Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Gaudinski MR, Berkowitz NM, Idris AH, et al. A monoclonal antibody for malaria prevention. N Engl J Med 2021;385:803-14. DOI: 10.1056/NEJMoa2034031

A Monoclonal Antibody For Malaria Prevention

Supplementary Appendix

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Supplemental Methods:

Quantification of CIS43LS Serum Concentrations

Serum concentration of CIS43LS was performed on the MSD (Meso Scale Discovery) platform. The anti-idiotype, anti-ID 1-1, antibody solution diluted in 1X PBS was applied at a concentration of 1 ug/mL to the uncoated MSD 96 well plate surface. Plates were agitated for a brief time to ensure solution was fully coated over well bottom surface. Plates were sealed and placed at 4ºC overnight. The next day the plates were washed with wash buffer (1XPBS+ 0.05% Twwen-20) then blocked for 1 hour with 5% MSD Blocker A blocking solution. The blocking solution was washed, and reference or test samples in duplicate 2-fold 8-point serial dilutions were applied to the wells and allowed to incubate with shaking for one hour. Plates were washed to remove unbound sample. Sulfo-tag labeled anti human IgG detection antibody at a concentration of 2 ug/mL was applied to the wells and allowed to associate with complexed 1-1 – CIS43LS within the assay wells. Plates were washed to remove unbound detection antibody. A read solution containing ECL substrate was applied to the wells, and the plates were entered into the MSD Sector instrument. A current was applied to the plates and areas of well surface which form a full 1-1 – CIS43LS -anti human IgG-SulfoTag complex emitted light in the presence of the ECL substrate. The MSD Sector instrument quantitates the amount of light emitted and reports this ECL unit response as a result for each sample and standard of the plate. The amount of CIS43LS sandwiched by the Anti ID and Anti Human IgG antibodies is directly proportional to the concentration of reactive CIS43LS protein in the sample wells. Assuming the standard and test material are biologically similar, the responses generated from both materials can be compared to quantitate the concentration of CIS43LS in test samples. All data analysis was performed using Microsoft Excel and GraphPad Prism.

Population Pharmacokinetics (PK) Analysis

A population PK analysis was performed using a two-compartment model with first order SC absorption (ADVAN4 TRANS4) and the program in NONMEM 7.3 (ICON, Dublin). A bootstrap evaluation with 1000 replicates was used to generate confidence intervals of the population PK parameters. In addition to defining population PK parameters, this analysis was used to create a predictive model which may be useful for clinical design of futures studies. Allometric scaling was utilized to normalize PK parameters to 70kg (e.g. CL and Q - $(WT/70)^{0.85}$; Vss and Vc - $(WT/70)^{1.0}$) [1]. Only dose level following IV administration was formally assessed as a potential covariate for PK parameters due to the relatively small number of study participants. The final population PK model was used to simulate 1000 virtual participants and generate prediction intervals following IV administration. These prediction intervals were compared to dose adjusted observed concentrations from the study participants (observed concentration x adjustment dose/actual mg/kg dose).

Junctional Epitope Sequence Conservation

Of the 6500 sequences of PfCSP, there were 8 alleles that varied in the junctional epitope (NPDPNANPNVDPNAN) of the 3D7 Pf strain (see table below). Six of the 8 variants occur in Asia which is geographic region with limited transmission of *P. falciparum*. Moreover, the mutations exist in isolates from Africa, are not critical contact sites, based on binding to CIS43LS using alanine scanning mutagenesis and structural analysis of CIS43 binding to the junctional epitope[2].

Position	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116			
Junctional Sequence	N	P	D	P	N	A	N	P	N	V	D	P	N	A	N	P			
Polymorphism																	Allele Frequency		Location
D111V											V						1/161	0.006211185	India
103DP104 > 103VL104			V	L													1/161	0.006211185	India
P102A		A															1/161	0.006211185	India
N105P					P												1/161	0.006211185	India
N105H					H												1/161	0.006211185	India
V110A®										A							1/5258	0.000190186	Ghana
D111N®											N						1/5260	0.000190114	Ghana
P102V✓		V															1/5264	0.00018997	Thailand

Adapted from Supplementary Figure 10 (Kisalu, NK et al., Nat Med, 2018)[2]

References:

1. Deng R, Iyer S, Theil FP, Mortensen DL, Fielder PJ, Prabhu S. Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: what have we learned? MAbs 2011;3:61-6.
2. Kisalu NK, Idris AH, Weidle C, et al. A human monoclonal antibody prevents malaria infection by targeting a new site of vulnerability on the parasite. Nat Med 2018;24:408-16.

