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Original article

DOES IVACAFTOR INTERFERE WITH THE ANTIMICROBIAL ACTIVITY OF COMMONLY USED ANTIBIOTICS AGAINST PSEUDOMONAS AERUGINOSA? – RESULTS OF AN IN VITRO STUDY

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Running Title: Does ivacaftor effect antibiotic susceptibility?

\textbf{Key words:} ivacaftor; 	extit{Pseudomonas aeruginosa}; cystic fibrosis; antibiotic susceptibility; microbiology

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What is known and objective

Within cystic fibrosis (CF), ivacaftor is a novel potentiator of defective cystic fibrosis transmembrane conductance regulator (CFTR) protein, which corrects the gating defect and increases ion-function of activated cell-surface CFTR. Bacteria also regulate their physiology employing ion channels, however little is known about the potential effects of ivacaftor on bacterial ion channels, which in turn, may have a potential effect on transport across the bacterial cell membrane. Therefore, any change in the ability to transport molecules across cell membranes in bacteria could have an important role in bacterial transport physiology. One area where this could be particularly important is in the movement of antibiotics, both into and out of the bacterial cell. An in vitro study was therefore performed to examine the influence of ivacaftor at therapeutic concentration on antibiotic susceptibility of 11 commonly used anti-pseudomonal antibiotics against a population of clinical Pseudomonas aeruginosa [PA], from CF and non-CF sources.

Method

PA (n=80; including 70 ivacaftor-naïve clinical PA from sputa from adult CF patients and 10 control PA from non-CF clinical blood culture sources) were examined. Antibiotic susceptibility was determined by standard disk diffusion assay using CLSI criteria and measuring zone size (mm), against four classes of anti-pseudomonal antibiotics, including beta-lactams (temocillin, ceftazidime, piperacillin/tazobactam, imipenem, meropenem and aztreonam), aminoglycosides (gentamicin, tobramycin, amikacin), fluoroquinolone (ciprofloxacin) and polymyxin (colistin), in the absence and presence of ivacaftor (5µM), as previously determined. In addition, all CF and non-CF PA were examined phenotypically in vitro, as previously described3, for changes linked to bacterial virulence, including (i) growth
density, (ii) pigmentation, (iii) presence of adhesins and (iv) change to mucoidy, in the
presence/absence of ivacaftor at therapeutic concentration.

Results and Discussion
Antibiotic susceptibility did not decrease significantly with any of the antibiotics examined
with CF PA isolates or with non-CF PA control organisms. There was a statistically
significant increase in zone size (CF PA & amikacin, gentamicin, temocillin & ciprofloxacin;
Non-CF PA & amikacin, gentamicin & aztreonam), however at a population level, this did
not translate into a shift in CLSI category to a more susceptible phenotype. None of the PA
isolates examined were susceptible to ivacaftor alone and additionally, no changes were
noted with the four phenotypic parameters examined in the presence of ivacaftor.

What is new and conclusion
This study showed that antibiotic susceptibility of commonly used anti-pseudomonal antibiotics was
not negatively affected by ivacaftor, in a population of ivacaftor-naive Pseudomonas aeruginosa.
1. WHAT IS KNOWN AND OBJECTIVE

Cystic fibrosis (CF) is an autosomal recessive genetic disorder characterised by defective mucociliary clearance and chronic airway infection [1]. The most commonly isolated pathogen from CF airways is the opportunistic Gram-negative bacterium Pseudomonas aeruginosa (PA) [2]. Chronic PA infection is associated with significant morbidity and mortality in CF patients [3] and necessitates multiple antibiotic courses, which increase the risk of the development of antibiotic resistance [2]. Many of the PA strains isolated from CF airways are of the mucoid phenotype, capable of producing large quantities of alginate which enable the formation of a matrix in which microcolonies can exist. These microcolonies are thought to provide protection against the host immune response and antibiotic therapy; conversion of PA in patients to the mucoid phenotype is associated with increased morbidity and mortality [4]. Typically, initial colonisation is with non-mucoid forms of PA, with the mucoid form predominating later.

