

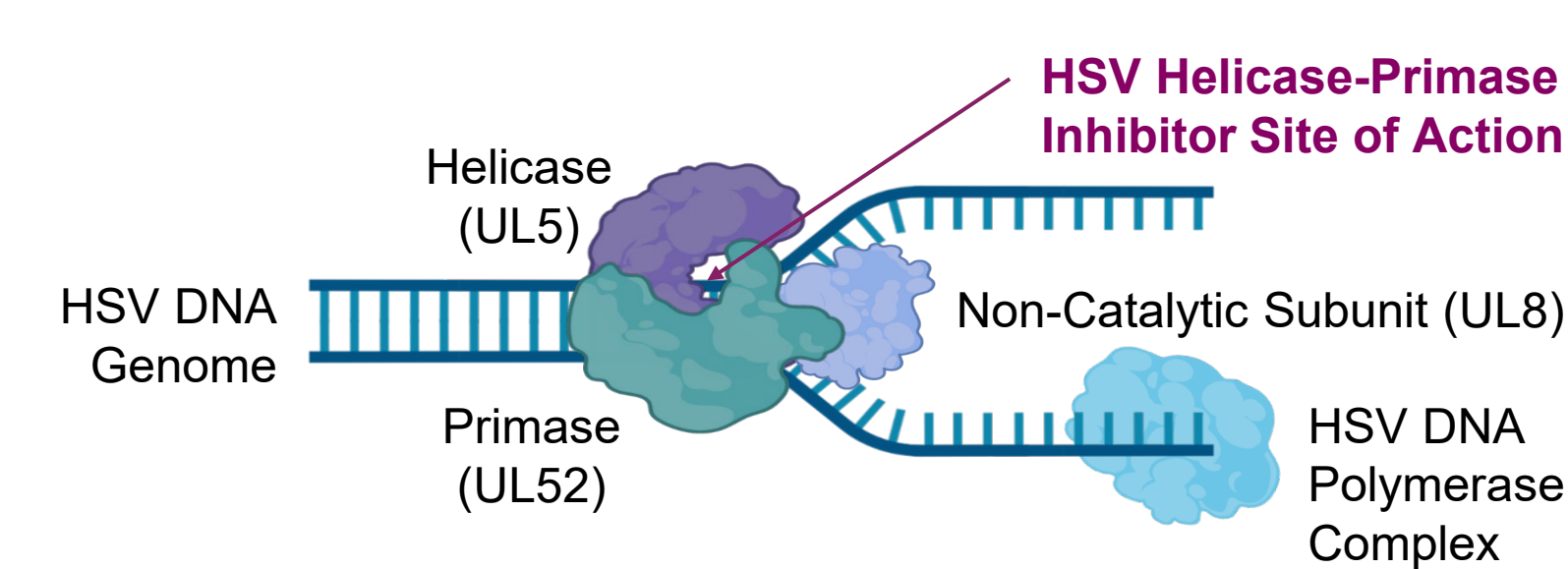
Preclinical Characterization of ABI-1179, a Potent Helicase Primase Inhibitor for the Treatment of Recurrent Genital Herpes

Aesop Cho¹, Varsha Nair¹, Lindsey May¹, Pui Yan Ho¹, Xiaolin Zhang¹, Kelly Wang¹, Judy Mwangi¹, Ariel Lewis-Ballester¹, Emma Abernathy¹, Fernando J. Bravo³, David I. Bernstein³, Qing Yao¹, Heidi Contreras², Ran Yan², Michel Perron², Annapurna Sapre¹, Arabinda Nayak¹, Christopher Richards¹, Hamed Ghaffari¹, Ting Wang¹, Roberto Mateo¹, Meghan M. Holdorf¹, Simon P. Fletcher¹, Alexandre Mercier¹

¹Gilead Sciences, Inc., Foster City, CA, USA; ²Assembly Biosciences, Inc., South San Francisco, CA, USA; ³Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, OH, USA

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:**



Created with BioRender.com.

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. IC₅₀s 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 EC₅₀s 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 EC₅₀s 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 CO₂ hydratase activity of the CA as previously described⁸
- 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. IC₅₀s for ABI-1179 are near the assay's lower limit. IC₅₀: 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.

