FINAL CLINICAL STUDY REPORT

1. TITLE PAGE

Study Title:	PLASMA LEVELS OF OSELTAMIVIR IN H1N1 INFECTED PATIENTS SUPPORTED BY EXTRACOPOREAL MEMBRANE OXYGENATION: A SINGLE-CENTRE COHORT STUDY
Short Title:	Plasma levels of Oseltamivir and Oseltamivir Carboxylate during ECMO
Investigational Product:	Oral Oseltamivir
Indication studied:	Treatment of H1N1 infections in ECMO patients
Study Sponsor:	University Hospitals of Leicester NHS Trust, Trust Headquarters, Level 3, Balmoral Building, Leicester Royal Infirmary, Infirmary Road, Leicester, LE1 5WW, United Kingdom
Protocol Number:	UHLHM0002
Report Number:	TAM001/01FINAL
EudraCT No:	2009-018200-17
Study Initiation Date (Date First Subject Enrolled):	27th November 2010
Study Completion Date (Date Last Subject Completed):	23rd February 2011
Investigator:	Principal Investigator Dr Raghu Ramaiah, Intensive care Consultant, Department of ECMO Email : raghu.ramaiah@uhl-tr.nhs.uk Tel: +44 (0) 7814845963
Author of Report:	Dr Hussain Mulla Centre for Therapeutic Evaluation of Drugs Department of Pharmacy University Hospitals of Leicester Tel: 0116 2502708 …….
GCP Statement:	The study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents.
Report Date	March 2012

2. SYNOPSIS

Title of study:
Plasma Levels Of Oseltamivir In H1N1 Infected Patients Supported By Extracoporeal Membrane Oxygenation: A Single-Centre Cohort Study

Investigators:
Dr Raghu Ramaiah

Study centre:
University Hospitals of Leicester, Glenfield Hospital, Leicester, LE3 9QP, UK.

Publication (reference):
The study has not yet been published

Studied period (years): < 1 year

Objectives:
Primary objective: To determine the pharmacokinetics of the prodrug oseltamivir and its primary metabolite, oseltamivir carboxylate, in ECMO adults and children.

Methodology:
A single centre cohort, multiple dose, rich sampling, pharmacokinetic modelling study of 75 mg oral oseltamivir phosphate administered twice daily to suspected or confirmed H1N1 influenza infected adult patients receiving ECMO treatment. Blood samples were taken from time zero until up to 12 hours post dose on 2 occasions (Day 1 and 5). Plasma oseltamivir phosphate and oseltamivir carboxylate concentrations were determined and population PK parameters estimated using a non-linear, mixed effects modeling approach.

Number of subjects (planned and analysed):
At least 20 patients (15 adults, 5 children) were planned to complete the study. 14 adult patients (male and female) were screened, eligible and entered. 12 subjects completed the study. For 2 patients blood samples were taken on Day 1 only due to early decannulation from ECMO.

Diagnosis and main criteria for inclusion:
All ECMO patients treated with oral oseltamivir therapy for suspected or confirmed H1N1 influenza infection were eligible for the study. Patients were recruited following written informed consent.

Test product, dose and mode of administration:
Multiple dose of 75 mg twice daily oral oseltamivir phospate given orally.

Duration of treatment:
There were no modifications to the treatment of care received by the ECMO patients. The clinical decision to initiate (or continue from the referring hospital) and terminate oseltamivir treatment was undertaken by the attending ECMO clinician.

Criteria for evaluation:
Primary:
Pharmacokinetic parameters:
Oseltamivir phosphate:

- Clearance (CLM),
- Volume of distribution (V)

Oseltamivir Carboxylate:

- Clearance (CLO)
- Volume of distribution (VM)

Secondary:
Pharmacokinetic parameters (Oseltamivir phosphate and oseltamivir carboxylate):

- Maximum plasma concentration (Cmax)
- Area under the curve (AUC)
- Half-life ($t_{1/2}$)

Statistical Methods:

A population-PK approach was applied in which all data from different individuals were fitted simultaneously using a non-linear, mixed effects modelling approach.

Both the parent drug (oseltamivir) and the primary metabolite (oseltamivir carboxylate) were evaluated and characterised. These data were exported from Microsoft Excel datasets as comma delimited data

files and used for analyses in NONMEM. Standard ADVAN subroutines were used. The PK analysis of oseltamivir phosphate and oseltamivir carboxylate was conducted simultaneously.

All analyses were performed using NONMEM v7.2 (Icon Development Solutions, USA). Data generated by NONMEM was evaluated using PDx-POP (version 4.0, ICON Development Solutions, USA) which has been developed as a graphical interface tool for NONMEM. This was used in conjunction with R v2.13.0 (R Foundation) software to generate output and graphics. Other supporting graphical software was used to aid visualisation.

All other supporting summaries and any secondary analyses were undertaken using Microsoft Excel 2007 (Microsoft Corporation, USA).

PK Results:

Population PK Parameters (n=14)* [a]	**Mean**	**BSV (%)** [b] ***BOV (%)***	**Eta Shrinkage (%)**
Oseltamivir			
KA (per hour)	1.39 (12.3)	15.6 (195)	
V/F (L)	59.7 (7.4)	94.6 (40.6) *88.9 (30.7)*	2.93
CLM/F (L/h)	97.5 (4.3)	69.9 (37.4)	1.94
Q1/F (L/h)	42.1 (10.7)	129 (46.1)	11.7
VP/F (L)	176 (4.7)	64.7 (56.1)	21.9
Bioavailability	0.25 fixed	NE	
Alpha Half-Life (hr) [c,d]	0.23 (0.14)	NE	
Beta Half-Life (hr) [c,d]	5.63 (4.95)	NE	
Oseltamivir Carboxylate			
V_M/F (L)	350 (3.94)	37.9 (81.9) *117.4 (26.8)*	21.3
CLO/F (L/h)[e]	1274 (12.8) x $CR^{-1.03\ (20.7)}$	34.8 (45.0)	10.6
K_{trans} (metabolic compartment)[f,g]	0.46 (56.0)	68.4 (106)	
Bioavailability	0.75 fixed	NE	
Half-Life (hr)[c,d]	36.4 (66.5)	NE	
Residual Error Models	*Oseltamivir*	*Oseltamivir Carboxylate*	
Additive Error (nM)	0.24 (261)	57.6 (40.2)	
Proportional Error (%CV)	0.32 (14.7)	0.05 (13.6)	

[a] All figures in parentheses are parameter precision and are expressed as percent relative standard error (100% x SE/Parameter Estimate)
[b] BSV / BOV = Between Subject / Between Occasion Variability and is calculated as $(variance)^{1/2}$*100%
[c] Derived from each individuals empirical Bayes estimates of model parameters
[d] Arithmetic mean (sd)
[e] CR = Serum Creatinine (µmol/L).
[f] First order rate constants for transit from metabolic compartment.
[g] Mean transit times (hours) =$(n + 1)/K_{trans}$ = 4.3 hours
NE= not estimated

Table 2: Secondary Pharmacokinetic Parameter Summary for Oseltamivir Carboxylate Day 5 of ECMO

Statistic (N=14)	AUC_{0-12} (ng/ml.h)	Cmax,m (ng/ml)	Tmax (h)
Mean	5370.9	557.11	4.96
SD	3608.8	331.48	1.95
CV (%)	67.2	59.5	39.3
Median	4346.6	509.14	4.50
Minimum	644.1	54.35	2.50
Maximum	13660.4	1277.20	9.00

SD:Standard Deviation; CV:Coefficient of Variation

Conclusions:

In conclusion, an integral pharmacokinetic model developed for oseltamivir and oseltamivir carboxylate in critically ill adult patients supported on ECMO, describes the pharmacokinetics of both analytes satisfactorily. The PK parameter estimates for the parent oseltamivir are similar to previous reports. In contrast, the elimination clearance of oseltamivir carboxylate was reduced compared to healthy and ambulatory adult subjects. Similar to previous reports, oseltamivir carboxylate clearance was significantly influenced by renal function and hence systemic exposure (AUC and Cmax) correlated with serum creatinine. The steady state volume of distribution of oseltamivir caboxylate was substantially greater than previously reported in healthy adult volunteers, but comparable to a previous report in critically ill adults.

From a therapeutic perspective, mean systemic exposure of oseltamivir carboxylate following the administration of oral oseltamivir 75mg twice daily in adult ECMO patients is comparable to those in ambulatory patients and far in excess of concentrations required to maximally inhibit neuraminidase activity of the H1N1 virus. Therefore, dosage adjustment for ECMO *per se* is not necessary; however it may be necessary to increase doses in individual subjects with impaired enteral absorption or convert to an intravenous neurodaminase inhibtor such as zanamivir. Finally, as revealed in other patient populations, dosages could be reduced in those subjects with renal dysfunction.

3. TABLE OF CONTENTS

4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

GLOSSARY

AUC_{0-12}	Area under the plasma metabolite concentration-time curve from time zero to 12 hours (also referred to as AUC)
BSV	Between Subject Variability
CLM	Formation clearance of oseltamivir carboxylate (also referred to as CLM/F)
CLO	Elimination clearance of oseltamivir carboxylate (also referred to as CLO/F)
$C_{max,}$	Maximum plasma concentration
CV	Coefficient of variation (%)
CWRES	Conditional Weighted Residuals
D	drug dose
DV	Dependent Variable (plasma metabolite concentration)
F	Bioavailability
FOCE	First order conditional estimation
IPRED	Individual predicted values
IWRES	Individual Weighted Residuals
K_{trans}	First order rate constant for transit of oseltamivir carboxylate from the metabolic compartment
Ka	Absorption rate constant
kg	Kilogram
L	Litre
LLOQ	The lower limit of quantification
MCMC	Markov Chain Monte Carlo
Min(s)	Minute(s)
mL	Millitre
MOF	Maximum likelihood of Objective Function
ng	Nanogram
nM	Nanomolar concentration
NONMEM	Nonlinear mixed effects modelling tool
NS	Not statistically significant
OBS	Observations
OFV	Objective function value (-2 × log likelihood)
PDx-POP	A Graphical Interface for the NONMEM System
PK	Pharmacokinetic
PRED	Population predicted values
Q1	Inter-compartmental clearance for oseltamivir
RES	Residuals
Rin	Zero order infusion rate
RSE	Relative standard error
SD	Standard Deviation
SE	Standard error
SIG	Statistically significant
$T_{max,}$	Time to the maximum concentration
$t_{1/2}$	Half life
$t_{1/2,\alpha}$	Distributional half life
$t_{1/2,\beta}$	Elimination half life
V	Volume of distribution of the central compartments of the oseltamivir model (also referred to as V/F)
VP	Volume of distribution of the peripheral compartment of the oseltamivir model (also referred to as VP/F)

VM	Volume of distribution of oseltamivir carboxylate (also referred to as VM/F)
VPC	Visual Predictive Check
WT	Bodyweight
Yrs	years

5. ETHICS

5.1 Independent Ethics Committee

Prior to enrolment of subjects into this study, the final protocol (including subject information sheet and consent form) were reviewed and approved by the Independent Ethics Committee (IEC) associated with the study centre. There were no protocol amendments.

5.2 Ethical Conduct of the Study

The study was conducted according to the protocol and in accordance with:

- the Declaration of Helsinki (1996)
- ICH GCP requirements
- Statutory Instruments (UK Law): SI 2004 No. 1031, 2006 No 1928.

5.3 Patient Information and Consent

Patients relatives / guardians gave written informed consent at the start of the study. The study procedures, and the risks and benefits of participating in the study, were first described to the relatives / guardians by the investigator and they were given study subject information sheets to read, prior to giving consent.

Relatives / guardians were given the opportunity to ask questions and were informed of their right to withdraw from the study at any time, for any reason.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

This was a single centre study, designed and reported by Dr Hussain Mulla in conjunction with the principal investigator, Dr Raghu Ramaiah. The study was sponsored, managed and monitored by University Hospitals of Leicester NHS Trust.

The following Contract Research Organisations were appointed to provide the services, as indicated, for this study:

- Pharmacokinetic analyses: PRA International - Early Development Services, Bioanalytical Laboratory

All personnel involved with the study are listed in the Trial Master File.

7. INTRODUCTION

Glenfield Hospital, Leicester is an internationally recognised Extra-Corporeal Membrane Oxygenation (ECMO) centre and is the only centre in the UK provisioned by the Department of Health to provide ECMO for adults and children with H1N1 influenza infection (Swine Flu). To date, this service has provided ECMO support for adults and children with severe H1N1 infection, all of whom have been treated with oral oseltamivir using doses recommended by the marketing authorisation holder (Roche) and literature reports. However, for many drugs, ECMO is known to affect the plasma levels of the drug, so doses recommended for the general patient population may not be appropriate to this group of patients. Apart from a limited case report of plasma oseltamivir levels in three children treated on ECMO, the pharmacokinetics of oseltamivir in ECMO patients has not been investigated (1). In order to define the optimum dose for this group of very sick Swine

Flu patients, it is important that the pharmacokinetics of oseltamivir and the active metabolite oseltamivir carboxylate are investigated.

This study was conducted to determine the pharmacokinetics of oseltamivir and oseltamivir carboxylate in patients undergoing ECMO treatment and hence the optimum dosage for treatment of H1N1 infections in ECMO patients. The study was a single centre, observational, multiple dose, population pharmacokinetic study using oral oseltamivir administered at 75 mg twice daily for suspected and confirmed H1N1 infection. The study was planned to be conducted in 20 ECMO patients, 15 adults and 5 children.

8. STUDY OBJECTIVE

Primary objectives

1. To determine the pharmacokinetics of oral oseltamivir and its primary metabolite, oseltamivir carboxylate, in ECMO adults.

2. Determine optimum dosage of oral oseltamivir phosphate for treatment of H1N1 infections in ECMO patients.

9. STUDY DESIGN

9.1 Overall Study Design

The study was a single-centre, observational, multiple dose, pharmacokinetic study in ECMO patients on the intensive care unit with suspected or confirmed H1N1 infection treated with oral oseltamivir. There were no modifications to the treatment of care received by the ECMO patients. The clinical decision to initiate oseltamivir treatment (or continue from the referring hospital) was undertaken by the attending ECMO clinician.