Outside of pulmonary exacerbation, the relative microbiological stability of the cystic fibrosis (CF) lung is a fine equilibrium of multiple factors. In microbiological terms, these include the presence/absence of bacterial pathogens, numbers of organisms present, antibiotic resistance, carriage/expression of bacterial virulence determinants, relative phase of bacterial growth (e.g. presence of senescent cells/persisters), host/environmental stress responses and relative nutritional/starvation status of bacterial pathogens, iron sequestration and competition and the dynamic flux from the co-habiting microbiome of the lung. Several other host (patient) related factors can also contribute to this instability in this two host [patient & bacterium] system [5].
In microbiological terms, what is not that well understood is the sequencing and interactions of these factors, when they occur together in a particular pattern, what precipitates the tipping of the relatively stable CF lung into a pulmonary exacerbation. In addition, the relative contribution and interaction of the human host undoubtedly plays a significant part in driving this stable equilibrium to a state of relative instability and hence onward to a pulmonary exacerbation.

Ivacaftor is a novel potentiator of defective CFTR protein, which corrects the gating defect and increases ion-function of activated cell-surface CFTR [6]. Bacteria also regulate their physiology employing ion channels, however little is known about the potential effects of ivacaftor on bacterial ion channels, which in turn, may have a potential effect on transport across the bacterial cell membrane. Therefore, any change in the ability to transport molecules across cell membranes in bacteria could have an important role in bacterial transport physiology. One area where this could be particularly important is in the movement of antibiotics, both into and out of the bacterial cell. An in vitro study was therefore performed to examine the influence of ivacaftor at therapeutic concentration on antibiotic susceptibility of 11 commonly used anti-pseudomonal antibiotics against a population of clinical Pseudomonas aeruginosa [PA], from CF and non-CF sources.
2 MATERIALS AND METHODS

2.1 Bacterial isolates

*Pseudomonas aeruginosa* isolates were obtained from the Northern Ireland Health and Social Care (HSC) Microbiology Culture Repository (MicroARK) (www.microark.com). These consisted of 80 clinical PA isolates, including 70 ivacaftor-naïve clinical PA from sputa from adult CF patients and 10 control PA from non-CF clinical blood culture sources. All isolates were taken from frozen storage at -80°C and were passaged twice prior to employment, by subculturing onto Columbia Blood agar (CM0331 Oxoid Ltd., Basingstoke, UK), supplemented with 5% (v/v) defibrinated horse blood and were incubated for 48h at 37°C under aerobic conditions.

2.2 Antibiotic susceptibility testing

Antibiotic susceptibility of these isolates to commonly employed anti-pseudomonal antibiotics was determined, both with and without ivacaftor. Briefly, standard disk diffusion assays using CLSI criteria [7] was performed individually on each isolate described above, by inoculating a 0.5 McFarland Standard onto Mueller-Hinton Agar (MHA; CM0337), followed by incubation for 24h, as detailed above. MHA with ivacaftor (MHA_{ivacaftor}) was prepared in accordance with the manufacturer’s instructions, with the additional step of including ivacaftor to a final concentration of 5µM. Ivacaftor was obtained directly from Vertex Pharmaceuticals Inc., Boston, MA., USA, as dry powder, which was reconstituted in DMSO and added to MHA post autoclaving and prior to plate pouring. Antibiotic susceptibility was performed by measuring zone size (mm), against four classes of anti-pseudomonal antibiotics, including beta-lactams (temocillin, ceftazidime, piperacillin/tazobactam, imipenem, meropenem and aztreonam), aminoglycosides
(gentamicin, tobramycin, amikacin), fluoroquinolone (ciprofloxacin) and polymyxin (colistin), in the absence and presence of ivacaftor (5μM). Antibiotic disk concentrations are detailed in Table 1.

2.3 Altered phenotype studies

2.3.1 Colony growth & proliferation

P. aeruginosa (n=80), as detailed above, were examined in this experiment. Bacterial growth in the presence of ivacaftor (5mM) was examined, whereby basal unsupplemented MHA was compared to ivacaftor-supplement MHA (MHAivacaftor). MHA and MHAivacaftor plates were streaked with a fresh inoculum (20μl) of each isolate, suspended at a concentration equivalent to a McFarland 0.5 standard [1.5x10^8 cfu/ml]. All plates were incubated aerobically at 37°C for 48h before examination.

