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***PSEUDOMONAS AERUGINOSA* IN PATIENTS WITH c.1652G>A
(G551D) -CFTR TREATED WITH IVACAFTOR -
CHANGES IN MICROBIOLOGICAL PARAMETERS**

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Running Title: Does ivacaftor change microbiological parameters *in vivo*?

Key words: ivacaftor; *Pseudomonas aeruginosa*; cystic fibrosis; antibiotic susceptibility;
microbiology;

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50 **SUMMARY**

51

52 *What is known and objective:* The CFTR potentiator, Ivacaftor (IVA), has been widely used in the
53 treatment of cystic fibrosis (CF) patients with the G551D mutation. To date, there has been limited
54 information on the microbiological status of patients on this therapy and no data on the effect (if any)
55 on the *in vivo* antibiotic susceptibility of *Pseudomonas aeruginosa* isolated from patients on therapy.
56 Whilst IVA intervention is not designed *per se* as anti-infective, the effect (if any) of this molecule to
57 CF patients' microbiological status merits careful monitoring. Therefore, it was the aim of this
58 observational study to examine the effect in patients, both before and after commencement of IVA
59 therapy, on several commonly reported microbiological markers in CF patients, including (i) bacterial
60 density, (ii) frequency (rate) of isolation of bacterial pathogens, particularly *Pseudomonas aeruginosa*
61 and (iii) antimicrobial susceptibility of these isolates to commonly prescribed oral and iv antibiotics.
62 In addition, we wished to examine the requirements for these antibiotics in CF patients, before and
63 after commencement of IVA therapy.

64

65 *Methods:* Archived data from 15 adult patients with the c.1652G>A (G551D) mutation were followed
66 from two years pre-IVA therapy to two years after commencement of IVA therapy. The
67 microbiological parameters examined included (i) oral antibiotic courses taken, (ii) intravenous (iv)
68 antibiotic courses taken, (iii) rate of isolation of non-mucoid *Pseudomonas aeruginosa* (NM-PA) and
69 mucoid *Pseudomonas aeruginosa* (M-PA), (iv) density of NM-PA and M-PA and (v) antimicrobial
70 susceptibility of NM-PA and M-PA to 11 antibiotics [aminoglycosides, beta-lactams, polymyxin and
71 fluoroquinolone]

72

73 *Results and discussion:* Following commencement of IVA therapy, patients required less iv antibiotic
74 courses but no change in number of oral antibiotics courses. There was significant reduction in both
75 the rate of isolation and density of M-PA ($p=0.02$; $p=0.006$, respectively). In contrast, there was no
76 significant reduction in both the rate of isolation and density of NM-PA ($p=0.90$; $p=0.07$, respectively).

77 Antimicrobial susceptibility in NM-PA and M-PA was not significantly reduced within any of the
78 antibiotics classes or individual antibiotics examined. Increased susceptibility was noted in the
79 beta-lactam class for NM-PA and M-PA, in particular with ceftazidime.

80

81 *What is new and conclusion:* Overall, (i) the requirement for less iv antibiotic therapy, (ii) a reduction
82 in the rate and density of M-PA, and (iii) no reduction in antibiotic susceptibility, indicates that
83 microbiological parameters with patients on IVA therapy were not detrimentally affected.

84

85

86

87

88 ***What is known and objective:***

89 The dominant feature of cystic fibrosis (CF)-related disease is the deterioration in patients' lung
90 function due to the chronic presence of bacterial pathogens, particularly *Pseudomonas aeruginosa*.
91 Any change in the status of the patient's microbiology may have a significant effect in clinical
92 outcome, in either a positive or negative manner. Outside of pulmonary exacerbation, the relative
93 microbiological stability of the cystic fibrosis (CF) lung is a fine equilibrium of multiple factors. In
94 microbiological terms, these include the presence/absence of bacterial pathogens, numbers of
95 organisms present, antibiotic resistance, carriage/expression of bacterial virulence determinants,
96 relative phase of bacterial growth (e.g. presence of senescent cells/persisters), host/environmental
97 stress responses and relative nutritional/starvation status of bacterial pathogens, iron sequestration and
98 competition and the dynamic flux from the co-habiting microbiome of the lung. Several other host
99 (patient) related factors can also contribute to this instability in this two host [patient & bacterium]
100 system.