Once cannulated on to ECMO, patients were prescribed a dosing regimen of 75 mg twice daily of oral oseltamivir as per dose defined in the SmPC. Once written informed consent had been obtained, a study team member assigned a study number to the patient and recorded all subsequent information in a case report form. Concomitant medications were recorded throughout the study duration.

Blood sampling was performed by ECMO specialists and intensive care nurses working in the unit. Blood samples were obtained from the ECMO circuit or via an indwelling central venous catheter pre-dose and at 0.5, 1 ,2 3, 5, 7, 9, and 12 hours post dose on two separate occasions, if possible.

9.2 Selection of Study Population

In order to develop a robust population pharmacokinetic model (i.e. precisely estimated parameters) data from a minimum of 150-200 blood samples were required. It was anticipated that the required number of samples would be obtained from 15 adults and 5 children in total.

9.2.1 Inclusion Criteria

1. All ECMO patients treated with oseltamivir therapy for suspected or confirmed H1N1 infection.
2. Provision of informed consent from relative/carer or parent.

9.2.2 Exclusion Criteria

1. Any clinically significant medical condition or abnormality, which, in the opinion of the investigator, might compromise the safety of the patient.

9.3 Treatments

9.3.1 Treatments administered

Each patient received 75 mg twice daily oral oseltamivir. Once recuited into this study, the medication was prepared for dosing by the critical care nurse using the oseltamivir capsules. The capsules are opened and dissolved in water and administered through the nasogastric tube using an enteral dosing syringe. The actual dose and time of dosing was recorded on a study specific blood sampling log.

9.3.2 Concomitant Therapy

For each patient, current drug therapy on study enrolment and any changes to treatment during the course of the study were recorded on the case report form.

9.4 Pharmacokinetic variables

The primary objective of the study was to determine the pharmacokinetic profile of oseltamivir in ECMO patients receiving multiple dose treatment.

The primary variables for this determination were the following PK parameters of the parent drug oseltamivir and its major metabolite oseltamivir carboxylate:

Primary pharmacokinetic parameters:
Oseltamivir:

- Absorption Function
- Clearance (CLM),
- Volume of distribution (V)

Oseltamivir Carboxylate:

- Clearance (CLO)
- Volume of distribution (VM)

The following PK parameters were also determined but were considered as secondary variables.

Secondary pharmacokinetic parameters (oseltamivir and oseltamivir carboxylate):

- Time to maximum concentration (Tmax),
- Maximum plasma concentration (Cmax)
- Area under plasma concentration time curve (AUC)
- Half-life ($t_{1/2}$)

9.5 Drug Concentration Measurements

Blood sampling was carried out the following time points:

pre-dose and at 0.5, 1 ,2 3, 5, 7, 9, and 12 hours post dose on Day 1 and Day 5 of ECMO

Since this was a population pharmacokinetic design, a sample at each time point was not insisted upon. Samples were taken when possible, normal clinical duties and procedures permitting.

Following each time point blood samples (2ml for adults, 1ml for children) were collected in EDTA/Sodium Fluoride collection tubes. The tube was inverted 8-10 times to ensure the anticoagulant was well mixed with the blood. Samples were then either centrifuged immediately or stored in a refrigerator (2-8^{o}C) for a maximum of 1 hour before centrifuging. Samples were centrifuged at 4°C and 1500g for 10 minutes. The supernatant plasma was then transferred to a storage tube (labelled with Subject ID, Sample Number, Collection time), before storing at –20°C prior to analysis.

Samples were shipped from the study sites in a single batch on dry ice with a temperature logger to ensure temperature remained below -20^{o}C. A total of 186 samples were received at the laboratory. In all cases, the samples were received at PRA International Laboratories (Netherlands) frozen and in good condition and were stored, after receipt, at -80°C.

Analysis was done using a highly sensitive and robust LC-MS/MS method that was developed and validated for the routine determination of oseltamivir and oseltamivir carboxylate. The full analytical and validation reports are stored in the Sponsors file - a copy can be found in Appendix .

Laboratory data were received by the pharmacokineticist (HM) on an Excel spread sheet from PRA, these data were entered and reconciled before performing the PK modelling.

9.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1 Statistical and Analytical Plans

The primary analyses for the final analysis were to be performed using NONMEM v7.2 (Icon Development Solutions). User written ordinary differential equation models were to be implemented. Post processing of NONMEM output was to be undertaken with the statistical package, R v2.13.0.

Descriptive statistics (number of patients, arithmetic mean, SD, minimum, median and maximum) were to be calculated for demographic continuous variables and the number and percentage of patients in each category were to be presented for categorical variables for each age group and all patients.
For the PK analysis, statistical significance was to be declared at the 5% level (two sided).

9.7.1.1 Analysis Populations

The primary population was the PK population. This was to include those patients who had at least one successful blood sample taken and satisfactorily completed the study without significant

protocol deviation/violations that were likely to affect the determination of the pharmacokinetic parameters. This population was to be used to analyse the primary variable (PK data) and summarise all relevant PK results.

Any deviations from the protocol were to be assessed and documented on a case-by-case basis. Patients with deviations that were considered to be likely to have a serious impact on the PK results were to be excluded from the PK population. The precise reasons for excluding patients from the PK population were to be fully defined and documented before commencing the statistical analysis.

9.7.1.2 Pharmacokinetic analysis

A population-PK approach was to be applied to the analysis of the data. In the population approach, all data from different individuals are fitted simultaneously using a non-linear, mixed effects modelling approach and *post hoc* individual kinetic parameters can be calculated with as few samples as one per individual.

Oseltamivir and oseltamivir carboxylate were to be evaluated and characterised. These data were transferred from Excel to comma delimited files and used for analyses in NONMEM.

Primary parameters of interest were Systemic Input Function, formation/elimination clearance and volume of distribution (Ka/Rin/Dn, CL/Fm and V/Fm, respectively). The number of model parameters depended on the complexity of the model under investigation. Other parameters of secondary interest including Tmax, Cmax, AUC and Elimination half-life ($t_{1/2}$) were to be calculated once an appropriate model had been selected.

Preliminary analyses were to involve characterising the PK profile using all the data collected. Both single and multi-compartment models were to be investigated and the parameters of these models were to be estimated using the NONMEM software. Nonlinear mixed models take the general form of y=f(x;b)=b0+b0*b1*x1+…, where x is vector of independent variables, b is vector of model parameters). By definition the model parameters are either fixed (theta(θ)) or random (eta(η)/epsilon(ε)) effects. In a general format of the drug concentration versus time curve, the concentration Ct will be related to time (t) by $Ct = D/V \exp^{(-CL/V)*t}$ where D=drug dose, CL=clearance and V=volume of distribution. Both CL and V are fixed effects.

In this investigation the fixed effects would be the primary parameters of interest. The actual parameters estimated, systemic input function, formation/elimination clearance and volume of distribution (Ka/Rin/Dn, CL/Fm and V/Fm, respectively) would be dependent on the model under investigation. There are in addition two Random components. The first random effect component is 'between patient' variation (assumed to be log-normally distributed) and this estimate (η) quantified the amount of 'biological variation' associated with each of the fixed effect parameters (eg: lnCLi= θ CL + ηCL where θ CL is the population mean and ηCL is the between patient variability). The second random component is 'within patient' variability (assumed to be normally distributed) and this estimate (ε) quantified the amount of residual error or outstanding unexplained 'noise'. The models used here investigated both additive and proportional error structures. The NONMEM output identifies between patient variability as Omega (ω^2) and within patient variability as Sigma (σ^2).

A number of model fitting algorithms are available within NONMEM VII, including first order conditional estimation (FOCE), stochastic approximation expectation maximisation (SAEM) and full Markov Chain Monte Carlo (MCMC) Bayesian Analysis methods. The method(s) of choice would be dependent on the nature of the data and complexity of the model. It was planned that if

appropriate more than one estimation method, i.e. a composite analysis, could be required to define the optimum model. Nevertheless, the most appropriate model which best explains the data would be selected. This involved both statistical and graphical methods but ultimately the most parsimonious model which best explains the data was to be selected.

Comparisons were to be based on improvements in model fitting. For example with FOCE, the objective function (-2 × LogL) details the amount of variation explained in the model. If there is an increase of more than 7.88 when two models are compared then this will be considered to be a statistically significant ($p<0.05$) and relevant change. With Markov Chain Monte Carlo Bayesian analysis, full and reduced models will be evaluated by comparing the 95% confidence intervals.

However, other selection criteria were also to be taken into consideration when evaluating models and graphical methods such as diagnostic 'goodness of fit' plots might indicate potential biasing problems with particular models.

'Structural' fixed effects parameters were to be evaluated with plots of drug concentration values (DV) vs predicted concentration values (IPRED-individual prediction, PRED-population prediction values). Points would be uniformly distributed along the line of identity if the model adequately explains the data.

Unexplained 'random' variability was to be investigated using diagnostic residual plots. Population predicted values (PRED) were to be plotted against both residual (RES) and conditional weighted residuals (CWRES). Unaccounted heterogeneity may be explained in displays with particular shape and form.

Once an appropriate model was selected, the primary parameter estimates along with estimates of precision (%SE) were to be given. Population fixed effect estimates as well as 'Between patient' estimates of variability were to be given. Results from other models were to be summarised. In addition, other secondary PK parameters such as Tmax, Cmax, AUC and half-life ($t_{1/2}$) may then be derived individually for each patient from the primary model parameters and then summarised and displayed.

After selection of an appropriate primary model an assessment of other potentially influencing patient characteristics was to be performed. The initial analysis was to include e.g. patient age and body weight as continuous covariates and sex as a dichotomous covariate. Subsequent analyses were to involve backward selection of covariates by removing each in turn from the model and evaluating the influence on the model fit. Only significant covariates were to be retained in the final selected model (eg: $\ln CLli=\mu CL +b1*Ageib2 +b3*Body\ weighti+ b4*Sexi + \eta CL$).

The covariates were to be plotted against the estimated random effects (η) for each of the fixed effects (Ka/Rin/Dn, CL/F, V/F) both using the primary selected model parameter estimates and the covariate model selected parameter estimates in order to evaluate obvious trends within the covariates of interest.

After selection of the most appropriate covariate model a final assessment of model appropriateness was to be undertaken using visual predictive checks and / or posterior predictive checks.

9.7.2 Determination of Sample Size

The sample size is a balance between pragmatism (recruitment rate in the next H1N1 flu season) and the need to develop a model of oseltamivir and oseltamivir carboxylate pharmacokinetics in ECMO patients. The model requires parameters (clearance, volume of distribution, area under the curve) to be estimated precisely with tight confidence intervals. Our assumption is that on average 16 blood samples will be collected from each adult participant and 8 blood samples from each child participant (although neonates will only have 2 samples taken). This will provide up to 280 oseltamivir plasma level data points and given this assumption and previous experience of modelling such data, unbiased estimates of pharmacokinetic parameters and their variability in the population should be possible.

9.8 Changes in the Conduct of the Study

9.8.1 Decision to not to recruit children into the study.

Recruitment into the study of adult subjects occurred in the winter of 2010/2011 (December – January). Due to the considerably reduced numbers of children diagnosed with severe swine flu infection in the UK, it became apparent that children with suspected or confirmed swine flu were not being referred to the ECMO unit during this period. Thus a decision was made by the PI that the study would proceed without children.

10. STUDY PATIENTS

10.1 Disposition of Patients

A total of 14 adult patients (male and female) were screened and entered the study between 27 November 2010 and 18th January 2011.

The last patient completed the study on 22nd January 2011 and since no more referrals to the ECMO unit were anticipated, the study was closed.

10.2 Protocol Deviations

Due to the emergency nature of the treatment (and the lag in relatives/carers arriving to Glenfield Hospital from referring hospital), written consent for 4 subjects was taken after the taking of blood samples had already begun.

Subject 008 - samples taken 15th December 2010 and assent received 17th December 2010
Subject 010 - samples taken 19th December 2010 and assent received 23rd December 2010
Subject 012 - samples taken 29th December 2010 and assent received 7th January 2011-12-01
Subject 014 - samples taken 18th January 2011 and assent received 20th January 2011.

Consent for subject 007 was taken collectively on a University Hospitals of Leicester surgical procedure consent form rather than the study specific consent form. The reason for this was that the investigator did not have the study specific consent form at hand. However, the assent process was also documented in the medical notes.

Although these were breaches of the protocol, in the context of the emergency nature of ECMO treatment, it was not considered that rights, safety and well being of the subject were compromised. Therefore, data from these subjects was not excluded from the PK analysis.

Subject 009 and 011 had PK samples collected on Day 1 of ECMO only since they were decannulated from ECMO before Day 5 sampling. These subjects were also not excluded from the PK analysis.

11. PHARMACOKINETIC EVALUATION

11.1 Data Sets Analysed

The PK population:
The primary population was the PK population. This included those patients who had at least one successful blood sample taken and satisfactorily completed the study without significant protocol deviation/violations that were likely to affect the determination of the pharmacokinetic parameters. This population was used to analyse the primary variable (PK data) and summarise all relevant PK results.

There was no major protocol violations that could affect the primary outcome measures. Therefore all 14 subjects who provided blood samples have been included in the PK population.

Table 11.1 summarises the subject disposition and patient populations.

Table 11.1: Subject disposition and patient populations

	Adults (mean [range])	**Child (aged 0 – 18 years)**
All enrolled	14	0
Age	38.8 (27 – 57) years	-
Weight	97.1 (65 – 121) years	-
Sex (M/F)	8/6	-
Serum Creatinine (μmol/L)	99.7 (36 .0 – 250)	
Creatinine Clearance (ml/min)*	147 (19 – 358)	
Number of blood samples (range)	13.3 (6 – 16)	-
Early withdrawal	2 subjects	-
Primary reason for withdrawal	Decannulation from ECMO	-

*Calculated using the Modified Jelliffe Formula

As discussed in Section 10.1 only adults were recruited into the study; no child during the 2010/2011 flu season was referred to the ECMO unit with suspected or confirmed H1N1 infection. All adult ECMO patients were defined as suspected or confirmed H1N1 associated respiratory failure. Subject 002 was 27 weeks pregnant at the time of cannulation. Subject 006 had been pregnant and had delivered by emergency C-section just prior to admission to the ECMO unit. Subject 004 had Grave's disease which had been treated, subject 009 had bronchial asthma. The

remaining subjects had no known associated risk factors, co-morbidities or significant medical histories.