2.3.2 Pigment production

All clinical CF isolates were plated onto MHA and MHAivacaftor as described in 2.2.1 above and were examined visually for evidence of enhanced pigment production, after 48h incubation.

2.3.2 Adhesin & mucoid production

All clinical CF isolates were plated onto MHA and MHAivacaftor, as described in 2.3.1 above and were examined after 48h incubation for evidence of (i). an adhesion phenotype and (ii) a mucoid phenotype. The adhesion phenotype was defined at examination, where the organism had an altered moist and “sticky” phenotype in comparison with the unsupplemented control, which was stringy on examination with an inoculation loop, but which fell short of mucoidy. The mucoid phenotype was defined at visual examination, where the organism produced
large amounts of extracellular polysaccharide material.

2.4 Statistical analyses

Mean antibiotic susceptibility, expressed as zone sizes (mm) ± standard error of the mean [SEM] were calculated for each of the 11 antibiotics tested. Paired t-tests were calculated for each antibiotic, where the Null hypothesis was that there was no significant difference in antibiotic susceptibility zone sizes, with and without ivacaftor. A p value <0.05 (5%) was considered statistically significant.

3 RESULTS

Antibiotic susceptibility data of 80 PA isolates against 11 antibiotics, with and without ivacaftor, is shown (Table 1). None of the PA isolates examined were susceptible to ivacaftor alone. Antibiotic susceptibility did not decrease significantly in the presence of ivacaftor, with any of the antibiotics examined with CF PA isolates or with non-CF PA control organisms (Table 1). In some cases, there was a statistically significant increase in zone size (CF PA & amikacin, gentamicin, temocillin & ciprofloxacin; Non-CF PA & amikacin, gentamicin & aztreonam), however at a population level, this did not translate into a shift in CLSI susceptibility category [sensitive (S), intermediate (I) or resistant (R)].

Phenotypic parameters examined including (i). colony growth & proliferation, (ii). pigment production and (iii). adhesin & mucoid production, were unaffected in the presence of ivacaftor (5 μM).
The 21st century is witnessing the development of many new pharmacological interventions in CF, in order to ameliorate the effects of cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction and thus improve patient outcomes. One such intervention is the use of ivacaftor, an effective potentiator of CFTR function in CF patients with appropriate genotype [6].

In this *in vitro* study, we set out to investigate potential alteration to antibiotic susceptibility with commonly employed antipseudomonal antibiotics, in the presence of ivacaftor at therapeutic concentrations. Our experience of ivacaftor from the current study does not indicate that this molecule has potent antimicrobial properties. However, we did see statistically significant increases in susceptibility in CF PA, with amikacin, gentamicin, temocillin and ciprofloxacin, as well as with amikacin, gentamicin and aztreonam within the non-CF PA isolates (Table 1). At this stage, we are unable to speculate as to why there were positive antibiotic/ivacaftor combinations with PA susceptibility *in vitro* and this is currently the subject of ongoing investigations. Although we observed some enhanced antibacterial activity in CF PA and non-CF PA, with certain classes of anti-pseudomonal antibiotic *in vitro*, we need to be cautious, not to over-emphasise this activity in the *in vivo* setting.

Previously, there have been several reports describing antibacterial activity of ivacaftor against a wide range of bacteria, as summarised in Table 2.

There has been a report that ivacaftor exhibits antibacterial properties *in vitro* against *Staphylococcus aureus, Streptococcus pneumoniae* and PA [19]. With regard to ivacaftor
activity against PA, this was only observed whenever PA cells had undergone prior permeabilization (0.2mM EDTA/pH8.0/15mins) and not with non-permeabilized cells. In addition, these workers also demonstrated additional antibacterial activity with ciprofloxacin in the presence of ivacaftor. Our data largely supports the findings of Reznikov and colleagues relating to increased antibiotic susceptibility against PA with ciprofloxacin/ivacaftor combinations [19].

