cohorts, see Table 2.

Primary outcome

Prevalence ratios for a positive culture between the ivacaftor group and their matched comparators were calculated and are presented in Figure 2 & Table 2. In 2014 ivacaftor use was associated with reduced rate of positive culture for *P. aeruginosa* (Adjusted PR [95% CI] 0.78 [0.68, 0.89], *p*<0.001) and *Aspergillus* spp. (Adjusted PR 0.56 [0.40, 0.77], p<0.001), however estimates were smaller for *S. aureus* (Adjusted PR 0.92 [0.76, 1.1], *p*=0.39), see

Table 2. Prevalence ratio for *P. aeruginosa* continued to decrease throughout the study period such that by 2016 there was a 32% reduction (Adjusted PR 0.68 [0.58, 0.79], p<0.001). After three years of treatment, less pronounced changes were observed for *S. aureus* (Adjusted PR 0.85 [0.70, 1.01], *p*=0.08).

Independence of improved *P. aeruginosa* rates from reductions in sampling.

In 2016 there were quantitative data available for the number of respiratory cultures performed. The ivacaftor group had fewer sputum samples in that year than their comparators (median [IQR]: 2 [0-6] vs. 4 [1-7], *p*<0.001) but similar total annual cough-swabs. The association between ivacaftor use and reduced *P. aeruginosa* remained when sputum sample count was included into the multivariable model for 2016, see Table S1. Furthermore, a restricted analysis including only "sputum producers" (those individuals with ≥3 sputum samples) found results consistent with the primary analysis, see Figure S3.

Combination of increased clearance and reduced acquisition contribute to improved *P. aeruginosa* rates.

Next we investigated whether reduced acquisition, increased clearance or both drove the changes in *P. aeruginosa* prevalence. In those individuals with 2 years of documented *P. aeruginosa* growth prior to 2013, there were significantly higher rates of clearance in the ivacaftor group by the end of the study period (33/87 [37.9%] vs. 432/1872 [22.8%], *p*<0.001), Figure 3a. Furthermore, in those subjects without *P. aeruginosa* growth in the 2 years prior to 2013, fewer subjects receiving ivacaftor had a subsequent growths (49/134 [36.6%] vs. 1157/2382 [48.6%], *p*=0.01), Figure 3b.

Characteristics of individuals with change in *P. aeruginosa status*

Next we tested the hypothesis that subjects with a change in *P. aeruginosa* infection status following ivacaftor initiation may have different clinical characteristics to those who did not. Those who cleared *P. aeruginosa* whilst receiving ivacaftor were younger (25.1 ± 8.8 years vs. 29.0 ± 8.3 years, p=0.03) and had a greater sweat chloride responses 6-8 weeks post-ivacaftor initiation, see Table 3. Those who acquired *P. aeruginosa* whilst receiving ivacaftor had poorer lung function at baseline (FEV1 80.9 ± 20.4 % predicted vs. 90.1 ± 18.1 % predicted, p=0.005), but no other significant differences, see Table 4.

Antibiotic usage

We compared antibiotic treatments between each group at baseline and across the study period. Rates of anti-pseudomonal antibiotic usage were similar at baseline see Table 1. Median years receiving inhaled antibiotics was also similar between groups (median [IQR] 2 years [1-4] vs. 3 years [1-4], p=0.40), and in no year was inhaled antibiotic use significantly greater in the ivacaftor group, Figure S4. Finally, in an analysis restricted only to those who did not receive inhaled colistimethate during the study period, we found our primary analysis was robust, suggesting the previously reported *in-vitro* synergism between ivacaftor and colistimethate was not acting as a confounder, see Figure S5. (8)

Discussion:

We used longitudinal data from the UK CF Registry to investigate changes in sputum microbiology associated with ivacaftor use in people with CF aged six years and above. Ivacaftor use was associated with early and sustained reductions in positive respiratory cultures for *P. aeruginosa* such that the likelihood of a positive culture was reduced by 32%

after three years of treatment. This association persisted even when adjusted for the reduced sampling seen in those receiving ivacaftor. These findings have implications for the need for ongoing chronic suppressive antimicrobial therapy in those receiving ivacaftor. Significant reductions in *S. aureus* were also observed, but only from the second year of treatment onwards and absolute reductions in prevalence were smaller than for *P. aeruginosa*. Early reductions in *Aspergillus* spp. were also observed, however the relatively low frequency in the ivacaftor group means this finding must be interpreted with caution. No association with BCC infection was observed although given the low prevalence of BCC infection, our study is likely underpowered in this regard.

