Preclinical Characterization of ABI-1179, a Potent Helicase Primase Inhibitor for the Treatment of Recurrent Genital Herpes

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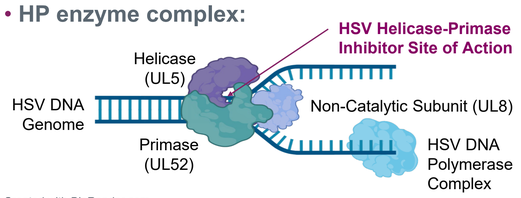
Introduction

- Recurrent genital herpes (RGH) is a sexually transmitted disease caused predominantly by herpes simplex virus type 2 (HSV-2)[1,2]
  - People living with RGH can suffer from painful recurring genital ulcers and psychological distress[1,2]
- Current standard-of-care (SOC) treatment is limited to nucleoside analogues (NAs; eg, acyclovir), which are only partially effective in preventing recurrences and require daily dosing[3,4]
- Targeting the HSV helicase-primase (HP) enzyme complex is a clinically validated mechanism (pritelivir) capable of further reducing HSV shedding rates and lesions compared with SOC[5,6]
- ABI-1179 is a promising long-acting oral HP inhibitor (HPI) with potent anti-HSV activity
- **HP enzyme complex:**



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Methods

- **Helicase unwinding assay:**
  - Recombinant UL5/UL52/UL8 from HSV-1 and HSV-2 (UL8 from HSV-1) was incubated at room temperature with fluorescently labeled forked DNA substrate in the presence or absence of compound. Reactions were initiated by the addition of ATP. IC_{50}s were determined by measuring the reduction in fluorescence signal
- **HSV and clinical isolate antiviral assays:**
  - Retinal epithelial (ARPE-19), human keratinocyte (HaCat), and neonatal human dermal fibroblast (NHDF) cells were infected with either HSV-1 or HSV-2 and treated with compound. HSV DNA EC_{50}s were measured by qPCR using gene-specific primers
- **Viral resistance determination:**
  - Vero cells infected with HSV-1 or HSV-2 were treated with escalating doses of compound until presence of full cytopathic effect (CPE). The cells and supernatant were processed for deep sequencing using gene-specific primers
- **Phenotypic assessment of resistant mutations:**
  - A bacmid encoding HSV-2 MS strain with an mCherry reporter was used to generate mutant constructs via *en passant* mutagenesis.[7] Cellular mCherry signal was used to determine EC_{50}s following infection of ARPE-19 cells with recombinant viruses
- **Carbonic anhydrase (CA) hydratase assay:**
  - The potency of ABI-1179 against CAI and CAII was determined in an absorbance-based assay monitoring the CO_2 hydratase activity of the CA as previously described[8]
- ***In vivo* efficacy study:**
  - Guinea pigs were vaginally infected with HSV-2, and acute disease was allowed to resolve. At 14 days post-infection, animals were given chow formulated with ABI-1179 (0.04% weight/weight) or left untreated (15 animals per group). Animals were examined for genital skin disease and scored 5 days a week throughout the study using a lesion scoring scale from 0 to 4, where 0 represents no disease and 4 represents severe disease

Results

Figure 1. ABI-1179 Potently Inhibits the DNA Unwinding Activity of the HSV HP Complex

Compound	IC_{50} (nM)		$K_{i,\ app}$ (nM)	
	HSV-1	HSV-2	HSV-1	HSV-2
ABI-1179	0.17 ± 0.05	0.16 ± 0.07	0.03 ± 0.02	0.03 ± 0.01
Pritelivir	11 ± 3	30 ± 6	5 ± 1	8 ± 0

Data are mean ± SD. IC_{50}s for ABI-1179 are near the assay's lower limit.
IC_{50}, half-maximal inhibitory concentration; $K_{i,\ app}$, inhibitor constant, apparent; SD, standard deviation.

