

# Pre-clinical characterization of ABI-5366: a highly potent long-acting helicase-primase inhibitor for the treatment of high recurrence genital herpes

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## Introduction

- About 12% of individuals in the US will be infected with HSV-2 in their lifetime<sup>1</sup>
- In the US and EU4/UK, ~4 million people have high-recurrence (>3 recurrences/year) genital herpes<sup>2-4</sup>
- Genital herpes is characterized by painful recurrent lesions<sup>5</sup> and is reported in >80% of individuals following primary genital HSV-2<sup>6</sup>
- Approved antivirals for HSV-2 are only partially effective, and it has been >20 years since an antiviral was approved to treat HSV-2<sup>7</sup>
- There is an unmet medical need for a potent, long-acting antiviral that improves adherence and offers convenient dosing
- We identified an HSV HPI, ABI-5366 (5366), that is suitable for development as a long-acting antiviral

## Objective of the Analysis

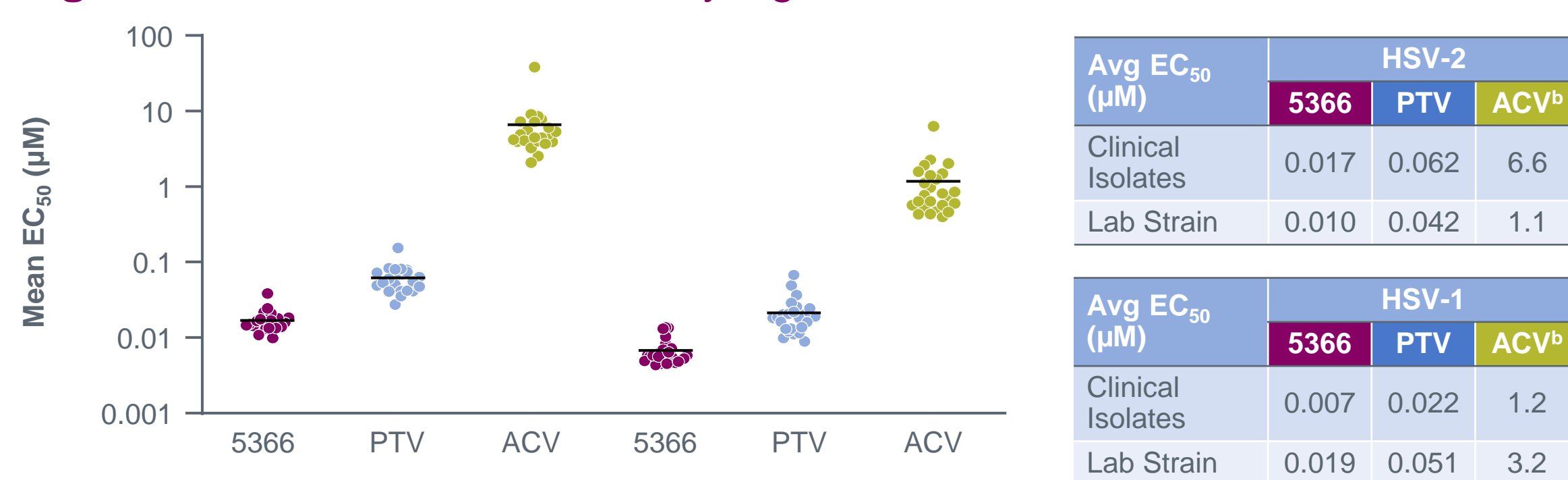
- To characterize 5366, a potent HSV HPI and potential long-acting antiviral