The reductions in *P. aeruginosa* seen here are in keeping with previous smaller studies, where the odds of a positive culture were reduced in the year following ivacaftor initiation. (10-12) We found reduced *S. aureus* following ivacaftor initiation, a finding also reported by a French study of 2 years ivacaftor experience but not observed in the G551D Observational (GOAL) Study, a prospective observational study in the US which reported culture results after one year of ivacaftor treatment. (10, 12) Here, differences to the GOAL study may be partly explained by the much larger cohort and longer follow up period in our study, particularly as significant reductions in *S. aureus* culture positivity only occurred in the latter two years of our follow-up period. (10) The GOAL study lacked a comparator group and our use of the UK CF Registry allowed us to confirm changes were limited to those receiving ivacaftor rather than in the wider CF population. Furthermore, GOAL was a US study and comparisons between different countries and healthcare systems are challenging, particularly given Bessanova *et al* (13) recently reported reduced *S. aureus* in people receiving ivacaftor in the US but not in the UK, where baseline *S. aureus* prevalence was half

of that in the US. The same study found similar reductions in *P. aeruginosa* and *Aspergillus* spp. as seen here but only included one year of follow up. We were able to include the three years post-ivacaftor initiation, the largest follow up of microbiological outcomes in this discrete patient group to date. The large dataset and longer follow up allowed us to show, for the first time, that changes in *P. aeruginosa* sputum positivity appear to be driven both by increased clearance and also reduced acquisition.

The question arises as to why ivacaftor is associated with a pronounced effect against *P. aeruginosa* in the CF lung. Although the quinolone ring in its chemical structure may confer innate antibacterial properties, in-vitro studies have demonstrated most activity against Gram-positive organisms such as *S. aureus* rather than *P. aeruginosa,* although when used in combination with colistimethate a synergistic effect against *P. aeruginosa* has been reported. (5-7) We found earlier and larger reductions in *P. aeruginosa*, regardless of colistimethate use, suggesting a direct bactericidal effect from ivacaftor is not the predominant mechanism for the changes we observed. Furthermore, clearance of *P. aeruginosa* was associated with a greater sweat chloride response to ivacaftor and any antimicrobial effect seems more likely related directly to CFTR restoration.

CFTR restoration has been associated with improved mucociliary clearance, which could explain some of the changes we observed. (14) However, increased elimination of bacteria by this mechanism should be species agnostic and might even favour elimination of species such as *S. aureus* that do not form biofilms as readily, we found the opposite. Alternatively, restoration of CFTR function has recently been associated with increased airway surface liquid pH, mirroring changes within the gut where pH normalised following ivacaftor

initiation, and this may restore activity of some pH dependent innate antimicrobial peptides. (14-16) Equally, such changes to regional growth conditions could disproportionately affect *P. aeruginosa* since it is well adapted to the CFTR-defective lung, where it gains a selective advantage via a number of complex physiological changes driven by mutations resulting in different genotypic and phenotypic traits. (17, 18) Whilst these traits allow it to successfully establish chronic infection, rapid and dramatic restoration of CFTR function could potentially render it vulnerable in an environment it is no longer adapted to survive. In keeping with this, ivacaftor initiation has previously been implicated in reductions of mucoid but not non-mucoid *P. aeruginosa*. (11) Finally, functional CFTR plays a specific role in the innate immune response to *P. aeruginosa* and hence ivacaftor may exert influence on *P.* aeruginosa by restoring that function. (19, 20) Interestingly, CFTR has also been implicated in the immune response to *Aspergillus* spp., for which we also found reductions following ivacaftor initiation. (21)

Our findings of reduced CF pathogens are relevant clinically given the morbidity associated with chronic infection by these species (22-24), and imply there may be potential to safely reduce the treatment burden in some patients. Indeed, one third of individuals with chronic *P. aeruginosa* infection prior to ivacaftor initiation were culture negative at the end of the study period. We found that older people with CF were less likely to eradicate pathogens, a finding supported by a smaller study by Hisert *et al* (25) of older adults with CF. In that study, although clearance of *P. aeruginosa* was not observed, bacterial load was initially reduced following ivacaftor initiation yet increased in the second year of treatment and the authors speculated that *P. aeruginosa* could diversify to survive in a CFTR restored

environment, suggesting an on-going need for anti-pseudomonal therapies in those who remain culture positive.