11.2. Concomitant Medication during the Study

Concomitant medication was defined as all medications which started before, at the same time or after the initiation of oseltamivir and continued during treatment. A wide variety of drugs were administered to the patients, ranging from antibiotics, antifungals, analgesics, inotropes and sedatives. All medications were considered for any potential or theoretical interactions with oseltamivir and included in the PK dataset for exploration as covariates in the PK model.

11.3 Measurements of Treatment Compliance

Apart from subject 003, all other subjects were already being administered oseltamivir from their referring hospital. The number of days that subjects had been on oseltamivir treatment prior to recruitment into this study ranged from 3 to 12 days. The doses prescribed were either 75mg or 150mg twice daily.

Once recuited into this study, the medication was prepared for dosing by the critical care nurse using the oseltamivir capsules. The capsules are opened and dissolved in water and administered through the nasogastric tube using an enteral dosing syringe. The actual dose and time of dosing was recorded don study specific blood sampling log. All patients received the protocol stipulated dose of 75mg twice daily.

11.4 Pharmacokinetic Results

11.4.1 Analysis of pharmacokinetics of Oseltamivir and Oseltamivir Carboxylate

Fourteen patients aged from 27 to 57 years provided blood samples at protocol specified sampling times following dosing with oseltamivir phosphate 75mg twice daily. However, two subjects had samples from Day 1 of ECMO only, a consequence of early decannulation from ECMO. Although 9 sampling time points were scheduled for both Day 1 and 5 of ECMO (18 sampling time points in total), samples were not able to be taken at every time point due to overriding clinical considerations. Hence, the mean (range) number of samples, 13.3 (6-16), was lower than scheduled.

A total of 186 samples were available for evaluation of the pharmacokinetic profiles for oseltamivir and oseltamivir carboxylate. The blood samples were obtained from baseline (pre-dose) up to 13 hours post dosing. The ‘Time after dose’ versus plasma concentration profiles for both analytes is shown below (Figures 11.4.1.1 – 11.4.1.4).

Figure 11.4.1.1 Plasma Oseltamivir Concentrations versus Time after Dose, Day 1 of ECMO

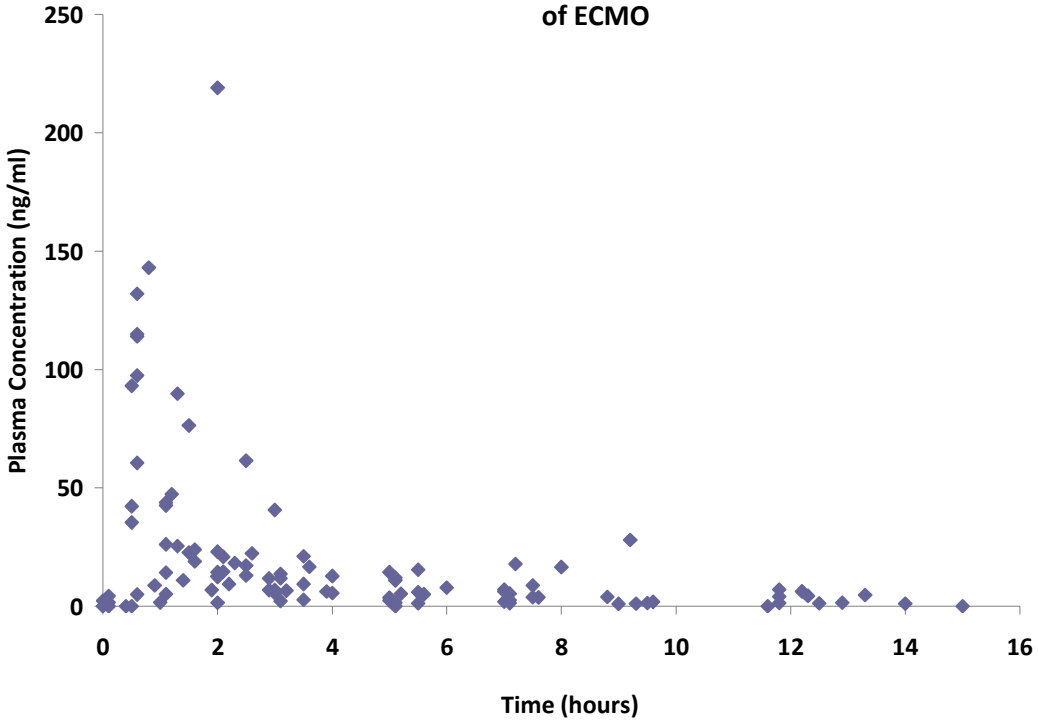


Figure 11.4.1.2 Plasma Oseltamivir Concentrations versus Time after Dose, Day 5 of ECMO

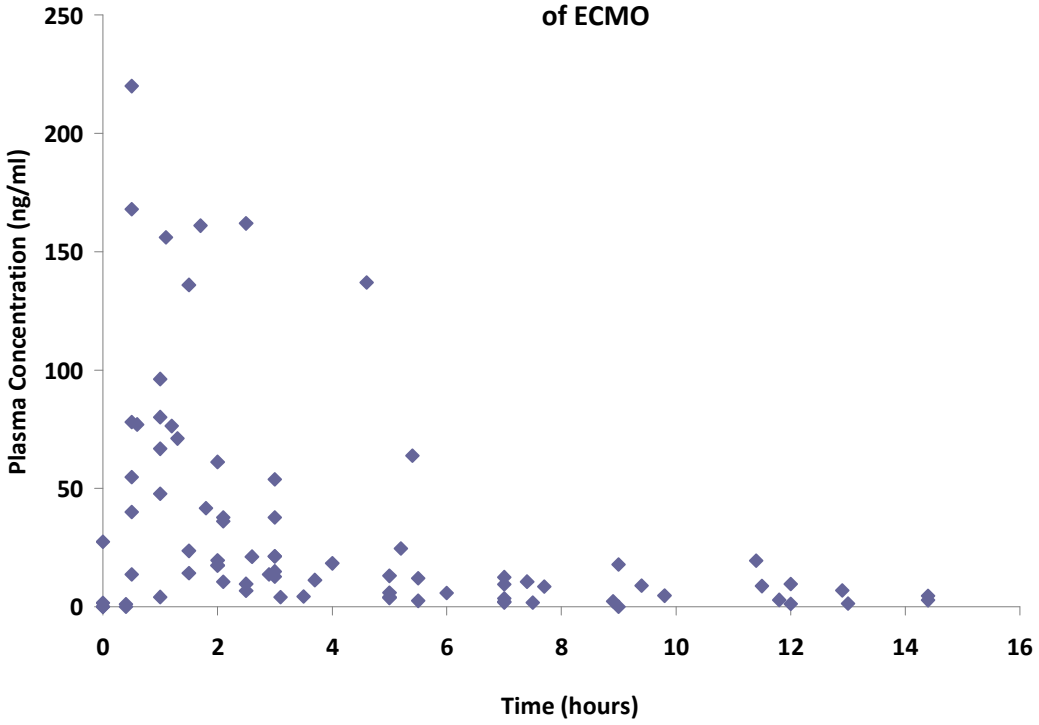


Figure 11.4.1.3 Plasma Oseltamivir Carboxylate Concentrations versus Time after Dose, Day 1 of ECMO

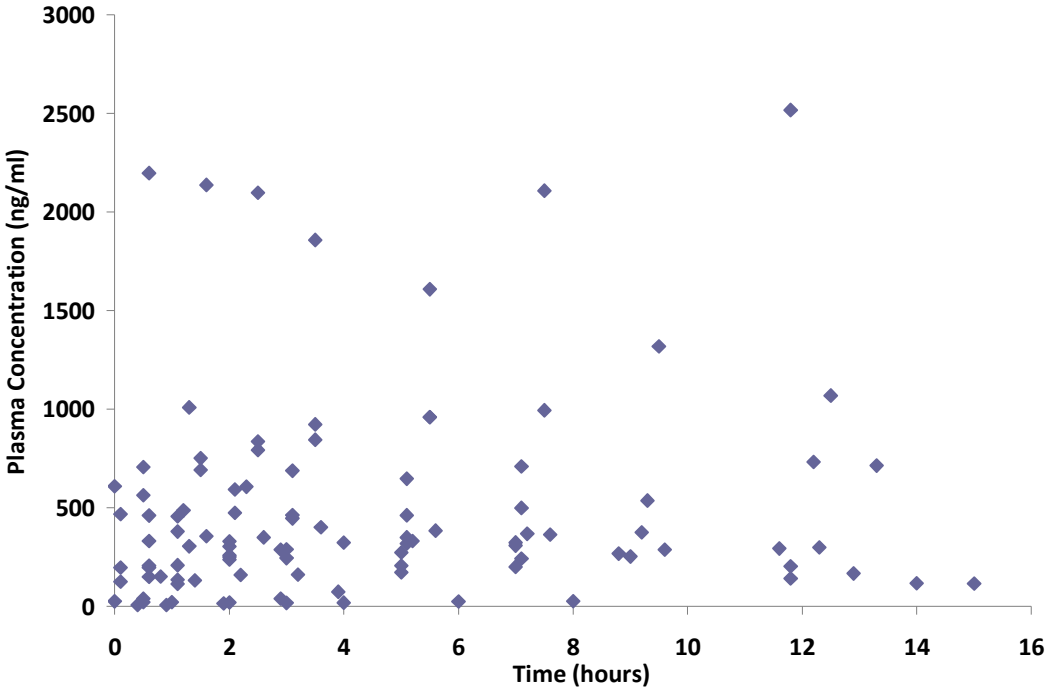
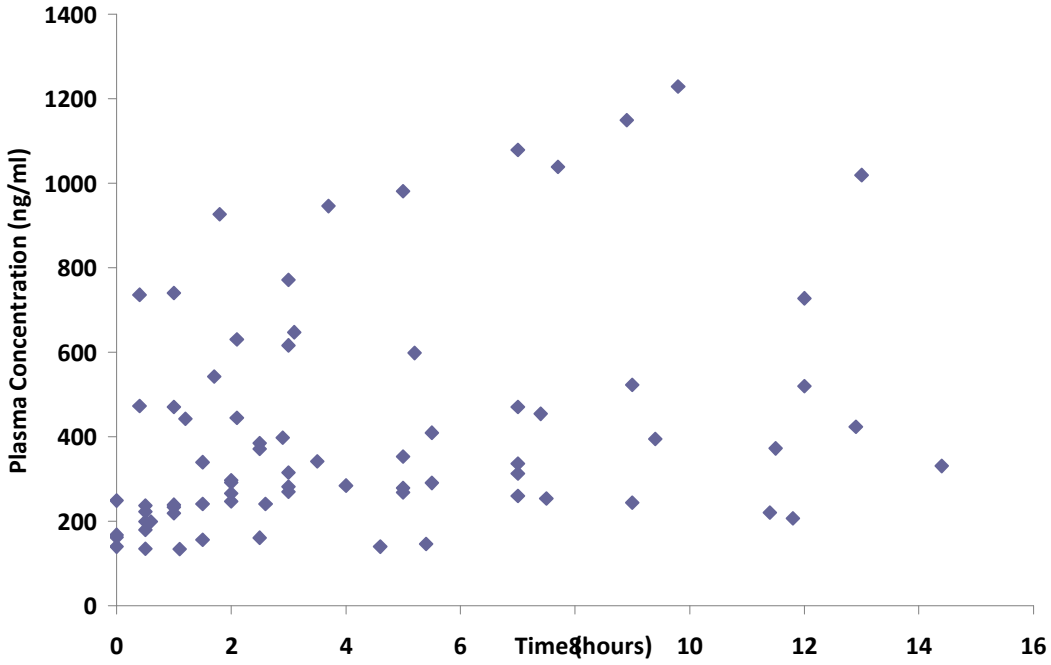


Figure 11.4.1.4 Plasma Oseltamivir Carboxylate Concentrations versus Time after Dose, Day 5 of ECMO



The PK analyses of oseltamivir and oseltamivir carboxylate were to be conducted simultaneously using a single integral model and similar to that that reported by Rayner et al (2008). The PK parameter estimates from the final selected PK model are summarised in Table 11.4.1.1 below.

The parent oseltamivir (prodrug) is rapidly hydrolysed by plasma, liver and gut esterases, so that following oral dosing the majority of the dose reaches the systemic circulation as oseltamivir carboxylate (active drug). Thus the best structural pharmacokinetic model, as adjudged by the improvement in OFV, diagnostic plots (OBS vs PRED, OBS vs IPRED, CWRES vs Time/PRED, Absolute IWRES versus Time/PRED) and reasonable precision of parameter estimates, was one that encompassed first order drug absorption from the dosing compartment, with a fraction F absorbed as the parent and a fraction 1-F absorbed as the metabolite. The latter accounted for first pass metabolism of oseltamivir to oseltamivir carboxylate. The parent (oseltamivir) profile was described using a two compartment model with the elimination of oseltamivir to oseltamivir caboxylate. Since there is a delay in the appearance of the oseltamivir carboxylate in the systemic circulation, a metabolism compartment was introduced to account for the delay in the disappearance of oseltamivir and the reappearance of the molecule as oseltamivir carboxylate. The disposition of oseltamivir carboxylate was best described using a 1 compartment model (Figure 11.4.1.5).

