Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment

Tara N. Guhr Lee[a,b,*], Deborah M. Cholon[b], Nancy L. Quinney[b], Martina Gentzsch[b,c], Charles R. Esther Jr[a,b]

[a] Division of Pediatric Pulmonology, Department of Pediatrics, University of North Carolina School of Medicine, 450-D MacNider Hall, Campus Box 7217, Chapel Hill, NC 27599-7217, USA
[b] Marsico Lung Institute/Cystic Fibrosis Research Center, University of North Carolina, Chapel Hill, NC 27599, USA
[c] Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 20 December 2019
Revised 17 April 2020
Accepted 21 April 2020
Available online xxx

Keywords:
CFTR modulator
Ivacaftor
F508del
Pharmacokinetics

ABSTRACT

Background: Current dosing strategies of CFTR modulators are based on serum pharmacokinetics, but drug concentrations in target tissues such as airway epithelia are not known. Previous data suggest that CFTR modulators may accumulate in airway epithelia, and serum pharmacokinetics may not accurately predict effects of chronic treatment.

Methods: CF (F508del homozygous) primary human bronchial epithelial (HBE) cells grown at air-liquid interface were treated for 14 days with ivacaftor plus lumacaftor or ivacaftor plus tezacaftor, followed by a 14-day washout period. At various intervals during treatment and washout phases, drug concentrations were measured via mass spectrometry, electrophysiological function was assessed in Ussing chambers, and mature CFTR protein was quantified by Western blotting.

Results: During treatment, ivacaftor accumulated in CF-HBEs to a much greater extent than either lumacaftor or tezacaftor and remained persistently elevated even after 14 days of washout. CFTR activity peaked at 7 days of treatment but diminished with further ivacaftor accumulation, though remained above baseline even after washout.

Conclusions: Intracellular accrual and persistence of CFTR modulators during and after chronic treatment suggest complex pharmacokinetic and pharmacodynamic properties within airway epithelia that are not predicted by serum pharmacokinetics. Direct measurement of drugs in target tissues may be needed to optimize dosing strategies, and the persistence of CFTR modulators after treatment cessation has implications for personalized medicine approaches.

1. Introduction

The basic defect in cystic fibrosis (CF) reflects loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR), a plasma membrane protein involved in chloride and bicarbonate transport across cellular membranes. A key therapeutic strategy for CF focuses on CFTR modulators, drugs designed to improve the function of abnormal CFTR. All currently approved CFTR modulators include the potentiator compound ivacaftor (discovered as VX-770, [1]), which improves the gating efficiency of CFTR. Therapies for patients with F508del mutations also include the correctors lumacaftor (discovered as VX-809, [2]) or tezacaftor (discovered as VX-661), which improve folding and maturation of CFTR bearing the F508del mutation. Combinations of a potentiator and a corrector improve CFTR function and clinical outcomes in patients homozygous for the F508del mutation, and next-generation therapies with novel correctors such as elexacaftor (discovered as VX-445) in combination with the approved ivacaftor/tezacaftor therapy were even more efficacious in clinical trials [3–6] (ClinicalTrials.gov identifiers NCT03525548, NCT03525444, NCT03460990, and NCT03447249). Several other companies are developing potentiators and correctors as well, including the novel amplifier compounds such as PTI-428 (Proteostasis Therapeutics) [7].

Because a lack of validated CF animal models precluded *in vivo* pharmacodynamic studies, dosing of ivacaftor and other CFTR

* Corresponding author at: Division of Pediatric Pulmonology, Department of Pediatrics, University of North Carolina School of Medicine, 450-D MacNider Hall, Campus Box 7217, Chapel Hill, NC 27599-7217, USA.
E-mail address: taraguhr@email.unc.edu (T.N. Guhr Lee).

Please cite this article as: T.N. Guhr Lee, D.M. Cholon and N.L. Quinney et al., Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2020.04.010

