The Pharmacokinetic Interaction between Ivacaftor and Ritonavir

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Declaration

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Summary

Ivacaftor is the first small molecule for the treatment of cystic fibrosis (CF) that directly targets the inherited defect in the cystic fibrosis transmembrane conductance regulator (CFTR). In phase III clinical trials ivacaftor showed a significant relative improvement in mean percentage predicted forced expiratory volume in 1 second ($ppFEV_1$) of 10.6% compared to placebo. There were also significant improvements in number of exacerbations, hospitalisations, weight and quality of life. The enthusiasm at the introduction of ivacaftor was tempered by its high cost—in Ireland, the cost of ivacaftor is almost €30 million per annum for approximately 130 patients.(1) There is also large variability in the clinical response to ivacaftor; 25% of patients in the phase III trial had an improvement in $ppFEV_1$ of 5% or less, some had no improvement at all. The drivers behind this variability in response were not discovered, but of note the relationship of variability in plasma levels of ivacaftor to response was not reported.(2) The aim of this study was to investigate the pharmacokinetic (PK) interaction between ivacaftor and ritonavir to explore the possibility of using ritonavir to boost plasma levels of ivacaftor in clinical practice. A liquid chromatography mass spectrometry (LC-MS) assay was developed to measure ivacaftor and its main active metabolite hydroxymethyl-ivacaftor (M1) in plasma. (3) A clinical trial to investigate the PK interaction between ivacaftor and ritonavir in vivo was completed with 12 healthy volunteers. This consisted of 3 single-dose studies of ivacaftor: ivacaftor 150mg alone (study A), ivacaftor 150mg with ritonavir 50mg daily (study B) and ivacaftor 150mg with ritonavir 50mg daily after two weeks of ritonavir treatment at 50mg daily (study C). The resulting PK profiles were analysed using non-compartmental analysis (NCA) to produce PK parameters for ivacaftor and M1 in each of studies A, B and C. Ivacaftor and M1 data were then fit to a compartmental PK model to allow dose simulation to ascertain the dose of ivacaftor with ritonavir that would be equivalent to standard dosing of ivacaftor alone. A budget-impact model was then constructed to estimate to potential cost saving of the implementation of ritonavir boosting of ivacaftor in clinical practice.

The ivacaftor and M1 assays met validation criteria. Inter-day and intra-day accuracy, as represented by mean bias, and precision, as represented by the coefficient of variation (CV), were all within 15%. Significantly higher exposure to ivacaftor was found when ivacaftor was administered with ritonavir in both studies B and C compared with ivacaftor alone in study A. Area under the concentration–time curve extrapolated to

infinity ($AUC_{0\text{-inf obv}}$) (95% CI) was 10.94 (8.259–14.48) µg.hr.mL^{-1} in study A compared to 215.6 (146.4–317.4) µg.hr.mL^{-1} in study B and 216 (165.5–281.8) µg.hr.mL^{-1} in study C. Maximum concentration (C_{max}) (GM [95% CI]) was 0.9944 (0.7819–1.265) µg in study A, 1.812 (1.323–2.482) µg in study B and 2.267 (1.863–2.757) in study C respectively. Elimination half-life ($T_{1/2}$) (GM [95% CI]) of ivacaftor in study A was 7.121 (5.59–9.07) hours compared to 79.24 (65.5–96.1) hours in study B and 65.99 (57.43–75.82) hours in study C. $AUC_{0–12}$ of M1 was significantly decreased in study B and in study C in comparison to study A. $AUC_{0–12}$ (GM 95% CI) was 11.77 (8.620 – 16.07) in study A, 2.961 (2.014 – 4.355) in study B and 0.6999 (0.4187 – 1.170) in study C. Ivacaftor data was found to fit best a one-compartment model with lag time absorption. Dosing simulation using this model showed that exposure to ivacaftor with 75mg twice weekly or 50mg three times weekly dosing with ritonavir was not reduced in comparison to ivacaftor 150mg alone twice daily. Median $AUC_{504\text{-}672}$ was 133.24 µg.hr.mL^{-1} for ivacaftor alone compared to 196.99 µg.hr.mL^{-1} for ivacaftor 75mg twice weekly with ritonavir 50mg daily and 293.96 µg.hr.mL^{-1} for ivacaftor 50mg three times weekly with ritonavir 50mg daily. Budget-impact modelling showed that the base case—uptake of ivacaftor plus ritonavir in 25% of adult population on ivacaftor in year 1 growing to 75% by year 5—resulted in a 5-year budget saving of €49,021,152. Scenario analyses resulted in a range of savings from €1,001,953 should only 1 patient take up ivacaftor plus ritonavir dosing to €176,303,641 should all patients on ivacaftor be switched to ivacaftor plus ritonavir over 5 years. As we move into an era of ultra-high-cost drugs for orphan diseases, we need innovative solutions to overcome the challenges of providing healthcare that is both equitable and at an acceptable opportunity cost. The potential benefits of using a CYP3A4 inhibitor in the CF setting warrants further exploration given the proof of the concept that ivacaftor plasma levels can be significantly increased with the use of a small dose of ritonavir.

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Abbreviations

ABC	ATP binding cassette
ABPA	Allergic bronchopulmonary aspergillosis
ACN	Acetonitrile
ACN:H_2O	Acetonitrile water
ADME	Absorption distribution metabolism excretion
AEs	Adverse events
AGP	α-1 acid glycoprotein
AIC	Akaike's information criterion
AR	Adverse reaction
AUC	Area under the plasma concentration–time curve
$AUC_{0\text{-inf obv}}$	Area under the time–concentration curve from time zero to infinity
$AUC_{0–12}$	Area under the time–concentration curve from time zero to 12 hours
$AUC_{504–672}$	Area under the time–concentration curve from time 504 to 672 hours equalling 1 week at steady state
BCRP	Breast cancer resistance protein
BCS	Biopharmaceutics classification system
BD	Twice daily
CaCC	Ca^{2+} activated chloride conductance
CBA	Cost benefit analysis
CEA	Cost-effectiveness analysis
CF	Cystic Fibrosis

CFI	Cystic fibrosis Ireland
CFQ-R	Cystic fibrosis questionnaire – revised
CFTR	Cystic fibrosis transmembrane conductance regulator
CI	Confidence interval
Cl^-	Chloride
CMA	Cost-minimisation analysis
C_{max}	Maximum concentration
COSS	Community Ophthalmic Services Scheme
CrCl	Creatinine Clearance
CRF	Case Report Form
CT	Computed tomography
C_{trough}	Minimum concentration
CUA	Cost-utility analysis
CV	Coefficient of variation
CYP	Cytochrome p-450
DALY	Disability adjusted life year
DMPA	Depot medroxyprogesterone acetate
DMSO	Dimethyl sulphoxide
DNDi	Drugs for Neglected Diseases Initiative
DoH	Department of Health
DPS	Drugs Payment Scheme
DSMB	Data safety monitoring board
DTSS	Dental Treatment Services Scheme
EC_{90}	90% effective concentration
ECG	Electrocardiogram

EEA	European Economic Area
EMA	European Medicines Agency
ENaC	Amiloride sensitive epithelial channel
ER	Endoplasmic reticulum
FDA	Food and Drug Administration
FMO	Flavin-containing monooxygenase
GCP	Good Clinical Practice
GMR	Geometric mean ratio
GMS	General medical services
GPVC	General Practitioner Visit Card
HCO^-	Bicarbonate
HDL	High-density lipoprotein
HIQA	Health information and quality authority
HIV	Human Immunodeficiency Virus
HPRA	Health Products Regulatory Agency
HSA	Human serum albumin
HSE	Health Services Executive
HT	High Tech Arrangements
HTA	Health Technology Assessment
HYE	Healthy years equivalent
ICH	International Conference on Harmonization
IPHA	Irish Pharmaceutical and Healthcare Association
IQR	Interquartile range
IS	Internal standard
IV	Intravenous

LCI	Lung clearance index
LC-MS	Liquid chromatography mass spectrometry
LDL	Low density lipoprotein
LFT	Liver function tests
LLOQ	Lower Limit of Quantification
LTI	Long Term Illness Scheme
LYG	Life years gained
M1	Hydroxymethyl-ivacaftor
M6	Ivacaftor carboxylate
MATE1	Multiantimicrobial extrusion protein 1
MATE2-K	Multiantimicrobial extrusion protein 2-K
MI	Metabolic intermediate
MSD	Membrane spanning domain
MT	Monday Thursday
MWF	Monday Wednesday Friday
MWFS	Monday Wednesday Friday Sunday
m/z	Mass to charge ratio
NBD	Nucleotide-binding domain
NCA	Non-compartmental pharmacokinetic analysis
NCPE	National Centre for Pharmacoeconomics
NHE3	Renal outer medullar K^+ channel, the sodium/proton exchanger
OAT1	Organic acid transporter 1
OAT3	Organic acid transporter 3
OATP1B1	Organic anion transporting polypeptide 1B1
OATP1B3	Organic anion transporting polypeptide 1B3

OCT2	Organic cation transporter 2
OD	Once daily
ODMS	Oncology drugs management system
OPAT	Outpatient Parenteral Antimicrobial Therapy
ORCC	Outwardly rectifying chloride channel
PCRS	Primary Care Reimbursement Service
PD	Pharmacodynamics
P-gP	P-glycoprotein
PI	Protease Inhibitor
PK	Pharmacokinetic
$ppFEV_1$	Percentage predicted forced expiratory volume in 1 second
PWCF	Persons with cystic fibrosis
PXR	Pregnane X receptor
QALY	Quality adjusted life year
RPM	Revolutions per minute
SAE	Serious adverse event
SC	Schwartz criterion
SD	Standard deviation
SPC	Summary of product characteristics
SpR	Specialist registrar
SSR	Sum of the square of the residuals
SUSAR	Suspected unexpected serious adverse reactions
$T_{1/2}$	Elimination half life
TBME	methyl tert-butyl ether
TDM	Therapeutic Drug Monitoring

TDS	Three times daily
T_{max}	Maximum concentration
ULN	Upper limit of normal
Vd	Volume of distribution
V_d/F	Volume of distribution/fraction of drug absorbed
WWCF	Women with cystic fibrosis

CHAPTER 1

1 Introduction

In 2012, ivacaftor became the first disease-modifying agent available for the treatment of CF, a congenital condition linked to a number of distinct genetic mutations that lead to either qualitative or quantitative defects in the CFTR, a chloride channel on the surface of cells that secrete mucus and sweat.(4) In Ireland there are currently over 1,100 patients with the condition.(5) A defect in the CFTR leads to the production of thick, viscous secretions that precipitate repeated infections in the respiratory tract, damage to the pancreas and poor absorption of nutrients, ultimately resulting in a much-shortened life span—the median age at death for patients with CF in Ireland was 30.5 years in 2015.(5) Ivacaftor is a small molecule that binds to the CFTR with the G551D mutation—a mutation that results in the transcription of a CFTR that is present at the cell surface but has an open probability that is approximately 100 times less than that of the normal channel—and increases the open probability of the channel, thus partially normalising the movement of ions and water across the cell membrane and decreasing the viscosity of secretions.(3) The G551D mutation is associated with a severe phenotype of CF and is present in approximately 11% of persons with CF (PWCF) in Ireland.(5) The results of the pivotal trial of ivacaftor in patients 12 years or older with the G551D mutation, the STRIVE study, reported a striking mean 10.4% increase in the primary endpoint, percentage predicted forced expiratory volume in one second ($ppFEV_1$), at 24 weeks in the treatment group; a relative increase compared to standard treatment of 10.6%. This improvement was sustained throughout the total 48 weeks of the trial. Several other improvements were demonstrated with ivacaftor therapy such as increased weight, decreased pulmonary exacerbations and improved quality of life.(6)

The enthusiastic reception among the CF community to the development of ivacaftor has been tempered by the enormous cost of the drug in clinical practice.(7) In Ireland, ivacaftor costs approximately €360 per tablet and the current recommended dosing schedule requires lifelong treatment in those over 6 years of age for whom it is indicated at a dose of one tablet twice daily. The drug acquisition cost to treat one patient with ivacaftor is €234,804 per annum, making it one of the most expensive drugs reimbursed in Ireland at present.(1) Across the globe, the extremely high cost of ivacaftor has far-reaching effects whether the drug is provided through socialised healthcare or through private health insurance as, ultimately, the cost is borne either by all tax payers or by those who can afford to invest in health insurance.(8) Thus, as is the case with many new high-cost treatments, in addition to the many PWCF who are affected by the cost barrier to the acquisition of ivacaftor, a larger population is affected by the opportunity cost of providing treatments that may be clinically effective but are not cost effective. Any solutions that may be available to reduce the burden of such high-cost treatments therefore not only benefit the patients in need of these treatments, but also have the potential to improve healthcare on a broader scale.

The metabolic pathways for ivacaftor have been elucidated; the drug is primarily metabolised by the cytochrome p450 (CYP) 3A4 enzyme system in the liver.(9) Inhibition of the CYP system to 'boost' plasma levels of drugs metabolized by these enzymes has been used in other disease areas for decades, primarily with the use of ritonavir as a CYP inhibitor to increase plasma levels of other protease inhibitors (PIs) in the treatment of HIV. Use of ritonavir in this way led to both simplified dosing regimens and improved clinical effectiveness of PIs, which had previously showed highly variable clinical effectiveness due to large interindividual variability in plasma concentrations.(10, 11) While ivacaftor was shown in the STRIVE study to result in significant improvements in

many aspects of the CF disease process for a sizable proportion of patients, it is noteworthy that an improvement in $ppFEV_1$ of 5% or more (deemed by the sponsor to be clinically significant) was seen in only 75% of the treatment group in the trial. It was not clear from a subgroup analysis why 25% of patients did not have this improvement. The numbers in the subgroup analysis were small and thus the drivers behind this variation were unclear, but notably it was not reported if variability in drug levels contributed to the difference in clinical outcome.(6) The inherent variability in pharmacokinetic parameters of ivacaftor that is expected given its metabolism by CYP3A4 is seen in the large standard deviations of the major pharmacokinetic parameters reported in the Summary of Product Characteristics (SPC) for ivacaftor—after oral administration of a single 150mg dose to healthy volunteers in a fed state, the mean (±SD) for area under the concentration–time curve (AUC) and C_{max} were 10.6 (5.26) µg*hr/mL and 0.768 (0.233) µg/mL, respectively.(12)

Given these issues with the clinical and cost effectiveness of ivacaftor treatment, this study will investigate the pharmacokinetic interaction between ivacaftor and ritonavir *in vivo* in healthy volunteers to establish whether a CYP inhibitor can be used to alter the metabolism of ivacaftor in such a way that will allow for a longer dosing interval of the drug while at the same time as increasing plasma ivacaftor levels.

1.1 Aims and Objectives

The aim of this thesis is to investigate the PK interaction between ivacaftor and ritonavir in vivo.

The objectives are as follows:

- Develop an LC-MS assay to measure ivacaftor and M1 in plasma

- Design and execute a clinical trial in healthy volunteers to explore the interaction between ritonavir and ivacaftor in vivo
- Use this healthy volunteer, single-dose PK interaction data to build a predictive pharmacokinetic model to explore potential dosing regimens for the combination of ivacaftor plus ritonavir
- Assess the budget impact of implementing ivacaftor boosting with ritonavir in clinical practice.

1.2 Overview of thesis

Chapter 2 provides background on the main themes explored in this thesis: CF, ivacaftor and ritonavir pharmacology and the principles of PK and pharmacoeconomic analysis. Chapter 3 outlines the validation of the LC-MS assay for the measurement of ivacaftor in plasma. Chapter 4 describes the design of the clinical trial in healthy volunteers to investigate the PK interaction between ivacaftor and ritonavir in healthy volunteers along with the outcomes of this trial. Chapter 5 explores these interaction data in more detail, applying a compartmental pharmacokinetic model and using this to simulate multi-dose PK profiles from the single-dose data from chapter 4. Chapter 6 describes the construction of a budget impact model to assess the budgetary implications of adopting the proposed ritonavir-boosted dosing of ivacaftor shown in chapter 5. Finally, chapter 7 briefly outlines the broader context of this work and the future directions that may be considered on foot of the findings herewith.

1.3 Conclusion

The introduction of targeted small molecules for the treatment of CF is welcome progress in the treatment of this complex and devastating condition. However, provision

of such high-cost therapies brings with it new, broad-reaching challenges. The budget impact of ivacaftor not only affects the limited resources available for other essential aspects of care for PWCF, the growing portion of the health budget demanded by these new, high-cost therapies erodes the resources available for healthcare for the population as a whole.(13) Investigating the pharmacokinetic features of ivacaftor and the possibility of using drug interactions to improve its clinical and cost effectiveness therefore has implications well beyond the real potential benefits for PWCF. The information gleaned by this study will be of interest to PWCF, clinicians in any field of practice and policy makers alike.

CHAPTER 2

2 Background

2.1 Cystic Fibrosis

CF is an inherited, multi-system disorder caused by either qualitative or quantitative defects in the CFTR, a chloride channel on the surface of cells that secrete mucus and sweat. These mutations lead to the production of thick, viscous secretions which, over time, cause tissue damage, particularly to the respiratory and gastrointestinal tract. This damage leads to a poor quality of life with repeated hospitalisations for respiratory infections, chronic dysfunction of the gastrointestinal tract and, ultimately, a much-shortened life span.(14)

2.1.1 Epidemiology

There are currently over 1,100 PWCF in Ireland. New-born screening was instituted in Ireland in 2011 and each year around 40 new diagnoses are made. There are six groups of genetic mutations that lead to the clinical phenotype of CF (discussed below). The most common mutation in the Irish population is the ΔF508 mutation, which results in a CFTR that misfolds and is destroyed before reaching the cell surface; 76% of PWCF in Ireland in 2015 had at least 1 copy of this mutation. The next most common mutation is G551D which results in a CFTR that reaches the cell surface but has a much-reduced open probability in comparison to the wildtype channel. In 2015 8% of PWCF in Ireland had a least one copy of this mutation.(15) This is higher than the prevalence in other countries, for example the prevalence of G551D mutations is 3–4% in the US. (16)

The median age of death for PWCF was 30.5 years in 2015. Around one third of PWCF were admitted to hospital at least once over the course of 2015 with a mean duration of stay in hospital of 21 days in children and 31 days in adults. Twenty-two persons were on

or added to the Irish lung-transplant list and there were no deaths of recipients of double-lung transplants between 2010 and 2015.(12)

2.1.2 Clinical Presentation

In Ireland at present, most new cases of CF are diagnosed at neonatal screening.(5) PWCF who were born prior to neonatal screening are still identified based on the typical clinical presentation. Failure to thrive (33.3%) and respiratory symptoms (46.7%) were the most common presenting complaints that lead to a diagnosis of CF in patients aged 10 or over (that is, patients born before neonatal screening). Other presenting symptoms included meconium ileus, rectal prolapse, sinus disease/nasal polyps, malnutrition and family history.(5)

2.1.3 Complications

The main organ systems affected by CF are the respiratory and gastrointestinal/hepatobiliary/pancreas systems. Most patients ultimately die from the respiratory complications of the disease. The most common complications in adults in 2015 were sinus disease, CF-related diabetes and gastrointestinal reflux disease.(5)

2.1.4 Treatment

Until the introduction of ivacaftor in 2011 all treatments for CF aimed to reduce the downstream effects of the production of abnormal secretions; no treatment could correct the underlying defect in the CFTR. Ivacaftor was the first available treatment to correct the underlying defect in the CFTR and to partially normalise secretions.(6) Ivacaftor did not replace the standard treatment for CF but was added to the established standard of care. Standard treatment for CF ideally consists of management by a multi-

disciplinary team including a CF physician, nurse specialist, physiotherapist, dietician, social worker and psychologist. There are no recent figures available regarding the cost of the provision of healthcare for PWCF in Ireland, but data from a UK cohort suggests that the cost of direct healthcare for a PWCF is €20,854 per annum.(17) Notably, this figure was based on data that predated the introduction of small molecules for the treatment of CF and consideration of this cost could be expected to substantially increase that figure.

2.1.4.1 Treatment of lung disease

Treatment of lung disease involves the use of antibiotics, in the acute and chronic setting, agents to promote airway clearance, anti-inflammatory agents, vaccinations, supplementary oxygen, ventilatory support and lung transplantation.

Oral azithromycin is recommended for all PWCF over 6 years of age with pseudomonas aeruginosa persistently present in the airways. Inhaled tobramycin, aztreonam and colistin are also indicated for the treatment of pseudomonas colonisation. Inhaled dornase alpha and inhaled hypertonic saline both promote the clearance of airway secretions and both confer a moderate to substantial benefit for PWCF, reducing the risk of exacerbations and improving lung function and quality of life for those over 6 years of age.

Chronic use of ibuprofen is recommended for those between 6 and 17 years of age to slow the loss of lung function.(18) Routine vaccination with the annual influenza vaccine and 5-yearly pneumococcal vaccination is recommended. Definitive data do not exist that show a survival benefit with the use of supplementary oxygen once significant hypoxemia is present, but supplementary oxygen is recommended given the benefits proven in other chronic respiratory conditions such as emphysema and chronic

bronchitis once hypoxia is well established. Lung transplantation is indicated when overall predicted survival has fallen below 30%. The median survival post transplantation is 8.5 years.(19)

2.2 Ivacaftor

The latest agents for the treatment of CF are the small molecules directed at the defect in the CFTR itself, the first of which was ivacaftor, introduced to the treatment of CF in 2011.

A discussion of the pharmacological properties of ivacaftor first requires a review of the features of the PK of xenobiotics in humans.

2.2.1 Overview of the Pharmacokinetics of Xenobiotics in Humans

PK is the study of the rates of change of xenobiotics in humans. Xenobiotics are defined as any substance that is foreign to the body. The change refers to the processes involved in changing the *location* of a xenobiotic (absorption, distribution and elimination) and in changing its *chemical structure* (metabolism). The physiological systems present in humans that manage xenobiotics evolved long before the advent of pharmaceuticals; these systems were predominantly involved in the management of xenobiotics absorbed via the gastrointestinal tract in food. The evolutionary struggles between those who ate and those who were eaten have resulted in a complex physiological system that in the 21st century processes man-made pharmaceuticals, many of which are plant and microorganism based.(20)

2.2.1.1 Absorption

Absorption is the process by which a drug proceeds from the site of administration to the site of measurement. Absorption can take place through varied routes such as the gastrointestinal tract, skin and respiratory tract (note there is no absorption step with intravascular administration of a drug). There are evolved systems in place to reduce the potential toxicity of xenobiotics absorbed through these routes. Primarily, in the gastrointestinal tract the intestinal epithelial cells form a functional as well as a physical barrier to foreign compounds. Efflux transporters such a P-glycoprotein (P-gP) limit the absorption of substrates. Transporters also exist that aid the absorption of certain molecules that do not readily cross the membrane. Drugs vary widely in their absorption characteristics depending on their molecular structure, solubility and interaction with cellular transporters in vivo.(21, 22) The absorption of a drug is characterised by its bioavailability, that is the fraction of the drug that reaches the systemic circulation unchanged. Bioavailability is one of the features of a drug that will influence the choice of route of administration, for example a drug with low oral bioavailability may be administered intravenously to ensure adequate systemic exposure.(23)

The Biopharmaceutics Classification System (BCS) exists to describe the oral bioavailability of drugs. It is based on two key parameters: solubility (a drug must be in soluble form before it can be absorbed in the GI tract) and permeability. Class I drugs have highly permeability and high solubility, class II drugs high permeability and low solubility, class III low permeability and high solubility and class IV both low permeability and solubility. Class IV drugs will particularly demonstrate variable absorption after oral doses and are a biopharmaceutical challenge to those developing oral formulations of these drugs.(24)

2.2.1.2 Distribution

Distribution is the process of reversible transfer of drug to and from the site of measurement (usually the plasma). Drugs demonstrate different patterns of distribution: some stay in the vascular compartment tightly bound to plasma proteins; some distribute throughout the body water; some specifically concentrate in a certain organ or tissue (for example fat). The factors that affect drug distribution include the molecular size of the drug, its lipophilicity, drug transporters, organ perfusion, plasma binding and individual body composition.(22)

2.2.1.3 Metabolism

Broadly speaking, drug metabolising enzyme systems transform lipophilic chemicals, which cross cell membranes easily and accumulate in tissues, into hydrophilic species to facilitate their removal from the circulation. In chemically transforming these xenobiotics to facilitate their elimination they are also often rendered biologically inactive. These generalisations do not always apply—certain chemical species are excreted as lipophilic compounds which are actively transported out of the circulation, and some chemical alterations to xenobiotics render them more active or more toxic or carcinogenic than their parent compound—however, in the main, these enzyme systems facilitate inactivation and excretion of xenobiotics.(22)

There are two main phases of drug metabolism: phase I consisting of oxidation, reduction or hydrolysis and phase II synthetic reactions consisting of conjugation of moieties such as glutathione or glucuronide to the products of phase I metabolism. Some species proceed directly to phase II metabolism depending on the functional groups present on the parent compound and how well they facilitate conjugation. Xenobiotic metabolism predominantly takes place in the GI tract. Xenobiotic metabolising enzymes

are present in the small and large intestine and to a greater extent in the liver. These enzymes exist in smaller quantities also in the respiratory tract (presumably to deal with inhaled chemical species), as well as in the kidneys and the brain.(22)

On the cellular level, phase I metabolism takes place in enzymes that are located primarily on the endoplasmic reticulum (ER). Lipophilic species readily cross the membrane of the ER and come into contact with the enzymes embedded in this membrane, namely the CYP and Flavin-containing monooxygenase (FMO) superfamilies and microsomal epoxide hydrolase (the other form, soluble epoxide hydrolase is expressed in the cytosol). Phase II metabolism predominantly takes place in the cytosol, except glucuronidation which takes place on the luminal surface of the ER.(25)

The metabolic pathway of ivacaftor demonstrates phase I and phase II metabolism of the parent compound into 11 distinct metabolites. As an example, the parent compound is converted to the M1 metabolite (with ⅙ of the pharmacological activity of the parent compound) by oxidation (phase I metabolism) and this metabolite is in turn converted to M1-sulphate by phase II sulfation.(2)

2.2.1.3.1 The *CYP* superfamily enzyme system

This enzyme system takes its name from the wavelength of maximal absorption of these membrane-bound haem proteins which is around 450nm.(26) Only 3 families of *CYP* enzymes are responsible for the metabolism of most xenobiotics, although over 50 individual CYP enzymes have been identified in humans (see table 1).

P450 sequences that demonstrate more than 40% homology are considered within the same family, those with greater than 55% homology within the same subfamily. Greater than 97% homology is considered allelic variation.(26) The enzymes are named based on

Table 1. CYP enzyme families responsible for the metabolism of most xenobiotics.

Families of CYP enzyme responsible for the metabolism of most xenobiotics (*Subject to clinically significant polymorphisms)
CYP 1A1
CYP1A2
CYP 1B1
*CYP 2A6**
CYP 2B6
CYP 2C8
*CYP 2C9**
*CYP 2C19**
*CYP 2D6**
CYP 2E1
CYP 3A4
CYP 3A5

CYP, cytochrome p450. Adapted from(25)

the family, subfamily and gene number; for example, *CYP* family 3, subfamily A and gene 4 is *CYP3A4*, the enzyme responsible for the metabolism of about 50% of all xenobiotics. These enzymes are involved in N-dealkylation, O-dealkylation, aromatic hydroxylation, N-oxidation, S-oxidation, deamination and dehalogenation reactions. *CYP*s consist of a peptide with bound heme and require 0_2 and NADPH to carry out the above reactions.(25)

2.2.1.4 Inhibition and induction of the enzymes in the CYP superfamily

The mechanism of inhibition of CYP450 enzymes can be broadly divided into three categories: reversible inhibition, quasi-irreversible inhibition and irreversible inhibition. Reversible inhibition is probably the most common mechanism and it is a result of a compounds competing for occupancy of the active site of an enzyme. The strength of the competitive inhibition of a compound is dictated by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron in the active site of the enzyme and also by the lipophilicity of the compound and its ability to bind hydrophobic regions

of the enzyme. Ketoconazole contains a nitrogen lone electron pair that binds to the haem iron in the CYP3A4 enzyme and is also strongly lipophilic; therefore, ketoconazole is a potent competitive inhibitor of CYP3A4. Quasi-irreversible inhibition involves the production of a metabolic intermediate (MI) that binds to the active site of the enzyme. Some MIs can be displaced from the active site by highly lipophilic molecules in vitro, but this does not appear to happen as readily in vivo, hence the term 'quasi' irreversible. Macrolide antibiotics are an example of such an inhibitor. Irreversible inhibition of CYP enzymes is through oxidization of substrates to reactive products that irreversibly alter the active site of the enzyme, often through complexation with the heme group. Because these inhibitors require oxidization before inhibition occurs they are also known as mechanism-based inhibitors or suicide inhibitors. Chloramphenicol is an example of a purely mechanism-based inhibitor. (27)

Many CYP enzymes are subject to induction by certain xenobiotics. The main mechanism by which this occurs is through activation of transcription factors, leading to increased synthesis of enzyme. The main receptors that bind xenobiotics and lead to enzyme induction are the aryl hydrocarbon receptor, the constitutive androstane receptor, both in the cytosol, and the pregnane X receptor (PXR) located in the nucleus. Activation of these receptors leads to heterodimerisation in the nucleus with other factors such as such as the retinoid X receptor, and these heterodimers bind to the xenobiotic response elements located on the promotor regions of the various CYP enzymes ultimately leading to transcription of CYP enzymes. Most enzyme induction is mediated by this mechanism, though in rarer cases induction is through another mechanism, such as the enzyme stabilisation that leads to enzyme induction with isoniazid.(28)

2.2.1.5 Elimination

Elimination is the irreversible loss of drug from the site of measurement. This can be through metabolic processes as described above or through excretion of the parent compound by the kidneys, liver or to a lesser extent through the lungs, breast milk and sweat. Metabolites produced by the chemical processes above are excreted through these processes also.(22)

2.2.1.5.1 Elimination by the kidney

Elimination by the kidney is an important route by which many hydrophilic drugs are removed from the body. Drugs can move from the blood into the urine through filtration and by active secretion by the tubules. Some drugs are also actively transported back from the urine into the plasma in the tubules. Kidney function, protein binding of drug, urinary pH and urine flow will affect the rate at which a drug is eliminated through the kidney by any mechanism.(25) Inhibition of active transport of drugs into the urine from plasma was used early in the development of modern pharmacology in 1951 when probenecid was used to slow the elimination of penicillin to maintain adequate plasma concentrations of the drug for wounded soldiers during world war II.(29)

2.2.1.5.2 Elimination by the liver

As well as the metabolic processes described above, the liver contributes to the elimination of drug through biliary excretion. About 1L of bile per day is drained into the GI tract. Drugs and their metabolites are eliminated through the bile only to the extent to which they are not resorbed back into the hepatic circulation from the GI tract in a process called enterohepatic recycling. Drugs excreted in bile tend to be larger molecules (molecule weight greater than 500-600) and can be cations, anions or non-ionized

molecules. Conjugated molecules can be deconjugated when emptied into the lumen of the intestine by gut bacteria and therefore reabsorbed in their original form, requiring repeated rounds of metabolism and excretion to eliminate them completely through the faeces.(23)

2.2.2 Non-compartmental pharmacokinetic parameters

The behaviour of a drug as it journeys through the above process can be described by a few key PK parameters. Some of these parameters are descriptive and some require calculation based on concentration–time data collected in a PK profile.

2.2.2.1 Descriptive pharmacokinetic parameters

2.2.2.1.1 Maximum concentration

The maximum concentration measured in an individual PK profile. Note that it is not necessarily the maximum concentration reached, it is the maximum concentration measured.(22)

2.2.2.1.2 Minimum concentration

The minimum concentration measured.(22)

2.2.2.1.3 Time to maximum concentration

The time taken to reach the maximum concentration measured.(22)

2.2.2.2 Calculated pharmacokinetic parameters

The two most important calculated parameters are the V_d and the clearance.

2.2.2.2.1 Volume of distribution

The apparent V_d is the *theoretical* volume that would be necessary to contain the total amount of an administered drug at the same concentration that it is observed in the plasma (it is not a physiological volume as such). It is calculated as follows:

$$V_d = \frac{Amount\ of\ drug\ in\ the\ body}{C}$$

where V_d is the volume of distribution and C is the concentration in the blood or plasma. The V_d can far exceed the actual total body water as when a drug is distributed it is not necessarily homogenously so—concentrations in the fat compartment for instance can be many times higher than that measured in the plasma compartment. The V_d has a profound effect on the rate at which a drug can be eliminated from the circulation as most elimination takes place for drug that is present in the plasma, therefore only the fraction of the drug that is in the plasma is available for elimination and excretion.(22)

2.2.2.2.2 Clearance

Systemic clearance refers to the volume of blood or plasma cleared of a compound per unit time. The clearance of a drug administered intravenously (IV) can be calculated as follows:

$$CL_s \frac{Dose^{IV}}{AUC_{0-\infty}^{IV}}$$

where $AUC_{0-\infty}^{IV}$ is the area under the time–concentration curve from zero to infinity after an IV dose of drug.

Clearance can also be calculated for an oral dose of a drug if the bioavailability is known as follows:

$$\frac{CL_S}{F^{po}} = \frac{Dose^{po}}{AUC_{0-\infty}^{po}}$$

Total systemic clearance is the sum of the clearance from the various organ systems. Renal clearance, hepatic clearance, and any other clearance can be calculated separately and summed to give the overall clearance.(21)

It is noteworthy that these calculations apply when clearance is constant over a concentration range. If clearance is saturable the rate of elimination is proportional to the maximum elimination capacity, and at concentrations close to or above the maximum elimination capacity steady state is never reached—as long as dosing continues, concentrations increases. Most drugs used in clinical practice exhibit first order elimination however, with the notable exceptions of phenytoin, ethanol and aspirin.(30)

2.2.2.2.3 Elimination half life

$T_{1/2}$ is the time required for the plasma concentration of a drug to be reduced by half after the point at which pseudo-equilibrium of distribution has been reached. It can be calculated as follows:

$$T_{1/2} = \frac{0.693}{\lambda_z}$$

where λ_z is the slope of the terminal phase. $T_{1/2}$ is a hybrid parameter that depends on both the clearance and the V_d of a particular drug. $T_{1/2}$ can also be expressed with the following equation:

$$T_{1/2} \frac{0.693\ x\ volume\ of\ distribution}{plasma\ clearance}$$

Plasma $T_{1/2}$ is most useful for determining rational dosing of a drug, as the accumulation after multiple doses is a function of the plasma terminal half-life.(31)

2.2.2.2.4 Area under the time–concentration curve

The AUC can be used as a parameter to express the exposure of tissues to a drug of interest. It is dependent on the amount of drug that enters the system (bioavailability) and the clearance. AUC can be calculated in a defined timeframe, for example $AUC_{0\text{-}t}$, or it can be extrapolated to infinity $AUC_{0\text{-}\infty}$. Using NCA, AUC is calculated using the linear or the log trapezoidal rule.(32)

2.2.3 Ivacaftor Pharmacokinetics

The PK of ivacaftor were studied in 17 sub-studies done as part of the phase I clinical trials of ivacaftor, sponsored by Vertex Pharmaceuticals®. These were conducted in healthy volunteers, PWCF and those with hepatic and renal impairment. The two primary studies conducted to explore absorption, distribution, metabolism and excretion (ADME) of ivacaftor were VX05-770-001 and VX05-770-003. VX05-770-001 was a 7-panel, double-blind, placebo-controlled dose escalation randomised study in male and female healthy volunteers and PWCF. VX05-770-003 was an open-label, non-randomised, single-dose, mass balance study which investigated the ADME of ivacaftor and was conducted in healthy male volunteers.(9)

2.2.3.1 Absorption

Ivacaftor is almost completely insoluble in water (<0.05µg/mL) and therefore no intravenous formulation exists to assess the absolute bioavailability of ivacaftor in humans. In-vitro studies with the Caco-2 assay showed high apparent permeability of

ivacaftor which may suggest that human intestinal absorption is high. Bioavailability in mice, rats, rabbits and dogs of the aqueous suspension of the amorphous form ranged from 30-100%.(9)

According to the SPC for ivacaftor, after one oral 150mg dose in the fed state the mean (±SD) C_{max} is 0.768µg/mL (±0.233), mean AUC (±SD) is 10.6 (±5.26) µg.hr/L and median (range) T_{max} is 4 (3–6) hrs. Absorption of ivacaftor is increased by approximately 2.5 times when it is administered with a high fat meal in comparison to the fasting state. T_{max} is also delayed by 3–5 hours. It is recommended in the SPC for patients to take ivacaftor with a high fat meal.(2)

2.2.3.2 Distribution

Ivacaftor is highly bound to plasma proteins (>98%). At the standard dose of 150mg every 12 hours for 7 days, the volume of distribution (V_d) (±SD) of ivacaftor was 353 (±122) L. This large V_d suggests good tissue penetration. Results from a mass balance study shows that radioactivity in plasma was significantly higher than that in blood, suggesting that ivacaftor does not bind to red blood cells.(9)

2.2.3.3 Metabolism

The primary route of drug elimination is by hepatic metabolism, with only 2.52% of the drug eliminated unchanged, primarily in the faeces (87.8%). The two major metabolites of ivacaftor are M1 and ivacaftor carboxylate (M6), together accounting for approximately 65% total dose eliminated. These metabolites are produced by the CYP enzyme system, specifically through oxidation by CYP3A4 and CYP3A5. Glucuronidation and sulfation also play a minor role in the hepatic metabolism of ivacaftor. There is no evidence of enterohepatic circulation at oral doses up to

200mg. M1 has one sixth of the potency of the parent compound while M6 has just one fiftieth of the activity of the parent compound and is considered pharmacologically inactive. (9, 33)

2.2.3.4 Elimination

The $T_{1/2}$ of ivacaftor is estimated to be 12 hours following a single oral dose in the fed state.(34) Five single-dose studies in healthy volunteers reported in the submission to the Office of New Drug Quality in the US showed a $T_{1/2}$ (SD) of 13.3 (2.7) hrs, 11.7 (3.1) hrs, 10.8 (1.1) hrs, 12.9 (2.62) hrs and 11.87 (2.70) respectively. These studies included both PWCF and healthy volunteers taking ivacaftor at doses ranging from 150mg to 275 mg. One study in PWCF given a single 100mg dose reported a $T_{1/2}$ of 6.56 (1.4) hrs although the reason behind this inconsistency in $T_{1/2}$ of ivacaftor between this and the other single dose studies is not discussed. Two studies of ivacaftor 150mg at steady state in healthy volunteers reported a $T_{1/2}$ (SD) of 14.08 (4.05) hrs and 14.7 (3.68) hrs respectively.(9)

Table 2. Key PK parameters of ivacaftor 150mg in fed state in healthy volunteers as reported in the summary of product characteristics.

PK parameter	Mean	Standard deviation	Coefficient of variation
C_{max}	0.768µg/mL	0.233 µg/mL	30.34%
AUC	10.6 µg.hr/L	5.26 µg.hr/L	49.62%
$T_{1/2}$	12	Not available	Not available
Clearance	17.3 L/hr	8.4 L/hr	48.55%

C_{max}, maximum concentration; AUC, area under the time-concentration curve; $T_{1/2}$, elimination half-life.(12)

The mean clearance of ivacaftor (SD) was estimated to be 17.3 (8.4) L/hr in healthy subjects.(34) This represents a CV (%) of the mean clearance of ivacaftor of 48.55%.

The factors driving the variability in PK parameters were explored using population PK analysis. The potential effect of weight, gender, disease status and age were assessed. This analysis showed that weight is the most significant predictor of ivacaftor PK parameters. As weight increases clearance of ivacaftor increases accordingly. Age, gender and disease status did not significantly affect ivacaftor disposition.(2)

2.2.3.5 Pharmacokinetic drug interactions with ivacaftor

Drug interactions were assessed in vitro and in vivo during drug development. In vitro experiments showed that ivacaftor is predominantly metabolised by CYP 3A4 and 3A5 enzymes. These studies also suggested that ivacaftor is an inhibitor of CYP2C8, CYP2C9 and CYP3A as well P-gP and potentially CYP2D6. M1 was also found to inhibit the above enzymes as well as P-gP. [3] The magnitude of these interactions is summarised in table 3.

Table 3. Drug interactions with ivacaftor.

Co-administered drug	Effect on ivacaftor exposure (AUC)
Ketoconazole	Increased 8.5 times
Fluconazole	Increased 3 times
Rifampicin	Decreased 9 times
Co-administered drug	**Effect of ivacaftor on co-administered drug exposure (AUC)**
Digoxin	Increased 1.3 times
Midazolam	Increased 1.5 times
Warfarin	Unpredictable effect on international normalised ratio: monitor

AUC, area under the curve. Table adapted from (2) and (12)

A recent case report published clinical findings in two siblings with CF and the G551D mutation on ivacaftor. One sibling was being treated with ivacaftor 150mg twice weekly in conjunction with itraconazole 200mg daily for the treatment of allergic bronchopulmonary aspergillosis (abpa) and the other sibling was treated with ivacaftor

150mg BD. The patient on ivacaftor 150 twice weekly demonstrated a greater increase in $ppFEV_1$ than his sibling (47%–84% in comparison to 55%–71%) which suggests that the decreased dose of ivacaftor when administered with a strong CYP3A inhibitor did not result in any negative effect on clinical outcome, though caution must be applied when extrapolating clinical outcomes from a case to report to the wider population, particularly given the large variability demonstrated in the disposition of ivacaftor between individuals.[5]

Ivacaftor is extensively bound to plasma proteins in vivo, primarily human serum albumin (HSA) and α-1 acid glycoprotein (AGP). To investigate the potential of drug–drug interactions based on displacement of ivacaftor from plasma proteins an in-vitro study using commercial preparations of HAS and AGP explored the drug-drug plasma binding interactions of ivacaftor in the presence of several other common medications. Docusate, montelukast, ibuprofen, dicloxacillin, omeprazole and loratidine were found to significantly displace ivacaftor, potentially increasing free drug concentrations and leading to higher exposure of tissues to unbound ivacaftor. [6] A study to assess the pharmacodynamic (PD) implications of these interactions has not yet been published.

2.2.4 Background to Ivacaftor pharmacodynamics: Cystic fibrosis transmembrane conductance regulator physiology.

The CFTR gene is located on the long arm of chromosome 7 and encodes a protein that is a member of the adenine triphosphate (ATP) binding cassette (ABC) superfamily of transporters. It is unique among the ABC transporters in that it is an ion channel, as opposed to the other transporters in the family that are channels for much larger molecules. The protein consists of two homologous halves, each with six transmembrane domains and a nucleotide binding domain (NBD), and these two halves

are linked by a regulatory domain. This homodimer creates an anion selective ion channel which is impermeable to cations and larger molecules. In vivo it provides a pathway for chloride (Cl^-), gluconate and bicarbonate (HCO^-) transport. It also acts as a regulator of other ion channels including the amiloride sensitive epithelial channel (ENaC), outwardly rectifying chloride channel (ORCC), renal outer medullary K^+ channel, the sodium/proton exchanger NHE3, Ca^{2+} activated chloride conductance channel (CaCC) and an aquaporin channel.[1, 2]

CFTR activity is regulated by both phosphorylation and ATP hydrolysis. Various kinases may phosphorylate the regulatory domain and the more extensive the phosphorylation the greater the open probability of the channel. ATP hydrolysis is a prerequisite for opening and closing of the channel. NBD-1 hydrolyses ATP to open the channel while NBD-2 hydrolyses ATP to close it, though there are also complex interactions between these domains. Phosphorylation of the regulatory domain is thought to exert its effect by altering the affinity of the NBDs for ATP.[2]

The CFTR is found in the epithelium of the intestines, pancreas, lungs, sweat glands and kidneys and regulates the movement of ions and water and the regulation of pH in these tissues. It is also found in other cell types such as smooth muscle, immune cells and cardiac myocytes.[3]

2.2.4.1 Mutations in the cystic fibrosis transmembrane conductance regulator gene

There are 6 broad classes of mutation in the CFTR gene that lead to the clinical manifestations of CF. The severity of the phenotype is related the class of mutation present, though this is not the only determinant of severity.[3] Class I mutations result from nonsense, frame-shift or splice site mutations in the CFTR gene. Non-functional CFTR protein is present on the surface of cells with this class. Class II mutations result

from improper maturation of mutated CFTR proteins. Wildtype CFTR folds into a protease resistant structure in the endoplasmic reticulum and is glycosylated and transported to the cell surface. CFTR with a class II mutation fails to fold into this protease resistance structure and is marked for ubiqutination soon after synthesis. Little or no CFTR with a class II mutation is present at the cell surface. ΔF508, the commonest mutation causing CF, is an example of a class II mutation. Class III mutations are those that interfere with either the phosphorylation of the R domain or binding of ATP to the NBD. The G551D mutation, which is partially corrected by ivacaftor, is an example of a class III mutation. Class IV mutations result in defective conductance of the CFTR due to changes in the sequence of amino acids in the pore of the channel. Class V mutations are not associated with mutations in the CFTR itself, but with splice sites, such that the absolute number of CFTR that is trafficked successfully to the cell surface is reduced. It is usually associated with a partial CF phenotype, such as congenital bilateral absence of the vas deferens. Class VI mutations affect the regulatory functions of the CFTR rather than ion transport. Some common CF genotypes can be associated with a CFTR that malfunctions in more than one of the above ways, for example the ΔF508 mutation results in the transcription of a protein that predominantly does not reach the cell surface, but when it does it also demonstrates sub-normal Cl^- conductance.[3]

2.2.5 Ivacaftor Pharmacodynamics

Ivacaftor increases the open probability of CFTR proteins that carry any one of several different mutations, namely G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R or R117H. These are class III CFTR mutations except R117H which demonstrates features of both class III and class IV mutations, resulting in decreased conductance of anions, predominantly Cl^- and HCO_3^- in addition to decreased open

probability.(34) Most of the information in the literature regarding the mechanism of action of ivacaftor pertains to the G551D mutation. The exact mechanism by which ivacaftor increases the open probability of the mutant CFTR has not been elucidated, though ongoing research, continuing since the launch of the drug in 2011 is constantly adding to the understanding of this elusive mode of action.

