

SUPPLEMENTARY MATERIAL

Prospective PBPK modeling

The described PBPK model was used to predict plasma and lung ISF concentrations during the evaluation of the candidate mAbs. Physiological parameters, including organ volumes/weights, plasma volume, cellular volumes, and plasma flow rates, were taken from the International Commission on Radiological Protection (ICRP) database.¹ Subcellular volumes, i.e. the interstitial volume and vascular volume, were derived using the fractions provided in the Shah and Betts model, and lymphatic flow rate was assumed to be 0.2% of the plasma flow rate.² Jones et al. estimated parameters describing the neonatal Fc receptor (FcRn)³, namely: (i) FcRn concentration in the endosomal space, (ii) the rate of pinocytosis and exocytosis per unit endosomal space of vascular endothelial, (iii) the rate at which FcRn unbound antibody molecules are degraded in the endosomal space, and (iv) the proportionality constant between the rate at which antibody transfers from the lymph node compartment to the plasma/blood compartment. The following parameters were fixed as per Shah and Betts: (i) the pinocytotic uptake rate of endothelial cells, (ii) the probability of mAb degradation in the absence of FcRn binding, (iii) endosomal transit time, (iv) volume of endosome, (v) equilibrium constant for mAb-FcRn binding (pH 6.0), (vi) dissociation rate constant for mAb-FcRn binding (pH 6.0) and (vii) degradation rate of FcRn-mAb complex at (pH 7.4).² All mAbs were assumed to have a molecular weight (MW) of 150 kDa, which is consistent with the known MW of IgG.⁴

AC-SIN scores for each mAb determine a correlation that calculates the dissociation rate between the mAb and the interstitial and vascular cellular membranes. AC-SINS, via the vascular and interstitial cellular membrane dissociation rates, affect the rate and fraction of mAbs recycled during intracellular transportation through the FcRn pathway (figure 1).

A typical patient was simulated using organ blood flow rates and organ volumes consistent with a 71 kg male. Patient-to-patient (interindividual) variability in mAb exposure was simulated with the PBPK model by introducing physiologic variability to relevant parameters (organ volumes, blood flows, endothelial cell number) for each “virtual” individual.^{5,6} A representative patient population (n=1000, 500:500 male:female) was generated through an algorithmic sampling of the National Health and Nutrition Examination Survey (NHANES) database NCHS⁷ (age: 25-65, weight: 45-120 kg, 50% male/female) with corresponding individualized physiologic values matched from the ICRP database.¹

Individual demographic characteristics (i.e. covariates), including age, sex, height, and weight, were randomly sampled from the NHANES database while preserving the boundaries mentioned above.⁷ These virtual patient demographics were used to scale the physiological parameters (e.g., volumes and flow

rates), as described previously.^{2,5} The interindividual variability in the number of endothelial cells was also accounted for using allometric scaling of individuals body weight, as described by Ucciferri et al.⁶

The efficacy metric for projecting dose ranges was to maintain lung ISF concentrations at or above the calculated IC₉₀ (conservatively determined as nine times the reported IC₅₀ values) for 14, 21, and 28 days post-administration. This assumed that maintaining lung ISF concentrations would also at least maintain target concentrations in plasma. A simulated IV infusion of a candidate mAb was administered over two hours to the central plasma compartment. Candidate mAbs were first characterized within the PBPK model, using only the AC-SINS scores for the typical patient. The dosing projections in this typical patient were simulated, and the dose (mg) expected to maintain a concentration above the IC₉₀ over 14, 21, and 28 days in both the plasma and lung ISF was determined.

The virtual population of subjects described above were then simulated and rank ordered based on individual plasma mAb concentrations on day 21. The individual ranked above 10% of all individuals in the virtual population was taken to represent the 10th percentile of PK response. This individual was then used to optimize the mAb dose, using the same methods as the typical patient, to determine the dose predicted to meet or exceed the calculated IC₉₀ in 90% of patients over 14, 21, and 28 days.

The dose was optimized using a nonlinear least square method which minimized the quadratic error between the model predicted concentrations and the IC₉₀ value on a given day. This optimization was performed, independently, for plasma and lung ISF mAb concentrations at days 14, 21, and 28. Dose predictions were evaluated for each of the target IC₉₀ values (i.e. one per assay laboratory) and for the geometric mean of the three values. Importantly, the derivation of IC₉₀ assumed a Hill coefficient of one and so the results may represent a more conservative projection of possible doses.