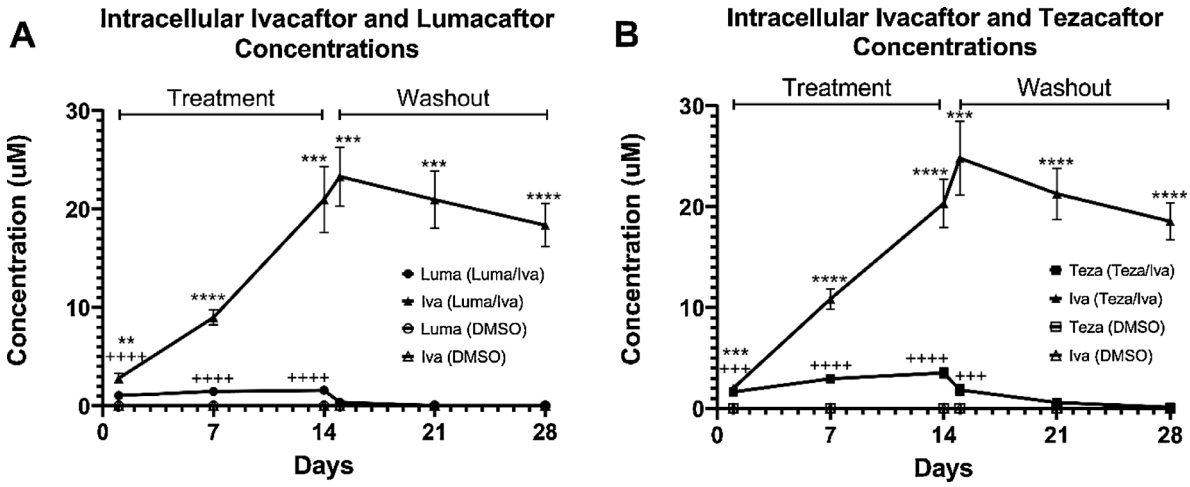

Fig. 1. MS analysis of CFTR modulator intracellular concentrations during treatment and washout phases in CF-HBE (F508del/F508del) cells. Ivacaftor continually accrued during the treatment period, and only moderately declined during the washout period. (A) Lumacaftor or Ivacaftor was measured in treated (Luma/Iva) or untreated (DMSO) samples. Lumacaftor displayed minimal accrual over the treatment phase and was undetectable within 7 days of washout. (B) Tezacaftor or Ivacaftor was measured in treated (Teza/Iva) or untreated (DMSO) samples. Tezacaftor also only accrued to a minimal extent during the treatment phase and diminished throughout the two-week washout phase. Both tezacaftor and lumacaftor concentrations were rapidly eliminated from CF-HBEs within 1 day of beginning washout (day 15), while ivacaftor levels were still increasing to maximum after treatment cessation. *, + indicate level of significance between vehicle and treatment groups at the specified time point.

modulators in clinical trials were based on other criteria including pharmacodynamic effects within *in vitro* cell culture systems, serum pharmacokinetic studies, and early dose ranging (Phase II) studies. While these approaches resulted in clinically effective dosing regimens, they did not account for the pharmacokinetic properties of these drugs within their primary target tissue, airway epithelia. Currently approved CFTR modulators are very hydrophobic and protein bound, and animal studies of ivacaftor have indicated accumulation within the lung and other tissues [8]. Our own studies [9] suggested that both ivacaftor and lumacaftor accumulate within cultured airway epithelia resulting in instability and turnover of CFTR at the cell surface. These findings suggest that the pharmacokinetic properties of these drugs within target tissues, and thus their optimal dosing, may vary substantially from serum pharmacokinetics. Indeed, genotype-phenotype studies [10] suggest that even the modest restoration of CFTR activity observed *in vitro* with modulators should lead to far more clinical efficacy than was observed in the clinical trials [11,12].

To better understand the pharmacokinetic properties of CFTR modulators in their primary target tissues, we exposed primary human bronchial epithelia (HBE) homozygous for the F508del CFTR mutation to ivacaftor in combination with either lumacaftor or tezacaftor, reflecting two of the currently approved therapeutics. Intracellular drug concentrations were measured using an established mass spectrometric method to determine whether and to what extent these drugs accumulate within CF-HBE during prolonged drug exposure and washout phases. The impact of these drugs on both mature CFTR expression and physiological activity were assessed.

2. Materials and methods

2.1. Cell culture

Primary HBE cells from 3 different patients homozygous for the F508del genetic mutation, prepared as described previously [13], were gifted by Dr. Scott H. Randell (Marsico Lung Institute, The University of North Carolina at Chapel Hill, USA). The cells were obtained under protocol #03-1396 approved by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board. Cells were expanded in BEGM (Lonza) and then cultured at air-liquid interface with a plating density of 250,000 cells on 12 mm Millicell inserts (Millipore) in a modified BEGM [14] until differentiated. Cells were treated with a combination of ivacaftor (VX-770, Selleck Chemicals) + lumacaftor (VX-809, Selleck Chemicals) or ivacaftor + tezacaftor (VX-661, Selleck Chemicals) daily with fresh media and drug changes, and DMSO was used as a vehicle control. Ivacaftor was administered at a 1 μM concentration, and lumacaftor and tezacaftor were used at 3 μM concentrations. Exposure to drug or vehicle lasted for a period of 1 day, 7 days, or 14 days. After a 14 day chronic exposure period to either of the dual combination therapies, cells underwent a washout phase lasting either 1 day, 7 days, or 14 days (15, 21, and 28 days after initiation of treatment). Cultures were functionally analyzed in Ussing chambers, or lysed/processed and assayed for drug concentrations and CFTR protein levels.