101
102 In microbiological terms, what is not well understood is the sequencing and interactions of these
103 factors, when they occur together in a particular pattern, what precipitates the tipping of the relatively
104 stable CF lung into a pulmonary exacerbation or alternatively the interactions which drive the CF lung
105 to a more stable situation, thereby reducing infection and exacerbations. In addition, the relative
106 contribution and interaction of the human host undoubtedly plays a significant part in driving this
107 stable equilibrium to a state of relative instability and hence onwards to a potential pulmonary
108 exacerbation.

109
110 The 21st century is witnessing the development of many new pharmacological interventions in CF, in
111 order to ameliorate the effects of cystic fibrosis transmembrane conductance regulator (CFTR)
112 dysfunction and thus improve patient outcomes. One such intervention is the use of CFTR
113 correctors/potentiators, as an effective intervention, which has revolutionized CF care in those patients
114 with a genetic profile of their alleles which are predicted to benefit from such interventions.¹

115 Ivacaftor [N-(2,4-Di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide]
116 (VX-770; Kalydeco®) [IVA] is an orally bioavailable CFTR-potentiator molecule, that is designed to
117 increase the time that activated CFTR channels at the cell surface remain open, in CF patients with
118 specific mutations, including G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, or
119 S549R. IVA therapy with genetically appropriate CF patients has been shown to improve lung
120 function, as well as improve (i) risk of pulmonary exacerbations, (ii) patient-reported respiratory
121 symptoms, (iii) weight, (iv) concentration of sweat chloride [1] and (v) quality of life.²

122

123 Whilst IVA intervention is not designed *per se* as anti-infective, the effect (if any) of this molecule to
124 CF patients' microbiological status merits careful monitoring. Therefore, it was the aim of this study to
125 examine the effect in patients, both before and after commencement of IVA therapy, on several
126 commonly reported microbiological markers in CF patients, including (i) bacterial density, (ii)
127 frequency (rate) of isolation of bacterial pathogens, particularly *Pseudomonas aeruginosa* and (iii)
128 antimicrobial susceptibility of these isolates to commonly prescribed oral and iv antibiotics. In
129 addition, we wished to examine the requirements for these antibiotics in CF patients, before and after
130 commencement of IVA therapy.

131

132

133

134

135 **Materials and methods**

136

137 *Patient population*

138 A retrospective analysis was conducted on 15 adult patients, who were receiving therapeutic
139 treatment for their cystic fibrosis with oral ivacaftor. Each patient had a confirmed diagnosis of cystic
140 fibrosis, with at least one copy of the G551D mutation. Patients had an age range of 16-43 years and
141 there were nine males (age range: 16 – 43years) and seven females (age range: 18-32 years). Male
142 patients treatment duration ranged from 18-30 months, with a mean treatment duration of 24 months
143 and female patients treatment durations ranged from 18-29 months, with a mean treatment duration of
144 23.4 months.

145

146 *Antibiotic treatment and microbiological parameters analysed*

147 Each parameter was examined (i) two years prior to commencement of ivacaftor therapy and (ii). two
148 years post ivacaftor therapy.

149

150 Archived microbiological data was analysed from the patients' clinical microbiology record file, with
151 particular reference to (i). total *Pseudomonas aeruginosa* (PA), (ii). non-mucoid (NM) PA and (iii).
152 Mucoid (M) PA. The specific microbiological parameters investigated included (i). incidence of (PA
153 or NM PA or M PA)/respiratory specimen, (ii). relative culture density of PA [NM PA or M
154 PA]/respiratory specimen, (iii). antibiotic susceptibility.

155

156 The rate of isolation of PA was defined as frequency of PA isolated from sputum and was presented as
157 rate/respiratory specimen. The density of PA isolated from respiratory specimen was quantitatively
158 recorded by taking the laboratory semi-quantitative result (i.e. none, +, ++, +++) and converting these
159 values into a fully quantitative value, ranging from 0, 1, 2 and 3, respectively, and expressed per
160 respiratory specimen.

161

162 Antibiotic susceptibility was defined by employment of the Relative Resistance Index (RRI)³ of PA
163 isolates, within the following classes of antibiotics (agents analysed): aminoglycosides (gentamicin,
164 tobramycin, amikacin); β -lactams (temocillin, ceftazidime, piperacillin/tazobactam, aztreonam),
165 carbapenems (imipenem, meropenem), polymyxin (colistin) and fluoroquinolone (ciprofloxacin).
166 Relative Resistance Index (RRI) values were calculated for each NM-PA and M-PA isolate in each
167 patient, at each visit to the CF Unit, as either an in-patient or out-patient. Antibiotic susceptibility was
168 routinely recorded as sensitive (S), intermediate (M) or resistant (R). Subsequently, for the purposes
169 of this analysis, RRI values were assigned, as follows: a value of 1 for sensitivity, 2 is
170 intermediate/moderate resistance and 3 for resistant.

