

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

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24 FF, DN and MJW contributed to the conception, design of the study and manuscript
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43

44 **Abbreviations:**

45

46 **AMR** Antimicrobial resistance

47 **BCC** *Burkholderia cepacia* complex

48 **BMI** Body mass index

49 **CF** Cystic fibrosis

50 **CFRD** Cystic fibrosis related diabetes

51 **CFTR** Cystic fibrosis transmembrane conductance regulator

52 **FEV1** Forced expiratory volume in one second

53 **GOAL** G551D Observational Study

54 **PR** Prevalence ratio

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57 **At a glance commentary:**

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

66
67 This study includes the longest follow-up of respiratory infection in people receiving
68 ivacaftor and provides evidence of sustained reductions in infection important CF
69 pathogens. For the first time we have shown that lower post-ivacaftor *P. aeruginosa*
70 prevalence appear to be driven by a combination of reduced acquisition and increased
71 clearance of infection. These findings have important implications for antibiotic stewardship
72 in people receiving ivacaftor.

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

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77
78

Abstract

79 **Rationale:**

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

83 **Objectives:**

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

85 **Methods:**

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

92 **Results**

93 Ivacaftor use was associated with early and sustained reduction in *P. aeruginosa* rates (2016
94 Adjusted Prevalence Ratio [95% CI] 0.68 [0.58, 0.79], $p < 0.001$) via a combination of
95 increased clearance in those with infection (Ivacaftor: 33/87 [37.9%] vs. Non ivacaftor:
96 432/1872 [22.8%], $p < 0.001$) and reduced acquisition in those without infection (49/134
97 [36.6%] vs. 1157/2382 [48.6%], $p = 0.01$). The improved prevalence of *P. aeruginosa* infection
98 was independent of reduced sampling in the ivacaftor cohort. Ivacaftor was also associated
99 with reduced prevalence of *Staphylococcus aureus* and *Aspergillus* spp. but not *Burkholderia*
100 *cepacia* complex.

101 **Conclusion**

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

105

106

107 **Introduction:**

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

113

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

119

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

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

133

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

136 **Methods:**

137 Study design

138 We undertook a retrospective cohort study utilising data from the UK CF Registry for the
139 period 2011-2016. The UK CF Registry Steering Committee approved the study. The UK CF
140 Registry is a Research Ethics Committee approved (Huntingdon Research Ethics Committee
141 07/Q0104/2) database holding demographic, clinical care, medication and health outcome
142 data with excellent coverage of the CF population. The UK CF Registry data includes the
143 annual presence or absence of key CF pathogens in respiratory cultures for each subject. For
144 2016, additional data including the number of respiratory cultures and number positive for
145 *P. aeruginosa* were available.

146 Population

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

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

155 Outcomes

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

159 Primary outcome analysis and adjustment

160 Prevalence of a positive respiratory culture for *P. aeruginosa*, *Staphylococcus aureus*,
161 *Aspergillus* spp. and the *Burkholderia cepacia* complex (BCC) were calculated annually 2011-
162 2016. The two years preceding ivacaftor initiation in 2013 were included to allow more
163 robust comparison of pre-ivacaftor microbiological trajectories. Annual prevalence ratios
164 were calculated using two approaches. Firstly, the unadjusted ratio between the annual
165 prevalence of each pathogen in the treatment and comparator cohorts. Secondly, a log-
166 binomial regression model adjusted for known confounders identified from a review of the
167 literature, see Supplement. Visual representation of identified potential associations in a
168 direct acyclic graph was used to identify variables that were confounders, but not mediators
169 or colliders, for inclusion in the model. Results from both unadjusted and adjusted analyses
170 are presented as prevalence ratios with 95% confidence intervals.

