

1 **Can cystic fibrosis patients finally catch a breath with Orkambi?**

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4 Elena K. Schneider¹, Felisa Reyes-Ortega¹, Jian Li^{1,2†}, Tony Velkov^{1†*}

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6 **Affiliations:** ¹Drug Delivery, Disposition and Dynamics, Monash Institute of

7 Pharmaceutical Sciences; Monash University, Parkville, Victoria 3052, Australia.

8 ²Monash Biomedicine Discovery Institute, Department of Microbiology, Monash

9 University, Clayton, Victoria 3800, Australia.

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12 [†]Joint senior authors

13 **Correspondence:** Tony Velkov, Phone: +61 3 9903 9539. Fax: +61 3 9903 9583. E-

14 mail: Tony.Velkov@monash.edu OR Jian.Li@monash.edu

15 Email:

16 Schneider EK: elena.schneider@monash.edu

17 Reyes-Ortega F: felisareyes@ugr.es

18 Li J: Jian.Li@monash.edu

19 Velkov T: Tony.Velkov@monash.edu

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1 **Introduction**

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3 Cystic fibrosis is a life limiting disease caused by defective or deficient cystic fibrosis

4 trans-membrane conductance regulator (CFTR) activity. The recent FDA approval of

5 lumacaftor combined with ivacaftor (Orkambi) targets patients with the F508del-

6 CFTR. The question remains, is this breakthrough combination therapy the ‘magic-

7 bullet’ cure the vast majority of patients with CF? This review covers the

8 contemporary clinical and scientific knowledge-base for Orkambi and highlights the

9 emerging issues from recent conflicting literature reports.

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Accepted Article

1 Cystic fibrosis (CF)

2 CF is an autosomal recessive genetic disease with an incidence of 1:3,500 in
3 Caucasian populations while less common in non-white populations (1-4). The
4 disease affects the exocrine mucus glands of the lung, liver, pancreas, and intestines
5 causing progressive multi-system failure, such as loss of lung function and pancreatic
6 insufficiency (1, 4-6). Even though a slew of medications such as antibiotics, anti-
7 inflammatories and mycolytics have increased the life expectancy of patients with CF,
8 there is still a long way to go until people with CF reach appreciable levels of quality
9 of life (7). With the inevitable progression of the disease patients often require more
10 intensive therapy, which prior to the development of cystic fibrosis transmembrane
11 conductance regulator (CFTR) drugs, only targeted the symptoms (8). In the clinical
12 setting, the most common methods to measure CFTR function are the assessments of
13 sweat chloride concentration and nasal potential difference (9). Sweat chloride is a
14 promising biomarker as sweat glands do not seem to be subjected to the secondary
15 damage from CF abnormalities which can occur at an early age (unlike *i.e.* lung and
16 gastrointestinal tract tissue). Notably, sweat chloride concentration has been proposed
17 as an index of CFTR function (10). This suggestion arises from the assumption that
18 greater residual CFTR function leads to lower sweat chloride concentrations, as well
19 as protection against severe lung disease. However, sweat chloride concentration
20 alone does not necessarily predict a milder pulmonary course of the disease (10).
21 Clinical manifestations of CF include chronic lung infection and inflammation with
22 the loss of lung function that eventually results in respiratory failure (4). Thick mucus
23 accumulation in the lung promotes bacterial infections (4, 7). Major pathogens in CF
24 lungs commonly include *Pseudomonas aeruginosa* followed by *Haemophilus*
25 *influenzae*, *Staphylococcus aureus*, *Burkholderia pseudomallei*, and

1 *Stenotrophomonas maltophilia* (4, 7, 11). Thick mucus also obstructs the pancreatic
2 duct eventually leading to complete closure by scar tissue which results in exocrine
3 pancreatic insufficiency (4, 12, 13). Insufficient secretion of pancreatic enzymes leads
4 to malabsorption of fats and protein that results in deficiencies of fat soluble vitamins
5 (*i.e.* A, D, E, and K), leading to cachexia that predisposes patients to infection (4). In
6 general, patients with CF with pancreatic sufficiency are associated with milder
7 expression of the CF phenotype (1, 3, 12). One consequence of pancreatic
8 insufficiency namely, distal intestinal obstructive disease is caused by partial or
9 complete bowel obstruction caused by inspissated faeces (4). This condition
10 necessitates surgery in 10% of newborns with CF (13). Gastro-intestinal reflux
11 disease or rectal prolapses are also more common in patients with CF than in the
12 general population (14). The second most common cause of death in patients with CF
13 is liver disease (15). One in four patients with CF over the age of four years display
14 liver function related issues and 6-8% suffer from potentially fatal liver disease (15).
15 The spectrum of liver abnormalities is very broad ranging from neonatal cholestasis,
16 benign increase in circulating liver enzymes, cholelithiasis, steatosis, focal biliary
17 cirrhosis, or multilobular biliary cirrhosis with portal hypertension (15).
18 In the US and Europe CF affects ~30,000 and ~35,800 people with a prevalence of
19 0.797/10,000 (US) and 0.737/10,000 (EU), respectively (2, 16, 17). Ireland is the
20 unfortunate exception with a prevalence of 2.98/10,000 (2). The mean annual health
21 cost for patients with CF is highly dependent on the severity and progression of the
22 disease (18). The base annual cost, not including the cost of Kalydeco or Orkambi, for
23 patients with CF with mild, moderate, and severe disease are US \$10,151, US
24 \$25,647, and US \$33,691, respectively (18). In Europe the annual cost per patient
25 varies depending on the country *i.e.* €21,144 in Bulgaria, €30,123 in Italy, or €53,256

1 in Germany (19). The majority of costs are accounted for by hospitalized inpatients
2 (58%), followed by pharmaceuticals (29%), medical services (10%), complications
3 (2%), and diagnostic testing (1%) (18).