One hypothesis regarding the mechanism of ivacaftor in cells with the G551D mutation was published by Jih and Hwang in 2013.(35) In this paper it is proposed that the CFTR exists not only in the open and closed states, but in several different forms of an open and closed state, some of which are regulated by ATP and some of which are ATP independent. As described above, ATP hydrolysis is associated with channel opening and further ATP hydrolysis with channel closure. However, the channel can also exist in an open state after hydrolysis of the second ATP molecule and similarly can be closed after hydrolysis of the first. The degree of stability of the open channel post hydrolysis of the first ATP molecule is proposed to be affected by ivacaftor such that the channel is found to be much more likely to be in the open state after hydrolysis of the first ATP molecule and more likely to remain open after the hydrolysis of the second. Therefore, the presence of ivacaftor increases the likelihood that the channel will cycle several times between a state of being bound to one ATP molecule and being bound to a second ATP molecule without closing before finally closing post hydrolysis of an ATP molecule that results in phosphorylation of a distinct site.(35)

It has also been shown that ivacaftor increases the open probability of G551D-CFTR in the absence of ATP, suggesting a non-ATP dependent opening mechanism is also augmented by this potentiator. Eckford et al. (2012) have shown that the magnitude of the increase in the open probability of the G551D-CFTR far exceeds the increase in

hydrolysis of ATP on addition of the potentiator, again pointing towards an ATP-independent mechanism.(35, 36)

These studies suggest that the binding site for ivacaftor is likely an allosteric site rather than the NBD. This may account for the relatively few side effects experienced from a potentiator of a type of channel that is ubiquitous and highly conserved. Interestingly, ivacaftor does not potentiate CFTR in mice, suggesting the allosteric site may be uniquely human.(36)

2.2.6 Ivacaftor Clinical Evidence

Consideration is given below to the randomised clinical trials that were undertaken to gain regulatory approval for ivacaftor in clinical practice, namely the STRIVE and ENVISION studies. The clinical effectiveness of ivacaftor was first assessed in a multicentre, randomised, double-blind, placebo-controlled phase III trial, the STRIVE study, the results of which were published in 2011. Subjects were eligible for inclusion if they were 12 years of age or older, had the G551D mutation on at least one allele, and had an FEV_1 of 40%–90%. Usual care was continued for all subjects except for hypertonic saline, which is not approved in the United States, and therefore its use would have resulted in treatment differences across countries. The primary efficacy endpoint was the change in $ppFEV_1$ through week 24. Secondary endpoints were $ppFEV_1$ through week 48, time to first pulmonary exacerbation through weeks 24 and 48, score in the respiratory domain of the cystic fibrosis questionnaire-revised (CFQ-R) through weeks 24 and 48, change in weight from baseline to week 24 and 48 and change from baseline in sweat chloride through week 24 and 48. Tertiary endpoints were number and duration of pulmonary exacerbations, total number of days spent in hospital for pulmonary exacerbation and need for antibiotic treatment for clinically diagnosis sinus or lung

disease. The study was designed to have 80% power to detect a change of 4.5% in the $ppFEV_1$. Analysis was on an intention to treat basis.

The study showed a significant increase in the primary endpoint with ivacaftor treatment, $ppFEV_1$, of 10.4%, a relative increase of 10.6% in comparison to placebo. This improvement was sustained throughout the 48 weeks of the trial. To put this in context, current CF pulmonary guidelines from the American Thoracic Society list inhaled aztreonam and dornase alpha as therapies with a high certainty of substantial benefit in moderate to severe CF; inhaled aztreonam shows an improvement of 6.3–10.3% in $ppFEV_1$, and dornase alpha improved $ppFEV_1$ up to a maximum of 9.4%.(37) Respiratory symptoms were shown to improve from baseline to 48 weeks with an increase in the CFQ-R score of 5.9 points (relative effect 8.6 points; P<0.001). By week 48 subjects in the Ivacaftor group had gained 3.1kg, a treatment effect of 2.7 kg (P<0.001). Through week 24 sweat chloride decreased by 48.7mmol/L in the ivacaftor group compared to 0.8mml/L in the placebo group (treatment effect of -47.9mmol/L in the ivacaftor group, P<0.001). Ivacaftor resulted in a 55% reduction in the risk of pulmonary exacerbation, with 99 exacerbations in the placebo group compared to 47 exacerbations in the Ivacaftor group. Thirty-one events lead to hospitalisation in the placebo group as compared to 21 events in 11 subjects in the ivacaftor group. This incidence of adverse events (AEs) was similar in both groups through week 48. There was a higher incidence of interruption of study drug due to AEs in the ivacaftor group, but all subjects randomised to ivacaftor except one were subsequently able to recommence the drug. The rate of serious AEs was lower in the ivacaftor group.(6)

The baseline characteristics of subjects in both groups appear to be well matched overall by visual inspection, but no statistical comparison of the two groups is reported. The primary endpoint in this trial, $ppFEV_1$, is consistent with the endpoint used in most

pivotal studies of the treatments for cystic fibrosis.(38) FEV_1 is considered an appropriate surrogate endpoint for these trials as low values are strongly associated with increased mortality and decreased quality of life.(39, 40) There are a number of caveats to be considered with this endpoint, however. Prior to the introduction of ivacaftor there were several interventions already established in clinical care that slowed the decline in lung function in PWCF, as discussed above. Many PWCF have preserved lung function well into their third decade. This trial allowed inclusion of those with $ppFEV_1$ of 40–90%, thus excluding those with preserved lung function. The clinical effectiveness of ivacaftor for PWCF and preserved lung function is therefore not known. The clinically meaningful change in FEV_1 is also not agreed. This test is subject to significant intra-patient variability, as much as 12% in trials lasting weeks as reported by the American Thoracic Society and the European Respiratory Society.(41) The mean relative improvement in $ppFEV_1$ of 10.6% is statistically significant but may represent a clinically meaningful improvement of the same magnitude. Those with $ppFEV_1$ of less than 40% were also excluded, representing 15.4% of the adult population with CF in Ireland in 2015.(5) Thus those at the extremes of lung function are not represented in this trial and the clinical utility of ivacaftor in these groups is not known. Lastly, it is reported in the supplementary material published with the paper above that the Knudson standards were used to calculate the $ppFEV_1$ from the absolute FEV_1, based on the age, sex and height of the participant, but this standard is not consistently used in the CF literature which may lead to incorrect comparisons between the clinical effectiveness of ivacaftor and other treatments if $ppFEV_1$ is the primary endpoint assessed. It would provide more useful information if the absolute change in FEV_1 was reported alongside the $ppFEV_1$ at the 24 and 48 week timepoints.

The use of sweat chloride as a clinical endpoint in this trial also warrants mention. It has been noted by other authors that even though there was a large change in sweat chloride seen with ivacaftor treatment this did not correlate with an improvement in lung function.(42) The clinical relevance of changes in sweat chloride in the assessment of CFTR modulators is not clear as yet.

Subjects and their carers were blinded to the results of their spirometry and sweat chloride over the full course of the study. Blinding of participants and investigators to other endpoints, that is weight, time to first pulmonary exacerbation and use of antibiotics was not possible. In particular this may have affected the subjective-reported respiratory symptoms in CFQ-R.(43)

The variability in the improvement in $ppFEV_1$ in the treatment group is explored but not explained. Analysis of subgroups in the treatment arm did not reveal why some subjects experienced a large improvement in $ppFEV_1$ of almost 40% whereas some did not experience improvement at all. The data collected in the trial regarding plasma concentrations of ivacaftor as outlined in the trial protocol is not reported, and the possible relationship of variable ivacaftor plasma levels to variable outcomes is not considered. Visual inspection of figure 2 in the supplemental material published alongside the paper reporting the changes in $ppFEV_1$ discussed above shows that these data are not normally distributed but skewed to the right. This is also not discussed.(44)

The results of the ENVISION study on the efficacy and safety of ivacaftor ages 6 to 11 years in CF with the G551D mutation was published two years later in 2013. As this trial was in a younger age group the inclusion criteria allowed for those with a $ppFEV_1$ of 40-105%. The minimum sample size of 30 patients was based on pragmatic considerations of the available population rather than a formal power calculation. Endpoints were similar to the STRIVE study with the primary endpoint the absolute change from baseline

through week 24 in the $ppFEV_1$ and secondary endpoint of $ppFEV_1$ at week 48, weight at week 24 and 48, sweat chloride at week 24 and 48, and CFQ-R at week 24 and 48 as reported by the patient and by the care-giver. No formal analysis was performed on pulmonary exacerbations. At week 24 the mean treatment effect was a relative improvement in $ppFEV_1$ of 12.5%. At 48 weeks the relative mean improvement was 10% in the ivacaftor group. Those in the ivacaftor group gained a mean of 2.8kg, a treatment effect of 1.9kg. There was a significant improvement in both the patient reported and care-giver reported CFQ-R at week 24 (relative improvement 6.1 points and 5.9 points respectively). No formal analysis of pulmonary exacerbation rates was planned as it was expected that rates would be low; there were three pulmonary exacerbations in the placebo group and four in the ivacaftor group. Adverse events in both groups were similar.(45)

The limitations discussed regarding the STRIVE study above also broadly apply to the ENVISION study. It is reported that a stratified analysis of improvement in $ppFEV_1$ showed that improvements were more significant in those with a lower $ppFEV_1$ at enrolment, as may be expected. Those who entered the trial with a $ppFEV_1$ of ≥90% had a non-significant mean (95% CI) improvement in lung function of 6.9 % (-3.8–17.6). As there are now several interventions available to clinicians at present to preserve the lung function of PWCF, more sensitive markers of lung function are needed for studies such as this to assess the benefit of new treatments prior to the establishment of lung damage evident in a change in $ppFEV_1$, particularly in children. The lung clearance index (LCI), which measures ventilation inhomogeneity is a more sensitive measure of damage to lung parenchyma that the FEV_1. The clinically meaningful change in LCI is yet to be established and there are as yet no studies that correlate this surrogate marker to hard endpoints such as survival. However, such endpoints will need to be explored in future

trials of treatments for CF as the paradigm shifts from treatment of damaged tissue to prevention from a young age. (38) Ivacaftor was granted regulatory approval from the FDA in January 2012, and was granted this with an exemption from the requirement to assess the safety and efficacy of ivacaftor in PWCF under 6 years old given the orphan designation of the drug.(46) In May 2012 the EMA also made a positive recommendation for ivacaftor for PWCF and at least one G551D mutation over 6 years of age based on the data discussed above, a summary of which is in tables 4 and 5.(47)

Table 4. Summary of results from STRIVE study of ivacaftor in patients with CF and the G551D mutation 12 years and older.

Endpoint	Week 24		
	Ivacaftor	Placebo	Difference (95% CI)
Change from baseline in $ppFEV_1$ (%)	10.4	-0.2	10.6 (8.6, 12.6)
Change from baseline in sweat chloride (mmol/L)	-48.7	-0.8	-47.9 (-51.3, -44.5)
Change from baseline in CFQ–R (points)	5.7	-2.7	8.6
Number of pulmonary exacerbations	18	35	Rate ratio 0.38 (0.22, 0.64)
Change in weight from baseline (kg)	3.0	.2	2.8 (1.8, 3.7)

CF, cystic fibrosis; $ppFEV_1$ percentage predicted forced expiratory volume in one second; CFQ–R, cystic fibrosis questionnaire–revised.

Table 5. Summary of results from the ENVISION study of ivacaftor in patients with CF and the G551D mutation aged 6–12 years.

Endpoint	Week 24		
	Ivacaftor	Placebo	Difference (95% CI)
Change from baseline in $ppFEV_1$ (%)	12.6	0.1	12.5 (6.6, 18.3)
Change from baseline in sweat chloride (mmol/L)	-55.5	-1.2	-54.3 (-61.8, -46.8)
Change from baseline in CFQ–R (child version) (points)	6.3	0.3	6.1 (-1.4, 13.5)
Change from baseline in CFQ-R (caregiver version) (points)	4.9	-1.1	5.9 (0.5, 11.4)
Change in weight from baseline (kg)	3.7	1.8	1.9 (0.9, 2.9)

$ppFEV_1$ percentage predicted forced expiratory volume in one second CFQ–R, cystic fibrosis questionnaire–revised.

2.2.7 Compartmental pharmacokinetic models

Compartmental PK involves mathematically conceptualising the human body as a series of compartments through which a drug traverses after entering the body before it is irreversibly eliminated. A compartment is not a physiological entity such as the liver or heart, it is a concept of a space in which drug will instantaneously distribute and behave in a uniform manner. An example of a two-compartmental model could incorporate one compartment consisting of the circulation and all well perfused organs into which drug distributes rapidly and a second compartment of fat, bone and connective tissue into which drug distributes more slowly.(22)

Below are diagrammatic representations of one and two compartment models applied to a drug with extravascular administration.

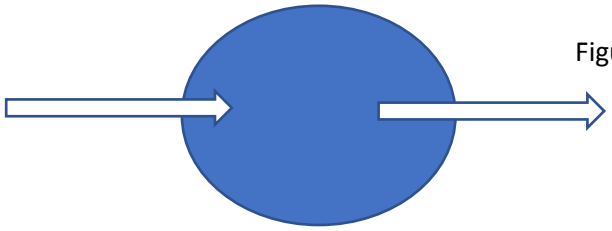


Figure 1. One-compartment PK model.

In a one-compartment model drug enters the central compartment from outside the body (absorption from the gut in the case of oral administration of a drug) and then leaves the central compartment irreversibly. A one-compartment model treats the entire body as one 'bucket' in which drug behaves identically in all tissue it is exposed to.

In a two-compartment model drug enters from outside the body (absorption from the gut in the case of oral administration). Drug can leave the central compartment by moving into the peripheral compartment or by leaving the body. Drug can circulate between each compartment but cannot re-enter the body once eliminated. This model considers the whole body to be divided into two 'buckets' within each of which drug behaves in an identical way.(20)

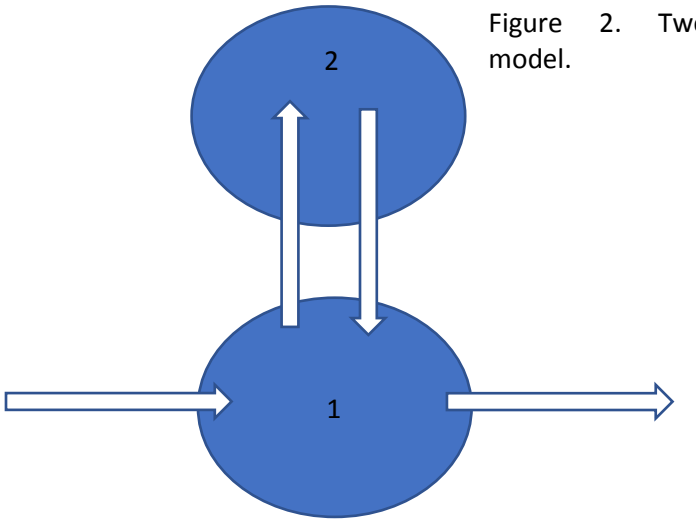


Figure 2. Two-compartment PK model.

Compartmental models are just that—mathematical models that can describe an experimentally obtained data set, in this case a concentration–profile of a drug. The parameters obtained from these models differ from those calculated by NCA in that NCA is purely observational, it calculates parameters directly from observed data, whereas those calculated from a compartmental model are observational *and* predictive; the model derived from the observational data can be used to simulate concentrations after multiple doses from single dose data as an example. The PK parameters that are obtained from non-compartmental pharmacokinetics are calculated directly from the concentration–time data obtained. In compartmental pharmacokinetics the PK parameters are obtained from a mathematical model containing a number of parameters that is dependent on the number of compartments in the best-fitting model.(20)

The current study explores the PK properties of a drug that is orally administered and for which there is no IV formulation. Therefore, the following discussion of compartmental models and their parameters focuses on models with extravascular administration.

2.2.7.1.1 One-compartment model, extravascular administration

A typical plasma drug concentration–time curve for a one-compartment model with

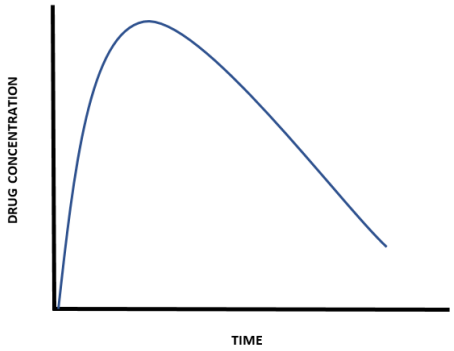


Figure 3. Theoretical example of a time–concentration curve of a drug demonstrating a one-compartment model

This curve is described by the following bi-exponential equation:

$$C = \frac{K_a . F . D_{po}}{V_d . (K_a - K)} [e^{-K.t} - e^{-K_a . t}]$$

where K_a is the first order absorption rate constant, K is the first order elimination rate constant, F is the absorbable fraction, D_{po} is the administered dose, V_d is the volume of distribution and C is the concentration at time t (assuming K_a >>K). This formula assumes first order absorption, which is typically the case with oral administration of drugs. The timecourse of the concentration in plasma of some compounds suggests a time delay between administration and the onset of absorption referred to as the lag time. To take this lag time into account the curve can be described as follows:

$$C = \frac{K_a . F . D_{po}}{V . (K_a - K)} [e^{-K.(t - t_{lag})} - e^{-K_a . (t - t_{lag})}]$$

Therefore, the primary parameters derived from fitting of a dataset to a one-compartmental model are K_a, K, V/F and t_{lag}. Unless a drug is available in both an oral and intravenous formulation V must always be described proportional to F, as these

parameters cannot be measured independently. AUC, absorption half-life, elimination half-life, T_{max} and C_{max} are secondary parameters that are calculated from these primary parameters.(20, 22)

2.2.7.1.2 Two-compartment model, extravascular administration

A typical plasma drug concentration–time curve for extravascular administration of a drug that demonstrates a two-compartment model is shown in figure 4.

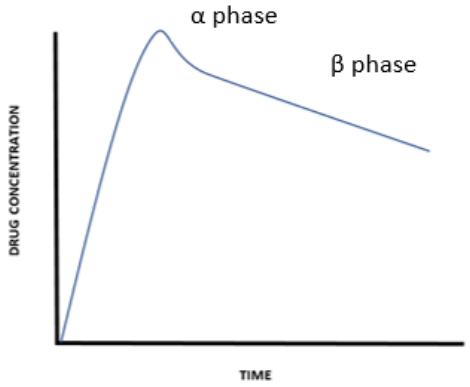


Figure 4. Theoretical example of a concentration–time curve of a drug demonstrating a two-compartment model

This curve clearly shows three distinct phases: an absorption phase, a distribution phase and a post-distribution phase. This curve can be described with the use of a multiexponential model, which again can include a t_{lag}.

$$C_t = \frac{K_a.F.D_{po}}{V_c}.\left\{\left\{\frac{(k_{21}-\alpha).e^{-\alpha.(t-t_{lag})}}{(K_a-\alpha).(\beta-\alpha)}\right\}+\left\{\frac{(k_{21}-\beta).e^{-\beta.(t-t_{lag})}}{(K_a-\beta).(\alpha-\beta)}\right\}+\left\{\frac{(k_{21}-K_a).e^{-K_a.(t-t_{lag})}}{(\alpha-K_a).(\alpha-K_a)}\right\}\right\}$$

Primary model parameters are A, α, B, β where -α and -β refer to the initial slope and terminal slope post the maximum measure concentration respectively when data are plotted on a semilogarithmic scale. Other parameters reported for a two-compartment model are K_{01} K_{10}, K_{12}, K_{21}, AUC, K_{01}_T½, K_{10}_T½, Alpha_T½, Beta_T½, V1/F, CL/F, V2/F, CLD2/F, T_{max} and C_{max}. The parameters that are listed as descriptive in NCA above, for

example C_{max} and T_{max} are calculated from the primary model parameters in the compartmental analysis.

2.2.7.1.3 Rate processes

The *rate of change* of the ADME processes described above control the ultimate concentration of a drug at the site of action. There are many orders and types of processes that describe this rate of change, but the most commonly encountered in PK analysis are zero order and first order processes.

A zero-order process, for the sake of argument drug elimination after an IV bolus, can be described by the following:

$$Y = Y_0 - K_0 t$$

where Y is the concentration of drug at time t, Y_0 is the concentration at time 0 and K_0 is the zero-order elimination rate constant. This equation shows that the elimination of drug is *constant* and as such the rate of elimination does not depend on the concentration of drug present.

This can be contrasted with the equation describing a first order elimination process:

$$Y = Y_0 e^{-Kt}$$

where Y is the concentration of drug at time t, Y_0 is the initial concentration and K is the first-order elimination rate constant. It can be seen here that elimination is proportional to the initial concentration.(22) The same principles can be applied to drug absorption and are discussed further below regarding the PK modelling of ivacaftor.

2.3 Ritonavir

2.3.1 Pharmacodynamics

Ritonavir was the one of the earliest PIs to show efficacy in clinical trials for the treatment of HIV infection. The two earliest clinical trials of ritonavir in the treatment of HIV (M94-247 and M94-245) showed a significant reduction in viral load coupled with an increase in CD4 cell count in those in the ritonavir alone arm. M94-247 compared ritonavir 600mg BD to placebo and M94-245 compared ritonavir 600mg BD to zidovudine 200mg three times daily (TDS) and to ritonavir 600mg plus zidovudine 200mg TDS. Interestingly, M94-245 did not show a benefit of combination therapy over and above ritonavir alone, though since this many studies have shown that combination therapy is most effective in the treatment of HIV infection.(48, 49) Ritonavir inhibits both HIV-1 and HIV-2 aspartyl proteases, resulting in the production of HIV particles with immature morphology that are unable to initiate new rounds of infection. It is approximately 500 times more specific for HIV protease than the human form of the enzyme. Currently, ritonavir is primarily used as a PK enhancer in the treatment of HIV and more recently hepatitis C.(50)

2.3.2 Pharmacokinetics

2.3.2.1 Absorption

As there is no parenteral formulation of ritonavir the exact bioavailability cannot be determined, but it has been shown the absorption increases when ritonavir is taken with food. C_{max} increases with repeated dosing less than would be expected based on single dose profiles, which may, at least in part, be due to autoinduction of metabolism (see below), but C_{max} and minimum concentration (C_{trough}) levels have been shown to stabilise at 2 weeks.(49)

2.3.2.2 Distribution

Ritonavir is 98-99% bound to plasma proteins, with equal affinity for AAG and HSA. The apparent V_d of ritonavir is approximately 20 – 40 L after a single 600mg dose, with tissue distribution predominantly into the liver, pancreas, adrenals, kidneys and thyroid and minimal distribution into brain tissue.(49)

2.3.2.3 Metabolism

Ritonavir is primarily metabolised by CYP3A4 and to a lesser extent by CYP2D6 in the liver. With repeated dosing ritonavir both induces and inhibits its own metabolism, as well as having profound effects on the metabolism of other xenobiotics (discussed below).(49)

2.3.2.4 Elimination

Ritonavir and its metabolites are primarily eliminated though the hepatobiliary system.(59)

2.3.3 Pharmacokinetics drug interactions of ritonavir

Ritonavir demonstrates PK interaction with many other drugs commonly used in clinical practice. This is due to the extensive number of drug metabolising enzymes and transporters that are either inhibited or induced in the presence of ritonavir. Cytochromes CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are all inhibited by ritonavir, with coincident activation of PXR also resulting in induction of CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4.(51, 52) Enzymes involved in glucuronidation are also induced by ritonavir. Transporters P-gP, breast cancer resistance

protein (BCRP), organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1 and OATP1B3), multiantimicrobial extrusion protein 1 and 2-K (MATE1 and MATE2-K), organic acid transported 1 and 3 (OAT1 and OAT3) and organic cation transporter 2 (OCT2) are all also inhibited by ritonavir.

These interactions result in clinically relevant changes in plasma concentrations of drugs coadministered with ritonavir. Table 6 contains some examples of drug interactions with ritonavir by the various mechanisms described above.(50)

The ability of ritonavir to inhibit the efflux and metabolism of other drugs was utilised early in the life of ritonavir to simplify dosing regimens and increase plasma levels of other PIs. Ritonavir has predominantly been used as such as a PK enhancer since the late 1990s, with the nature of the interaction between ritonavir and another PI, namely saquinavir, appearing in the literature in 1997 in the work by Merry et al.(10) Currently

Table 6. Examples of drug interactions with ritonavir.

Drug co-administered with ritonavir	Dose of co-administered drug	Dose of ritonavir	Mechanism of interaction	Effect on co-administered drug	Recommendation
Buprenorphine	16mg OD	100mg BD	CYP3A4 inhibition	AUC increased by 57%	Monitor adverse effects
Theophylline	3mg/kg TDS	500mg BD	CYP1A2 induction	AUC decreased by 43%	Increase dose of theophylline
Methadone	5mg single dose	500mg BD	Induction of glucuronidation	AUC decreased by 36%	Increase dose of methadone based on clinical symptoms
Rivaroxaban	10mg single dose	600mg BD	CYP3A4 and P-gP inhibition	AUC increased by 153%	Avoid concomitant use
Digoxin	500mcg single dose	300mg BD	P-gP inhibition	AUC increased by 86%	Reduced digoxin dose by 30-50% based on monitoring

OD, once daily; BD, twice daily; TDS, three times daily.

the key PI regimens boosted with ritonavir include ritonavir-boosted atazanavir, ritonavir-boosted darunavir, ritonavir-boosted lopinavir and ritonavir-boosted fosamprenavir.(53)

Despite its use for over two decades as a pharmacological booster, the exact mechanism by which ritonavir inhibits the CYP enzyme remains unclear. The published data on ritonavir inhibition of CYP suggest inhibition by mechanism-based, competitive or mixed competitive-noncompetitive CYP3A4 inactivation.(54-57) Studies based on more modern imagining techniques available in recent years suggest the predominant method of inhibition by ritonavir is indeed through mechanism-based inhibition. The details of the nature of the proposed mechanism-based inhibition are also debated, with evidence for MI complex formation, heme modification and apoprotein adduction all presented by different groups.(55, 56, 58) The induction effects of ritonavir are also still under investigation, with the balance of induction and inhibition different depending on the co-administered drug in question.(59)

2.4 Beneficial drug–drug interactions

Outside the area of HIV there has been use of PK drug interactions to improve the clinical efficacy and effectiveness of drug therapy. Ritonavir is now also utilised to boost PIs in the treatment of hepatitis C.(60) The use of probenecid to slow the renal elimination of procaine penicillin is an early example, where it was used in the 1950s to improve the clinical efficacy and reduce dosing burden of the drug.(29) The use of probenecid was also later used to overcome problems with the development of microbial resistance to penicillin in the treatment of gonococcal infections in men.(61) Ketoconazole, another strong CYP3A4 inhibitor, has been used in the area of organ transplantation to reduce the dose of immunosuppression with ciclosporin required. Results from such studies in renal transplantation have been variable,(62, 63) but one study in heart transplantation

showed a cost saving and a decreased rate of organ rejection with the use of ketoconazole boosting of ciclosporin.(64) Ketoconazole boosting of tacrolimus has also successfully been used in Mexico to reduce to financial burden of renal transplantation without any deleterious clinical effects.(65)

2.5 Healthcare in Ireland

In Ireland, healthcare policy and expenditure are governed by the Department of Health (DoH) and administered through the Health Services Executive (HSE). Healthcare is funded primarily through taxation (69% in 2015), with a smaller contribution from out-of-pocket payments and voluntary private health insurance.(66)

2.5.1 Reimbursement of Health Technologies in Ireland

Health Technologies are reimbursed in Ireland through hospitals, the Primary Care Reimbursement Service (PCRS) and through other local schemes. The PCRS consists of distinct schemes that provide reimbursement of health technologies and services, outlined in table 5. The majority of spending on health technologies, as opposed to health services, takes place in the general medical services (GMS), drugs payment scheme (DPS), long term illness (LTI) and high tech (HT) schemes.

2.5.1.1 General Medical Services

In 2016, €1,026.74 million was spent health technologies reimbursed by the GMS. This scheme caters for persons who cannot access medical services for themselves or their dependents without undue hardship.(1)

2.5.1.2 Drugs Payment Scheme

In 2016, €65.3 million was spent on health technologies reimbursed by the DPS. This scheme provides for payment to pharmacists for the supply of medicines to individuals or families above a threshold of €144 per calendar month. (1)

Table 7. Schemes for reimbursement of health technologies in Ireland

General Medical Services (GMS)
Drugs Payment Scheme (DOS)
Long Term Illness Scheme (LTI)
Dental Treatment Services Scheme (DTSS)
European Economic Area (EEA)
High Tech Arrangements (HT)
Primary Childhood Immunisation Scheme
Health (amendment) Act 1996
Methadone Treatment Scheme
HSE Community Ophthalmic Services Scheme (HSE-COSS)
Immunisations for GMS Eligible Persons
General Practitioner Visit Card (GPVC)
Discretionary Hardship Arrangements
Centralised reimbursement of selected high cost drugs administered or dispensed to patients in hospitals
Centralised reimbursement of Outpatient Parenteral Antimicrobial Therapy (OPAT)
Redress for Women Resident in Certain Institutions

2.5.1.3 Long Term Illness Scheme

The LTI scheme allows for the provision of health technologies completely free of charge for a limited list of 16 conditions (including CF) regardless of the income of the person with the illness or the cost of the technologies required to treat the illness. In 2016, €207.45 million was spent of health technologies reimbursed through the LTI scheme.(1)

2.5.1.4 High Tech Scheme

The HT scheme caters for drugs that are usually only initiated in hospitals such as anti-neoplastics, anti-rejection drugs and for the new small molecule therapies for CF such as ivacaftor. In 2016, €611.74 million was spent on technologies reimbursed by the HT

scheme. The increase in the cost of high tech drugs over the last 5 years is one of the major drivers of the increase in overall spending on drugs in Ireland during that time period.(1) While spending on the DPS, GMS and LTI was reduced from 2012 to 2015 with the introduction of a variety of cost saving measures the expenditure in the HT scheme has risen steadily. In the time period from 2011 to 2016 spending in the HT scheme has increased by 76%.(13) The spending on this scheme is a focus of a recent review published by the Irish Government Economic & Evaluation Service which assesses the sustainability of pharmaceutical expenditure at the current rate of growth.(13) There is an urgent need to control the growth of spending in this scheme, which will reach approximately €750 million by 2020 should it continue on its current trajectory. Reimbursement of ivacaftor through the HT scheme cost €29.9 million in 2016.(1)

2.5.1.5 Hospitals

Twenty-one percent of the spend on pharmaceuticals is through hospitals. The spend on hospital pharmaceuticals has been on an upward trajectory over the last number of years, with an annual mean increase of 9% per year from 2012 to 2016. This main driver for this has been new oncology drugs. In absolute terms €4 million was spent on new oncology drugs in hospitals in 2012 growing to €23 million in 2016. This rate of growth is expected to continue given the number of new oncology drugs that have been approved in 2016.(13) The oncology drugs management system (ODMS), established in 2012, is the central funding mechanism by which cancer drugs are paid for in individual hospitals. Other drugs provided in the hospital setting are paid for through the individual hospital budgets.(67)

2.5.2 Decision process for the reimbursement of new health technologies in Ireland

The HSE must operate within the resources supplied to it by Dáil Éireann each year. To this end it must make informed decisions regarding the addition of new technologies to the list of reimbursable items, considering the following criteria (as outlined in the Health [Pricing and Supply of Medical Goods] Act 2013)(68):

- The health needs of the public
- The cost-effectiveness of meeting health needs by supplying the item concerned rather than providing other health services
- The availability and suitability of items for supply or reimbursement of both under section 59 of the act of 1970
- The proposed costs, benefits and risks of the item or listed item relative to the therapeutically similar items or listed items provided in other health service settings and the levels of certainty in relation to the evidence of those costs, benefits and risks.
- The potential or actual budget impact of the item or listed item
- The clinical need for the item or listed item
- The appropriate level of clinical supervision required in relation to the item to ensure patient safety
- The efficacy (performance in trial), effectiveness (performance in real situations) and added therapeutic benefit against existing standards of treatment
- The resources available to the Executive.

The ultimate decision on reimbursement of items is made by the HSE Leadership Team. The HSE Leadership Team is advised by the HSE Drugs Committee which comprises

representations from a range of HSE National Directorates as well as representation from the PCRS, HSE Corporate Pharmaceutical Unit (the interface between the industry and the HSE) and the National Centre for Pharmacoeconomics (NCPE).(69)

The NCPE was established in 1998, with the aim of promoting pharmacoeconomic expertise in Ireland through practice, research and education. With the inception of the Irish Pharmaceutical Healthcare Association/HSE agreement (IPHA/HSE) in 2006, submission of pharmacoeconomic data prior to reimbursement was agreed between the pharmaceutical industry and the HSE.(67) This agreement updated in 2016.(70) With the inception of the Health Act 2013 and the establishment in law of cost effectiveness as a criterion in the decisions relating to the reimbursement of new drugs, the number of pharmacoeconomic assessments of new drugs submitted to the NCPE has increased significantly.(67)

The first step in the NCPE process involves the submission of a Rapid Review, containing summary information on the target population, the indication, clinical evidence, comparators, economic considerations including budget impact estimates, other indications for which the drugs is being investigated and the outcomes of health technology assessment (HTA) from other jurisdictions by the applicant pharmaceutical company. On foot of this submission reimbursement may be recommended, not recommended or a full pharmacoeconomic evaluation may be requested. A price reduction may also be recommended in order to move to recommendation of reimbursement. The submission of a full pharmacoeconomic assessment provides significantly more information on which to base one of the recommendations above.(67)

2.5.2.1 Types of economic evaluation in HTA

The types of economic evaluation presented by the applicant pharmaceutical company to the NCPE are highlighted in the 'Guidelines for the Economic Evaluation of Health Technologies in Ireland' published by the Health Information and Quality Authority (HIQA) in 2018. Economic evaluation of health technologies allows comparison of both the costs and consequences of existing health technologies with one or more potential alternatives. These economic evaluations can be divided into two major types: cost-effectiveness analysis (CEA) and cost-benefit analysis (CBA). (Cost-utility analysis [CUA] can be considered a subtype of cost-effectiveness analysis). The evaluation of costs is similar is both types of economic evaluation; the key difference between them is in how the consequences of the health technology are assessed.(71)

2.5.2.2 Cost-Utility Analysis

CUA is widely considered to be the gold standard for conducting economic evaluations of health technologies. CUA analysis measures the costs of alternative technologies in monetary units and the consequences in a common unit of health improvement, such as Quality Adjusted Life Years (QALY). The QALY is one of several common units of health improvement such as the Disability Adjusted Life Year (DALY) and the Healthy Years Equivalent (HYE). The QALY is calculated by multiplying the duration of time spent in a health state by the health-related quality of life associated with that health state. It is independent of the specific nature of any particular condition.(72) This allows for comparison of technologies that have distinct clinical effects and as such, this type of analysis gives information on the opportunity cost of an intervention. In Ireland, as is outlined in the IPHA/HSE agreement, the cost-effectiveness threshold is set at €45,000 per QALY gained, although the cost-effectiveness ratio of €20,000 per QALY gained in

also of interest to the HSE.(70) In general, technologies that exceed this threshold will not be recommended for reimbursement, though in some circumstances such as orphan diseases and cancer therapeutics drugs that exceed this threshold will proceed to price negotiations lead by the HSE-CPU and informed by the NCPE.(69)

2.5.2.3 Cost-effectiveness analysis

CEA measures the costs of alternative technologies in monetary units and the consequences of health technologies in natural units. Should the primary effect of a health technology be the lengthening of life, outcomes can be measured in life-years gained (LYG). Surrogate markers can also be used, for example a reduction in blood pressure measured in mmHg, but there must be an established validated link between the surrogate marker and an important patient outcome, such as extension of life. As the consequences are measured in natural units, in this case in mmHg, it is not possible to compare the value for money of the hypertension intervention to an intervention outside of this treatment area. Therefore, it is not possible to assess the *opportunity cost* of the interventions in question when CEA alone is employed. In addition, there is no established willingness-to-pay threshold for CEA, therefore the information provided by such an analysis may fall short of what is required to inform decision making.

CEA can be used in situations where the cost of alternatives is being considered within a limited framework, such as a policy of the national treatment of hypertension. CEA can also be presented as an additional analysis complementary to the primary CUA. In limited circumstances it may be used when a CUA is considered unsuitable.(71)

In CBA both the costs and consequences of health technologies are presented in monetary terms. It is rarely used as it is difficult to accurately express health outcomes in purely monetary terms. Cost minimisation analysis (CMA) may be applied when

alternative technologies being assessed are considered clinically equivalent in their outcome. This situation applies in a limited number of scenarios, such as comparing drugs of the same pharmacological class.(71)

2.5.3 Case Study: Pharmacoeconomic assessment of ivacaftor.

Ivacaftor underwent a full pharmacoeconomic assessment in a number of countries when it was introduced to the market, including in Ireland where it was assessed by the NCPE. A summary report of their findings was published in 2013 prior to the decision not to reimburse ivacaftor at the originally proposed price.(73)

The economic dossier prepared by Vertex Pharmaceuticals® contained an economic model based on 4 studies: STRIVE, ENVISION, PERSIST and DISCOVER (a study of ivacaftor in patients homozygous for the F508del mutation). The base case analysis assumed the improvement in the primary endpoint, $ppFEV_1$ remained stable over time with ivacaftor treatment and declined for standard of care. Two scenarios were also considered, one in which the $ppFEV_1$ slope for the ivacaftor group declined at 50% of that of standard of care and another in which the $ppFEV_1$ slope for the ivacaftor group declined at the same rate as the standard of care group. The base case produced an ICER of €449,035/QALY, far in excess of the €45,000/QALY threshold. The most conservative scenario—one in which the $ppFEV_1$ was assumed to decline at the same rate of that in the standard of care group with the only benefit the initial improvement in $ppFEV_1$—estimated the ICER at €855,437/QALY. Using the most conservatively estimated ICER for reimbursement decisions minimises the financial risk undertaken by the HSE. Given the lack of long-term data and the cost per QALY far in excess of the threshold, ivacaftor was not recommended for reimbursement at the submitted price.(73)

This unfavourable assessment of the value for money of ivacaftor for PWCF and the G551D mutation was consistent with the outcome in other countries. The Pharmaceutical Benefits Scheme in Australia(74), the Canadian Agency for Drugs and Technologies in Health(75), the National Institute for Health Care and Excellence in England(76) and Scottish Medicines Consortium(77) all recommended against reimbursement of ivacaftor at the originally proposed priced as it far exceeded the threshold for cost effectiveness, even in the most optimistic scenario. After price negotiations in 2013, agreement was reached to reimburse ivacaftor in Ireland. The budget impact of this decision has been significant, with most recent data from 2016 showing that the annual cost of ivacaftor reached €29.9 million.(1) Since the reimbursement of ivacaftor for CF and the G551D mutation in patients 6 years and over, further submissions have been made by Vertex Pharmaceuticals® to expand the indication to those patients aged 2 years and older and to include those with the R117H mutation. Reimbursement was not recommended for either indication, but again was agreed after confidential price negotiations.(78, 79)

CHAPTER 3

3 Validation of the Liquid Chromatography–Mass Spectrometry Method for the Quantification of Plasma Concentrations of Ivacaftor and its Active Metabolite Hydroxymethyl-Ivacaftor.

3.1 Introduction

Therapeutic drug monitoring (TDM) of ivacaftor is not routinely used in clinical practice. At the time of commencement of the current study no assay was readily available for the measurement of ivacaftor and M1 in plasma except for that used by the manufacturer in the original clinical trials of the product. Therefore, it was necessary to develop an original method to quantify concentrations of ivacaftor in plasma. As LC-MS is a simple and robust method for detection of a wide variety of chemical compounds this technology was used for the development of an assay to detect ivacaftor and M1 in plasma.

LC-MS is a chemical analytical technique the combines the separation technology of liquid chromatography with the mass analysis capabilities of mass spectrometry. There are a number of different liquid chromatography techniques including paper chromatography, thin layer chromatography and column liquid chromatography. Column liquid chromatography is typically used with MS technology. The column consists of a solid phase of tightly packed inert material such as silica or charcoal over which the mobile phase containing the sample is run at low pressure. The solid phase adsorbs the molecules in the sample and retards their progress through the column to the detector at different rates, thus achieving separation of analytes.(80) Detection of the molecules separated out by column liquid chromatography is then achieved with MS through conversion of the analytes to a charged state with subsequent analysis of the ions produced based on the mass to charge ratio (m/z). Positive or negative ions can be

created depending on which is found to be most suitable for any given compound of interest. These ions are then detected by a quadropole analyser which consists of four parallel metal rods with varying voltages running through them. The voltages can be varied across a large range of values to produce a mass spectrum (scanning mode) or they can be set to monitor one or a small number of specific values (ion selection mode). Using ion selection mode results in greater sensitivity of detection of compounds.(81) There are a large number of factors that may affect the magnitude of the instrument response to the detection of an analyte of interest and as such absolute MS responses will be subject to significant day to day variation. Use of internal standards is therefore usually required to achieve reliable quantitative results. Stable isotopes of analytes of interest, such as deuterated analogues, are ideal internal standards, though any molecule with almost identical chemical properties can be utilised once no interference with measurement of the analyte of interest is found.(81) Use of internal standards can also correct for any variability in preparation of individual samples prior to measurement with LC-MS.

The validation of the LC-MS method for the quantification of ivacaftor in plasma was based on the European Medicines Agency (EMA) guidelines for method validation. These guidelines require an assay to meet prespecified standards in 9 key areas: selectivity, lower limit of quantification, calibration curve, accuracy, precision, carry-over, dilution integrity (if dilution of samples is to be utilised) stability and matrix effects. These 9 key areas of performance are explored by spiking blank plasma (that is plasma that does not contain the analyte of interest) with a reference standard of the analyte, which is a highly characterised, pure form of the analyte of interest. Selectivity refers to the ability of the method to differentiate the analyte of interest from any other compounds present in matrix.(3) The selectivity of a method is greatly improved by the use of MS in

detection, as should multiple compounds elute from the column at the same time these will be detected separately once they have different mass to charge ratios.(81) The lower limit of quantification is the lowest concentration of a compound that can be detected with acceptable accuracy and precision (discussed below). The LLOQ should also show an instrument response of at least 5 times that of the blank sample. The calibration curve is a plot of the instrument response against known concentration of analyte. The simplest mathematical relationship, that is the simplest weighting scheme, that adequately describes the curve should be ultilised. The accuracy of a method is the closeness of the measured concentration to the known concentration and the precision is the closeness of repeated measurements of the analyte to each other. This must be evaluated within and between analytical runs. Carry-over refers to residual detection of analyte in a sample from a sample run previously. This is assessed by analysing blank plasma directly after a sample of high concentration to assess for any detection of the compound of interest in the blank sample. Dilution integrity must be explored if it is expected that samples with a concentration higher than the maximum concentration on the calibration curve will be analysed. Dilution with plasma or another matrix as appropriate must be shown not to affect the accuracy and precision of the assay. The stability of the analyte at low and high concentrations in short-term and long-term storage and on the bench must be shown. Finally, if MS is used for detection, matrix effects—that is the effect of any compounds present, in this case, in plasma on the detection of the compound of interest—must be explored as this method of detection is vulnerable to interferences caused by the presence of incidental ions supressing the detection of the analyte of interest. The pre-specified, acceptable values that must be obtained in each of these domains to validate an assay for a particular compound are outlined in the methods section below.(3, 82)

3.2 Objectives

The objective of this study was to validate an assay that adequately measures the concentration of ivacaftor and M1 in plasma according to the EMA guidelines for bioanalytic method validation.

3.3 Materials

The following materials were used in the completion of the experiments described below:

- Ivacaftor reference standard, Selleck Chemicals LLC (purity 99.81%)
- Internal standard (IS) (1-((4-amino-6,7-dimethoxyquinazolin-s-yl)amino-3-((7-methyl-2,3-dihydro-1*H*-inden-4-yl)xoy)propan-2-ol, prepared in-house
- Hydroxymethyl-ivacaftor (M1), prepared in-house
- Dimethyl Sulfoxide (DMSO), Sigma Aldrich, Ireland
- Acetonitrile, Sigma Aldrich, Ireland
- High performance liquid chromatography water, Millipore Ireland
- Vortex mixer, Fisher Scientific, Ireland
- Sonicator, Ultra Sonik 300, Sigma, Ireland
- Phosphate buffer, Sigma Aldrich, Ireland
- Formic acid, Sigma Aldrich, Ireland
- ISOLUTE® SLE+ Supported Liquid Extraction Columns, Biotage, Sweden
- Ritonavir 80mg/mL oral solution, AbbVie
- Ivacaftor 150mg tablets, Vertex Pharmaceuticals®, UK

- GraphPad Prism version 7.2, U.S.A.
- Microsoft® Excel® 2016

3.4 Methods

3.4.1 Liquid chromatography–mass spectrometry system parameters

LC-MS analyses were performed on an Agilent 1100 high performance liquid chromatography (HPLC) system equipped with a G13795 degasser, G1312A BinPump, a G1313A ALS and G1316A column oven (COLCOM) (Agilent, Little Island, Cork). Separation was obtained on an Allure PFP Propyl column (5 µm, 50 x 2.1 mm) Restek (Bellefonte, PA, USA). Mobile phase A consisted of 0.1% formic acid (FA) in water and mobile phase B consisted of 0.1% FA in acetonitrile (ACN). The Agilent LC-MSD settings were as follows: capillary voltage 3500 V, drying gas (N_2) 12 L/min at 350 °C, nebulizer gas (N_2) pressure 50 psi, SIM*m/z* 425, 409 and 393, fragmentor voltage 50 V. Both positive electrospray mode and negative electrospray mode were explored. The injection volume was 20.0 µL, flow rate was 0.4 mL/min and the column temperature was 30°C. Injection volume and column temperature were chosen as per advice from engineers at Restek Corporation. Flow rate of 0.4mL/min is the maximum flow rate possible on the LC-MS system with a high volume of samples and was chosen to optimise run time. A gradient elution of mobile phase A and B was used.

3.4.2 Preparation of stock and working solutions

Ivacaftor powder was dissolved in DMSO and stored in the freezer at -80˚C at a concentration of 1mg/mL and stock solution was made fresh every 6 months as per ivacaftor powder product insert.(83) Working solution was made up in acetonitrile water

(ACN:H_2O) plus 0.1% FA to a concentration of 100µg/mL. ACN:H_2O plus 0.1% FA was chosen as this was also the mobile phase used for separation of analytes on the LCMS column. IS was diluted in DMSO, a suitable universal solvent, and stored at a concentration of 200µg/mL at -80 ˚C for up to 6 months. Working solution of IS was made up in ACN:H_2O 0.1% formic acid to a concentration of 1µg/mL. M1 was stored in methanol, a suitable solvent for the polar M1 molecule, at a concentration of 2µg/mL for up to 6 months at -80 ˚C. Working solution was made up in ACN:H_2O 0.1% formic acid to a concentration of 100µg/mL. All working solutions and phosphate buffer were freshly prepared daily.