2.2. Functional measurements of CFTR

Change in short-circuit current (ΔI_{sc}) was measured from CF-HBE cultures in Ussing chambers as previously described [13,15,16] in a bilateral Krebs bicarbonate-Ringers solution. Amiloride (100 μM, Sigma-Aldrich) was added to the apical bath to inhibit the epithelial sodium channel ENaC. Bilateral addition of forskolin (10 μM, Sigma-Aldrich) followed to stimulate CFTR channel activity. CFTR inhibitor-172 (10 μM, Sigma-Aldrich) was then apically introduced to inhibit CFTR. Transepithelial resistance ($\Omega \bullet cm^2$) was measured to assess monolayer integrity. UTP (100 μM, GE Healthcare) response for Ca^{2+} channel activity was assessed as an internal control.

2.3. Mass spectrometric analysis of drugs

Ivacaftor, lumacaftor, and tezacaftor were organically extracted from cell lysates using an equal volume of methyl tert-butyl ether (MTBE), with ivacaftor and lumacaftor concentrations measured via liquid chromatography/tandem mass spectrometry (LC/MS-MS) as previously described [9,17]. Tezacaftor concentrations were measured in a similar manner, using selected reaction monitoring of the transition of *m/z* 521.5→449.1 (collision energy 15 eV) in tandem mass spectrometry, which generated a single peak at run time 8.7 min. DMSO-treated CF-HBE lysates were spiked with known CFTR modulator concentrations for reference.

Please cite this article as: T.N. Guhr Lee, D.M. Cholon and N.L. Quinney et al., Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2020.04.010

Table 1
CFTR Modulator Intracellular Concentration in HBE (F508del/F508del) Cells. Averages of ivacaftor, lumacaftor, and tezacaftor concentrations (μM) ± standard error of the mean. *p*-values were calculated for each modulator in comparison to the corresponding DMSO vehicle control.

	DMSO	Iva (Luma/Iva)	*p*-value	Iva (Teza/Iva)	*p*-value	Luma (Luma/Iva)	*p*-value	Teza (Teza/Iva)	*p*-value
1	0.00 ± 0.00	2.80 ± 0.49	0.0247	2.01 ± 0.34	0.0059	1.05 ± 0.27	0.0598	1.64 ± 0.11	<0.0001
7	0.00 ± 0.00	8.98 ± 0.76	<0.0001	10.86 ± 1.01	<0.0001	1.47 ± 0.13	<0.0001	2.95 ± 0.24	<0.0001
14	0.00 ± 0.00	20.96 ± 3.35	0.0041	20.33 ± 2.40	0.0005	1.59 ± 0.11	<0.0001	3.53 ± 0.51	0.0021
15	0.00 ± 0.00	23.30 ± 2.98	0.0009	24.81 ± 3.65	0.0024	0.36 ± 0.04	0.0006	1.84 ± 0.55	0.1268
21	0.00 ± 0.00	20.95 ± 2.90	0.0016	21.28 ± 2.53	0.0005	0.03 ± 0.01	0.2937	0.59 ± 0.17	0.1217
28	0.00 ± 0.00	18.36 ± 2.18	0.0005	18.55 ± 1.83	0.0001	0.01 ± 0.00	0.3995	0.12 ± 0.03	0.0781