Addressing selection bias and confounding in observational studies of drug effects is inherently challenging. We used a multivariable log-binomial regression model to adjust for known confounders but there remains potential for residual confounding particularly with respect to unmeasured imbalance between groups. Misclassification is an inherent risk in registry based studies, and in attempt to mitigate this we only included individuals with two consecutive years of a similar microbiological status in our clearance and acquisition analyses. Since we excluded those under the age of 6 and those with incomplete microbiological data, including those who died during the study period, our findings may not be generalisable to young children and those with more severe disease. Equally, a larger proportion of patients were excluded from the potential comparator group than the ivacaftor group due to insufficient data (most likely due to continued ivacaftor prescription being dependent on regular follow up) and thus we cannot exclude a sampling bias in that cohort. Given the registry based nature of this study there is also a risk of bias from inter-centre variation. For example respiratory culture sampling, processing and laboratory assessment may differ between centres particularly with regard low volume, or low quality specimens.

There is a risk of indication bias in that ivacaftor use is dictated by genotype, where some are associated with increased bacterial colonisation. (26) However, groups had similar rates of class I-III mutations and, importantly, had comparable sputum microbiology in both pre-ivacaftor years suggesting they were on similar microbiological trajectories. With regards to

363 microbiology, for most of the study period the UK CF Registry recorded only the presence or
364 absence of each pathogen in that year, hence for the most part we are unable to determine
365 if the proportion of positive samples changed over time. Quantitative data for *P. aeruginosa*
366 was available for 2016, which allowed us to confirm the changes we observed were
367 consistent even when adjusted for reduced sampling in the ivacaftor group.
368
369 The strengths of this study lie in the large sample size and the longest follow-up period to
370 date. The large dataset afforded us by utilising the UK CF registry allowed us to demonstrate
371 for the first time that both increased clearance and reduced acquisition contributed to
372 reductions in *P. aeruginosa* infection following ivacaftor initiation.
373
374 **Conclusion**
375 In summary, we utilised national registry data to compare changes in respiratory
376 microbiology in the years after ivacaftor initiation. We found ivacaftor was associated with
377 reductions in lung infections by important CF pathogens.
378

379 **Acknowledgements**
380 We would like to thank the UK CF Registry Steering Committee for provision of data.
381

382 **Author contributions:**
383 FF, DN and MJW contributed to the conception, design of the study and manuscript
384 preparation. SC contributed to the design and statistical methodology. CW contributed to
385 the interpretation of results and manuscript preparation. FF and SC performed the statistical
386 analyses. FF is the guarantor of the data.
387

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Figure 1: Annual prevalence (± 95% confidence interval) of a positive respiratory culture for *P. aeruginosa* (A), *S. aureus* (B), *Aspergillus* spp. (C), *B. cepacia* complex (D) in the ivacaftor (n=276) and non-ivacaftor groups (n=5296). Dotted black line represents ivacaftor initiation in 2013.

Figure 2: Adjusted prevalence ratio ± 95% confidence intervals between the ivacaftor (n=276) and non-ivacaftor users (5296) for a positive respiratory culture for *P. aeruginosa* (A), *S. aureus* (B), *Aspergillus* spp. (C), *B. cepacia* complex (D).

Figure 3: Kaplan-meier curve for clearance of *P. aeruginosa* infection in previously infected individuals (A) and acquisition of *P. aeruginosa* infection in previously uninfected individuals (B).

Table 1: Baseline characteristics of included subjects (n=5572) by ivacaftor use. Data are taken from year 2012, the year prior to ivacaftor initiation, and are presented as count (%) and mean (SD).