3.4.3 Preparation of plasma standards

Pooled whole blood samples were obtained from the Irish Blood Transfusion Services for all experiments except matrix effects and selectivity, which required plasma samples from 6 separate individual volunteers. Plasma obtained from the Irish Blood Transfusion Service was deemed suitable as PWCF, that is those with a chronic illness requiring ongoing treatment with medications, on ivacaftor would not meet the criteria to donate blood in Ireland and therefore this plasma would not contain analyte of interest.(84) For matrix effects experiments, blood was collected from healthy volunteers not on ivacaftor in 5mL EDTA blood-collection tubes. These volunteers were on no other medications, were fasting on collection of samples and none had lipaemic plasma.

Blood samples were centrifuged at 2500 revolutions per minute (RPM) at room temperature for 10 minutes, at which point visual inspection confirmed separation of plasma supernatant, which was then removed, as per local lab procedures.

Plasma samples were spiked with known concentrations of ivacaftor and M1, and IS was added to all samples. These were mixed with the vortex mixer after spiking. These

prepared plasma standards were used to validate the assay for the measurement of ivacaftor and M1 as described below.

3.4.4 Ionisation

Both positive and negative electrospray mode were explored during assay validation.

3.4.5 Ivacaftor assay validation

3.4.5.1 Selectivity

Selectivity was evaluated by analysing 6 blank samples from volunteers not on ivacaftor, 6 samples spiked with 0.125µg/mL ivacaftor, the lower limit of quantification (LLOQ), and 6 samples spiked with IS. Absence of interfering compounds was accepted where the background response in the blank sample was less than 20% of the LLOQ for the analyte and less than 5% for the IS.(3)

3.4.5.2 Carry over

Carry over was assessed by analysis of blank samples after the analysis of samples of high concentration in 6 separate plasma samples. This was considered acceptable if detection of the analyte of interest was less than 20% of the LLOQ in the blank sample after analysis of a sample of high concentration.(3)

3.4.5.3 Lower Limit of Quantification

The LLOQ for this study was 0.125µg/mL. This lower limit was selected as it was sufficiently far enough below the 90% effective concentration (EC_{90}) of ivacaftor published at the time, 0.423 µg/mL. This was considered acceptable if the detection of the analyte at the LLOQ was at least 5 times that of background interference.(85)

3.4.5.4 Calibration Curve

The calibration curve was evaluated over the range 0.125 µg/mL to 8 µg/mL. The weighting factor that gave the smallest absolute values of relative error was used to describe the concentration-response relationship for the calibration curve. The acceptance criterion for each back-calculated standard concentration was within ±15% of the nominal concentration for all values except for the LLOQ for which it should be within ± 20%. Weighting factors of 1/x, 1/y, $1/x^2$ and $1/y^2$ were assessed to ascertain which weighting factor would give the smallest absolute value of relative error.(3)

3.4.5.5 Accuracy and Precision

Accuracy and precision were evaluated using five replicates of spiked samples at four concentration levels (0.125 µg/mL, 0.375 µg/mL, 4 µg/mL, 6 µg/mL) over three analytical runs. Accuracy was expressed as a percentage of the nominal value. As per EMA guidelines, a mean concentration within 15% of the nominal value was deemed acceptable, except for the LLOQ which was acceptable within 20% of the nominal value. The precision of the method was expressed as the CV. The intra-assay and inter-assay CV was deemed acceptable at below 15% except for the LLOQ, which was accepted at below 20%.(3)

3.4.5.6 Matrix Effect

As mass spectrometric methods were used, matrix effects were assessed using 6 lots of blank matrix from individuals not taking ivacaftor. The CV of the normalised matrix factor was deemed acceptable if it was below 15%. As this study was planned for healthy

volunteers it was not anticipated that analysis of lipaemic or haemolysed samples would be necessary, therefore matrix effects with such samples were not explored.(3)

3.4.5.7 Stability

Bench top stability and stability 4°C was assessed by analysing low and high concentration samples (0.375 µg/mL and 6 µg/mL) at 3, 4 and 6 hours at room temperature or 4°C and comparing response to samples processed immediately after spiking. Stability over 24 hours in dry ice was also assessed in a similar manner. Long term stability of the analyte in matrix stored in the freezer was assessed using a bracketing approach with low and high concentration samples (0.375 µg/mL and 6 µg/mL) at -20°C and -80 °C reanalysed in triplicate over 2 months. Samples were considered stable if 85–115% of the response for fresh samples of identical concentration was found on LC-MS analysis at the predetermined incremental timepoints.(3)

3.4.6 Modification of the ivacaftor assay

Once the validation process was complete, exploratory analysis of a small selection of volunteer samples containing ivacaftor was carried out. The method was then modified to reduce the upper limit of quantification to 4µg/mL, the LLOQ to 0.0625 µg/mL and to reduce the run time from 25 minutes to 20 minutes. A partial validation of these changes was then carried our consisting of a repeat of the accuracy and precision experiment as described above.

3.4.7 M1 assay

The validation of the assay for M1 consisted of a partial validation because very limited quantities of the standard of M1 were available. Experiments to validate the M1 LLOQ of 0.625µg/mL, accuracy and precision of the assay, matrix effects and sample stability after 2 months storage in plasma at -20 ˚C and -80˚C were completed as described above. Information regarding the remaining aspects of assay validation, namely benchtop and refrigerator storage of M1 in plasma for up to six hours, was available in the Biopharmaceutical Review of ivacaftor published by the FDA. As this stability is independent of the assay used it was deemed appropriate to use this published information to preserve M1 standard for analysis of samples in the study that follows (see chapter 4).(2)

3.4.8 Analysis of study samples

Samples were collected from volunteers in 5mL EDTA blood-collection tubes either by venepuncture or through an in-dwelling venous cannula. These were centrifuged at 2500 RPM at room temperature for 10 minutes immediately after collection and were aliquoted into fractions of 300µL. These were frozen at -80˚C until analysis.

3.4.9 Sample preparation

Spiked samples or thawed patient samples were mixed 1:1 with 300µL of phosphate buffer, 20µL of IS at a concentration of 1µg/mL was added to each sample and these were mixed with the vortex mixer. Then 550µL was placed on the ISOLUTE®SLE+ supported Liquid Extraction column and drawn onto the column with the application of a vacuum. After 5 minutes columns were washed through with 5mL of methyl tert-butyl ether (TBME). Above sample preparation was undertaken as per the product literature

for the ISOLUTE®SLE+ supported Liquid Extraction column.(86) Samples were then blown to dryness and reconstituted with 200µL of ACN:H2O 1:1 plus 0.1% FA. This was then centrifuged at 18,000 RPM at 4˚C for ten minutes to remove any remaining particulate matter. The supernatant was then transferred to Agilent® autosampler vials with 150µL glass inserts. All patient samples were analysed in duplicate. Low and high concentration quality-control samples were included with each sample run.

3.5 Results

3.5.1 Validation of the method for the quantification of ivacaftor in plasma.

3.5.1.1 Ionisation

Negative electrospray mode was found to give approximately ten times more sensitivity in the detection of ivacaftor and M1; however, it was not suitable for the measurement of the IS. Five samples at low and high concentrations of ivacaftor with IS were measured using negative ionisation. At a concentration of ivacaftor of 3µg/mL interference with the measurement of IS was noted such that the peak area of IS was smaller in the presence of higher concentrations of ivacaftor (see table 8).

Table 8. Peak area of Ivacaftor in the presence of high and low concentrations of ivacaftor measured using negative ionisation.

	Mean peak area of 20µL of IS at a concentration of 1µg/mL ± SD mAU*min n=5
Measured with Ivacaftor 3µg/mL	522422 ± 56071
Measured with Ivacaftor 0.125µg/mL	903444 ± 32843

IS, internal standard; SD, standard deviation.

Therefore, positive electrospray mode was utilised for assay validation.

3.5.1.2 Selectivity

No interfering peaks were found at the retention time of the analyte or IS. Examples of chromatograms of blank plasma and blank plasma spiked with ivacaftor, M1 and IS are shown in figures 5 and 6.

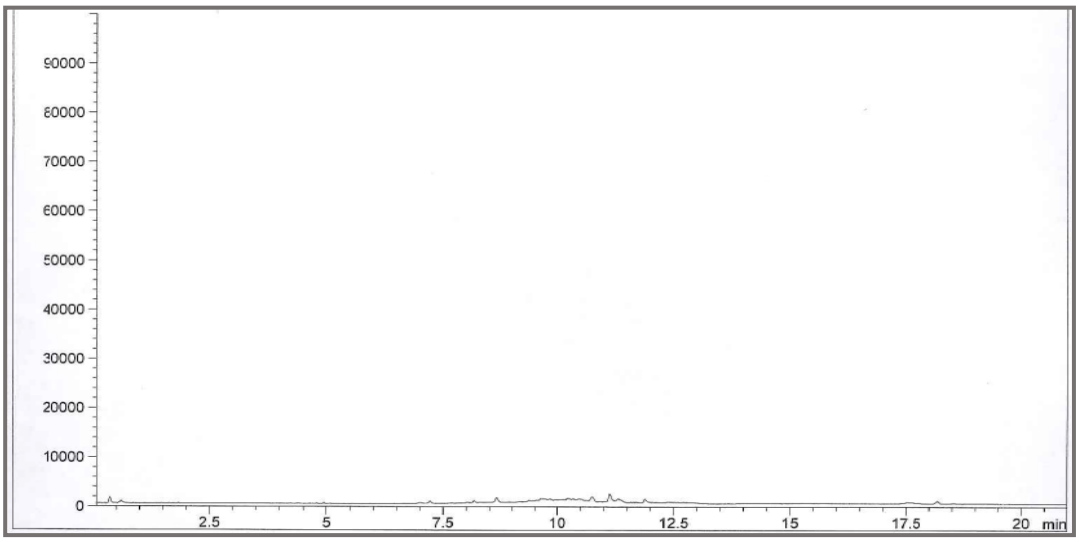


Figure 5. Chromatogram of blank plasma.

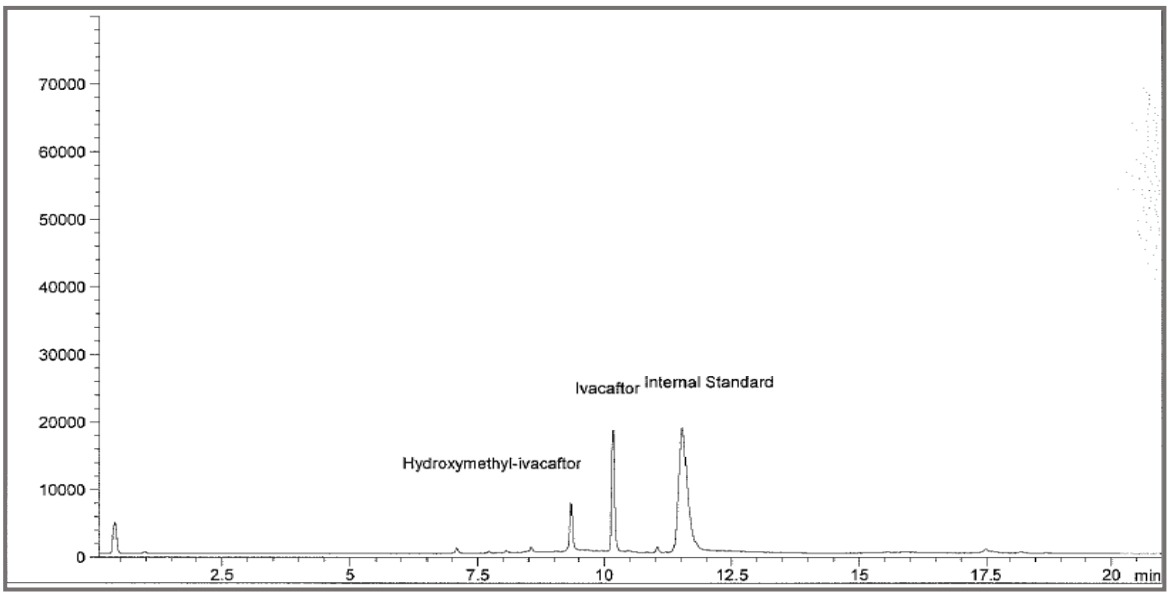


Figure 6. Chromatogram of blank plasma spiked with ivacaftor, M1 and IS.

3.5.1.3 Lower limit of quantification

A LLOQ of 0.125µg/mL was selected. The accuracy and precision of the LLOQ of ivacaftor was within 20% and the baseline interference was less than 20% in all samples analysed as part of the validation process.

3.5.1.4 Linearity of the calibration curve

The calibration curve was found to be linear over the range 0.125 µg/mL to 8 µg/mL. The mean correlation coefficient was 0.9947 ± 0.0026. This was calculated from all calibration curves used in the assay validation process (n=15). Visual inspection shows the lowest mean absolute sum of relative error, the mean absolute sum of residuals and the mean percentage error of the LLOQ is obtained with $1/x^2$ weighting. FDA guidelines dictate that the simplest weighting scheme that best describes the relationship between plasma concentration and instrument response be used. Comparison of 1/x and $1/x^2$ weighting using Student's t-test shows non-significant improvement in mean absolute sum of relative error and mean absolute sum of residuals but a significant improvement in mean error of LLOQ ($p=0.049$). Therefore $1/x^2$ weighting was applied to the calibration curve (see table 9).

Table 9. Mean absolute sum of relative error, mean absolute sum of residuals and mean percentage error of LLOQ for each weighting scheme assessed in 15 calibration curves.

	No weighting	**1/x**	**1/y**	**$1/y^2$**	**$1/x^2$**
Mean absolute sum of relative error % ±SD	52.64 ± 36.38	33.44 ± 22.57	52.56 ± 29.07	64.84± 22.33	27.62 ± 14.30
Mean absolute sum of residuals ±SD	0.32 ± 0.20	0.21± 0.13	0.32 ± 0.16	0.38 ± 0.19	0.17 ± 0.10
Mean error of LLOQ % ±SD	30.84 ± 20.83	20.39 ± 13.76	29.69 ± 17.95	27.47± 12.02	11.57 ± 7.0

LLOQ, lower limit of quantification; SD standard deviation. Data from 15 standard curves measured as part of the validation process.

3.5.1.5 Carry over

No carry over was found in blank samples analysed after samples of a high concentration (3µg/mL) of ivacaftor and M1.

3.5.1.6 Accuracy and precision

Inter-assay and intra-assay accuracy and precision were within 15% and the CV was less than 15% for all concentrations evaluated (see table 10).

Table 10. Accuracy and precision of the measurement of ivacaftor.

Nominal Ivacaftor concentration	**Ivacaftor concentration found ± SD µg/mL**			**Mean percentage of nominal concentration ± SD %**	**CV ± SD %**
	Run 1 n=5	Run 2 n=5	Run 3 n=5		
6 µg/mL	5.866 ± 0.188	5.897 ± 0.139	5.952 ± 0.467	98.42 ±4.692	4.77
4 µg/mL	3.796 ± 0.24	4.598 ± 0.185	4.326 ± 0.281	106.0 ±10.24	9.66
0.375 µg/mL	0.425 ± 0.02	0.358 ± 0.019	0.409 ± 0.019	105.8 ±9.391	8.88
0.125 µg/mL	0.108 ± 0.012	0.099 ± 0.005	0.127 ± 0.008	89.03 ±11.53	12.95

SD, standard deviation; CV, coefficient of variation.

3.5.1.7 Matrix effect

The CV of the normalised matrix factor calculated from 6 lots of matrix was less than 15% (see table 11).

Table 11. Normalised matrix factor and CV of normalised matrix factor.

	0.375 µg/mL	**6 µg/mL**
Mean normalised matrix factor ± SD n=6	0.5780 ± 0.01851	0.9663 ± 0.06594
CV of normalised matrix factor n=6	3.2%	6.82%

CV, coefficient of variation; SD, standard deviation.

3.5.1.8 Stability

Stability was assessed on dry ice for 24 hours to provide for a contingency plan should samples need to be transported from other centres for analysis. Ivacaftor was found to be stable when stored in plasma on dry ice for 24 hours. Stability on dry ice was assessed by calculating the mean concentration of three samples at high and low concentrations (see table 12).

Table 12. Stability of ivacaftor on dry ice for 24 hours.

Nominal Concentration	Mean recovered concentration µg/mL ± SD n=3	% recovery ± SD n=3
0.375 µg/mL	0.365 ± 0.013	97.32 ± 3.34
6 µg/mL	5.248 ± 0.249	87.47 ± 4.15

SD, standard deviation.

Ivacaftor was found to be stable at room temperature and at 4°C and for 6 hours. A ratio of the peak area of ivacaftor to IS was compared at 3, 4, and 6 hours (no calibration curve was run in this analysis) to that of freshly prepared samples (see table 13 and 14).

Table 13. Stability of ivacaftor at room temperature.

Concentration	Ratio of peak area of ivacaftor to IS in fresh samples	Ratio of peak area of ivacaftor to IS at 3 hours (% recovery)	Ratio of peak area of ivacaftor to IS at 4 hours (% recovery)	Ratio of peak area of ivacaftor to IS at 6 hours (% recovery)
0.375µg/mL	0.1769	0.1869 (105.68)	0.1720 (97.28)	0.1901 (107.53)
6 µg/mL	3.4143	3.6795 (107.77)	3.4197 (100.16)	3.4557 (101.22)

IVA, ivacaftor; IS internal standard.

Table 14. Stability of ivacaftor at 4°C

	Ratio of peak area of IVA to IS in fresh samples	Ratio of peak area of IVA to IS at 3 hours (% recovery)	Ratio of peak area of IVA to IS at 4 hours (% recovery)	Ratio of peak area of IVA to IS at 6 hours (% recovery)
0.375µg/mL	0.1769	0.1811 (102.44)	0.1720 (97.28)	0.1759 (99.49)
6 µg/mL	3.4143	3.5270 (103.30)	3.502 (102.57)	3.3066 (96.85)

IVA, ivacaftor; IS internal standard.

A longer-term stability study of ivacaftor was conducted over a 2-month period. Ratio of the peak area of ivacaftor to internal standard of freshly prepared samples was compared to samples stored in the freezer for 2 months. Mean recovery was within ± 15% for each concentration at both -20°C and -80°C at two months (see table 15).

Table 15. Stability of ivacaftor at -20°C and -80°C for two months.

Nominal concentration	Mean ratio of peak area of ivacaftor to IS in fresh samples	Mean ratio of peak area of ivacaftor to IS at 2 months at -80°C (% recovery)	Mean ratio of peak area of ivacaftor to IS at 2 months at -20°C (% recovery)
0.375µg/mL	0.265	0.273 (96)	0.282 (104)
6 µg/mL	3.840	3.768 (102)	3.864 (100)

IS, internal standard.

3.5.1.9 Modification of the ivacaftor assay

Accuracy and precision experiments were repeated as a partial validation after the run time was reduced to 20 minutes and concentration range was changed to 0.0625 µg/mL to 4 µg/mL.

The CV% of the LLOQ was less than 20% and baseline interference less than 20% in all LLOQ samples analysed in the partial validation of the modified assay (see table 16).

Table 16. Accuracy and precision of modified ivacaftor assay.

Nominal Ivacaftor concentration	**Ivacaftor concentration found µg/mL**			**Mean percentage of nominal concentration %**	**Mean CV %**
	Run 1 n=5	Run 2 n=5	Run 3 n=5		
3 µg/mL	2.6 ± 0.324	2.83 ± 0.066	2.86 ± 0.174	92.25 ± 4.63	6.95 ±5.09
2 µg/mL	2.02 ± 0.187	2.15 ± 0.125	2.13 ± 0.076	104.93 ± 3.5	6.2 ± 2.87
0.125 µg/mL	0.142 ± 0.0177	0.144 ± 0.007	0.14 ± 0.023	114.8 ± 1.29	11.03±5.66
0.0625 µg/mL	0.0544 ± 0.0086	0.058 ± 0.004	0.056 ± 0.007	89.55 ± 2.37	11.5 ± 4.43

CV, coefficient of variation.

3.5.2 Validation of the method for the measurement of hydroxymethyl-ivacaftor in plasma

3.5.2.1 Selectivity

No interfering peaks were found at the retention of the analyte or IS. Typical retention time of M1 was 9.4 minutes (see figures 5 and 6).

3.5.2.2 Carry over

No carry over was found in blank samples analysed after samples of a high concentration (3µg/mL) of M1.

3.5.2.3 Lower limit of quantification

A LLOQ of 0.625µg/mL was selected. This lower limit was selected as at half the original LLOQ of ivacaftor it was low enough to detect the predicted lower concentrations of M1

in the presence of ritonavir but not so low that accuracy and precision would not be feasible on the LC-MS system in use. The accuracy and precision of the LLOQ of M1 was within 20% and the baseline interference was less than 20% in all samples.

3.5.2.4 Linearity of the calibration curve

The calibration curve was found to be linear over the range 0.0625 µg/mL to 4 µg/mL. The mean correlation coefficient (±SD) was 0.993 (±0.0057). This was calculated from all calibration curves used in the assay validation process as well as calibration curves subsequently used in sample analysis. Weighting of $1/x^2$, as was applied to the parent compound ivacaftor, was found to be sufficiently accurate and precise in the interpretation of the calibration curve for M1.

3.5.2.5 Accuracy and precision

Inter-assay and intra-assay accuracy and precision were within 15% and the CV was less than 15% for all concentrations evaluated (see table 17).

Table 17. Accuracy and precision of the measurement of M1.

Nominal M1 concentration	**M1 concentration found µg/mL n=5**			**Mean percentage of nominal concentration ± SD %**	**Mean CV ± SD %**
	Run 1	Run 2	Run 3		
3 µg/mL	2.618 ± 0.109	2.709 ± 0.126	2.671 ± 0.108	94.21 ± 6.97	4.45 ± 0.36
2 µg/mL	2.099 ± 0.083	2.17 ± 0.119	2.013 ± 0.075	104.68 ± 4.62	4.39 ± 0.97
0.125 µg/mL	0.144 ± 0.016	0.142 ± 0.014	0.144 ± 0.011	114.85 ± 10.95	9.54 ± 1.71
0.0625 µg/mL	0.06 ± 0.006	0.061 ± 0.005	0.056 ± 0.003	94.21 ± 6.79	7.35 ± 2.32

M1, hydroxymethyl-ivacaftor.

3.5.2.6 Matrix effect

The CV of the normalised matrix factor calculated from 6 lots of matrix was no greater than 15% (see table 18).

Table 18. Normalised matrix factor and CV of normalised matrix factor

	0.125 µg/mL	**3 µg/mL**
Mean normalised matrix factor n=6	0.99	0.96
CV of normalised matrix factor	2.17%	2.2%

CV, coefficient of variation.

3.5.2.7 Stability

A stability study of M1 was conducted over a 2-month period. Stability was expressed as a percentage of M1 peak area of extracted plasma stability standard over the M1 peak area of the equivalent freshly-prepared plasma stability standard. Mean recovery was within ± 15% for each concentration at both -20°C and -80°C at two months (see table 19).

Table 19. Percentage recovery of M1 for two months stored at -20°C and -80°C.

Nominal concentration	**M1 concentration recovery % n=3**	
	2 months at -80°C	**2 months at-20°C**
3 µg/mL	107.65 ± 1.3%	103.12 ± 5.7%
0.125 µg/mL	94.37 ± 3.8	97.3 ± 8.4%

M1, hydroxymethyl-ivacaftor.

3.6 Discussion, limitations and conclusion.

The above data show the establishment of an adequately functioning assay for the measurement of ivacaftor and M1 in plasma. The accuracy of both assays was within 15%, precision within 15% and the LLOQ was accurately measured without interference.

There was no interference in mass spectrometric methods from the matrix. $1/x^2$ weighting was found to accurately describe the relationship between plasma sample concentration and instrument response. Plasma samples of ivacaftor and M1 were found to be stable in the freezer up to 2 months and ivacaftor was found stable on the benchtop and in the fridge at 4°C for up to 6 hours. Given the very limited amounts of M1 available benchtop stability data from M1 was taken from the assay details published by Vertex®.(2) The number of spiked samples and analytical runs analysed in this study meets the criteria laid out by the EMA to show an assay is reliable and reproducible. There is, however, limited information available in the literature regarding assay validation for ivacaftor at present. In 2016, after the development of this assay, another ivacaftor assay was reported in the literature, developed by Schneider et al. This more recently developed assay has the advantage of also measuring lumacaftor, a small molecule developed recently for combination treatment with ivacaftor for those with the F508 mutation, in addition to measuring in sputum as well as plasma. It was interesting to note however that this more recent assay does not ultilise an internal standard, and therefore plasma concentrations measured could be open to variability in sample preparation, which is not the case for the assay presented here.(87) There is a small amount of information regarding the ivacaftor assay developed by Vertex Pharmaceuticals in the course of the clinical trials of ivacaftor available in the biopharmaceutical review of ivacaftor published by the FDA. The assay reported in this publication used a similar sample preparation method with liquid: liquid extraction with TBME, column chromatography and mass spectrometry for detection. This assay was more sensitive than the one presented here and concentrations as low as 2ng were detected. The range of the assay was more limited, from 2ng to 2µg, with the utilization of dilution for samples of higher concentrations but curve weighting was the same at

$1/x^2$. A strength of the Vertex assay is also the measurement of M6, the second most abundant metabolite of ivacaftor. This was not included in the study above as while M6 has relevance to PK studies of ivacaftor, it has negligible pharmacological activity as opposed to M1 which has one sixth of the pharmacological activity of ivacaftor. The assay reported by Vertex Pharmaceuticals also provides reassurance that there is no interference in measurement of ivacaftor or M1 from the M6 metabolite. An internal standard was used, but it is not specified what molecule this internal standard is.(2) Deuterated ivacaftor and M1 may perform better as an IS than the molecule in the study presented here. Use of deuterated molecules may also allow for the development of a more sensitive assay using negative ionization.(88)

Interference from concomitant medications was not studied in this assay validation. As this assay was intended to be used purely for the measurement of plasma samples containing ivacaftor from healthy volunteers known to be on no other medications other than ritonavir this was considered acceptable in this case. Ritonavir was not expected to cause appreciable interference with the detection of ivacaftor, M1 or internal standard as it has a molecular weight different to that of all compounds measured here and it is expected to elute from the column at a different timepoint given its chemical structure. It is not reported in either the ivacaftor biopharmaceutical review or in the paper describing the assay developed by Schneider et al whether there was any exploration for interference from typical concomitant medications found in plasma of PWCF,(2, 87) which may warrant further exploration, particularly should therapeutic drug monitoring be considered in the future.

The assay described here adequately measures the concentration of ivacaftor and M1 in plasma. This assay is used for the measurement of ivacaftor and M1 in the plasma

samples of volunteers taking ivacaftor in the absence and presence of ritonavir as described in chapter 4.

CHAPTER 4

4 Clinical Trial: The Pharmacokinetic Interaction between Ivacaftor and Ritonavir in Healthy Volunteers

4.1 Introduction.

At time of commencement of this clinical trial no data were available in the literature on the interaction between ivacaftor and ritonavir. Studies of drug interactions in vivo are usually carried out in a healthy volunteer population. This study was carried out in healthy volunteers as per the EMA guideline on the investigation of drug interactions that recommends that drug interaction studies be carried out in healthy volunteers unless there is clinical concern regarding toxicity of the investigational products,(89) a concern that is not present for ivacaftor or ritonavir at the doses used in this study.(2, 90) In phase I studies, the characteristics of drug interactions are generally assumed to be similar in healthy volunteers and the target patient population for a drug.(89) The present study is strengthened by the availability of data from phase I and II studies of ivacaftor that show no significant differences in PK parameters between populations of healthy volunteers and PWCF.(2) The definition of 'healthy volunteer' is not consistent and detailed in the literature. The Royal College of Physicians in the UK defined a healthy volunteer as an 'individual who is not known to suffer of any significant illness relevant to the proposed study, who should be within the ordinary range of body measurements, such as weight, and whose mental state is such that he is able to understand and give valid consent to the study' in 1986 and this definition has not been updated.(91) The EMA presents a broad definition for healthy volunteers in PK studies: 'fasting, healthy, adult volunteers, in well-defined and controlled conditions'.(92) Thus the definition of a healthy volunteer can be tailored to the particular purpose of the trial and the nature of the investigational product as seen fit by the investigator, assuming these criteria are

found acceptable to the regulator.(93) In Ireland, the HPRA provide a suggested list of inclusion and exclusion criteria for healthy volunteers in their clinical trial protocol template,(94) and these are the basis for the inclusion and exclusion criteria in the present study.

EMA guidelines recommended that basic PK parameters, C_{max}, T_{max}, AUC, CL, $T_{1/2}$ and C_{trough} be reported for the investigational drug with and without the coadministration of the interacting drug. There are no prescribed data analysis methods to determine these parameters, that is, it is not stated that NCA or compartmental analysis is preferred in these guidelines. In contrast, the FDA Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry draft guideline states that NCA should be employed to estimate pharmacokinetic parameters in drug interaction studies.(95) NCA is usually favoured when the primary goal is to determine the exposure to and elimination of a compound, as is the case with drug interactions studies, as this method of analysis requires the least number of assumptions to be made with regard to the data.(32)

Ritonavir demonstrates multiple complex interactions with many drugs, some of which are discussed above (see chapter 2). This complexity arises from the different models of inhibition that have been demonstrated—mechanism-based, competitive or mixed competitive-noncompetitive CYP3A4 inactivation—(54-57) in addition to the induction effects of ritonavir.(59) The time dependencies of these interactions must be taken into account when designing an interaction study. The point of maximum effect of induction or inhibition must be estimated and the interaction assessed at this time.(89) It has been show that the magnitude of inhibition of metabolism of other substrates by ritonavir is equivalent in concomitant administration of both drugs compared to pre-treatment with ritonavir.(54) In contrast, it has been shown that the induction effect of ritonavir is

maximal after two weeks of daily administration of ritonavir.(54) The washout period required between studies must also be estimated based on the known pharmacokinetic parameters of each drug. The time-course for enzyme recovery to baseline post treatment with ritonavir has been shown to be approximately 3 days in a study exploring the effect of coadministration of triazolam with ritonavir.(96) This information, along with the known pharmacokinetic parameters of ritonavir and ivacaftor in isolation can be used to estimate the required washout period, but analysis of plasma samples in each PK study to show absence of both drugs at time zero is also required to ensure no carry-over from one study to the next.

4.2 Study objectives

The primary objective of this study was to investigate (i) the PK interaction between ivacaftor 150mg single dose and ritonavir 50mg on days 0, 1, 2 and 3 and (ii) the PK interaction between ivacaftor 150mg single dose and ritonavir daily after at least two weeks of continuous ritonavir treatment at 50mg daily to determine the effect of concomitant ritonavir treatment on $T_{1/2}$, $C_{max,}$, T_{max}, C_{trough}, AUC, clearance and V_d of ivacaftor and its major metabolite M1. The trial was designed such that if 50mg ritonavir did not result in a significant increase in ivacaftor $T_{1/2}$ the study could be repeated with ritonavir at a dose of 100mg.

Other objectives were investigation of whether treatment with ivacaftor in conjunction with ritonavir decreases the inter-individual variability in the PK parameters of ivacaftor and its major active metabolite M1 and to evaluate the effect that ritonavir treatment for two weeks has on fasting serum total cholesterol (TC), high-density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG) concentrations. (Measurement of

serum lipids was undertaken as derangement of lipids has been report as an adverse effect of ritonavir treatment.(97))

4.3 Methods

4.3.1 Clinical trial approval.

The clinical trial protocol entitled 'An Investigation of the Pharmacokinetic Interaction between Ritonavir (Norvir®) and Ivacaftor (Kalydeco®) in Healthy Volunteers' was submitted to the Health Products Regulatory Agency (HPRA). Version 4 of the protocol was approved on May 1, 2015. The trial was assigned a EudraCT number 2015-000483-34. Ethical approval for the trial was granted by the joint AMNCH and St James's Research Ethics Committee on May 11, 2015. The trial was funded by the Department of Pharmacology and Therapeutics, Trinity College Dublin. The principle investigator of the trial was the author, Dr Anne Marie Liddy and the sponsor was Prof Michael Barry. This study was conducted in accordance with the approved protocol, the International Conference on Harmonization (ICH), Good Clinical Practice (GCP) and the ethical principles underlying European Union Directive 2001/20/EC and 2005/28/EC. The trial was open to internal or external auditing and inspection procedures to ensure adherence to GCP.

4.3.2 Selection of study population

Healthy volunteers over the age of 18 able to give informed consent who were not on any regular medications were recruited for this trial. Recruitment was facilitated by placing poster advertisements in the Trinity College Campus and in St James's Hospital in July–September 2015. Detailed inclusion and exclusion criteria are contained in figure 7.

INCLUSION CRITERIA

- Able and willing to give written informed consent and to comply with the requirements of the study protocol.
- Aged 18 years or older.
- Judged to be in generally good health by the investigator based upon the results of the medical history, laboratory tests and physical examination (electrocardiogram [ECG] was not performed as neither ritonavir or ivacaftor have been associated with clinically significant increases in the QTc interval[23] laboratory tests consisted of full blood count, liver and renal function, lipid profile, HIV and hepatitis serology).
- If female and of child-bearing potential and if male with partner of child-bearing potential, willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study day (this is based on the predicted $T_{1/2}$ of ivacaftor in the presence of a strong inhibitor of CYP3A4 of 84 hours, as per dose adjustment recommended for co-administration of ivacaftor with ketoconazole and the elimination of a drug from circulation over 5 half-lives). Barrier or other non-hormonal methods of contraception must be used by women of childbearing potential as ritonavir can change the uterine bleeding profile and reduce the effectiveness of oestradiol-containing contraceptives.
- Female subjects – urine pregnancy test at screening must be negative.
- HIV negative status.
- Clinically acceptable laboratory parameters within 6 weeks prior to enrolment.

EXCLUSION CRITERIA

- Allergy/sensitivity to study medications or their ingredients.
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.
- Subjects who have participated in another study and received any other investigational agent within the time-frame for the PK elimination of that investigational agent.
- Subjects unable to provide written informed consent.
- Subjects who have any other significant disease or disorder which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
- Subjects who have a history of drug or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive, hepatitis B or C or HIV infection.
- Concurrent malignancy.
- Subjects requiring the chronic administration of medications throughout the time period of the study.
- AST or ALT ≥ 3 x upper limit of normal (ULN).
- Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula.

Figure 7. Clinical trial inclusion and exclusion criteria.

Volunteers had to meet criteria at screening and again at each visit. Healthy volunteer criteria were derived from the HPRA clinical trial protocol template.(94)

4.3.3 Trial design

This was a single-centre, open-label, sequential, crossover study. A balanced trial design was achieved by randomizing patients into three sequences of three periods representing a Latin square (each intervention, study A, B or C, is represented in each sequence and each period). This allowed for the adjustment of a potential period effect due to the sequential design of the trial.

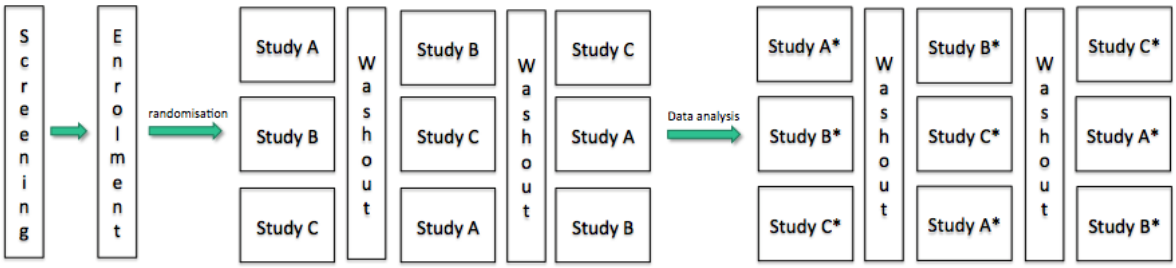


Figure 8. Study schema.

The interaction between ritonavir and ivacaftor was assessed using a 50mg dose of ritonavir on days 0, 1, 2, 3 both with ritonavir starting on day 0 along with the administration of ivacaftor and ritonavir starting two weeks prior to the administration of ivacaftor. If this dose of ritonavir did not result in a significant change in the PK parameters of ivacaftor and M1, the trial was designed to continue on to assess the effect of ritonavir 100mg in a similar manner. A washout period of 5 days was allowed for profiles with ivacaftor alone. A washout period of at least 10 days was allowed to elapse between PK sampling days with ritonavir and Ivacaftor combined to ensure that plasma Ivacaftor levels were negligible at the beginning of each study. These washout periods were based on both the concept that a drug is eliminated from the circulation

over five half-lives and that CYP3A4 inhibited by ritonavir recovers to baseline in 3 days.(96)

4.3.4 Detailed overview of study assessments and procedures

Figure 9 outlines the schedule of events throughout the study. Informed consent was obtained prior to any study procedures being carried out. The principle investigator, Dr Anne Marie Liddy obtained informed consent. The nature and purpose of the study was explained, and all questions related to the study answered. Prior to any study-related screening procedures being performed on the subject, the informed consent statement was reviewed, signed and dated by the subject and the principle investigator. Subjects were made aware that consent could be withdrawn at any time during the study.

The duration of the complete study was at least 10 weeks. Patients made 16 visits to the clinical trial centre, including screening and enrolment. PK profile sampling days consisted of 12 hours sampling for the PK profile of ivacaftor alone and for the PK profiles in the presence of ritonavir there were 12 hours in-house sampling plus return visits for sampling at 24, 36, 48, 72 and 84 hours (see figure 9).

Figure 9. Schedule of events.

Procedures	Visit 1 Screen	Visit 2 Baseline	Study A	Study B	Study C	Study C
Inclusion/Exclusion Criteria	x	x	x	x	x	x
Informed consent		x				
Medical history & examination		x				
Laboratory tests		x				
Pregnancy test		x				
Concomitant medications		x				
Fasting lipids		x				x
Observed administration of Ivacaftor 150mg one dose and PK profile			x			
Observed administration of Ivacaftor 150mg one dose plus ritonavir 50mg (or 100mg) and PK profile				x		
Administration of ritonavir 50mg (or 100mg) daily for two weeks					x	

4.3.5 Data collection

4.3.5.1 Demographics

The date of birth, gender and race were recorded at screening

4.3.5.2 Medical and Surgical History

Patients were assessed for any current or past medical or surgical history that may preclude them from the study due to either unacceptable risk to the volunteer's health or likelihood that this history will cause undue influence on the study results.

4.3.5.3 Concomitant Medication

All over-the-counter or prescription medication, vitamins and/or herbal supplements were recorded on the case report form (CRF). The indication for treatments was recorded. Ongoing treatment with medication resulted in exclusion from the clinical trial.

4.3.5.4 Physical Examination

The complete physical examination included the evaluation of the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, neurological systems. Height and weight were also recorded. Blood pressure, temperature, heart rate, and respiratory rate were recorded for all subjects at each visit.

4.3.5.5 Clinical Laboratory Tests

The following laboratory tests were completed at baseline:

- Haematology: haemoglobin, white cell count, red cell count and platelet count.
- Biochemistry: urea, creatinine, sodium, potassium, alkaline phosphatase, gamma glutamyl transferase, alanine transaminase and aspartate transaminase.

- Urine drug screen for cannabis, cocaine and opioids.
- HIV and hepatitis screen.
- Lipid profile: TC, LDL, HDL and TG.

All laboratory results were reviewed, and the reports signed by the investigator who recorded these in the CRF as normal, abnormal but not clinically significant, or abnormal and clinically significant. Any abnormal and clinically significant test results were referred to the volunteer's general practitioner or the appropriate hospital specialist.

4.3.5.6 Pregnancy Tests

Urine pregnancy test in women of childbearing potential was performed at screening.

4.3.5.7 Pharmacokinetic Profiles

For the PK profile of ivacaftor alone a cannula was inserted at time 0 (administration of ivacaftor) and samples drawn from this at time 0, 1, 2, 3, 4, 6, 8, 10 and 12 hours, equivalent to one standard dosing interval of ivacaftor alone. Standardised meals were given to all patients over this time.

For the PK profiles involving ivacaftor and ritonavir a cannula was placed at time 0 (administration of ivacaftor and ritonavir) and samples were taken from this at times 0, 1, 2, 3, 4, 6, 8, 10 and 12 hours. Standardised meals were given over this time. The cannula was then removed, and volunteers were asked to return to the study centre at times 24, 36, 48, 72 and 84 hours for collection of further plasma samples by venepuncture. Sampling time was extended to 84 hours in studies B & C to allow for adequate collection of samples in the terminal phase should the $T_{1/2}$ of ivacaftor be increased several-fold by ritonavir.

4.3.5.7.1 Standardised meal

Participants all received standardised meals for the first 12 hours of each study. This consisted of breakfast, lunch, dinner and two snacks. Table 20 shows the contents of each meal and snack. One participant did not eat ham for cultural reasons and for these meals ham was replaced with turkey.

Table 20. Meal schedule.

Timing	Breakfast Time 0	Snack 1	Lunch	Dinner	Snack 2
Meal contents	50mg Cornflakes 125ml full fat milk on cereal 250ml full fat milk to drink Large toffee muffin	125g full-fat yoghurt	Sandwich with ham and turkey on brown bread 1 packet crisps 250mg full fat milk	Medium salad with ham, 1 egg, 50mg coleslaw, lettuce and tomato 1 large bap with 50mg butter 1 chocolate pastry with full fat cream	Small cup of tea with milk 2 chocolate shortbread biscuits

4.3.6 Determination of sample size

Recruitment of n=12 subjects was determined to be adequate for this study. The primary outcome of this study was the comparison of $T_{1/2}$ of ivacaftor when ivacaftor was administered alone and when administered with ritonavir. The test statistic of interest was the mean difference in $T_{1/2}$ between these paired groups. A power analysis was conducted in R using the 'power.t.test' function. For a given effect, standard deviation and error rates (type I and II), this function calculates the number of patients needed to detect this effect within the given error rates. Type I error was set at 5%, type II at 10%. A minimum of a twofold increase in $T_{1/2}$ of ivacaftor when given in conjunction with ritonavir was expected, as a conservative estimation. The $T_{1/2}$ of ivacaftor in the literature is approximately 12 hours;(12) a $T_{1/2}$ of at least 24 hours was expected in the ritonavir group, which yields an effect size of 12 hours. Unfortunately, the literature does not report a standard deviation for the $T_{1/2}$ of ivacaftor. As a conservative assumption, the

standard deviation of the mean difference was set to 9 hours. Based on these inputs, the power analysis in R estimated that n=8 subjects were needed to have 90% power to detect a twofold increase in $T_{1/2}$ of ivacaftor with a type I error rate of 5%.

Due to the minimally invasive nature of this trial, a small drop-out rate was expected. However, to remain cautious and due to the small size of the study, we accounted for a 33% dropout rate in the sample size calculation. A sample size of n=12 also allowed for a balanced crossover design with 3 groups and 4 volunteers in each. We therefore proposed to recruit n=12 patients for this study. In the event of drop out of study participant(s), it was decided that the interaction effect would be calculated and presented with and without the incomplete data attained from participants who did not complete the study.

The study was also powered to detect the following effects in secondary outcome measures (type I error set at 5%, type II error set at 10%):

- A 1.5-fold increase in C_{max} of ivacaftor from 0.768 µg/mL to 1.152 µg/mL, assuming a standard deviation of 0.233 µg/mL.
- A 50% decrease in C_{max} of the M1 from 1.696 µg/mL to 848 µg/mL, assuming a standard deviation of 100µg/mL.
- A 1.5-fold increase in $T_{1/2}$ of the M1 from 21.2 hours to 31.8 hours, assuming a standard deviation of 9 hours.

4.3.7 End of trial

The trial was designed to terminate prematurely should significant inhibition (that is a two-fold increase in the $T_{1/2}$ of ivacaftor) with a 50mg dose of ritonavir be achieved. Premature termination of the study had to be mutually agreed upon by the principle investigator and the sponsor. It was also agreed that the trial should terminate

prematurely if any safety concerns arose at any point during the study. Termination was also deemed appropriate if the study conduct (for example recruitment rate, data quality, protocol compliance, drop-out rate) did not suggest completion of the trial with numbers allowing acceptable significance of results within a reasonable time frame.

4.3.7.1 Discontinuation/withdrawal of subjects from the study protocol

Subjects had the right to voluntarily withdraw from the study at any time for any reason without consequences. The investigator also had the right to discontinue a subject from the study treatment or withdraw a subject from the study at any time if it was deemed in the best interest of the subject.

Subjects were obliged to discontinue the investigational medicinal products and be withdrawn from the study in the following circumstances:

- withdrawal of consent by the subject
- any medical condition that the investigator or sponsor determines may jeopardize the subject's safety if she or he continues receiving the study treatment
- pregnancy
- ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- AE which requires discontinuation of the study medication
- lack of compliance with the study and/or study procedures (for example, dosing instructions, study visits)
- lost to follow-up after three documented attempts to contact the subject

4.3.8 Storage and disposition of study treatments

Both ivacaftor and ritonavir were stored as per the product SPC throughout the study. Ivacaftor was stored below 30˚C and ritonavir was stored below 25˚C in a tightly closed bottle. Study treatments were stored and locked in a secure cabinet until they were dispensed for subject use. Temperature of the storage unit was assessed by research nurses in the Department of Pharmacology and Therapeutics daily.

4.3.9 Overdose of study treatment

Protocols to deal with overdose of either study treatment were available on TOXBASE®.

4.3.10 Safety Assessment

During the study, safety monitoring consisted of reports of AEs from volunteers, vital signs monitoring, physical examination and laboratory measurement of lipids. The known side-effect profiles of ivacaftor and ritonavir are available in the respective SPC for each product. Any new information regarding AEs that was discovered during the trial was reported to the HPRA and the manufacturer. This information was also contemporaneously reported to the participants in the trial.

4.3.11 Safety Reporting

Safety reporting was planned as per the standards dictated by the HPRA.

4.3.11.1 Definitions

Pre-defined definitions of safety events were outlined in detail in the clinical trial protocol as per the HPRA guidelines.

4.3.11.1.1 Adverse event

An AE is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

4.3.11.1.2 Adverse reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose. The phrase 'responses to a medicinal product' means that a causal relationship between a study medication and an AE is at least a reasonable possibility, that is the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as ARs.