171

172 Additionally, the number of antibiotic courses, both oral and intravenous (IV), were analysed and
173 expressed as the mean courses of antibiotics given per month over the two years prior to
174 commencement of ivacaftor therapy and the two years after commencement of ivacaftor therapy.

175

176 *Statistical analysis*

177 Student t-tests were used to compare pre-ivacaftor and post-ivacaftor microbiology findings. Where
178 unpaired t-tests were employed, F values were calculated to determine equal or unequal variance. P
179 values and confidence intervals (CIs) were two-sided, where $p \leq 0.05$ (5%) was deemed significant.
180 Error bars were calculated as \pm standard error of the mean (SEM).

181

182

183 **Results**

184

185 *Antibiotic therapy*

186 There was no significant difference between the number of oral antibiotic courses/month before and
187 after IVA therapy (0.1 v 0.07; p=0.41) (Figure 1). There was a significant reduction in the number of
188 iv antibiotic courses/month, following IVA therapy (0.15 v 0.02; p=0.0003) (Figure 2). There was no
189 requirement for iv antibiotics in 6/15 (40%) patients, both prior to and following commencement of
190 IVA therapy. Five of the patients who required iv antibiotics pre-IVA therapy (33.3%) subsequently
191 did not require iv antibiotics, following commencement of IVA therapy. For the remainder of the
192 patients, 4/15 (26.7%) required less courses of iv antibiotics following commencement of IVA
193 therapy.

194

195 All patients who were taking nebulized anti-pseudomonal antibiotics for suppressive therapy
196 continued to take these after commencement of IVA therapy and thus there was no change in the rate
197 of inhaled antibiotics with the *Pseudomonas*-positive patients.

198

199 *Rate & density of Pseudomonas aeruginosa isolation from respiratory specimens*

200

201 *Non-mucoid Pseudomonas aeruginosa*

202 Eleven patients from the total 15 patients included in this study were culture-positive for NM-PA,
203 prior to commencement of IVA therapy. Overall at a population level, there was no significant
204 difference in either the rate (p=0.90) or density (p=0.07) of NM-PA, following commencement of IVA
205 therapy (Table 1). When examined statistically, at individual patient level, the density of NM-PA was
206 significantly lower (before v after commencement IVA therapy) in one patient (p=0.02), even though
207 this patient had a constant rate of NM-PA isolation (Figure 3). One patient acquired an NM-PA after
208 commencement of IVA therapy, but this isolate appeared to be transient, as it was only reported in a
209 single specimen, whereby the remaining six specimens were negative (data not shown). The other

210 three patients remained NM-PA free.

211

212 *Mucoid Pseudomonas aeruginosa*

213 Nine patients in this study were culture-positive for M-PA, prior to commencement of IVA therapy.

214 Overall, at a population level, there was a significant lowering in both the rate ($p=0.02$) and density

215 ($p=0.006$) of M-PA, following commencement of IVA therapy (Table 1 & Figure 4). Interestingly, a

216 reduced density of M-PA isolation was statistically observed in three of the nine patients (33.3%) and

217 additionally two patients became negative for M-PA after commencement of IVA therapy. None of

218 the six patients previously negative for M-PA prior to IVA therapy gained M-PA, after

219 commencement of therapy.

220

221 *Other microorganisms*

222 There was no significant difference in both the rate ($p=0.37$) and density ($p=0.43$) of *Staphylococcus*

223 *aureus* in patients ($n=7$), before v after commencement of IVA therapy. Additionally, in one patient,

224 there was no significant difference in the rate or density of *Burkholderia cenocepacia*, before v after

225 commencement of IVA therapy. For other non-*Pseudomonas* organisms, Table 2 details a qualitative

226 comparison of microorganisms cultured in patients before and after commencement of IVA therapy.