	Ivacaftor		Non-ivacaftor	
n	276		5296	
Age	21.43	(10.4)	23.4	(12.0)
Male	154	(55.8)	2815	(53.2)
CFRD	47	(17)	1281	(24.2)
Pancreatic enzymes	255	(92.4)	4538	(86.5)
FEV1, *predicted*	81.07	(22.5)	72.91	(23.3)
FEV1, *litres*	2.5	(1.0)	2.16	(1.01)
Body mass index				
Adults, kg/m2	23.17	(3.4)	22.47	(3.76)
Children, percentile	55.48	(26.8)	48.34	(28.5)
Annual IV days	13.14	(20.8)	19.56	(28.9)
Microbiology				
P. aeruginosa	128	(46.4)	2536	(47.9)
Chronic	102	(37.0)	2068	(39.0)
S. aureus	89	(32.2)	1489	(28.1)
Chronic	58	(21)	996	(18.8)
B. cenocepacia	4	(1.4)	48	(0.9)
B. multivorans	3	(1.1)	100	(1.9)
Aspergillus spp.	34	(12.3)	733	(13.8)
Genotype				
Class I-III	232	(84.1)	3977	(75.1)
Phe508del homozygous	2	(0.7)	2881	(54.4)
Phe508del heterozygous	200	(72.5)	1783	(33.7)
Nebulised anti-pseudomonal				
Any	135	(48.9)	2401	(45.3)
Tobramycin	67	(24.3)	1200	(22.7)
Colistimethate	103	(37.3)	1793	(33.9)
Oral Macrolide	139	(50.4)	2505	(47.3)

Abbreviations: CFRD= Cystic fibrosis related diabetes; FEV1= Forced expiratory volume in 1 second; IV= intravenous

Table 2: Annual prevalence of *P. aeruginosa, S. aureus, Aspergillus* spp. And BCC in ivacaftor (n=276) and non-ivacaftor (n=5296) users. Unadjusted and adjusted[a] prevalence ratios are presented with 95% confidence intervals.

P. aeruginosa								
	Ivacaftor	**Non-ivacaftor**	**Unadjusted Prevalence Ratio**			**Adjusted Prevalence Ratio**		
Year	Culture-positive (%)	Culture-positive (%)	PR [95% CI]		*p*	PR [95% CI]		*p*
2011	101 (36.6)	2250 (42.5)	0.89	[0.74,1.01]	0.69	0.92	[0.8,1.05]	0.25
2012	128 (46.4)	2536 (47.9)	0.97	[0.85,1.10]	0.54	1.01	[0.9,1.13]	0.80
2013	135 (48.9)	2691 (50.8)	0.96	[0.85. 1.09]	0.37	0.99	[0.88,1.1]	0.82
2014	112 (40.6)	2806 (53)	0.77	[0.66,0.89]	0.003	0.78	[0.68,0.89]	<0.001
2015	102 (37)	2825 (53.3)	0.69	[0.59,0.81]	<0.001	0.72	[0.61,0.82]	<0.001
2016	99 (35.9)	2901 (54.8)	0.65	[0.56,0.77]	<0.001	0.68	[0.58,0.79]	<0.001
***Aspergillus* spp.**								
	Ivacaftor	**Non-ivacaftor**	**Unadjusted Prevalence Ratio**			**Adjusted Prevalence Ratio**		
Year	Culture-positive (%)	Culture-positive (%)	PR [95% CI]		*p*	PR [95% CI]		*p*
2011	26 (9.4)	525 (9.9)	0.95	[0.65,1.38]	0.92	0.93	[0.62,1.32]	0.70
2012	34 (12.3)	733 (13.8)	0.89	[0.65,1.23]	0.53	0.87	[0.62,1.17]	0.38
2013	33 (12)	888 (16.8)	0.71	[0.51 0.99]	0.04	0.69	[0.49,0.94]	0.03
2014	32 (11.6)	1055 (19.9)	0.58	[0.42,0.81]	<0.001	0.56	[0.4,0.77]	<0.001
2015	31 (11.2)	1042 (19.7)	0.57	[0.41, 0.80]	<0.001	0.56	[0.39,0.77]	<0.001
2016	13 (4.7)	897 (16.9)	0.28	[0.16,0.47]	<0.001	0.27	[0.15,0.44]	<0.001
S. aureus								
	Ivacaftor	**Non-ivacaftor**	**Unadjusted Prevalence Ratio**			**Adjusted Prevalence Ratio**		
Year	Culture-positive (%)	Culture-positive (%)	PR [95% CI]		*p*	PR [95% CI]		*p*
2011	67 (24.3)	1248 (23.6)	1.03	[0.83,1.28]	0.77	1.04	[0.83,1.27]	0.73
2012	89 (32.2)	1489 (28.1)	1.15	[0.96,1.37]	0.15	1.14	[0.94,1.34]	0.16
2013	92 (33.3)	1681 (31.7)	1.05	[0.88,1.25]	0.6	1.04	[0.87,1.22]	0.67
2014	83 (30.1)	1699 (32.1)	0.94	[0.78,1.13]	0.51	0.92	[0.76,1.1]	0.39
2015	71 (25.7)	1741 (32.9)	0.78	[0.64,0.96]	0.01	0.77	[0.62,0.94]	0.01
2016	83 (30.1)	1844 (34.8)	0.86	[0.72,1.04]	0.12	0.85	[0.7,1.01]	0.08
BCC								
Year	**Ivacaftor**	**Non-ivacaftor**	**Unadjusted Prevalence Ratio**			**Adjusted Prevalence Ratio**		
Year	Culture-positive (%)	Culture-positive (%)	PR [95% CI]		*p*	PR [95% CI]		*p*
2011	9 (3.3)	172 (3.2)	1.00	[0.52,1.94]	0.99	0.98	[0.47,1.78]	0.96
2012	10 (3.6)	184 (3.5)	1.04	[0.56,1.95]	0.87	1.02	[0.51,1.8]	0.95
2013	12 (4.3)	205 (3.9)	1.12	[0.64,1.98]	0.63	1.1	[0.59,1.85]	0.75
2014	11 (4)	219 (4.1)	0.96	[0.53,1.74]	0.99	0.93	[0.59,1.75]	0.82
2015	13 (4.7)	227 (4.3)	1.10	[0.64,1.90]	0.76	1.06	[0.59,1.75]	0.82
2016	14 (5.1)	233 (4.4)	1.15	[0.68,1.95]	0.55	1.11	[0.62,1.8]	0.70

