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[1]
- In the US and EU4/UK, ~4 million people have high-recurrence (>3 recurrences/year) genital herpes[2-4]
- Genital herpes is characterized by painful recurrent lesions[5] and is reported in >80% of individuals following primary genital HSV-2[6]
- Approved antivirals for HSV-2 are only partially effective, and it has been >20 years since an antiviral was approved to treat HSV-2[7]
- 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

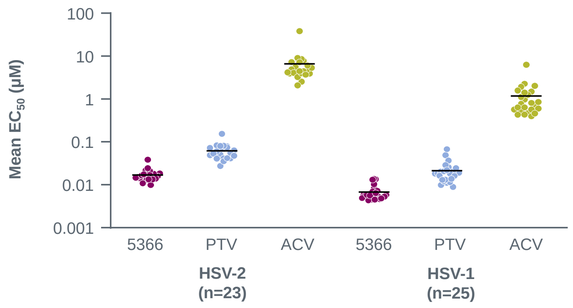
- To characterize 5366, a potent HSV HPI and potential long-acting antiviral

Methods

- **HSV-1 and HSV-2 antiviral assay:** Five-day multicycle replication assay measuring virally-induced CPEs in Vero cells. EC_{50} 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_{50} 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 LC-MS. hERG inhibition 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[a]



Avg EC_{50} (µM)	HSV-2		
	5366	PTV	ACV[b]
Clinical Isolates	0.017	0.062	6.6
Lab Strain	0.010	0.042	1.1

Avg EC_{50} (µM)	HSV-1		
	5366	PTV	ACV[b]
Clinical Isolates	0.007	0.022	1.2
Lab Strain	0.019	0.051	3.2

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

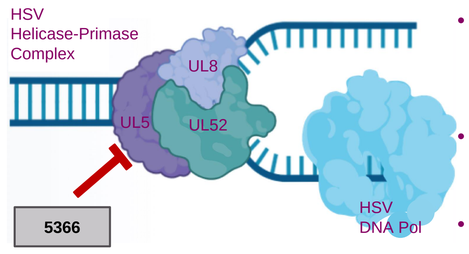
- 5366 demonstrated broad antiviral activity against both HSV-1 and HSV-2 lab strains and clinical isolates (**Figure 1**)
  - None of the clinical isolates tested exhibited reduced susceptibility against 5366
- No significant differences in 5366 antiviral activity were observed between lab strains and 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

A

Virus Isolates	Mutation Detected	EC_{50} (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

B

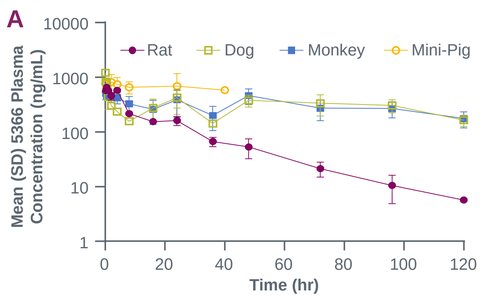


- Resistance selection with 5366 identified mutations in UL5 (helicase) and indicated that 5366 acts on the helicase-primase complex (**Figure 2**)
- 5366-resistant isolates were cross-resistant to PTV but were sensitive to ACV
- The K355 mutations were previously observed with PTV

(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

A



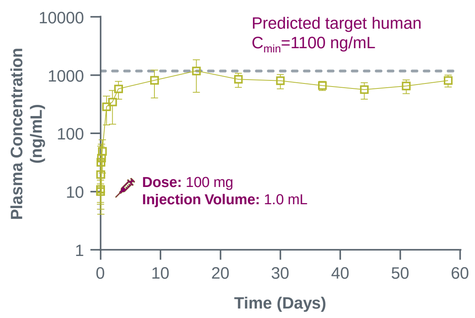
- 5366 had an extremely low clearance rate (CL=0.002–0.02 L/hr/kg) in all preclinical species tested (**Figure 3A**)
- Using allometric scaling, human PK modeling predicted 5366 will have an extremely low human plasma clearance of 0.0009 L/hr/kg (CL=0.063 L/hr), making it an ideal candidate for a long-acting therapeutic (**Figure 3B**)

B

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

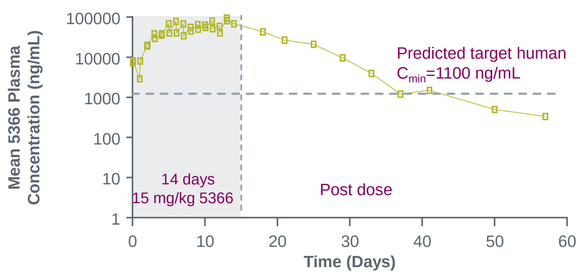
[a]Denotes predicted values using four species. [b]Calculated using an estimated human weight of 70 kg.