4.3.11.1.3 Serious adverse event (SAE)

Any untoward medical occurrence or affect that at any dose:

- results in death,
- is life-threatening*,
- requires hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect
- important medical events**

*Regarding a life-threatening event, this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

**Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events (hereinafter referred to as 'important medical events') should also be considered as 'serious' in accordance with the definition.

4.3.11.1.4 Suspected unexpected serious adverse reactions

An AR, the nature or severity of which is not consistent with the applicable product information (for example the investigator's brochure for an unauthorised investigational medicinal product or SPC for an authorised medicinal product).

4.3.11.2 Evaluation of adverse events and serious adverse events

4.3.11.2.1 Assessment of seriousness

Medical and scientific judgement was exercised in deciding whether an event is serious in accordance with the above criteria.

4.3.11.2.2 Assessment of causality

All AEs judged by either the investigator or the sponsor as having a reasonable suspected causal relationship to an investigational medicinal product qualified as ARs. The investigator/sponsor made an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions:

- Unrelated: where an event is not considered to be related to the study medication.

- Possibly: Although a relationship to the study medication cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship make other explanations possible.
- Probably: The temporal relationship and absence of a more likely explanation suggest the event could be related to the study medication.

All AEs/SAEs judged as having a reasonable suspected causal relationship (for example possibly, probably) to the study medication were considered as ARs/SARs. All AEs/SAEs judged as being related (for example possibly, probably) to an interaction between the study medication and another medication were also considered to be ARs/SAR. Alternative causes such as natural history of the underlying disease, concomitant therapy, other risk factors and the temporal relationship of the event to the treatment were also considered.

4.3.11.2.3 Assessment of severity

The investigator made an assessment of severity for each AE/SAE and recorded this on the CRF according to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with every day activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities.

4.3.11.2.4 Assessment of expectedness

The expectedness of an AR was determined by the sponsor according to the information available of both investigational medicinal products in their respective SPCs.

4.3.11.3 Reporting procedures for all adverse events

All AEs occurring during the study observed by the investigator or reported by the subject, whether or not attributed to the study medication, were recorded on the CRF. The following information was recorded: description, date of onset and end date, severity, assessment of relatedness to the study medication, other suspect medication or device and action taken. AEs considered related to the study medication as judged by an investigator or the sponsor were followed until resolution or until the event was considered stable. It was planned that any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed-up for congenital abnormality.

4.3.11.4 Reporting procedures for serious adverse events

Reporting protocols for AEs were put in place prior to commencement of the trial. It was planned that the investigator reports all serious AEs immediately to the sponsor. The immediate report would then be followed by detailed, written reports. The immediate and follow-up reports identify subjects by unique code numbers assigned to the latter. The immediate report should be made by the investigator within a very short period of time and under no circumstances should this exceed 24 hours following knowledge of the serious AE. All SAE information must be recorded on an SAE forms and sent expeditiously to the sponsor. Additional information received for a case (follow-up or corrections to the original case) needed to be detailed on a new SAE form and sent expeditiously to the sponsor.

The sponsor kept detailed records of all AEs which were reported to him by the investigator or investigators. It was planned that the sponsor reports all SUSARs to the

competent authorities (the HPRA in Ireland) and the ethics committees concerned, with fatal or life-threatening SUSARs reported within 7 days, SUSARs which are not fatal and not life-threatening reported within 15 days. The sponsor was also obliged to inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

4.3.11.5 Data safety monitoring board (DSMB)

A DSMB is usually employed for studies that are conducted on multiple sites and that involve outcomes of major morbidity and mortality.(98) As this study was conducted in a single centre, the medicines being used in the trial were already in common clinical use and the primary endpoints were pharmacokinetic, a DSMB was not established.

4.3.12 Data handling

Volunteer data was collected in hard copy format and then stored in a password-protected file in the Trinity Centre for Health Sciences, St James's Hospital. Data generated from sample analysis was stored in a password-protected file in the Trinity Centre for Health Sciences, St James's Hospital. Data was coded and only Dr Anne Marie Liddy and Prof Michael Barry had access to the key to re-identify the data. Hard copies of data were stored in a locked filing cabinet in the Trinity Centre for Health Sciences or in St James's Hospital. All information obtained in connection with this study remains confidential. Participants will not be identified in any publication or public presentation of data from the study. Source documents for this study consisted of data collection forms and participant medical records kept for the purpose of the clinical trial (participants were healthy volunteers, so many had no established medical record in this or any other healthcare institution) and results of analysis of plasma samples which are

stored on a computer (as coded data) in the laboratory in the Department of Pharmacology and Therapeutics. These documents contained data that were also stored on the CRFs. On all study-specific documents other than the signed consent, the subject was referred to by the study subject code. Data will be retained for the length of the study and for ten years after the completion of the study as the Trinity College Dublin policy on good research practice. Ten years after study completion all electronic files will be deleted from the secure server and all hard copies of data will be shredded in the Department of Pharmacology and Therapeutics, Trinity Centre for Health Sciences, Trinity College Dublin. In the event that trial-related monitoring was undertaken, direct access was agreed to be granted to authorised representatives from the sponsor, host institution and the regulatory authorities.

4.3.13 Data analysis

Formal statistical advice was provided by Dr Susanne Schmitz and Professor Cathal Walsh from the National Centre for Pharmacoeconomics, Ireland. Demographic data relating to the volunteers in the trial were reported descriptively and using means ± SD. Median (IQR) values for plasma lipids obtained before and after two weeks of ritonavir treatment were reported and the Wilcoxon signed rank test was used to compare these values to assess for any significant difference. All samples taken at the times specified in the trial protocol were analysed using the LC-MS assay described above. Each sample was analysed in duplicate and the mean ratio of analyte to IS was used to calculate the concentration of the sample from the standard curve.

PK profiles from each study for each individual volunteer were then analysed in WinNonlin version 6.4, Pharsight, U.S.A.® using NCA analysis. Values for $T_{1/2}$, clearance, area under the time–concentration curve from time 0 to 12 hours ($AUC_{0\text{-}12}$), $AUC_{0\text{-inf obv}}$,

V_d, C_{max} and T_{max} were obtained and as PK data is typically non-normally distributed the median for each of these parameters is the summary statistic reported. All data were log transformed and compared between studies using the Wilcoxon signed-rank test. Log transformation of pharmacokinetic data is recommended as the primary comparison of interest is the ratio rather than the difference between the means of the parameters of interest.(99) Given the small size of the group, data were assumed to be non-normally distributed even after log transformation and were analysed using the non-parametric Wilcoxon signed-rank test. The geometric mean for each parameter in each study was reported along with the GMR between each of study A, B and C. As an enzyme inhibitor is expected to increase a drug concentration by a certain percentage rather than by a certain amount, the geometric mean is a most appropriate summary statistic.(100) The CV for each of these parameters was also reported. The effect of mealtimes on plasma concentrations of ivacaftor was also described. A comparison of the data obtained in this study was made to the data published for single doses of ivacaftor up to 800mg. The main PK parameters for M1 were also reported and analysed as for the parent compound.

4.4 Results

4.4.1 Participants

15 participants in total were recruited for the clinical trial (baseline features in table 21). 11 volunteers recruited were white European and one was Asian in origin and 2 were female. Participant number 1 was excluded at screening due to vasovagal syncope secondary to venepuncture. Participants 7 and 8 withdrew consent after screening prior to the first study. The 12 other participants met all inclusion criteria and all completed

studies A, B and C. Therefore 12 participants, volunteers 2–6 and 9–15, ultimately contributed PK data to the study.

Table 21. Volunteer characteristics,n=12, 2 female.

	Weight Kg	Height m	BMI kg/m^2	Age years
Mean ± SD	71.25 ± 13.93	1.78 ± 0.1	23.07 ± 3.62	23 ± 3.99

SD, standard deviation.

4.4.2 Adverse events

3 participants reported mild nausea while taking ritonavir 50mg daily for two weeks. One participant reported numbness at the tip of the index finger lasting for approximately 5 days while on ritonavir for 14 days. This resolved spontaneously. One participant reported coryza while on ivacaftor during studies B and C. These events were classified as mild and expected AEs and were reported to the manufacturer and the HPRA. No other AEs of any other classification occurred. One volunteer found ritonavir liquid unpalatable; this was resolved by taking ritonavir liquid with a small amount of chocolate milk as per instructions in SPC for Norvir® liquid.(50)

4.4.2.1 Lipids

Fasting lipid profiles before and after treatment with ritonavir for two weeks were available for 11 out of 12 participants. One volunteer was not fasting at screening and therefore did not have a fasting lipid profile available before treatment. Using the Wilcoxon signed ran test, no significant difference was seen in total cholesterol or HDL before and after treatment with ritonavir (p=0.5771 and p=0.22207 respectively) but a statistically significant difference was seen in LDL and TG before and after ritonavir 50mg

for two weeks (p=0.0217 and 0.0410 respectively). These differences, while statistically significant, may not be of clinical significance given their small magnitude.

Table 22. Mean lipid profile before and after ritonavir 50mg for two weeks.

	Before ritonavir mmol/L Median (IQR)	After ritonavir 50mg daily for 2 weeks mmol/L Median (IQR)	Significant difference
Total cholesterol	4.43 (3.92–4.72)	4.52 (3.95–4.72)	No (p=0.5771)
HDL	1.62 (1.34–1.75)	1.37 (1.2–1.79)	No (p=0.2207)
LDL	2.24 (2.07–2.66)	2.85 (2.26–3.15)	Yes (p=0.0217)
TG	0.98 (0.58–1.14)	0.74 (0.63–0.78)	Yes (p=0.0410)

IQR, interquartile range; HDL, high density lipoprotein; LDL, low density lipoprotein; TG triglycerides.

4.4.3 Non-compartmental pharmacokinetic analysis of ivacaftor with and without concomitant administration of ritonavir

4.4.3.1 Concentration–time curves of ivacaftor

The median and interquartile-range concentration–time profiles of ivacaftor in studies A, B and C are shown in figure 10.

Figures 11, 12 and 13 spaghetti plots show approximately even distribution of PK profiles in study A, one outlier in study B and clustering of profiles in the mid-range in study C with low and high outliers. Volunteer 2 was a male with a BMI of 25.25kg/m^2. In study C volunteer 2 data points were not outliers.

In study C three volunteers that clustered in the high range of the data were a female with a BMI of 22.41 kg/m^2, a male with a BMI of 24.45 kg/m^2 and a male with a BMI of 24.63 kg/m^2. These BMIs were close to the study mean of 23.07 kg/m2. One volunteer whose data was at the bottom of the range of data was Malaysian (all other volunteers were white Irish) and had the highest BMI in the group 32.67kg/m^2.

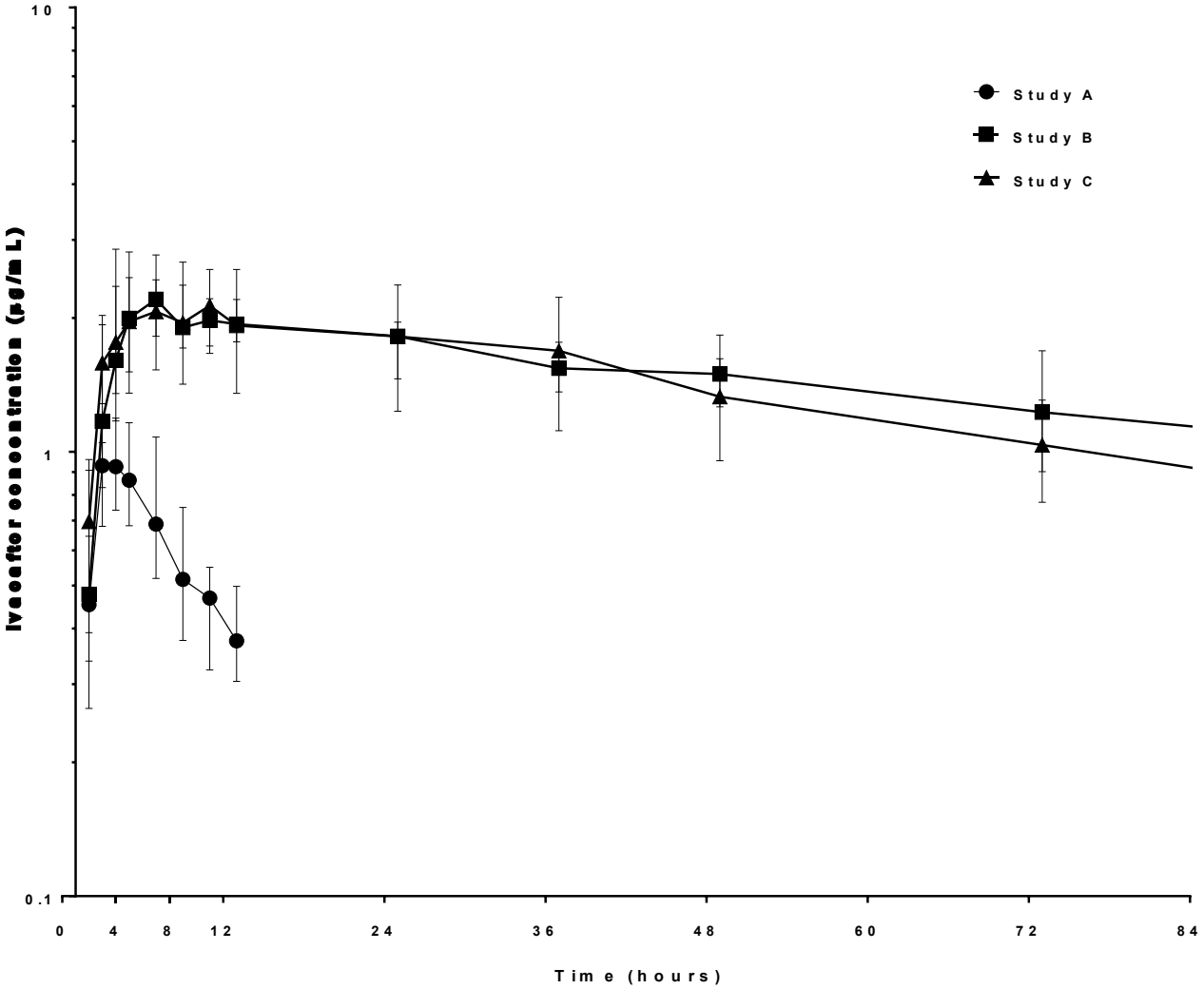


Figure 10. Concentration-time curve of ivacaftor in studies A, B and C (median with IQR)

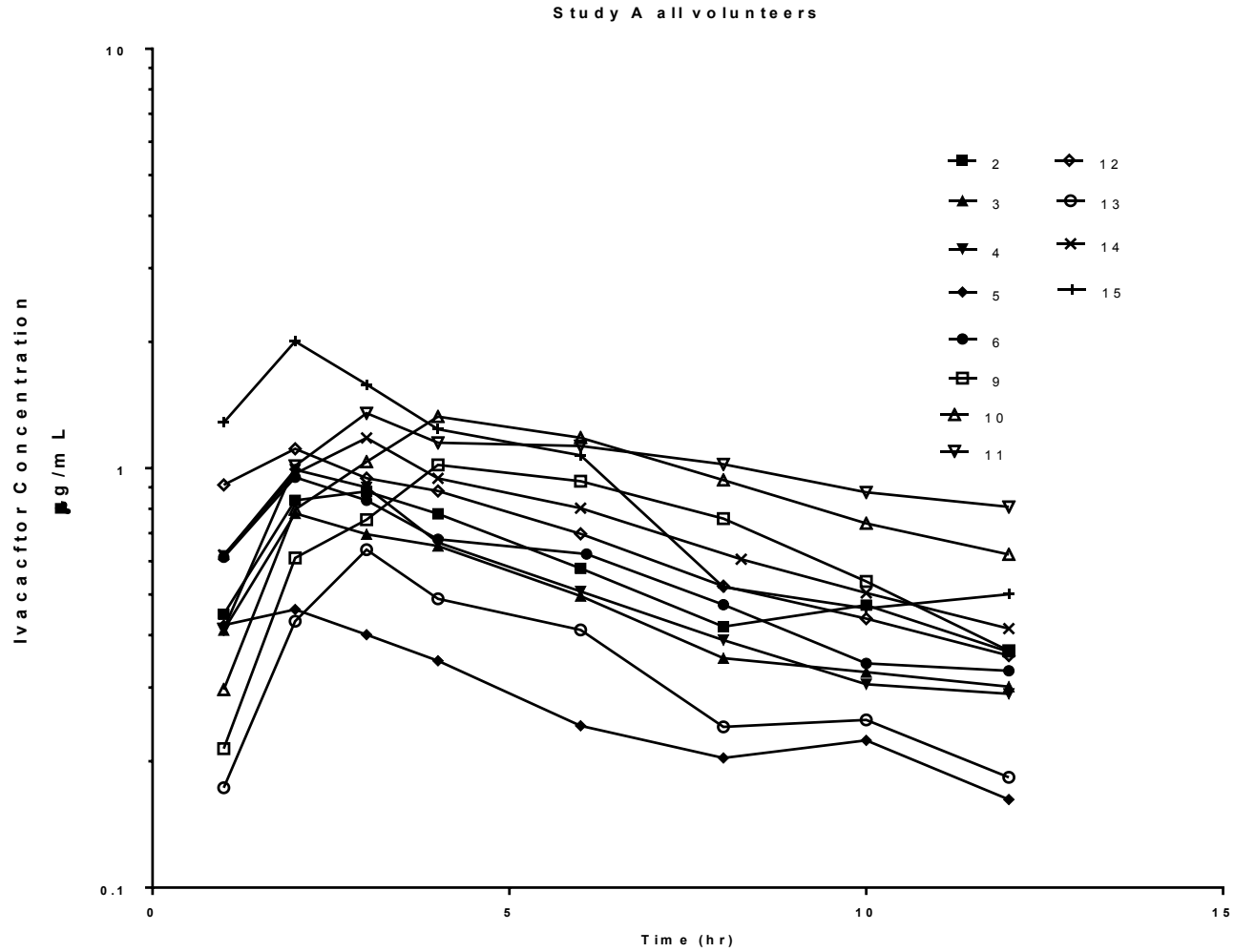


Figure 11. Spaghetti plots of data from all volunteers in study A, ivacaftor 150mg single dose.

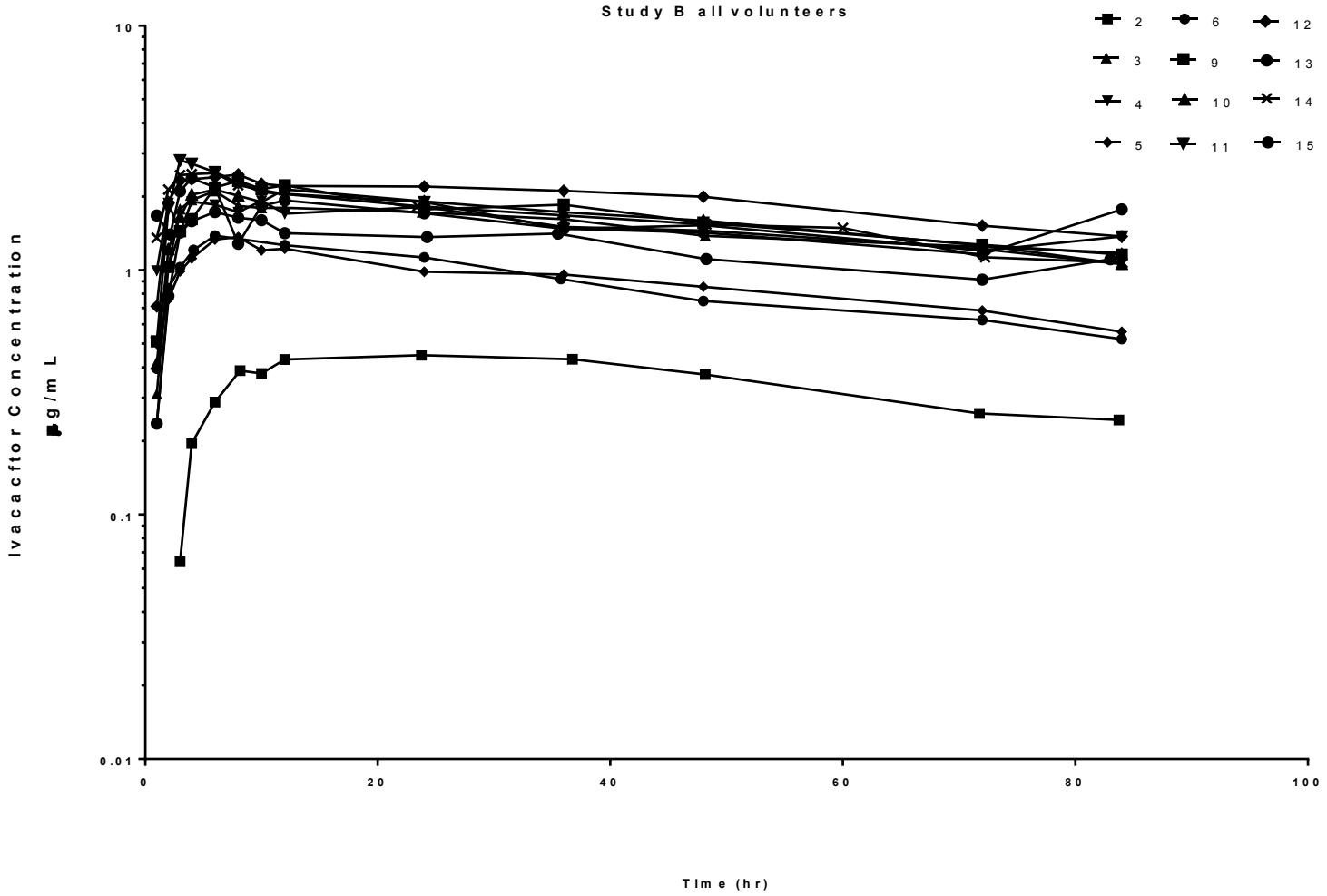


Figure 12. Spaghetti plots of data from all volunteers in studies B, ivacaftor 150mg plus ritonavir 50mg daily.

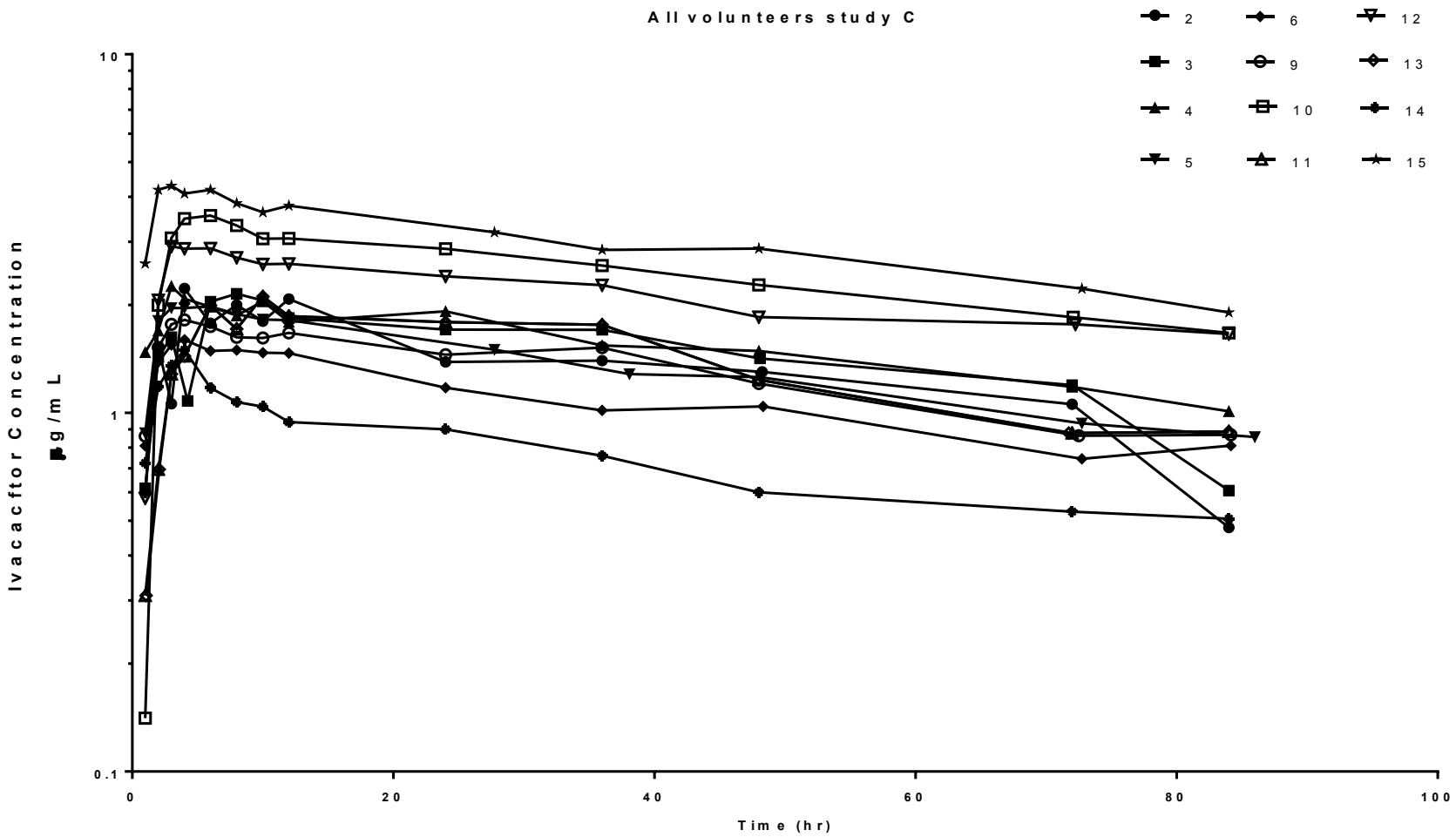


Figure 13. Spaghetti plots of data from all volunteers in studies C, ivacaftor 150mg plus ritonavir 50mg daily after two weeks of ritonavir 50mg daily.

4.4.3.2 Elimination half life

$T_{1/2}$ was calculated for each PK profile using at least three terminal concentrations. $T_{1/2}$ (GM [95% CI]) of ivacaftor in study A was 7.121 (5.59–9.07) hrs compared to 79.24 (65.5–96.1) hrs in study B and 65.99 (57.43–75.82) hrs in study C. $T_{1/2}$ of ivacaftor was significantly prolonged by coadministration of ritonavir in both study B and study C in comparison to study A (GMR [95% CI] 11.14 [8.72 – 13.26] and 9.27 [6.68 – 12.85] respectively; p=0.0005 and 0.0005 respectively). There was no significant difference in $T_{1/2}$ between study B and study C (GMR [95% CI] 0.83[0.63-1.1]; p=0.2036).

4.4.3.3 Area under the time–concentration curve from time zero to infinity

Significantly higher exposure to ivacaftor, as represented by $AUC_{0\text{-inf obv}}$, was found when ivacaftor was administered with ritonavir in both studies B and C compared with ivacaftor alone in study A (p= 0.0045 and 0.0005 respectively). $AUC_{0\text{-inf obv}}$ (GM [95% CI]) was 10.94 (8.259–14.48) $\mu g.hr.mL^{-1}$ in study A compared to 215.6 (146.4–317.4) $\mu g.hr.mL^{-1}$ in study B and 216 (165.5–281.8) $\mu g.hr.mL^{-1}$ in study C respectively (GMR [95% CI] study A:study B 19.71 [13.18–31.33] and study A:study C 19.74 [14.0–27.92] respectively).

4.4.3.4 Area under the time–concentration curve from time zero to 12 hours

$AUC_{0–12}$ was also significantly increased in study B and in study C in comparison to study A. $AUC_{0–12}$ (GM [95% CI]) was 7.024 (5.48–9.0) in study A, 16.65 (11.4 – 24.32) in study B and 21.5 (17.38 – 26.59) in study C [GMR study A:B 2.37 (1.62 – 3.47). A:C 3.06 (2.36 – 3.87) and B:C 1.29 (0.856 – 1.95)].

4.4.3.5 Volume of distribution/fraction of drug absorbed (V_d/F)

The Volume of distribution/fraction of drug absorbed (V_d/F) was significantly different between study A and study B, and between study A and study C but there was no significant difference between studies B and C.

Table 23. Median Vd/F in study A, B and C.

	Study A	**Study B**	**Study C**
Median ± IQR Vd/F (L)	140.7 (90.55–208.9)	71.37 (64.39–100.6)	64.06 (55.53–80.13)

Vd/F, volume of distribution/fraction of drug absorbed.

This is likely not a true reflection of a change in V_d however, as there is most likely a change in F with the addition of ritonavir. As F for ivacaftor is not known (the drug is practically insoluble in water and therefore no IV formulation is available for PK study in humans)(2) it is not possible to quantify any change in F with the addition of ritonavir. However, given that V_d/F is calculated:

$$\frac{V_d}{F} = \frac{dose}{t_{1/2}.AUC_{0-infob}}$$

if the V_d is assumed to remain unchanged, the ratio of F in the absence of ritonavir to F in the presence of ritonavir can be calculated as follows:

$$\frac{F_A.\ dose}{T_{1/2A}.AUC_{0-infobA}} = \frac{F_B.\ dose}{T_{1/2B}.AUC_{0-infobB}}$$

$$\therefore$$

$$\frac{F_A}{F_B} = \frac{T_{1/2A}.AUC_{0-infobA}}{T_{1/2A}.AUC_{0-infobA}}$$

By substituting in the known values for $T_{1/2}$ and $AUC_{0\text{-inf obv}}$ in each study the ratio of the F_A:F_B can be calculated. This can also be calculated for F_A:F_C and F_B:F_C. There is a significant increase in F when both study B and study C are compared to study A, with no significant difference between study B and study C.

Table 24. Comparison of fraction of ivacaftor absorbed between each study A, B and C.

F Study B: F Study A Median ± IQR	F Study C: F study A Median ± IQR	F Study C: F Study B Median ± IQR
1.714 1.381 – 2.71	2.227 1.58 – 3.207	1.185 0.9622 – 1.477

IQR, interquartile range.

4.4.3.6 Maximum concentration

C_{max} (GM [95% CI]) in study A was 0.9944 (0.7819–1.265) µg/mL, 1.812 (1.323–2.482) µg/mL in study B and 2.267 (1.863–2.757) µg/mL in study C respectively. C_{max} of ivacaftor was significantly increased in the presence of ritonavir in both study B and study C (GMR [95% CI] 1.82 [1.34–2.48] and 2.28 [1.84–2.83] respectively; p=0.0049 and 0.0005 respectively) with no significant difference in C_{max} of ivacaftor between study B and study C (p=0.2661).

4.4.3.7 Time to maximum concentration

Median T_{max} of ivacaftor was significantly later in the presence of ritonavir (median [range] 2.5 [2–4] hrs, 6.5 [3–23.75] hrs and 4 [3–10] hrs in studies A, B and C respectively; p=0.001).

4.4.3.8 Clearance

Clearance of ivacaftor (GM [95% CI]) in study A was 13.72 (10.36–18.16) L/h, 0.6958 (0.4726–1.024) L/h in study B and 0.6946 (L0.5323–0.9063) in study C respectively.

Clearance of ivacaftor was significantly decreased in the presence of ritonavir in both study B and study C (GMR [95% CI] 0.05 [0.03–0.08] and 0.05 [0.04–0.07] respectively; p= 0.0005) with no significant difference in clearance of ivacaftor between study B and study C (p>0.9999).

4.4.3.9 Outliers

All volunteers were found to have an increase in plasma levels of ivacaftor with the addition of ritonavir except for volunteer 2 study B. The reason for this outlier could not be determined.

4.4.3.10 Individual level analysis

An analysis of individual level data shows that for all volunteers $AUC_{0\text{-inf obv}}$ and $T_{½}$ consistently increased with the addition of ritonavir (that is values from study B and study C are both larger than study A). All volunteers except for volunteer 2, study B also consistently showed an increase in $AUC_{0\text{-}12}$ and C_{max} with the addition of ritonavir. Correspondingly all volunteers showed a decrease in clearance with the addition of ritonavir in both studies B and C in comparison to study. The change from study B to study C was not consistent between volunteers. AUC, $AUC_{0\text{-}12}$, $T_{1/2}$, C_{max} and clearance either increased or decreased or stayed almost identical.

4.4.3.11 Coefficient of variation of pharmacokinetic parameters

The CV of $AUC_{0\text{-inf obv}}$, $AUC_{0\text{-}12}$ and C_{max} was similar between studies A, B and C. The CV in $T_{1/2}$ of ivacaftor reduced with the addition of ritonavir, reducing to almost half when comparing study C to study A, as shown in table 26.

Table 25. Summary of PK parameters of for study A, B and C.

	Study A Median (range)	Study B Median(range)	Study C Median (range)	GMR Study A:Study B (CI)	GMR Study A:Study C (CI)	GMR Study B:Study C (CI)
AUC_{0-12} µg.hr.mL^{-1}	7.024 (5.48–9.0)	16.65 (11.4–24.32)	21.5 (17.38 – 26.59)	2.37 (1.62–3.47)	3.06 (2.36–3.97)	1 .29 (0.856–1.95)
$AUC_{0\text{-inf obv}}$ µg.hr.mL^{-1}	10.94 (8.26 – 14.48)	215.6 (146.4–317.4)	216 (165.5 – 281.8)	19.71 (13.18–31.33)	19.77 (14.0–27.93)	1.00 (0.68–1.47)
C_{max} µg/mL	0.9944 (0.7819–1.265)	1.812 (1.323–2.482)	2.267 (1.863 – 2.757)	1.82 (1.34–2.48)	2.28 (1.84–2.83)	2.54 (0.65–9.9)
$T_{1/2}$ hrs	7.12 (5.59–9.07)	79.34 (65.5 –96.1)	65.99 (57.43 – 75.82)	11.14 (8.72–13.26)	9.27 (6.68–12.85)	0.83 (0.63–1.1)
Clearance L/h	13.72 (10.36–18.16)	0.6958 (0.4726–1.024)	0.6946 (0.5323–0.9063)	0.05 (0.03–0.08)	0.05 (0.04–0.07)	1.00 (0.68–1.46)
T_{max} hrs	2.5 (2–4)	6.5 (3-23.75)	4.0 (3-10)			

PK, pharmacokinetic; $AUC_{0–12}$, area under the time-concentration curve from 0 to 12 hours; $AUC_{0\text{-inf obv}}$, area under the time-concentration curve from 0 to infinitiy; C_{max} maximum concentration; $T_{1/2}$ elimination half-life; T_{max} time to maximum concentration; GMR, geometric mean ratio; CI, confidence interval.

Table 26. CV of PK parameters

	Study A	Study B	Study C
$AUC_{0\text{-inf obv}}$	45.12%	41.86%	45.82%
$AUC_{0\text{-}12}$	37.46%	33.13%	34.59%
C_{max}	37.46%	33.13%	34.59%
$T_{1/2}$	43.96%	33.48%	22.32%
Cl/F	46.39%	89.84%	37.46%

CV, coefficient of variation; PK, pharmacokinetic; $AUC_{0\text{-inf obv}}$, area under the time-concentration curve from 0 to infinity; $AUC_{0–12}$, area under the time-concentration curve from 0 to 12 hours; C_{max} maximum concentration; $T_{1/2}$ elimination half-life; Cl/F, clearance over fraction of drug absorbed.

4.4.3.12 Effect of timing of meals

Examining the median concentrations of ivacaftor in the presence of ritonavir shows multiple peaking in both acute and chronic dosing of ritonavir. Mapping the median

plasma concentrations to the timing of meals, it appears that eating a high fat dinner (as per the trial protocol) may increase plasma level of ivacaftor. This is seen with an increase in plasma levels of ivacaftor in studies B and C and a flattening out of the slope of the elimination in study A post dinner. At treatment doses ivacaftor has not been reported to demonstrate intrahepatic circulation, though ivacaftor is found in bile after a 150mg dose is taken by mouth.(9)

4.4.4 Non-compartmental pharmacokinetic analysis of hydroxymethyl-ivacaftor with and without concomitant administration of ritonavir

The median and IQR concentrations of M1 are shown in figure 20. Several samples contained concentrations of M1 that were below the LLOQ. Each volunteer in study C had at least 1 sample (1-hour sample) that was below the LLOQ, with 4 volunteers missing 1 further sample, 2 missing a further 2 samples and 2 missing a further 3 samples. Volunteer 9, study C contained 8 of 13 samples that were below the LLOQ. The graph above represents the median and IQR of the samples that were available for analysis at each timepoint.

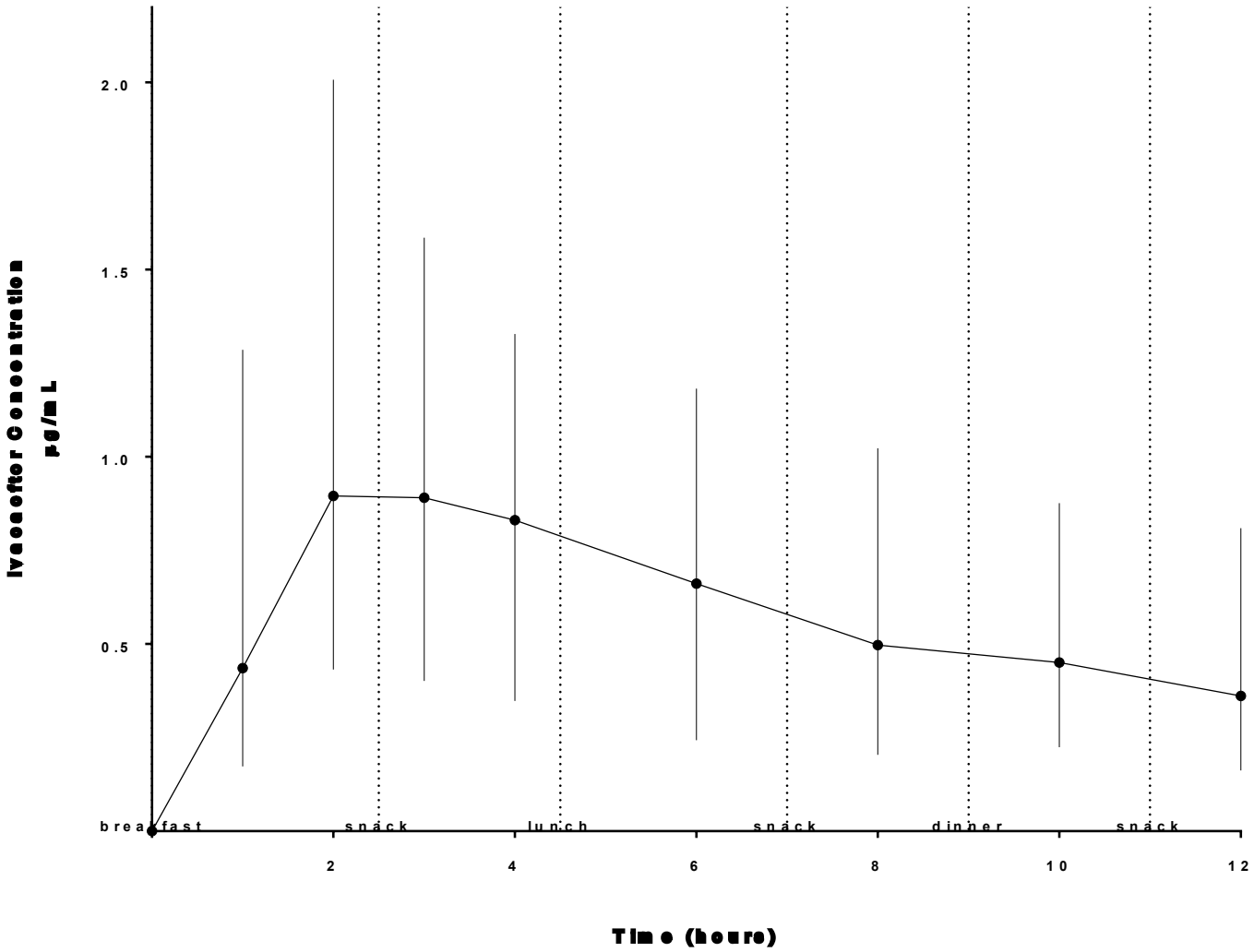


Figure 14. Effect of timing of meals on concentrations of ivacaftor in study A.

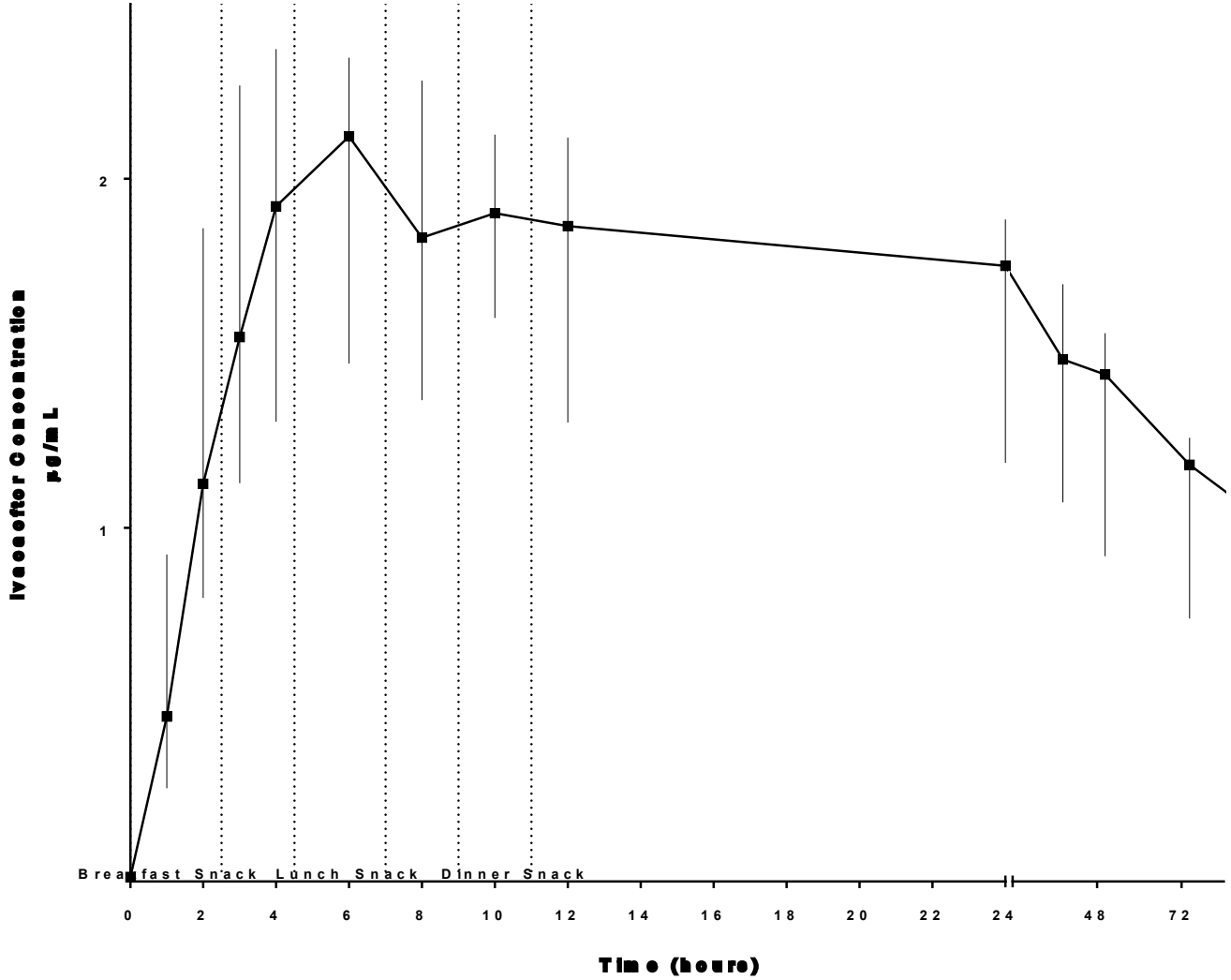


Figure 15. Effect of timing of meals on concentrations of ivacaftor in study B.

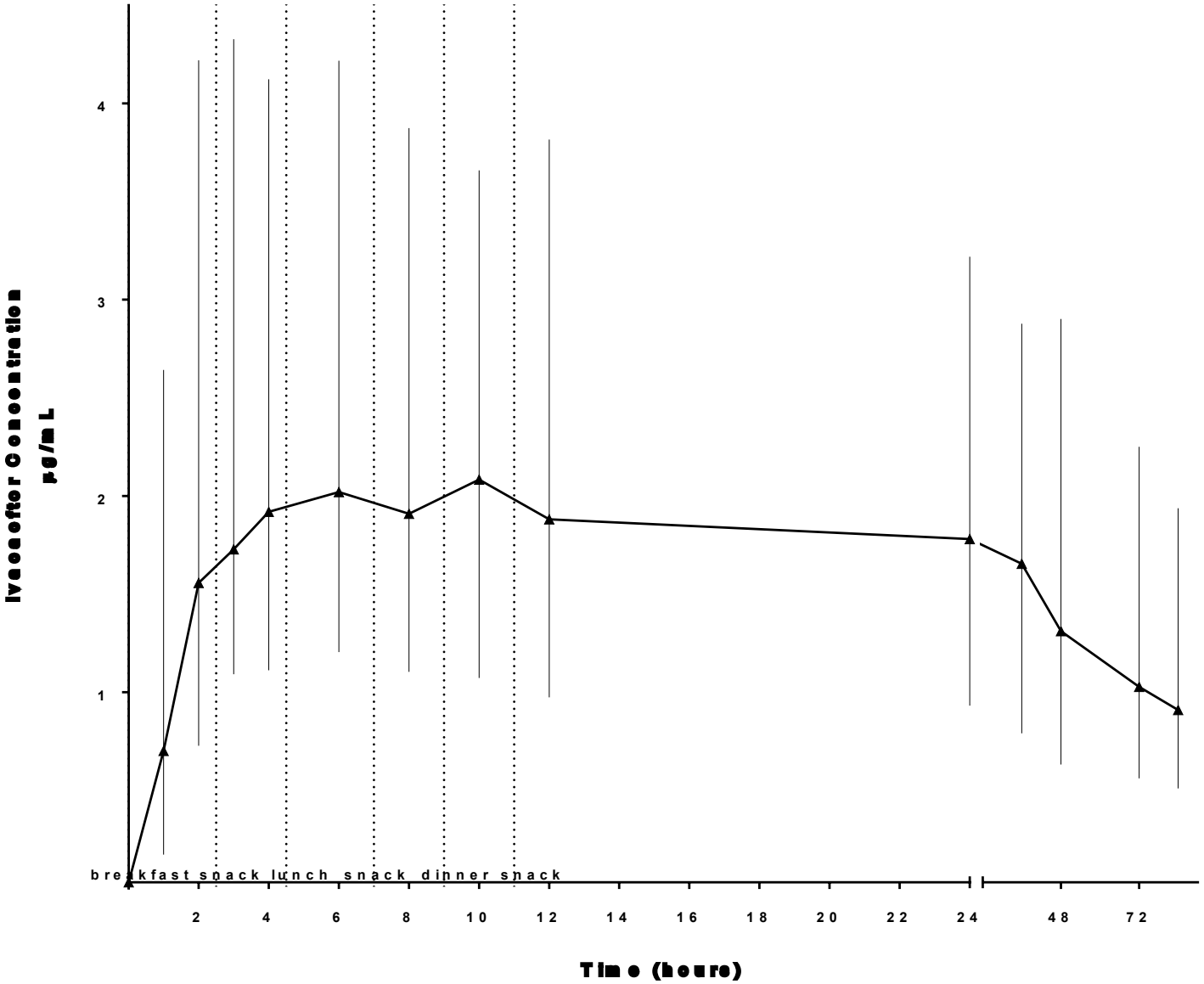


Figure 16. Effect of timing of meals on concentrations of ivacaftor in study C.