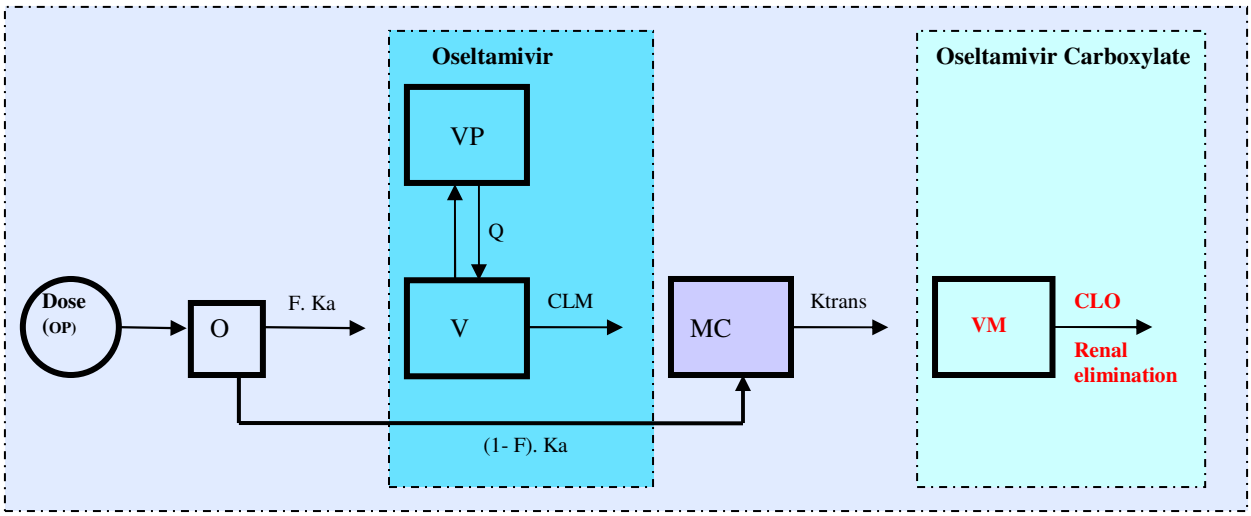


Figure 11.4.1.5: Structural Pharmacokinetic Model

Dose is the oral dose of oseltamivir phosphate (OP) administered, O is the parent drug (oseltamivir) available for absorption in the gut, Ka is the first order absorption rate constant, F is fraction of parent drug escaping first pass metabolism and hence absorbed into the systemic circulation; 1-F is the fraction of parent drug undergoing first pass metabolism. V and VP are parameters describing the central and peripheral volume of distribution of oseltamivir whilst VM, is the volume of distribution parameter for oseltamivir carboxylate. CLM is the clearance of oseltamivir to oseltamivir carboxylate, Q is the intercompartmental clearance of oseltamivir and CLO is the renal clearance of oseltamivir carboxylate. MC is the metabolic compartment and K_{trans} is the first order rate constant for transit of oseltamivir carboxylate.
A number of assumptions were made during model development. First, since esterases are ubiquitous in the human body and in literature reports less than 5% of oseltamivir has been recovered in urine it was assumed in the model that oseltamivir is fully converted to oseltamivir carboxylate (i.e. no clearance of unchanged drug). Second, since only one parameter of either F/1-F or V/VM is identifiable in the model, it was decided to fix F to 0.25 in the model, based on previous literature reports. That is to say, the model assumed that 75% of the absorbed dose

underwent first pass metabolism to the metabolite (oseltamivir carboxylate) and 25% was absorbed as unchanged drug, oseltamivir (2,3).

After the structural base model had been established, the contributions of various discrete and continuous factors were evaluated on the model's ability to describe the observed data.
In order to identify possible trends, the selected base model was used to generate covariate plots of the between subject random effects (etas) for each of the fixed effect parameters V,VP,CLM,Q CLO. This clearly suggested a correlation of CLO with creatinine clearance (CRCL) and serum creatinine (CR), but correlation with other covariates was highly unlikely (Appendix I, Figures 13-15). These two covariates were introduced into the model as power functions. Both CRCL and CR were found to be statistically significant with a decrease in the maximum likelihood objective function, MOF > 3.84 ($p<0.05$). However, since CRCL and CR are significantly correlated ($r^2=0.76$, Appendix 1, Figure7), only one covariate effect on CLO was to be selected. This was on the basis of the covariate that produced the highest statistically significant effect as well as a model that converged without difficulties and parameter estimates that were precise (i.e. 95% confidence intervals did not include zero). This turned out to be CR. Thus, in the final covariate model the population estimate of the elimination clearance of oseltamivir carboxylate was shown to decrease non-linearly with serum creatinine. Figure 13 (Appendix I) shows that the clearance of oseltamivr carboxylate reduces by >50 % when serum creatinine increases to >150 μmol/L. The PK parameter estimates from the final selected PK model are summarised in Table 11.4.1.1 .

The goodness-of-fit (GOF) diagnostic plots for the final population PK model are shown in Appendix 1. There was no apparent bias in these diagnostic plots, suggesting that the model was adequate in describing parent and metabolite plasma profiles in the study population.

11.4.2 Visual Predictive Checks

The ability of the final covariate model to simulate (and hence describe) the observed data is illustrated by the Visual Predictive Check (see Figures 16-17, Appendix I). The final covariate model was used to simulate 2000 times the predicted plasma concentrations of each oseltamivir and oseltamivir carboxylate in the study population.

The oseltamivir VPC plot shows that overall, the model predicted median, 5^{th} and 95^{th} percentiles 'capture and envelope' the observed data reasonably well though perhaps the 95^{th} percentile around Tmax is slightly wider than the observations suggest. The predictive capability of the final is supported since 89% of the observed concentrations were within the 90% prediction interval of the model-predicted concentration range in the population.

The oseltamivir carboxylate VPC plot shows that the model predicted median, 5^{th} and 95^{th} percentile 'capture and envelope' majority of the data reasonably well with 91% of the observed data lying within the 90% prediction interval. However, very high plasma concentrations were observed with one subject (ID 13) on Day 1 of ECMO that appear to be an outlier and are not explained by the model. This subject had 48hours pre-ECMO exposure to oseltamivir and was admitted with renal dysfunction. Data on renal function prior to ECMO was not available and therefore the estimate of variability of oseltamivir carboxylate PK parameters may be underestimated and imprecise.

11.4.3 Secondary Parameter Derivation

The half-life of oseltamivir and oseltamivir carboxylate were calculated for each subject from the individual empirical Bayes estimates of model parameters from the final covariate model. These are summarised in Table 11.4.1.1. For oseltamivir the median (SD) $t_{1/2\alpha}$ was 0.23 (0.14), $t_{1/2\beta}$ was 5.63

(4.95) hours. The median (range) elimination half-lives for oseltamivir carboxylate was 8.3 (2.5 - 247) hours.

Other secondary PK parameters including Tmax and Cmax were derived for each individual by using their empirical Bayes estimates of primary PK parameters to simulate plasma concentration time profiles on a fine time grid. The simulated population (i.e. demography, sample size) was the same as the clinical study dataset.

The distribution of AUC and Cmax for oeltamivir and oseltamivir carboxylate is shown in Table 11.4.3.1 -2. The mean (range) AUC and Cmax for oseltamivir was 223 (43 - 782) ng.h/ml and 545 (6.9 - 5034) ng/ml respectively, and reveals high between subject variability (18-fold difference in AUC). For oseltamivir carboxylate, the median (range) AUC and Cmax were 5371 (644 - 13660) ng.h/ml and 557 (54 - 1277) ng/ml respectively and again shows significantly between subject variability (twenty-one and twenty-four fold respectively).

Plots of oseltamivir carboxylate AUC and Cmax versus CR suggest a strong relationship (r^2= 0.37 and 0.29 respectively, Figures 18-19, Appendix I). This is explained by the effect of CR on metabolite clearance (CLO) in the model, as identified during the covariate analysis.

11.4.4 Pharmacokinetic-Safety Assessment

There were no safety concerns attributable to oseltamivir raised during the conduct of the study. No exploration of the relationship between oseltamivir and oseltamivir carboxylate with adverse effects or vital signs was necessary or undertaken.

Table 11.4.1.1.1: Population Pharmacokinetic Parameters of the analytes from the Final Covariate Model

Population PK Parameters (n=14)* [a]	**Mean**	**BSV (%)** [b] ***BOV (%)***	**Eta Shrinkage (%)**
Oseltamivir			
KA (per hour)	1.39 (12.3)	15.6 (195)	
V/F (L)	59.7 (7.4)	94.6 (40.6) *88.9 (30.7)*	2.93
CLM/F (L/h)	97.5 (4.3)	69.9 (37.4)	1.94
Q1/F (L/h)	42.1 (10.7)	129 (46.1)	11.7
VP/F (L)	176 (4.7)	64.7 (56.1)	21.9
Bioavailability	0.25 FIX	NE	
Alpha Half-Life (hr) [c,d]	0.23 (0.14)	NE	
Beta Half-Life (hr) [c,d]	5.63 (4.95)	NE	
Oseltamivir Carboxylate			
V_M/F (L)	350 (3.94)	37.9 (81.9) *117.4 (26.8)*	21.3
CLO/F (L/h)[e]	1274 (12.8) x $CR^{-1.03\ (20.7)}$	34.8 (45.0)	10.6
K_{trans} (metabolic compartment)[f,g]	0.46 (56.0)	68.4 (106)	
Bioavailability	0.75 fixed	NE	
Half-Life (hr)[c,d]	36.4 (66.5)	NE	
Residual Error Models	*Oseltamivir*	*Oseltamivir Carboxylate*	
Additive Error (nM)	0.24 (261)	57.6 (40.2)	
Proportional Error (%CV	0.32 (14.7)	0.05 (13.6)	

[a] All figures in parentheses are parameter precision and are expressed as percent relative standard error (100% x SE/Parameter Estimate)
[b] BSV / BOV = Between Subject / Between Occasion Variability and is calculated as $(variance)^{1/2}$*100%
[c] Derived from each individuals empirical Bayes estimates of model parameters
[d] Arithmetic mean (sd)
[e] CR = Serum Creatinine (µmol/L).
[f] First order rate constants for transit from metabolic compartment.
[g] Mean transit times (hours) =(n + 1)/K_{trans} = 4.3 hours
NE= not estimated

Table 11.4.3.1 Secondary Pharmacokinetic Parameter Summary for Oseltamivir Day 5 of ECMO

Statistic (N=14)	AUC_{0-12} (ng/ml.h)	Cmax (ng/ml)	Tmax (h)
Mean	222.9	545.14	0.73
SD	182.7	1417.27	0.63
CV (%)	82.0	260.0	86.3
Median	170.4	111.00	0.50
Minimum	43.3	6.93	0.25
Maximum	781.9	5033.70	2.50

SD:Standard Deviation; CV:Coefficient of Variation

Table 11.4.3.2 Secondary Pharmacokinetic Parameter Summary for Oseltamivir Carboxylate Day 5 of ECMO

Statistic (N=14)	AUC_{0-12} hrs (ng/ml.h)	Cmin (ng/ml)	Cmax (ng/ml)	Tmax (h)
Mean	5371	483	557	4.96
SD	3609	336	332	1.95
CV (%)	67.2	69.6	59.5	39.3
Median	4346	322	509	4.50
Minimum	644	146	54.4	2.50
Maximum	13660	1230	1277	9.00

SD:Standard Deviation; CV:Coefficient of Variation

12 DISCUSSION AND OVERALL CONCLUSIONS

Oseltamivir is a neuraminidase (NA) inhibitor which is licensed for the prophylaxis and treatment of influenza. It is a potent and selective inhibitor of influenza A NA subtypes. It is the only orally available neuraminidase inhibitor and it is strongly recommended by WHO for cases of suspected or confirmed H1N1 infection. It is a pro-drug and requires conversion to oseltamivir carboxylate to the deliver the antiviral effect. The licensed dose of 75mg twice daily has been shown to achieve plasma concentrations that exceed the IC50 of H1N1 1000-fold, and has also shown to be effective in treating uncomplicated acute H1N1 influenza in adults.

ECMO provides life support to critically ill patients. It has previously been reported that ECMO can alter pharmacokinetics as a consequence of the expanded circulating volume and interaction of drugs with the polymeric components of the circuit and oxygenator. However, to date there are no reports of a pharmacokinetic investigation of oral oseltamivir in patients supported on ECMO and whether the licensed doses provide therapeutic levels of the active metabolite. Thus, this population pharmacokinetic study aimed to determine whether subjects receiving ECMO support achieved therapeutic levels of the active metabolite, oseltamivir carboxylate, for treating H1N1 infection with the licensed oral dose of 75mg twice daily.

Data on plasma concentration profiles of oseltamivir and oseltamivir carboxylate from 14 adults with suspected or confirmed H1N1 infection was modelled simultaneously using an integral multi-compartment model. The profiles reflected considerable variability in the systemic exposure to oseltamivir and oseltamivir carboxylate, and is probably a reflection of the variability in drug absorption in these critically ill patients.

The final pharmacokinetic model assumed that 75% of the absorbed dose underwent first pass metabolism to the metabolite (oseltamivir carboxylate) and 25% was absorbed as unchanged drug (oseltamivir). The parent, oseltamivir, profile was described using a two compartment model with the elimination of oseltamivir to oseltamivir caboxylate. Since there is a delay in the appearance of the oseltamivir carboxylate in the systemic circulation, a metabolism compartment was introduced to account for the delay in the disappearance of oseltamivir and the reappearance of the molecule as oseltamivir carboxylate. The median Tmax for oseltamivir carboxylate on Day 5 of ECMO was 4.5 hours, is not too dissimilar to that reported in healthy volunteers following multiple oral doses (2.7 – 3.9 hours) but a wide range was observed in this study (2.5 – 9 hours) reflecting delayed oseltamivir absorption following nasogastric administration in some subjects. The disposition of oseltamivir carboxylate was best described using a 1 compartment model.

The population mean estimate of oseltamivir clearance was 97.5 L/h, similar to a previous population PK report in non-critically ill subjects of 133 L/h. The median Cmax and AUC on Day 5 of ECMO (111 ng/ml and 170.4 ng/ml.h respectively) are also similar to previous reports following multiple 75mg twice daily dosing (65.2 ng/ml and 112 ng/ml.h respectively).

Only renal function was found to be a significant covariate affecting the elimination clearance of oseltamivir carboxylate. This is anticipated since oseltamivir carboxylate is not further metabolised but eliminated entirely by renal excretion. The population mean renal clearance of oseltamivir carboxylate for a patient with a normal serum creatinine of 88µmol/L was 12.7 L/h, lower than the previously reported value of 18.8 L/h in healthy adult subjects. Nevertheless, both these values exceed glomerular filtration rate (7.5 L/h) indicating that tubular secretion occurs in addition to glomerular filtration. Consequently, the oseltamivir carboxylate AUC on D5 was significantly correlated with serum creatinine (r^2=0.37). The median (range) estimate of metabolite steady state volume of distribution was 179 (61 – 13003) L, significantly greater than previously reported value of 23 to 26 L following intravenous administration of oseltamivir carboxylate in healthy adult volunteers. Indeed, Rayner et al (2008) in their population pharmacokinetic study of pooled oseltamivir PK data estimated a mean population value of 67L (4). However, Ariano et al (2010) in their study of oseltamivir carboxylate PK in critically ill, ventilated adult patients also estimated median (range) volume of distribution in patients with normal renal function to be 148 (89 – 234) L (5). As a consequence of the enlarged volume of distribution in critically ill adult ECMO patients, the estimated mean half-life of oseltamivir carboxylate of 36.4 hours is significantly longer than the 6-10 hours reported in previous studies.

