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

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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 United States and European Union, >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

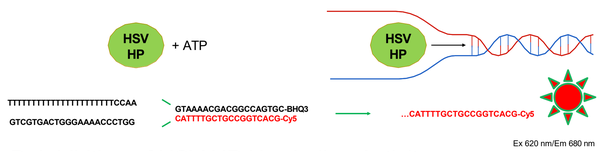
Methods

- **Biochemical unwinding assay:**
  - Recombinant UL5/UL52/UL8 from HSV-1 and HSV-2 (UL8 from HSV-1) was incubated with fluorescently labeled forked DNA substrate and ATP in the presence or absence of compound. IC_{50}s were determined by measuring the reduction in fluorescence signal
- **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 deep sequencing using gene-specific primers
- **Combination studies:**
  - Vero cells were infected with HSV-1 or HSV-2 and treated with various ratios of ABI-5366 and acyclovir for 5 days. Synergy analyses were performed using both CompuSyn[12] and MacSynergy II[13] software
- **Virus specificity assays:**
  - MRC-5 cells were infected with VZV (Oka strain), B95-8 cells were infected with EBV (B95 strain), and PBMCs were infected with HHV-6 (SF strain); all were treated with compounds. Viral replication levels were measured by qPCR, and EC_{50}s were calculated
  - MCR-5 cells were infected with hCMV (AD169 strain), HEp-2 cells were infected with RSV (A2 strain), and both were treated with compounds; antiviral activity was evaluated by CTG assay
  - HCV-1b replicon Huh7 cells were treated with compounds, and EC_{50}s were determined by reporter assay
  - HepG2–NTCP cells were infected with HBV from AD38 cells or HDV and treated with compounds, and EC_{50}s were determined by ELISA
  - RPTEC/TERT1 cells were infected with BK virus (MM strain) and treated with compounds. EC_{50}s were determined by immunofluorescence of SV-40 T antigen
- **Cytotoxicity assays:**
  - CC_{50}s were determined in 8 cell types/lines (NCI-H226, MOLT-4, HEK293, HeLa-H1, HepG2, Huh-7, PBMC, and MT-4) representative of different tissues. Log-phase cells were treated with compounds, and cell viability was measured using the CTG assay
- **Carbonic anhydrase (CA) esterase assay:**
  - CA activity in the presence or absence of compounds was determined in biochemical assays by monitoring accumulation of nitrophenolate at 405 nm, which colorimetrically tracks the hydrolysis of 4-nitrophenyl acetate over time
- **Pharmacokinetic (PK) studies:**
  - PK studies were performed in male beagle dogs (n=3). For oral dosing, 100 mg of ABI-5366 (2 units of 50 mg) 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 by LCMS

Results

Figure 1. ABI-5366 Biochemical Activity Against HSV HP Complex

A. ABI-5366 Biochemical Unwinding Assay



B. Inhibition of HSV HP Unwinding Activity

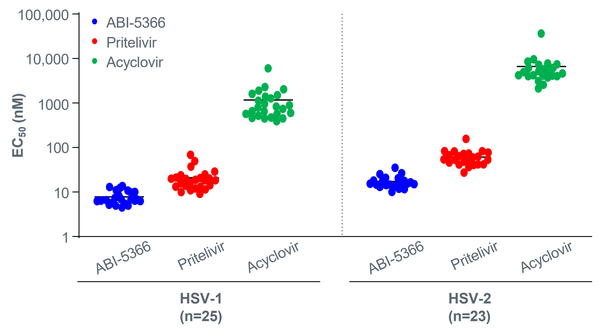
HPI	HSV-1 IC_{50} (nM)	HSV-2 IC_{50} (nM)
Pritelivir	11	30
ABI-5366	1.3	2.4

Em, donor molecule emission; Ex, acceptor molecule excitation; HP, helicase primase; HPI, HP inhibitor; IC_{50}, half-maximal inhibitory concentration.

- ABI-5366 exhibits approximately 10× more potent inhibition of HSV HP unwinding activity compared with pritelivir (**Figure 1**)
- Substrate competitive studies (ATP and DNA) indicate that ABI-5366 is a noncompetitive HPI

Figure 2. 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 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 3. 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.

- Cryo-EM data demonstrate that ABI-5366 is modeled into the density between UL5/UL52 from HSV-1 (data not shown)
- Resistance selection with ABI-5366 identified the K355N and K355R variants in the UL5 gene (**Figure 3A**), which are observed in pritelivir resistance selections[14]
- ABI-5366–resistant isolates remain sensitive to acyclovir
- ABI-5366 retains potency against an NA-resistant mutant (**Figure 3B**)

Figure 4. Combination Studies With ABI-5366 and Acyclovir

A. Combination Index (CompuSyn) Summary

Virus	Combination Index ED_{50}	ED_{75}	ED_{90}	ED_{95}	Synergism/ Antagonism
HSV-1	0.96	0.94	0.93	0.94	Nearly additive
HSV-2	1.01	0.93	0.87	0.84	Additive to slight synergism

B. MacSynergy II Summary

Virus	Values of Synergism (μM²%)	Values of Antagonism (μM²%)	Synergism/ Antagonism
HSV-1	59	−4	Minor synergy
HSV-2	50	−16	Minor synergy

$ED_{50/75/90/95}$, 50%/75%/90%/95% effective dose.

