The Helicase-Primase Inhibitor ABI-5366 is a Novel, Potent, Long-Acting Inhibitor for the Treatment of Recurrent Genital Herpes

Heidi Contreras, Kirsten Stray, Dinara Azimova, Michael Shen, Zhixin Zong, Gene Schulze, Yanhong Zhu, Hassan Pajouhesh, Michael A. Walker, Kathryn Kitrinos, Michel J. Perron, William Delaney, Ran Yan

[1]Assembly Biosciences, Inc., South San Francisco, CA, USA

Introduction

- An estimated 13% or 491 million people worldwide aged 15 to 49 years are living with herpes simplex virus type 2 (HSV-2) infection[1]
  - In the US and EU, >4 million people with initial symptomatic genital herpes infection have 3+ recurrences per year[2-7]
- Recurrent genital herpes (RGH) is typically caused by HSV-2 infection, resulting in painful lesions that often last a week or more[1,8]
- Standard-of-care RGH-suppressive therapies, nucleoside analogues (NAs), are limited by suboptimal efficacy in most patients[9]
- The helicase-primase (HP) enzyme complex is essential for viral replication and is a clinically validated target[10,11]
  - HP inhibitors (HPIs) are a novel class of antivirals with improved efficacy compared with NAs, as measured by reduced viral shedding and symptoms[10,11]
- ABI-5366 is a promising long-acting oral HPI with potential anti-HSV activity

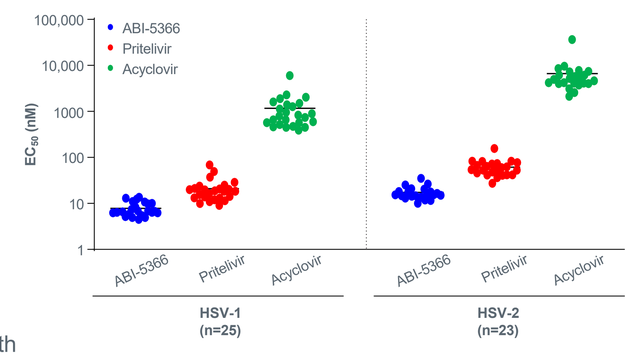
Methods

- **Cytopathic effect reduction assay:** Vero cells were infected with HSV and treated with compounds for 5 days. Virally reduced cytopathic effects and EC_{50}s were measured by CellTiter-Glo (CTG)
- **ABI-5366 resistance selection:** Vero cells were infected with HSV-2 clinical isolates and selected with escalating doses of ABI-5366. The cells and supernatant were processed for next-generation sequencing using gene-specific primers
- **Tissue distribution studies:** Rats received a single PO dose of ABI-5366 or pritelivir at 15 mg/kg via gavage needle. The concentration of ABI-5366 or pritelivir in plasma and tissues was determined using liquid chromatography with mass spectrometry (LC-MS)
- **Preclinical pharmacokinetic (PK) studies:** Sprague Dawley rats, beagle dogs, cynomolgus monkeys, and mini-pigs were IV dosed with 0.1-0.25 mg/kg ABI-5366, and compound plasma levels were monitored for 120 hours. Dogs were dosed with 15 mg/kg ABI-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 1µM ABI-5366 for 45 minutes, and metabolism was monitored using LC-MS
- **GSH trapping:** Human LMs and GSH were incubated with 10 µM ABI-5366 for 60 minutes. 5366 metabolism was monitored using LC-MS
- **CYP and hERG inhibition:** Human LMs were incubated with 10 µM ABI-5366 using diclofenac (2C9), bufuralol (2D6), testosterone (3A4T), and midazolam (3A4M) as probe substrates. ABI-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 ABI-5366
- **Caco efflux:** Caco-2 cells were incubated with 10 µM ABI-5366 and were tested in both the apical-to-basolateral and basolateral-to-apical directions after 90 minutes. ABI-5366 was monitored using LC-MS
- **Time-dependent inhibition:** Human LMs were incubated with 10 µM ABI-5366 using phenacetin (1A2), diclofenac (2C9), S-mephenytoin (2C19), bufuralol (2D6), and midazolam (3A4M) as probe substrates. 5366 metabolism was monitored using LC-MS
- **PK studies after PO and IM dosing:** PK studies were performed in male beagle dogs (n=3). For oral dosing, 100 mg of ABI-5366 was administered via oral gavage. For intramuscular (IM) dosing, 400 mg of ABI-5366 (2 units of 200 mg) was administered via 2 IM injections. Plasma samples were collected at each time point and were analyzed LC-MS
- **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 an ABI-5366 SC injection was assumed to be between 50% and 100%

Results

Figure 1. ABI-5366 Exhibits Broad Activity Against HSV-1 and HSV-2 Clinical Isolates

A. Clinical Isolate Sensitivity



B. Mean Antiviral Activity

Virus	Strain	EC_{50} (nM)		
		ABI-5366	Pritelivir	Acyclovir
HSV-1	Laboratory strain (HF)	18 ± 5 (n=44)	66 ± 23 (n=35)	3380 ± 1070 (n=7)
	Clinical isolates	7 ± 3 (n=25)	21 ± 13 (n=25)	1174 ± 1211 (n=25)
HSV-2	Laboratory strain (G)	10 ± 3 (n=86)	38 ± 12 (n=62)	1080 (n=1)
	Clinical isolates	17 ± 6 (n=23)	62 ± 26 (n=23)	6606 ± 7173 (n=23)

In panel A, the horizontal line indicates the mean. In panel B, EC_{50}s are mean ± SD.
EC_{50}, half-maximal effective concentration; SD, standard deviation.