Supplemental Figures:

Figure S1. CIS43LS Serum Concentrations for Part A and Part B Participants.

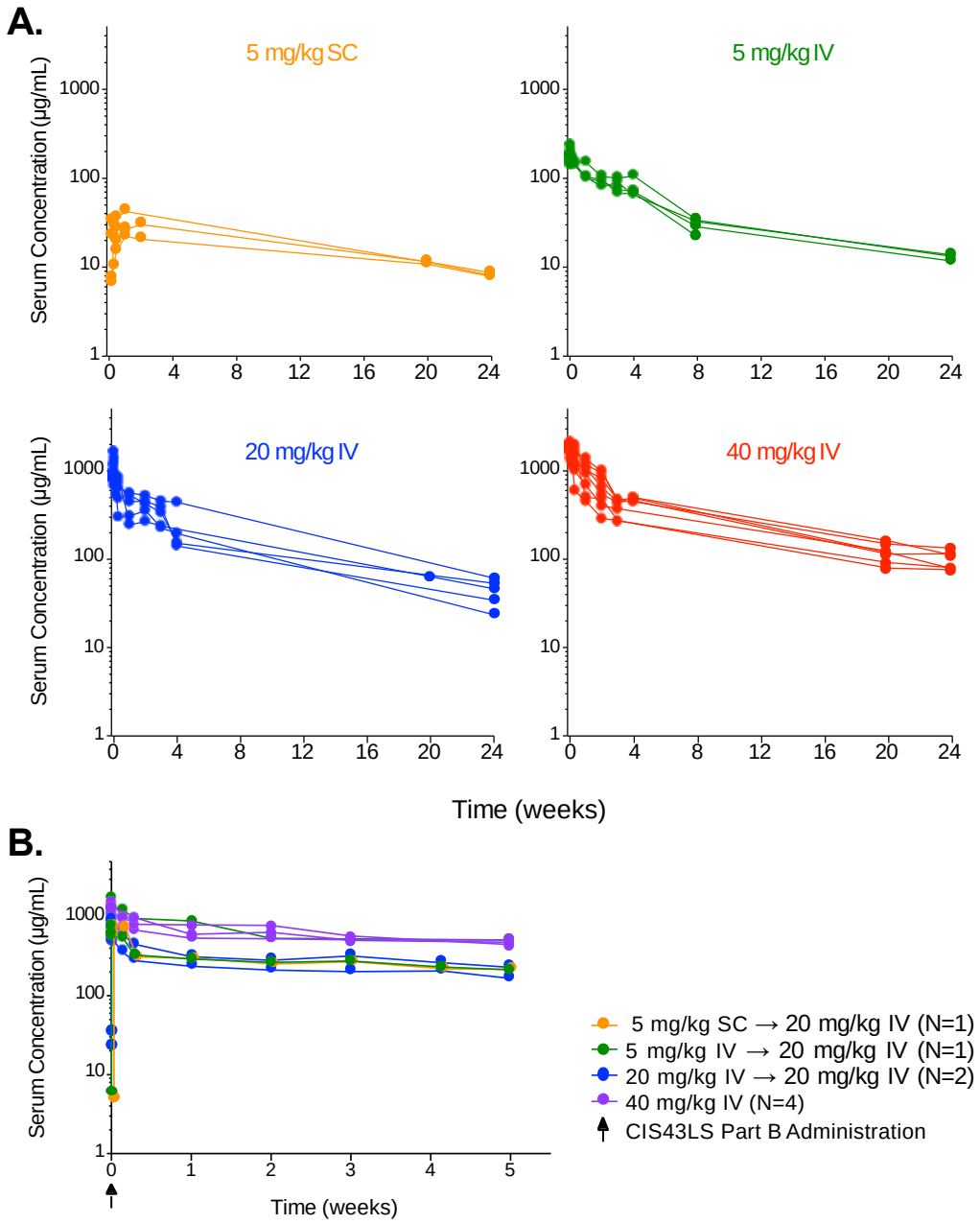

Figure S1. CIS43LS Serum Concentrations for Part A and Part B Participants.
A. Serum concentrations of CIS43LS for all Part A participants by dose group following a single administration. **B.** Serum concentrations of CIS43LS for Part B participants which includes new participants who received 40 mg/kg IV (purple) and four Part A participants who received a second dose of 20 mg/kg IV (blue, green, orange). Dose, route, and group size (N) are specified in the legend.