## Methods

- HSV-1 and HSV-2 antiviral assay:** Five-day multicycle replication assay measuring virally-induced CPEs in Vero cells. EC<sub>50</sub> values were generated by CTG
- 5366 resistance selection:** Vero cells were infected with an HSV-2 clinical isolate and then 5366 was added at concentrations 10 times greater than its EC<sub>50</sub> value. When plaque formation was observed (~96 hours postinfection), 5366 concentration was doubled until more plaque formation was observed (~144 hours postinfection). Once 100% CPE was observed (~192 hours postinfection), the cells and supernatant were processed for antiviral activity and sequencing using UL5-specific primers
- Preclinical PK:** Sprague Dawley rats, beagle dogs, cynomolgus monkeys, and mini-pigs were IV dosed with 0.1–0.25 mg/kg 5366 and compound plasma levels were monitored for 120 hours. Dogs were dosed with 15 mg/kg 5366 PO QD for 14 days, and compound plasma levels were monitored for 57 days
- Metabolic stability:** Cynomolgus monkey and human LMs were incubated with 1 μM 5366 for 45 minutes. 5366 metabolism was monitored using LC-MS
- GSH trapping:** Human LMs and GSH were incubated with 10 μM 5366 for 60 minutes. 5366 metabolism was monitored using LC-MS
- CYP and hERG inhibition:** Human LMs were incubated with 10 μM 5366 using diclofenac (2C9), bufuralol (2D6), testosterone (3A4T), and midazolam (3A4M) as probe substrates. 5366 metabolism was monitored using CHO cells expressing hERG channels of P29 in the presence of 10 μM 5366
- Caco efflux:** Caco-2 cells were incubated with 10 μM 5366 and were tested in both the apical-to-basolateral and basolateral-to-apical directions after 90 minutes. 5366 was monitored using LC-MS
- Time-dependent inhibition:** Human LMs were incubated with 10 μM 5366 using phenacetin (1A2), diclofenac (2C9), S-mephenytoin (2C19), bufuralol (2D6), and midazolam (3A4M) as probe substrates. 5366 metabolism was monitored using LC-MS
- Non-GLP toxicology study in rats and dogs:** For 7-day toxicology studies, 5366 was delivered PO to rats (0, 10, 100, and 300 mg/kg) and dogs (0, 1, 10, and 100 mg/kg) QD for 7 days. Changes in clinical signs, body weight, food consumption, gross pathology, clinical pathology, and organ weight were monitored
- Simulated human PK:** Human PK was simulated using a one-compartmental model in which human clearance and volume of distribution values were predicted using allometric scaling of rat, dog, monkey, and mini-pig IV PK data. The absorption rate constants were estimated based on monkey SC PK data. Bioavailability of a 5366 SC injection was assumed to be between 50% and 100%