Whilst these data are interesting, it is important to consider such potential antimicrobial effects in vivo. To date, there has been a paucity of published data detailing the concentration of ivacaftor in sputum in CF patients being treated with ivacaftor. Some recent data from a group in Victoria, Australia [20], described ivacaftor concentrations in CF sputum as 0.15 ±0.05 µg/ml (after 2.5h) and 0.10±0.03 µg/ml (overnight). Therefore, it is unlikely that ivacaftor concentrations in the lumen of the airways or in sputum of a CF patient being treated with ivacaftor, would ever reach concentrations, that would be antimicrobial per se to PA in sputum.

Ivacaftor has been developed to act as a CFTR potentiator in CF patients with certain CF genotypes. It is important to examine the effect (if any) of any novel therapy in CF, which alters lung physiology and to assess this physiological alteration in terms of the second host system in CF patients, namely the bacteria and microbiome. Overall, whilst we did not find ivacaftor alone to be antimicrobial against CF PA, it is important to note that it did not negatively alter antibiotic susceptibility, indeed where a statistical difference was observed, an increase in susceptibility was noted.
In conclusion, this study showed that antibiotic susceptibility of commonly used anti-
pseudomonal antibiotics was not negatively affected by ivacaftor, in a population of
ivacaftor-naive Pseudomonas aeruginosa.

Acknowledgement

This work was supported by Vertex Pharmaceuticals Inc, Boston, USA, through an
Investigator Initiated Study (IIS) Grant.

Transparency declarations

None to declare
REFERENCES

1. Fodor AA, Klem ER, Gilpin DF et al. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. *PloS one* 2012; 7: e45001.


## TABLE 1: Antibiotic susceptibility as determined by standard disk diffusion assay using CLSI criteria in the presence and absence of ivacaftor (mean zone size expressed in mm ± SEM [standard error of mean])

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Ivacaftor (5 µM)</th>
<th>Amikacin (30 µg)</th>
<th>Gentamicin (10 µg)</th>
<th>Tobramycin (30 µg)</th>
<th>Aztreonam (30 µg)</th>
<th>Ceftazidime (10 µg)</th>
<th>Imipenem (10 µg)</th>
<th>Meropenem (10 µg)</th>
<th>PIP/TAZ (30/6 µg)</th>
<th>Temocillin (30 µg)</th>
<th>Ciprofloxacin (5 µg)</th>
<th>Colistin (10 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF PA</td>
<td>without</td>
<td>11.2 ± 0.9</td>
<td>7.3 ± 0.5</td>
<td>15.1 ± 0.7</td>
<td>16.2 ± 1.4</td>
<td>9.3 ± 1.1</td>
<td>17.4 ± 1.1</td>
<td>25.0 ± 1.4</td>
<td>12.3 ± 1.4</td>
<td>8.7 ± 1.2</td>
<td>18.7 ± 1.2</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(n=70) with</td>
<td>13.5 ± 0.6</td>
<td>9.2 ± 0.6</td>
<td>15.3 ± 0.6</td>
<td>16.3 ± 1.4</td>
<td>9.9 ± 1.2</td>
<td>17.7 ± 1.1</td>
<td>24.3 ± 1.5</td>
<td>12.0 ± 1.4</td>
<td>9.2 ± 1.2</td>
<td>19.5 ± 1.2</td>
<td>9.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>0.34</td>
<td>0.78</td>
<td>0.08</td>
<td>0.5</td>
<td>0.18</td>
<td>0.55</td>
<td>0.01*</td>
<td>0.04*</td>
<td>0.5</td>
</tr>
<tr>
<td>Non-CF PA</td>
<td>without</td>
<td>15.6 ± 0.7</td>
<td>9.0 ± 0.8</td>
<td>14.8 ± 0.6</td>
<td>17.4 ± 1.5</td>
<td>16.6 ± 2.1</td>
<td>19.8 ± 1.7</td>
<td>30.2 ± 1.6</td>
<td>19.6 ± 3.4</td>
<td>0</td>
<td>28.8 ± 0.4</td>
<td>7.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(n=10) with</td>
<td>16.6 ± 0.7</td>
<td>10.6 ± 0.7</td>
<td>16.9 ± 0.8</td>
<td>19.8 ± 1.3</td>
<td>17.6 ± 2.2</td>
<td>21.3 ± 2.0</td>
<td>29.2 ± 1.7</td>
<td>17.2 ± 2.3</td>
<td>0</td>
<td>29.6 ± 0.8</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>0.01*</td>
<td>0.01*</td>
<td>0.14</td>
<td>0.01*</td>
<td>0.34</td>
<td>0.17</td>
<td>0.21</td>
<td>0.48</td>
<td>-</td>
<td>0.42</td>
<td>0.59</td>
</tr>
</tbody>
</table>