4.4.4.1 Area under the time–concentration curve from time zero to 12 hours

AUC $_{0–12}$ of M1 was significantly decreased in study B and in study C in comparison to study A. AUC $_{0–12}$ (GM [95%CI]) was 11.77 (8.620 – 16.07) in study A, 2.961 (2.014 – 4.355) in study B and 0.6999 (0.418– 1.170) in study C [GMR study A:B 0.25 (0.23 – 0.27). A:C 0.06 (0.049 – 0.073) and B:C 0.24 (0.21 – 0.268)]. There was a significant difference between all studies (p=0.0005).

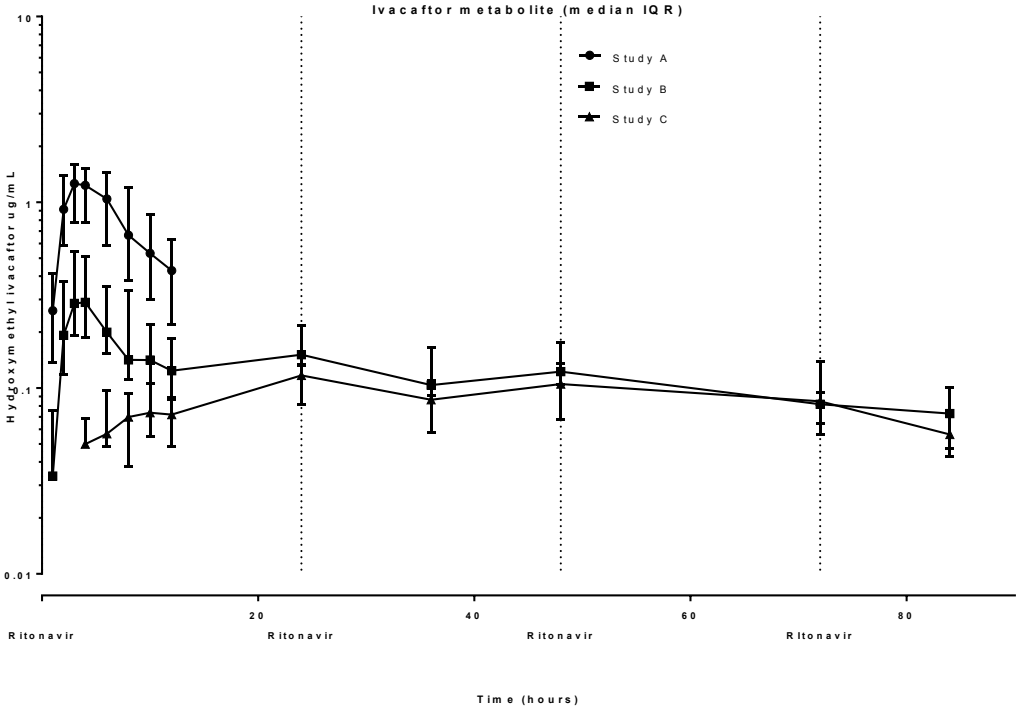


Figure 17. Median concentrations of M1 in studies A, B and C.

4.4.4.2 Maximum concentration

C_{max} (GM [95% CI]) of M1 was 1.650 (1.253 – 2.175) µg/mL in study A, 0.4401 (0.3001 – 0.6454) µg/mL in study B and 0.1532 (0.1081 – 0.2170) in study C [GMR A:B 0.27 (0.24 – 0.29), A:C and A:B 0.09 (0.086 – 0.1) B:C 0.35 (0.33 – 0.36) respectively]. There was a significant difference in C_{max} between all three studies (p=0.0005)

Table 27. PK parameters of M1.

	Study A median (range)	**Study B median (range)**	**Study C median (range)**	**GMR Study B: Study A**	**GMR Study C: Study A**	**GMR Study C: Study B**
$AUC_{0–12}$ (µg.hr.L^{-1})	11.77 (8.620 – 16.07)	2.961 (2.014 – 4.355)	0.6999 (0.4187– 1.170)	0.25 (0.23 – 0.27)	0.06 (0.049 - 0.073)	0.24 (0.21 – 0.268)
C_{max} µg/mL	1.650 (1.253 – 2.175)	0.4401 (0.300 – 0.645)	0.1532 (0.1081– 0.217)	0.27 (0.24 – 0.29)	0.09 (0.086 – 0.1)	0.35 (0.33 – 0.36)

$AUC_{0–12}$, area under the time-concentration curve from 0 to 12 hours; C_{max} maximum concentration; GMR, geometric mean ratio.

4.5 Discussion

The PK data obtained from this healthy volunteer study show that ritonavir at a dose of 50mg once daily, in both acute and chronic dosing, significantly increases plasma concentrations of ivacaftor. $AUC_{0–inf\ obv}$ (95% CI) was 10.94 (8.259–14.48) µg.hr.mL^{-1} in study A compared to 215.6 (146.4–317.4) µg.hr.mL^{-1} in study B and 216 (165.5–281.8) µg.hr.mL^{-1} in study C. Maximum concentration (C_{max}) (GM [95% CI]) in study A was 0.9944 (0.7819–1.265) µg, 1.812 (1.323–2.482) µg in study B and 2.267 (1.863–2.757) in study C respectively. Elimination half-life ($T_{1/2}$) (GM [95% CI]) of ivacaftor in study A was 7.121 (5.59–9.07) hours compared to 79.24 (65.5–96.1) hours in study B and 65.99 (57.43–75.82) hours in study C. These data provide proof of concept that concomitant

dosing of ivacaftor and ritonavir could be used in clinical practice to increase the dosing interval of ivacaftor while, given the magnitude of inhibition, also potentially allow patients to maintain higher plasma levels than with ivacaftor alone at the recommended dose of 150mg twice daily.

$T_{1/2}$ of ivacaftor in the presence of ritonavir was increased to a larger extent than had been predicted prior to the commencement of the study (in the statistical plan a doubling of the $T_{1/2}$ of ivacaftor was assumed). This could have implications for the washout period between studies should ivacaftor have not reached negligible levels after the completion of one study prior to the commencement of the next. This concern was address by inspection of the T_0 sample from each volunteer in each study, none of which contained detectible levels of ivacaftor, therefore potential underestimation of the washout period is unlikely to have affected the results.

As with the addition of any new drug to a patient's treatment, the balance of beneficial and undesirable effects must be assessed; if ritonavir were to be used as a PK enhancer, side effects would have to be minimal in order to make it a practical choice for this purpose. In the early years of HIV treatment, the use of ritonavir was associated with significant and debilitating side effects such as abdominal pain, nausea, vomiting, severe diarrhoea, parasthesias, rashes and peripheral neuropathy. Doses used for treatment (as opposed to those used for PK enhancement) were up to 1,200mg per day.[6] The side effects of ritonavir used at doses for PK enhancement are reported to be much less frequent and less severe.[16] Notably, the doses used for PK enhancement are typically at least twice that used in the current study. The side effects of ritonavir have also been shown to be dose related, and it was for this reason that the smallest dose of ritonavir reported in the literature to provide significant boosting of other PIs (50mg once daily) was used.[13, 17] It was encouraging that no volunteer experienced side

effects of ritonavir that compelled them to stop taking the drug, and for those who did experience side effects these were mild and transient. This suggests that ritonavir will be tolerable for use in PK enhancement of ivacaftor in clinical practice for patients with CF.

A small increase in plasma lipids was shown to occur when volunteers took ritonavir 50mg for two weeks. This study was not powered to detect any change in lipids per se, this is the result of an exploratory analysis that constitutes a signal that it would be prudent to monitor lipids in patients who are on ritonavir 50mg daily. Ritonavir used in treatment doses or in combination with other PIs is known to be associated with a derangement of the lipid profile and, at the extreme end of the spectrum, lipodystrophy, leading to abnormalities in energy metabolism.(49) Studies with boosting doses of ritonavir have suggested that ritonavir at a smaller boosting dose does not contribute significantly to the side effect profile of the boosted PI.(11). Considering that the side effects of ritonavir tend to be dose related, the metabolic side effects of ritonavir 50mg daily are unlikely to present a clinically significant problem. Based on the small increase in LDL (0.3mmol/L) shown in this study it could be recommended to monitor lipids carefully in those on ritonavir-boosted ivacaftor and perhaps consider patients with multiple cardiovascular risk factors unsuitable for ritonavir treatment. This is an area that could be explored further in a clinical trial of ritonavir boosting of ivacaftor in PWCF.

As discussed above, the large change in V_d/F between study A and studies B and C was most likely due to a change in the proportion of the ivacaftor dose absorbed rather than a true change in V_d. This cannot be tested in humans as an IV formulation of ivacaftor does not exist.(9) In animals, the amorphous form of ivacaftor was shown to have 30-100% oral bioavailability. The apparent permeability in the caco-2 assay was shown to be high, suggesting that intestinal absorption may be high after administration in humans. Ivacaftor is not a substrate for PgP, and given that ivacaftor is metabolised by CYP3A4

and this enzyme is abundant in the intestinal mucosa, it must be assumed that a certain amount of the ivacaftor is metabolised in the intestinal epithelium before reaching the systemic circulation and that inhibition of this CYP3A4 is likely to be the underlying mechanism by which ritonavir increases absorption of ivacaftor. However, the absorption profile of ivacaftor is complex, so it is difficult to draw conclusions with any certainly.

Previous studies of ritonavir have suggested that it takes approximately three days for enzymes to recover after irreversible inhibition by ritonavir.(54) The dose of ritonavir used in this study is unlikely to accumulate to any extent given that it is administered once every 24 hours and the $T_{1/2}$ of ritonavir is around 5hours.(101) The inhibition that persists throughout the dosing interval of the ritonavir is likely due to irreversibly inhibited enzyme rather than any ongoing competitive inhibition by ritonavir.

A trend towards an increase in the level of M1 between doses of ritonavir can be seen on inspection of the M1 PK profiles in study B and C. There is likely some recovery of CYP3A4 enzyme taking place during the dosing interval. This strengthens the case for daily administration of ritonavir, as opposed to administration of ritonavir only at 0hrs with ivacaftor. Given the data that drug metabolising enzyme usually recovers within three days, without daily administration of ritonavir levels of ivacaftor would become very low at the end of a dosing interval that was, for example, twice weekly for both ritonavir and ivacaftor.

The median $AUC_{0\text{-}12}$ for studies B and C are not statistically significantly different, but the GMR does not demonstrate bioequivalence (GMR study B: study C 1.29).(102) This is also true for the for median C_{max} of ivacaftor between studies B & C. Median $AUC_{0\text{-}inf\ obv}$, $T_{1/2}$ and clearance do fall within bioequivalence criteria. This is a logical finding considering that CYP3A4 enzymes will be much more active in the gut and liver of volunteers in study

B than those in study C, given that volunteers in study C have been exposed to ritonavir before ivacaftor and therefore have inhibition of CYP already established in these organs. The primary aim of comparing studies B & C was to show that the induction effect of ritonavir would not be problematic in chronic dosing. This is an important finding as some drug interactions with ritonavir show change over time. Alprazolam is an example; one study showed significant inhibition of alprazolam metabolism with acute dosing of ritonavir, but when ritonavir was used daily for 12 days this effect disappeared. It is hypothesised that this was due to the induction effect of ritonavir.(18) It was therefore important to establish that such a situation would not arise with the inhibition of the metabolism of ivacaftor. As the induction effects of ritonavir are maximal at two weeks [18] the similar level of inhibition that exists between study B and study C suggests that any induction effects of ritonavir will not be clinically significant should ritonavir be used in the long-term as a PK enhancer.

It was a curious finding that the coefficient of variation of C_{max} and $AUC_{0\text{-}inf\ obs}$ did not change, while that of $T_{1/2}$ showed a marked reduction. There are insufficient data in the current study to fully explore this phenomenon, though it could be hypothesised that the variability in drug absorption seen in other pharmacokinetic studies of ivacaftor contributed to this.(9) While absorption of ivacaftor may have remained variable with co-administration of ritonavir, thus affecting the variability of C_{max} and $AUC_{0\text{-}inf\ obs}$, the variability in elimination was smoothed out and this was seen in the reduction in the variability in $T_{1/2}$.

It was interesting to note that in study B volunteer 2 was on outlier, with significantly lower concentrations of ivacaftor throughout the study in comparison to other volunteers, but was not an outlier in study C. Volunteer 14 data was the lowest in study C but was on the higher end of the spectrum in study B. This may be due to many factors,

but the role of both the variable absorption of ivacaftor and the induction effect of ritonavir warrant mention. It has been shown that intraindividual and interindividual absorption of ivacaftor is subject to significantly variability and this is seen in the CV of all of the PK parameters discussed above. Variable absorption of ivacaftor could explain the absence of a change in the CV of C max and AUC $_{0\text{-inf obv}}$ between studies A, B & C whereas more consistent enzyme inhibition between volunteers could account for the reduction in the variability of $T_{1/2}$ from study A to study C of almost 50%. The induction effect of ritonavir could be expected to be negligible at a low dose of 50mg daily based on the literature available to date.(59) There may be variability within this however, and variability in this induction /inhibition balance could contribute to the variability in PK parameters still present in study C. Further studies are needed to clarify these points.

Single-dose escalation studies of ivacaftor are reported in the biopharmaceutical review of Kalydeco® that was submitted to the FDA.(2) Visual comparison of median data of 150mg ivacaftor plus ritonavir shows a similar peak concentration to that of a single dose of either 500mg, 675mg or 800mg of ivacaftor. As can be observed in the graph below, the double peaking effect is evident with higher doses of ivacaftor, 675mg and 800mg single doses. Ivacaftor demonstrates solubility limited absorption; peak concentration of ivacaftor plateaus at the 500 mg dose. The elimination of ivacaftor is much slower in the presence of ritonavir in comparison to the 500mg, 675mg and 800mg single dose, as expected.(2)

The appearance of multiple peaking in the concentration–time profile of ivacaftor, in single-dose escalation studies shown above and particularly in the presence of ritonavir, further suggests a complex absorption profile for ivacaftor. Ivacaftor is a class II compound based on BCS, which means it demonstrates poor solubility and high permeability.(9) Differences in intestinal pH and other local conditions may mean that

ivacaftor is absorbed in an erratic fashion as it traverses the gut.(103) It is stated in the product literature that ivacaftor does not undergo significant enterohepatic circulation. However, it is also noted that ivacaftor is present in the bile in animal studies post dosing. It may be that with higher doses/concentrations of ivacaftor enterohepatic circulation is of a significant enough magnitude to result in a double-peak phenomenon.(9) This may also explain the association of the double peak with meal-times in this study (though the association with food in the dose escalation studies is not discussed by the authors). This phenomenon may be worth further study to clarify this issue.

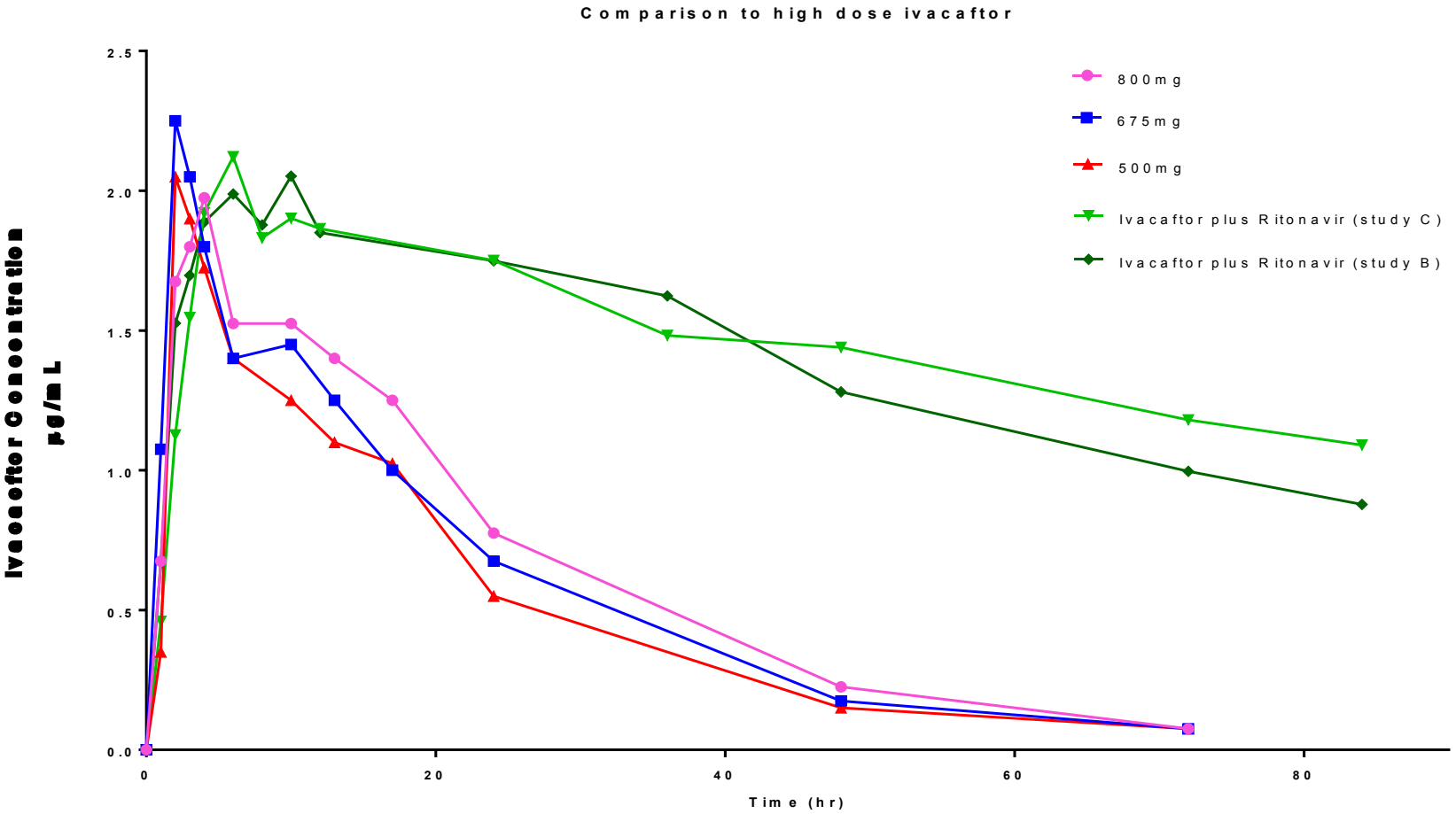
4.6 Limitations

As a double-peaking phenomenon is noted in the absorption of ivacaftor in this study, future studies could further elucidate this by incorporating richer sampling in the absorption phase of ivacaftor. Study A in particular contains very few plasma concentrations prior to T_{max}. Ritonavir was only studied at one dose and at one dosing interval in this study. It may be the case that a smaller dose of ritonavir and/or a larger dosing interval may still result in significant inhibition of the metabolism of ivacaftor while minimising further systemic exposure to ritonavir. This could also be explored in future studies of this PK interaction. Cross resistance of HIV virus to PIs is a well-known challenge in the treatment of HIV. While regular HIV testing mitigates the risk of a patient contracting HIV being exposed to PIs at a subtherapeutic dose, this risk cannot be completely eliminated. As with most PK studies, the numbers in this study are small and while statistically powered to detect the differences described, the results may not be generalisable to a larger population. Finally, the PK/PD relationship of ivacaftor is not currently well characterised(2) and while the changes in plasma concentration are

measured in this study there is no way to extrapolate this reliably to any changes that may occur in clinical efficacy.

4.7 Conclusion

Combination of ivacaftor and ritonavir results in inhibition of the metabolism of ivacaftor: $AUC_{0-12,}$ $AUC_{0-inf\ obv}$, C_{max} and $T_{½}$ of ivacaftor are significantly increased in the presence of ritonavir and clearance of ivacaftor is significantly decreased. Absorption of ivacaftor is also likely increased. These data show that should ivacaftor be combined with a small 50mg daily dose of ritonavir in clinical practice the dosing interval of ivacaftor could be increased, leading to a sizable decrease in the cost of ivacaftor treatment. The pharmacokinetic time–concentration curves from this study will be used in the next study to determine the best fit compartmental model for ivacaftor metabolism and to simulate steady state concentrations in different dosing regimens of ivacaftor in combination with ritonavir. Ketoconazole results in an increase in AUC of ivacaftor of 8.5 times compared to ivacaftor alone and consequent to this twice weekly dosing of ivacaftor 150mg with ketoconazole is established in clinical practice.(12) Given the larger magnitude of the increase in AUC of ivacaftor in the presence of ritonavir shown here, the time–concentration curves presented in this study will be used in the next chapter to explore the steady state concentrations of ivacaftor 50mg twice weekly, ivacaftor 75mg twice weekly, ivacaftor 50mg three times weekly and ivacaftor 75mg three times weekly in the presence of ritonavir 50mg daily to establish a dosing regimen of ivacaftor plus ritonavir equivalent to standard dosing of ivacaftor 150mg twice daily alone.



Adapted from (2).

Figure 18. Comparison of ivacaftor plus ritonavir to single doses of ivacaftor.

CHAPTER 5

5 PK modelling and dose simulation

5.1 Introduction

The data presented in chapter 4 show that ritonavir at a small dose of 50mg daily will significantly increase the plasma concentration and slow the elimination of ivacaftor. To translate these data into a new dosing regimen for ivacaftor, multi-dose simulation must be undertaken using data from the single-dose PK profiles. Fitting PK data to a compartmental model provides a description of the absorption and the elimination of ivacaftor in the absence and presence of ritonavir which can be used to simulate multi-dose concentrations from these single-dose data.

Determining the best-fit compartmental pharmacokinetic model to the PK profiles obtained from the previous study allows for the most accurate estimation of model parameters such as K_{01}, K_{10} V/F and Cl/F (as described in chapter 2). These parameters are estimated using non-linear regression, a process in which an algorithm is applied to an initial set of estimated parameters and an iterative process refines the calculation of these parameters until there is a negligible difference between successive calculations. WinNonlin® uses the Gauss-Newton method as the default algorithm for the iterative estimation of model parameters, which is based on mathematical methods originally developed by Sir Isaac Newton in the 18th century.(22)

Trials of different types of models are necessary to determine the best-fit model. The route of administration will be determined by the drug itself, but other parameters such as the number of compartments and the absorption features should be explored and a number of tests of fit then employed to determine the best fit overall. For orally administered drugs, WinNonlin® allows for exploration of first order input with or without a lag time and with either one or two compartments, with first order

elimination.(22) Goodness-of-fit is then determined by visual analysis of the curve fitting to data points and of the scatter of residuals. If residuals are not scattered randomly then a weighting scheme may be applied to improve fit. The accuracy and precision of the parameter estimates, the sum of the squares of the residuals (SSR) and the correlation between parameters provide objective data on goodness-of-fit, and the acceptable numbers for these will usually depend on the ultimate purpose of modelling. Models that are found acceptable based on the above parameters can then be compared using the F-test for comparison of nested models. This allows testing of whether a model with a greater number of input parameters provides more accurate information than a model with fewer parameters. Akaike's information criterion (AIC) and the Schwartz criterion (SC) are formulas that relate the SSR to the number of output parameters, with a lower value associated with the best fit model.(20, 22)

Once the best fit model is determined the output parameters that describe the PK curve can be used to simulate drug concentrations after multiple doses. Any variety of doses and dosing intervals of ivacaftor with and without ritonavir 50mg can be simulated. These data can then be used to determine the dose of ivacaftor plus ritonavir that is at least bioequivalent to usual dosing of ivacaftor alone.(20)

5.2 Objective

The aim of this study was to fit the single-dose data obtained in the previous study to a suitable pharmacokinetic model to obtain model parameters that can be used to calculate predicted, steady-state concentrations at different doses to identify the weekly dose of ivacaftor that may be equivalent to standard twice daily ivacaftor 150mg.

5.3 Methods

Data were analysed using WinNonlin version 6.4, Pharsight, U.S.A., GraphPad Prism version 7.2, U.S.A. and Microsoft® Excel® 2016. Data were first visually examined by means of a spaghetti plot of studies A, B and C. The presence of trends in the various PK parameters was assessed.

5.3.1 Model fitting

Individual volunteer profiles from studies A and C were explored by application of 4 different PK models:

- One-compartment model with 1st order absorption
- One-compartment model with 1st order absorption including lag time
- Two-compartment model with 1st order absorption
- Two-compartment model with 1st order absorption including lag time

Initial estimates for the one-compartment model were calculated by WinNonlin® and initial estimates for the two-compartment model were calculated both by WinNonlin® and by the author and models using both estimates were assessed. All scenarios were assessed with uniform weighting and data was generated using the Gauss-Newton method in WinNonlin®.

Model fitting was assessed by visual analysis of the plots of the observed versus predicted data and visual analysis of the residual plots. The sum of the square of the residuals (SSR), Akaike's information criterion (AIC), Schwartz criterion (SC) and the CV% of final parameters were also assessed. A correlation coefficient >0.9 and CV% less than 50% were considered acceptable. The condition number (that is the square root of the ratio of the largest eigenvalue to the smallest eigenvalue) was considered acceptable if it

was less than or equal to $10^{\text{number of parameters estimated}}$, as is commonly accepted in PK studies of this type.(20)

All models that were considered acceptable based on the above criteria were then compared to ascertain the best fit model overall. In order to compare models, the F-test and a comparison of AIC and SC values were used.

The F-test for comparison of nested models is calculated as follows:

$$F = \frac{(WRSS_j - WRSS_k)/(np_k - np_p)}{WRSS_k/(n - np_k)}$$

where k denotes the model with the greatest number of parameters, j denotes the model with the smaller number of parameters, WRSS is the weighted residual sum of squares, np is the number of parameters and n is the number of observations.(20) Two models are nested if one is a special case of the other, as in the case with a one-compartment model with lag time which is a special case of a one compartment model. If the more complicated model (that is the model including lag time absorption) is a better fit then it would be expected that the decrease in the SSR going from the simpler to the more complicated model would be larger than the decrease in the degrees of freedom (the number of degrees of freedom is equal to the number of data points minus the number of parameters).(104)

AIC and SC values were compared between models by calculation of the difference in these values between two models (for example one-compartment model and one-compartment model with lag time) and assessing if this difference was statistically significant using a student's t-test. AIC was calculated as follows:

$$AIC = N_{obs} \,.\, ln\,(WRSS) + 2N_{par}$$

and SC was calculated as follows:

$$SC = N_{obs} \,.\, ln(WRSS) + N_{par} \,.\, ln(N_{obvs})$$

where N_{obs} is the number of observations, *ln*(WRSS) is the natural log of the weighted sum of squares of the residuals (W=1 if no weighting is applied) and N_{par} is the number of parameters.

Final parameters and secondary parameters were reported for the model deemed to be the best fit for the data overall.

5.3.2 Dosing simulation

Once the best-fit model was ascertained, four different dosing scenarios were simulated:

- 50mg ivacaftor Monday and Thursday (50mg MT) with ritonavir 50mg daily
- 75mg ivacaftor Monday and Thursday (75mg MT) with ritonavir 50mg daily
- 50mg ivacaftor Monday, Wednesday and Friday (50mg MWF) with ritonavir 50mg daily
- 75mg ivacaftor Monday, Wednesday and Friday (75mg MWF) with ritonavir 50mg daily

All the above scenarios were simulated for each individual PK profile using data from study C. Predicated data at steady state were then used to calculate C_{min}, C_{max} and $AUC_{504\text{-}672}$ (that is, AUC over one week at steady state). The median and upper and lower quartile values of these parameters from data from the 12 volunteers is reported. An assessment of individual level data for ivacaftor $AUC_{504\text{-}672}$ in all scenarios and dosing simulations is also shown.

Dosing simulation was also undertaken with data from study A to calculate weekly exposure to ivacaftor with a dose of ivacaftor 150mg BD without ritonavir (that is, current standard dosing). Ivacaftor 150mg BD was also simulated using the smallest value for K_{10} obtained from all 12 volunteers in study A then applied to each simulation to calculate a 'worst case scenario' of maximum accumulation of ivacaftor for

comparison to the dosing simulations with ivacaftor combined with ritonavir as described above. Lastly an adjusted $AUC_{504-672}$ for ivacaftor 150mg BD was calculated with a correction for the ⅙ pharmacological activity of M1 to account for the loss of this activity when comparison is made with dosing scenarios with ritonavir, in which the levels of the active metabolite are negligible. This was done by calculating the $AUC_{504-672}$ of M1 at steady state, dividing this value by 6 to reflect the ⅙ activity of M1 and then adding this on to the $AUC_{504-672}$ of the parent compound to give combined $AUC_{504-672}$ of ivacaftor and M1 that reflects the pharmacological activity of both compounds.

All dosing regimens were simulated based on administration at 9am, except simulations of ivacaftor 150mg BD which assumed administration at 9am and 9pm. MWF morning dosing was assumed to be the easiest model for patients taking ivacaftor three times per week, rather than spacing dosing evenly throughout the week.

5.4 Results

5.4.1 Model fitting

Table 29 contains a summary of assessment of fit parameters obtained for each model to which the PK data were fitted. In study A the median sum of squares of the residuals was minimised with application of one-compartment model with lag time, the condition number was minimised with application of the one-compartment model and the correlation of observed versus prediction concentrations was highest with a one compartment model with lag time. The CV% of the output parameters all met acceptability criteria with application of a one compartment model and with application of the one compartment model with lag time all output parameters except K01 met

Table 28. Summary of assessment-of-fit parameters obtained for each model to which the PK data were fitted.

	Study A				Study B					
	One-compartment model	One-compartment model with lag time	Two-compartment model	Two-compartment model with lag time	One-compartment model	One-compartment model with lag time	Two-compartment model	Two-compartment model with lag time	Two-compartment model with lag time and calculated estimates	Two-compartment model with lag time and calculated estimates
Sum of squares of residuals	0.037 (0.02669 – 0.08477)	0.01481 0.005823 – 0.02165	0.02502 0.01847 – 0.09969	0.02791 0.00543 – 0.1545	0.219 0.02907 – 0.3733	0.1039 0.01759 – 0.2261	0.04674 0.02384 – 0.6656	0.04037 0.009613 – 0.1507	0.2968 0.1441 – 0.7518	0.1609 0.06419 – 0.2188
Condition number	2005	34555	551700	8759	3430	3770	551700	224400	197650	232850
Number of parameters	3	4	5	6	3	4	5	6	5	6
Correlation observed vs predicted concentration	0.9705 (0.9662 – 0.9820)	0.9913 (0.9734 – 0.9857)	0.9801 (0.9734– 0.9857)	0.9904 (0.9799 - 0.9837)	0.9721 (0.9504-0.9837)	0.9913 (0.9678-0.9942)	0.9717 (0.953-0.9951)	0.9949 (0.9864-0.9972)	0.9736 (0.9445-0.9851)	0.99 (0.9794-0.9934)
V/F (CV)	17.71 (14.63-11915)	24.25 (8.02 -52.78)			4.892 (4.349 – 7.608)	3.77 (2.655 –5.428)				
K01 (CV)	36.47 (31.41– 11886)	668 (51.72– 5743)			17.08 (13.24– 29.31)	14.66 (12.27– 43.6)				
K10 (CV)	29.66 (23.01– 11889)	16.46 (11.9– 55.03)			18.9 (13.11–28.79)	12.29 (8.863– 19.08)				
T lag (CV)		34.36 (18.96–922.5)		68.78 (25.72–399.1)		14.31 (13.04–40.86)		19.65 (13.91– 92.98)		26.95 (14.96–54.2)
A (CV)			12372 (1507–161693)	2323 (927.9–40403)			7185 (2626–12125)	2323 (214.7–3403)	244.5 (22.42–3200)	235.2 (14.65–1934)
B (CV)			1158 (87.51–1976)	389.7 (219.6–2979)			23.38 (9.096–59.41)	16.01 (9.313–558.8)	59.8 (19.78–864953)	1076 (10.77– 4385358604)
K01 (CV)			1170 (374.9–5762)	781 (240.4–3434)			565.3 (221.9–1344)	240.4 (30.3–305)	320.3 (62.36–410.5)	210.9 (23.33–466.7)
Alpha (CV)			12372 (1507–161693)	2323 (927.9–40403)			7185 (2626–12125)	2323 (214.7–3403)	3200 (244.5–193564)	1934 (235.2– 92025000000)
Beta (CV)			5489 (129.7–159433)	600.8 (189.5–6606)			75.66 (15.86–4195)	43.56 (25.15–7667672)	371.7 (37.64–119306)	13459 (37.11–28477428)

	Study C					
	0ne-compartment model	One-compartment model with lag time	Two-compartment model	Two-compartment model with lag time	Two-compartment model with lag time and calculated estimates	Two-compartment model with lag time and calculated estimates
SSR	0.1716 0.0483 – 0.575	0.06342 0.02452 – 0.5579	0.1762 0.02754 – 0.3713	0.06101 0.009608- 0.3713	0.2732 0.06054 – 0.6659	0.08393 0.0275 – 0.2055
Condition number	2937	3208	522400	533900	285400	1562000
Number of parameters	3	4	5	6	5	6
Correlation observed vs predicted data	0.9868 (0.9637–0.9918)	0.9953 (0.9898–0.9973)	0.9646 (0.9556–0.995)	0.9935 (0.9547–0.9969)	0.9772 (0.9536–0.9916)	0.9935 (0.9801–0.9963)
V/F (CV)	6.004 (2.98-8.221)	2.802 (1.775-5.86)				
K01	16.94 (12.96–25.17)	26.44 (22–52.57)				
K10	17.27 (10.59–25.66)	11.65 (6.813–15.86)				
T lag (CV)		31.49 (11.2–143.1)		28.99 (6.99–392.5)		35.72 (8.758–155.4)
A(CV)			2970 (40.93–8400)	173 (51.59–4583)	2518 (41.38–7690)	642 (51.2–6464)
B(CV)			12.74 (5.75–21.52)	9.77 (5.402–151.6)	9.77 (6.872–2028)	27.12 (6.704–114678596)
K01 (CV)			730.1 (259.5–1326)	368.8 (28.22–1284)	258 (46.91–762.7)	139.7 (39.11–504.9)
Alpha (CV)			8400 (2970–43363)	4583 (173–22194)	7690 (2518–25311)	6464 (642–376311065)
Beta (CV)			29.46 (16.2–70.76)	22.19 (12.35–85.97)	55.22 (18.63–1106)	24.6 (15.07–1992023)

CV, coefficient of variation; V/F, volume of distribution/fraction of drug absorbed; K01, absorption rate constant; K10, elimination rate constant; T lag, lag time.

SSR, sum of the square of the residuals; CV, coefficient of variation; V/F, volume of distribution/fraction of drug absorbed; K01, absorption rate constant; K10, elimination rate constant; T lag, lag time.

acceptability criteria (this is related to the complex absorption features of ivacaftor, see discussion below). The CV% of none of the output parameters met acceptability with application of the two-compartment model either with or without lag time absorption.

In study B, application of the two-compartment model with lag time using estimates calculated by WinNonlin® results in the minimum median value for SSR and the highest correlation of observed versus predicted concentrations, however the CV% of the output parameters was unacceptably large for several of the parameters. The median condition number was also far above acceptability at 224,400. The CV% of some parameters was improved by manual calculation of initial estimates but these were still far above acceptability criteria. Both the one compartment model and the one compartment model with application of lag time met acceptability criteria.

Output from model comparisons for study C was similar. Outputs from application of a one-compartment model and a one-compartment model with lag time met acceptability criteria, whereas all condition numbers and the CV% for most output parameters with the application of a two-compartment model and the two-compartment model with application of lag time did not meet acceptability criteria.

Based on these outputs the one-compartment model and one-compartment model with lag time were deemed acceptable overall and these were then compared to ascertain which represented the best fit for PK profiles in study A and in study C using the F-test and a comparison of the AIC values.

Based on the F-test, the best fit model for 1 of the profiles in study A was one-compartment model, and one-compartment model with lag time for the remaining 11 profiles. There was a significant difference between the mean AIC values for the one-compartment model and the one-compartment model with lag time ($p=<0.0001$); the mean AIC for the one-compartment model was -23.16 and for the one-compartment

model with lag time was -30.94. Based on the F-test the best fit model for study C was a one-compartment model with lag time for 10 PK profiles and a one-compartment model for 2 profiles. There was a significant difference between the mean AIC values for the one-compartment models and the 1 compartment models with lag time (p=0.015); the mean AIC for the one-compartment model was -9.79 and for the one-compartment model with lag time was -20.29 (see table 29). The one-compartment model with lag time was therefore identified as the best fit model overall and this model was used in all subsequent dosing simulations.

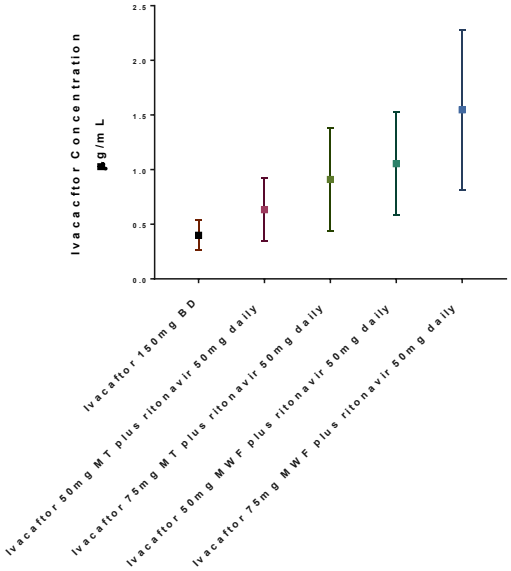
Table 29. Results of F test for comparison of nested models to compare the application of a 1-compartment model and a 1-compartment model with lag time to PK data from study A and study C.

	Result of F test comparing 1-compartment model to a 1-compartment model with lag time absorption	
	Study A	Study C
Volunteer 2	6.71699209	0.2161117
Volunteer 3	18.855267	18.184927
Volunteer 4	9.23508472	0.0300598
Volunteer 5	0.32278568	73.773427
Volunteer 6	9.14151976	6.781439
Volunteer 9	14.3471364	6.6633389
Volunteer 10	15.1271351	138.57824
Volunteer 11	11.354124	2.850566
Volunteer 12	8.54858755	42.355356
Volunteer 13	5.88918883	3.3713609
Volunteer 14	2.70108992	2.3189274
Volunteer 15	5.31118248	12.745178

5.4.2 Dosing simulation

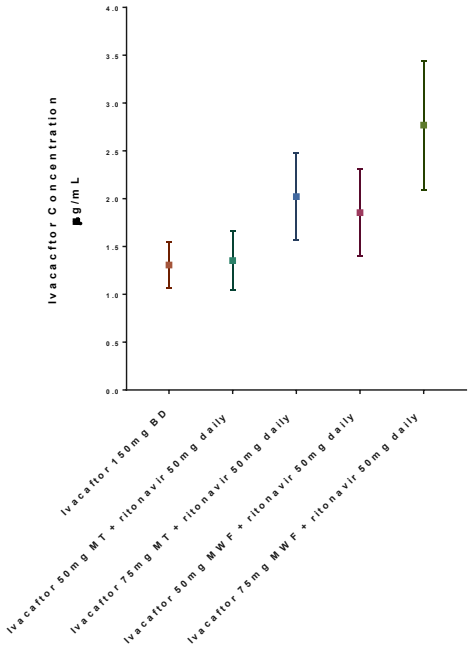
Four ivacaftor dosing scenarios were simulated using a one-compartment model with lag time: 50mg MT, 75mg MT, 50mg MWF and 75mg MWF all with ritonavir 50mg daily. The median and upper and lower quartile concentrations of the predicted data were used to calculate the exposure to ivacaftor over 7 days at steady state, $AUC_{504-672}$. Figures 19–22 show the median, upper quartile and lower quartile for study A and the 4 dosing

scenarios. Peak and trough concentrations were higher in all dosing scenarios with ritonavir in comparison to ivacaftor 150mg twice daily (figures 19 and 20).



BD, twice daily; MT, Monday and Thursday; MWF, Monday, Wednesday and Friday; C_{min}, minimum concentration.

Figure 19. Predicted trough concentrations of ivacaftor at steady state for each dosing scenario.



BD, twice daily; MT, Monday and Thursday; MWF, Monday, Wednesday and Friday; C_{max}, maximum concentration

Figure 20. Predicted peak concentrations of ivacaftor at steady state in each dosing scenario.

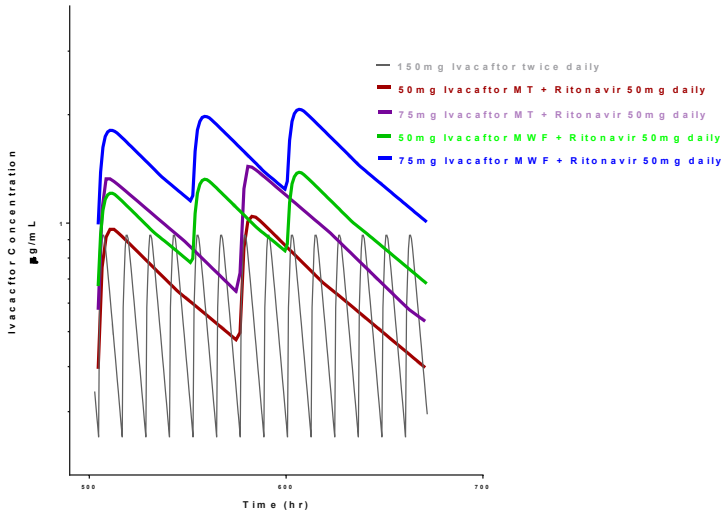


Figure 21. Lower quartile concentration–time profiles of predicted data for each dosing scenario.

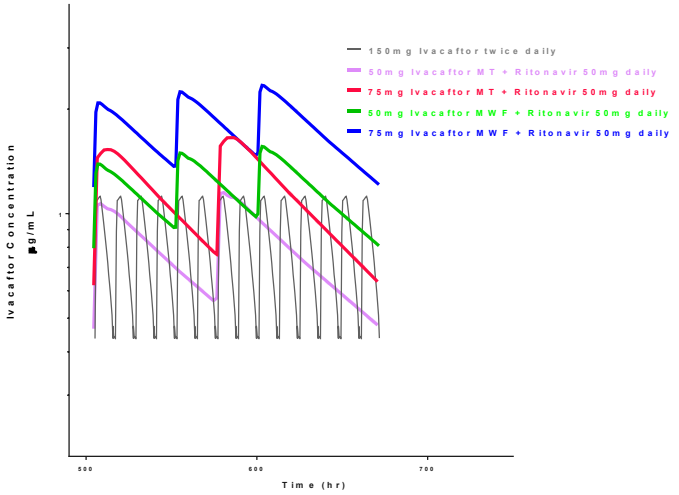


Figure 21. Median concentration–time profiles of predicted data for each dosing scenario.

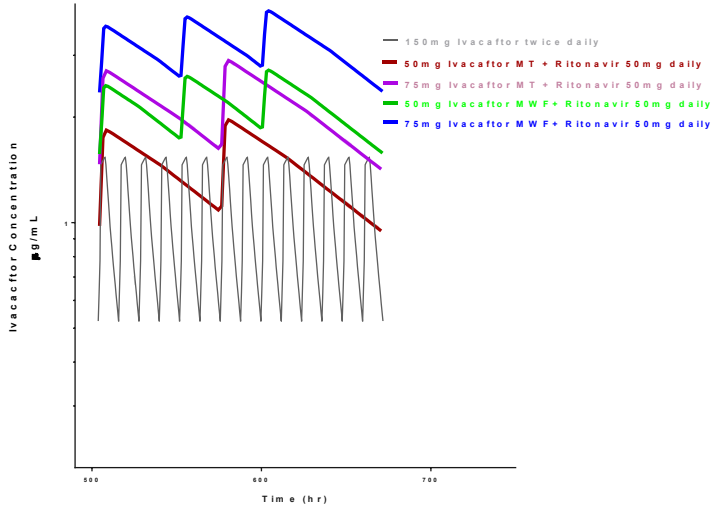


Figure 23. Upper quartile concentration–time profiles of predicted data for each dosing scenario.

5.4.2.1 Ivacaftor corrected for activity of M1

M1 demonstrates one sixth of the pharmacological activity of ivacaftor.(9) The addition of ritonavir in study C led to very low and undetectable levels of M1 in volunteer plasma. To correct for the loss of pharmacological activity of M1 with the addition of ritonavir, a corrected ivacaftor $AUC_{504\text{-}672}$ was calculated to incorporate the activity of the M1. This corrected $AUC_{504\text{-}672}$ is shown in table 29 below. Correction for the activity of M1 in the ivacaftor 150mg twice daily dose modelling increases the $AUC_{504\text{-}672}$ such that the ivacaftor 50mg MT with ritonavir 50mg daily results in reduced exposure, but all other dosing scenarios have an equivalent or higher $AUC_{504\text{-}672}$.

Table 30. Ivacaftor $AUC_{504\text{-}672}$ for all dosing scenarios and regimens.