4 At present, there is no cure for CF. Due to the complex disease manifestations and
5 multi-organ involvement, patient management usually requires large number of
6 medications for symptomatic treatment of respiratory infections, inflammation, the
7 clearance of mucus, and nutritional maintenance (1, 4). In recent years, medical and
8 technological improvements like early diagnosis, specialist care, advanced treatment
9 methods, and lung transplants have resulted in an increase in median survival age to
10 now ~37.5 years in total (in the US). This is a marked improvement from the 1980s
11 when the median survival age was ~20 years (4). This shift furthermore impacts on
12 socioeconomic aspects such as independent adults with this lethal disease entering the
13 workforce or wishing to start their own families.

14 Notwithstanding, there is still a high unmet medical need for effective treatment that
15 targets the underlying mechanism of the diseased state itself (20). The progressive
16 nature of CF combined with the life-long healthcare costs are major factors in favour
17 for the development of novel CF therapeutics that modulate CFTR function (18).

18 Orkambi, the novel lumacaftor-ivacaftor combination is the first CF therapeutic that
19 actually treats the disease itself as opposed to only managing the symptoms.

20 Excitingly, this new therapeutic potentially represents the ‘magic bullet’ patients with
21 CF and respiratory clinicians have been waiting for. Worryingly, a number of
22 conflicting reports have emerged that overshadow the clinical efficacy of this
23 purported wonder drug (21, 22). Can Orkambi live up to its promise of allowing
24 patients with CF to finally ‘catch a breath’? This review surveys the current clinical
25 and pre-clinical knowledge-base on Orkambi, and provides a critical point-of-view of

1 the benefits and potential caveats of this revolutionary new CFTR corrector-
2 modulator combination.

3

4 **Mode of action**

5 An understanding of the mode of action of Orkambi necessitates the background into
6 the biology and mechanism of the CF disease state itself.

7 CF is caused by mutations in the gene that encodes the CFTR protein, which is
8 expressed in epithelial cells of various tissues including the lung (3). The full length
9 CFTR protein is a multi-domain membrane protein consisting of two membrane
10 spanning domain (MSD1&2) and two nucleotide binding domains (NBD1&2) linked
11 together by a phosphorylation regulated (R) domain (**Figure 1A**) (23). The MSD
12 forms an anion-selective pore and the dimerized NBDs mediate the ATP-binding, -
13 hydrolysis and ATP-dependent gating by the phosphorylation of the CFTR protein
14 (23). CFTR function is regulated by the cyclic adenosine monophosphate (cAMP)-
15 dependent protein kinase A. The CFTR functions to conduct hydrochloride across the
16 cytoplasmic membrane (24, 25). A deficiency in bicarbonate secretion results in poor
17 solubility of luminal mucus; leading to the production of abnormal mucus secretion
18 which damages the lungs, liver, pancreas, and intestine resulting in multi-organ
19 failure (4, 5, 13). Furthermore, due to a mutation in the CFTR the majority of men
20 with CF suffer from associated congenital bilateral absence of the vas deferens which
21 results in obstructive azoospermia (26).

22 One in 22 people of European descent carry one gene for CF making it one of the
23 most common autosomal recessive genetic diseases (3). More than 1900 CFTR
24 protein mutations have been identified and are categorized into functional classes I, II,
25 III, IV, V, and VI (**Figure 1B**) (1, 3). Broadly speaking, the class I, II, and III

1 mutations lead to the classic CF phenotype with pancreatic insufficiency and a non-
2 functional CFTR; while IV, V, and VI mutations produce a partially functional CFTR
3 and are associated with a milder form of the disease (1, 3, 13). Class I mutations result
4 in the expression of a defective CFTR with a complete loss of function (13). The most
5 common mutation found in 70% of patients with CF (Caucasians of European
6 descent) is the class II F508 deletion. Class III mutations (*i.e.* G551D) result in
7 defective regulation of channel opening (13, 27). The G551D mutation produces a
8 CFTR protein that is localized on the epithelial membrane but fails to open (28). The
9 G551D-CFTR is missense mutation targeted by Kalydeco is seen in approximately
10 4% of patients with CF and is the third most common mutation found in class III (3).
11 A clinical study conducted by Vertex convincingly demonstrated the clinical efficacy
12 of ivacaftor in a cohort of 167 G551D-CFTR CF patients (16, 29). Patients received
13 150 mg ivacaftor or placebo every 12 h with fat containing food for 48 weeks. The
14 ivacaftor treatment group displayed a 10.6% improvement in ppFEV1 in patients ≥ 12
15 years ($P < 0.0001$) and 12.5% ($P < 0.0001$) in patients aged 6-11 years compared to
16 placebo which continued through a 48-week period.

17 The class II mutation causes aberrant processing of the CFTR protein, which leads to
18 either its trafficking to the incorrect location or the presentation of non-functional
19 mature glycoform of the CFTR (**Figure 1B**) (13). The F508del mutation is by far the
20 most common mutation in the population with CF, with an overall prevalence of 70%
21 and about 50% homozygous patients (16), positioning itself as a major target for
22 CFTR corrector/modulator therapeutics (28). This mutation produces a severe defect
23 in the processing and trafficking of CFTR resulting in little to no CFTR protein
24 presentation on the cell surface (16, 25, 30-36), and additionally leads to abnormal
25 opening of the channel in the limited amounts of CFTR that actually makes it to cell

1 surface (28). Class IV mutations result in defective ion conductance by the CFTR
2 (13). Class V mutations marginally decrease the amount of functional CFTR at cell
3 surface and class VI mutations impact on the stability of the CFTR at the cell surface
4 (37-40). Notwithstanding, patients with class V and VI mutations still retain some
5 functional CFTR expression (**Figure 1B**).

6 Serendipitously, the concept for the development of CFTR modulators originated
7 from background research on the class II F508del-CFTR mutation. The F508del
8 mutation in NBD1 leads to a decrease in the thermostability of the CFTR (23, 41, 42).

9 Moreover, the F508del mutation perturbs the native interactions between NBD1 and
10 the coupling helix of MSD2 (23). The breakthrough finding was that mis-folding of
11 the defective F508del-CFTR could be corrected by lowering the temperature (<30°C)

12 or by employing chemical chaperones (e.g. glycerol) (43-48). For the first time, this
13 indicated that defective CFTR function could be restored, this in turn led to extensive
14 efforts by Vertex to identify small molecule CFTR modulators (43-48).