171 Acquisition and clearance analyses

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

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

183 Sensitivity analyses

184 To address the robustness of our primary analysis we performed a number of further
185 analyses. Quantitative sample data, i.e. number of respiratory samples per year, was only
186 available for the year 2016 and consequently could not be included in the analysis
187 throughout. To test the impact of this variable on effect estimates we performed two
188 sensitivity analyses by firstly including total sputum samples in a regression model for 2016
189 data and secondly, a restricted analysis, where all subjects providing < 3 sputum samples in
190 2016 were excluded. Given an in-vitro synergism between colistimethate and ivacaftor has
191 been reported, (8) we performed a separate second restriction analysis excluding those
192 receiving inhaled colistimethate preparations at any stage during the study period.

193 Statistical analyses

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

199 data. Unadjusted p -values are presented throughout with statistical significance considered
200 <0.05 .

201 **Results:**

202 Study cohort

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

207 Reduced prevalence of CF pathogens following ivacaftor initiation

208 In 2012 the ivacaftor cohort had similar *P. aeruginosa* prevalence as their matched
209 comparators (128/276, 46.4% vs. 2536/5296, 47.9%), see Table 2, however following
210 ivacaftor initiation in 2013 the annual prevalence of *P. aeruginosa* fell in the ivacaftor cohort
211 (48.9%, 40.6%, 37.0% and 35.9% for 2013-2016 respectively), see Figure 1. Smaller
212 reductions were observed for *S. aureus* (prevalence 33.3%, 30.1%, 25.7%, and 30.1% for
213 2013-2016 respectively). From 2013 to 2016, *Aspergillus* spp. prevalence in the ivacaftor
214 cohort fell from 12.0% to 4.7%, but for the same time-period there was $<1\%$ change in BCC
215 prevalence in both cohorts, see Table 2.

216 Primary outcome

217 Prevalence ratios for a positive culture between the ivacaftor group and their matched
218 comparators were calculated and are presented in Figure 2 & Table 2. In 2014 ivacaftor use
219 was associated with reduced rate of positive culture for *P. aeruginosa* (Adjusted PR [95% CI]
220 0.78 [0.68, 0.89], $p<0.001$) and *Aspergillus* spp. (Adjusted PR 0.56 [0.40, 0.77], $p<0.001$),
221 however estimates were smaller for *S. aureus* (Adjusted PR 0.92 [0.76, 1.1], $p=0.39$), see

222 Table 2. Prevalence ratio for *P. aeruginosa* continued to decrease throughout the study
223 period such that by 2016 there was a 32% reduction (Adjusted PR 0.68 [0.58, 0.79],
224 $p<0.001$). After three years of treatment, less pronounced changes were observed for *S.*
225 *aureus* (Adjusted PR 0.85 [0.70, 1.01], $p=0.08$).

226

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

228 In 2016 there were quantitative data available for the number of respiratory cultures
229 performed. The ivacaftor group had fewer sputum samples in that year than their
230 comparators (median [IQR]: 2 [0-6] vs. 4 [1-7], $p<0.001$) but similar total annual cough-
231 swabs. The association between ivacaftor use and reduced *P. aeruginosa* remained when
232 sputum sample count was included into the multivariable model for 2016, see Table S1.
233 Furthermore, a restricted analysis including only “sputum producers” (those individuals with
234 ≥ 3 sputum samples) found results consistent with the primary analysis, see Figure S3.

235 Combination of increased clearance and reduced acquisition contribute to improved *P.* 236 *aeruginosa* rates.

237 Next we investigated whether reduced acquisition, increased clearance or both drove the
238 changes in *P. aeruginosa* prevalence. In those individuals with 2 years of documented *P.*
239 *aeruginosa* growth prior to 2013, there were significantly higher rates of clearance in the
240 ivacaftor group by the end of the study period (33/87 [37.9%] vs. 432/1872 [22.8%],
241 $p<0.001$), Figure 3a. Furthermore, in those subjects without *P. aeruginosa* growth in the 2
242 years prior to 2013, fewer subjects receiving ivacaftor had a subsequent growths (49/134
243 [36.6%] vs. 1157/2382 [48.6%], $p=0.01$), Figure 3b.