With regards to the use of the PBPK model along with the mAb-specific AC-SINS values, there are several characteristics that determine the PK properties of mAbs. The physicochemical properties known to be associated with mAb in vivo clearance (CL), for example, include: non-specific charge-based interactions (e.g., isoelectric point), self-association, and human neonatal Fc receptor (hFcRn) binding affinity. Chung et al.⁸ reported no apparent association between CL and either combined antibody variable region (Fv) charge or isoelectric point (pI) ($R^2 < 0.25$). However, one particular in vitro assay, the AC-SINS assay, was shown to be well-correlated with in vivo mAb clearance (Spearman correlation coefficient of 0.7 between AC-SINS and CL)⁹ and appears to offer a practical solution for screening mAb candidates during early-stage antibody discovery. The PBPK model used here specifically included the association of AC-SINS with clearance. The training and test data used to develop that published, open access model included 31 IgG mAbs, representing a wide range of AC-SINS scores (0-25). The resulting model was well in agreement between the observed and fitted data, suggesting that the model accounted

for the correct whole-body catabolic capacity in human. Thus, model validation demonstrated that this PBPK accurately predict in vivo PK for antibodies a priori using in vitro data.⁴

Verification of the PBPK model translation from Berkeley Madonna to R/mrgsolve^{9,10} was completed by resimulating and matching the scenarios reported by Jones et al.,³ (results not shown).

Viral dynamic model equations

$\text{conc} = \text{centr}/\text{vc}$; # drug concentration in nM

$\text{lungconc} = \text{delay} * 0.15$; # lung concentration is 15% that of serum for mAbs

$\text{fb} = \text{lungconc}/(\text{lungconc} + \text{IC50})$; # fraction virus bound, IC50 = 1.1 nM

$\text{virusfree} = \text{VL} * (1 - \text{fb})$; # virus not bound to drug, available for infecting target cells

$d/\text{dt}(\text{centr}) = -\text{K10} * \text{centr} - \text{K12} * \text{centr} + \text{K21} * \text{peri}$; # mAb PK central cmpt

$d/\text{dt}(\text{delay}) = \text{keo} * (\text{conc} - \text{delay})$; # Assume 2 hour delay for mAb steady state in lung relative to serum

$d/\text{dt}(\text{peri}) = \text{K12} * \text{centr} - \text{K21} * \text{peri}$; # mAb PK peripheral cmpt

$d/\text{dt}(\text{dft}) = -\text{beta} * \text{dft} * \text{virusfree}$; # dft is fraction of uninfected target cells. $\text{dft}_0 = 1$

$d/\text{dt}(\text{VL}) = \text{gamma} * \text{dft} * \text{virusfree} - \text{delta} * \text{VL}$; # total virus $\text{VL}_0 = 21.8$

Table S1 PKPD parameters used in the viral dynamic model^a

Parameter	Typical value (% CV)
Clearance (L/day)	0.137 (30)
Central volume (L)	3.30 (30)
Peripheral volume (L)	2.71 (30)
Intercompartmental clearance (L/day)	0.429 (30)
Viral load at onset of symptoms (copies/mL)	22 (200)
Rate constant for viral infection ($([\text{copies/ml}]^{-1}\text{day}^{-1})$)	1.35×10^{-5} (175)
Rate constant for viral replication (day^{-1})	3.8 (8)
Death rate of infected cells (day^{-1})	0.7 (5)
Bamlanivimab IC ₅₀ (nM)	1.1

^aPK parameters are taken from Chakraborty et al, 2012, variability of 30% was assumed for the unstudied monoclonal antibody.¹¹ Viral dynamic parameters adapted from Kim et al, 2020. Viral load at symptom onset from throat swabs.¹² CV, coefficient of variation; IC50, half maximal inhibitory concentration; L, liter; mL milliliter; nM, nanomolar.