15 Mechanistically, CFTR modulators fall into three classes: **1**) suppressors that prevent
16 premature termination of protein synthesis (*i.e.* ataluren), **2**) correctors that partially
17 correct folding and processing defects (*i.e.* lumacaftor), and **3**) potentiators that
18 increase channel gating and conductance (*i.e.* ivacaftor).

19 Eckford *et al* (49) were the first to report that ivacaftor binds directly to the CFTR
20 protein causing CFTR channel opening via a non-conventional ATP-independent
21 mechanism (49). In CHO (Chinese hamster ovary) and human CFPAC-1 (Human

22 Caucasian pancreatic adenocarcinoma) cell culture models lumacaftor has been
23 shown to increase of the amount of functional CFTR present at cell surface by ~34%
24 (50, 51). Van Goor *et al* (32) showed that in cultured human bronchial epithelial cells

25 lumacaftor improved F508del-CFTR trafficking to the cell surface and furthermore

1 enhanced chloride secretion up to 14% (32). Furthermore, Ren *et al* (50) proved that
2 lumacaftor stabilized the F508del-CFTR *N*-terminal domain (the MSD-1 region) and
3 partially restored the function. Coincidentally, Kopeikin *et al* (51) have shown that the
4 main effect of ivacaftor on F508del-CFTR is to increase the ATP-dependent opening
5 rate of the channel. The authors purport that this results from the destabilization of the
6 closed-state, thereby shifting the balance towards the open state (**Figure 2**) (51). On a
7 purely mechanistic level, there is substantial evidence in favour of combining the
8 CFTR potentiator ivacaftor with the CFTR corrector lumacaftor. However, as will be
9 outlined in the proceeding discussion, on a pharmacokinetic level, the combination of
10 ivacaftor with lumacaftor may not be a marriage made in heaven.

11

12 **Orkambi - ‘the promised land of clinical efficacy’**

13 The three pillars of CF therapy are **1)** maintaining lung function, **2)** reducing the
14 frequency of pulmonary exacerbations, and finally **3)** improving nutritional status.

15 (16, 52). Ivacaftor (VX-770) is the first FDA-approved CFTR potentiator (53-55).

16 Ivacaftor functions as a G551D-CFTR potentiator producing an increased channel
17 opening probability to enhance chloride influx (49, 56). Although Vertex’s first-of-

18 its-kind CFTR potentiator drug Kalydeco (ivacaftor) represents a major breakthrough

19 in CF therapy, its target patient collective is limited to around 4% of patients with CF

20 (G551D mutation) [glycine (G) in position 551 is replaced by aspartic acid (D)] (3,

21 21). Additionally Ivacaftor was later approved for other gating mutations such as

22 G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, or S549R (57).

23 To solve this limitation, Vertex has developed the novel corrector-potentiator

24 combination Orkambi (lumacaftor-ivacaftor) which targets the vast majority of CF

25 patients carrying the homozygous F508del-CFTR. The F508del is an in frame

1 deletion of the CFTR gene which results in the loss of phenylalanine (F) at position
2 508 (25, 30-36). Lumacaftor (VX-809) is a first of its kind CFTR corrector that
3 facilitates processing and trafficking of the F508del-CFTR protein to increase the
4 amount at the epithelial cell surface (**Table 1**) (16). This strategy combines a CFTR
5 corrector which rescues F508del-CFTR to the cell surface with a potentiator that
6 increases the channel gating open probability (50), effectively expands the treatment
7 window to ~28% of the CF population (58). In the US alone, Orkambi now expands
8 the patient collective from only 1,950 patients that were treatable with Kalydeco to
9 15,000 patients with homozygous F508del-CFTR mutation (16, 25, 30-32). In the
10 Vertex clinical phase 3 studies 103 and 104 (TRAFFIC and TRANSPORT) (**Table 2**)
11 involving 1,108 homozygous F508del-CFTR patients, Orkambi was shown to
12 decrease sweat chloride secretion and increase ppFEV1 (percent predicted forced
13 expiratory volume in 1 second) over placebo (16). According to Vertex's FDA
14 briefing, Orkambi proved to be superior over placebo in all key secondary endpoints
15 such as change in ppFEV1 (5.3% difference to placebo), change in Body Mass Index
16 (0.36% difference to placebo), change in CF questionnaire revised respiratory domain
17 score (2.9% difference to placebo), reduction in sweat chloride (-11% compared to
18 placebo), and number of pulmonary exacerbations (0.7 % vs 1.2 % placebo) (16, 59).
19 Notably, the ppFEV1 was sustained over a 48-week period (16, 50). Encouragingly,
20 patients receiving Orkambi experienced less lung infections requiring hospitalization
21 [-61% (P<0.0001)] or intravenous (IV) antibiotics [-56% (P<0.0001)] (16).

22 *Orkambi phase 2 efficacy results*

23 Lumacaftor by itself went into a phase 2 clinical study, where patients with CF with
24 homozygous F508del-CFTR mutations were randomly assigned 25-200 mg/day

1 lumacaftor for 4 weeks (**Table 2**) (43). The study findings revealed that lumacaftor
2 produced a dose-dependent, rapid and sustained reduction in sweat chloride levels
3 ($P=0.0013$). The reduction in sweat chloride values was rapid and sustained, with
4 changes seen within 7 days of lumacaftor therapy. The mean change from baseline in
5 sweat chloride concentration (mmol/l) were +2.2 in the placebo group, -0.5 (25 mg), -
6 3.7 [50 mg (95% CI -7.1 to -0.28, $P=0.03$)], -2.3 (100 mg); and -6.6 [200 mg (95% CI
7 -10.27 to -2.83, $P=0.0008$)] (20). However, the study did not show sufficient clinical
8 benefit due to increased pulmonary exacerbation rates, and disappointing FEV1-
9 scores [percentage changes relative from baseline FEV1: placebo +0.07, lumacaftor
10 25 mg (-2.46), 50 mg (-2.15), 100 mg (+0.32), and 200 mg (+0.47)] and CFQ-R
11 scores [respiratory domain score after 28 days: placebo +4.5, lumacaftor 25 mg (-5.2),
12 50 mg (-6.3), 100 mg (-1.3), and 200 mg (+2.2)] (20, 43). Lumacaftor alone did not
13 show sufficient clinical benefit, including no improvement in lung function or quality
14 of life, over the course of the phase 2 study. Therefore, lumacaftor monotherapy did
15 not proceed into the phase 3 trials.