- ABI-5366 exhibits potent activity against both HSV-1 and HSV-2 laboratory strains and clinical isolates (**Figure 2**)
- ABI-5366 is ~4-fold more potent than pritelivir and ~400-fold more potent than acyclovir against HSV-2 clinical isolates (**Figure 2B**)

Figure 2. ABI-5366 Targets the HP Complex

A. Activity Against HPI Resistance Selection Viruses

Virus Isolates	Mutation Detected	EC_{50} (µM)			
		ABI-5366	Fold Change	Acyclovir	Fold Change
HSV2-IS18	-	0.02	-	5	-
HSV2-IS18R1	UL5 K355R	>50	>2874	5	1.0
HSV2-IS18R2	UL5 K355N	>50	>2874	6	1.3
HSV2-IS18R3	UL5 K355R	>50	>2874	8	1.6
HSV2-IS22	-	0.02	-	4	-
HSV2-IS22R	UL5 K355N	>50	>2294	3	0.7
HSV2-IS27	-	0.02	-	9	-
HSV2-IS27R	UL5 K355N	>50	>2381	3	0.4
HSV2-IS28	-	0.02	-	3	-
HSV2-IS28R	UL5 K355N	>50	>2959	4	1.2

B. Activity Against NA Resistance Selection Viruses

Virus Isolates	Mutation Detected	EC_{50} (µM)			
		ABI-5366	Fold Change	Acyclovir	Fold Change
HSV2-IS53	-	0.01	-	2	-
HSV2-IS53 ACVR4	UL23 T288M	0.02	1.9	>100	>48

EC_{50}, half-maximal effective concentration; HP, helicase primase; HPI, HP inhibitor; NA, nucleoside analogue.

- ABI-5366 is predicted to bind at the UL5/UL52 interface based upon cryo-EM modeling (data not shown)
- Resistance selection with ABI-5366 identified the K355N and K355R variants in the UL5 gene (**Figure 2A**), which were observed in pritelivir resistance selections[14]
- ABI-5366–resistant isolates remain sensitive to acyclovir
- ABI-5366 retains potency against an NA-resistant mutant (**Figure 2B**)

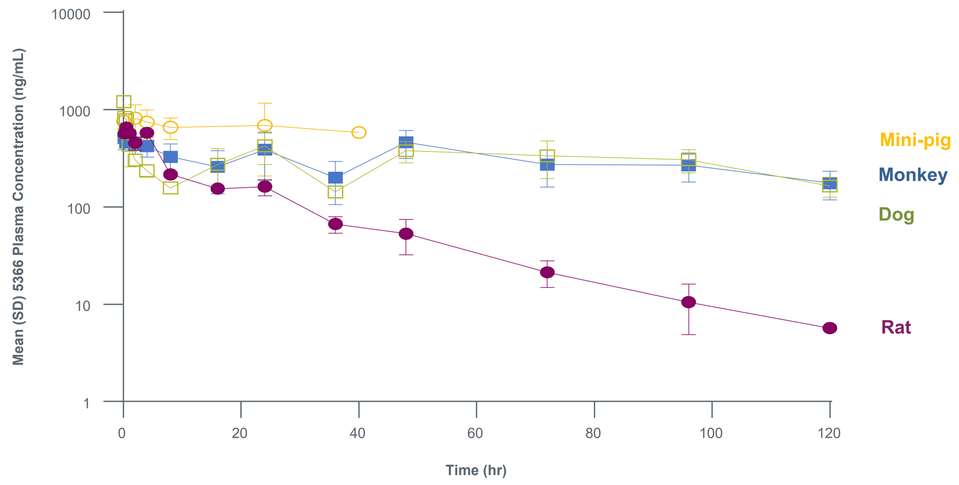
Table 1. Tissue Distribution of ABI-5366 after a single 15 mg/kg oral dose in Sprague-Dawley rats

Ratio $AUC_{last\text{-}tissue}/AUC_{last\text{-}plasma}$		
Tissue	ABI-5366	Pritelivir
Brain	0.041	0.027
Lung	0.704	0.335
Liver	1.70	0.824
Kidney	0.747	0.396
Heart	0.884	0.300
Bone marrow	0.399	0.158
Ganglia	0.125	0.062

- ABI-5366 distribution to rat brain and ganglion as well as all other tissues is comparable or greater than the distribution of pritelivir
- Distribution is high in the liver; moderate distribution was observed to the heart, lung, kidney, and bone marrow tissues

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

A. Plasma Concentration



- ABI-5366 had an extremely low clearance rate (CL = 0.0018-0.02 L/hr/kg) in all preclinical species tested (**Figure 3A**)
- Using allometric scaling, human PK modeling predicts ABI-5366 will have an extremely low human plasma clearance of 0.00086 L/hr/kg (**Figure 3B**)

B. Allometric Scaling

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

[a]Denotes predicted values using four species.