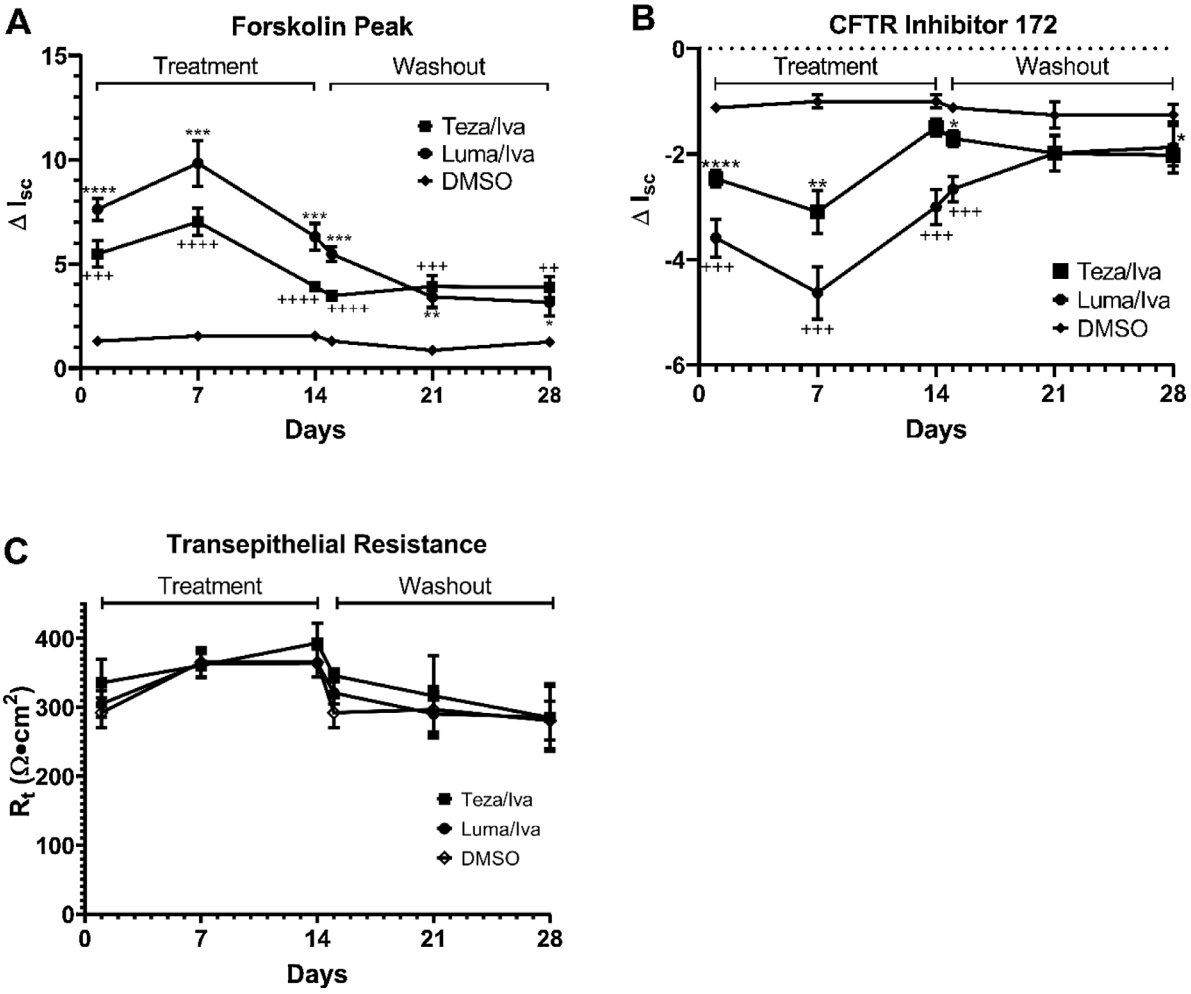

Fig. 2. Ussing chamber measurements of CFTR activity in CF-HBEs (F508del/F508del) treated with CFTR modulators. (A) Forskolin peaks measured by short-circuit current (I_{sc}) were elevated in both treatment arms (Teza/Iva, Luma/Iva) compared to vehicle (DSMO) control for the duration of both the treatment and washout phases. Forskolin peaks reached maximum at day 7 of the treatment arms. (B) CFTRinh172 inhibition was accentuated in both treatment arms compared to vehicle (DMSO) control during both treatment and washout phases. CFTRinh172 inhibition reached maximum at day 7 of the treatment arm. (C) Transepithelial resistance (R_t) demonstrates that the cell monolayer remained intact over the course of the treatment period, indicating no toxic effects of the drugs compared to the vehicle (DMSO) control group. *, + indicate level of significance between vehicle and treatment groups at the specified time point.

2.4. CFTR protein maturation by immunoprecipitation/ western blot

Western blot analysis of endogenous CFTR protein was performed as described previously [9,18]. Briefly, whole-cell lysates of fully differentiated CF-HBE cultures were prepared and then CFTR was immunoprecipitated. Samples were separated on 4 to 20% gradient SDS–polyacrylamide gel electrophoresis gels (Bio-Rad) and then transferred to nitrocellulose. Blots were probed with mouse monoclonal anti-CFTR antibodies and then with IRDye 680–goat anti-mouse immunoglobulin G (Molecular Probes). Anti-actin (Cell Signaling) was used as a loading control. Protein bands were visualized using a Sapphire Biomolecular Imager (Azure Biosystems).

2.5. Statistics

Data analysis was performed using GraphPad PRISM (version 8.1) software. Statistical significance was calculated using the Student's *t*-test or ANOVA with alpha at $p = 0.05$.

3. Results

Primary HBEs homozygous for F508del CFTR mutation were cultured at air-liquid interface as described [14]. Cells were exposed to a combination of either ivacaftor plus lumacaftor or ivacaftor plus tezacaftor with drug and media exchanges daily for a total

Please cite this article as: T.N. Guhr Lee, D.M. Cholon and N.L. Quinney et al., Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2020.04.010