Figure S2. PK Modelling for IV Administration of CIS43LS with 95% Prediction Intervals

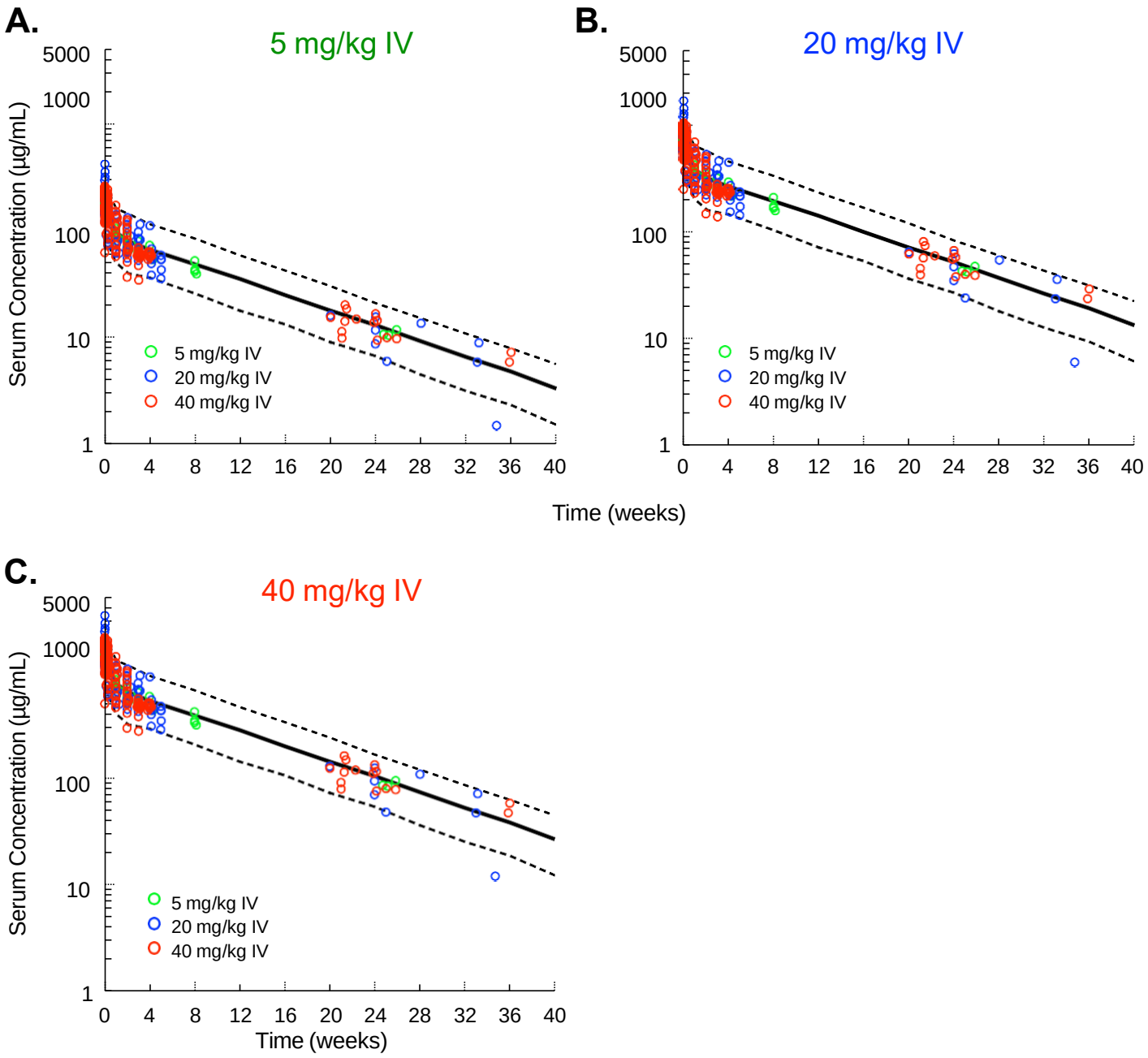

Figure S2. PK Modelling for IV Administration of CIS43LS with 95% Prediction Intervals. Predicted median CIS43LS concentrations are depicted (solid lines) with 95% prediction intervals (5th - 95th percentiles, dashed black lines) based on Monte Carlo simulations using the population pharmacokinetic model following IV doses of 5 mg/kg **(A)**, 20 mg/kg **(B)**, and 40 mg/kg **(C)**. Observed CIS43LS concentrations (normalized for each of the respective doses) are overlayed for comparison.

Supplemental Tables:

Table S1. Baseline Demographic Characteristics of VRC 612 Study Participants.