The large variability in estimates of clearance and volume of distribution for both parent and metabolite largely reflect the underlying variability in oral bioavailability in this study population. Impaired drug absorption in critically ill and septic shock patients as consequence of decreased gut motility, impaired gut perfusion, oedema of the bowel wall has previously been reported (6). In addition, the considerably larger estimated steady state volume of distribution in this study population may also be a reflection of their critically ill nature. Critical illness has previously been shown to inflate the volume of distribution of aminoglycosides as a consequence of oedema, leaky capillary syndrome and third spacing. Furthermore, the ECMO circuit will necessarily expand the circulating volume.

Dosages explored in human experimental influenza studies and in phase III clinical trials were selected on the basis of exposure sufficient to have an antiviral effect *in vitro* and clinical efficacy in experimental influenza infection of laboratory animals. Clinical efficacy in humans was shown to be similar at both 75mg and 150mg twice daily in field trials of treatment of naturally acquired influenza. The drug was well tolerated in clinical testing and there was no relationship between drug exposure and adverse events or laboratory abnormalities at dosages up to 1000 mg/day. Table 12.1 below shows a comparison of the AUC and Cmax values of the active metabolite observed in phase III influenza field trials and healthy volunteers with those observed in adult ECMO subjects in this study. The 50% maximal inhibitory concentration (IC50) of oseltamivir carboxylate for H1N1 has been reported by laboratories to range from 0.09 – 0.186 ng/ml. Thus the minimum oseltamivir carboxylate plasma concentrations estimated in this study population are between 1000- to 5000- fold higher than the IC50 for H1N1.

In addition, a dose of 75mg twice daily results in steady state mean systemic exposures in ECMO patients that is in excess of those observed in non-ECMO patients and healthy volunteers. This is as a consequence of reduced renal elimination of the active metabolite in EMCO patients as discussed above. This would therefore suggest that the licensed dose of 75mg twice daily provides therapeutic levels of oseltmaivir carboxylate. However, it should be noted that there is considerable between subject variability in both AUC and Cmax in critically ill ECMO patients, reflecting the variability

in bioavailability and renal function. It may be prudent therefore to increase the dose in those subjects where enteral absorption maybe sub-optimal or convert to an intravenous neurodaminase inhibtor such as zanamivir.

Table 12.1 Comparison of Systemic Oseltamivir Carboxylate Exposure following Twice Daily Dosing

Dose	Ambulatory Adults*		ECMO		
	Day 7 Cmax (ng/ml)	Day 7 AUC (ng.h/ml)	Day 5 Cmin (ng/ml)	Day 5 Cmax (ng/ml)	Day 5 AUC (ng.h/ml)
75mg**	335	2976	483	557	5371
150mg***	786	6229			

*Similar active metabolite profiles have been observed following administration of oral oseltamivir in healthy volunteers and patients with experimentally induced or naturally acquired influenza (2).
**Midpoint of 50mg and 100mg dose taken from Table V in He et al (2).
***Midpoint of 100mg and 200mg dose taken from Table V in He et al (2).

9. CONCLUSIONS

In conclusion, an integral pharmacokinetic model developed for the oseltamivir and oseltamivir carboxylate in critically ill adult patients supported on ECMO, describes the pharmacokinetics of both analytes satisfactorily. The PK parameter estimates for oseltamivir are similar to previous reports. In contrast, the elimination clearance of oseltamivir carboxylate was reduced compared to healthy and ambulatory adult subjects. As with previous findings, oseltamivir carboxylate clearance was significantly influenced by renal function and hence systemic exposure (AUC and Cmax) correlated with serum creatinine. The steady state volume of distribution of oseltamivir caboxylate was substantially greater than previously reported in healthy adult volunteers, but comparable to a previous report in critically ill adults.

From a therapeutic perspective, mean systemic exposure of oseltamivir carboxylate following the administration of oral oseltamivir 75mg twice daily in adult ECMO patients is comparable to those in ambulatory patients and far in excess of concentrations required to maximally inhibit neuraminidase activity of the H1N1 virus. Therefore, dosage adjustment for ECMO *per se* is not necessary; however it may be necessary to increase doses in individuals with impaired enteral absorption or convert to an intravenous neurodaminase inhibtor such as zanamivir. Finally, as revealed in other patient populations, dosages could be reduced in those subjects with renal dysfunction.

12 REFERENCES

1. Wildschut ED, de Hoog M, Ahsman MJ, Tibboel1 DJ, Osterhaus ADME, Fraai PLA. Plasma Concentrations of Oseltamivir and Oseltamivir Carboxylate in Critically Ill Children on Extracorporeal Membrane Oxygenation Support. PLoS ONE 5(6): 1-3.

2. He G, Massarella J, Ward P. Clinical Pharmacokinetics of the Prodrug Oseltamivir and its Active metabolite Ro 64-0802. Clin Pharmacokinet 1999; 37(6): 471-484.

3. Davies BE. Pharmacokinetics of oseltamivir: an oral antiviral for the treatment and prophylaxis of influenza in diverse populations. J Antimicrob Chemother 2010; 65 Suppl 2: ii5–10

4. Rayner CR, Chanu P, Gieschke R, Boak LM, Jonsson EN. Population Pharmacokinetics of Oseltamivir When Coadministered With Probenecid. Journal of Clinical Pharmacology, 2008;48:935-947

5. Ariano RE, Sitar DS, Zelenitsky SA, Zarychanski R, Pisipati A, Ahern S, Kanji S, Rello J, Kumar A. Enteric absorption and pharmacokinetics of oseltamivir in critically ill patients with pandemic (H1N1) influenza. CMAJ • MARCH 9, 2010 • 182(4)

6. Power BM, Forbes AM, van Heerden PV, et al. Pharmacokinetics of drugs used in critically ill adults. Clin Pharmacokinet 1998; 34: 25-26.