## Results

**Figure 1. 5366 Has Broad Activity Against HSV-1 and HSV-2 Clinical Isolates<sup>a</sup>**

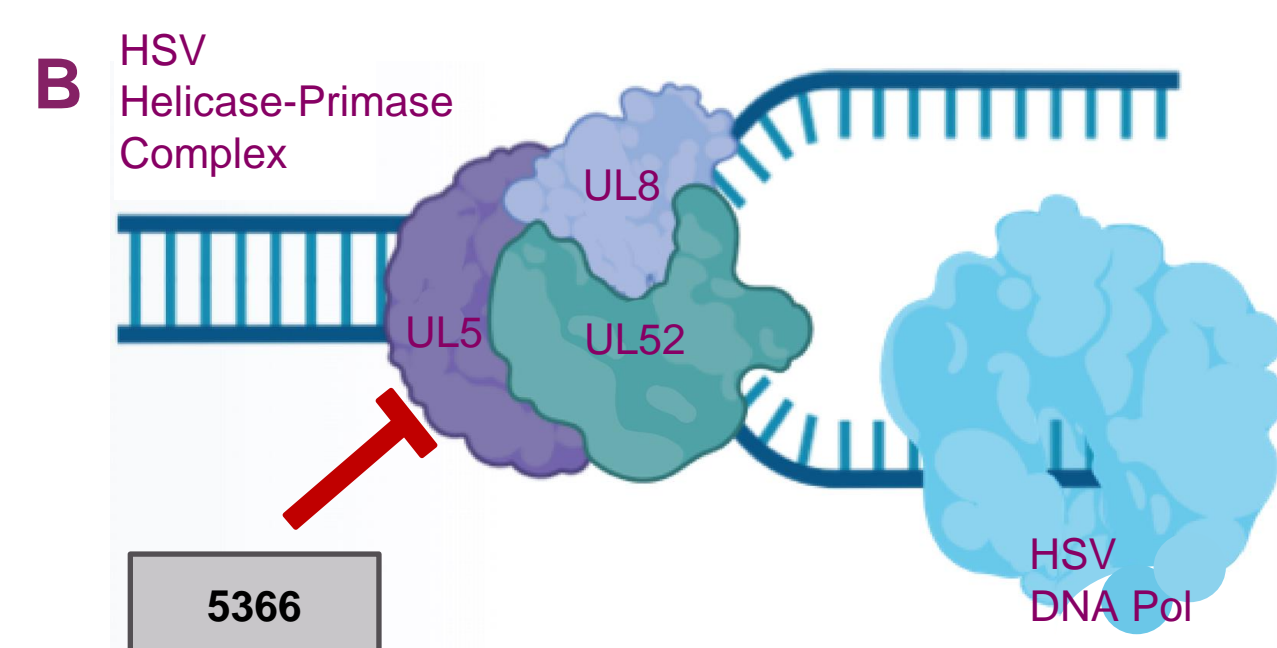


<sup>a</sup>The number of clinical isolates tested is noted underneath the respective virus. <sup>b</sup>Data generated by Assembly Biosciences, Inc. in-house assays. <sup>c</sup>ACV is a standard-of-care treatment for HSV.

- 5366 demonstrated broad antiviral activity against both HSV-1 and HSV-2 lab strains and clinical isolates (**Figure 1**)
  - None of the clinical isolates tested exhibited reduced susceptibility against 5366
- No significant differences in 5366 antiviral activity were observed between lab strains and clinical isolates
- 5366 is ~4× more potent than PTV and ~400× more potent than ACV against HSV-2 clinical isolates

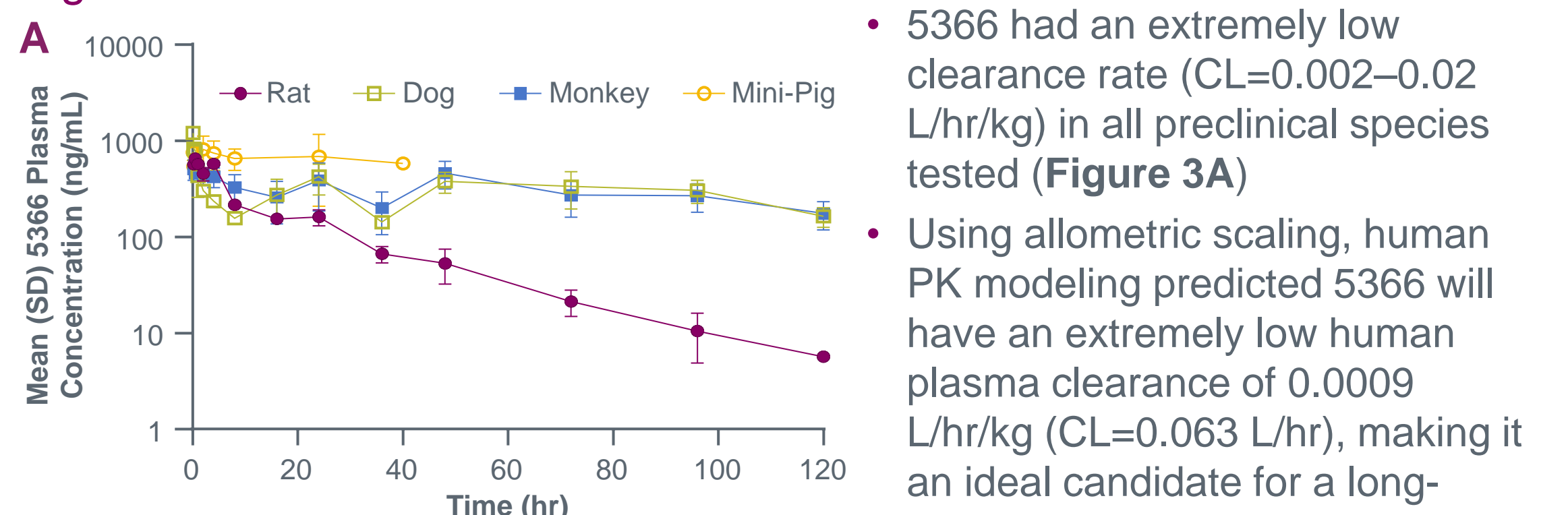
**Figure 2. In Vitro HSV-2 Resistance Studies With 5366 Identify Mutations in UL5 Resulting in Resistance to 5366 and PTV but Not ACV**