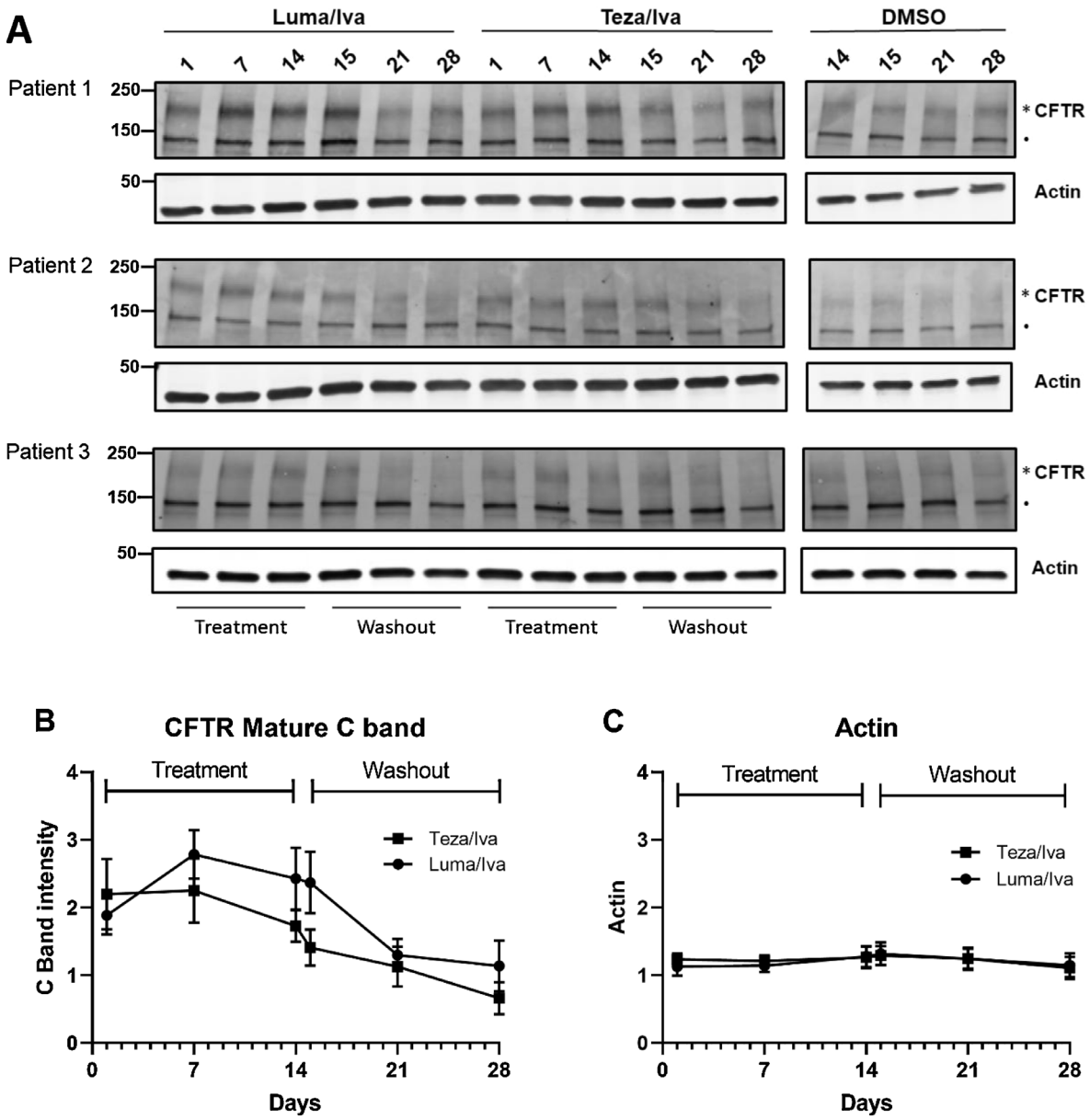

Fig. 3. CFTR protein maturation in CF-HBE (F508del/F508del) cells during treatment and washout phases. (A) Mature CFTR protein (band C, ∗) and immature CFTR protein (band B, •) visualized by Western blot analysis of HBE cultures derived from 3 CF (F508del/F508del) patients. Numbers at the top of the lanes represent days since treatment start. Actin is shown as a loading control. (B) Mature C band quantification, normalized to DMSO-treated control cells, shows increased amounts of mature CFTR protein in lumacaftor plus ivacaftor-treated cells and tezacaftor plus ivacaftor-treated cells during the treatment phase, which parallels CFTR function shown in Fig. 2. (C) Quantification of actin in lumacaftor plus ivacaftor-treated cells and tezacaftor plus ivacaftor-treated cells, normalized to DMSO-treated control cells, indicates a stable amount of loading.

of 14 days, with cells recovered for drug concentration measures, CFTR protein, and CFTR functional measures at 1, 7, and 14 days of treatment. A parallel set of cultured CF-HBEs were exposed to drug as above for 14 days, then drug was removed and cells were recovered at 1, 7, and 14 days into the washout period.

3.1. Intracellular CFTR modulator concentrations during treatment and washout phases

In cells treated with ivacaftor plus lumacaftor, measured intracellular ivacaftor concentrations increased continually during the two-week treatment period, with concentrations at 14 days >7-fold higher than on day 1 (Fig. 1a, Table 1, Iva (Luma/Iva)). In contrast, lumacaftor intracellular concentrations increased modestly, with a 1.5-fold increase over the treatment period (Fig. 1a, Table 1, Luma (Luma/Iva)). Similar findings were observed in the ivacaftor plus tezacaftor-treated cells, with substantial increases in ivacaftor during treatment (10 fold; Fig. 1b, Table 1, Iva (Teza/Iva)) but much more modest increases in tezacaftor (2.15 fold; Fig. 1b, Table 1, Teza (Teza/Iva)). During the washout period, both lumacaftor and tezacaftor concentrations decreased significantly within one day of washout, and were nearly undetectable by the end of the washout period (Fig. 1, Table 1). In contrast, in both treatment groups ivacaftor intracellular concentrations declined slowly during the washout phase and remained substantially above concentrations on treatment day 1 even after two weeks of washout (Fig. 1). No measurable drug was observed in vehicle (DMSO)-treated control cells.