227

228 *Antibiotic susceptibility in PA isolates*

229 The Relative Resistance Index [RRI]³ was employed as a semi-quantitative method to determine the

230 susceptibility of NM-PA and M-PA. In total, 366 isolates of PA were analysed for antibiotic

231 susceptibility against 11 antibiotics, including 220 NM-PA ($n=109$ & $n=111$ before and after

232 commencement of IVA therapy, respectively), as well as 146 M-PA ($n=77$ & $n=69$ before and after

233 commencement of IVA therapy, respectively). This equated to approximately 9-10 PA isolates per

234 patient before, as well as after commencement of IVA therapy.

235

236 Figure 5a shows antibiotic susceptibility, as expressed as RRI, for total PA (NM-PA + M-PA) against

237 11 antibiotics within four antibiotic classes. Figure 5b shows antibiotic susceptibility, as expressed as
238 RRI, for NM-PA and Figure 5c for M-PA.

239

240 There were no statistically significant differences in antibiotic susceptibility for the aminoglycosides,
241 polymyxin (colistin) or fluoroquinolone (ciprofloxacin) in NM-PA and M-PA (Figure 5a-c). Whilst
242 Figure 5a-c shows a general lowering of the RRI value, this was only statistically significant in one
243 class of antibiotics, namely certain β -lactam antibiotics, before and after commencement of IVA
244 therapy. For total PA, there is a significant increase in susceptibility relating to ceftazidime
245 ($p=0.002$), tazocin ($p=0.004$), azteonam ($p=0.045$) and meropenem ($p=0.005$). Regarding NM-PA,
246 three β -lactams showed a significant increase in susceptibility, namely ceftazidime ($p=0.03$), tazocin
247 ($p=0.002$) and meropenem ($p=0.02$), whilst only ceftazidime showed a significant increase in
248 susceptibility ($p=0.04$), with M-PA (see Figure 5a-c).

249

250

251

252 **Discussion**

253

254 With any novel non-antimicrobial pharmacological intervention in CF, it is important to carefully
255 monitor the microbiological status of patients on therapy, in order to ensure that the intervention is not
256 having a deleterious effect on the patients' microbiological parameters, which could potentially
257 manifest in clinical deterioration. In this observational study, our objective was to retrospectively
258 examine the microbiology of CF patients, who had received IVA therapy. This was achieved by
259 examining microbiological data routinely gathered as part of the patient CF care pathway, from two
260 years preceding initiation of IVA therapy and continuing to approximately two years after
261 commencement of IVA.

262

263 Overall, there was a significant reduction in the requirement for iv antibiotic courses when patients
264 commenced IVA therapy ($p= 0.0003$) (Figure 2). Of the nine patients who required iv antibiotics,
265 prior to the commencement of IVA therapy, five of these did not require any iv antibiotics, whilst on
266 IVA therapy and the remaining four patients required a reduced number of iv antibiotic courses. We
267 did not observe any patient, who did not routinely receive iv antibiotics subsequently requiring iv
268 antibiotics whilst on IVA therapy.

269

270 In contrast, there was no statistical difference in requirement for oral antibiotics before versus after
271 commencement of IVA therapy ($p=0.41$). Nine patients from the 15 patients examined in this study
272 required oral antibiotics, prior to commencement of IVA therapy and seven of these patients
273 continued to require oral antibiotics whilst on IVA therapy. Following commencement of therapy,
274 there were two patients who did not require any oral antibiotics and one patient who did require oral
275 antibiotics on therapy, who did not require oral antibiotics before commencement of IVA.

276

277 Similarly, in a recent study from the Czech Republic, a 21% reduction on antibiotic therapy (per
278 patient-year) was reported ($p<0.001$), however it is not clear whether this therapy included oral, ivs or

279 both.⁴ In our study we observed an overall significant reduction (86.7%) in the number of iv
280 antibiotic courses per month and a 20% reduction in the number of oral antibiotic courses per month,
281 although not statistically significant.

282

283 The question does however remain from our study, as to why iv antibiotic usage went down, whilst
284 oral usage remained unaltered. One possible explanation is a lower clinical threshold for starting an
285 oral antibiotic in healthier patients versus that for commencement of an iv antibiotic in sicker patients.
286 Consequently, oral antibiotics may be an interesting first choice for examining antimicrobial
287 stewardship within cystic fibrosis.