Virus Isolates	Mutation Detected	EC <sub>50</sub> (µM)					
		5366	Fold Change	PTV	Fold Change	ACV	Fold Change
HSV-2-IS18	NA	0.0174	NA	0.0554	NA	6.3	NA
HSV-2-IS18R1	UL5 K355R	>50	>2874	2.11	38	4.9	0.78
HSV-2-IS18R2	UL5 K355N	>50	>2874	>50	>903	6.2	1.00



(A) Comparison of HSV-2 resistance to 5366, PTV, or ACV under selective pressure. (B) Representation of 5366 targeting the HSV helicase-primase complex.

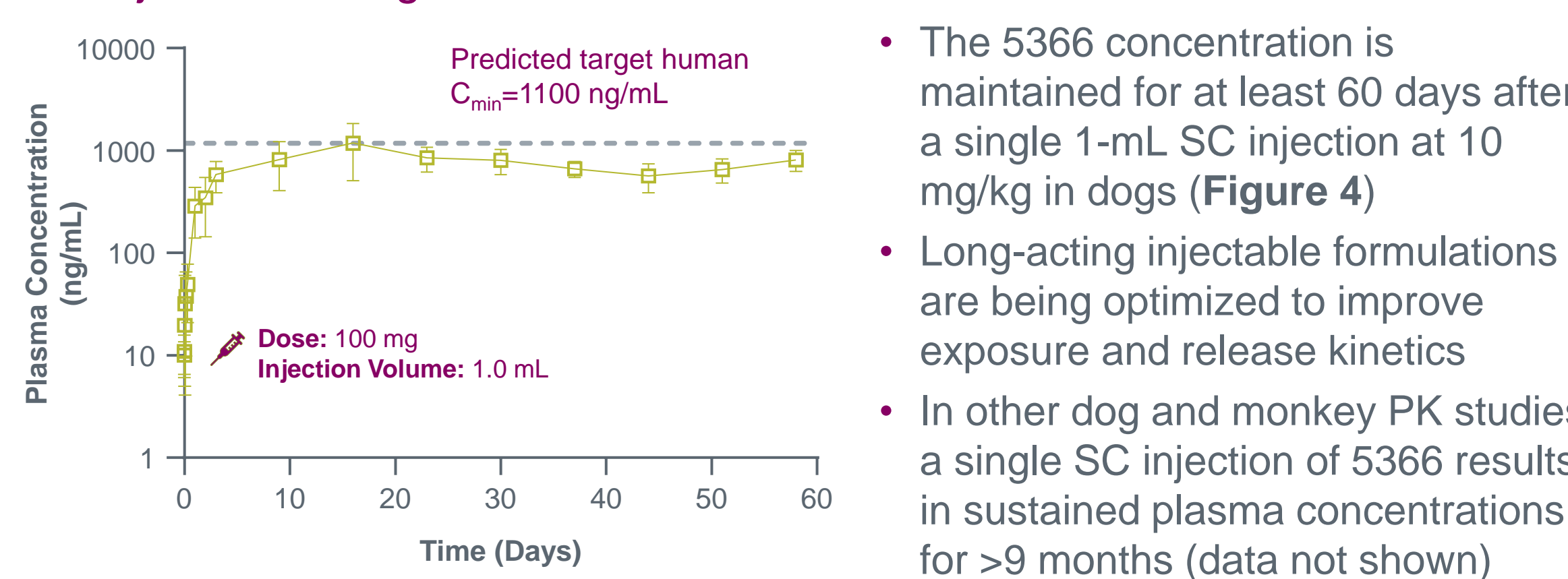
**Figure 3. 5366 Shows Low Clearance in Rats, Dogs, Monkeys, and Mini-Pigs After IV Administration**