Table 2. ABI-5366 ADME Properties

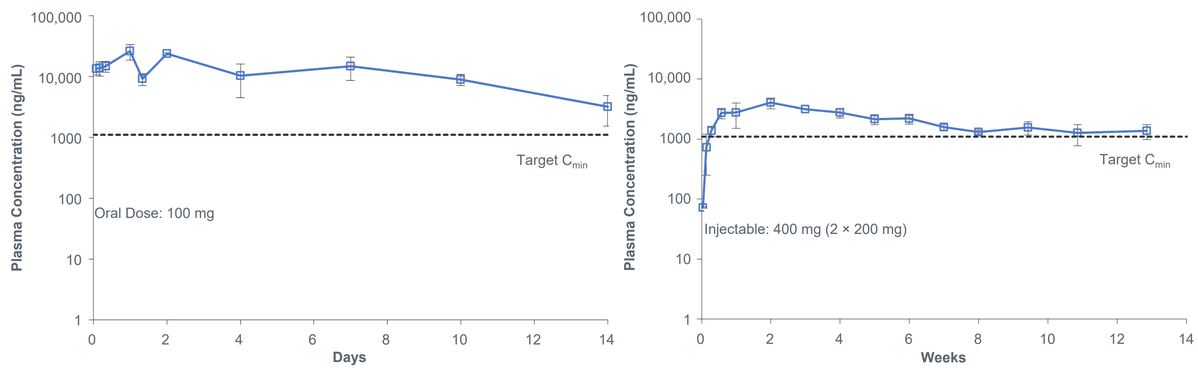
ADME Property	ABI-5366
LMs in h/r/d/m (% Remaining After 45 Minutes)	96/92/100/100
GSH Trapping in LMs (GSH Adduct Formation)	No GSH adduct
Plasma Protein Binding in h/r (% Bound)	99.8/99.8
CYP Inhibition at 10 µM (2C9/2D6/3A4M/3A4T), % Inhibition	31.5/−9/3.7/4.6
Time-Dependent Inhibition (1A2/2C9/2C19/2D6/3A4M),%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

- ABI-5366 demonstrates a favorable ADME profile, with no expected drug-drug interaction liabilities (**Table 2**)
- ABI-5366 exhibits excellent stability in rat, dog, cynomolgus monkey and human LMs, with no GSH adduct formation
- ABI-5366 does not inhibit any CYP450 enzymes tested and does not demonstrate time-dependent inhibition
- In vitro toxicology studies show ABI-5366 is not genotoxic and does not have mitochondrial toxicity, suggesting that ABI-5366 has low potential for off-target pharmacological effects

Figure 4. PK Profile After PO and IM Dosing

A. PK Profile After Single PO Dose of 100 mg in Dogs (N=3)

B. PK Profile After IM Dose of 400 mg in Dogs (N=3)



C_{min}, minimum blood plasma concentration; IM, intramuscular; PK, pharmacokinetic; PO, oral.

- In dog PK studies, an oral or injectable dose of ABI-5366 results in sustained therapeutic plasma concentrations for approximately 2 weeks and more than 3 months, respectively, demonstrating the long-acting potential of ABI-5366 (**Figure 4**)

Conclusions

- ABI-5366 potently inhibits both HSV-1 and HSV-2 replication and exhibits broad activity against HSV clinical isolates
- ABI-5366 targets UL5/UL52 and maintains potency against NA-resistant HSV mutants
- ABI-5366 has broad tissue distribution comparable or greater than pritelivir
- Low clearance is projected for ABI-5366
- ABI-5366 has favorable ADME properties and low potential for drug-drug interactions
- ABI-5366 has favorable oral and IM preclinical PK, demonstrating its long-acting potential
- These results support the clinical development of ABI-5366; a Phase 1a/1b study is ongoing
  - Phase 1a observations suggest $t_{1/2}$ ~ 20 days
  - Once weekly and once monthly doses are planned to be evaluated in Phase 1b

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Contact: mperron@assemblybio.com

DISCLOSURES

HC, **KS**, **DA**, **MS**, **ZZ**, **GS**, **YZ**, **HP**, **MAW**, **KK**, **MJP**, **WD**, and **RY** are employees and stockholders of Assembly Biosciences, Inc.

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