TABLE S1. BASELINE DEMOGRAPHIC CHARACTERISTICS OF VRC 612 STUDY PARTICIPANTS.

CATEGORY	SUBCATEGORY	5 mg/kg SC (N=4)	5 mg/kg IV (N=4)	20 mg/kg IV (N=9)*	40 mg/kg IV (N=12)	PART A CONTROLS (N=8)	PART B CONTROLS (N=8)
GENDER no.(%)	**MALE**	2 (50.0%)	2 (50.0%)	6 (66.7%)	6 (50.0%)	1 (12.5%)	2 (25.0%)
	FEMALE	2 (50.0%)	2 (50.0%)	3 (33.3%)	6 (50.0%)	7 (87.5%)	6 (75.0%)
AGE† no.(%)	**18-20**	0 (0.0%)	1 (25.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	21-30	4 (100.0%)	3 (75.0%)	6 (66.7%)	9 (75.0%)	5 (62.5%)	5 (62.5%)
	31-40	0 (0.0%)	0 (0.0%)	2 (22.2%)	2 (16.7%)	2 (25.0%)	3 (37.5%)
	41-50	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	1 (12.5%)	0 (0.0%)
	MEAN (SD)	23.5 (3.7)	26.5 (5.1)	26.8 (5.5)	29.8 (8.1)	30.5 (8.9)	30.1 (4.5)
	RANGE	[21.0, 29.0]	[19.0, 30.0]	[20.0, 37.0]	[22.0, 50.0]	[23.0, 50.0]	[26.0, 38.0]
RACE no.(%)	**ASIAN**	2 (50.0%)	0 (0.0%)	3 (33.3%)	3 (25.0%)	1 (12.5%)	1 (12.5%)
	BLACK OR AFRICAN AMERICAN	1 (25.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	1 (12.5%)
	WHITE	1 (25.0%)	4(100.0%)	5 (55.6%)	9 (75.0%)	6 (75.0%)	3 (37.5%)
	MULTIRACIAL	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (12.5%)	3 (37.5%)
ETHNICITY no.(%)	**NON-HISPANIC/LATINO**	4 (100.0%)	3 (75.0%)	8 (88.9%)	12 (100.0%)	7 (87.5%)	7 (87.5%)
	HISPANIC/LATINO	0 (0.0%)	1 (25.0%)	1 (11.1%)	0 (0.0%)	1 (12.5%)	1 (12.5%)
WEIGHT (kg)	**MEAN (SD)**	70.5 (15.1)	67.1 (8.8)	69.7 (14.8)	72.6 (13.9)	67.0 (15.0)	74.9 (16.0)
	RANGE	[48.8, 82.4]	[60.6, 80.0]	[48.8, 95.9]	[53.9, 92.9]	[49.8, 92.0]	[54.7, 102.9]
EDUCATION no.(%)	**COLLEGE/UNIVERSITY**	4 (100.0%)	3 (75.0%)	7 (77.8%)	5 (41.7%)	2 (25.0%)	3 (37.5%)
	ADVANCED DEGREE	0 (0.0%)	1 (25.0%)	2 (22.2%)	7 (58.3%)	6 (75.0%)	5 (62.5%)

*Includes 4 participants from Part A who received CIS43LS 20 mg/kg IV in Part B
† Age represents age at initial enrollment day.

Table S2. Pharmacokinetic (PK) Parameters of CIS43LS.

TABLE S2. PHARMACOKINETIC (PK) PARAMETERS OF CIS43LS.

PK PARAMETERS BY DOSE GROUP

DOSE GROUP	C_{max} (µg/mL)	T_{max} (Days)	C_{7D}	C_{4WK}	C_{24WK}
	Mean (SD, N)				
5 mg/kg SC	---*	---*	29.4 (9.3, 4)	---*	8.0 (0.4, 3)
5 mg/kg IV	198.4 (28.2, 4)	0.10 (0.08)	114.3 (25.2, 4)	77.3 (19.5, 4)	12.8 (1.0, 3)
20 mg/kg IV	934.6 (292.6, 9)	0.07 (0.07)	356.1 (118.6, 9)	230.1 (2.5, 8)	43.5 (14.9, 5)
40 mg/kg IV	1764.4 (259.6, 12)	0.25 (0.5)	825.3 (293.1, 12)	473.2 (22.8, 5)	96.8 (23.8, 6)

PK PARAMETERS FOR ALL GROUPS (N=29)

PK PARAMETER	VALUE	BOOTSTRAP (BS) MEDIAN	BS 5th – 95th PERCENTILE†
$T_{1/2b}$ (days)	56	58.9	51.9 - 77.0
CL (mL/day)	44.2	43.4	39.6 - 47.9
V_{dss} (L)	3.45	3.54	3.25 - 4.13

C_{max} = maximum serum concentration; T_{max} = time to maximum serum concentration; C_{7D}, C_{4WK}, C_{24WK} = concentration on day 7, weeks 4 and 24, respectively.