Dosing Regimen	$AUC_{504\text{-}672}$ (hr*mcg/mL)		
	Lower Quartile	Median	Upper Quartile
Ivacaftor 150mg twice daily	96.61	133.24	169.73
Ivacaftor 150mg twice daily corrected for activity of M1	115.65	169.48	221.1
Ivacaftor 150mg twice daily simulated with maximum accumulation	243.49	290.9	473.46
Ivacaftor 50mg MT plus ritonavir 50mg daily	114.04	131.58	239.63
Ivacaftor 75mg MT plus ritonavir 50mg daily	170.22	196.99	365.15
Ivacaftor 50mg MWF plus ritonavir 50mg daily	169.63	196.17	358.35
Ivacaftor 75mg MWF plus ritonavir 50mg daily	253.32	293.96	532.53

M1, hydroxymethyl-ivacaftor; MT, Monday and Thursday; MWF, Monday, Wednesday and Friday; $AUC_{504–672}$, area under the time–concentration curve over one week at steady state.

5.4.2.2 Ivacaftor dosing adjusted for smallest K_{10}

A comparison of the data from study A NCA to published data shows that the mean $T_{1/2}$ in the current study (7.121 [5.59–9.07] hrs) is shorter than that reported in the literature (12 hours).(12) In order to model a scenario of maximum possible accumulation of, and therefore exposure to, ivacaftor with 150mg twice daily dosing, all dosing simulations were rerun using the minimum K_{10} obtained from the 12 volunteers (K_{10} = 0.059). Data obtained from this scenario are shown in table 29. In this scenario ivacaftor 75mg MWF with ritonavir 50mg daily shows similar exposure over 7 days.

5.4.2.3 Individual profiles

The individually calculated $AUC_{504\text{-}672}$ for all 4 dosing scenarios is contained in table 31. All dosing regimens except 50mg MT lead to exposures equivalent or above steady state exposure of that seen in the standard dosing of 150mg twice daily without ritonavir (note that volunteers 1, 7 and 8 did not progress past the initial screening step; therefore the 12 volunteers participating are numbered 2–6 and 9–15). Analysis of the individual PK profiles and dosing simulations shows that the largest increase in ivacaftor concentrations with the addition of ritonavir was seen in volunteer 15 and the smallest for volunteer 14. The maximum accumulation of ivacaftor in dosing simulation from study A was seen in volunteer 11 as discussed above. To meet an $AUC_{504\text{-}672}$ equivalent or above that seen in standard dosing of ivacaftor volunteers 11 and 14 require ivacaftor 150mg MWF plus ritonavir 50mg daily. When the ivacaftor $AUC_{504\text{-}672}$ is adjusted for the activity of M1, equivalent $AUC_{504\text{-}672}$ with ritonavir 50mg daily requires administration of ivacaftor 150mg MT for volunteers 11 and 14. Finally if the dosing simulation using the maximum accumulation scenario for ivacaftor 150mg BD dosing is compared to dosing with ritonavir, to obtain and equivalent or higher $AUC_{504\text{-}672}$ for ivacaftor with ritonavir

50mg daily volunteers 2, 6, 10 and 11 require ivacaftor 150mg MT, volunteer 9 requires ivacaftor 150mg MWF and volunteer 14 requires ivacaftor 150mg Monday, Wednesday Friday and Saturday (MWFS).

5.5 Discussion

These data suggest that the dosing interval of ivacaftor can be significantly increased and the dose reduced in the presence of a very small dose of ritonavir, without decreasing overall exposure to ivacaftor. Median $AUC_{504-672}$ of ivacaftor 150mg bd at steady state without ritonavir is predicted to be 133.24 hr*mcg/mL. In combination with ritonavir 50mg daily, median $AUC_{504-672}$ of ivacaftor is 131.58 hr*mcg/mL, 196.99 hr*mcg/mL, 196.17 hr*mcg/mL and 293.96 hr*mcg/mL for ivacaftor 50mg twice weekly, ivacaftor 75mg twice weekly, ivacaftor 50mg three times weekly and ivacaftor 75mg three times weekly respectively. When the median $AUC_{504-672}$ is corrected for the activity of M1 ivacaftor 75mg twice weekly in combination with ritonavir 50mg daily is the lowest dose of ivacaftor that results in equivalent or higher predicted exposure compared to ivacaftor 150mg twice daily (median $AUC_{504-672}$ 169.48 hr*mcg/mL versus 196.99 hr*mcg/mL respectively). The doses of ivacaftor described above (50mg and 75mg) are readily available as a powder formulation, which has been shown to have the same bioavailability as the ivacaftor tablet used in the study above at equivalent doses.(34)

The best fit model, a one-compartment model with absorption lag time, was different to that in the Clinical Pharmacology and Biopharmaceutics Review of Ivacaftor submitted to the FDA.(9) The model used in this was a two-compartment model with zero-order input

Table 31. Predicted $AUC_{504-672}$ (h*ug/mL) for each modelling and dosing scenario explored.

Volunteer Number	Predicted $AUC_{504-672}$ (h*ug/mL)									
	Ivacaftor 150mg BD	Ivacaftor $AUC_{504-672}$ adjusted for M1	Ivacaftor 150mg BD maximum accumulation	Ivacaftor 50mg MT+ Ritonavir 50mg OD	Ivacaftor 75mg MT+ Ritonavir 50mg OD	Ivacaftor 50mg MWF + Ritonavir 50mg OD	Ivacaftor 75mg MWF + Ritonavir 50mg OD	Ivacaftor 150 mg MT + Ritonavir 50mg OD	Ivacaftor 150mg MWF + Ritonavir 50mg OD	Ivacaftor 150mg MWFS + Ritonavir 50mg OD
2	133.69	173.53	278.69	108.75	163.13	162.47	243.71	323.91		
3	105.49	146.1	241.41	196.09	294.13	291.80	437.71			
4	107.03	139.93	272.33	170.78	256.18	254.44	381.66			
5	63.54	79.73	119.7	125.62	188.43	188.07	282.11			
6	134.06	152.1	249.73	108.01	160.29	161.49	239.66	321.38		
9	134.46	218.96	512.59	134.97	202.45	201.67	302.51	401.48	605.02	
10	203.10	254.2	620.54	257.81	386.71	384.34	576.51	773.43		
11	351.87	402.96	351.85	124.92	184.54	186.65	275.73	366.65	551.47	
12	150.78	221.78	296.45	258.69	380.57	386.21	568.17			
13	69.01	90.34	191.04	124.59	186.89	186.16	279.24			
14	165.75	181.83	342.41	59.77	90.16	89.65	135.24	179.22	268.94	392.38
15	189.07	220.04	609.48	289.13	433.7	433.13	649.70			

BC, twice daily; $AUC_{504–672}$, area under the time-concentration curve over 1 week at steady state; MT, Monday and Thursday; MWF, Monday Wednesday and Friday; Monday, Wednesday, Friday and Saturday MWFS; OD, once daily.

to the absorption compartment and first-order absorption for adults and a two-compartment model with lag-time absorption for those aged 6-11 years old. Furthermore, as can be observed in the data plots above, absorption of ivacaftor often features a double peak. This can also be seen in the single dose escalation studies, particularly for doses above 675mg.(9) It may be the case that with richer sampling in the absorption phase, coupled with longer sampling time, a different model may be a superior fit, but for the purposes of the current study that focuses on characterising the inhibition of the metabolism of ivacaftor by ritonavir, one-compartment model with lag-time first order absorption does work adequately to describe the data.

It is of utmost clinical importance that if a patient were to transfer from ivacaftor 150mg BD dosing to a dosing regimen using ritonavir 50mg daily with reduced frequency of ivacaftor, exposure to ivacaftor would not be decreased—a situation which could theoretically lead to a decline in clinical condition. The short sampling time in study A (12 hours) could have resulted in the underestimation of $T_{1/2}$ of ivacaftor, leading then to an underestimation of accumulation of ivacaftor. To assess the effect of this scenario, all data were dose simulated based on the smallest K_{10} value from all volunteers (K_{10} = 0.059; volunteer 11).(12) Modelling this shows that the median exposure was still equivalent with three-times weekly dosing of ivacaftor 75mg even in this theoretical situation of maximum accumulation of ivacaftor at standard 150mg BD dosing.

A dosing regimen with ritonavir was sought that could provide equivalent exposure to ivacaftor while also maintaining trough levels above a minimum that has been shown to be effective. The EC_{90} for ivacaftor in the treatment of CF is not yet known; however, in the biopharmaceutical review of Orkambi that was submitted to the FDA in 2013, a reviewer reanalysed the PK/PD data available for ivacaftor from the STRIVE study and showed that there was a trend of improvement in $ppFEV_1$ up to the third quartile of

trough concentrations of ivacaftor, that is to a plasma concentration of approximately 550ng/mL.(2) The confidence intervals were wide, but in the absence of robust PK/PD data it is reasonable to use this trough level as a reference point in the dosing simulations. The lower quartile C_{min} is maintained above this level in all dosing scenarios except 50mg MT. This is a significant improvement in trough concentration in comparison to the standard 150mg BD dosing in which simulations show a trough level below 550ng/mL in 9 out of 12 volunteers.

Ivacaftor animal studies have shown the drug to be safe at doses several-fold above those intended to be used in clinical practice. In human studies, ivacaftor has been shown to be safe in doses up to 250mg BD (data from initial dose finding study #101).(9) There was no increase in adverse effects between a group treated with a 250mg BD dose compared to a group treated with the 150mg BD dose. This suggests that the increase in exposure associated with the increase in dose from 150mg BD to 250mg BD is not accompanied by an increase in toxic effects. Therefore, ivacaftor does not appear to have a narrow therapeutic window. Moreover, the concentrations of ivacaftor measured in this dose escalation study exceed 10 µg/mL in some cases. These concentrations are almost twice what is predicted to be the peak concentration of ivacaftor administered with ritonavir from the present study, providing reassurance that the potential increased peak doses shown in the above study will be safe in clinical practice.

In the initial stages of the STRIVE study, there was a safety signal suggesting that liver function tests (LFT) may be adversely affected by ivacaftor. Measurement of LFT was undertaken once every two weeks subsequent to this signal to clarify this effect of ivacaftor on the liver. Final analysis of these data showed that ivacaftor treatment did not cause an increase in liver enzymes. Given that the dose of ritonavir proposed in this study is extremely small and that once daily (OD) ritonavir will not accumulate to any

significant extent given its short $T_{1/2}$ (approximately 5 hours for ritonavir 100 mg daily at steady state), it is unlikely that this combination will have an adverse effect on liver function.(50) Assuming a similar $T_{1/2}$ for ritonavir 50mg daily means that over a 24 hour period the dose of ritonavir will be almost completely eliminated, that is, accumulation of ritonavir with chronic use will be minimal. An initial monitoring period of LFTs would be reasonable with ivacaftor and ritonavir treatment to confirm safety from a liver function perspective.

Treatment with inhibitors of the CYP system presents challenges in all clinical scenarios when it must be utilised, though it is encouraging that drug interactions of boosted PIs have been managed successfully in clinical practice for many years since the advent of this drug regimen for the treatment of HIV. Furthermore, the case of treatment of cystic fibrosis for those on strong CYP inhibitors is already faced in standard clinical practice when treatment with itraconazole is needed, usually for ABPA. Treatment with itraconazole requires patients to take ivacaftor 150mg twice weekly rather than twice daily, along with appropriate adjustment of other regular medications, so there is already established clinical experience in managing concomitant medications for PWCF who are on strong CYP inhibitors.

Table 30 contains a list of medications that may be used in the treatment of cystic fibrosis along with a prediction of the likelihood of an interaction with ritonavir. This is not an exhaustive list of medications that may be used in CF, indeed a person with CF may have many other concomitant conditions but contained are those deemed to be most frequently prescribed. It is advantageous that the vast majority of medications routinely used in the treatment of CF are not subject to clinically significant alterations in metabolism in the presence of ritonavir, making ivacaftor coadministered with ritonavir a practical option in the treatment of CF. Cross-resistance is a known feature of the

treatment of HIV with PIs. Theoretically, any patient on ritonavir-boosted ivacaftor could contract HIV and develop a resistance mutation that will render their strain of HIV resistant to ritonavir as well as other PIs such as indinavir and saquinavir.(105, 106) All patients starting on ritonavir boosting should therefore be tested for HIV. Ongoing routine HIV testing should then take place throughout the time course that any person with CF is on ritonavir as indicated by sexual health screening guidelines. This would not represent a significantly increased burden of testing for patients on ritonavir boosting.

Table 32. Pharmacokinetic drug interactions with ritonavir.

Medication			Metabolism	Interaction potential
Drugs used in the maintenance of lung health				
	Salbutamol		Sulfation (Salbutamol ➔ salbutamol4–O-sulfate)(107)	B: interaction; no action needed
	Azithromycin		Excreted Unchanged in urine and bile	C: interaction; monitor therapy
	Inhaled DNase		Metabolised by proteases in pulmonary fluid	A: no known interaction
	Inhaled hypertonic saline		No metabolism	A no known interaction(108)
	Ibuprofen		Excretion unchanged in the urine	A: no known interaction(109)
	Systemic glucocorticoids			
		Prednisolone	Excreted unchanged in the urine & as glucoconjugates	D: interactions; consider modification of therapy(50)
		Hydrocortisone	Hepatic CYP 3A4, primarily metabolised to	C: interaction; monitor carefully
	Inhaled glucocorticoids			
		Fluticasone	Hepatic CYP 3A4	D: interaction; consider modification
		Budesonide	Hepatic CYP 3A4	C: interactions; monitor patient carefully
		Beclomethasone	Hydrolysed by esterases	A: no known interaction
	Inhaled tobramycin		Minimal systemic exposure after inhalation. Eliminated unchanged by the kidneys	A: no known interaction
	Montelukast		Extensively metabolised by P450 2C8	A: no known interaction
	Inhaled N-acetylcysteine		De-acetylated to cysteine in the liver and then metabolised to glutathione. 1/3 eliminated unchanged in the urine	A: no known interaction
	Inhaled iptratropium			A: no known interaction
	Inhaled aztreonam		Excreted mostly unchanged in the bile, biotransformation in the gut lumen and excretion through the kidneys	A: no known interaction

Drugs used in the treatment of pulmonary exacerbations			
	Aminoglycoside antibiotics	Excreted unchanged in the urine	Unlikely
	Beta lactam antibiotics	Predominantly excreted unchanged in the urine	Unlikely
Drugs for the treatment of Pseudomonas Aeruginosa			
	Oral ciprofloxacin	Excreted predominantly unchanged in the urine and bile.	C: interactions; monitor patient carefully
	Nebulised colistin	Excreted predominantly unchanged in urine(110)	A: no known interaction
Drugs for the treatment of ABPA			
	Itraconazole	CYP 3A4 is the major enzyme involved in the metabolism of itraconazole(111)	D: interactions; modify therapy
Drugs used in the maintenance of GI function			
	Proton pump inhibitors for GORD (for example lansoprazole)	Mainly metabolised by CYP2C19 with come contribution by CYP 3A4	A: no known interaction(112)
	Pancreatic enzymes	Proteolysed in the gut lumen(113)	A no known interaction
Drugs used to treat diabetes associated with CF - insulins			
	Insulin glargine	Proteolytic cleavage to active M1 metabolite(114) Cleared by metabolic degradation by a receptor mediated process	C: interaction; monitor patient
	Insulin lispro	Metabolic degradation receptor mediated(115)	C: interaction; monitor patient
Drugs used to treat osteoporosis			
	Cholecalciferol	Metabolised in the liver to the active metabolite 1,25-dihydroxycolecalciferol(116)	A: no known interaction
	Calcium	No metabolism	A: no known interaction
	Bisphosphonates	No metabolism. Renal elimination.(117)	A: no known interaction
Drugs used to treat small bowel bacterial overgrowth			
	Metronidazole		X interaction identified

Adapted from (18, 118)unless otherwise stated.

5.6 Limitations

The above data are simulations using single-dose data to predict concentrations at steady state. More information regarding the elimination of ivacaftor at steady state in the presence of ritonavir would be obtained if PK profiles were obtained from healthy volunteers at steady state after multiple doses of ivacaftor and ritonavir. The above data were all collected from healthy volunteers, and while PK studies have suggested that PK in healthy volunteers is equivalent to that in PWCF it would be prudent to examine the effect of ritonavir inhibition in this cohort. This could be achieved by completing PK profiles on individual patients before and after the introduction of ritonavir to ensure adequate exposure to ivacaftor with reduced dosing. Richer sampling in the absorption phase may also provide more detailed information on the absorption profile of ivacaftor, especially in the presence of ritonavir. Measurement of individual steady state PK profiles for patients on a ritonavir plus ivacaftor regimen would also mitigate any potential issues that could arise from the modelling of single dose data for a drug that demonstrates such variable absorption. Furthermore, longer sampling times, particularly in study A, may reveal the presence of a second or third compartment which could improve model fit. Finally, the simulations above provide only information regarding predicted steady state concentrations of ivacaftor, there is no information regarding the pharmacodynamic effects of the combination, which would require study of a larger population of PWCF on ivacaftor plus ritonavir.

5.7 Conclusion

Addition of ritonavir 50mg daily to ivacaftor treatment results in a significant increase in the dosing interval coupled with a reduction in dose of ivacaftor to obtain an equivalent exposure to ivacaftor. Use of ritonavir boosting of ivacaftor has a clinical precedent in

the use of twice weekly dosing of ivacaftor when concomitant treatment with itraconazole is required and based on the data available for both ivacaftor and ritonavir is likely to be safe and practical in everyday practice. A 75mg ivacaftor twice weekly plus ritonavir 50mg daily dosing regimen is used in the next chapter to explore the budget impact of concomitant dosing of ivacaftor plus ritonavir in the population of those on ivacaftor for treatment of CF with the G551D mutation.

CHAPTER 6

6 Budget impact analysis

6.1 Introduction

The proposed dosing schedule of ivacaftor plus ritonavir presented in the preceding chapters allows for much reduced dosing of ivacaftor without any reduction in exposure to the active ingredient in vivo. This offers the possibility of a significant cost saving in ivacaftor treatment. The PCRS statistical analysis of claims and payments 2016 reported that the overall expenditure on ivacaftor was €29.88 million Jan – Dec 2016 inclusive. This represents 5.66% of the total HT scheme expenditure, but only 0.29% of the overall number of prescriptions written for the scheme, highlighting the current, particularly high-cost nature of ivacaftor treatment.(1)

At the time of writing, clinical efficacy data pertaining to twice weekly ivacaftor treatment with ritonavir is not available; however, based on a model of clinical efficacy proportional to overall drug exposure, no detrimental effect on clinical efficacy is expected. In this context, a formal budget impact analysis of the change in overall cost with this new dosing regimen is the most appropriate method to assess its financial impact. This comprehensive analysis also takes into account all of the extra expenses that may be involved in implementing the new regimen safely and effectively for the patient population.

6.2 Methods

This budget impact (BI) model was prepared as per the Guidelines for the Budget Impact Analysis of Health Technologies in Ireland published by the Health Information and Quality Authority, 2014 and the Guidance on Budget Impact Analysis of Health

Technologies in Ireland 2018.(71, 119) Data were analysed using Microsoft® Excel® 2016.

6.2.1 Perspective

The perspective taken in this budget impact model is that of the payer, the HSE.

6.2.2 Technology assessed

The technology being assessed is that of a combination treatment of ivacaftor with ritonavir for the treatment of CF with the G551D mutation. The base case is the lowest dose of ivacaftor in conjunction with 50mg ritonavir daily that leads to equivalent exposure to the ivacaftor 150mg BD regimen in the analyses described above, that is ivacaftor 75mg twice weekly. The comparator is the usual dose of ivacaftor used in clinical practise for patients who do not require concurrent treatment with CYP3A4 inhibitors, namely ivacaftor 150mg twice daily.

6.2.3 Time Frame

This BI analysis is presented over a 5-year time horizon without discounting.

6.2.4 Target population

Data describing the Irish population with the G551D mutation on ivacaftor since the drug was approved in 2013 were obtained from the PCRS. Complete data on the number of patients on ivacaftor each year were available from 2013 to 2016. Based on the mean number of new patients per year starting ivacaftor, these data were extrapolated to 2022.

6.2.5 Cost of the intervention

The cost due to the intervention was calculated based on 1) the cost of ivacaftor paediatric formulation 50mg or 75mg or ivacaftor tablets 150mg 2) the cost of daily ritonavir 50mg and 3) the cost of the additional monitoring necessary to switch to from ivacaftor daily to ivacaftor plus ritonavir in addition to the ongoing monitoring necessary after the commencement of ritonavir. It is not anticipated that dispensing ivacaftor plus ritonavir will lead to any extra costs in comparison to ivacaftor alone as there is a single patient care fee in the community pharmacy in both scenarios therefore the cost of administration is not considered in this budget impact model. Given the minimal side effects of the boosting dose of ritonavir and wide therapeutic index of ivacaftor for the purposes of this analysis it was assumed that the cost of treating AEs will remain unchanged.

The cost of the paediatric formulation of ivacaftor was obtained from the PCRS. In the clinical trial described in Chapter 5, ritonavir liquid measured to a dose of 50mg was used rather than ritonavir sachets. In the time between completion of the trial and completion of the budget impact analysis, ritonavir liquid was discontinued and replaced with ritonavir sachets at a dose of 100mg. These two formulations have been shown to be bioequivalent at the same dose and therefore it was considered appropriate to substitute ritonavir liquid for ritonavir sachets in this analysis.(120) The cost of 100mg ritonavir sachets was obtained from the records of the Pharmacy Department in St James's Hospital in 2017 (cost quoted is that prior to any confidential discounts that may apply).

The cost of switching from ivacaftor alone to ivacaftor plus ritonavir is mainly driven by the cost of obtaining a full PK profile over 1 dosing interval of ivacaftor alone and a

further PK profile over one dosing interval of ivacaftor plus ritonavir to ensure at least identical exposure. The cost of the PK profile was calculated using the following costs:

- Half-day, day-ward stay for first the complete ivacaftor alone profile and for the first 12 hours of the ivacaftor plus ritonavir PK profile
- 5 further blood samples taken by CF nurse specialist to complete the 48 hour PK profile (cost of time calculated based on the midpoint of pay scale with associated non-pay costs added in accordance with methods outlined by the Regulatory Impact Analysis guidelines issued by the department of the Taoiseach(71, 121))
- Total itemised cost of consumables for each plasma sample for PK analysis (Costs of all consumables were obtained from suppliers in Ireland and were based on the consumables used in the LC-MS assay described in chapter 3)
- Personnel in biochemistry laboratory where samples are analysed (3 hours mid-grade lab technician(126))
- PK data analysis (time of mid-grade pharmacology and therapeutics specialist registrar(126))

Cost for all patients on the ivacaftor plus ritonavir regimen also includes 6-monthly HIV test, plasma lipids and glucose levels and 3-monthly liver function tests. These costs were obtained from the NCPE.

6.2.6 Base Case

The base case scenario consisted of a total eligible population of all patients on ivacaftor above the age of 18, 25% of whom were considered likely to take up

treatment with ritonavir in the first year. In the STRIVE study 25% of those in the ivacaftor arm had a suboptimal (5% or less) improvement in ppFEV$_1$.(6) A group of patients who have a suboptimal response to ivacaftor were considered the most likely and most clinically appropriate group to initially move to ivacaftor plus ritonavir dosing given the possibility that an increase in plasma levels may improve their clinical response, though there is no evidence that an improvement would be guaranteed given the uncertainty around the PK/PD relationship of ivacaftor. For the purposes of the budget impact model it is assumed that the clinical response remains the same. An annual increase in uptake of 10% of the total population per year up to a maximum of 65% in 2022 was applied, though due to the absence of data available on the attitudes of the total population on ivacaftor to the use of dosing regimen with ritonavir this assumption is fully explored in scenario analyses (described below).

6.2.7 Areas of relative certainty

The price of ivacaftor is set until the patent expires in 2026. The price of the ritonavir sachets is not expected to change over the time horizon. Based on horizon scanning it is not expected that new, high-cost therapies will be introduced for the treatment of CF with the G551D mutation in the next 5 years.(122, 123)

6.2.8 Analysis of uncertainty

The predominant uncertainty lies in the size of the population that will take up treatment with ivacaftor and ritonavir over the 5-year time horizon. A number of scenarios in this regard were explored. The other significant area of uncertainty is the PK modelling and simulation that results in the final dosing scenarios that are fed into

the BI model. To explore this, scenarios were modelled based on the different dosing simulations described in the chapter above

6.2.8.1 Male-only population

Given the issues discussed in the preceding chapter regarding the interaction of ritonavir with contraceptive methods, a scenario in which only male adult patients sought the intervention was explored. The percentage uptake of 25% in year 1 and annual increments of 10% were applied as before.

6.2.8.2 Adult population excluding those patients with CF related diabetes

Given concerns regarding the metabolic effects of ritonavir at treatment doses, a scenario in which patients with established CF related diabetes are excluded is explored. Projections of the size of the future population of those with CF and the G551D mutation who will develop CF related diabetes is based on data from the CF registry 2015.(5)

6.2.8.3 Complete adult population

A scenario in which all patients over the age of 18 switch to ivacaftor plus ritonavir in year 1 and continue for 5 years.

6.2.8.4 Complete population above 6 years old

This scenario includes the total adult population and those over the age of 6 on ivacaftor.

6.2.8.5 Uptake steady at 25%

This scenario assumes that the population who likely have a suboptimal response to ivacaftor (25% based on the clinical trial data(6)) take up concomitant dosing of ivacaftor plus ritonavir and this population then remains static for 5 years.

6.2.8.6 One patient only

This scenario illustrates the minimum potential saving should ivacaftor plus ritonavir dosing only be taken up by one patient who remains on this regimen for 5 years. Thus, a range of budget impact from a population of only 1 patient to the complete population of ivacaftor over 6 years old can be appreciated.

6.2.8.7 Individually-tailored dosing simulations compared to ivacaftor 150mg BD.

In the previous chapter an individual-level analysis shows that twice weekly ivacaftor at a dose of either 50mg, 75mg or 150mg is sufficient so that each volunteer in the study achieves equivalent or increased exposure to ivacaftor 150mg BD. To assess the effect that these tailored dosing schedules may have on the BI the base case population was modelled using the proportions of these doses needed in the study group.

6.2.8.8 Individually-tailored dosing simulations based on ivacaftor exposure corrected for M1

This scenario results in 4 volunteers requiring ivacaftor three times per week, rather than twice per week as described in the median analysis of the group. This finding is extrapolated out to the whole population such that an identical ratio of patients will be on twice weekly and three times weekly ivacaftor.

6.2.8.9 Individually-tailored dosing simulations based on modelling highest K_{10} in study A

Individual modelling using maximum K_{10} in study A (which reflects the expected accumulation ratio of approximately 2 based on the $T_{1/2}$ of approximately 12 hours) shows that 4 out of 12 patients will require ivacaftor three times weekly and 1 will require ivacaftor 4 times weekly with the others taking ivacaftor twice weekly as before. Again, this finding is extrapolated out to the whole population such that an identical ratio of patients will be on twice weekly, three times weekly and 4 times weekly ivacaftor dosing.

6.3 Results

6.3.1 Comparator

The comparator is standard treatment with ivacaftor 150mg BD in the population of patients with CF and the G551D mutation in which this treatment is indicated.

6.3.2 Eligible population

Complete data describing the number of patients on ivacaftor with the G551D mutation were available from 2013 to 2016, along with data up to March 2017. The median increase in patients each year was 7. This increase was used to calculate the projected increase in population from 2018 to 2022. The proportion of males to females and adults to children were assumed to remain constant. Table 33 contains the projected population figures from 2018 to 2022.

Table 33. Projected population with at least one G551D mutation in Ireland

	2018	2019	2020	2021	2022
Total population of patients with the G551D mutation eligible for ivacaftor treatment	162	169	176	183	190

6.3.3 Cost of the Intervention

6.3.3.1 Cost of Ivacaftor

The annual cost of standard dosing of ivacaftor 150mg tablets and of the 50mg and 75mg paediatric formulations, including the patient care fee paid to the pharmacy dispensing drugs reimbursed through the HT scheme, is contained in table 34.

Table 34. Annual cost of ivacaftor.

	Total annual cost including patient care fee
Ivacaftor 150mg BD tablets	€235,549
Ivacaftor paediatric granules 75mg	€234,219
Ivacaftor paediatric granules 50mg	€234,219

BD, twice daily.(124)

6.3.3.2 Cost of ivacaftor plus ritonavir

The base case describes treatment with ivacaftor 75mg twice weekly with ritonavir 50mg daily. The total annual cost for drug acquisition including patient care fee was calculated to be €34,478.9 (patient care fee is a fixed fee of €62.03 per patient per month. Data obtained from PCRS).(124) Annual cost of other dosing regimens is also shown in table 35.

Table 35. Annual cost of dosing scenarios

		Ritonavir 50mg daily	**Total Cost**
Ivacaftor 75mg or 50mg twice weekly	€34,077.91	€400.99	€34,478.90
Ivacaftor 75mg or 50mg three times weekly	€50,744.62	€400.99	€51,145.61
Ivacaftor 150mg twice weekly	€34,288	€400.99	€34,688.99
Ivacaftor 150mg three times weekly	€51,059.76	€400.99	€51,460.75
Ivacaftor 150mg 4 times weekly	€67,832	€400.99	€68,232.99

6.3.3.3 Cost of monitoring

The total cost per PK profile is contained in table 34. The cost for yearly basic monitoring includes LFT x3, glucose x2, HIV test x2, lipid profile x2. LFTs were calculated as three times yearly as once yearly LFTs are expected as part of standard care with ivacaftor treatment, giving a total of LFTs 4 times yearly.

Table 36. Cost of monitoring

Cost per 12-hour PK profile	Cost per 72-hour profile	Cost for yearly basic monitoring
€812.33	€1003.91	€106

PK, pharmacokinetic.

6.3.3.4 Base case

The gross and incremental budget impact of the base case as described above are contained in table 37.

The number of eligible patients consists of the adult population in each given year from 2018 to 2022. Treatment cost is the cost of ivacaftor granules 75mg twice weekly, ritonavir 50mg daily and increased monitoring, as described above.

The net budget impact of ritonavir boosting of ivacaftor is a saving of €4,952,411 in year 1 increasing to €15,032,168 in year 5, with a cumulative 5-year saving of €49,021,152.

Table 37. 5-year budget impact of incremental uptake of ivacaftor 75mg twice weekly and ritonavir 50mg daily in the adult population.

	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	0.25	25	€936,317	€5,888,728	-€4,952,411
Year 2 (2019)	0.35	36	€1,276,602	€8,479,769	-€7,203,167
Year 3 (2020)	0.45	48	€1,694,489	€11,306,358	-€9,611,870
Year 4 (2021)	0.55	61	€2,146,960	€14,368,497	-€12,221,537
Year 5 (2022)	0.65	75	€2,634,016	€17,666,184	-€15,032,168
Cumulative 5-year cost					**-€49,021,152**

BD, twice daily.

6.3.4 Analysis of uncertainty

Tables 38–46 below contain the net budget impact for all scenarios described in the analysis of uncertainty.

Table 38. 5-year budget impact of dosing regimen adjust for the pharmacological action of M1

	Number of patients eligible for treatment	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	98	0.25	25	€1,076,519	€5,888,728	-€4,812,209
Year 2 (2019)	102	0.35	36	€1,478,493	€8,479,769	-€7,001,275
Year 3 (2020)	106	0.45	48	€1,963,677	€11,306,358	-€9,342,681
Year 4 (2021)	110	0.55	61	€2,489,054	€14,368,497	-€11,879,443
Year 5 (2022)	114	0.65	75	€3,054,623	€17,666,184	-€14,611,561
Cumulative 5-year cost						**-€47,647,169**

Table 39. 5-year budget impact of dosing regimens based on individual modelling extrapolated to total adult population

	Number of patients eligible for treatment	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	98	0.25	25	€955,804	€5,888,728	-€4,932,924
Year 2 (2019)	102	0.35	36	€1,304,664	€8,479,769	-€7,175,105
Year 3 (2020)	106	0.45	48	€1,731,904	€11,306,358	-€9,574,454
Year 4 (2021)	110	0.55	61	€2,194,509	€14,368,497	-€12,173,987
Year 5 (2022)	114	0.65	75	€2,692,479	€17,666,184	-€14,973,706
Cumulative 5-year cost						**-€48,830,175**

Table 40. 5-year budget impact of dosing regimen based on a fixed uptake of 25% of the adult population

	Number of patients eligible for treatment	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	98	0.25	25	€936,317	€5,888,728	-€4,952,411
Year 2 (2019)	102	0.25	26	€902,075	€6,124,277	-€5,222,202
Year 3 (2020)	106	0.25	27	€936,660	€6,359,826	-€5,423,166
Year 4 (2021)	110	0.25	28	€971,245	€6,595,376	-€5,624,131
Year 5 (2022)	114	0.25	29	€1,005,830	€6,830,925	-€5,825,095
Cumulative 5-year cost						**-€27,047,005**

Table 41. 5–year budget impact of uptake amongst adult population excluding those with diabetes mellitus

	Number of patients eligible for treatment	% uptake	number of patients treated	total treatment cost	total cost of ivacaftor 150mg BD	incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	77	0.25	20	€749,054	€4,710,983	-€3,961,929
Year 2 (2019)	81	0.35	29	€1,026,652	€6,830,925	-€5,804,273
Year 3 (2020)	87	0.45	40	€1,411,868	€9,421,965	-€8,010,097
Year 4 (2021)	92	0.55	51	€1,791,135	€12,013,005	-€10,221,870
Year 5 (2022)	97	0.65	64	€2,245,309	€15,075,144	-€12,829,835
Cumulative 5-year cost						**-€40,828,004**

Table 42. 5–year budget impact of dosing regimens based on uptake amongst adult male population only

	Number of patients eligible for treatment	% uptake	number of patients treated	total treatment cost	total cost of ivacaftor 150mg BD	incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	52	0.25	13	€486,885	€3,062,139	-€2,575,254
Year 2 (2019)	55	0.35	20	€711,772	€4,710,983	-€3,999,210
Year 3 (2020)	59	0.45	27	€953,867	€6,359,826	-€5,405,960
Year 4 (2021)	62	0.55	35	€1,233,414	€8,244,219	-€7,010,806
Year 5 (2022)	66	0.65	43	€1,510,093	€10,128,612	-€8,618,520
Cumulative 5-year cost						**-€27,609,749**

Table 43. 5–year budget impact of dosging regimen based on modelling of the maximum accumulation of ivacaftor 150mg BD.

	Number of patients eligible for treatment	% uptake	number of patients treated	total treatment cost	total cost of ivacaftor 150mg BD	incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	98	0.25	25	€1,147,934	€5,888,728	-€4,740,794
Year 2 (2019)	102	0.35	36	€1,581,330	€8,479,769	-€6,898,439
Year 3 (2020)	106	0.45	48	€2,100,792	€11,306,358	-€9,205,566
Year 4 (2021)	110	0.55	61	€2,663,305	€14,368,497	-€11,705,192
Year 5 (2022)	114	0.65	75	€3,268,866	€17,666,184	-€14,397,318
Cumulative 5-year cost						**-€46,947,309**

Table 44. 5–year budget impact of uptake of ivacaftor plus ritonavir dosing regimen in entire population on ivacaftor with incremental uptake starting at 25%

	Number of patients eligible for treatment	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	162	0.25	41	€1,535,560	€9,657,514	-€8,121,954
Year 2 (2019)	172	0.35	61	€2,167,034	€14,368,497	-€12,201,462
Year 3 (2020)	183	0.45	83	€2,933,638	€19,550,577	-€16,616,940
Year 4 (2021)	194	0.55	107	€3,769,411	€25,203,756	-€21,434,346
Year 5 (2022)	206	0.65	134	€4,711,806	€31,563,583	-€26,851,776
Cumulative 5-year cost						**-€85,226,478**

Table 45. 5–year budget impact with uptake of ritonavir plus ivacaftor dosing by one patient only

	Number of patients eligible for treatment	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	162	0.01	1	€37,453	€235,549	-€198,096
Year 2 (2019)	172	0.01	1	€34,585	€235,549	-€200,964
Year 3 (2020)	183	0.01	1	€34,585	€235,549	-€200,964
Year 4 (2021)	194	0.01	1	€34,585	€235,549	-€200,964
Year 5 (2022)	206	0.01	1	€34,585	€235,549	-€200,964
Cumulative 5-year cost						**-€1,001,953**

Table 46. 5–year budget impact with 100% uptake of ivacaftor plus ritonavir dosing regimen in the complete population on ivacaftor

	Number of patients eligible for treatment	% uptake	Number of patients treated	Total treatment cost	Total cost of ivacaftor 150mg BD	Incremental cost of ivacaftor plus ritonavir
Year 1 (2018)	162	100	162	€6,067,334	€38,158,958	-€32,091,624
Year 2 (2019)	172	100	172	€5,864,922	€39,807,802	-€33,942,880
Year 3 (2020)	183	100	183	€6,107,017	€41,456,646	-€35,349,630
Year 4 (2021)	194	100	194	€6,349,111	€43,105,490	-€36,756,379
Year 5 (2022)	206	100	206	€6,591,205	€44,754,334	-€38,163,129
Cumulative 5-year cost						**-€176,303,641**

A summary of incremental savings for the above scenarios is contained in table 48.

Table 47. Budget impact summary

Scenario	Incremental cost
Base case	-€49,021,152
Adjusted for individual modelling	-€48,830,175
Adjusted for action of M1	-€47,647,169
Adjusted for maximum accumulation of ivacaftor 150mg BD	-€46,947,309
All patients, incremental uptake	-€85,226,478
Adult men only	-€27,609,749
All adults without diabetes	-€40,828,004
Capped at 25% uptake	-€27,047,005
1 patient only	-€1,001,953
All patients 100% uptake	-€176,303,641

6.4 Discussion

The results of this study show that significant savings are possible with the implementation of ritonavir boosting of ivacaftor, even with uptake of the dosing regimen in a small percentage of the population on ivacaftor. The base case, uptake in 25% of the adult population on ivacaftor growing to 75% over 5 years, offers the possibility of saving €49,021,152 over this time. Of the two predominant areas of uncertainty in the model—the dosing regimen of ritonavir boosted ivacaftor and the size of the population that may utilise boosting—the model is most sensitive to changes in the size of the population. The budget impact ranges from a 5-year saving of €176,303,641 should ritonavir boosting of ivacaftor be universally adopted to a saving of €1,001,953 seen with treatment of only one patient with ritonavir-boosted ivacaftor over 5 years. Uncertainty regarding the specific dosing regimen for each individual patient has a much smaller effect on savings. It is incorporated into the costing that each patient will have a bespoke dosing regimen based on PK profiles done before and

after implementation of ritonavir boosting. The base case assumes a dosing regimen of ivacaftor twice weekly with ritonavir daily, but in the scenario where the individualised dosing regimen of the patient population is extrapolated from the individualised dosing regimen from the clinical trial, the savings change only from €49,021,152 to €48,830,175 over 5 years.

The exploration of a wide variety of scenarios showing the effect of changes in the population on the potential savings associated with the ivacaftor plus ritonavir regimen show a very wide range of a 5-year budget saving of €1m to €176m. The population scenarios are based on known features of the medications involved, such as the interaction with hormonal contraceptives that prompted the exploration of a population that excluded females and the exclusion of those with CF associated diabetes to avoid any theoretical increase in metabolic adverse effects with the addition of ritonavir. Even taking these constraints on the population that will be suitable for ritonavir boosting of ivacaftor into account, along with the assumption that uptake will start at only a quarter of that population, savings are sizeable.

The magnitude of the savings of over €1 million should the ritonavir boosted ivacaftor population be only 1 single patient serves to illustrate to significant budget impact and opportunity cost of ivacaftor treatment. This can be contextualised by considering the cost of proposed new facilities for PWCF that have been recommended by patient groups and clinicians. CF Ireland (CFI) have published two reports in the last 5 years outlining the urgent need for improved infrastructure and services in Beaumont Hospital, Dublin, and Cavan General Hospital. The assessment of needs for a dedicated inpatient facility in Beaumont Hospital was published in 2012 and outlines the projected cost for a new build, 14 single-bed, CF-dedicated unit of €1,666,667. Projected staffing costs for the unit are in the order of €1,074,571 per annum.(125)

Should 3 patients switch to ritonavir boosted ivacaftor the savings would exceed the projected capital and staffing costs for the unit for one whole year. The Cavan General Hospital proposal describes the development of 2 new adult inpatient isolation rooms, development of a paediatric inpatient ward and expansion of the outpatient facilities for PWCF at a total cost of €1,429,210, less than the potential savings to be made from two patients switching to ivacaftor plus ritonavir boosting.(126)

Given the issues discussed in the previous chapter regarding drug interactions with various contraceptive methods for women, a scenario that excludes all females from the ivacaftor plus ritonavir dosing was explored. This is considered a conservative approach as in the base case even at the rate of maximum uptake of 65% of adult patients there will be 35% of patients not on ivacaftor plus ritonavir, many of whom will be those on contraception. Those females under 18 are also automatically excluded in this scenario. The analysis of uncertainty therefore comprehensively considers the large effect of uncertainty in the population that will take up treatment with ivacaftor plus ritonavir and the much smaller degree of uncertainty in the modelling of the PK data and resulting dosing regimens.

6.5 Limitations

The budget impact analysis above does not account for any change in the effectiveness of ivacaftor that may be seen with the addition of ritonavir. Based on the data that is shown in chapter 5 and the recent analysis of ivacaftor trough levels in the STRIVE study published by the FDA(2), it is plausible that the increase in the trough levels of ivacaftor that accompanies a switch to ritonavir boosting may result in clinical improvement in some patients. It would be beneficial if ritonavir boosting of ivacaftor was taken up by a cohort of patients and a study of the clinical effect and potential side effects of this new

regimen were conducted, albeit in an open-label non-randomised study. This analysis also assumes that the price of ivacaftor will not change over the time horizon. A static price is the most likely scenario, but other groups are working with similar molecules such as Concert Pharmaceuticals®, who had a deuterated analogue of the ivacaftor molecule as far as phase II clinical trials. Concert Pharmaceuticals® has since been purchased by Vertex Pharmaceuticals®,(127) so this particular development is unlikely to change the price of ivacaftor, but another such competitor may change the landscape, if not in the 5-year time horizon of this study in the time up to the 2026 expiration of Kalydeco® patent exclusivity.

6.6 Conclusion

This research predicts that ritonavir boosting of ivacaftor could result in significant savings to the health budget without impacting the clinical effectiveness of the drug. The base case scenario, uptake in the adult population of ritonavir boosting of ivacaftor of 25% increasing by 10% annually, shows a 5-year budget saving of over €46m. The 5-year budget impact ranges from a saving of €1 million should only 1 patient on ivacaftor switch to the ivacaftor plus ritonavir regimen to a saving in excess of €176 million in the best-case scenario of universal uptake of ritonavir boosting in those on ivacaftor. Further study in the CF population of this dosing regimen of ivacaftor is warranted.

CHAPTER 7

7 Conclusion and future work

The introduction of ivacaftor in 2013 marked a point of transformational change in the management of CF in Ireland and across the globe. This change was brought about not only by the significant clinical impact of the drug in the small subgroup of PWCF for whom it was indicated, but also by the broader effect of the implicit and explicit promise of future, better therapies for all patients with CF—a message full of hope but also one ringing dissonant with societal implications of the ultra-high cost of ivacaftor and other such promised future therapies.

The challenges surrounding the development and provision of new therapies in any disease area are global. There are many avenues being explored to make access to medicines affordable, for example those aiming to improve transparency regarding the cost of the development of therapies to ensure appropriate return on the investment of public funds, particularly in early research. Overall change in the model of drug development is also afoot, with groups such as the Drugs for Neglected Diseases Initiative (DNDi) producing therapies for a fraction of the $1bn figure traditionally espoused by the industry. One innovation stream this group has used is the repurposing of established therapies in novel combinations, for instance their combination of an artemisinin-based drug with amodiaquine for the treatment of malaria, which was approved in 2007.(128) The repurposing of established therapies is gaining interest in the developed world also, indeed it was the overall theme of the first issue of the *British Journal of Pharmacology* in 2018, which includes original research articles on topics such as the use of rifampicin for neuroprotection in acute brain injury and ibuprofen and other non-steroidal anti-inflammatory drugs for the treatment of pancreatitis.(129) The work presented here is built on this principle: improvement in

the utilisation of a drug using a novel combination with another established therapy, in this case the use of the well-established practise of ritonavir boosting.

At the time of development of the assay used in this thesis there was no other assay for the measurement of ivacaftor and M1 reported in the literature. Since then a number of assays have been reported as part of larger projects designed to assess the clinical pharmacokinetics and real-world effectiveness of ivacaftor. As yet, the results of assay validation have only been reported, no results are available for any of the PK/PD studies which are ongoing.(87, 130) As more information is discovered regarding the PK/PD relationships of ivacaftor in vivo this will be valuable to further explore the potential of the findings reported here. At the time of writing, there is no other literature on the interaction between ivacaftor and ritonavir, so the results of the clinical trial above remain the primary launching point for further clinical studies exploring this interaction. The predictive PK model generated from these data will also be valuable, well beyond the data generated and presented here, to form a basis for dosing regimens that may be used to test any new PKPD target that is clarified in future studies. The use of ritonavir boosting of ivacaftor elucidated here could also have a role in the optimisation of the clinical use of ivacaftor in individuals who may be maintaining suboptimal levels at standard dosing.

Until the patent for ivacaftor expires in 2026 the critical issue of the high cost of ivacaftor will persist. With a growing population of those in whom it is indicated, both from the incident rate of CF with the G551D mutation, improved survival (a clearly welcome consequence of ivacaftor treatment) and expansion of the indications to other mutations this problem can only increase. This study is a crucial launching point for clinical studies of ritonavir boosting in patients on ivacaftor to improve the cost effectiveness of the treatment. Such studies have been successful in other disease

areas, for example, the use of ketoconazole to spare cyclosporine in heart transplant patients.(64) The positive results from this trial are encouraging, more so when one considers how well placed ivacaftor is for this kind of study. As opposed to cyclosporine, ivacaftor does not appear to have a narrow therapeutic index. Furthermore, ritonavir is used in this study at a very small dose, much smaller than even for traditional boosting, and therefore the adverse effects could be expected to be minimised even more than those of ketoconazole in prior studies.

A clinical trial of ritonavir boosting of ivacaftor for PWCF on this treatment is the next step to assessing the feasibility of this treatment regimen. This could be modelled on the clinical studies already completed for ketoconazole boosting of cyclosporine and tacrolimus in organ transplantation. Coupled with the ongoing study of the PK/PD characteristics of ivacaftor there is ample opportunity to improve the utilisation of this medication, both for a potentially substantial pharmacoeconomic benefit prior to the expiration of patent exclusivity and beyond this to maximise the clinical benefit accrued by each person who requires this treatment.