PIP/TAZ= piperacillin/tazobactam;

Highlighted boxes = statistically significant result
<table>
<thead>
<tr>
<th>Title of Paper</th>
</tr>
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<tbody>
<tr>
<td><strong>Pseudomonas aeruginosa</strong> in cystic fibrosis patients with c.1652G&gt;A (G551D)-CFTR treated with ivacaftor - Changes in microbiological parameters.</td>
</tr>
<tr>
<td>• 15 adult CF patients</td>
</tr>
<tr>
<td>• Assessed 2y pre and 2y after initiation IVA therapy</td>
</tr>
<tr>
<td>Retrospective observational, single centre, clinical microbiology study</td>
</tr>
<tr>
<td>Study Findings relating to Ivacaftor</td>
</tr>
<tr>
<td>Following initiation of IVA therapy:-</td>
</tr>
<tr>
<td>• patients required less IV antibiotic therapy</td>
</tr>
<tr>
<td>• a reduction in the rate and density of M-PA was observed</td>
</tr>
<tr>
<td>• Increased susceptibility was noted in the beta-lactam class for NM-PA and M-PA, in particular with ceftazidime.</td>
</tr>
<tr>
<td>• no reduction in antibiotic susceptibility indicated that microbiological parameters with were not detrimentally affected.</td>
</tr>
<tr>
<td>Year [Reference]</td>
</tr>
<tr>
<td>2018 [8]</td>
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<table>
<thead>
<tr>
<th>Title of Paper</th>
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<tbody>
<tr>
<td>Retrospective observational study of French patients with cystic fibrosis and a Gly551Asp-CFTR mutation after 1 and 2 years of treatment with ivacaftor in a real-world setting.</td>
</tr>
<tr>
<td>• 57 patients with CF</td>
</tr>
<tr>
<td>• Assessed at initiation, 1y and 2y IVA therapy</td>
</tr>
<tr>
<td>Retrospective observational, multicentre, medical and clinical microbiology study</td>
</tr>
<tr>
<td>Study Findings relating to Ivacaftor</td>
</tr>
<tr>
<td>Following initiation of IVA therapy:-</td>
</tr>
<tr>
<td>• Mean absolute change in FEV1% predicted improved from baseline over two years</td>
</tr>
<tr>
<td>• Statistically significant benefits were observed with increased body mass index, fewer <em>Pseudomonas aeruginosa</em> and <em>Staphylococcus aureus</em> positive cultures.</td>
</tr>
<tr>
<td>• Decrease administration of IV antibiotics and maintenance treatment prescriptions (including azithromycin, Dornase alpha and nutritional supplements).</td>
</tr>
<tr>
<td>• No significant adverse events reported.</td>
</tr>
<tr>
<td>Year [Reference]</td>
</tr>
<tr>
<td>2018 [9]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Title of Paper</th>
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<tbody>
<tr>
<td>Lumacaftor (VX-809) restores the ability of CF macrophages to phagocytose and kill <em>Pseudomonas aeruginosa</em>.</td>
</tr>
<tr>
<td>• Human CF (del508Phe/del508Phe) monocyte derived macrophages (MDMs)</td>
</tr>
<tr>
<td>Basic science study</td>
</tr>
<tr>
<td>Study Findings relating to Ivacaftor</td>
</tr>
<tr>
<td>• Lumacaftor alone restored the ability of CF MDMs to phagocytose and kill <em>P. aeruginosa</em> to levels observed in MDMs obtained from non-CF donors (WT-CFTR).</td>
</tr>
<tr>
<td>• IVA reduced the ability of lumacaftor to stimulate phagocytosis and killing of <em>P. aeruginosa</em>.</td>
</tr>
<tr>
<td>• Lumacaftor had no effect on <em>P. aeruginosa</em>-stimulated cytokine secretion by CF MDMs</td>
</tr>
<tr>
<td>• IVA (5 µM) alone, or in combination with lumacaftor, reduced the secretion of several pro-inflammatory cytokines.</td>
</tr>
<tr>
<td>• The clinical efficacy of Orkambi may be related in part to the ability of lumacaftor to stimulate phagocytosis and killing of <em>P. aeruginosa</em> by macrophages.</td>
</tr>
<tr>
<td>Year [Reference]</td>
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<td>2017 [10]</td>
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<table>
<thead>
<tr>
<th>Title of Paper</th>
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<tbody>
<tr>
<td>Ivacaftor for the p.Ser549Arg (S549R) gating mutation - The Israeli experience.</td>
</tr>
<tr>
<td>• 8 CF patients (3 females, 5 males) carrying, p.Ser549Arg (S549R) mutation</td>
</tr>
<tr>
<td>Retrospective observational medical and clinical microbiology study</td>
</tr>
<tr>
<td>Study Findings relating to Ivacaftor</td>
</tr>
<tr>
<td>After one year of IVA therapy:-</td>
</tr>
<tr>
<td>• Significant improvement was found in FEV1,), FVC, and FEF25-75,</td>
</tr>
<tr>
<td>• Sweat chloride concentration decreased and BMI increased</td>
</tr>
<tr>
<td>Year [Reference]</td>
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</table>
CORK Study in Cystic Fibrosis: Sustained Improvements in Ultra-Low-Dose Chest CT Scores After CFTR Modulation With Ivacaftor.