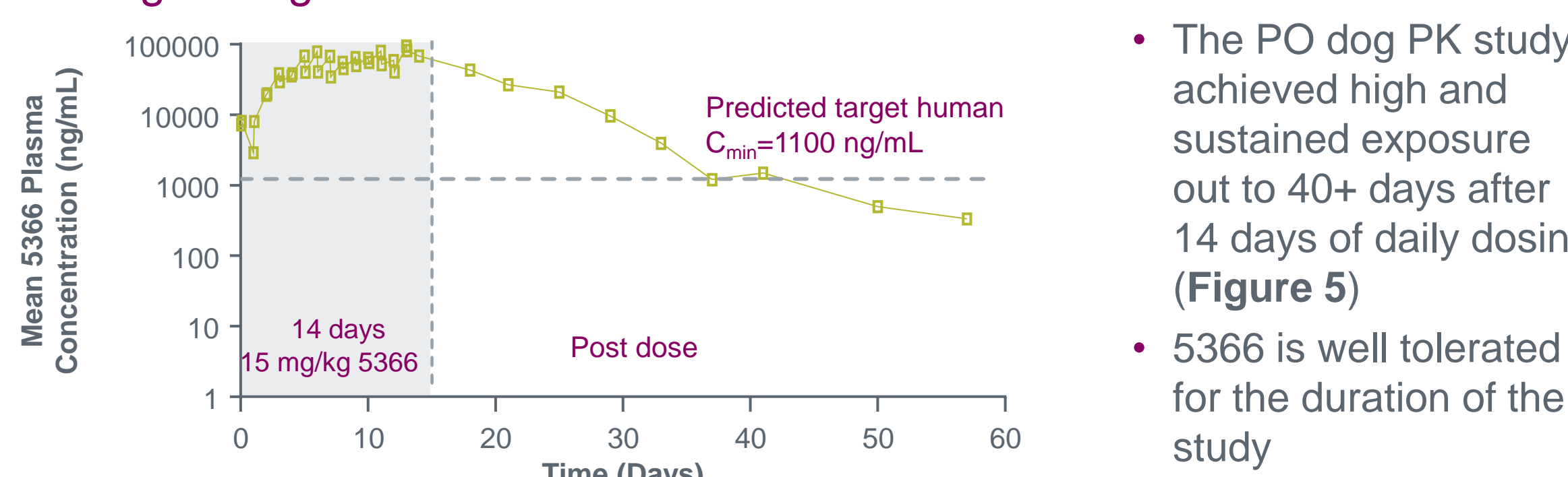
Parameter	5366				
	Rat	Dog	Monkey	Mini-Pig	Human
CL (IV, L/hr/kg)	0.02	0.0023	0.004	0.0018	0.00086 <sup>a,b</sup>
Half-life (hr)	20	55	71	134	182 <sup>a</sup>

<sup>a</sup>Denotes predicted values using four species. <sup>b</sup>Calculated using an estimated human weight of 70 kg.

**Figure 4. 5366 Concentration Is Maintained for ~2 Months After a Single SC Injection in Dogs**



**Figure 5. 5366 Demonstrates Sustained Exposure 40+ Days After PO Dosing in Dogs**



**Table 1. 5366 Demonstrates a Favorable ADME Profile**

ADME Property	5366
LMs in h/r/d/m (% Remaining After 45 Minutes)	96/92/100/100
GSH Trapping in LMs (GSH Adduct Formation)	No GSH adduct
Plasma Protein Binding in h/r (% Bound)	99.8/99.8
CYP Inhibition at 10 μM (2C9/2D6/3A4M/3A4T), % Inhibition	31.5/-9/3.7/4.6
Time-Dependent Inhibition (1A2/2C9/2C19/2D6/3A4M), % Inhibition	0/0/0.2/4.8/3.5
Caco Papp (A-B)/Papp (B-A), ×10 <sup>-6</sup> cm/s	26.3/28.5
hERG EC <sub>50</sub> , μM	>30