16 In further phase 2 trials, study 102 efficacy results studies reported by Boyle *et al* (37)
17 (**Table 2**) showed that Orkambi therapy was able to achieve an improvement in lung
18 function (measured as FEV1) for a collective of 312 CF patients suffering from
19 homozygous F508del-CFTR mutation (initial lung function 40-90% FEV1) (37, 60).
20 In cohort 1 (**Table 2**), 62 homozygous F508del-CFTR patients received 200 mg
21 lumacaftor once daily, combined with 150 or 250 mg doses of ivacaftor (twice daily).
22 Both of these treatment groups showed a reduction of sweat chloride [~ 9.1 mmol/L
23 ($p<0.001$)] compared to placebo; however, no significant changes in FEV1 were
24 observed (60, 61). In cohorts 2 and 3, mean sweat chloride concentrations did not
25 decrease significantly between day 28 and day 56 [cohort 2: $p=0.889$ (Lumacaftor 200

1 mg once per day, ivacaftor 250 mg every 12 h); $p=0.664$ (Lumacaftor 400 mg once
2 per day, ivacaftor 250 mg every 12 h); $p=0.218$ (Lumacaftor 600 mg once daily,
3 ivacaftor 250 mg every 12 h) and cohort 3: $p=0.544$ (Lumacaftor 400 mg-Ivacaftor
4 250 mg every 12 h)]; compared to baseline. However a pre-planned secondary
5 analysis, Boyle *et al* observed that the total change in sweat chloride over the entire
6 study period of 56 days decreased significantly in the lumacaftor 400 mg and the
7 lumacaftor 600 mg group for 28 days followed by the addition of ivacaftor 250 mg for
8 a further 28 days: -9.1 mmol/L [95% CI -13.3 to -4.9 ; $p<0.001$; change from
9 baseline] and -8.9 mmol/L [-13.1 to -4.7 ; $p<0.001$ (change from baseline)),
10 respectively. For the patients from cohort 3 the total change in sweat chloride over the
11 entire study period was -10.3 mmol/L (-16.7 to -4.0 $p=0.002$). These reductions were
12 significant compared with placebo [-11.1 , -18.5 to -3.7 , $p=0.00$;(treatment vs.
13 placebo)] (60). Most importantly, in cohort 2, patients treated with lumacaftor 600 mg
14 once daily, displayed an improvement in FEV1 (change from baseline) of 5.6%
15 ($p=0.013$) (60). In cohort 3, FEV1 only improved during the combination period by
16 7.7% ($p=0.003$). These dose ranging studies suggest that lumacaftor should be dosed
17 at a higher ratio to ivacaftor, which leads us to the phase 3 studies.

18 *Orkambi phase 3 efficacy results*

19 The multi-centre phase 3 studies led by Wainwright, Elborn, Ramsay, and Boyle *et al*
20 (58) corroborated the phase 2 findings (58). They conducted two phase 3,
21 randomized, double-blind, placebo-controlled studies to investigate the effectiveness
22 of lumacaftor combined with ivacaftor in 1,108 patients with CF 12 years or older that
23 had the homozygous F508del-CFTR mutation and a FEV1 of 40-90% (58). In
24 contrast to the phase 2 study, the patients were given a high dose of lumacaftor 600

1 mg once daily or 400 mg every 12 hours in combination with ivacaftor 250 mg every
2 12 hours for an extended duration of 24 weeks (58). The non-placebo patients
3 displayed an increase in FEV1 within 15 days of the initiation of therapy that was
4 sustained throughout 24 weeks. The reported findings in The New England Journal of
5 Medicine showed a significant improvement of predicted FEV1 (change from
6 baseline) in the lumacaftor-ivacaftor group over placebo (3.3% for the 600 mg once
7 daily and 2.8% for the 400 mg twice daily, respectively [$p < 0.001$ for all groups]); less
8 pulmonary exacerbations (39% fewer exacerbations in the 400 mg twice daily
9 lumacaftor group compared to placebo [$p < 0.001$] and 30% fewer exacerbation in the
10 600 mg once daily lumacaftor group compared to placebo [$p = 0.001$]). Similarly to the
11 phase 2 studies, the phase 3 study patients displayed an overall lower rate of
12 hospitalization and/or the use of IV antibiotics (58, 61).

13

14 **Pharmacokinetics**

15 Orkambi is prescribed as an oral dose twice daily every 12 h in the form of a tablet
16 (200 mg lumacaftor and 125 mg ivacaftor) (16, 32-36). After administration,
17 lumacaftor and ivacaftor are absorbed in the gastrointestinal tract and exhibit peak
18 plasma concentrations (T_{max}) in ~3-6 h and ~4 h, respectively (29). The systemic
19 exposure of lumacaftor is approximately 2-fold higher in healthy individuals
20 compared to patients with CF (**Table 3**) (59). The difference in AUC might be
21 attributed to absorption difficulties in patients with CF due to deficiencies in
22 pancreatic enzymes, impaired bowel movement or thick mucus accumulation.
23 However, experimental data is needed to validate these postulates. The exposure of
24 ivacaftor in healthy or patients with CF is similar (29). The exposure of lumacaftor
25 and ivacaftor is increased when given with high-fat containing food (29). After a

1 single dose with high-fat containing food, lumacaftor exposure was ~2-fold higher
2 and ivacaftor exposure was ~3-fold higher compared to those administration on a
3 fasting stomach, respectively (**Table 3**) (59). It is a common phenomenon that the oral
4 absorption of lipophilic drugs is improved by dietary fats, which leads us to the
5 postulate that the oral absorption (the oral bioavailability of Orkambi ranging from
6 30-100%) (29) of Orkambi could be optimized via a lipid-based formulation (16, 29,
7 53). Presently, there is very little information regarding the peak and steady-state
8 plasma concentrations of Kalydeco or Orkambi. Schneider et al., have recently
9 published a HPLC-LC/MS method for the monitoring of exposure-response
10 relationships of ivacaftor and lumacaftor (62). Given the noted metabolism of
11 ivacaftor and lumacaftor monitoring of exposure-response relationships is requisite to
12 achieve optimizing dosage regimens for Kalydeco or Orkambi therapy. Hence, there
13 remains the question of whether current dosage regimens are achieving optimal
14 exposure levels and/or overdosing is an issue.