3.2. Functional CFTR activity during treatment and washout phases

To assess CFTR functional activity, we performed Ussing chamber measurements on CF-HBE during treatment (days 1, 7, and 14) and washout (days 15, 21, and 28) phases. Peak forskolin responses,

Please cite this article as: T.N. Guhr Lee, D.M. Cholon and N.L. Quinney et al., Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2020.04.010

measured as changes in short-circuit currents (ΔI_{sc}), were elevated relative to DMSO-treated controls throughout the treatment period in both ivacaftor/lumacaftor- and ivacaftor/tezacaftor-treated cells (Fig. 2a). Interestingly, forskolin responses reached maximum peak at treatment day 7, and were significantly lower at treatment day 14 relative to day 7 (Fig. 2a), similar to results observed in previous studies [9,19]. During the washout phase, peak forskolin responses declined but remained above baseline even after 14 days of washout (Fig. 2a).

The forskolin-induced currents observed in the treatment phase were effectively inhibited by CFTR inhibitor-172 (CFTRinh172), consistent with functionally active CFTR protein (Fig. 2b). As with the forskolin responses, inhibition of CFTR channel activity peaked at day 7 of treatment in both lumacaftor and tezacaftor dual treated cells, but were reduced after 14 days of treatment (Fig. 2b). CFTRinh172 significantly inhibited forskolin-induced currents during the washout phase, but not consistently at all time points. Transepithelial resistance ($\Omega \bullet cm^2$) over the course of the 28-day treatment period remained high and did not significantly differ over time or among treatment or control groups (Fig. 2c), consistent with a lack of toxic effects.

3.3. CFTR protein maturation during treatment and washout phases

CFTR immunoblots were generally consistent with functional studies, showing increases in the mature, complex glycosylated C band throughout the treatment period in both treatment groups, and decreases during washout to a level consistent with the DMSO controls (Fig. 3a and b). Actin staining as a loading control was similar at all time points (Fig. 3a and c).

4. Conclusions

The introduction of CFTR modulator therapies represents a significant advancement in the treatment and management of CF. Ivacaftor is a pillar of all currently approved regimens, including the recently approved triple combination therapy, and understanding its pharmacokinetic properties could have substantial therapeutic implications. We observe extensive accumulation and persistence of ivacaftor in CF-HBEs exposed to concentrations similar to those observed in serum. Our findings are consistent with other *in vitro* and animal model studies that show high ivacaftor concentrations in lung and tracheal tissues following oral administration [8], as well as a recent study that demonstrated epithelial ivacaftor accumulation in patients with CF on modulator therapy [20].

Our findings have two important implications. First, they suggest the pharmacokinetic and pharmacodynamic properties of CFTR modulators in airway epithelia are complex and pose considerable challenges to optimizing dosing strategies. In particular, the propensity of ivacaftor to accumulate within airway epithelia suggests the possibility that dosing regimens based on serum pharmacokinetics may not accurately predict its pharmacodynamic effects. For example, it is possible that longer dosing intervals for ivacaftor may be sufficient to maintain pharmacologically relevant concentrations within airway epithelia, with the added benefit of minimizing systemic side effects. The concentrations we used in our treatment media are of similar magnitude to the concentrations that are reported in patient plasma. Furthermore, since ivacaftor has been shown to destabilize corrected F508del CFTR [9,19] as well as other non-gating mutations [21], accumulation within airway epithelia with frequent dosing may offset the positive impacts. While these effects, if present, do not prevent clinical benefit from CFTR modulator therapy with current dosing, they may be limiting these agents' potential.

The second implication relates to precision medicine. A substantial number of CFTR modulators are currently in preclinical or clinical trials, suggesting a future in which multiple potential therapies are available for individual patients, most of whom will already be on modulator therapy. Testing of biological cells or tissues from individual patients could be used to tailor the most effective drug combination. One attractive precision medicine approach is to use organoids from epithelial cell biopsies, which can be utilized without the need for expansion, reducing the turnaround time from weeks to days [22,23]. However, our data suggest that this strategy must be used cautiously in patients already on modulator therapy, since ivacaftor persists in epithelia much longer than its serum half-life would predict.