288

289 There was no significance difference in either the rate of isolation or density of NM-PA after
290 commencement of IVA therapy. This was in contrast to M-PA, where there was a significant reduction
291 in both the rate of isolation and density. To date, there have been no other reports which have divided
292 total PA into NM-PA and M-PA components, however the study by Heltshe et al. reported significant
293 reduction in the percentage of patients with total PA ($p=0.004$) and M-PA ($p=0.05$), following 1 year
294 post IVA therapy.⁵ More recently, a further study reported that IVA caused a marked reduction in PA
295 density, which commenced 48 hours post commencement of IVA therapy and which continued in the
296 first year of therapy. Following this, PA density rebounded in 6/7 patients, commencing at day 210 on
297 IVA therapy.⁶

298

299 There is a relative paucity of data describing the fate of bacterial numbers in patients commencing
300 IVA therapy. Recently published data from the 2013 NACFC Meeting demonstrated that appropriate
301 patients on ivacaftor had improved mucociliary clearance⁷ and demonstrated that mucociliary
302 clearance increased from $8.5 \pm 1.7\%$ at baseline to $18.7 \pm 2.3\%$ and $17.7 \pm 1.7\%$ at one and three
303 months post-treatment, respectively ($p < 0.001$ for each comparison to baseline). Dramatic
304 improvements in peripheral lung clearance were also demonstrated ($1.5 \pm 1.8\%$ vs. $12.2 \pm 2.0\%$, $9.1 \pm$
305 2.4% at baseline, one month and three months, respectively; $p < 0.05$ for each comparison to baseline).

306 Current research, which is part of the GOAL observational study, from Sagel's group in Colorado,
307 also presented at the recent NACFC meeting.⁸ Induced sputum was collected pre- and post-ivacaftor
308 treatment in 14 subjects (age 27 ± 14 yrs; FEV1 $84 \pm 23\%$ predicted; 6 females). Sputum bacterial
309 diversity did not change significantly with treatment [Shannon Diversity: mean change (SE) 0.13
310 (0.14), $p=0.34$]. The combined relative abundance (RA) of traditional CF bacterial pathogens
311 including *Pseudomonas*, *Staphylococcus*, *Stenotrophomonas*, *Achromobacter*, and *Burkholderia*
312 trended down with treatment [mean change (SE) -13.9 (8.2), $p=0.11$]. *Prevotella* RA significantly
313 increased with treatment [mean change (SE) 8.8 (3.0), $p=0.01$]. By qPCR, neither total bacterial load
314 changed significantly between paired samples [mean change (SE) -0.18 (0.16) \log_{10} gene
315 copies/mL, $p=0.28$], nor did *Pseudomonas* load [mean change (SE) -0.76 (0.66) \log_{10} gene
316 copies/mL, $p=0.27$]. There were no significant changes in any sputum markers of inflammation,
317 including neutrophil elastase activity [mean change (SE) -0.1 (0.1) \log_{10} $\mu\text{g/mL}$, $p=0.29$].

318

319 On first examination of the Sagel's data,⁸ it would appear that nothing significant happened to the
320 total bacterial counts nor the PA counts, which is counter- intuitive to data of Donaldson,⁷ with
321 improved mucociliary clearance in patients on ivacaftor. The problem here is that the Sagel group
322 measured bacterial numbers with an inappropriate methodology, namely enumeration solely via a
323 molecular means qPCR. Where DNA is the target for qPCR determination, it will count total bacteria
324 in the sputum specimen and consequently this will include both living, as well as dead bacterial cells.
325 Given the persistence of DNA from living or dead bacterial cells, therefore any changes (+ or -) in
326 culturable bacterial numbers would be effectively missed by using this methodology. The
327 consequences of this would thus create an uncertainty as to whether or not the culturable bacteria in
328 CF sputum in patients on ivacaftor remained constant or not.

329

330 On considering the dynamics of growth of bacteria and bacterial CF respiratory pathogens in the CF
331 airways in patients on ivacaftor, the published data regarding bacterial numbers counted either via
332 molecularly^{6,8} or conventionally-acquired⁶ does not give any indication if such numbers are present

333 due to (i) bacterial cells being in the stationary phase in a relative state of senescence/dormancy but
334 which remain culturable, (ii). actively metabolising where numbers dying off equals numbers being
335 generated de novo and (iii). have switched phenotypically from culturable sessile & planktonic
336 vegetative cells to non-culturable persister cells. Scenarios (i) and (ii) would show no significant
337 change in numbers, even though the physiology leading to this point is completely different. In this
338 regard, due to the now functional ciliated escalator, bacteria need to work extremely hard to maintain
339 their numbers constant, i.e. the Red Queen Hypothesis, as per the data of Sagel.⁸

340

341 The consequences of which modality is happening is profound. In the case of (i) above, bacterial
342 cells are not actively dividing nor dying, therefore they will not have a high metabolic turnover and
343 should not be as visible to the host immune system. However, in the case of (ii) above, whilst there
344 does not appear to be much happening, in terms of changes in bacterial counts, there is an important
345 occult metabolism taking place, which would be very visible to the host's immune system, which
346 would drive inflammatory processes. Therefore, it is important to understand the microbiological
347 mechanisms underpinning the fate of constant bacterial populations within the CF airways in patients
348 on IVA.