*Could not be determined due to truncated sample collections

$T_{½β}$ = βeta half-life; CL = clearance; V_{dss} = volume of distribution

† Bootstrap 90% confidence intervals for population PK parameters

Table S3. Controlled Human Malaria Infection Mosquito Score/Salivary Gland Rating and Outcome.

TABLE S3. CONTROLLED HUMAN MALARIA INFECTION MOSQUITO SCORE/SALIVARY GLAND RATING AND OUTCOME.

SUBJECT	CIS43LS DOSE/ROUTE		MOSQUITO SCORING			SALIVARY GLAND RATING*		DAY OF POSITIVE PCR‡
	Part A	Part B	Total # Used	# Fed	Qualifying Bites†	Average	Raw Ratings	
1	5 mg/kg IV	20 mg/kg IV	6	6	5	3.4	0,3,4,4,3,3	Negative
2	5 mg/kg SC	20 mg/kg IV	9	8	5	3.2	0,3,3,0,NF,0,3,4,3	Negative
3	20 mg/kg IV	20 mg/kg IV	9	6	5	2.6	NF,3,3,3,0,NF,2,NF,2	Negative
4	20 mg/kg IV	20 mg/kg IV	10	9	5	2.6	NF,2,3,0,0,2,0,0,2,4	Negative
5	40 mg/kg IV	---	12	11	5	2.2	NF,2,1,0,0,0,0,0,2,2,2,3	Negative
6	40 mg/kg IV	---	9	6	5	2.8	NF,NF,NF,2,3,3,2,0,4	Negative
7	---	40 mg/kg IV	7	7	5	3.2	4,4,3,0,0,3,2	Negative
8	---	40 mg/kg IV	7	5	5	3.2	4,3,2,NF,NF,4,3	Negative
9	---	40 mg/kg IV	12	9	5	3.4	NF,NF,NF,4,3,0,0,0,0,4,3,3	Negative
10	Control		8	6	5	3.4	2,4,0,NF,NF,4,3,4	9
11	Control		11	9	5	3	3,2,0,0,NF,2,0,NF,4,0,4	Negative
12	Control		7	5	5	2.8	2,3,3,NF,NF,3,3	9
13	Control		6	6	5	3	4,2,3,3,0,4	8
14	Control		7	6	5	3.2	4,3,2,0,NF,3,4	9
15	Control		8	7	5	3.4	NF,0,2,3,0,4,4,4	8

CHMI using *Anopholes Stephensi* mosquitoes infected with *Plasmodium falciparum* -3D7 strain.
*Salivary gland rating is based on number of sporozoites (spz) observed after dissection. NF = mosquito did not feed, 0 = no spz observed, 1 = 1-10 spz, 2 = 11-100 spz, 3 = 101 – 1000 spz, 4 > 1000 spz.
† A qualifying bite was defined as a bite with a mosquito bearing a salivary gland rating of 2 or greater. All challenged subjects were required to have 5 qualifying bites.
‡ Volunteers were treated with atovaquone/proguanil on day of positive PCR, or at day 21 if untreated by that time point.

Table S4. Serum CIS43LS at Time of CHMI.

TABLE S4. SERUM CIS43LS AT TIME OF CHMI.

SUBJECT	CIS43LS DOSE/ROUTE		CIS43LS SERUM CONCENTRATION @ CHMI	TIME FROM LAST ADMINISTRATION†
	PART A	PART B	(µg/mL)	(Weeks)
1	5 mg/kg IV	20 mg/kg IV	210.1	5.1
2	5 mg/kg SC	20 mg/kg IV	217.4	5.1
3	20 mg/kg IV	20 mg/kg IV	230.7	5.0
4	20 mg/kg IV	20 mg/kg IV	167.7	5.1
5	40 mg/kg IV	---	57.0*	36.1
6	40 mg/kg IV	---	46.2*	36.0
7	---	40 mg/kg IV	453.7	4.1
8	---	40 mg/kg IV	422.3	4.0
9	---	40 mg/kg IV	493.8	4.0

*Serum concentration in Part A participants who received a single CIS43LS administration prior to CHMI.
†Time span from last CIS43LS administration until time of CHMI.