- ABI-1179 is a highly potent inhibitor of HSV-1 and HSV-2 HP complexes ($K_{i,\ app}$ <0.05 nM) compared with pritelivir ($K_{i,\ app}$ 5–8 nM; **Figure 1**)

Figure 2. ABI-1179 Demonstrates Low Potential for Off-Target CA Inhibition

Compound	CO_2 Hydratase IC_{50} (nM)	
	CAI	CAII
ABI-1179	>100,000	6600 ± 750
Pritelivir	451 ± 170	1800 ± 194
Acetazolamide[a]	354 ± 54	15 ± 2

The data are mean ± SD of at least 3 independent experiments done with 10 replicates.
[a]Assay positive control; acetazolamide is a well-known CA inhibitor and contains sulfonamide pharmacophore.
IC_{50}, half-maximal inhibitory concentration; SD, standard deviation.

- ABI-1179 does not inhibit CAI, whereas pritelivir inhibits CAI with an IC_{50} of 451 nM (**Figure 2**)
- ABI-1179 also displays weaker CAII inhibition than pritelivir (**Figure 2**)
- Inhibition of CAs is not anticipated at the projected human efficacious dose

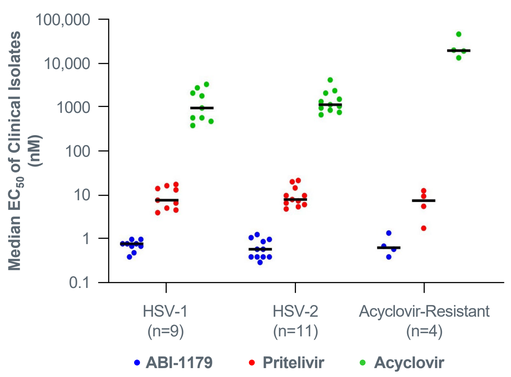
Figure 3. ABI-1179 Is a Potent Inhibitor of HSV-1 and HSV-2 Laboratory Strains

Virus (Strain)	Compound	ARPE-19 EC_{50} (nM)	HaCat EC_{50} (nM)	NHDF EC_{50} (nM)
HSV-1 (KOS)	ABI-1179	0.95 ± 0.15	-	-
	Acyclovir	2410 ± 390	-	-
HSV-2 (MS)	ABI-1179	1.07 ± 0.30	1.27 ± 0.13	0.89 ± 0.23
	Acyclovir	3620 ± 1400	224 ± 80	161 ± 24

Data are mean ± SD.
ARPE-19, human retinal epithelial cells; EC_{50}, half-maximal effective inhibitory concentration; HaCat, human keratinocytes; NHDF, neonatal human dermal fibroblasts; SD, standard deviation.

- ABI-1179 has potent antiviral activity against HSV-1 and HSV-2 replication in ARPE-19 cells. Similar potency is observed in other physiologically relevant cell lines (**Figure 3**)
- ABI-1179 is >2500-fold more potent than acyclovir in ARPE-19 cells against HSV-1 and HSV-2, and >150-fold more potent against HSV-2 in HaCat and NHDF cells

Figure 4. ABI-1179 Is a Potent Inhibitor of HSV-1 and HSV-2 Clinical Isolates

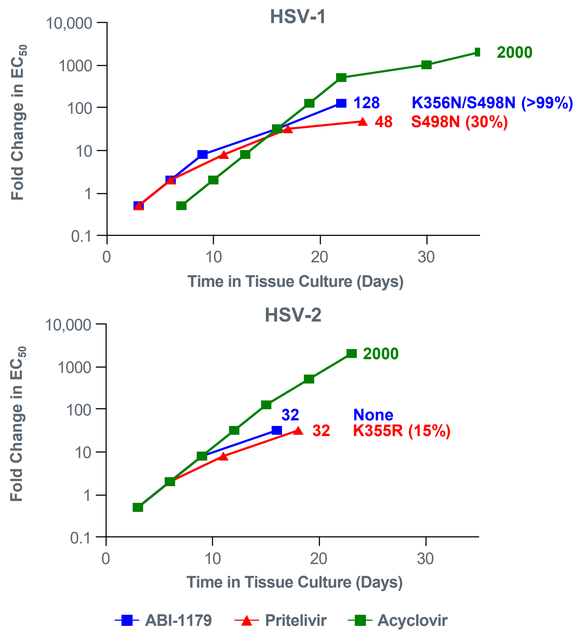


n denotes the number of clinical isolates tested. Each point represents an individual isolate and the horizontal line depicts the median EC_{50} across all clinical isolates tested.
EC_{50}, half-maximal effective inhibitory concentration.