References:

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3. Jones, H.M. et al. A Physiologically-Based Pharmacokinetic Model for the Prediction of Monoclonal Antibody Pharmacokinetics From In Vitro Data. *CPT Pharmacometrics Syst. Pharmacol.* 8, 738–47 (2019).
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5. Willmann, S. et al. Development of a physiology-based whole-body population model for assessing the influence of individual variability on the pharmacokinetics of drugs. *J. Pharmacokinet. Pharmacodyn.* 34, 401-31 (2007).
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8. Chung, S. *et al.* An in vitro FcRn-dependent transcytosis assay as a screening tool for predictive assessment of nonspecific clearance of antibody therapeutics in humans. *MAbs* 11, 942-55 (2019).
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10. Baron, K.T. & Gastonguay, M.R., Simulation from ODE-based population PK/PD and systems pharmacology models in R with mrgsolve. *Omega* 2015; 2: 1x.
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12. Kim, K.S. *et al.* Modelling SARS-CoV-2 Dynamics: Implications for Therapy. *medRxiv* published online. Doi:10.1101/2020.03.23.20040493 (2020).

```
# Script name: COVID_viral_load_patients.R
# Analyst name: Emmanuel Chigutsa
# Date created: April 2020
# Software version: R version 3.5.0 for Windows
```

```
library(RxODE)
library(scales)
library(ggplot2)
require(gridExtra)
library(truncnorm)
library(dplyr)
library(gridExtra)
rm(list=ls())
```

```
##### define model #####
```

```
ode<-"
```

```
  KD=ec50;
  conc=centr/vc; # drug concentration in nM
  lungconc=delay*scalar; # lung concentration is x% that of serum for mAbs
  fb = lungconc/(lungconc+KD) ; # fraction virus bound, KD in nM
```

```
virusfree=VL*(1-fb); # virus not bound to LY, available for infecting target cells
```

```
  d/dt(centr) = -K10*centr - K12*centr + K21*peri; # mAb central compt
  d/dt(delay) = keo*(conc-delay);
  d/dt(peri) = K12*centr - K21*peri; # mAb peripheral compt
  d/dt(dft) = - beta*dft*virusfree; # VL is viral load, target is number of uninfected target cells, beta is
rate constant of infection
  # d/dt(infected) = beta*target*virusfree- delta*infected; # number of productively infected cells.
Delta is death rate of infected cells
  d/dt(VL)=gamma*dft*virusfree - delta*VL; # total virus. p is shedding rate (release) of virions per cell.
c is elimination rate of free and bound virus
  d/dt(vauc) = VL; # viral load auc
"
```

```
##### end of model #####
```

```
mod4<-RxODE(model = ode, modName = "mod4")
```

```
# list model parameters
```

```
npat=12000
```

```
# need function to chop off 5th and 95th tails #
```

```
chop<-function(x){
  x<-x[x>(quantile(x,0.05))&x<(quantile(x,0.95))]
}
```

```
##### viral kinetics
```

```
# VL0 = 21.8*exp(rnorm(n=npat, mean=0, sd=3.65))
```

```

VL0 = 21.8*exp(rnorm(n=npat, mean=0, sd=2))
VL0 = chop(VL0)
beta=(6.77e-5)*exp(rnorm(n=npat, mean=0, sd=1.75))/5 # infection rate constant ((copies/mL)-1)/day).
Divide by 5 to prolong profile
beta=chop(beta)
gamma=3.8*exp(rnorm(n=npat, mean=0, sd=0.0787)) # maximum rate constant for virus infection
gamma=chop(gamma)
delta=0.7*exp(rnorm(n=npat, mean=0, sd=0.0464)) # (/day) # death rate of infected cells. Paper has
value of 1.59, but slowed it down to be conservative
delta=chop(delta)
#####

ec50=1.1 #

## PK ### sourced from canakinumab paper, IgG1 antibody. Chakraborty et al. 2012.
cl = 0.137*exp(rnorm(n=npat, mean=0, sd=0.3)) # (L/d/70 kg)
cl=chop(cl)
vc = 3.3*exp(rnorm(n=npat, mean=0, sd=0.3)) # L/70 kg
vc=chop(vc)
vp = 2.71*exp(rnorm(n=npat, mean=0, sd=0.3)) # L/70 kg
vp=chop(vp)
q = 0.429 # L/day/70 kg
K10 = cl/vc
K12 = q/vc
K21 = q/vp
scalar = 0.15
keo=41 # Assume 2 hour for SS lung distribution from serum. 2 h is thalf*5. keo=0.693/thalf, convert to
days
dstart=runif(npat,0.5,3)
dstart=chop(dstart)

params.all<-cbind.data.frame(VL0=VL0,beta=beta,gamma=gamma,delta=delta,
                             vc=vc, cl=cl, vp=vp, q=q, K10=K10, K12=K12,
                             K21=K21,ec50=ec50,scalar=scalar,keo=keo,dstart=dstart)
params.all$ID<-1:length(params.all$dstart)
doses=c(0,700/70,1400/70) # mg/kg
wt=70 # typical weight
dft0=1
params.all<-params.all[1:10000,]

### day 0 #####
res <- NULL #Create an empty matrix for storing results
df.full<-NULL

for(jj in unique(doses)){
  amt=jj
  df_ jj <- do.call(rbind, lapply(1:nrow(params.all), FUN = function(uu) {