15 Ivacaftor and lumacaftor are both very hydrophobic drugs and as such are ~99%
16 bound to plasma proteins, which significantly limits the free (active) drug
17 concentration (1). Ivacaftor is highly bound to human serum albumin (HSA) and α 1-
18 acid glycoprotein (AGP) (1). Similarly, lumacaftor preferentially binds to HSA (29,
19 59). Schneider *et al* have investigated the impact of co-administered CF drugs on the
20 plasma protein binding of ivacaftor (1). Using surface plasmon resonance and
21 fluorimetric binding assays that measure the displacement of site-selective probes,
22 they have showed that interactions between ivacaftor are to be expected with
23 ducosate, montelukast, ibuprofen, dicloxacillin, omeprazole and loratadine due to
24 their ability to strongly compete for the ivacaftor binding sites on HSA and AGP. The
25 half-life of lumacaftor administered as Orkambi is about 26 h in patients with CF. The

1 half-life of ivacaftor administered as Kalydeco is 12 h and ~9 h when administered as
2 Orkambi (29, 59). Due to high plasma protein binding there is a strong possibility that
3 co-administered CF drugs could compete with ivacaftor or lumacaftor for the same
4 plasma protein binding sites and impact on the free drug concentration. This in turn
5 could lead to variable free drug plasma concentrations and thereby impact therapeutic
6 outcomes (1). Similarly, the shorter half-life of ivacaftor when administered as
7 Orkambi may be due to the displacement from its plasma protein binding sites by
8 lumacaftor, which in turn leads to increased free drug and clearance. Our group has
9 reported that such drug-drug interactions between ivacaftor and other CF drugs can be
10 expected with docusate, montelukast, ibuprofen, dicloxacillin, omeprazole, and
11 loratadine. Ideally, Kalydeco and Orkambi should be administered in a staggered
12 dosage regimen with these CF drugs to maximize free drug concentrations and
13 clinical efficacy (1).

14 Ivacaftor is heavily metabolized in humans, primarily by CYP3A4 into 11 metabolites
15 found in bile, urine, plasma, and faeces (53, 59). Ivacaftor is primarily metabolized
16 into active hydroxymethyl-ivacaftor (M1) and inactive ivacaftor-carboxylate (M6) by
17 oxidation (**Table 1**) (53, 59). A disconcerting aspect of the metabolism of Orkambi
18 are the potentially complex interactions between lumacaftor and ivacaftor themselves
19 and with the supplementing medications to treat the symptoms of this multisystem
20 disease (**Table 3**) (52). Notably, strong CYP3A4 inhibitors such as clarithromycin or
21 -azole antifungals have been reported to increase exposure of ivacaftor (16). The
22 product information inset states that in the first week of initiating Orkambi or
23 Kalydeco therapy in conjunction with these drugs, half of the dose of
24 ivacaftor/lumacaftor is recommended (**Table 3**) (59). Similarly, the concomitant use
25 of strong CYP3A4 inducers such as rifampin, rifabutin, phenobarbital,

1 carbamazepine, phenytoin, and St John's Wort (*hypericum perforatum*) are not
2 recommended as the exposure of ivacaftor is decreased by ~57% (59). Lumacaftor is
3 not heavily metabolized with the majority being excreted unchanged in the faeces (16,
4 29). The approximately 10% of lumacaftor that is metabolized occurs via oxidation
5 and glucuronidation (29). In the FDA and EMA reports for Orkambi, Vertex mentions
6 lumacaftor itself is an inducer of the cytochrome P450 drug metabolizing enzymes
7 (16, 29). Paradoxically ivacaftor is a substrate of cytochrome P450 3A4 (CYP3A4)
8 which leads us to another potential antagonistic drug-drug interaction between the
9 actual components of Orkambi themselves (*i.e.* lumacaftor *versus* ivacaftor) (29, 59).
10 To this end we have found that the steady-state plasma concentration of ivacaftor in
11 patients receiving Orkambi therapy is significantly lower than the levels we measured
12 in patients receiving Kalydeco therapy (62).

13

14 **Side effects**

15 In general oral administration of Orkambi showed an acceptable tolerability profile
16 when added to the standard therapy of patients with CF aged ≥ 12 years in the 24-
17 week TRAFFIC and TRANSPORT studies (58). However, to date the 120-week
18 extension of these trials, called PROGRESS study is still ongoing. Aside from the
19 benefits of Orkambi, it is also essential to examine the other side of the coin, its
20 potential caveats. Ironically, the major adverse effects reported for patients
21 undergoing Orkambi therapy *versus* placebo were dyspnoea (14%:7.8%) and
22 respiratory chest tightness (9.8%:5.9%) (16, 29, 59). Diarrhoea, nausea, and upper
23 respiratory tract infections have also been reported as adverse effects (16, 59).
24 Notwithstanding, Vertex suggests that most respiratory adverse effects resolve within
25 the first few weeks of treatment (16, 59). Additionally, elevation of liver enzymes and

1 hepatobiliary disorders were reported in some patients compared to placebo (0.9%)
2 (16). This could restrict Orkambi treatment, as up to 35% of patients with CF suffer
3 from liver disease, a known clinical manifestation as a result of CFTR dysfunction in
4 their biliary tract cells (15, 16, 63-66). Coincidentally, Vertex's pooled phase 2 studies
5 103 and 104 were discontinued due to the patients exhibiting elevated liver
6 transaminases (0.5%) and respiratory events (0.7%) (16). If the benefits of therapy
7 outweigh the risks, Orkambi could be used carefully at a lower dosage and
8 importantly, with close monitoring in patients with advanced liver disease (59). Thus,
9 liver function monitoring is recommended prior to and during the course of Orkambi
10 therapy.