349

350 To date, there have been no reports on the effect of IVA therapy on antibiotic susceptibility in PA. In
351 our study, overall, there was no decrease in antibiotic susceptibility observed in PA (NM-PA and
352 M-PA) in this study. Four classes of commonly employed antibiotics were examined, namely
353 aminoglycosides, β -lactams, a polymyxin and a fluoroquinolone. There was no difference in
354 susceptibility with the aminoglycoside, the polymyxin or the fluoroquinolone. It was interesting to
355 note the increase in susceptibility with the β -lactam class of antibiotics, in particular ceftazidime,
356 tazocin, aztreonam and meropenem. A rationale for observing a signal in only the β -lactam
357 antibiotics remains uncertain and this is the subject of a further study, which is underway.

358

359 Whilst our study reports statistical increase with certain antibiotic susceptibilities in PA, these values

360 are largely of academic interest only. These data did not demonstrate marked shifts in susceptibility
361 which altered the susceptibility designation, from sensitive, intermediate or resistant. Given this, IVA
362 therapy should not be construed in any manner in the antibiotic management of CF patients.
363 Importantly, antibiotic susceptibility from patients on IVA therapy is not reduced and therefore IVA
364 therapy does not appear to have a detrimental association with antimicrobial therapy.

365

366 ***What is new and conclusions***

367 Overall, although the findings of this study cannot directly correlate with the effect of IVA therapy
368 with microbiological parameters, it is of interest to note that microbiological parameters with patients
369 on IVA therapy were not detrimentally affected. Indeed, overall, (i) the requirement for less iv
370 antibiotic therapy, (ii) a reduction in the rate and density of M-PA, and (iii) no reduction in antibiotic
371 susceptibility, suggests an improvement in the microbiology status of patients on IVA therapy.

372

373

374

375

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377

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380

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414

415

416

417

418 **Figure & Table Titles**

419

420 **Figure 1: Number of oral antibiotic course per month for patients before and after**

421 **commencement of IVA therapy**

422

423 **Figure 2: Number of intravenous antibiotic course per month for patients before and after**

424 **commencement of IVA therapy**

425

426 **Figure 3a: Rate of non-mucoid PA isolation before and after commencement of ivacaftor (IVA)**

427 **therapy in patients who were chronically colonised prior to the commencement of IVA**

428 **therapy**

429

430 **Figure 3b: Density of non-mucoid PA isolation before and after commencement of ivacaftor**

431 **(IVA) therapy in patients who were chronically colonised prior to the commencement**

432 **of IVA therapy**

433

434 **Figure 4a: Rate of mucoid PA isolation before and after commencement of ivacaftor (IVA)**

435 **therapy in patients who were chronically colonised prior to the commencement of IVA**

436 **therapy**

437

438 **Figure 4b: Density of mucoid PA isolation before and after commencement of ivacaftor (IVA)**

439 **therapy in patients who were chronically colonised prior to the commencement of IVA**

440 **therapy**

441

442 **Figure 5: Antibiotic susceptibility, as expressed by Relative Resistance Index, against four**

443 **classes of antibiotic in (a) Total *Pseudomonas aeruginosa*, (b) Non-mucoid *P.***

444 ***aeruginosa* and (c) Mucoid *P. aeruginosa*, isolated from patients before and after**

445 **commencement of ivacaftor therapy.**

446 (Abbreviations: IVA= ivacaftor; M= mucoid; NM= non-mucoid; PA= *Pseudomonas aeruginosa*)

447 **Table 1: Comparison of the rate of isolation and density of non-mucoid and mucoid**
448 ***Pseudomonas aeruginosa* before and after commencement of ivacaftor therapy.**

449

450 **Table 2: Non-*Pseudomonas* organisms cultured from CF patients' respiratory specimens**
451 **before and after commencement of ivacaftor therapy**

452