- 5366 demonstrates a favorable ADME profile, with no expected drug-drug interaction liabilities (**Table 1**)
- 5366 exhibits excellent stability in rat, dog, cynomolgus monkey, and human LMs, with no GSH adduct formation; 5366 also does not inhibit any of the CYP450 enzymes tested and does not demonstrate inhibition
- In vitro toxicology shows that 5366 is not genotoxic (preliminary via Ames test) nor is it a mitochondrial toxin (Glu/Gal assay)
- 5366 demonstrates low potential for off-target pharmacologic effects

**Table 2. 5366 Is Well Tolerated in All PK Studies**

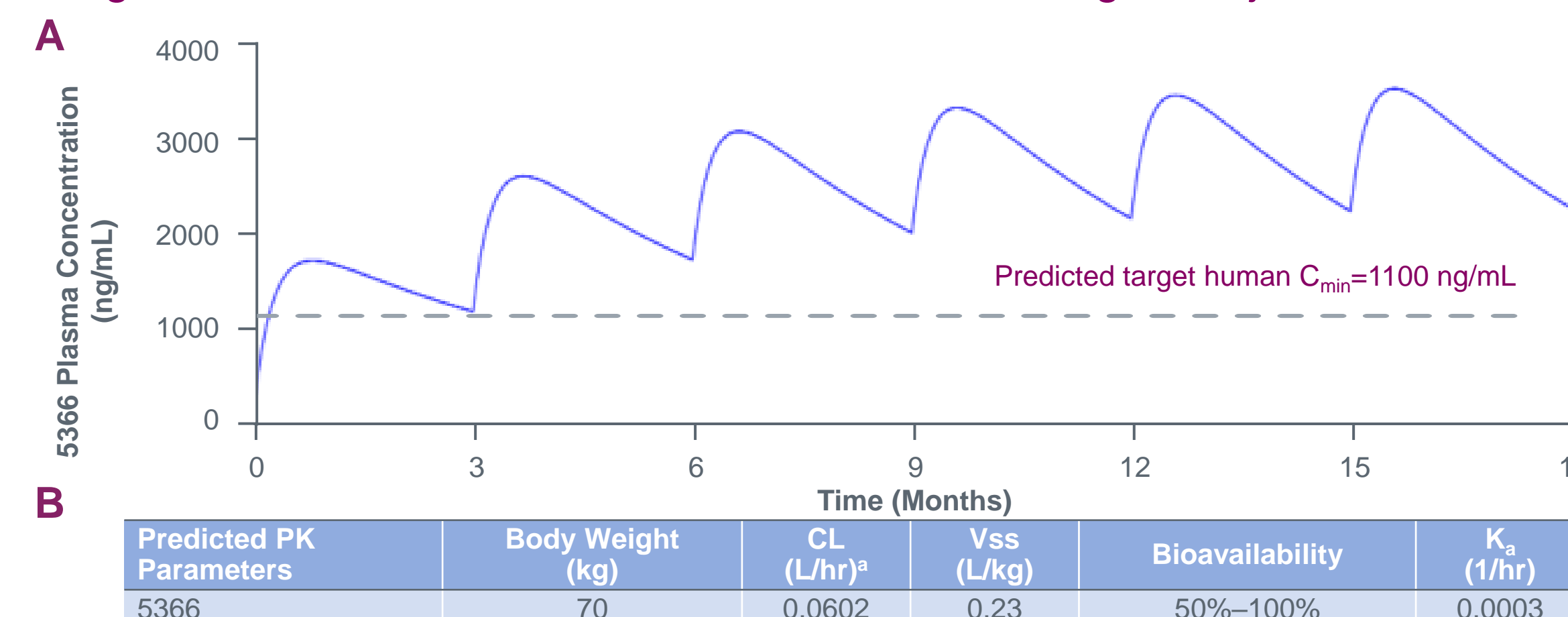
7-Day PK Study in Rats	PO Dose Level (mg/kg/day)	Mean C <sub>max</sub> (ng/mL)	Mean AUC <sub>0-24</sub> (ng-hr/mL)	Mean C <sub>min</sub> (ng/mL)	Fold Over Target C <sub>min</sub> (1100 ng/mL)
	100	47,710	651,736	16,206	14.7
	300	48,180	652,278	16,765	15.2