11 Due to their lipophilicity, ivacaftor and lumacaftor could potentially cross the blood
12 brain barrier. Pharmacodynamic studies in rats have shown that ivacaftor functions as
13 an inhibitor of the monoamine transporter and binds serotonin 5-HT_{2C} receptors (16),
14 suggesting that Kalydeco and Orkambi could possess neuropharmacological activity.

15 To date, no adverse data for Orkambi in pregnant women has been reported; currently
16 Orkambi is listed as a pregnancy category 'B' drug that requires additional human
17 trials (61, 67). Kaminski *et al* (68) were the first to report a successful uncomplicated
18 pregnancy during Kalydeco therapy for a single patient with CF (68). However,
19 breastfeeding mothers should remain cautious, as both ivacaftor and lumacaftor have
20 been detected in the milk of lactating rats (61, 67).

21 A beneficial side effect of ivacaftor is its antimicrobial activity (69-71). Schneider *et*
22 *al* have shown synergistic antibacterial activity of ivacaftor in combination with
23 polymyxin B against a panel of polymyxin-resistant Gram-negative pathogens that
24 commonly colonize the lungs of patients with CF, including strains that were resistant
25 to the quinolone antibiotic ciprofloxacin, which is structurally related to ivacaftor (69,

1 70). As polymyxins (in particular colistin) are commonly used in patients with CF for
2 treatment of lung infections caused by multidrug-resistant *P. aeruginosa*, such a
3 synergistic killing effect plays a key role in minimising any potential resistance to
4 polymyxins (70).

6 **Ivacaftor versus lumacaftor**

7 Although the aforementioned TRANSPORT and TRAFFIC clinical studies have
8 confirmed that Orkambi therapy improves F508del-CFTR function from day 1 to day
9 56 of therapy, the reasons for the discouraging long-term results (>24 weeks) were
10 not obvious from the available clinical data (TRANSPORT and TRAFFIC studies for
11 long term efficacy and safety are still ongoing). Recently, evidence has emerged from
12 a number of independent laboratories suggesting that prolonged exposure to ivacaftor
13 counteracts the corrector function of lumacaftor by destabilising the lumacaftor-
14 rescued mature glycoform of F508del-CFTR (21, 22, 72). Such inhibitory interactions
15 are especially concerning given that lumacaftor only partially restores (11-15%)
16 F508del-CFTR surface expression (21, 22). The *in vitro* studies by Gentsch *et al* (22,
17 43) suggested that chronic administration of ivacaftor caused a dose-dependent
18 reversal of lumacaftor-mediated CFTR correction in homozygous F508del human
19 primary bronchial epithelial cells. They reported that in cells chronically treated with
20 5 μ M lumacaftor, acute exposure to ivacaftor (5 μ M) resulted in an increase of CFTR
21 mediated ion transport within minutes (22). Whereas, chronic treatment (24-48 h) of
22 lumacaftor rescued cells with 5 μ M ivacaftor resulted in a decrease in channel
23 conductance (22). This result reflected the destabilization of corrected Δ F508 CFTR
24 by ivacaftor, dramatically increasing its turnover rate. Chronic ivacaftor treatment
25 furthermore reduced mature wild-type CFTR levels and function. These findings

1 demonstrate that chronic treatment with CFTR potentiators and correctors may have
2 unexpected effects that cannot be predicted from short-term studies. Furthermore, it
3 was demonstrated that the CFTR-mediated short circuit current increases after
4 addition of ivacaftor to corrector-rescued F508del-CFTR, albeit this effect was
5 transient (22). These findings may be an indicator of a rapidly decreasing quantity of
6 functional protein at the apical membrane (22). They also showed that the loss of
7 corrected-rescued function of F508del-CFTR treated with lumacaftor in combination
8 with ivacaftor was reflected in reduced chloride secretion responses (22). In their
9 Western blot the amount of mature F508del-CFTR was actually diminished and
10 instead the F508del-CFTR protein only appeared as an immature band B (22).
11 Therefore, it is essential that we gain further knowledge of the interaction and
12 interference between CFTR potentiators and CFTR correctors.

13 Another recent study by Veit *et al* (21) indicated that ivacaftor reduces the correction
14 efficacy of lumacaftor. Their results indicated that ivacaftor destabilizes the native
15 CFTR protein in immortalized and primary human respiratory epithelia (21). The
16 authors purport that destabilization is caused by ivacaftor itself which could further
17 play an essential role in the faulty assemblage of the CFTR protein. Furthermore, this
18 implies that the lumacaftor rescued F508del-CFTR is still defective and not
19 functionally equivalent to the native CFTR (73). These findings show that chronic
20 treatment with Orkambi may have unexpected long-term effects that are not
21 predictable from the clinical studies.

22

23 **Cost versus benefit**

24 Affordability is a key issue with Orkambi therapy (28). As the price is supposed to be
25 representative of the compensation Vertex would expect to cover their development

1 costs (due the small patient collective), we would assume the price of Kalydeco to
2 have gone down with the introduction of Orkambi (28). However, pricing for both
3 drugs remains extraordinary high, despite the low cost of goods (ivacaftor and
4 lumacaftor are easily accessible synthetically and therefore the cost of development is
5 presumably low). However, we must take into account the high development costs
6 which would help justify the high price of these drugs. Orkambi treatment is
7 estimated at \$259,000 USD per year, which is lower than the established pricing of
8 Kalydeco at \$307,000 USD per year (61, 74). Given the price differential, it would be
9 more economically prudent for patients with G551D-CFTR to simply adopt Orkambi
10 therapy. However, Orkambi has yet to be approved for patients with G551D-CFTR.
11 Moreover, given that patients with G551D-CFTR express CFTR on the epithelial cell
12 surface, this may not prove successful due to the increase in CFTR turnover rates
13 reported with Orkambi therapy (**Figure 3**) (21, 22, 45). As patients are forced to take
14 Orkambi or Kalydeco as a lifelong therapy, the impact on the health care budget is
15 staggering (40). In view of the modest clinical outcomes reported for Orkambi
16 therapy, the cost *versus* benefit becomes questionable.