```

```

ev <- eventTable() # put it in loop to empty it for each simulation. Otherwise RxODE stores the values
and they pile up. Stupid
# Specify sampling
ev$add.sampling(c(seq(0,7,0.5),seq(8,28,1)))
params <- params.all[uu,]
inits<-c(centr=0,delay=0,peri=0,dft=dft0,infected=0,VL=params.all[uu,"VL0"],AUCV=0) # define initial
conditions

ev$add.dosing(dose=1000000*amt*wt/150000,nbr.doses=1,dosing.to=1,start.time=params.all[uu,"dstart"]) # dose in mg, times 1 million = ng, divide by 150 kDa to get nanomoles
x <- mod4$run(params, ev, inits = inits)
x<-cbind(x,"ID"=params.all[uu,"ID"],"ARM"=jj,"START"=params.all[uu,"dstart"]) # append results
res <- rbind.data.frame(res,x) # append results into dataframe
})
)
df.full<-rbind(df.full,df_jj)
}
res<-df.full

data<-data.frame(res)
data$VL <- data$VL*(1 + rnorm(length(data$VL),mean=0,sd=0.2)) # add 20% residual error
names(data)

data.out<-cbind.data.frame(ID=data$ID,ARM=ifelse(data$ARM==0,"Control",
                                               ifelse(data$ARM==20,"LY dose 1","LY dose 2")),
                          TIME=data$time,VL=data$VL,VAUC=data$vauc,RAND_START=data$START)

# write.csv(data.out,"mild_moderate_patients_standard_variability_2.csv",quote=F,
na=".",row.names=F)

data.plot<-data%>%
  group_by(time,ARM)%>%
  summarise(
    lowpi=quantile(VL,0.025),
    med=quantile(VL,0.5),
    highpi=quantile(VL,0.975)
  )

d.p<-cbind.data.frame(xx=c(0.5,3),ymin=10e-6,ymax=10e6)

pdf("virtual_patients_plots_700_1400.pdf",width=7,height=6)
ggplot(data.plot,aes(x=time, y=med,fill=as.factor(ARM))) +
  geom_ribbon(data=d.p,aes(x=xx,ymin=ymin,ymax=ymax),alpha=0.3,inherit.aes = FALSE,fill='grey4')+
  geom_ribbon(aes(x=time,ymin=lowpi,ymax=highpi),alpha=0.3)+
  geom_line(cex=1.2,aes(color=factor(ARM)))+
  geom_hline(yintercept = 1,col="black",cex=1.2,lty="dashed")+
  theme_bw()+

```



```
theme(plot.title = element_text(hjust =
0.5),legend.position=c(0.8,0.8),legend.text=element_text(size=12),legend.title=element_text(size=12))+
scale_x_continuous(breaks=seq(0,28,7),limits=c(0,28),labels=seq(0,28,7))+
scale_y_log10(breaks = trans_breaks("log10", function(x) 10^x),
labels = trans_format("log10", math_format(10^.x)),limits=c(0.00001,10000000)) +
# ggtitle("Viral load over time - 95% prediction interval \nMild-moderate patients (standard
variability)") +
xlab("Time since onset of symptoms (days)") +
ylab("Viral load (copies/mL)")+
theme(axis.text=element_text(size=15),
axis.title=element_text(size=15),
plot.title=element_text(size=17))+
scale_fill_discrete(name="Study arms",
labels=c("Control", "700 mg", "1400 mg"))+
scale_color_discrete(name="Study arms",
labels=c("Control", "700 mg", "1400 mg"))
dev.off()
```