7-Day PK Study in Dogs	PO Dose Level (mg/kg/day)	Mean C <sub>max</sub> (ng/mL)	Mean AUC <sub>0-24</sub> (ng-hr/mL)	Mean C <sub>min</sub> (ng/mL)	Fold Over Target C <sub>min</sub> (1100 ng/mL)
	10	99,275	1,054,233	57,550	52.3
	100	111,825	1,399,749	68,550	62.3

- Results from the 7-day PO PK study in rats demonstrate that 5366 is well tolerated at all doses and no clinically relevant findings were observed at the highest doses evaluated: 300 mg/kg in rat, which is 15× the predicted HEC and 100 mg/kg in dog, which is 62× the predicted HEC (**Table 2**)
- Exposure plateaued between 10 and 100 mg/kg
- A 14-day PO PK study in dogs demonstrates that 5366 is well tolerated, with no drug-related clinical signs, gross pathology, or clinical pathology at any dose, including the highest dose (15 mg/kg, data not shown)
- In a rabbit study, single SC doses of 5366 up to 200 mg/injection are well tolerated, without significant injection-site irritation (data not shown)

**Figure 6. Predicted 5366 Plasma Concentration Is Maintained Above the Target Minimum Efficacious Concentration Following SC Injections**



<sup>a</sup>Calculated using an estimated human weight of 70 kg.

- Modeling of a 400-mg, SC injection of 5366 every 3 months is expected to achieve plasma concentrations 2-fold above the predicted efficacious concentration (trough concentration=1100 ng/mL; **Figure 6A**)
- Predicted human clearance using simple allometry show extremely low clearance (**Figure 6B**)

## Conclusions

- Resistance selection studies confirm that 5366 is an HSV HPI
- 5366 is projected to have an extremely low human clearance of 0.063 L/hr, supporting its potential as a long-acting therapeutic
- Single-dose SC and multi-dose PO PK studies with 5366 in animals demonstrate sustained plasma concentrations for over 1 month post dose
- 5366 has a favorable safety profile in preclinical studies to date, with good exposure margins and minimal potential for off-target effects
- A Phase 1a first-in-human study with 5366 is planned to begin in 1H-2024

## References

- McQuillan G, et al. *NCHS Data Brief*. 2018;304:1-8.
- Engelberg R, et al. *Sex Transm Dis*. 2003;30:174-77.
- Benedetti J, et al. *Ann Intern Med*. 1999;131:14-20.
- Benedetti J, et al. *Ann Intern Med*. 1994;121:847-54.
- Gupta R, et al. *Lancet*. 2007;370:2127-37.
- Hanfelt-Goade D. *Infectious Diseases*. 3rd Edition. Elsevier Inc. 2010;58:640-8.
- De Clercq E, et al. *Clin Microbiol Rev*. 2016;29:695-747.

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## Abbreviations

5366, ABI-5366; A-B, apical-to-basolateral; ACV, acyclovir; ADME, absorption, distribution, metabolism, and excretion; AUC<sub>0-24</sub>, area under the curve from time 0 to 24 hours; avg, average; B-A, basolateral-to-apical; CHO, Chinese hamster ovary; CL, clearance; C<sub>max</sub>, maximum concentration; C<sub>min</sub>, trough concentration; CPE, cytopathic effect; CTG, CellTiterGlo 2.0; CYP, cytochrome P; EC<sub>50</sub>, half-maximal effective concentration; GLP, good clinical practice; Glu/Gal, glucose/galactose; GSH, glutathione; h/r/d/m, human/rat/dog/mouse; HEC, human efficacious concentration; hERG, human ether-a-go-go-related gene; HPI, helicase-primase inhibitor; HSV, herpes simplex virus; IV, intravenous; K<sub>a</sub>, absorption rate constant; LC-MS, liquid chromatography-tandem mass spectrometry; LM, liver microsome; NA, not applicable; PK, pharmacokinetics; PO, by mouth; Pol, polymerase; PTV, pritelivir; QD, once daily; SC, subcutaneous; SD, standard deviation; V<sub>ss</sub>, volume of distribution at steady state.