APPENDIX I

Goodness of Fit plots for Oseltamivir

Figure 1

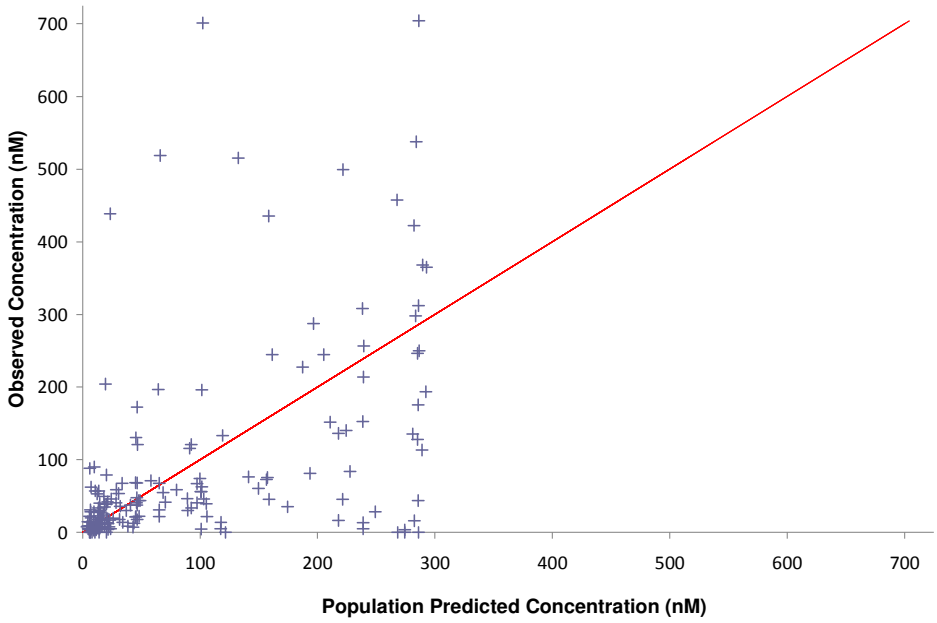


Figure 2

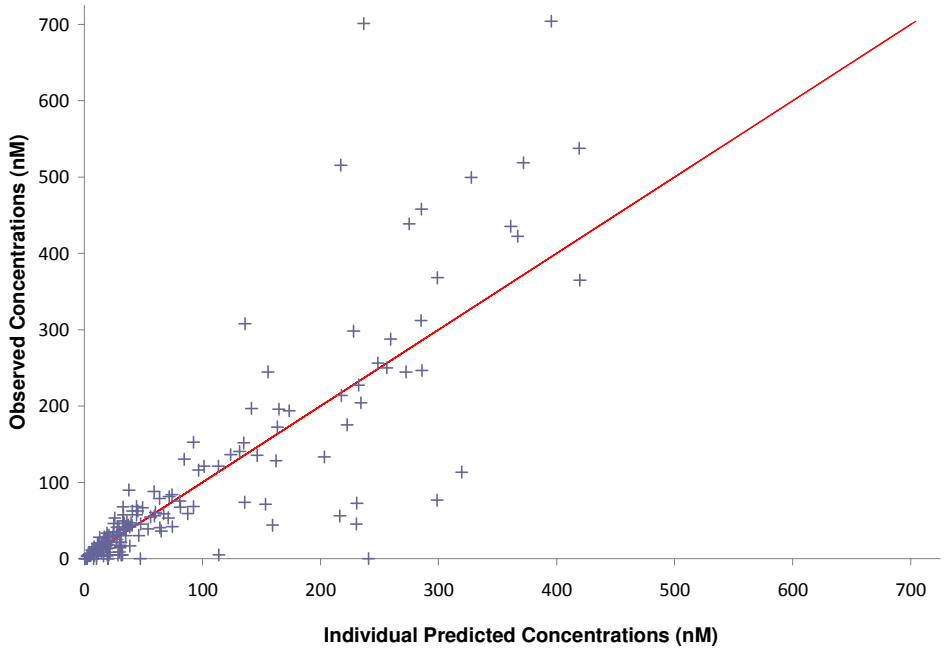


Figure 3

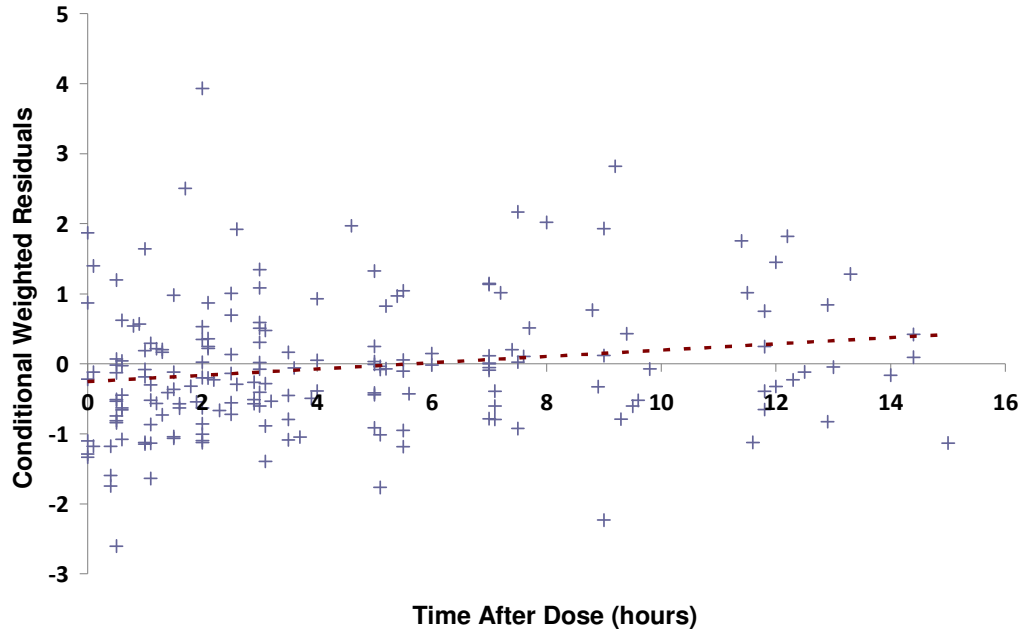


Figure 4

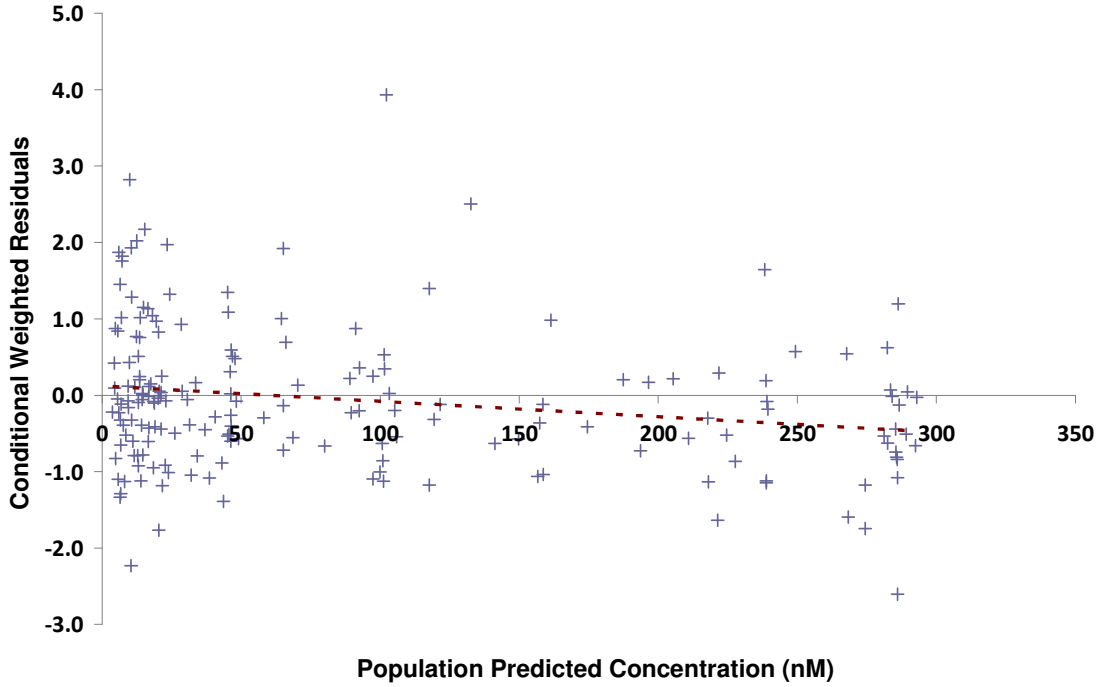
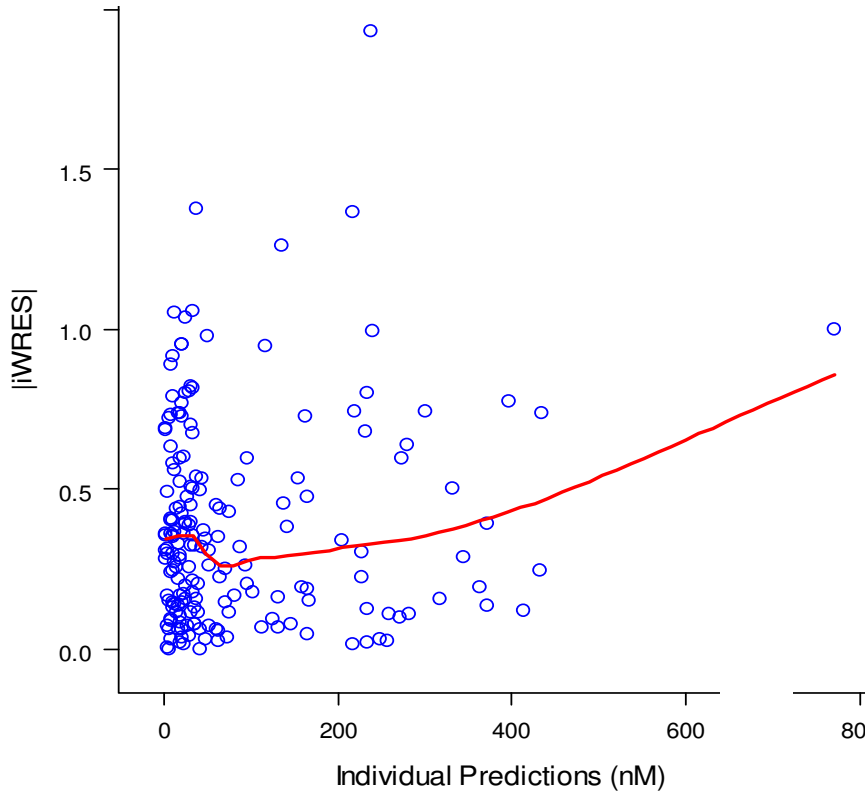