- ABI-1179 is active against HSV-1 and HSV-2 clinical isolates, including those with reduced susceptibility to acyclovir (**Figure 4**)
- ABI-1179 is >12-fold more potent than pritelivir against HSV-1 and HSV-2 clinical isolates and >1500-fold more potent than acyclovir
- All acyclovir-resistant HSV isolates tested remain susceptible to ABI-1179

Figure 5. ABI-1179 Has a High Barrier to Resistance *In Vitro*

A. Dose-Escalation Summary for HSV-1 and HSV-2



B. Genotyping of HSV-1 and HSV-2 From Resistance Selection

Gene/Protein	ABI-1179 22 Days (128× EC_{50})	Pritelivir 24 Days (48× EC_{50})	DMSO 20 Days
HSV-1			
UL5/helicase	K356N (>99%) S498N (>99%)	S498N (29.5%) V662I (72.8%)	- V662I (25.1%)
HSV-2			
UL5/helicase	-	K355R (14.5%)	-

In Panel A, frequencies of variants detected in ABI-1179– and pritelivir-treated cultures (≥15%) are indicated in parentheses. In Panel B, variant frequency (%) is compared with unpassaged virus.
DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective inhibitory concentration.

- ABI-1179 has a higher barrier to resistance for both HSV-1 and HSV-2 populations passaged in Vero cells compared with acyclovir (**Figure 5A**)
- The K356N and S498N variants of HSV-1 UL5 are present at the highest ABI-1179 passage concentration tested (128-fold EC_{50}; **Figure 5B**)
- For HSV-2, there are no variants in target genes UL5 and UL52 at the highest concentration of ABI-1179 tested (32-fold EC_{50}) that produced CPE (**Figure 5B**)
- Resistance selection data suggest that ABI-1179 binds at the UL5/UL52 interface, consistent with Cryo-EM structure data (not shown)

Figure 6. ABI-1179 Is More Resilient to Binding Site Variations than Pritelivir

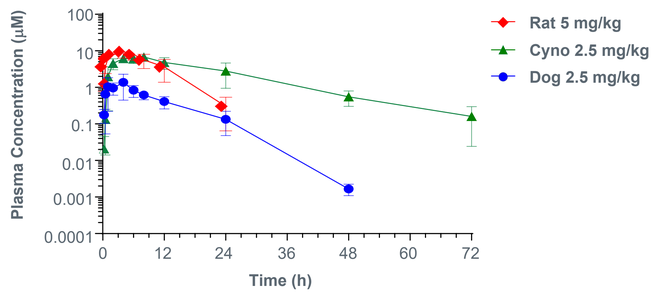
Constructs	EC_{50} (nM) [Fold Change From Wild Type]	
	ABI-1179	Pritelivir
Wild type	0.9	8.2
UL52 A906V	2.3 [3]	377 [46]
UL5 K355N	268 [306]	>2000 [>243]
UL5 K355T	10.7 [12]	562 [68]
UL5 K355R	2.2 [3]	319 [39]
UL5 L805I	1.4 [2]	22.2 [3]
UL5 S497N	2.4 [3]	22.5 [3]
UL5 K355R + UL5 L805I	>1000 [>1111]	>122,000 [>14,878]
UL5 K355R + UL5 L805I + UL52 A906V	>64,000 [>71,111]	>122,000 [>14,878]
UL5 K355N + UL5 S497N	>64,000 [>71,111]	>122,000 [>14,878]

EC_{50}, half-maximal effective inhibitory concentration; UL5, helicase; UL52, primase.