244

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

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

253 Antibiotic usage

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

262

263 **Discussion:**

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

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

277

278 The reductions in *P. aeruginosa* seen here are in keeping with previous smaller studies,
279 where the odds of a positive culture were reduced in the year following ivacaftor initiation.
280 (10-12) We found reduced *S. aureus* following ivacaftor initiation, a finding also reported by
281 a French study of 2 years ivacaftor experience but not observed in the G551D Observational
282 (GOAL) Study, a prospective observational study in the US which reported culture results
283 after one year of ivacaftor treatment. (10, 12) Here, differences to the GOAL study may be
284 partly explained by the much larger cohort and longer follow up period in our study,
285 particularly as significant reductions in *S. aureus* culture positivity only occurred in the latter
286 two years of our follow-up period. (10) The GOAL study lacked a comparator group and our
287 use of the UK CF Registry allowed us to confirm changes were limited to those receiving
288 ivacaftor rather than in the wider CF population. Furthermore, GOAL was a US study and
289 comparisons between different countries and healthcare systems are challenging,
290 particularly given Bessanova *et al* (13) recently reported reduced *S. aureus* in people
291 receiving ivacaftor in the US but not in the UK, where baseline *S. aureus* prevalence was half

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

298

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

309

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

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

329

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

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

341

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

358

359 There is a risk of indication bias in that ivacaftor use is dictated by genotype, where some
360 are associated with increased bacterial colonisation. (26) However, groups had similar rates
361 of class I-III mutations and, importantly, had comparable sputum microbiology in both pre-
362 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

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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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480

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

485

486

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

491

492

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

496

497

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

501
 502
 503

	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/m ²	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		
<i>P. aeruginosa</i>	128 (46.4)	2536 (47.9)
Chronic	102 (37.0)	2068 (39.0)
<i>S. aureus</i>	89 (32.2)	1489 (28.1)
Chronic	58 (21)	996 (18.8)
<i>B. cenocepacia</i>	4 (1.4)	48 (0.9)
<i>B. multivorans</i>	3 (1.1)	100 (1.9)
<i>Aspergillus spp.</i>	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)

504
 505 Abbreviations: CFRD= Cystic fibrosis related diabetes; FEV1= Forced expiratory volume in 1
 506 second; IV= intravenous
 507

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

<i>P. aeruginosa</i>								
Year	Ivacaftor	Non-ivacaftor	Unadjusted Prevalence Ratio			Adjusted Prevalence Ratio		
	Culture-positive (%)	Culture-positive (%)	PR [95% CI]	<i>p</i>	PR [95% CI]	<i>p</i>		
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
<i>Aspergillus</i> spp.								
Year	Ivacaftor	Non-ivacaftor	Unadjusted Prevalence Ratio			Adjusted Prevalence Ratio		
	Culture-positive (%)	Culture-positive (%)	PR [95% CI]	<i>p</i>	PR [95% CI]	<i>p</i>		
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
<i>S. aureus</i>								
Year	Ivacaftor	Non-ivacaftor	Unadjusted Prevalence Ratio			Adjusted Prevalence Ratio		
	Culture-positive (%)	Culture-positive (%)	PR [95% CI]	<i>p</i>	PR [95% CI]	<i>p</i>		
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		
	Culture-positive (%)	Culture-positive (%)	PR [95% CI]	<i>p</i>	PR [95% CI]	<i>p</i>		
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

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

512

513

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

517

Clearance of <i>P. aeruginosa</i>			
	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

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520

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

521 **Table 4:** Clinical characteristics of individuals without documented growth of *P.*
 522 *aeruginosa* in the two years prior to initiating ivacaftor with comparison based
 523 upon subsequent acquisition. Data are presented as mean (SD) or count (%).
 524

Acquisition of <i>P. aeruginosa</i>			
	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 <i>mmol/L</i>			
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

525
 526 **CFRD=Cystic fibrosis related diabetes. FEV1=Forced expiratory volume in 1 second.**
 527
 528
 529