The mechanisms that underlie the intracellular accumulation of ivacaftor are not fully understood. Ivacaftor is a very lipophilic drug, and lipophilicity is known to contribute to tissue accumulation [24]. In contrast, lumacaftor [25] and tezacaftor [26] are less lipophilic, which may explain why they have less propensity to accumulate with time. However, we cannot rule out the possibility that other mechanisms such as specific transporters contribute to drug accumulation. We also cannot rule out the possibility that ivacaftor accumulation was influenced by co-treatment with a CFTR corrector, since our study focused on treatments for F508del and therefore did not include an ivacaftor monotherapy arm. Epithelial ivacaftor accumulation was observed with both ivacaftor monotherapy and combination therapy in a small clinical study [20], but may not have had the same deleterious effects since the G551D CFTR mutation in these patients is resistant to ivacaftor-mediated destabilization [9].

While our findings raise concerns about the pharmacokinetics of CFTR modulators, further investigation is needed to determine if the effects we observed *in vitro* also occur *in vivo*. Ivacaftor and other CFTR modulators are highly hydrophobic and protein bound, which may alter the effective concentrations to which airway epithelia are exposed *in vivo*. However, a recent clinical study [20] suggested that ivacaftor concentrations are significantly higher in nasal epithelia relative to serum in patients on CFTR modulator therapy, consistent with our findings. Of note, our culture systems do not effectively mimic systemic drug metabolism, which is primarily based in the liver. Further study in animal models and/or human subjects will be needed to understand and optimize CFTR modulator dosing.

In summary, our data suggest that CFTR modulators, particularly ivacaftor, accumulate substantially within airway epithelia and can persist for prolonged periods of time even in the absence of drug exposure. These findings have important implications for drug treatments and suggest that quantitation of concentrations of CFTR therapeutics in target tissues may be necessary to optimize dosing strategies. The persistence of ivacaftor will also need to be accounted for in personalized medicine strategies that test biopsy samples from patients already on modulator therapy.

Disclosure statement

The authors have no competing interests to declare.

Funding

CRE was supported by NIH P30-ES10126, P01-HL108808, and P30-DK065988. MG was supported by CFF BOUCHE15R0, GENTZS18P0 and NIH P30-DK065988. Cells were distributed by Dr. Scott Randell, supported by CFF BOUCHE15R0 and NIH P30-DK065988. Antibodies for CFTR were provided by Tim Jensen through the University of North Carolina and Cystic Fibrosis Foundation antibody distribution program, supported by GENTZS18XX0.

Please cite this article as: T.N. Guhr Lee, D.M. Cholon and N.L. Quinney et al., Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2020.04.010

CRediT authorship contribution statement

Tara N. Guhr Lee: Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Visualization. **Deborah M. Cholon:** Formal analysis, Investigation, Resources, Writing - review & editing, Visualization. **Nancy L. Quinney:** Methodology, Formal analysis, Investigation, Resources. **Martina Gentzsch:** Conceptualization, Writing - review & editing, Funding acquisition. **Charles R. Esther Jr:** Conceptualization, Methodology, Investigation, Writing - original draft, Supervision, Funding acquisition.

Acknowledgments

None.

References

[1] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, et al. Rescue of CF airway epithelial cell function *in vitro* by a CFTR potentiator, VX-770. Proc Natl Acad Sci USA 2009;106(44):18825–30. doi:10.1073/pnas.0904709106.

[2] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, et al. Correction of the F508del-CFTR protein processing defect *in vitro* by the investigational drug VX-809. Proc Natl Acad. Sci. USA 2011;108(46):18843–8. doi:10.1073/pnas.1105787108.

[3] Davies JC, Moskowitz SM, Brown C, Horsley A, Mall MA, McKone EF, et al. VX-659-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. N Engl J Med 2018;379(17):1599–611. doi:10.1056/NEJMoa1807119.

[4] Keating D, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, et al. VX-445-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. N Engl J Med 2018;379(17):1612–20. doi:10.1056/NEJMoa1807120.

[5] Middleton PG, Mall MA, Drevinek P, Lands LC, McKone EF, Polineni D, et al. Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single Phe508del allele. N Engl J Med 2019;381(19):1809–19. doi:10.1056/NEJMoa1908639.

[6] Taylor-Cousar JL, Mall MA, Ramsey BW, McKone EF, Tullis E, Marigowda G, et al. Clinical development of triple-combination CFTR modulators for cystic fibrosis patients with one or two F508del alleles. ERJ Open Res 2019;5(2):00082–2019. doi:10.1183/23120541.00082-2019.

[7] Lommatzsch ST, Taylor-Cousar JL. The combination of tezacaftor and ivacaftor in the treatment of patients with cystic fibrosis: clinical evidence and future prospects in cystic fibrosis therapy. Ther Adv Respir Dis 2019;13:1753466619844424. doi:10.1177/1753466619844424.

[8] (CHMP) CfMPfHU. Kalydeco: EPAR- Public Assessment Report. 2012 06/08/2012. Contract No.: EMA/473297/2012.