a: Multivariable log- binomial regression model adjusted for age and genotype.

Table 3: Clinical characteristics of individuals with two years of consecutive *P. aeruginosa* growth with comparison based upon subsequent clearance. Data are presented as mean (SD) or count (%).

Clearance of *P. aeruginosa*			
	No	Yes	p
n	54	33	
Age, *years*	29.0 (8.3)	25.1 (8.8)	0.03
Male	30 (55.6)	16 (48.5)	0.68
CFRD	16 (29.6)	5 (15.2)	0.20
Pancreatic enzymes	50 (92.6)	31 (93.9)	0.99
FEV1, *%predicted*	70.3 (27.7)	77.0 (21.9)	0.24
FEV1, *litres*	2.5 (1.1)	2.7 (0.9)	0.54
Annual IV days	19.0 (25.5)	14.8 (22.3)	0.44
Sweat Chloride, *mmol/L*			
Pre-ivacaftor	107.4 (11.1)	103.7 (14.1)	0.47
6-8 weeks post-ivacaftor	58.2 (21.7)	39.8 (17.4)	0.03
Change	49.2 (16.9)	63.9 (11.7)	0.02

CFRD=Cystic fibrosis related diabetes. FEV1=Forced expiratory volume in 1 second.

Table 4: Clinical characteristics of individuals without documented growth of *P. aeruginosa* in the two years prior to initiating ivacaftor with comparison based upon subsequent acquisition**.** Data are presented as mean (SD) or count (%).

Acquisition of *P. aeruginosa*			
	No	Yes	p
n	85	49	
Age, years	17.1 (9.5)	18.9 (11.1)	0.31
Male	54 (58.7)	28 (49.1)	0.33
CFRD	11 (12.0)	8 (14.0)	0.91
Pancreatic enzymes	78 (84.8)	53 (93.0)	0.22
FEV1, % predicted	90.1 (18.1)	80.9 (20.4)	0.005
FEV1 (L)	2.5 (1.1)	2.3 (1.0)	0.28
Annual IV days	9.3 (19.6)	14.8 (20.4)	0.10
Sweat Chloride *mmol/L*			
Pre-ivacaftor	98.4 (30.3)	94.5 (21.6)	0.62
6-8 weeks post-ivacaftor	53.3 (18.1)	49.8 (17.1)	0.50
Change	47.1 (19.7)	43.8 (21.2)	0.58

CFRD=Cystic fibrosis related diabetes. FEV1=Forced expiratory volume in 1 second.