17

18 **Perspective-Can CF patients finally catch a breath?**

19 Orkambi represents a first-of-its-kind breakthrough CF treatment strategy. However,
20 since its release a number of key questions remain unanswered. First and foremost,
21 unlike the experience with Kalydeco, patients receiving Orkambi therapy displayed
22 only modest improvements in lung function and pulmonary exacerbations (16).
23 Secondly, antagonistic drug-drug interactions could potentially limit Orkambi's
24 clinical efficacy (57). Despite these concerns, the long-term benefits of Orkambi
25 require further assessment and we are all curiously awaiting the results of the

1 ongoing-longterm TRANSPORT and TRAFFIC studies for these two revolutionary

2 CF drugs.

3

4

5

Accepted Article

1 **Authorship Contributions**

2 *Wrote or contributed to the writing of the manuscript:*

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4
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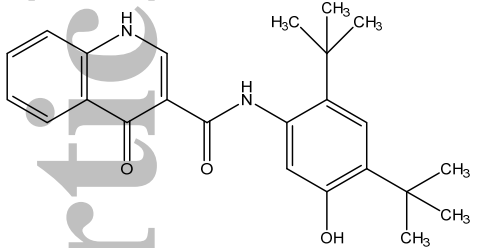
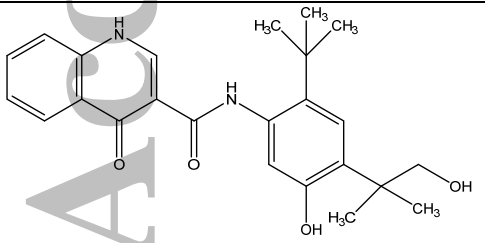
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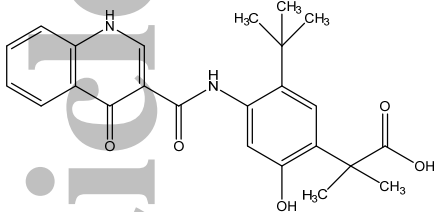
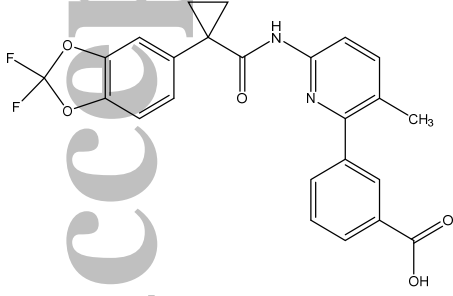
28 **Figure 1A.** Model of the proposed structure of the cystic fibrosis transmembrane
29 conductance regulator (CFTR) channel. The CFTR is a member of the ABC
30 transporter family that functions a Cl⁻ channel. It is located in the apical membrane of
31 epithelia. The CFTR is composed of five domains which include two membrane-
32 spanning domains (MSD-1 and -2), two nucleotide-binding domains (NBD-1 and -2),
33 and a regulatory (R) domain.
34

35 **Figure 1B.** Schematic diagram showing the outcomes of CFTR mutation classes I, II,
36 III, IV, V, and VI. Mutation that reduce the trafficking of functional CFTR to the cell
37 surface are classed as III and IV. Mutations that reduce the total quantity of cellular
38 CFTR are classed as I, II, V, VI.
39

40 **Figure 2.** Mutant F508del CFTR channel after prolonged Orkambi treatment. *Left,*
41 Orkambi therapy improves long-term CFTR function by acting to enhance the
42 trafficking of CFTR to the cell surface and enhancing Cl⁻ influx intracellularly. *Right,*
43 alternatively, prolonged Orkambi therapy could causes destabilization and accelerated
44 turnover of CFTR, resulting in less functional CFTR being presented on the cell
45 surface.

Table 1. Overview of the major metabolites of ivacaftor and lumacaftor

Chemical properties		
Ivacaftor		
	PubChem CID	16220172
	Chemical names	Ivacaftor; VX-770; Kalydeco; 873054-44-5; VX770; N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide
	Molecular formula	C ₂₄ H ₂₈ N ₂ O ₃
	Molecular weight	392.49 g/mol
	Metabolism	primarily metabolized by CYP3A4 into M1 and M6
	Plasma protein binding	99% plasma protein bound, primarily to HSA and AGP
Hydroxymethyl-Ivacaftor (M1)		
	PubChem CID	Not found
	Chemical names	N-(2-(tert-Butyl)-5-hydroxy-4-(1-hydroxy-2methylpropane-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide
	Molecular formula	C ₂₄ H ₂₈ N ₂ O ₄
	Molecular weight	408.49 g/mol
	Metabolism	Ivacaftor oxidation by CYP3A4 to M1
	Plasma protein binding	Highly bound but lesser extent than ivacaftor

	binding	
Ivacaftor-Carboxylate (M6)		
	PubChem CID	46917263
	Chemical names	UNII-38WUF8D79H; 38WUF8D79H; 1246213-24-0; 2-(5-(tert-Butyl)-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-methylpropanoic acid; Ivacaftor (m6); Ivacaftor carboxylate;
	Molecular formula	C ₂₄ H ₂₆ N ₂ O ₅
	Molecular weight	422.47 g/mol
	Metabolism	Ivacaftor oxidation by CYP3A4 to M6
	Plasma protein binding	Highly bound but lesser extent than ivacaftor
Lumacaftor		
	PubChem CID	16678941
	Chemical names	936727-05-8; Lumacaftor; VX-809; VX809; VX 809; 3-(6-(1-(2,2-Difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-3-methylpyridin-2-yl)benzoic acid;
	Molecular formula	C ₂₄ H ₁₈ F ₂ N ₂ O ₅
	Molecular weight	452.41 g/mol
	Metabolism	not extensively metabolized
Plasma protein binding	99% plasma protein bound, primarily to	

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	binding	HSA
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Table 2. Chronology of the randomized, double-blind, placebo-controlled Orkambi phase 2 and 3 studies in patients bearing a F508del-mutation of the CFTR