Figure 5



Goodness of fit plots for Oseltamivir Carboxylate

Figure 6

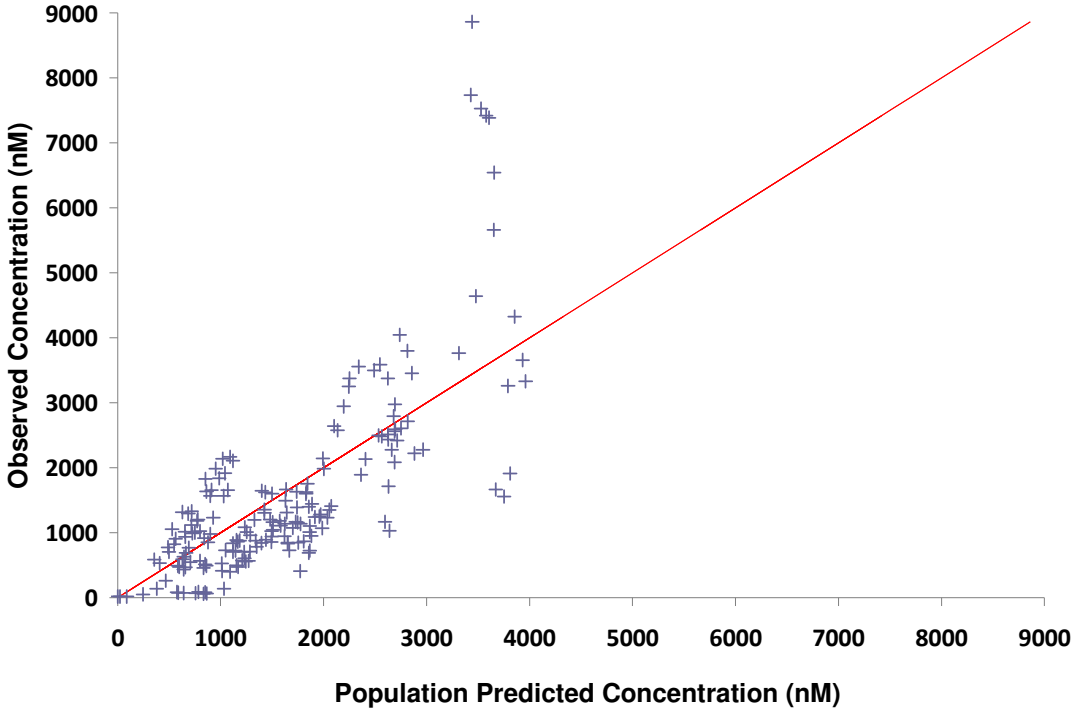


Figure 7

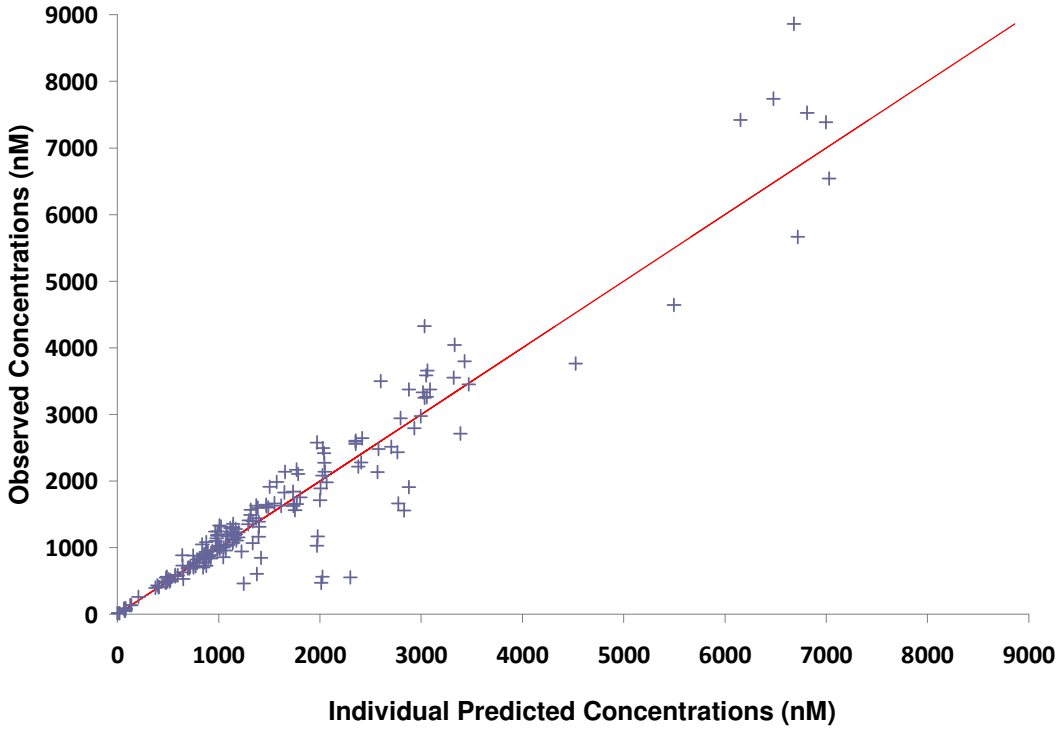


Figure 8

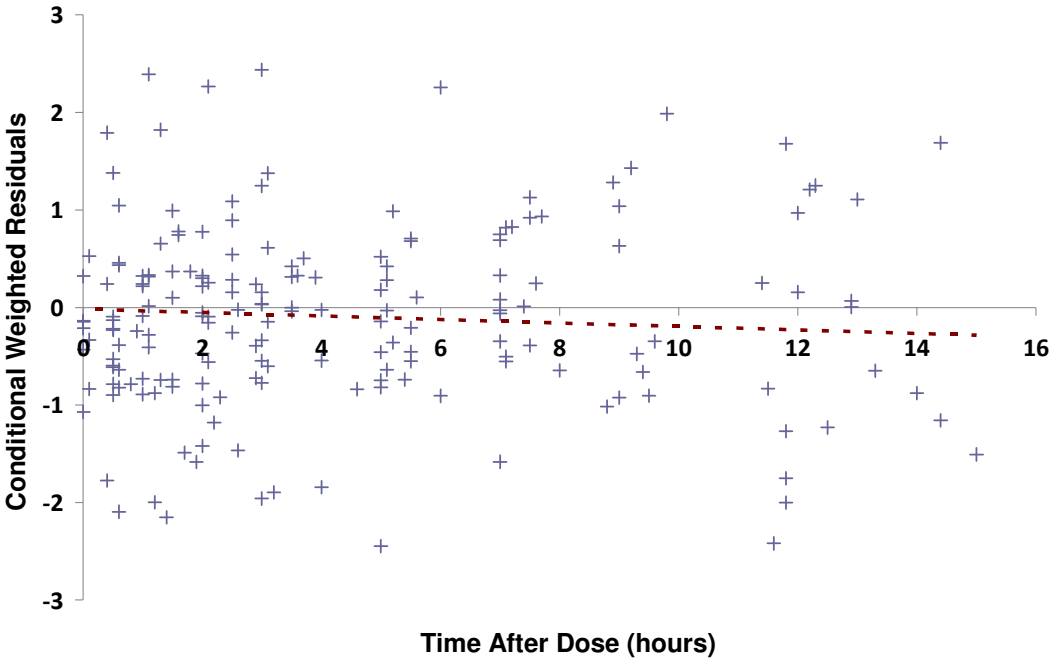


Figure 9

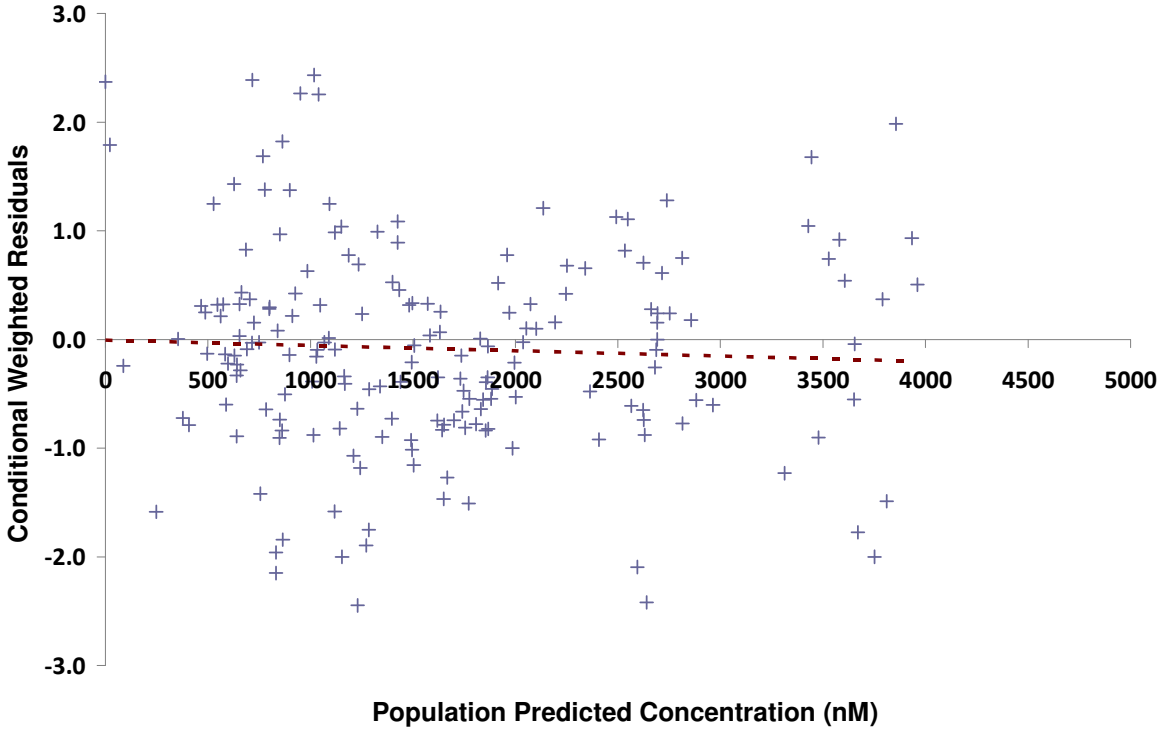


Figure 10

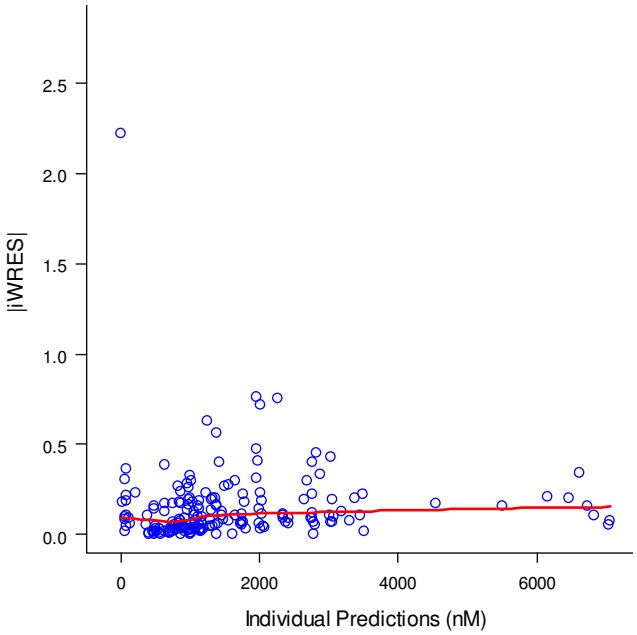
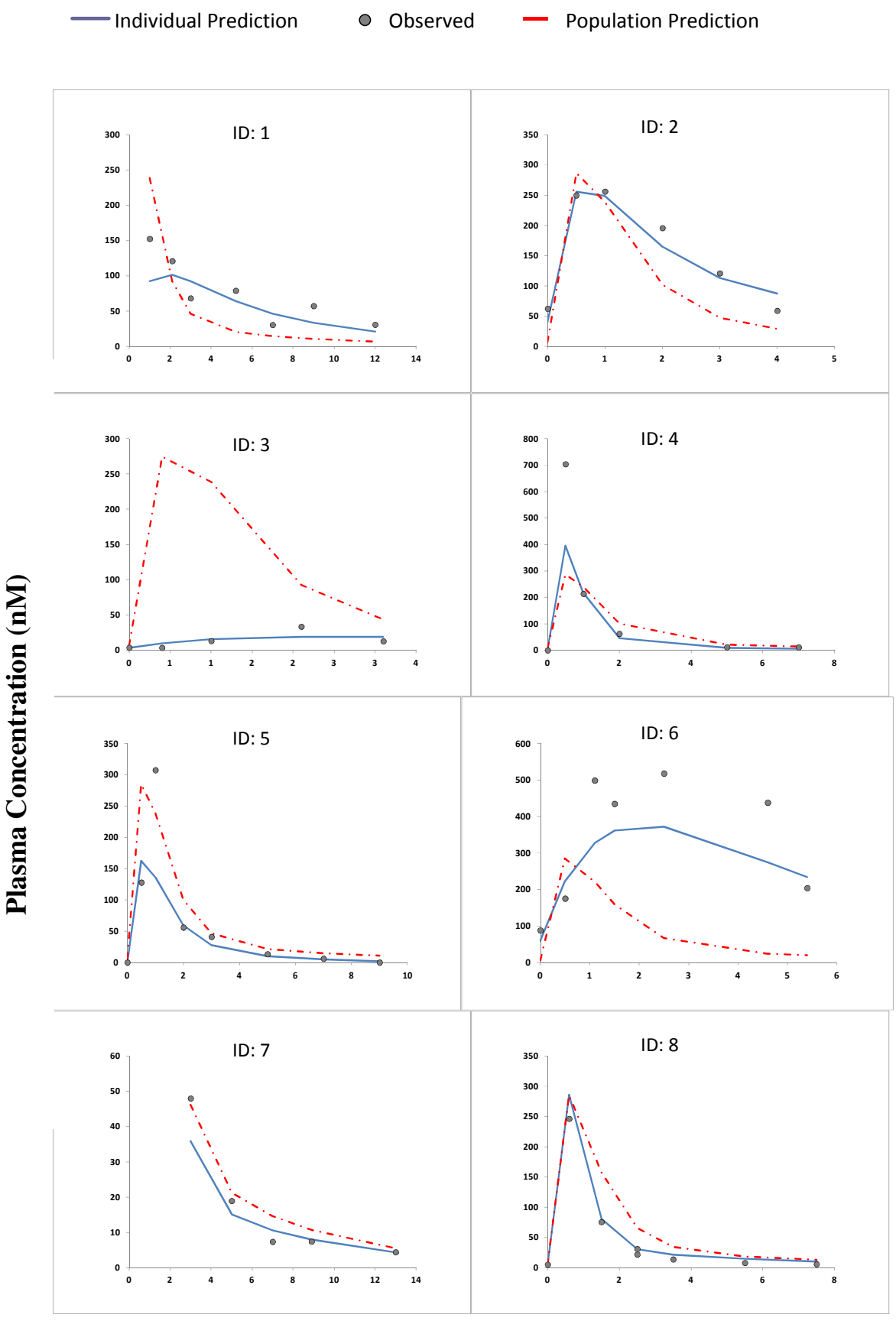

2.5
2.0
1.5
1.0
0.5
0.0
|iWRES|
0
2000
4000
6000
Individual Predictions (nM)

Figure 11: Individual Subject Model Predictions for Oseltamivir on Day 5 of ECMO



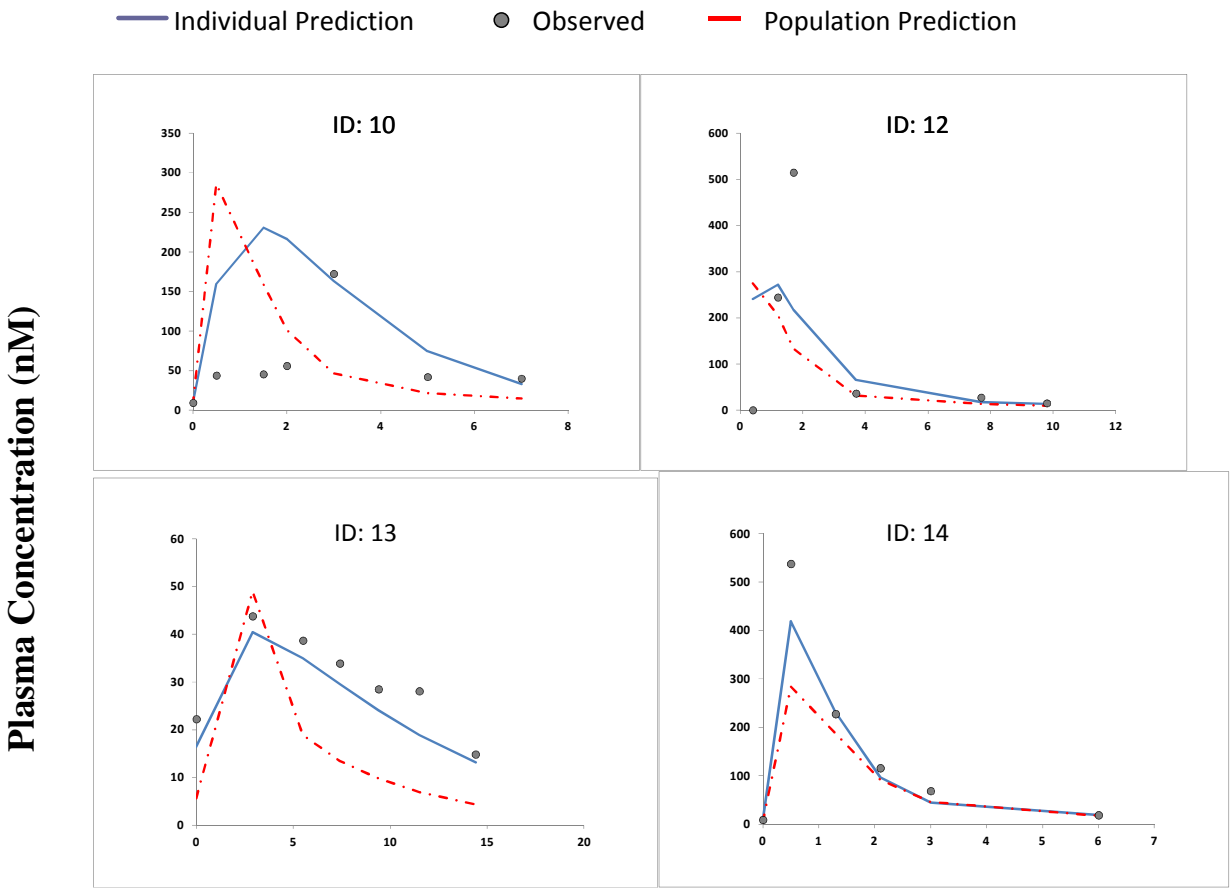
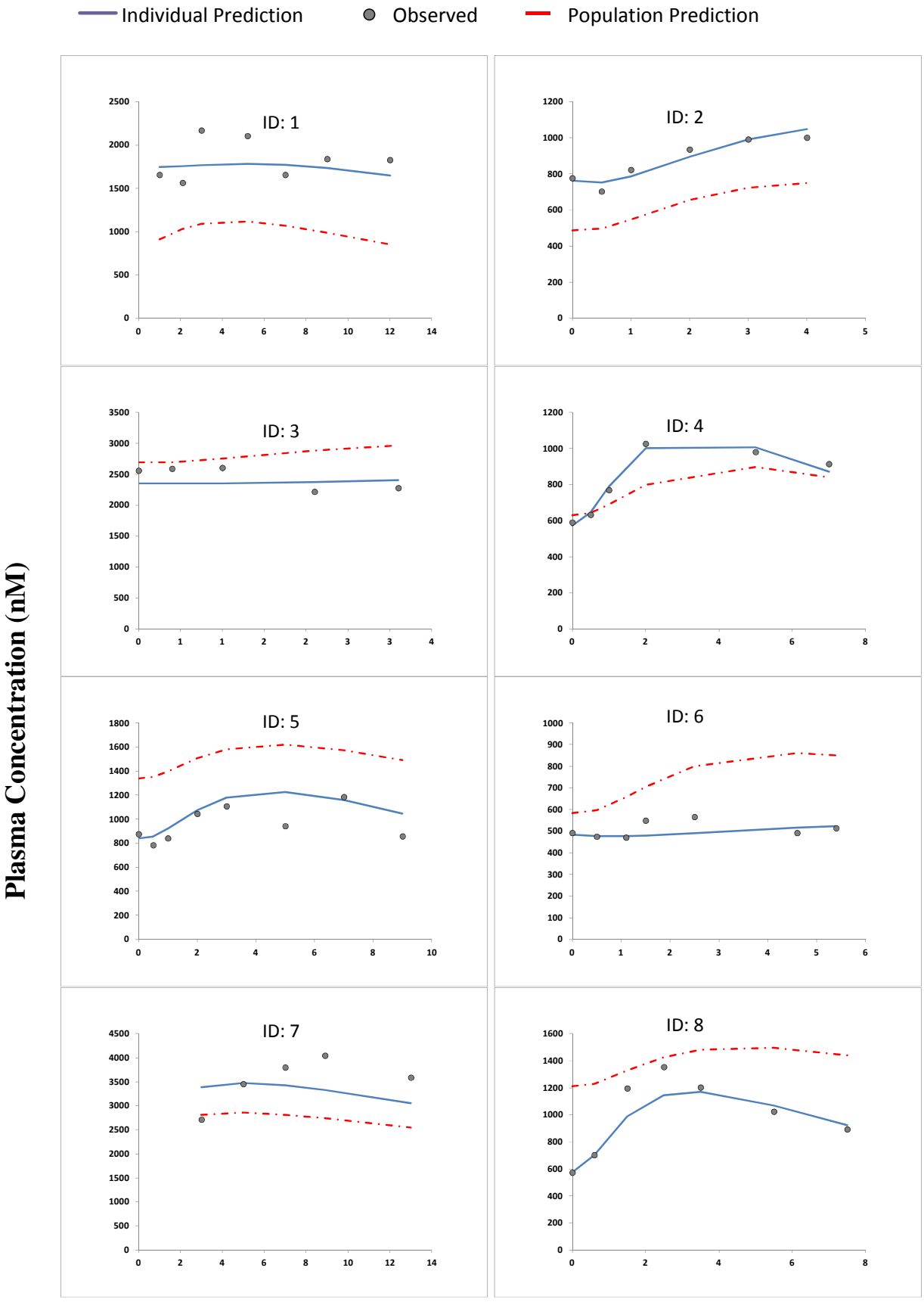
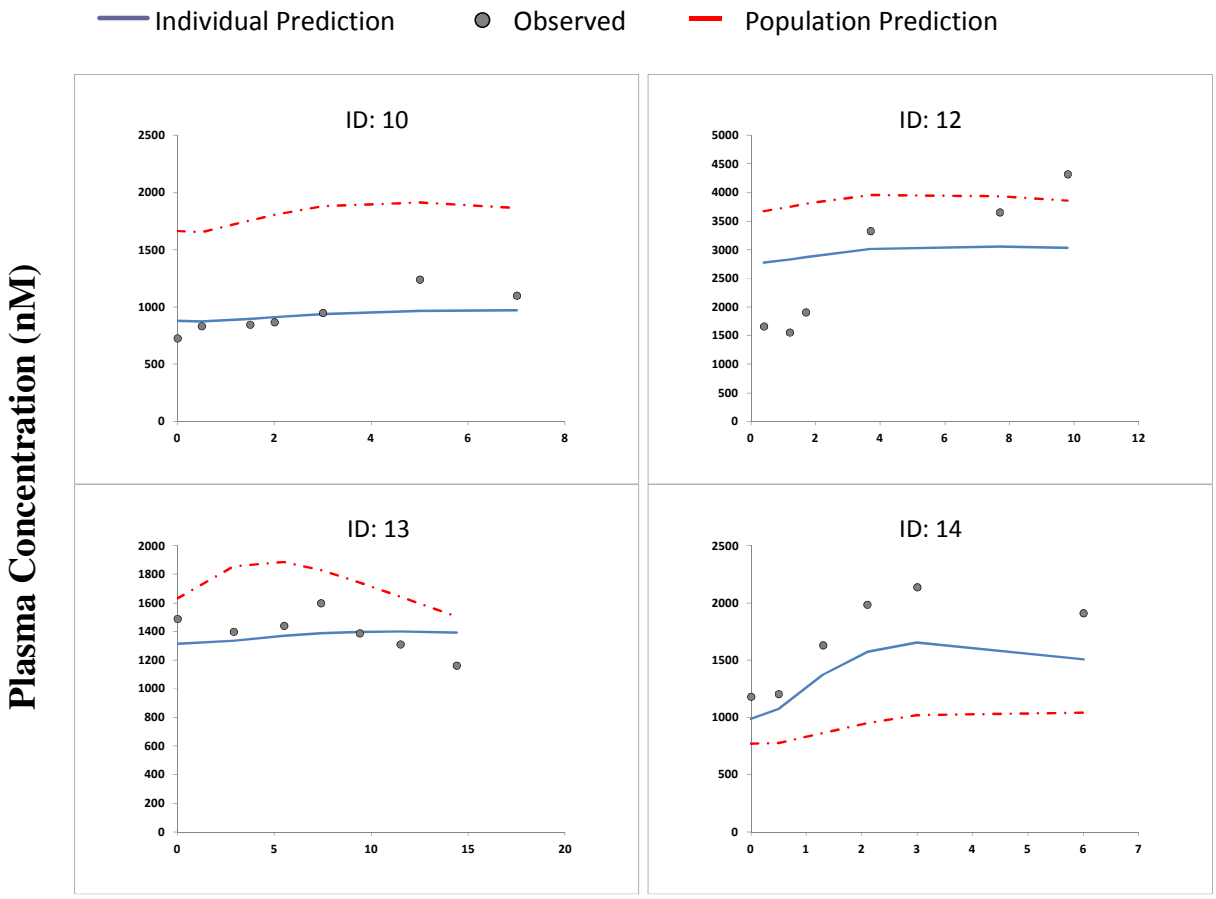