- 33 CF patients (paediatric & adult) with the G551D mutation
- Assessed at baseline and prospectively every 3 months for 1 year after initiation of IVA

Retrospective observational, single centre, medical and clinical microbiology study

Following initiation of IVA therapy:
- Significant improvements in FEV₁, BMI, sweat chloride levels and improvement in ultra-low-dose CT imaging scores were observed.
- Significant mean reductions in total Bhalla score, peribronchial thickening and extent of mucous plugging
- Reductions in circulating inflammatory markers (interleukin (IL)-1β, IL-6, and IL-8)
- Change in lung microbiota (30% reduction in the relative abundance of Pseudomonas spp., increase in the relative abundance of bacteria associated with more stable community structures).
- Early and sustained improvements on ultra-low-dose CT scores suggest it may be a useful method of evaluating treatment response as it paralleled improvement in symptoms and other markers,

Activity of innate antimicrobial peptides and ivacaftor against clinical cystic fibrosis respiratory pathogens.

- Clinical respiratory isolates
- Murine infection model

Basic Science

IVA demonstrated:
- Bactericidal activity against Streptococcus
- Bacteriostatic activity against Staphylococcus aureus but no activity against Pseudomonas aeruginosa, Achromobacter spp. and Stenotrophomonas maltophilia.
- Synergy with tobramycin antimicrobial activity against S. aureus and Streptococcus spp.
- Immunomodulatory but no antimicrobial activity in a P. aeruginosa in vivo murine infection model.

Pseudomonas eradication and clinical effectiveness of Ivacaftor in four Hispanic patients with S549N

- 4 paediatric Hispanic patients with S549N and F508del observed prior to initiation of IVA and following 24 months