[9] Cholon DM, Quinney NL, Fulcher ML, Esther CR Jr, Das J, Dokholyan NV, et al. Potentiator ivacaftor abrogates pharmacological correction of DeltaF508 CFTR in cystic fibrosis. Sci Transl Med 2014;6(246):ra96. doi:10.1126/scitranslmed.3008680.

[10] McCague AF, Raraigh KS, Pellicore MJ, Davis-Marcisak EF, Evans TA, Han ST, et al. Correlating cystic fibrosis transmembrane conductance regulator function with clinical features to inform precision treatment of cystic fibrosis. Am. J. Respir. Crit. Care Med. 2019;199(9):1116–26. doi:10.1164/rccm.201901-0145OC.

[11] Taylor-Cousar JL, Munck A, McKone EF, van der Ent CK, Moeller A, Simard C, et al. Tezacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del. N Engl J Med 2017;377(21):2013–23. doi:10.1056/NEJMoa1709846.

[12] Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. N Engl J Med 2015;373(3):220–31. doi:10.1056/NEJMoa1409547.

[13] Fulcher ML, Randell SH. Human nasal and tracheo-bronchial respiratory epithelial cell culture. Methods Mol Biol 2013;945:109–21. doi:10.1007/978-1-62703-125-7_8.

[14] Hild M, Jaffe AB. Production of 3-D airway organoids from primary human airway basal cells and their use in high-throughput screening. Curr Protoc Stem Cell Biol 2016;1237:IE.9.1–IE.9.15. doi:10.1002/cpsc.1.

[15] Grubb BR, Gabriel SE, Mengos A, et al. SERCA pump inhibitors do not correct biosynthetic arrest of deltaF508 CFTR in cystic fibrosis. Am J Respir Cell Mol Biol 2006;34(3):355–63. doi:10.1165/rcmb.2005-0286OC.

[16] Thomas EJ, Gabriel SE, Makhlina M, Hardy SP, Lethem MI. Expression of nucleotide-regulated Cl(-) currents in CF and normal mouse tracheal epithelial cell lines. Am J Physiol Cell Physiol 2000;279(5):C1578–86. doi:10.1152/ajpcell.2000.279.5.C1578.

[17] Trimble A, McKinzie C, Terrell M, Stringer E, Esther CR. Measured fetal and neonatal exposure to Lumacaftor and Ivacaftor during pregnancy and while breastfeeding. J Cyst Fibros 2018;17(6):779–82. doi:10.1016/j.jcf.2018.05.009.

[18] Gentzsch M, Ren HY, Houck SA, Quinney NL, Cholon DM, Sopha P, et al. Restoration of R117H CFTR folding and function in human airway cells through combination treatment with VX-809 and VX-770. Am J Physiol Lung Cell Mol Physiol 2016;311(3):L550–9. doi:10.1152/ajplung.00186.2016.

[19] Veit G, Avramescu RG, Perdomo D, et al. Some gating potentiators, including VX-770, diminish ΔF508-CFTR functional expression. Sci Transl Med 2014;6(246):246ra97. doi:10.1126/scitranslmed.3008889.

[20] Guimbellot JS, Ryan KJ, Anderson JD, Liu Z, Kersh L, Esther CR, et al. Variable cellular ivacaftor concentrations in people with cystic fibrosis on modulator therapy. J Cyst Fibros 2020;20(S1569-1993):30035–7. doi:10.1016/j.jcf.2020.01.011.

[21] Avramescu RG, Kai Y, Xu H, Bidaud-Meynard A, Schnur A, Frenkiel S, et al. Mutation-specific downregulation of CFTR2 variants by gating potentiators. Hum Mol Genet 2017;26(24):4873–85. doi:10.1093/hmg/ddx367.

[22] Barkauskas CE, Chung MI, Fioret B, Gao X, Katsura H, Hogan BL. Lung organoids: current uses and future promise. Development 2017;144(6):986–97. doi:10.1242/dev.140103.

[23] Dekkers JF, van der Ent CK, Beekman JM. Novel opportunities for CFTR-targeting drug development using organoids. Rare Dis (Austin, Tex) 2013;1:e27112. doi:10.4161/rdis.27112.

[24] National Center for Biotechnology Information. PubChem Database. Ivacaftor, CID=16220172, https://pubchem.ncbi.nlm.nih.gov/compound/16220172 (accessed on February 14, 2020)

[25] National Center for Biotechnology Information. PubChem Database. Lumacaftor, CID=16678941, https://pubchem.ncbi.nlm.nih.gov/compound/16678941 (accessed on February 14, 2020)

[26] National Center for Biotechnology Information. PubChem Database. Tezacaftor, CID=46199646, https://pubchem.ncbi.nlm.nih.gov/compound/Tezacaftor (accessed on February 14, 2020)

Please cite this article as: T.N. Guhr Lee, D.M. Cholon and N.L. Quinney et al., Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment, Journal of Cystic Fibrosis, https://doi.org/10.1016/j.jcf.2020.04.010