When Frederick Banting, Charles Best and James Collip developed insulin therapy for the treatment of diabetes in 1923 they sold the patent for its production to the University of Toronto for $1 apiece. The university then proceeded to grant the pharmaceutical industry the right to produce insulin royalty free to ensure that the benefits of the discovery reached as many patients as possible.(131) Today, development of new therapeutics often stands Janus-faced in the maelstrom of the provision of healthcare—there are still numerous unmet clinical needs, but increasingly a therapy for one will mean an opportunity cost that inevitably must displace a therapy for another. This new landscape requires a rethink of the model of a shareholder-driven, relentless search for new targets and new drugs as the only way forward for

therapeutics. This model undoubtedly has merits and has produced a panoply of therapies that otherwise would not exist; the rethink merely entails a broader look at our resources and a creative approach as to how we can optimally utilise them. Ritonavir boosting of ivacaftor represents one such potential opportunity to further enhance the use of this superlative discovery in the treatment of CF in the future.

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Appendix 1. Individual PK profiles.

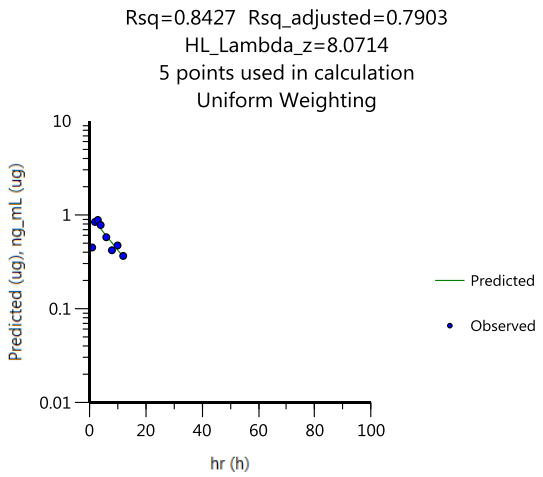


Figure 22. Study A volunteer 2.

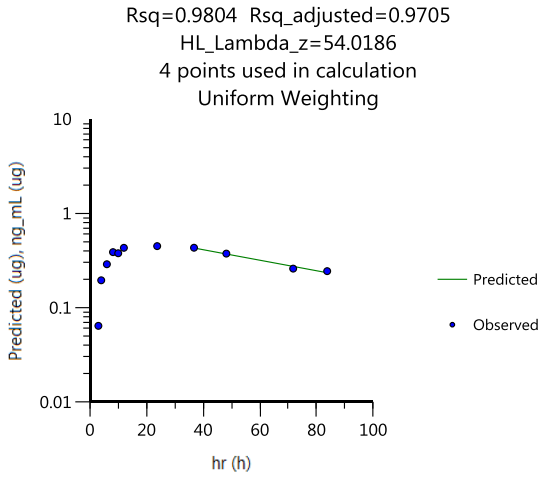


Figure 23. Study B volunteer 2.

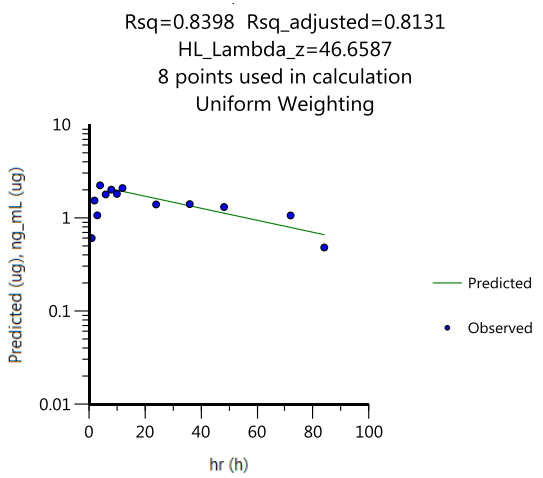
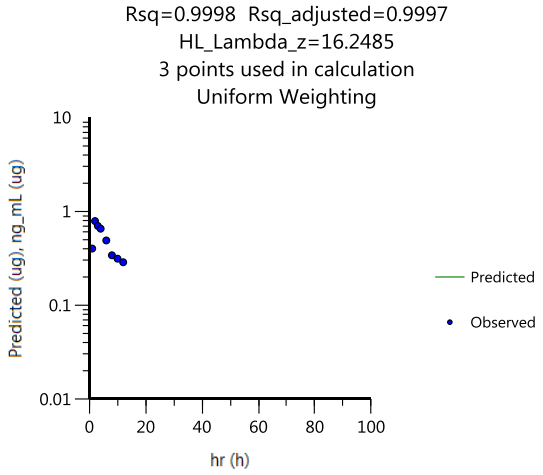


Figure 24. Study C volunteer 2.



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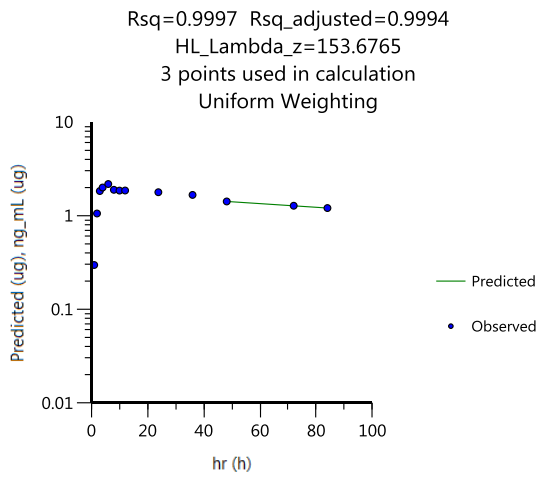

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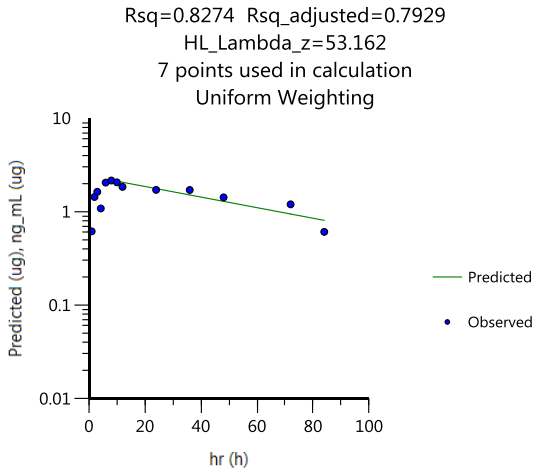

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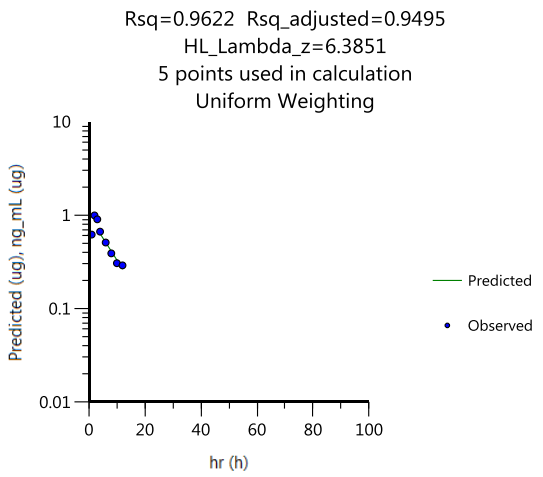
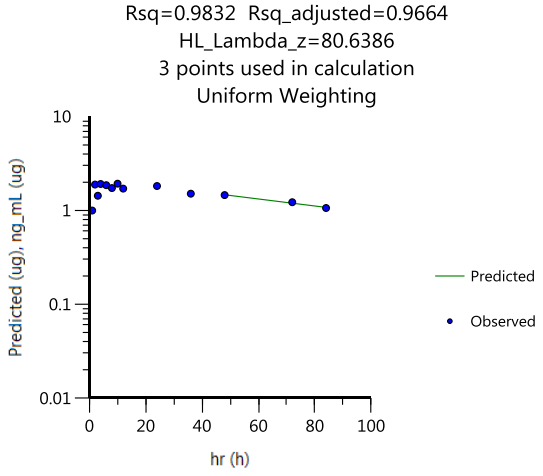


Figure 28. Study A volunteer 4.



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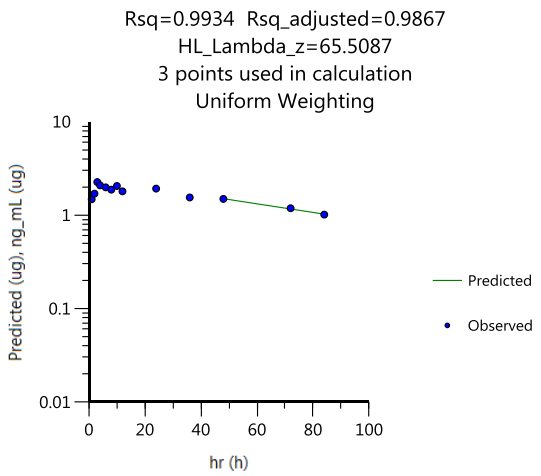

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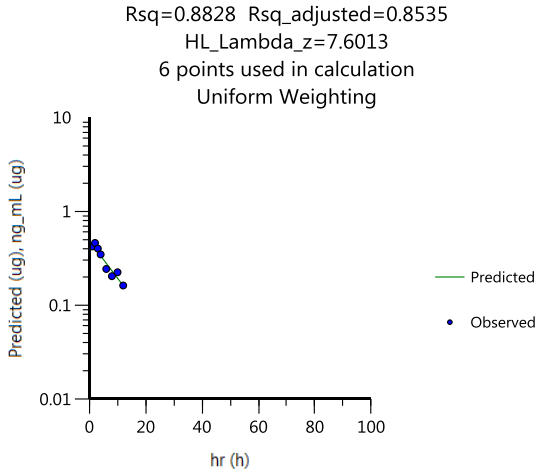

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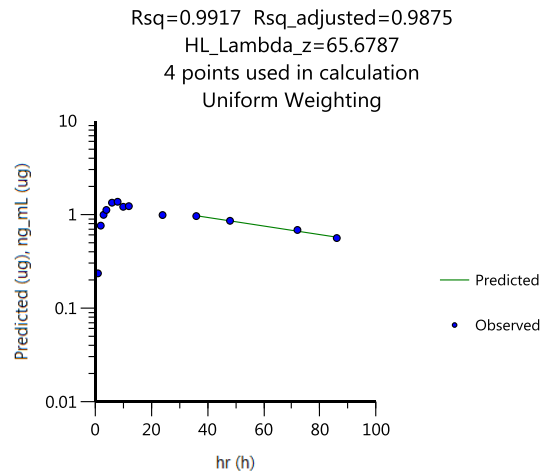

Figure 32. Study B volunteer 5**.**

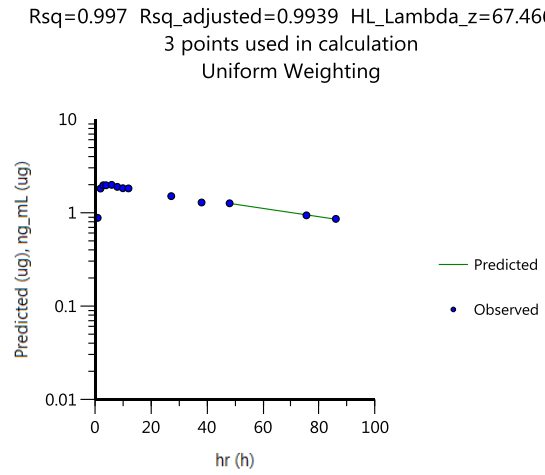

Figure 33. Study C volunteer 5.

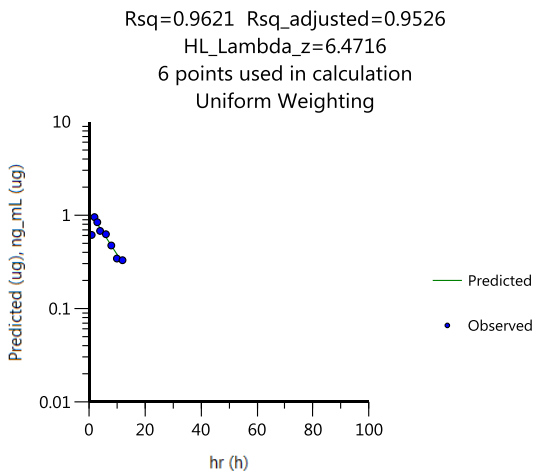

Figure 34. Study A volunteer 6.

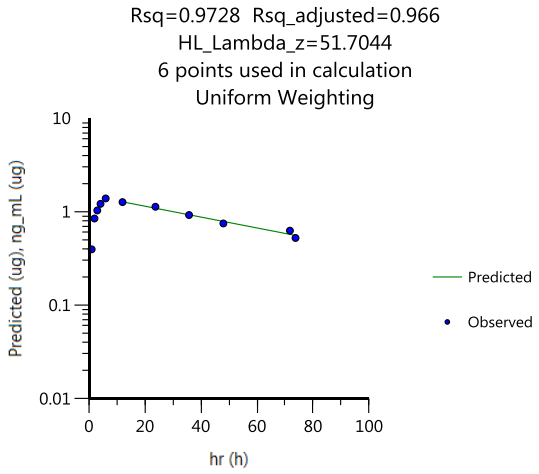

Figure 35. Study B volunteer 6.

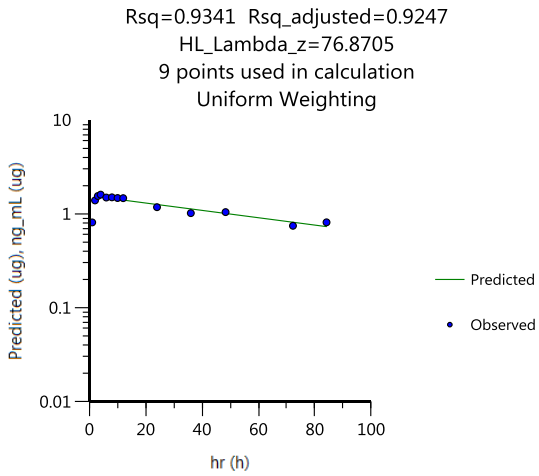

Figure 36. Study C volunteer 6.

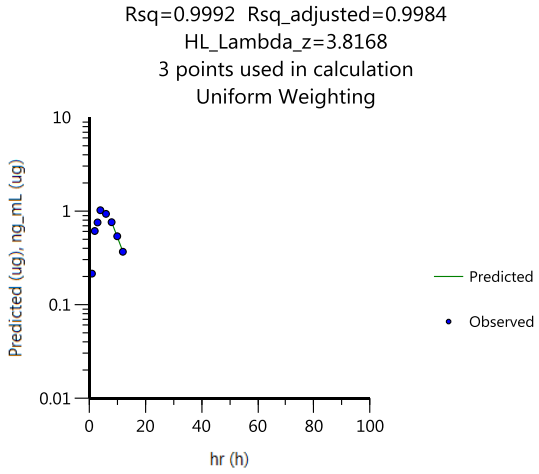

Figure 37. Study A volunteer 9.

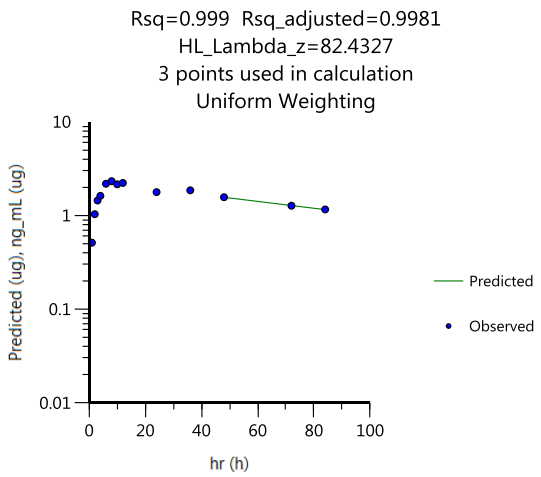

Figure 38. Study B volunteer 9.

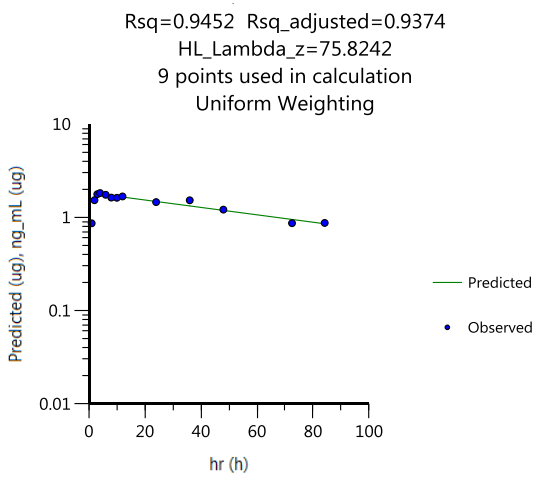

Figure 39. Study C volunteer 9.

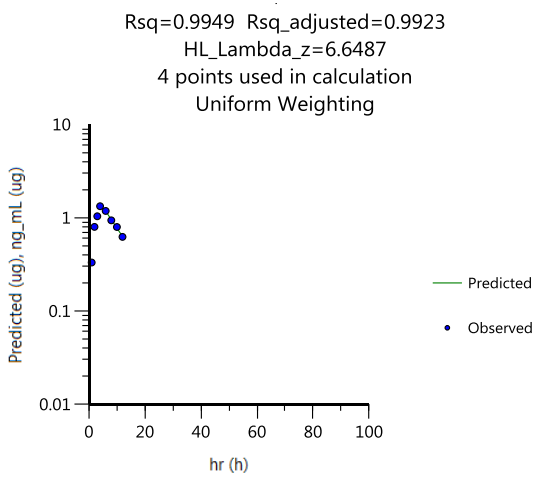

Figure 40. Study A volunteer 10.

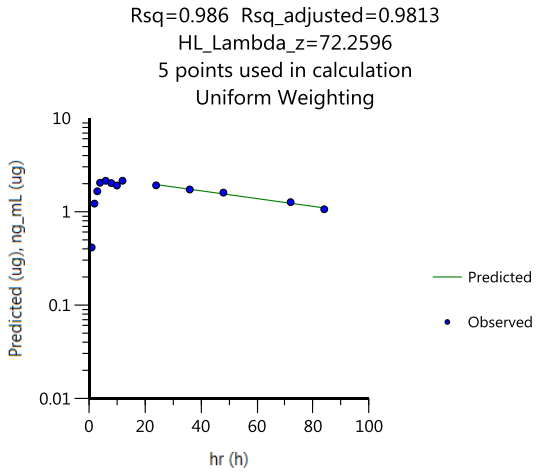

Figure 41. Study B volunteer 10.

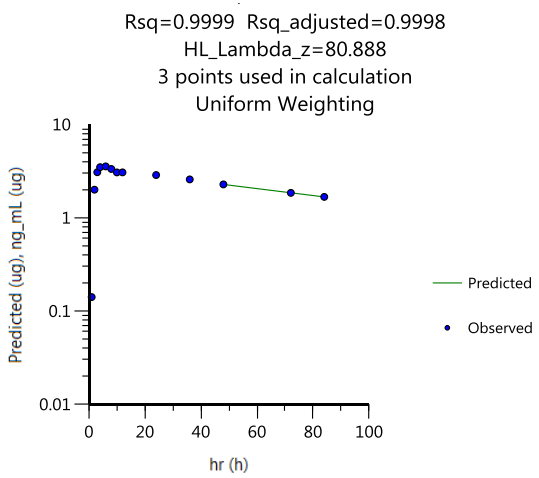

Figure 42. Study C volunteer 10.

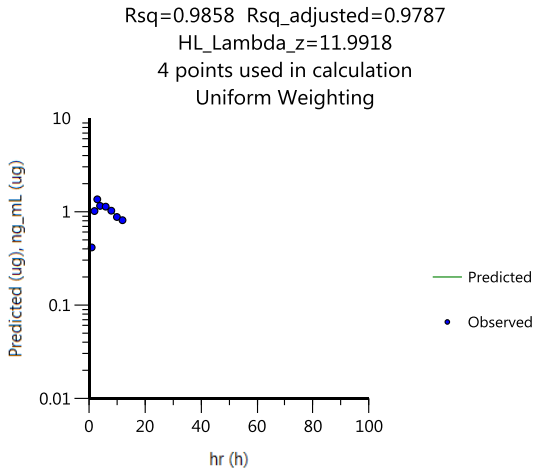

Figure 43. Study A volunteer 11.

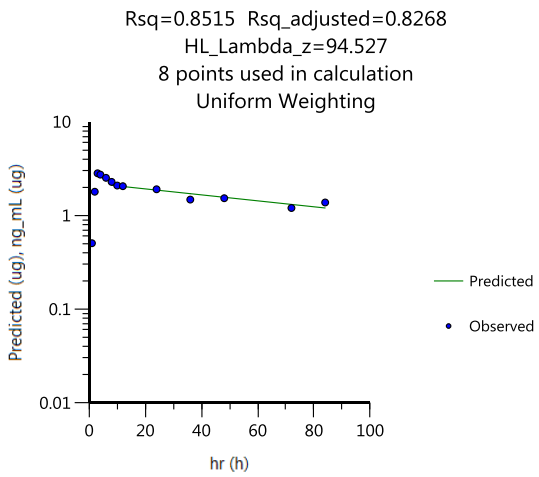

Figure 44. Study B volunteer 11.

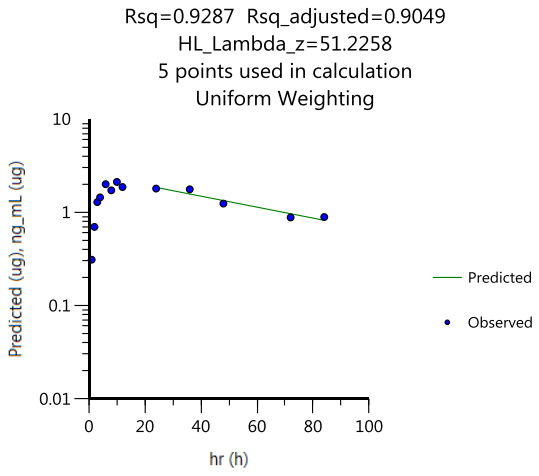

Figure 45. Study C volunteer 11.

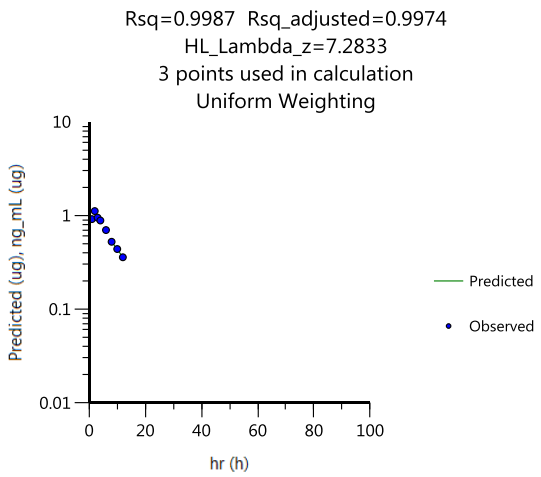

Figure 46. Study A volunteer 12.

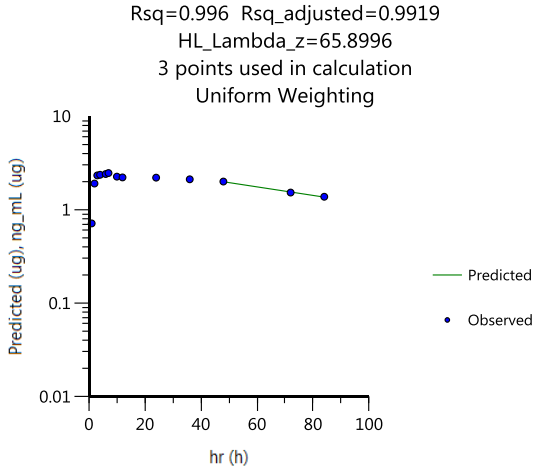

Figure 47. Study B volunteer 12.

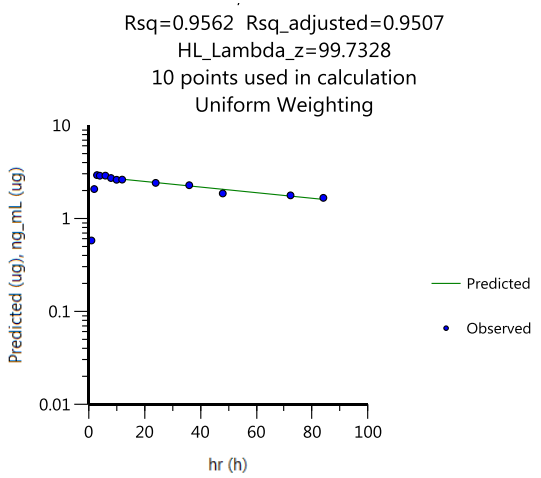

Figure 48. Study C volunteer 12.

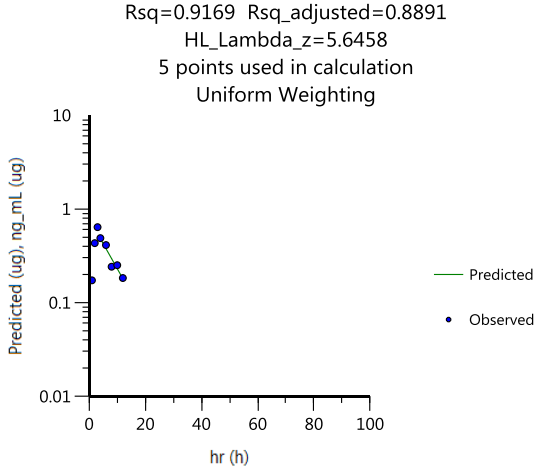

Figure 49. Study A volunteer 13.

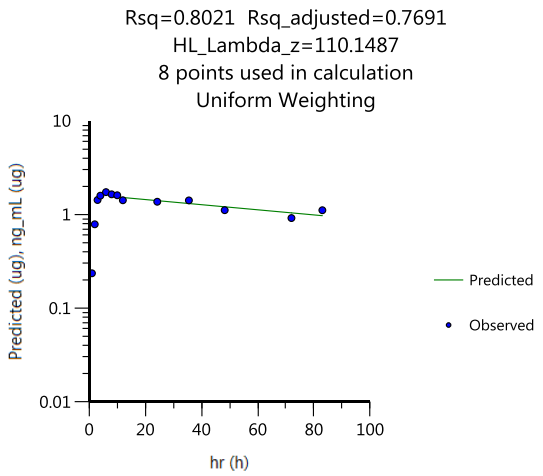

Figure 50. Study B volunteer 13.

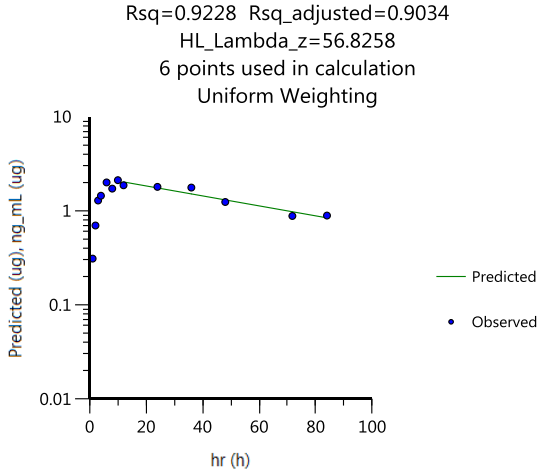

Figure 51. Study C volunteer 13.

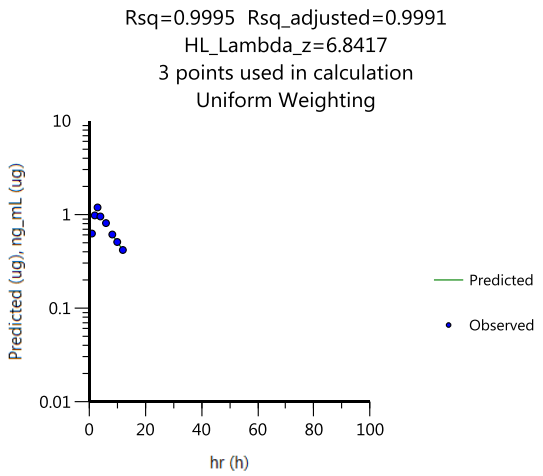

Figure 52. Study A volunteer 14.

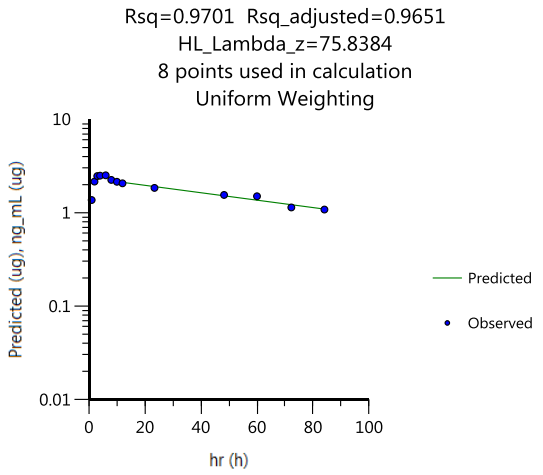

Figure 53. Study B volunteer 14.

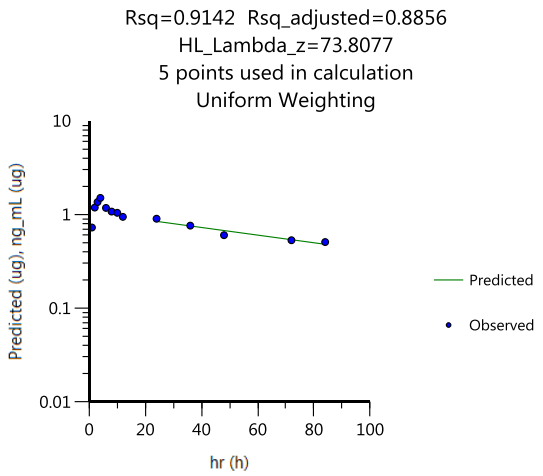

Figure 54. Study C volunteer 14.

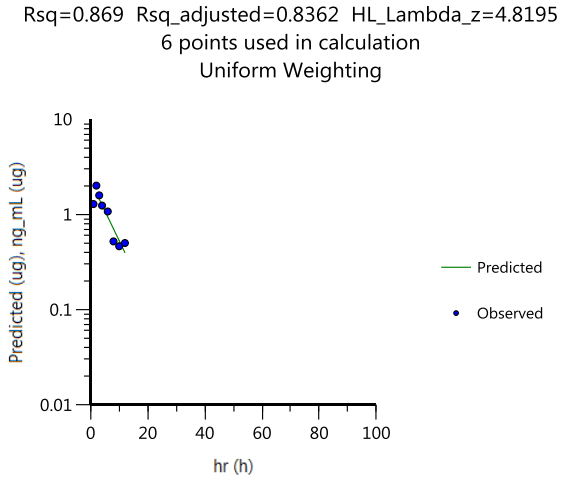

Figure 55. Study A volunteer 15.

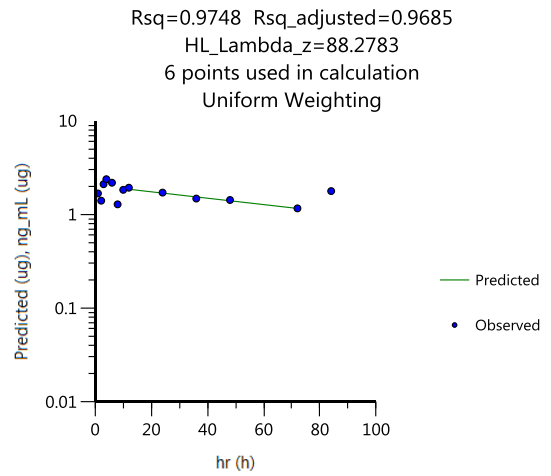

Figure 56. Study B volunteer 15.

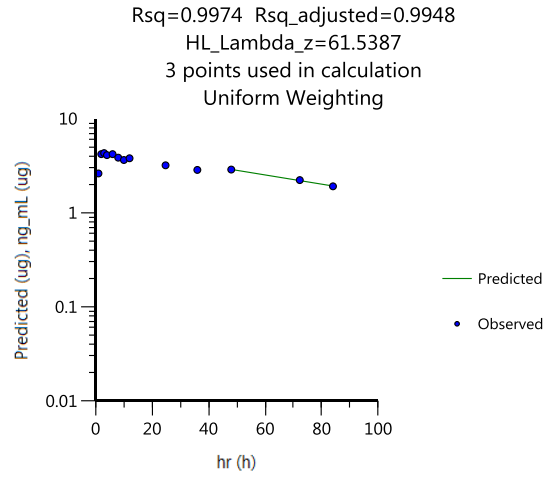

Figure 57. Study C volunteer 15.

Appendix 2. Clinical Trial Documents.

CRF 1: Visit 1. Screening Visit. **Date**

___/___/___

Volunteer **name**

_____

Inclusion criteria

Healthy volunteers over the age of 18 able to give informed consent who are not on any regular prescription medications will be recruited for this trial.
To be classed as 'healthy volunteers' each subject must meet each of the following criteria at screening (Visit 1) and must continue to fulfil these criteria at baseline (Visit 2):

- Able and willing to give written informed consent and to comply with the requirements of this study protocol
- Aged 18 years or older
- Judged to be in generally good health by the investigator based upon the results of the medical history, laboratory tests, physical examination (electrocardiogram [ECG] will not be performed as neither ritonavir or ivacaftor have been associated with clinically significant increases in the QTc interval.23)
- If female and of child-bearing potential and if male with partner of child-bearing potential, willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study
- Female subjects – urine pregnancy test at screening must be negative
- HIV negative status
- Clinically acceptable laboratory parameters within 6 weeks prior to enrolment

Exclusion criteria

Subjects are excluded from the study if any of the following criteria are met at Screening (Visit 1) or at Baseline (Visit 2):

- Allergy/sensitivity to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.

- Subjects who have participated in another study and received any other investigational agent within the time-frame for the pharmacokinetic elimination of that investigational agent
- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
- Subjects who have a history of drug (including cigarette smoking) or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive, hepatitis B or C or HIV infection
- Concurrent malignancy
- Subjects requiring the chronic administration of medications which either induce or inhibit enzymes in the cytochrome p450 system □ AST or ALT ≥ 3 x upper limit of normal (ULN)
- Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula
- Scheduled for procedures requiring general anaesthesia during the study

1. Inclusion/Exclusion criteria explained to Volunteer: Yes □ No □
2. Patient information leaflet given to volunteer to take away Yes □ No □

If no to 2. above give reason:

_____

_____

_____

_____

CRF 2: Visit 2. Baseline.

Date: ___/___/___

Volunteer

name_____

D.O.B_____

Gender □ M □ F

Volunteer number_____

Race_____

Medical History

Does the patient have a history of:

Any surgical procedure that would preclude the volunteer from the study due to either unacceptable risk to the volunteer's health or likelihood that this history would cause undue influence on study results (including a need for surgical procedures requiring anaesthetic during the course of the study) Yes □ No □

If yes, give details_____

_____

_____

Any medical condition that would preclude the volunteer from the study due to either unacceptable risk to the volunteer's health or likelihood that this history would cause undue influence on study results Yes □ No □

If yes, give details_____

_____

_____

Is the patient on any regular medications/supplements/alternative remedies: Yes □ No □

If yes, give details (incl. indications)

_____

Does the patient take any PRN medications: Yes □ No □

If yes, give details_____

_____

Can these medications be avoided during the time-frame of the study

Yes □ No □

Does the volunteer have any medication allergies: Yes □ No □

If yes, give details_____

_____

Has the volunteer recently participated in another study Yes □ No □

If yes, give details_____

_____

Does the volunteer use alcohol Yes □ No □

If yes, how many units per week? _____

Can alcohol be avoided 24 hours before each study commences and for the duration of each study? Yes □ No □

Does the volunteer currently smoke? Yes □ No □

Does the volunteer use recreational drugs Yes □ No □

If yes give details_____

_____

Has the volunteer signed the consent form Yes □ No □

Has the volunteer had blood drawn for FBC/U&E/LFT/Hepatitis/HIV/Fasting lipids

Yes □No □

For female volunteers

Is the volunteer known to be pregnant, considering becoming pregnant or breastfeeding? Yes □ No □

Has the volunteer had a urine HCG Yes □ No □
Result: Positive □ Negative □

If female and of child-bearing potential and if male with partner of child-bearing potential is the volunteer willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study day Yes □
No □

Has the volunteer had a urine drug screen Yes □ No □

BP _____
Temp _____
HR _____
Resp rate _____
Height _____
Weight _____

Respiratory exam Satisfactory □ Not Satisfactory □

If not satisfactory give details

_____
_____

Cardiovascular exam Satisfactory □ Not Satisfactory □

If not satisfactory give details

_____
_____

Gastrointestinal exam Satisfactory □ Not Satisfactory □

If not satisfactory give details

_____
_____

Neurological exam Satisfactory □ Not Satisfactory □

If not satisfactory give details

_____
_____

Dermatological/musculoskeletal exam Satisfactory □ Not Satisfactory □

If not satisfactory give details

_____
_____

CRF 3. Study A. **Date**

___/___/___

Volunteer

name_____

D.O.B_____

Gender □ M □ F

Volunteer

number_____

Inclusion Criteria

- Able and willing to give written informed consent and to comply with the requirements of this study protocol
- Aged 18 years or older
- Judged to be in generally good health by the investigator based upon the results of the medical history, laboratory tests, physical examination (electrocardiogram [ECG] will not be performed as neither ritonavir or ivacaftor have been associated with clinically significant increases in the QTc interval.23)
- If female and of child-bearing potential and if male with partner of child-bearing potential, willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study
- Female subjects – urine pregnancy test at screening must be negative
- HIV negative status
- Clinically acceptable laboratory parameters within 6 weeks prior to enrolment

Exclusion criteria

- Allergy/sensitivity to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.
- Subjects who have participated in another study and received any other investigational agent within the time-frame for the pharmacokinetic elimination of that investigational agent

- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
- Subjects who have a history of drug (including cigarette smoking) or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive, hepatitis B or C or HIV infection
- Concurrent malignancy
- Subjects requiring the chronic administration of medications which either induce or inhibit enzymes in the cytochrome p450 system
- AST or ALT ≥ 3 x upper limit of normal (ULN)
- Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula
- Scheduled for procedures requiring general anaesthesia during the study

Does the volunteer continue to meet the inclusion criteria? Yes □ No □

Is the patient suitable to start study A Yes □ No □

If no give details_____

_____

FBC □ Normal □ Abnormal but clinically insignificant □ Abnormal and clinically significant

U&E □ Normal □ Abnormal but clinically insignificant □ Abnormal and clinically significant

LFT □ Normal □ Abnormal but clinically insignificant □ Abnormal and clinically significant

Lipids □ Normal □ Abnormal but clinically insignificant □ Abnormal and clinically significant

Hepatitis B □ Negative □ Positive

Hepatitis C □ Negative □ Positive

HIV □ Negative □ Positive

Cockrauft-Gault eGFR:_____

Print and attach blood results to this CRF (FBC/U&E/LFT/Hepatitis/HIV/Fasting lipids)

BP _____
Temp _____
HR _____
Resp rate _____

150mg of Ivacaftor administered at: (time)_____

Was the volunteer fasting before administration of ivacaftor Yes □ No □

Meal 1 eaten at: (time)_____

Meal 2 eaten at: (time)_____

Meal 3 eaten at: (time)_____

			Time
Blood draws:	0 hrs	□	_____
	1 hr	□	_____
	2 hrs	□	_____
	3 hrs	□	_____
	4 hrs	□	_____
	6 hrs	□	_____
	8 hrs	□	_____
	10 hrs	□	_____
	12 hrs	□	_____

Did any adverse event occur during study A Yes □ No □

If yes fill out adverse event CRF

CRF 4. Study B. **Date ___/___/____**

Volunteer

name_____

D.O.B_____

Gender □ M □ F

Volunteer

number_____

Inclusion Criteria

- Able and willing to give written informed consent and to comply with the requirements of this study protocol
- Aged 18 years or older
- Judged to be in generally good health by the investigator based upon the results of the medical history, laboratory tests, physical examination (electrocardiogram [ECG] will not be performed as neither ritonavir or ivacaftor have been associated with clinically significant increases in the QTc interval.23)
- If female and of child-bearing potential and if male with partner of child-bearing potential, willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study
- Female subjects – urine pregnancy test at screening must be negative
- HIV negative status
- Clinically acceptable laboratory parameters within 6 weeks prior to enrolment

Exclusion criteria

- Allergy/sensitivity to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.
- Subjects who have participated in another study and received any other investigational agent within the time-frame for the pharmacokinetic elimination of that investigational agent
- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident)

which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.

- Subjects who have a history of drug (including cigarette smoking) or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive, hepatitis B or C or HIV infection
- Concurrent malignancy
- Subjects requiring the chronic administration of medications which either induce or inhibit enzymes in the cytochrome p450 system
- AST or ALT ≥ 3 x upper limit of normal (ULN)
- Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula
- Scheduled for procedures requiring general anaesthesia during the study

Does the volunteer continue to meet the inclusion criteria? Yes □ No □

BP _____
Temp _____
HR _____
Resp rate _____

150mg of Ivacaftor administered at: (time)_____

50 mg of ritonavir administered at: (time)_____

Was the volunteer fasting before administration of both drugs Yes □ No □

Meal 1 eaten at: (time)_____ Meal 2 eaten at: (time)_____

Meal 3 eaten at: (time)_____

	Date			Time
Blood draws:	___/___/___	0 hrs	□	_____
		1 hr	□	_____
		2 hrs	□	_____
		3 hrs	□	_____
		4 hrs	□	_____
		6 hrs	□	_____
		8 hrs	□	_____
		10 hrs	□	_____

	12 hrs	□	_____
___/___/___	**24 hrs**	□	_____
	36 hrs	□	_____
___/___/___	**48 hrs**	□	_____
___/___/___	**72 hrs**	□	_____
	84 hrs	□	_____

50mg of ritonavir administered at □24 hours □48 hours □72 hours

Has any adverse event occurred since study A Yes □ No □

Did any adverse event occur during study B Yes □ No □

If yes fill out adverse event CRF

CRF 5. Study C-1. **Date ___/___/____** **(No. of days since study B____)**

Volunteer name_____

D.O.B_____

Gender □ M □ F

Volunteer number_____

Inclusion Criteria

- Able and willing to give written informed consent and to comply with the requirements of this study protocol
- Aged 18 years or older
- Judged to be in generally good health by the investigator based upon the results of the medical history, laboratory tests, physical examination (electrocardiogram [ECG] will not be performed as neither ritonavir or ivacaftor have been associated with clinically significant increases in the QTc interval.23)
- If female and of child-bearing potential and if male with partner of child-bearing potential, willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study
- Female subjects – urine pregnancy test at screening must be negative
- HIV negative status
- Clinically acceptable laboratory parameters within 6 weeks prior to enrolment

Exclusion criteria

- Allergy/sensitivity to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.

- Subjects who have participated in another study and received any other investigational agent within the time-frame for the pharmacokinetic elimination of that investigational agent
- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
- Subjects who have a history of drug (including cigarette smoking) or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive, hepatitis B or C or HIV infection
- Concurrent malignancy
- Subjects requiring the chronic administration of medications which either induce or inhibit enzymes in the cytochrome p450 system
- AST or ALT ≥ 3 x upper limit of normal (ULN)
- Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula
- Scheduled for procedures requiring general anaesthesia during the study

Does the volunteer continue to meet the inclusion criteria? Yes □ No □

BP _____
Temp _____
HR _____
Resp rate _____

Has any adverse event occurred since study B Yes □ No □

If yes fill out adverse event form

Ritonavir liquid dispensed at a dose of 50mg/day for at least two weeks Yes No

□

Affix duplicate label here:

CRF 6. Study C-2. **Date**

___/___/____

Volunteer

name_____

_

D.O.B_____

Gender □ M □ F

Volunteer

number_____

Inclusion Criteria

- Able and willing to give written informed consent and to comply with the requirements of this study protocol
- Aged 18 years or older
- Judged to be in generally good health by the investigator based upon the results of the medical history, laboratory tests, physical examination (electrocardiogram [ECG] will not be performed as neither ritonavir or ivacaftor have been associated with clinically significant increases in the QTc interval.23)
- If female and of child-bearing potential and if male with partner of child-bearing potential, willing to ensure that they or their partner use effective contraception during the study and for 18 days after the last study
- Female subjects – urine pregnancy test at screening must be negative
- HIV negative status
- Clinically acceptable laboratory parameters within 6 weeks prior to enrolment

Exclusion criteria

- Allergy/sensitivity to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.

- Subjects who have participated in another study and received any other investigational agent within the time-frame for the pharmacokinetic elimination of that investigational agent
- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder (including uncontrolled diabetes, unstable ischemic heart disease, moderate to severe congestive heart failure, recent cerebrovascular accident) which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.