Case Series

IVA was highly efficacious with no further Pseudomonas positive cultures despite prior chronic colonization in three patients as well as notable improvements in lung function and growth rate (BMI), similar to G551D patients with more striking changes in airway infection status.
<table>
<thead>
<tr>
<th>Study Title</th>
<th>Participants/Methods</th>
<th>Findings</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Restoring Cystic Fibrosis Transmembrane Conductance Regulator Function Reduces Airway Bacteria and Inflammation in People with Cystic Fibrosis and Chronic Lung Infections. | 12 CF patients with G551D-CFTR mutations observed prior to initiation of IVA and over the following 2 year period | Retrospective observational, single centre, medical and clinical microbiology study Following initiation of IVA therapy:-  
  - A rapid decrease in sputum *Pseudomonas aeruginosa* density began within 48 hours and continued in the first year of treatment.  
  - No subject eradicated their infecting *P. aeruginosa* strain.  
  - After the first year *P. aeruginosa* densities rebounded.  
  - Sputum total bacterial concentrations also decreased, but less than *P. aeruginosa*.  
  - Sputum inflammatory measures decreased significantly in the first week of treatment and continued to decline over 2 years.  
  - Computed tomography revealed that ivacaftor decreased airway mucous plugging. | 2017 | [15] |
| An "Unlikely" Pair: The Antimicrobial Synergy of Polymyxin B in Combination with the Cystic Fibrosis Transmembrane Conductance Regulator Drugs KALYDECO and ORKAMBI. | 22 clinical isolates (16 polymyxin-resistant and 5 polymyxin-susceptible) of *P. aeruginosa*, including mucoid and non-mucoid strains, from patients with acute exacerbations of CF. | Basic Science  
  - Polymyxin B, ivacaftor or lumacaftor were ineffective when used individually against polymyxin-resistant (MIC, ≥ 4 mg/L) isolates.  
  - Concentrations of polymyxin B (2 mg/L) combined with ivacaftor (8 mg/L) or ivacaftor (8 mg/L)+lumacaftor (8 mg/L) and displayed synergistic killing activity against polymyxin-resistant *P. aeruginosa* isolates as demonstrated by 100-fold decrease in the bacterial count (CFU/mL) after 24 h.  
  - The combinations also displayed excellent antibacterial activity against *P. aeruginosa* under CF relevant conditions in a sputum medium assay.  
  - Lumacaftor with polymyxin B showed additivity against *P. aeruginosa*. Scanning and transmission electron micrographs revealed that the combinations produce outer membrane damage to *P. aeruginosa* cells that is distinct from the effect of each compound per se. Ivacaftor was also shown to be a weak inhibitor of the bacterial DNA gyrase and topoisomerase IV with no effect on either human type I or type IIα topoisomerases. Lumacaftor displayed the ability to increase the cellular production of damaging reactive oxygen species. | 2016 | [16] |
| *Pseudomonas aeruginosa* in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. | Cohort of 151 US patients with CF aged 6 years and older with at least 1 copy of the G551D mutation. *Pseudomonas aeruginosa* infection category in the year before and year after | A longitudinal observational clinical microbiology study with results linked with retrospective and prospective culture data in the US Cystic Fibrosis Foundation's  
  - *Pseudomonas aeruginosa* culture positivity was significantly reduced following IVA treatment.  
  - Reduction in *P. aeruginosa* was not associated with change in FEV1, body mass index, or hospitalizations. | 2015 | [17] |
| Clinical mechanism of the cystic fibrosis transmembrane conductance regulator potentiator ivacaftor in G551D-mediated cystic fibrosis. | ivacaftor was compared and correlated with clinical findings | National Patient Registry. | Following initiation of IVA therapy:-
- FEV1 predicted & BMI improved from baseline to 6 months
- Sweat chloride decreased from baseline to 6 months
- Significant improvement in hospitalization rate
- Significant improvement in *Pseudomonas aeruginosa* burden.
- Significant improvements in mucociliary clearance, gastrointestinal pH and microbiome | 2014 [18] |
| Antibacterial properties of the CFTR potentiator ivacaftor. | QC strains and clinical strains of *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. | Basic Science | IVA induced a dose-dependent reduction in bioluminescence of *S. aureus* and decreased the number of colony forming units.
- A similar but less robust effect in *P. aeruginosa* following outer membrane permeabilization.
- IVA inhibited the growth of respiratory isolates of *S. aureus* and *Streptococcus pneumoniae*.
- IVA exhibited positive interactions with ceftriaxone, vancomycin, trimethoprim/sulphamethoxazole, moxifloxacin and linezolid. | 2014 [19] |