- CompuSyn analysis of ABI-5366 inhibition from constant ratios of ABI-5366 and acyclovir yields the indicated combination index values, with a nearly additive effect observed for HSV-1 and additive to slight synergistic effects for HSV-2 (**Figure 4A**)
- MacSynergy II analysis of the ABI-5366 and acyclovir combination is within the 95% confidence interval, with minor synergistic effects detected (**Figure 4B**)

Figure 5. ABI-5366 Exhibits Activity Specific to HSV and Is Generally Not Cytotoxic

A. EC_{50} and HSV Selectivity Index

Virus (Strain)	ABI-5366 EC_{50} (μM)	HSV-1 Selectivity Index	HSV-2 Selectivity Index
VZV (Oka)	5	270	490
EBV (B95)	>10	>550	>1010
HHV-6B (SF)	>10	>550	>1010
HCMV (AD169)	>20	>1100	>2020
BK (MM)	14	760	1400
RSV (A2)	>20	>1100	>2020
HCV (1b)	>20	>1100	>2020
HBV (AD38)	2	103	190
HDV	7	404	742

B. Cytotoxicity Assay in Multiple Cell Lines

Cell Lines	ABI-5366 CC_{50} (μM)
NCI-H226	>30
MOLT-4	15 ± 7
HEK293	>30
HeLa-H1	>30
HepG2	>30
Huh-7	>30
PBMC	>30
MT-4	>30

EC_{50}s are mean, and CC_{50}s are mean or mean ± SD.
CC_{50}, half-maximal cytotoxic concentration; EC_{50}, half-maximal effective concentration; SD, standard deviation.

- ABI-5366 antiviral activity is specific for HSV, with limited to no activity against a panel of human viruses, including other herpesviruses (**Figure 5A**)
- ABI-5366 has a CC_{50} >15 μM for a variety of cell types that results in an HSV-1 and HSV-2 selectivity index of >829 and >1500, respectively (**Figure 5B**)

Figure 6. No Off-Target Effects of ABI-5366 Are Observed

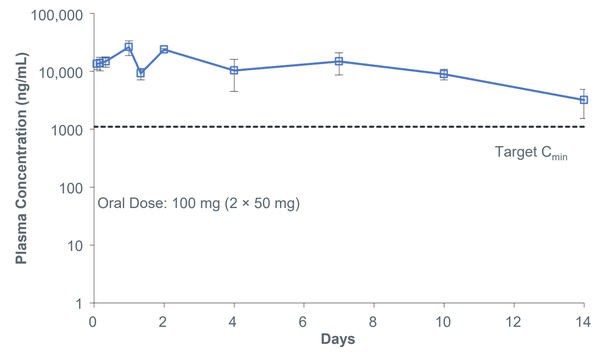
CA Esterase	ABI-5366 IC_{50} (μM)	Selectivity index (HSV-1/HSV-2)	Pritelivir IC_{50} (μM)	Selectivity index (HSV-1/HSV-2)	Acetazolamide[a] IC_{50} (μM)
CAI	2.6 ± 0.6	2015/1092	5.3 ± 0.2	498/175	0.03 ± 0.012
CAII	1.4 ± 0.2	1077/583	3.1 ± 1.5	288/101	0.02 ± 0.002
CAVII	6.8 ± 2.0	5192/2813	8.4 ± 1.1	794/279	0.09 ± 0.002

IC_{50}s are mean ± SD.
[a]Assay positive control; acetazolamide is a well-known CA inhibitor and contains sulfonamide pharmacophore.
CA, carbonic anhydrase; IC_{50}, half-maximal inhibitory concentration; SD, standard deviation.

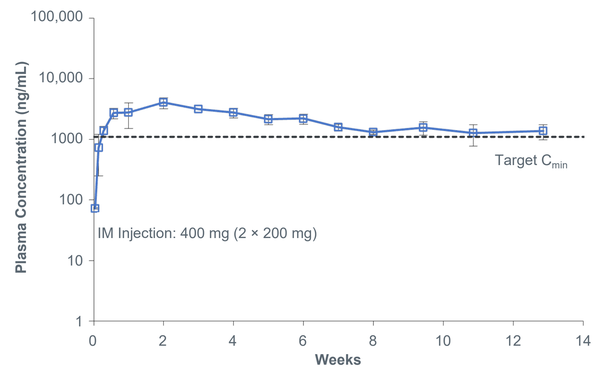
- No off-target effects of ABI-5366 are observed *in vitro*
  - ABI-5366 inhibits HSV over potential human off-target CAs I, II, and III with a selectivity index of >500 (**Figure 6**)
- A favorable safety profile of ABI-5366 is observed in rats and dogs in 28-day oral toxicity studies, with high safety margins relative to the predicted human equivalent dose (data not shown)

Figure 7. PK Profile in Preclinical Species

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 7**)

Conclusions

- ABI-5366 is a small-molecule inhibitor of HSV helicase-primase enzyme complex
- ABI-5366 potently inhibits both HSV-1 and HSV-2 replication and exhibits broad activity against HSV clinical isolates
- ABI-5366 has a favorable safety profile with minimal potential for off-target effects
- Oral and IM preclinical PK studies with ABI-5366 demonstrate the long-acting potential of ABI-5366
- These results support the clinical development of ABI-5366, and a Phase 1a/1b study is ongoing

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DISCLOSURES

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