- Subjects who have a history of drug (including cigarette smoking) or alcohol use that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Known history of, or documented positive, hepatitis B or C or HIV infection
- Concurrent malignancy
- Subjects requiring the chronic administration of medications which either induce or inhibit enzymes in the cytochrome p450 system
- AST or ALT ≥ 3 x upper limit of normal (ULN)
- Creatinine clearance (CrCl) < 60 mL/min measured by 24-hour urine collection or estimated by the Cockcroft and Gault formula
- Scheduled for procedures requiring general anaesthesia during the study

Does the volunteer continue to meet the inclusion criteria? Yes □ No □

BP _____
Temp _____
HR _____
Resp rate _____

Was ritonavir 50mg daily taken since last visit: Yes □ No □

How much ritonavir (in mls) was used by the patient on return _____

Does this represent >80% compliance Yes □ No □

150mg of Ivacaftor administered at: (time)_____

50 mg of ritonavir administered at: (time)_____

Was the volunteer fasting before administration of the above medicationsYes □ No □

Meal 1 eaten at: (time)_____

Meal 2 eaten at: (time)_____

Meal 3 eaten at: (time)_____

	Date			Time
Blood draws:	___/___/___	0 hrs	□	_____
		1 hr	□	_____
		2 hrs	□	_____
		3 hrs	□	_____
		4 hrs	□	_____
		6 hrs	□	_____
		8 hrs	□	_____
		10 hrs	□	_____
		12 hrs	□	_____
	___/___/___	**24 hrs**	□	_____
		36 hrs	□	_____
	___/___/___	**48 hrs**	□	_____
	___/___/___	**72 hrs**	□	_____
	___/___/___	84 hrs	□	_____

Fasting blood drawn for lipid profile at 0 hours Yes □ No □

Ritonavir 50mg administered at □24 hours □28 hours □72 hours

Has any adverse event occurred since study C-1 Yes □ No □

Did any adverse event occur during study C-2 Yes □ No □

If yes fill out adverse event form

CRF 12. Permanent discontinuation form

Volunteer

name_____

D.O.B_____

Gender ☐ M ☐ F

Volunteer

number_____

Reason for discontinuation:

☐ Withdrawal of consent by the subject

☐ Pregnancy

☐ A medical condition that may jeopardise the subject's safety

☐ Ineligibility

☐ An AE which requires discontinuation of the study medication

☐ Lack of compliance

☐ Lost to follow-up after three attempts to contact subject

Give details

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Adverse Event CRF **Date ___/___/___**

Volunteer

name_____

D.O.B_____

_____

Gender □ M □ F

Volunteer

number_____

1. Describe the nature of the adverse event

Date of onset of the adverse event ____/____/____

End date of adverse event ____/____/____

2.Is there a reasonable possibility that there is a causal relationship between the investigational medicinal product(s) and the adverse event Yes ☐ No ☐

Give details

Is this a serious adverse event i.e.

Did the adverse event result in death	Yes ☐ No ☐
Was the adverse event life-threatening	Yes ☐ No ☐
Did the adverse event require hospitalisation	Yes ☐ No ☐
Did the adverse event result in persistent disability or incapacity	Yes ☐ No ☐
Was the adverse event a congenital anomaly or birth defect	Yes ☐ No ☐
Was this adverse event an important medical event	Yes ☐ No ☐

If yes to any of the above fill out serious adverse event form.

Is this adverse event

Mild	Yes ☐ No ☐
Moderate	Yes ☐ No ☐
Severe	Yes ☐ No ☐

Is this adverse event:

Unrelated to the study medication(s)	Yes ☐ No ☐
Possibly related to the study medication(s)	Yes ☐ No ☐
Probably related to the study medications	Yes ☐ No ☐

Give details

Was this adverse event expected Yes ☐ No ☐

Was there another suspect medication or device Yes ☐ No ☐

Give details

What action was taken in response to this adverse event

Give details

ST. JAMES'S HOSPITAL
James's. St. Dublin 8. Ireland.

Trinity College Dublin
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The University of Dublin

An Investigation of the Pharmacokinetic Interaction between Ritonavir (Norvir®) and Ivacaftor (Kalydeco®) in Healthy Volunteers

Principal Investigator:	Dr. Anne Marie Liddy	+353 87996143
Sponsor:	Prof. Michael Barry	+353 1 4162291

You are being invited to volunteer to take part in a clinical research study in St James's Hospital.

Before you decide whether or not you wish to take part, you should read the information provided below carefully and, should you choose, discuss with your family, friends and GP.

You should clearly understand the risks and benefits of taking part so that you can make the decision that is right for you. You may take as much time as you need to make your decision and you may ask any questions you feel appropriate. This process is known as 'informed consent' and is your right as a potential research participant.

You can change your mind about the study at any point, even after it has started. You do not need to give a reason to opt out of any or all parts of the study.

Your participation in this study or withdrawal/refusal of consent to participate will not affect any future medical care you may need in this or any other hospital.

Why is this study being done?

This study is being done to get more information on how the body breaks down and eliminates a medication called ivacaftor (trade name: Kalydeco®) and how this process is affected by another medication called ritonavir (trade name: Norvir®)

Ivacaftor is used for the treatment of patients with a particular type of cystic fibrosis (CF). CF is a disease that children are born with that results in progressive organ damage to their lungs and gut over time and ultimately means a much-shortened life span.

Ivacaftor is a new treatment for CF that was introduced to Ireland in 2012 and it has given great benefit to some of the patients treated with it. It has been noticed, however, that some patients on ivacaftor do not get as much benefit as others. This may be because the level of the medication in their blood is too low, despite them being on the correct dose. This can be because individuals remove medications from the body at different rates, and someone who removes it very quickly may not get as much benefit as someone who removes it slowly.

We are testing the effect that another medication, ritonavir, has on the way the body breaks down and eliminates ivacaftor. We think that this medication will slow down the speed with which Ivacaftor is broken down in all individuals and, if this is the case, this will mean a combination of these two medications, ivacaftor and ritonavir, may be a more reliable and efficient way of using Ivacaftor so that all CF patients get the maximum benefit.

This would also reduce the cost of ivacaftor as it would mean it could be taken, say, once a day or once every two days instead of every 12 hours. This is important as ivacaftor is one of the most expensive medications the health service purchases for patients today.
The purpose of this study is therefore to assess, in healthy volunteers, how ivacaftor is affected by taking ritonavir with it. A study in healthy volunteers is the first step necessary before this drug combination can be tried in patients with CF already on the medication.

How do I know if I am suitable for the study?

There are certain criteria we use to see who is and is not suitable for the study – these are known as inclusion and exclusion criteria.

Inclusion criteria i.e. people who are suitable for the study:

- Volunteers able and willing to give written informed consent and to comply with the requirements of this study protocol
- Aged 18 years or older
- Judged to be in generally good health by the investigator based upon the results of the medical history, physical examination and laboratory tests.
- Willing to ensure that they or their partner use barrier or other non-hormonal forms of contraception if female and of child-bearing potential or if male with partner of child-bearing potential
- Not pregnant or breast-feeding
- HIV negative
- Clinically acceptable blood tests within 6 weeks prior to starting the trial.

Exclusion criteria i.e. people who are not suitable for the study:

- Allergic/sensitive to study medications or their ingredients
- Female subjects who are pregnant or breast-feeding or considering becoming pregnant during the study.
- Subjects who have participated in another study and received an investigational medication in the recent past
- Subjects unable to provide written informed consent
- Subjects who have any other significant disease or disorder which, in the opinion of the investigator, may either put the subject at risk by participation in the study, or may influence the result of the study.
- Subjects who have a history of drug or alcohol use that, in the opinion of the investigator, would interfere with the study.

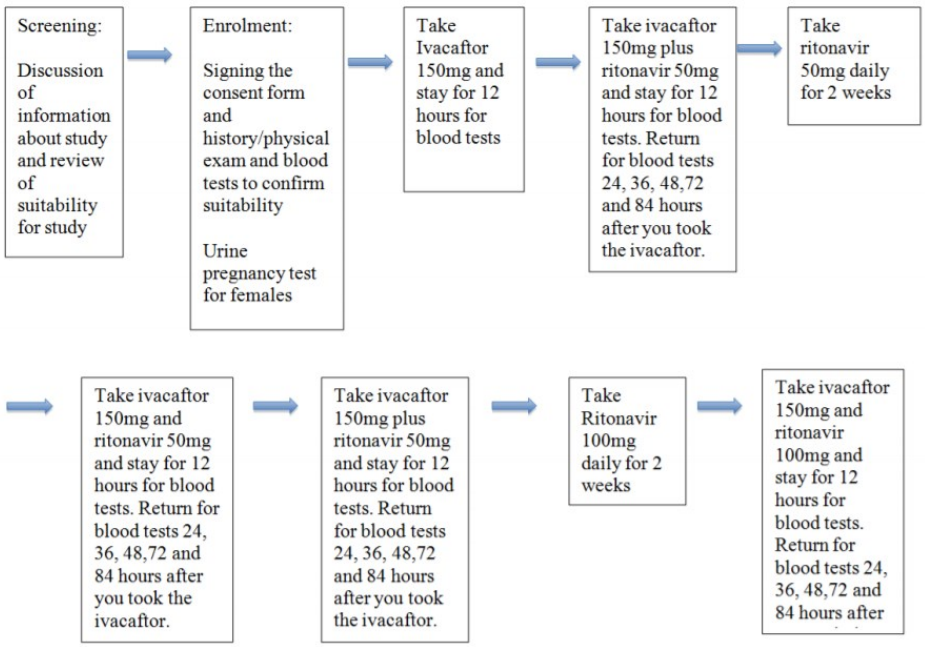
- HIV or hepatitis B/C positive (past or present)
- Being treated for/have a diagnosis of cancer
- Subjects requiring the long-term administration of medications
- Have abnormal liver function
- Have abnormal kidney function
- Scheduled for procedures requiring general anaesthesia during the study

Please note your participation in the trial may be stopped at any point should it be deemed to be in the best interest of your health and safety.

What will happen if I agree to take part?

If you agree to take part you will be asked to attend St. James's Hospital clinical trials unit for a visit to confirm that you are eligible for the study. You will then attend St James's Hospital for several study days in which you will take the various combinations of medications and have blood tests. To make sure you are eligible for the study we will ask you questions about your medical history, do a physical exam and then do some blood tests including a HIV and hepatitis test. We will then go on to do what we call our pharmacokinetic or 'PK' profiles. This means that we will take blood tests over a period of hours after you have taken ivacaftor with or without ritonavir to see what the blood levels of ivacaftor are over that time.

This will be organized as follows:



There will be 9 visits in total over at least 10 weeks. 5 of the visits will take 12 hours; the other 4 visits will be less than 1 hour.

Our aim is to find the smallest dose of ritonavir that will increase the blood levels of ivacaftor; therefore, if we find that 50mg of ritonavir is enough to increase the level of ivacaftor in the blood we will stop the study early and will not go on to test the 100mg dose. If, however, a dose of 50mg is not strong enough to increase the blood level of ivacaftor we will go on to do the part of the study using 100mg of ritonavir.

How are the blood tests taken?

When you are in St James's Hospital for 12 hours blood will be taken from a cannula – this is a small plastic line inserted in your arm. This means there will be only 1 needle necessary for the full 12 hours. When you return for blood tests after the twelve-hour period blood will be taken each time with a small needle. 10 mls (two teaspoons) of blood will be taken at each timepoint.

What are ritonavir (Norvir^{R}) and ivacaftor (Kalydeco^{R}) and how must I take them?

Ivacaftor (Kalydeco^{R}) is a medication used for the treatment of cystic fibrosis. It is a small blue tablet taken orally.
Ritonavir (Norvir^{R}) is a medication used for increasing the blood levels of other medications that are used for the treatment of HIV infection. In this study we will be using a smaller dose than is normally used in patients. It is in the form of a green liquid taken orally.

What will I eat when I am attending St James's Hospital?

Meals will be provided for you when you are in attendance in St James's hospital for 12 hours. Each volunteer will be given the same meal to make sure food intake does not affect the results.

During the time of the study and for a week beforehand you will be asked not to drink grapefruit juice or eat cruciferous vegetables (i.e. broccoli, cabbage, kale, brussels sprouts, cauliflower and turnips) as these foods can affect the blood levels of certain medications.

For 24 hours before the study visits and during the time we are taking blood tests you will be asked not to take any medication, not to drink any alcohol or caffeine and not to smoke as these can also affect the blood levels of medications.

Risks and benefits of taking part in the study

Will I be paid for participating in the study?

Yes you will be paid for participating in the study. You will be paid 10 euro per hour for the time you spend in St James's Hospital and 10 euro for each blood test you travel in to St James's Hospital for.

Apart from payment what are the benefits of participating in the study?

This study will benefit patients with cystic fibrosis who are on treatment with ivacaftor (Kalydeco^{R}). It will increase our knowledge of how the body breaks down and gets rid of this medication and it will also give us new information on how ivacaftor interacts with ritonavir and potentially how we can use this interaction to improve the benefit that patients get from the medication.

What are the risks involved in taking part in the study?

There are known side effects of both ivacaftor and ritonavir that may occur on taking these medications.
This study is designed so that healthy volunteers take the lowest dose possible of both of medications so that side effects will be kept to a minimum.

Ivacaftor

Very common side effects are inflammation/infection in the nose and sinuses, headache, sore throat/mouth, abdominal pain, diarrhoea and rash. Very common means that in the trial of this medication in patients with cystic fibrosis these side effects happened in more than one in ten people.

Ritonavir

Very common side effects with ritonavir include change in taste, pins and needles/pain in the lips and mouth, headache, dizziness, sore throat, cough, itch, rash, back and joint discomfort, feeling tired and flushing/feeling hot. These side effects happen in more than one in ten people, but with the use of doses higher than those being used in this study. Other studies have shown that the side effects of ritonavir are related to the dose you take so it is reasonable to assume that the side effects of ritonavir in this study will be less frequent and less severe.

Taking part in this study will also have the risks related to any blood test, for example: pain, bruising, minor bleeding. Insertion of the cannula may also result in skin infection. Any adverse event arising from taking part in the study will be addressed promptly and treated appropriately.

Note:

If you are a woman of childbearing age, you may participate in this study only if you are surgically sterilised or are using a reliable form of **non-hormonal** contraception (the medications being used in the study may affect how well the oral contraceptive pill works). You must not be pregnant or breast-feeding and you must have a negative pregnancy test before the study begins. The effects of ivacaftor and ritonavir on an unborn baby or child are unknown and may be harmful. If you should become pregnant, in spite of all precautions, please notify your doctor immediately

Is the study confidential?

All information obtained about you in the study is treated with the same respect for your confidentiality as any of your medical records. All information pertaining to you will remain private and confidential. You name will not be published or passed on to any third party. Only Dr. Anne Marie Liddy and Prof. Michael Barry will have access to the information pertaining to you as part of this study.

Will data be kept about me identify me?

All data is *coded* in this study – this means that there will be a number assigned to you information and only Dr. Anne Mare Liddy and Prof. Michael Barry will have the key to this code that will identify any stored data.

Will any information capable of identifying me appear in any publication or presentation?

Results of this clinical trial may be presented at conferences, published in academic journals and will also make up part of the PhD thesis for Dr. Anne Marie Liddy. However, **no** information that identifies you will ever be published or presented.

How long will you keep the information about me?

Information will be kept for ten years. This is in line with the research policies of Trinity College Dublin.

Compensation

The doctors treating you in this clinical trial are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights.

Permission from the Tallaght Hospital/St James's Hospital joint Research Ethics Committee and the HPRA has been given for this clinical trial.

Where can I get further information?

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from:

Dr Anne Marie Liddy +353879961493 liddyam@tcd.ie

ST. JAMES'S HOSPITAL
James's, St. Dublin 8, Ireland.

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

DO YOU WANT TO HELP PEOPLE WITH CYSTIC FIBROSIS?

WE ARE LOOKING FOR HEALTHY VOLUNTEERS FOR A RESEARCH STUDY!

This study is designed to learn more about a medication called Kalydeco which is used to treat a certain type of cystic fibrosis

Volunteers will be asked to take Kalydeco with and without another medication, ritonavir, on a few occasions so that we can check blood levels of Kalydeco and learn more about how it is broken down by the body

There will be payment for volunteers who take part in this study

Please contact Dr. Anne Marie Liddy for more information:

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The University of Dublin

ST. JAMES'S HOSPITAL
James's. St. Dublin 8. Ireland.

CONSENT FORM

Title of study: An Investigation of the Pharmacokinetic Interaction between Ritonavir (Norvir®) and Ivacaftor (Kalydeco®) in Healthy Volunteers

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form and patient information leaflet providing information on this study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement to keep for my own records.

PARTICIPANT'S NAME:_____

PARTICIPANT'S SIGNATURE:_____

Date: _____

Date on which the participant was first furnished
with this form and patient information leaflet: _____

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's signature:_____

Date:_____

Keep the original of this form in the participant's medical record, give one copy to the participant, keep one copy in the investigator's records.

HPRA
An tÚdarás Rialála Táirgí Sláinte
Health Products Regulatory Authority

1st May 2015

Department of Pharmacology & Therapeutics,
Trinity Centre for Health Sciences,
James's Street,
Dublin 8.

European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations, 2004

Re: CT Number: CT 900/566/1 - Ritonavir/Ivacaftor
Case number: 2159245
EudraCT number: 2015-000483-34
Protocol number: 7702015
Title of trial: An Investigation of the Pharmacokinetic Interaction Between Ritonavir (Norvir) and Ivacaftor (Kalydeco) in Healthy Volunteers

Dear Sir/Madam,

The Health Products Regulatory Authority has considered the application dated 1st March 2015 seeking authorisation to conduct the above clinical trial.

On the basis of the evidence available, the application is acceptable.

Please note that the date of this letter is the date of authorisation of the trial.

In accordance with Article 11 of Directive 2001/20/EC, confirmation of the authorisation of a clinical trial is mandatory for the updating of EudraCT, the EU database for clinical trials, and will be made public. Therefore, the Health Products Regulatory Authority requires that you provide the following information for this clinical trial as soon as it is available:

- Name of the responsible ethics committee
- Ethics committee opinion (favourable, not favourable, withdrawal)
- Date of the ethics committee opinion

If any changes are made to the EudraCT application form, you are reminded to provide the latest

version of the XML file to the Health Products Regulatory Authority for uploading to the EudraCT database. If in future, the XML file is updated as a result of a non-substantial amendment please submit the revised version of the XML file with the documentation for the next substantial amendment application.

Yours sincerely,

Sinead Murphy

Digitally signed by Sinead Murphy
DN: cn=Sinead Murphy, o=HPRA, ou=Business Process Co-ordination, email=sinead.murphy@hpra.ie, c=IE
Date: 2015.05.01 15:56:18 +01'00'

A person authorised in that behalf
by the Board of the said Authority

SJH/AMNCH Research Ethics Committee Secretariat
Claire Hartin Ph: 4142199
email: claire.hartin@amnch.ie

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING
THE NATIONAL CHILDREN'S HOSPITAL

TALLAGHT, DUBLIN 24, IRELAND
TELEPHONE +353 1 4142000

Dr. Anne Marie Liddy
Spr Pharmacology and Therapeutics
St. James's Hospital
James's Street
Dublin 8

11th May 2015

RE: An Investigation of the Pharmacokinetic Interaction between Ritovavir (Norvir) and Ivacaftor (Kalydeco) in Healthy Volunteers

REC Reference: 2015/04/03 / 2015-05 List 19 (1) please quote Reference on all correspondence

Dear Dr. Liddy,

Thank you for your correspondence dated 30th April in which you responded to the Committee's request for clarifications in relation to the above referenced study.

The Chairman of the Committee has reviewed your responses, is satisfied if the weekly web notice board is used and has given ethical approval on behalf of the Committee.

The following was reviewed and/or approved;

- Cover letter with responses
- Original Application Form
- Revised PIL
- HPRA letter

Full ethical approval is now in place for this study.

Yours sincerely,

Claire Hartin
Secretary
SJH/AMNCH Research Ethics Committee

Appendix 3. Presentations and publications.

Presentations:

'Development of an LCMS assay for the measurement of ivacaftor in plasma' at Pharmacology 2014, annual meeting of the British Pharmacological Society in London
Winner of GlaxoSmithKline Prize for the best Clinical Oral Communication at Pharmacology 2014

'The Pharmacokinetic Interaction between Ivacaftor and Ritonavir: Potential to improve the clinical and cost effectiveness of the treatment of cystic fibrosis in Ireland' at St Luke's Symposium, RCPI 2017. Finalist for the William Stokes Award for excellence in clinical research by higher specialist trainees.

Publications:

Liddy, A. M., McLaughlin, G., Schmitz, S., D'Arcy, D. M., and Barry, M. G. (2017) The pharmacokinetic interaction between ivacaftor and ritonavir in healthy volunteers. *British Journal of Clinical Pharmacology*, 83: 2235–2241. doi: 10.1111/bcp.13324.

British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2017) **83** 2235–2241 2235

DRUG INTERACTIONS

The pharmacokinetic interaction between ivacaftor and ritonavir in healthy volunteers

Correspondence Anne Marie Liddy, Department of Pharmacology and Therapeutics, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8, Ireland. Tel.: +35 31 416 2291; E-mail: liddyam@tcd.ie

Received 25 January 2017; **Revised** 13 April 2017; **Accepted** 27 April 2017

Anne Marie Liddy[1], Gavin McLaughlin[1], Susanne Schmitz[2], Deirdre M. D'Arcy[3] and Michael G. Barry[1]

[1]Department of Pharmacology and Therapeutics, Trinity College Dublin, Ireland, [2]Luxembourg Institute of Health, Strassen, Luxembourg, and [3]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland

Keywords cytochrome P450, drug interactions, pharmacoeconomics, respiratory medicine

AIMS

The aim of this study was to determine the pharmacokinetic interaction between ivacaftor and ritonavir.

METHODS

A liquid chromatography mass spectrometry (LC–MS) method was developed for the measurement of ivacaftor in plasma. An open-label, sequential, cross-over study was conducted with 12 healthy volunteers. Three pharmacokinetic profiles were assessed for each volunteer: ivacaftor 150 mg alone (study A), ivacaftor 150 mg plus ritonavir 50 mg daily (study B), and ivacaftor 150 mg plus ritonavir 50 mg daily after two weeks of ritonavir 50 mg daily (study C).

RESULTS

Addition of ritonavir 50 mg daily to ivacaftor 150 mg resulted in significant inhibition of the metabolism of ivacaftor. Area under the plasma concentration–time curve from time 0 to infinity ($AUC_{0\text{-inf obv}}$) increased significantly in both studies B and C compared to study A (GMR [95% CI] 19.71 [13.18–31.33] and 19.77 [14.0–27.93] respectively). Elimination half-life ($t_{1/2}$) was significantly longer in both studies B and C compared to study A (GMR [95% CI] 11.14 [8.72–13.62] and 9.72 [6.68–12.85] respectively). There was no significant difference in any of the pharmacokinetic parameters between study B and study C.

CONCLUSION

Ritonavir resulted in significant inhibition of the metabolism of ivacaftor. These data suggest that ritonavir may be used to inhibit the metabolism of ivacaftor in patients with cystic fibrosis (CF). Such an approach may increase the effectiveness of ivacaftor in 'poor responders' by maintaining higher plasma concentrations. It also has the potential to significantly reduce the cost of ivacaftor therapy.

DOI:10.1111/bcp.13324

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Ivacaftor is metabolized by cytochrome P450 3A4 and as such is subject to pharmacokinetic interactions with drugs that affect this enzyme.
- Ketoconazole has been shown to interact with ivacaftor such that a dose reduction of ivacaftor from 150 mg twice daily to 150 mg twice weekly is necessary.
- There is currently no literature published on the interaction between ritonavir and ivacaftor in healthy volunteers.

WHAT THIS STUDY ADDS

- This study investigates the pharmacokinetic interaction between ritonavir 50 mg daily and ivacaftor 150 mg in healthy volunteers.
- Exposure to ivacaftor is significantly increased and half-life of ivacaftor is significantly prolonged in the presence of ritonavir.
- This pilot study will inform the design of future studies in patients on ivacaftor therapy to explore the possibility of using ritonavir to increase the dosing interval of ivacaftor in clinical practice, thereby significantly reducing the budget impact of this high-cost drug.

Tables of Links

TARGETS	
Other Ion channels [2]	**Enzymes** [3]
CFTR	CYP3 family

LIGANDS	
Cobicistat	Ketoconazole
Ivacaftor	Ritonavir

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2, 3].

Introduction

In 2012 ivacaftor became the first disease-modifying agent available for the treatment of cystic fibrosis (CF), an inherited condition that may be caused by a number of distinct genetic mutations that lead to either qualitative or quantitative defects in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel on the surface of the cells that secrete mucus and sweat [4]. It is the most common inherited disease in Ireland, where there are currently over 1100 patients with the condition [5]. A defect in the CFTR leads to the production of thick, viscous secretions that precipitate repeated infections in the respiratory tract, damage to the pancreas and poor absorption of nutrients, ultimately resulting in a much-shortened life span — the median age at death for patients with CF in Ireland was 26.9 years in 2014 [5]. Ivacaftor is a small molecule that binds to the CFTR with the G551D mutation and increases the open probability of the channel, thus partially normalizing the movement of ions and water across the cell membrane and decreasing the viscosity of secretions [6]. The G551D mutation is associated with a severe phenotype of CF and is present in approximately 11% of persons with CF in Ireland [5].

The results of the pivotal trial of ivacaftor in patients 12 years or older with the G551D mutation, the STRIVE study sponsored by Vertex Pharmaceuticals, reported a striking average 10.4% increase in the primary endpoint, percentage predicted forced expiratory volume in 1 s ($ppFEV_1$), at 24 weeks in the treatment group; a relative increase compared to standard treatment of 10.6%. This improvement was sustained throughout the total 48 weeks of the trial. Several other improvements were demonstrated with ivacaftor therapy such as increased weight, decreased pulmonary exacerbations and improved quality of life [6].

While ivacaftor was shown in this trial to result in significant improvements in many aspects of the CF disease process for a sizable proportion of patients, it is noteworthy that an improvement in $ppFEV_1$ of 5% or more (deemed to be clinically significant) was seen in only 75% of the treatment group in the trial. It was not clear from a subgroup analysis why 25% of patients did not have this improvement. The numbers in the subgroup analysis were small and thus the drivers behind this were unclear, but it was not reported if variability in drug levels contributed to the difference in clinical outcome [6]. There is currently no sufficiently precise pharmacodynamic/pharmacokinetic model in the literature to inform the ideal plasma level of ivacaftor that patients need to maintain [7]; however, in an analysis published by the Food and Drug Administration in 2015, a linear relationship was seen between plasma ivacaftor levels and improvement in $ppFEV_1$ up to the third quartile of plasma concentrations in the treatment group, indicating that many patients may be maintaining suboptimal drug levels during the dosing interval [8]. Drugs such as ivacaftor that are metabolized by the mixed function oxidase CYP3A4 usually demonstrate significant inter-individual variability due to both genetic variations in the enzyme and environmental factors such as undetected inhibition/induction, disease

states and age [9, 10]. This variability is seen in the standard deviations of the major pharmacokinetic parameters reported in the summary of product characteristics for ivacaftor: after oral administration of a single 150 mg dose to healthy volunteers in a fed state, the mean (±SD) for area under the concentration–time curve (*AUC*) and maximum concentration (C_{max}) were 10 600 (5260) ng.h ml^{-1} and 768 (233) ng ml^{-1}, respectively [11].

There is existing clinical evidence that use of CYP3A4 inhibitors can overcome this problem of interindividual variability in CYP3A4 metabolism, increasing plasma levels throughout the dosing interval and also allowing for reduced dosing of the original drug, in this case, ivacaftor. The use of CYP inhibitors is well established in the treatment human immunodeficiency virus (HIV) infection for instance, with ritonavir boosting of other protease inhibitors now established as standard of care [12, 13]. The use of ritonavir for pharmacokinetic enhancement in the early years of HIV treatment enabled patients to take far fewer tablets over the course of the day, and those that did not achieve viral suppression due to low plasma levels of protease inhibitor became virally suppressed with the addition of ritonavir with the subsequent increase in plasma levels of co-administered protease inhibitors. The addition of a small dose of ritonavir to the medication regime for HIV meant that treatment became more convenient and more clinically effective for a larger number of patients [14].

In addition to clinical effectiveness, the issue of cost effectiveness is extremely pertinent in the case of ivacaftor. The drug acquisition cost to treat one patient with ivacaftor is €234 804 per annum [15]. Ivacaftor is one of the most expensive drugs reimbursed in Ireland at present and, due to the nature of the drug and the disease it treats, lifelong treatment is essential.

Given these issues with the clinical and cost effectiveness of ivacaftor treatment, this study investigates the pharmacokinetic interaction between ivacaftor and ritonavir *in vivo* in healthy volunteers to establish whether a CYP inhibitor can be used to alter the metabolism of ivacaftor in such a way that will allow for a longer dosing interval of the drug while at the same time increasing plasma levels of the drug.

Subjects and methods

Subjects

Subjects eligible for inclusion in this trial were healthy volunteers over the age of 18, able to give informed consent and not on any regular medications. To be classified as 'healthy volunteers', subjects underwent medical history, physical examination and laboratory tests including full blood count, urea and electrolytes, liver function tests, HIV and hepatitis serology, urine drug screen and pregnancy testing for women of child-bearing age. If female and of child-bearing potential or if male with a partner of child-bearing potential, volunteers had to be willing to use effective contraception during the study and up to 18 days after the last study day. The use of hormonal contraception was not permitted. Subjects were excluded if, in the opinion of the investigator, they had any medical condition that would put the subject at risk by participation in the study or influence the study result. Regular use of cigarettes or any substance that may induce or inhibit the cytochrome P450 system also resulted in exclusion. Volunteers were advised to avoid both drinking grapefruit juice and eating cruciferous vegetables for 1 week before the study and throughout the duration of the study. Over-the-counter medications, caffeine and alcohol were avoided 24 hours before the study commenced and throughout the duration of the study.

Study design

This was a single-centre, open-label, sequential, cross-over study with 12 healthy volunteers. A balanced trial design was achieved by randomizing subjects into three sequences of three periods representing a Latin square (Figure 1).

Study A consisted of administration of ivacaftor alone and measurement of ivacaftor plasma levels at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h. Study B consisted of administration of ivacaftor and ritonavir 50 mg at time 0 and further administration of ritonavir 50 mg at 24, 48 and 72 h. Ivacaftor levels were measured at 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 84 h. To investigate any induction effect of ritonavir, study C consisted of administration of 50 mg of ritonavir daily for 2 weeks prior to administration of ivacaftor 150 mg and ritonavir 50 mg at each of days 0, 1, 2 and 3. Ivacaftor levels

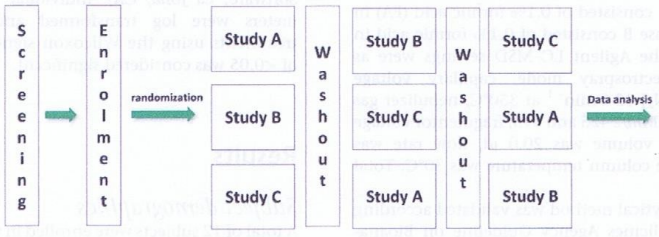

Figure 1

Study schema. Study A: Ivacaftor 150 mg. Study B: Ivacaftor 150 mg plus ritonavir 50 mg on days 0, 1, 2 and 3. Study C: Ritonavir 50 mg daily × 2 weeks then ivacaftor 150 mg plus ritonavir 50 mg on days 0, 1, 2 and 3

were measured at 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 84 h. Standardized, high-fat meals were given to all volunteers for the first 12 h of each study and water was freely available throughout. Ritonavir liquid was administered with a small amount of chocolate milk to improve palatability as required. It was written into the original trial protocol that should ritonavir 50 mg not result in significant inhibition of the metabolism of ivacaftor at interim analysis of data after the completion of studies A, B and C, studies A, B and C would then go on to be repeated in the same manner with ritonavir at a dose of 100 mg. The lower dose of ritonavir was investigated first as the overall aim of the study was to ascertain the lowest dose of ritonavir that would provide significant inhibition of ivacaftor metabolism, thus minimizing any potential adverse effects of long-term exposure to ritonavir, some of which have been shown to be dose related [16–18]. Potential induction effects of ritonavir were also predicted to be minimized by the use of the smallest dose feasible [19].

Blood samples were collected in 5 ml tubes containing ethylenediaminetetraacetic acid (EDTA). These were centrifuged for 10 min at room temperature immediately after collection and the plasma supernatant aliquoted into 300 µl fractions and frozen at −80°C.

The main pharmacokinetic parameters assessed in the study included $t_{1/2}$ of ivacaftor in the absence and presence of ritonavir, change in C_{max} and time to maximum concentration (t_{max}) and the area under the time–concentration curve (AUC) of ivacaftor in the presence and absence of ritonavir. Variability in the above pharmacokinetic parameters was also assessed in the absence and presence of ritonavir.

Ethical approval for the study was obtained from the Tallaght Hospital/St James's Hospital Joint Research Ethics Committee on 11 May 2015 (reference number 2015/04/03/2015-05 list 19[1]). The clinical trial protocol was submitted to the Health Products Regulatory Authority and approval for the study was obtained on 1 May 2015.

LC–MS analysis

LC–MS analyses were performed on an Agilent 1100 high performance liquid chromatography (HPLC) system equipped with a G13795 degasser, G1312A BinPump, a G1313A ALS and G1316A column oven (COLCOM) (Agilent, Little Island, Cork). Separation was obtained on an Allure PFP Propyl column (5 µm, 50 × 2.1 mm) Restek (Bellefonte, PA, USA). Mobile phase A consisted of 0.1% formic acid (FA) in water and mobile phase B consisted of 0.1% formic acid in acetonitrile (ACN). The Agilent LC–MSD settings were as follows: positive electrospray mode, capillary voltage 3500 V, drying gas (N_2) 12 l min^{-1} at 350°C, nebulizer gas (N_2) pressure 50 psi, SIM*m/z* 425 and 393, fragmentor voltage 50 V. The injection volume was 20.0 µl, flow rate was 0.4 ml min^{-1} and the column temperature was 30°C. Total run time was 25 min.

The ivacaftor analytical method was validated according to the European Medicines Agency Guideline on Bioanalytical Method Validation [20]. Plasma samples were spiked with known concentrations of ivacaftor to produce a standard curve from 0.125 µg ml^{-1} to 4 µg ml^{-1}. The method was shown to be sufficiently selective down to a concentration of 0.125 µg ml^{-1} and $1/x^2$ weighting was the simplest model that described the relationship between plasma concentration and instrument response. Inter-day and intra-day accuracy, as represented by mean bias, and precision, as represented by coefficient of variation, were all within 15%. Ivacaftor was shown to be stable for 2 months at −80°C as well as stable at room temperature for at least 6 h.

Plasma standards or thawed study samples were mixed 1:1 with 300 µl of phosphate buffer: 20 µl of internal standard was added to each sample, and 550 µl was then placed on an ISOLUTE® SLE+ Supported Liquid Extraction column and drawn onto the column with the application of a vacuum. After 5 min, columns were washed through with 5 ml of methyl tert-butyl ether. Samples were then blown to dryness and reconstituted with 200 µl of ACN:H_2O 1:1 plus 0.1% FA. This was then centrifuged at 35 199 × *g* at 4°C for 10 min to remove any remaining particulate matter. The supernatant was then transferred to Agilent autosampler vials with 150 µl glass inserts. All patient samples were analysed in duplicate.

Pharmacokinetic analysis

The pharmacokinetic parameters for ivacaftor were determined by non-compartmental methods using Phoenix WinNonlin Professional (version 6.4; Pharsight, Mountain View, CA) pharmacokinetic software. Parameters estimated included $AUC_{0\text{-inf obs}}$ area under the plasma concentration–time curve over 12 h (AUC_{0-12}), $t_{1/2}$, C_{max} and t_{max}. AUC was calculated using the linear trapezoidal rule. The $t_{1/2}$ of ivacaftor was calculated from at least three terminal concentrations.

Statistical analysis

A sample size of eight was originally calculated to have 90% power to detect a two-fold increase in the half-life of ivacaftor with a type 1 error rate of 5%. To account for a dropout rate of 20% in the context of the sequential crossover design with three sequences and three periods, a sample size of 12 was chosen.

Statistical analysis of both data from the bioanalytical method validation and pharmacokinetic parameters derived from WinNonlin pharmacokinetic software were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA). Individual pharmacokinetic parameters were log transformed and compared between treatments using the Wilcoxon signed-rank test. A P-value of <0.05 was considered significant.

Results

Subject demographics

A total of 12 subjects were enrolled in the study, two of whom were female. The median age of the subjects was 23 years (range 20–30 years), median body weight was 71.25 kg and median body mass index (BMI) was 23.1 kg m^{-2}. All subjects were Caucasian.

Safety and tolerability

Both ivacaftor and ritonavir were well tolerated in the study population. There were no deaths or serious adverse events reported during the study. Mild adverse events reported included nausea in three subjects during the 2 weeks of ritonavir treatment, lasting for 1 day each, coryza in the first 24 h of studies B and C in one subject and numbness in the index finger and thumb of one subject which lasted 5 days during ritonavir treatment for 2 weeks. All adverse events resolved spontaneously and did not require discontinuation of study drug.

Concentration–time profiles of ivacaftor

The median (± interquartile range) concentration–time profiles of ivacaftor in studies A, B and C are shown in Figure 2.

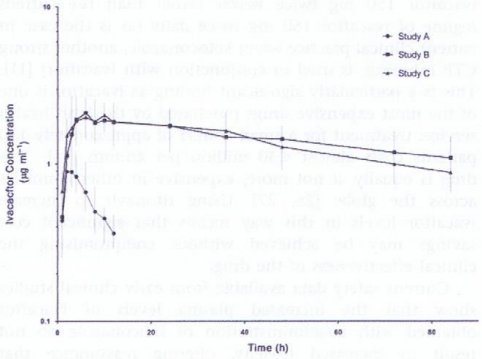

Figure 2

Concentration–time profiles of ivacaftor from studies A, B and C (median ± interquartile range)

Pharmacokinetic parameters

Ivacaftor pharmacokinetic parameters are summarized in Table 1. Significantly higher exposure to ivacaftor, as represented by $AUC_{0\text{-inf obv}}$ was found when ivacaftor was administered with ritonavir in both studies B and C compared with ivacaftor alone in study A (P = 0.0005 and 0.0005 respectively). $AUC_{0\text{-inf obv}}$ (95% CI) was 10.94 (8.259–14.48) μg.h.ml^{-1} in study A compared to 215.6 (146.4–317.4) μg.h.ml^{-1} in study B and 216 (165.5–281.8) μg.h.ml^{-1} in study C, respectively (GMR [95% CI] study A:study B 19.71 [13.18–31.33] and study A:study C 19.77 [14.0–27.92], respectively). C_{max} (GM [95% CI]) in study A was 0.9944 (0.7819–1.265) μg, 1.812 (1.323–2.482) μg in study B and 2.267 (1.863–2.757) μg in study C, respectively. C_{max} of ivacaftor was significantly increased in the presence of ritonavir in both study B and study C (GMR [95% CI] 1.82 [1.34–2.48] and 2.28 [1.84–2.83] respectively; P = 0.0049 and 0.0005 respectively) with no significant difference in C_{max} of ivacaftor between study B and study C (P = 0.2661). Median t_{max} of ivacaftor was significantly later in the presence of ritonavir (median [range] 2.5 [2–4] h, 6.5 [3–23.75] h and 4 [3–10] h in studies A, B and C, respectively; P = 0.001).

Elimination half-life (GM [95% CI]) of ivacaftor in study A was 7.121 (5.59–9.07) h compared to 79.24 (65.5–96.1) h in study B and 65.99 (57.43–75.82) h in study C. The $t_{1/2}$ of ivacaftor was significantly prolonged by coadministration of ritonavir in both study B and study C in comparison to study A (GMR [95% CI] 11.14 [8.72–13.26] and 9.27 [6.68–12.85] respectively; P = 0.0005 and 0.0005 respectively). There was no significant difference in $t_{1/2}$ between study B and study C (GMR [95% CI] 0.83[0.63–1.1]; P = 0.2036). The coefficient of variation of $AUC_{0\text{-inf obv}}$ and C_{max} was similar between studies A, B and C. The coefficient of variation in $t_{1/2}$ of ivacaftor reduced with the addition of ritonavir, reducing to almost half when comparing study C to study A, as shown in Table 2.

Discussion

The pharmacokinetic data obtained from this healthy-volunteer, drug-interaction study show that ritonavir at a

Table 1

Summary of PK parameters of ivacaftor in studies A, B and C

	Study A	Study B	Study C	Geometric mean ratio Study A: Study B	Geometric mean ratio Study A: Study C	Geometric mean ratio Study B: Study C
$AUC_{0\text{-}12}$ (μg.hr.ml^{-1})	7.024 (5.48–9.0)	16.65 (11.4–24.32)	21.5 (17.38–26.59)	2.37 (1.62–3.47)	3.06 (2.36–3.97)	1.29 (0.856–1.95)
$AUC_{0\text{-inf obv}}$ (μg.hr.ml^{-1})	10.94 (8.26–14.48)	215.6 (146.4–317.4)	216 (165.5–281.8)	19.71 (13.18–31.33)	19.77 (14.0–27.93)	1.00 (0.68–1.47)
C_{max} (μg)	0.9944 (0.7819–1.265)	1.812 (1.323–2.482)	2.267 (1.863–2.757)	1.82 (1.34–2.48)	2.28 (1.84–2.83)	2.54 (0.65–9.9)
$t_{1/2}$ (h)	7.12 (5.59–9.07)	79.34 (65.5–96.1)	65.99 (57.43–75.82)	11.14 (8.72–13.26)	9.27 (6.68–12.85)	0.83 (0.63–1.1)
Median time to t_{max} (range) (h)	2.5 (2–4)	6.5 (3–23.75)	4.0 (3–10)			

Table 2

Coefficient of variation (%) of key PK parameters

	Study A	Study B	Study C
$AUC_{0\text{-inf obv}}$	45.12%	41.86%	45.82%
C_{max}	37.46%	33.13%	34.59%
$t_{1/2}$	43.96%	33.48%	22.32%

dose of 50 mg administered once daily, in both acute and chronic dosing, significantly increases plasma concentrations of ivacaftor (Table 1). All key pharmacokinetic parameters (AUC_{0-12}, $AUC_{0\text{-inf obv}}$, C_{max}, $t_{1/2}$) were increased several fold by the presence of ritonavir. Additionally, the coefficient of variation of $t_{1/2}$ of ivacaftor was decreased by the use of ritonavir for 2 weeks prior to ivacaftor administration (Table 2). These data provide proof of concept that concomitant dosing of ivacaftor and ritonavir could be used in clinical practice to increase the dosing interval of ivacaftor while also allowing patients to maintain higher plasma levels than with ivacaftor alone at the recommended dose of 150 mg twice daily.

It was a curious finding that the coefficient of variation of C_{max} and $AUC_{0\text{-inf obv}}$ did not change, while that of $t_{1/2}$ showed a marked reduction. There are insufficient data in the current study to fully explore this phenomenon, though it could be hypothesized that the variability in drug absorption seen in other pharmacokinetic studies on ivacaftor contributed to this [21]. While absorption of ivacaftor may have remained variable with co-administration of ritonavir, thus affecting the variability of C_{max} and $AUC_{0\text{-inf obv}}$, the variability in elimination was smoothed out, and this was seen in the reduction in variability in $t_{1/2}$.

In the early years of HIV treatment, the use of ritonavir was associated with a number of significant and debilitating side effects such as abdominal pain, nausea, vomiting, severe diarrhoea, parasthesias, rashes and peripheral neuropathy. Doses used for treatment (as opposed to those used for pharmacokinetic enhancement) were up to 1200 mg per day [22]. The side effects of ritonavir used at doses for pharmacokinetic enhancement are reported to be much less frequent and less severe [23]. These side effects have also been shown to be dose related and it was for this reason that the smallest dose of ritonavir reported in the literature to provide significant boosting of other protease inhibitors (50 mg once daily) was used [16, 24]. It was encouraging that no volunteer experienced side effects of ritonavir that compelled them to stop taking the drug, and for those who did experience side effects, these were mild and transient. This suggests that ritonavir will be tolerable for use in pharmacokinetic enhancement of ivacaftor in clinical practice for patients with cystic fibrosis.

A comparison of study B and study C data suggests that similar inhibition is achieved with acute and chronic dosing of ritonavir. This is an important finding, as some drug interactions with ritonavir show change over time. Alprazolam is one example; one study showed there is significant inhibition of alprazolam metabolism with acute dosing of ritonavir, but when ritonavir was used daily for 12 days, this effect disappeared. It is hypothesized that this was due to the induction effect of ritonavir [25]. It was therefore important to establish that such a situation would not arise with the inhibition of ivacaftor. As the induction effects of ritonavir are maximal at 2 weeks [25] the equivalent level of inhibition that exists between study B and study C suggests that any induction effects of ritonavir will not be clinically significant should ritonavir be used in the long term as a pharmacokinetic enhancer.

The use of pharmacokinetic enhancement of ivacaftor with ritonavir in clinical practice not only has the potential to improve clinical outcomes for people with cystic fibrosis on ivacaftor treatment; increasing the dosing interval would significantly reduce the cost of ivacaftor. The magnitude of the pharmacokinetic enhancement demonstrated by this small dose of ritonavir opens the possibility of much reduced dosing of ivacaftor, such as ivacaftor 150 mg twice *weekly* rather than the current regime of ivacaftor 150 mg twice daily (as is the case in current clinical practice when ketoconazole, another strong CYP inhibitor, is used in conjunction with ivacaftor) [11]. This is a particularly significant finding as ivacaftor is one of the most expensive drugs purchased by the Irish health service: treatment for a small cohort of approximately 130 patients costs almost €30 million per annum [15]. This drug is equally, if not more, expensive in other countries across the globe [26, 27]. Using ritonavir to increase ivacaftor levels in this way means that significant cost savings may be achieved without compromising the clinical effectiveness of the drug.

Current safety data available from early clinical studies show that the increased plasma levels of ivacaftor obtained with co-administration of itraconazole do not result in increased toxicity, offering reassurance that increased plasma levels of ivacaftor resulting from co-administration of ritonavir will not be problematic [7]. Future studies can be carefully designed based on the information gleaned from the current study to explore the ideal ivacaftor plus ritonavir dosing regimen for patients with cystic fibrosis.

Based on the above findings, it appears that ivacaftor metabolism is particularly sensitive to the effects of CYP3A4 inhibitors. This opens the possibility of further studies exploring other inhibitors such as cobicistat, the newly marketed, specifically designed CYP3A4 inhibitor currently used in the treatment of HIV for pharmacokinetic boosting. Therefore, there is potential for a number of boosting agents to become available to patients on high-cost drugs such as ivacaftor in the future.

As we move into an era of ultra-high-cost drugs for orphan diseases, we need innovative solutions to overcome the challenges of providing healthcare that is both equitable and at an acceptable opportunity cost. The potential benefits of using a CYP3A4 inhibitor in the CF setting warrants exploration given the proof of the concept that ivacaftor plasma levels can be significantly increased with the use of a small dose of ritonavir.

Competing Interests

There are no competing interests to declare.

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Contributors

Author responsibilities were as follows: A.M.L., G.M.L., S.S., D.M.D. and M.G.B. all contributed to the preparation of the manuscript. A.M.L., S.S. and M.B. designed the research. A.M.L. performed the clinical research and was principal investigator of the clinical trial. G.M.L. and A.M.L. developed the LC–MS assay, and data analysis and interpretation were undertaken by A.M.L., S.S., D.M.D. and M.B. All authors approved the final version of the manuscript.

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