- Phenotypic assessment of UL5-K355 helicase variants, including those identified *in vitro* and in the clinic, reveals modest potency shifts for ABI-1179 compared with pritelivir (**Figure 6**)
- HSV-2 double and triple mutants display a high level of resistance against ABI-1179 and pritelivir (**Figure 6**)

Figure 7. ABI-1179 Has a Favorable Oral PK Profile in Preclinical Species, Which Supports Once-Weekly Oral Dosing

A. ABI-1179 Plasma Exposure in Preclinical Species



B. Rat and Human Plasma Protein Binding

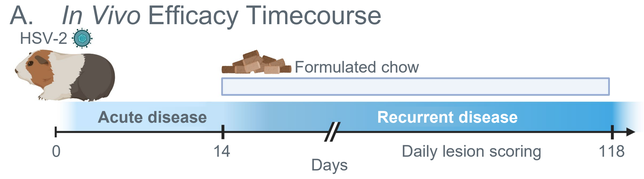
Species	%Free in Plasma (n=3)	*In Vitro* ^{3}H Hepatocyte Clearance (L/h/kg)	*In Vivo* Blood Clearance (L/h/kg)	Restriction Factor
Rat	0.34 ± 0.07	0.950 ± 0.087	0.139	6.8
Human	0.24 ± 0.04	0.051 ± 0.005	(0.0053)	(9.6)

Data are mean ± SD for plasma concentration, %free in plasma, and *in vitro* clearance; mean for *in vivo* clearance. Parentheses indicate projected value. Plasma free fraction data were generated with Dianorm equilibrium dialysis device.
Cyno, cynomolgus monkey; restriction factor, ratio of the *in vitro* predicted clearance to the observed *in vivo* clearance; SD, standard deviation.

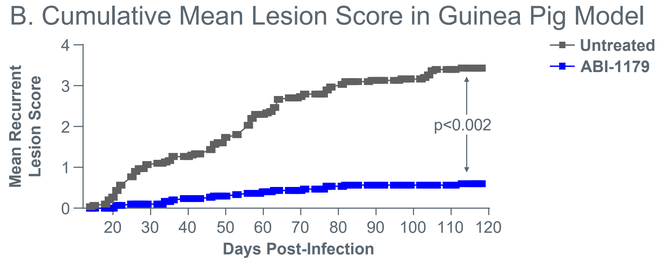
- *In vivo* systemic clearance is lower than *in vitro* predicted clearance in nonclinical species (**Figure 7A**)
- ABI-1179 shows a similar unbound fraction between rat and human plasma; therefore, the restriction factor observed in rats is used for human PK projections (**Figure 7B**)
- A once-weekly 250-mg dose of ABI-1179 is projected to achieve efficacious coverage in humans

Figure 8. ABI-1179 Reduces the Number of HSV Lesions in the Guinea Pig Model of Recurrent HSV Infection

A. *In Vivo* Efficacy Timecourse



B. Cumulative Mean Lesion Score in Guinea Pig Model



PK sampling at 21, 49, 77, and 105 days post-infection. ABI-1179 (0.04% weight/weight) plasma concentrations remain 8-fold greater than the guinea pig protein-adjusted EC_{95} (133 nM).
EC_{95}, 95% effective inhibitory concentration.

- Following latency establishment, ABI-1179 significantly reduces the development of lesions in a guinea pig model of recurrent HSV infection when treated with formulated chow at therapeutically relevant concentrations (**Figure 8**)

Conclusions

- ABI-1179 targets the HSV helicase-primase complex and is a potent inhibitor of HSV replication across clinical isolates or laboratory strains with a high barrier to resistance
- In a preclinical model of HSV recurrent disease, ABI-1179 significantly reduces the number of HSV lesions
- ABI-1179 demonstrates a favorable PK profile with a projected human oral dose of 250 mg, once weekly
- A Phase 1a/1b first-in-human study with ABI-1179 is planned to start in the second half of 2024

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DISCLOSURES

AC, **VN**, **LM**, **PYH**, **XZ**, **KW**, **JM**, **ALB**, **EA**, **QY**, **AS**, **AN**, **CR**, **HG**, **TW**, **RM**, **MMH**, **SPF**, and **AM** are employees and stockholders of Gilead Sciences, Inc. **HC** and **RY** are employees and stockholders of Assembly Biosciences, Inc. **FJB** has nothing to disclose. **DIB** is a consultant for Innovac Therapeutics.