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



- The 5366 concentration is maintained for at least 60 days after a single 1-mL SC injection at 10 mg/kg in dogs (**Figure 4**)
- Long-acting injectable formulations are being optimized to improve exposure and release kinetics
- In other dog and monkey PK studies, a single SC injection of 5366 results in sustained plasma concentrations for >9 months (data not shown)

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



- The PO dog PK study achieved high and sustained exposure out to 40+ days after 14 days of daily dosing (**Figure 5**)
- 5366 is well tolerated for the duration of the study

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^{-6} cm/s	26.3/28.5
hERG EC_{50}, 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

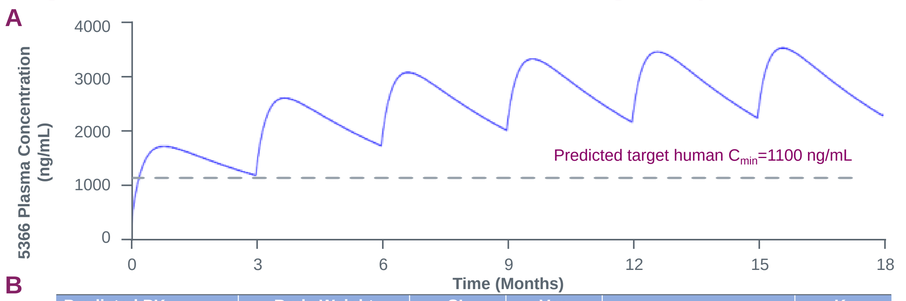
Table 2. 5366 Is Well Tolerated in All PK Studies

	PO Dose Level (mg/kg/day)	Mean C_{max} (ng/mL)	Mean AUC_{0-24} (ng•hr/mL)	Mean C_{min} (ng/mL)	Fold Over Target C_{min} (1100 ng/mL)
7-Day PK Study in Rats	10	18,852	280,191	7850	7.1
	100	47,710	651,736	16,206	14.7
	300	48,180	652,278	16,765	15.2
	PO Dose Level (mg/kg/day)	Mean C_{max} (ng/mL)	Mean AUC_{0-24} (ng•hr/mL)	Mean C_{min} (ng/mL)	Fold Over Target C_{min} (1100 ng/mL)
7-Day PK Study in Dogs	1	26,125	246,466	10,410	9.5
	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

A



B

Predicted PK Parameters	Body Weight (kg)	CL (L/hr)[a]	Vss (L/kg)	Bioavailability	K_a (1/hr)
5366	70	0.0602	0.23	50%–100%	0.0003

[a]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

1) McQuillan G, et al. *NCHS Data Brief.* 2018;304:1-8. **2)** Engelberg R, et al. *Sex Transm Dis*. 2003;30:174-77. **3)** Benedetti J, et al. *Ann Intern Med*. 1999;131:14-20. **4)** Benedetti J, et al. *Ann Intern Med*. 1994;121:847-54. **5)** Gupta R, et al. *Lancet.* 2007;370:2127-37. **6)** Hanfelt-Goade D, Infectious Diseases. 3rd Edition. Elsevier Inc. 2010;58:640-8. **7)** De Clercq E, et al. *Clin Microbiol Rev.* 2016;29:695-747.

Acknowledgments

- The authors would like to thank Jason Wu for contributions to formulation development for ABI-5366
- Writing and editorial support were provided by Gregory Suess, PhD, CMPP, of AlphaBioCom, a Red Nucleus company, and were funded by Assembly Biosciences, Inc.
- This study was sponsored by Assembly Biosciences, Inc.

Abbreviations

5366, ABI-5366; A-B, apical-to-basolateral; ACV, acyclovir; ADME, absorption, distribution, metabolism, and excretion; AUC_{0-24}, area under the curve from time 0 to 24 hours; avg, average; B-A, basolateral-to-apical; CHO, Chinese hamster ovary; CL, clearance; C_{max}, maximum concentration; C_{min}, trough concentration; CPE, cytopathic effect; CTG, CellTiterGlo 2.0; CYP, cytochrome P; EC_{50}, 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_a, 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; Vss, volume of distribution at steady state.

Presented at the 47th Annual International Herpesvirus Workshop (IHW) 2023; July 15–19, 2023