Individual Prediction
Observed
Population Prediction
ID: 10
ID: 12
ID: 13
ID: 14
Plasma Concentration (nM)
Time After Dose (hours)

Figure 12: Individual Subject Model Predictions for Oseltamivir Carboxylate on Day 5 of ECMO




Individual Prediction
Observed
Population Prediction
ID: 10
ID: 12
ID: 13
ID: 14
Plasma Concentration (nM)
Time After Dose (hours)

Covariate Analysis in the base structural model

Figure 13: Plasma Oseltamivir Carboxylate Clearance versus Serum Creatinine in adult ECMO patients

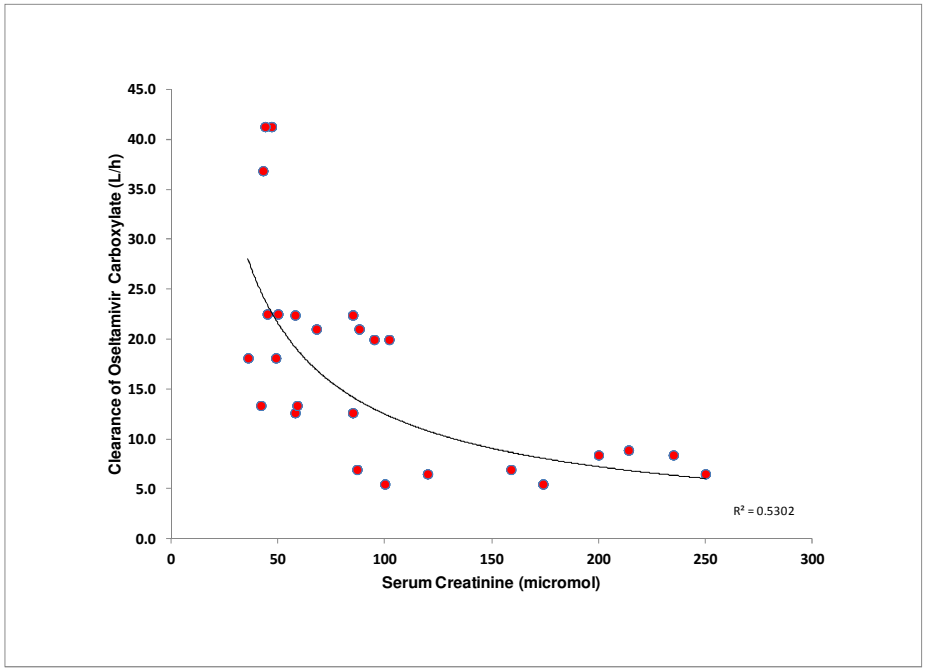


Figure 14: Plasma Oseltamivir Carboxylate Clearance versus Creatinine Clearance (calculated using the Modified Jellife Formula) in adult ECMO patients

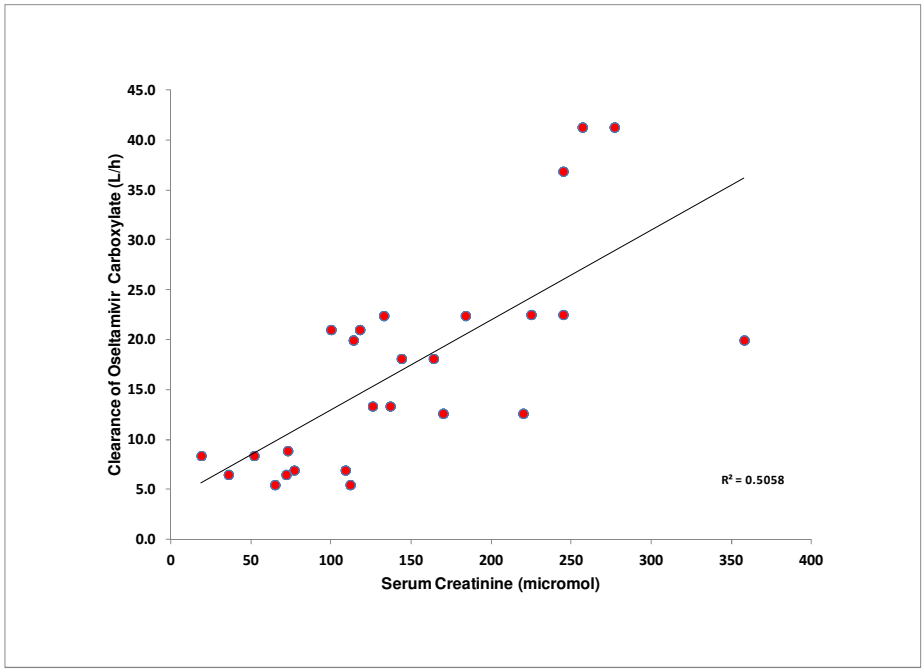
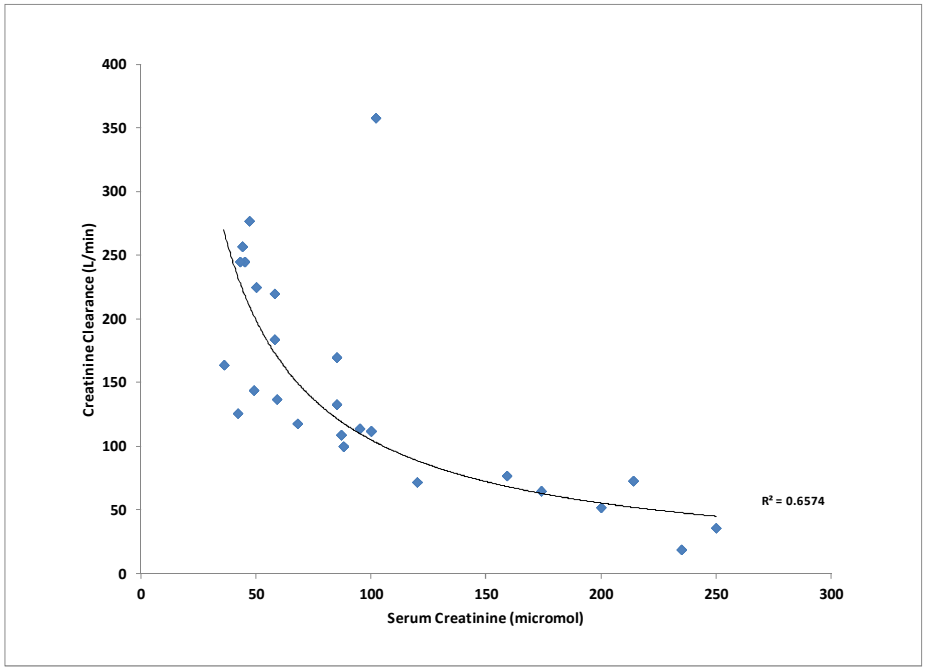


Figure 15: Serum Creatinine Clearance (calculated using the Modified Jellife Formula) versus Serum Creatinine in study subjects



Visual Predictive Check Plots for Final Covariate Model (n=2000 simulations)

Figure 16: Oseltamivir

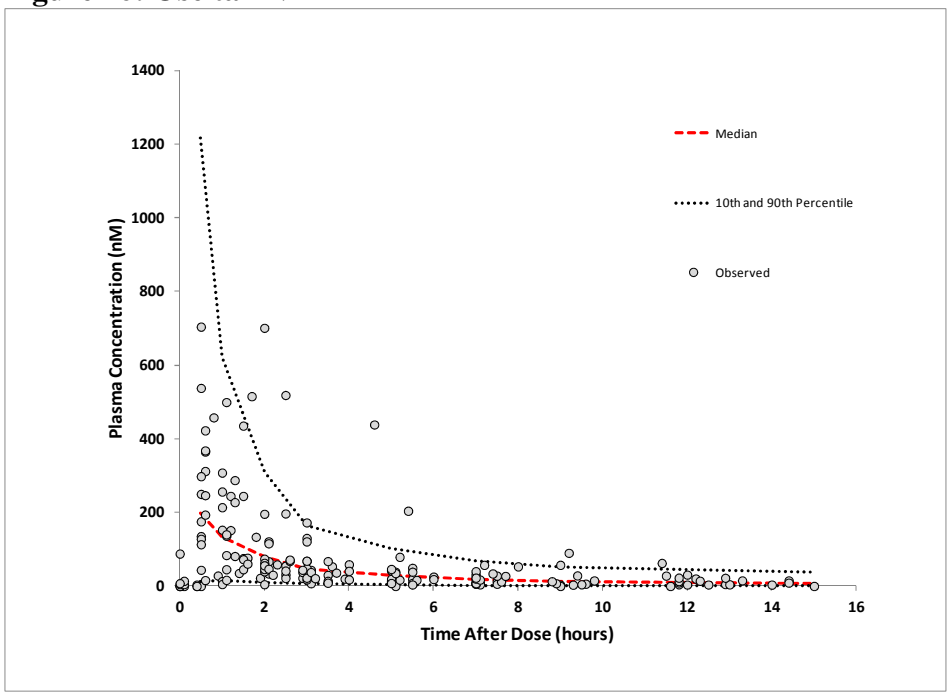


Figure 17: Oseltamivir Carboxylate

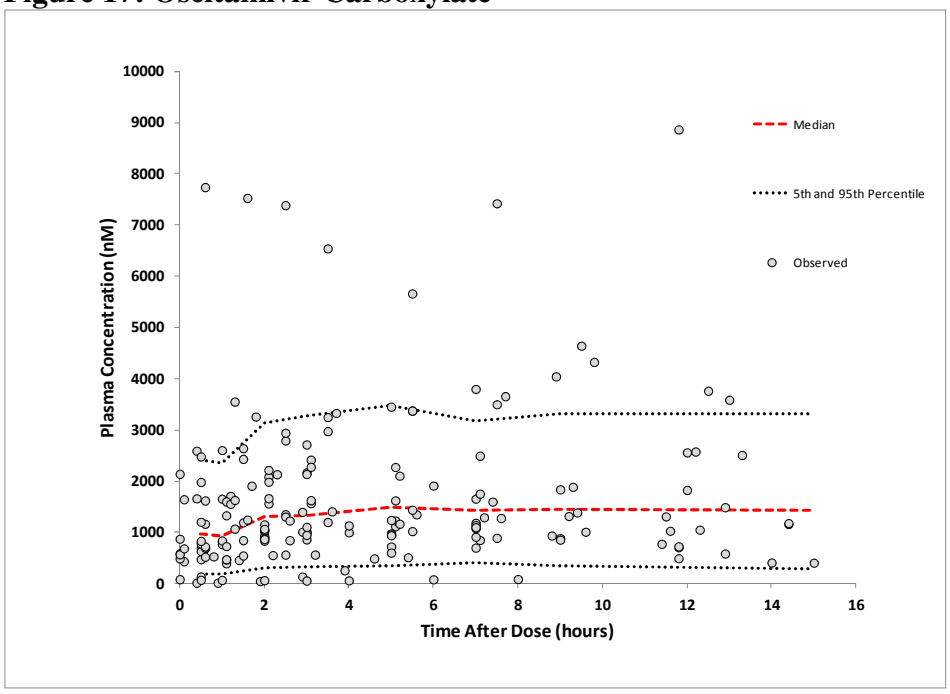


Figure 18: Oseltamivir Carboxylate Area Under the Curve on Day 5 of ECMO versus Serum Creatinine

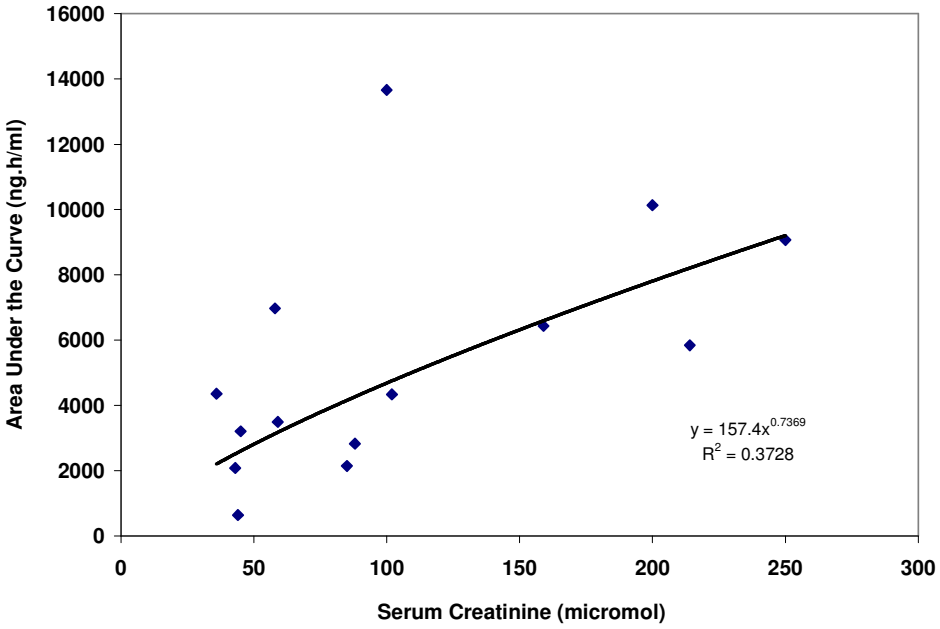


Figure 19: Maximum Oseltamivir Carboxylate Plasma Concentration on Day 5 of ECMO versus Serum Creatinine