Type	Name	Objectives	Treatment	Patient Collective	Duration	Endpoints
Phase 2A	101	Safety and tolerability of LUMA	LUMA 25, 50, 100, 200 mg qd; orally	89 patients with a homozygous F508del-CFTR mutation	28 d	<u>Primary endpoints:</u> <ul style="list-style-type: none"> • Change of CFTR function measured by sweat chloride concentration • Safety
Phase 2	102	Safety and tolerability of LUMA-IVA combination	<ul style="list-style-type: none"> • Cohort 1: LUMA 200 mg qd followed by LUMA 200 mg qd/ IVA 150 or 250 mg q12h • Cohort 2: LUMA 200, 400, 600 mg qd followed by 	312 patients with homozygous (cohort 1 &3) or heterozygous (cohort 4) F508del-CFTR mutation (cohort 2 mixed)	<ul style="list-style-type: none"> • Cohort 1: 14 d mono- followed by 7 d combination therapy • Cohort 2 & 3: 28 d mono- 	<u>Secondary endpoints:</u> <ul style="list-style-type: none"> • Absolute change in predicted FEV1 percentage • Change in sweat chloride concentration from baseline • Change in CFQ-R score

			<p>LUMA/IVA 250 mg q12h</p> <ul style="list-style-type: none"> • Cohort 3: LUMA 400 mg q12h followed by LUMA 400 mg q12h/ IVA 250 mg q12h • Cohort 4: LUMA 400 mg q/12h followed by LUMA 400 mg qd/ IVA 250 mg q12h 		<p>followed by 28 d combination therapy</p> <ul style="list-style-type: none"> • Cohort 4: 56 d of combination therapy 	
TRAFFIC and TRANSPORT studies						
Phase 3	103	Efficacy and safety of LUMA-IVA combination	LUMA 600 or 400 mg qd/ IVA 250 mg q12h	549 patients with a homozygous F508del-CFTR mutation	Up to 24 w + 5 d	<p><u>Primary endpoints:</u></p> <ul style="list-style-type: none"> • Absolute change from baseline in predicted FEV1 percentage at week 24 <p><u>Secondary endpoints:</u></p> <ul style="list-style-type: none"> • Relative change from baseline in predicted FEV1 percentage (for week 16 & 24) • Absolute change from baseline in BMI
Phase 3	104	Efficacy and safety of LUMA-IVA combination	LUMA 600 or 400 mg qd/ IVA 250 mg q12h	559 patients with a homozygous F508del-CFTR mutation	Up to 24 w + 5 d	

Phase 3	105	Long term efficacy and safety of LUMA-IVA combination	<ul style="list-style-type: none"> • Part A: LUMA 600 or 400 mg qd/ IVA 250 mg q12h • Part B: LUMA 400 mg qd/ IVA 250 mg q12h 	<ul style="list-style-type: none"> • Part A: 1031 patients with a homozygous F508del-CFTR mutation (including patients from study 103/104) • Part B: 119 patients with a heterozygous F508del-CFTR mutation 	~96 w	<p>percentage at week 24</p> <ul style="list-style-type: none"> • Absolute change from baseline in CFQ-R score at week 24 • Percentage of patients with a relative increase from baseline of $\geq 5\%$ in the percentage predicted FEV1 • Number of pulmonary exacerbations through week 24 • Safety
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Table 3. Pharmacokinetics of lumacaftor and ivacaftor (14, 22, 37)

		ORKAMBI	
		Lumacaftor	Ivacaftor
Absorption	single oral dose	exposure increases by 2-fold if taken with fat-containing food	exposure increases by 3-fold if taken with fat-containing food
	multiple oral dose in combination	exposure increase proportional to dose from 200 mg/24h to 400 mg/24h	exposure increase with dose from 150 mg/12h to 250 mg/12h
Distribution		99% plasma protein bound, primarily to HSA	99% plasma protein bound, primarily to HSA and AGP
Half-life		26 h	12 h (in combination with lumacaftor 9 h)
Metabolism		not extensively metabolized	primarily metabolized by CYP3A4 into M1 and M6

Excretion		51% unchanged in faeces; minimal urinary excretion	87·8% excreted in faeces after metabolic conversion to mainly M1 and M6; minimal urinary excretion of ivacaftor and metabolites
Drug-drug interactions	CYP3A4	inducer	Substrate
	CYP2C9	inhibitor	potential inhibitor
	P-glycoprotein	inducer/ inhibitor	weak inhibitor
Side effects (37, 73)	ORKAMBI (after 24 weeks of treatment)		
	14%	dyspnea, nasopharyngitis, nausea	
	8-12%	respiration abnormal, fatigue, upper respiratory tract infections, diarrhea	
	<7%	Influenza, rhinorrhea, flatulence, rash, blood	

		creatin phosphokinase increase	
	Ivacaftor (after 48 weeks of treatment)		
	24%	-	headache
	22-15%	-	Oropharyngeal pain, upper respiratory tract infection, nasal congestion, abdominal pain
	15-9%	-	Nasopharyngitis, diarrhoea, rash, nausea, dizziness
Potential drug-drug interactions for Orkambi (37)			
CF Drug Class	Interaction to be expected with Orkambi	Dose adjustment	Alternative treatment option (no drug-drug interactions to be expected due to different metabolism and/or route of elimination)
Antibiotics	Clarithromycin Erythromycin Telithromycin	Preferentially: seek alternative treatment option	Ciprofloxacin Azithromycin Levofloxacin Aztreonam Ceftazidim Colistimethate Colistin Tobramycin Sulfamethoxazol/ Trimethoprim

Antifungals	Itraconazole Ketoconazole Posaconazole Voriconazole	Preferentially: seek alternative treatment option	Fluconazole
Anti-allergies and systemic corticosteroids	Montelukast Prednisone Methylprednisone	Dose adjustment for Montelukast is not recommended. Increase dose of Prednisone and Methylprednisone	Budesonide Fluticasone Cetirizine
Anti-inflammatives	Ibuprofen	Increase dose of Ibuprofen	
Antidepressants	Citalopram Escitalopram Sertaline	Increase dose of these antidepressants	
Hormonal contraceptives	Estrogen Progesterone	Hormonal contraceptives should not be relied on.	
Proton pump inhibitors, H2-blockers, Antiacids	Omeprazole Esomeprazole Lansoprazole	Increase dose of the -prazoles	