^aAssay positive control; acetazolamide is a well-known CA inhibitor and contains sulfonamide pharmacophore. IC₅₀: half-maximal inhibitory concentration; SD, standard deviation.

- ABI-1179 does not inhibit CAI, whereas pritelivir inhibits CAI with an IC₅₀ 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

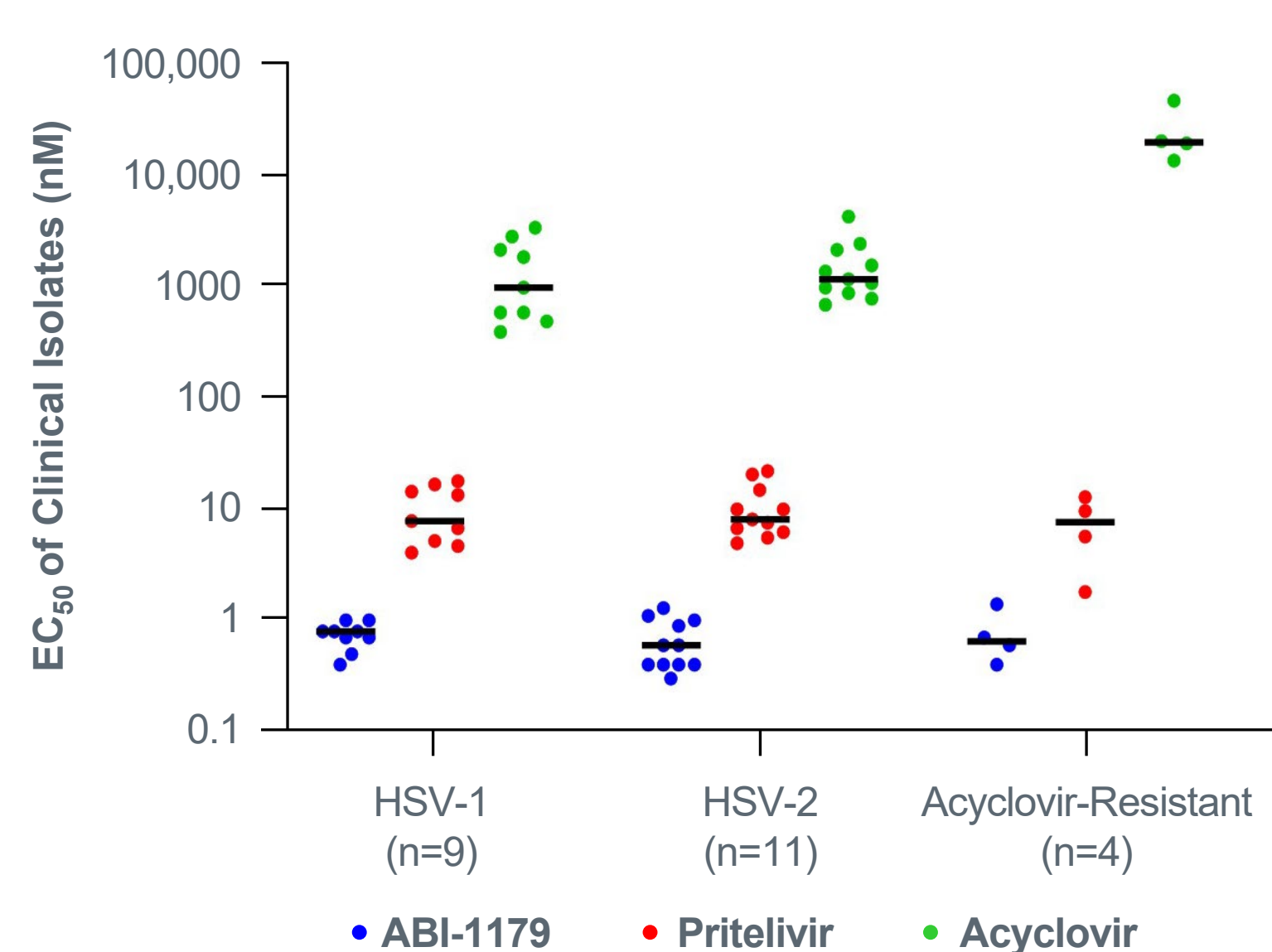
Figure 3. ABI-1179 Is a Potent Inhibitor of HSV-1 and HSV-2 Laboratory Strains

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; EC₅₀, 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

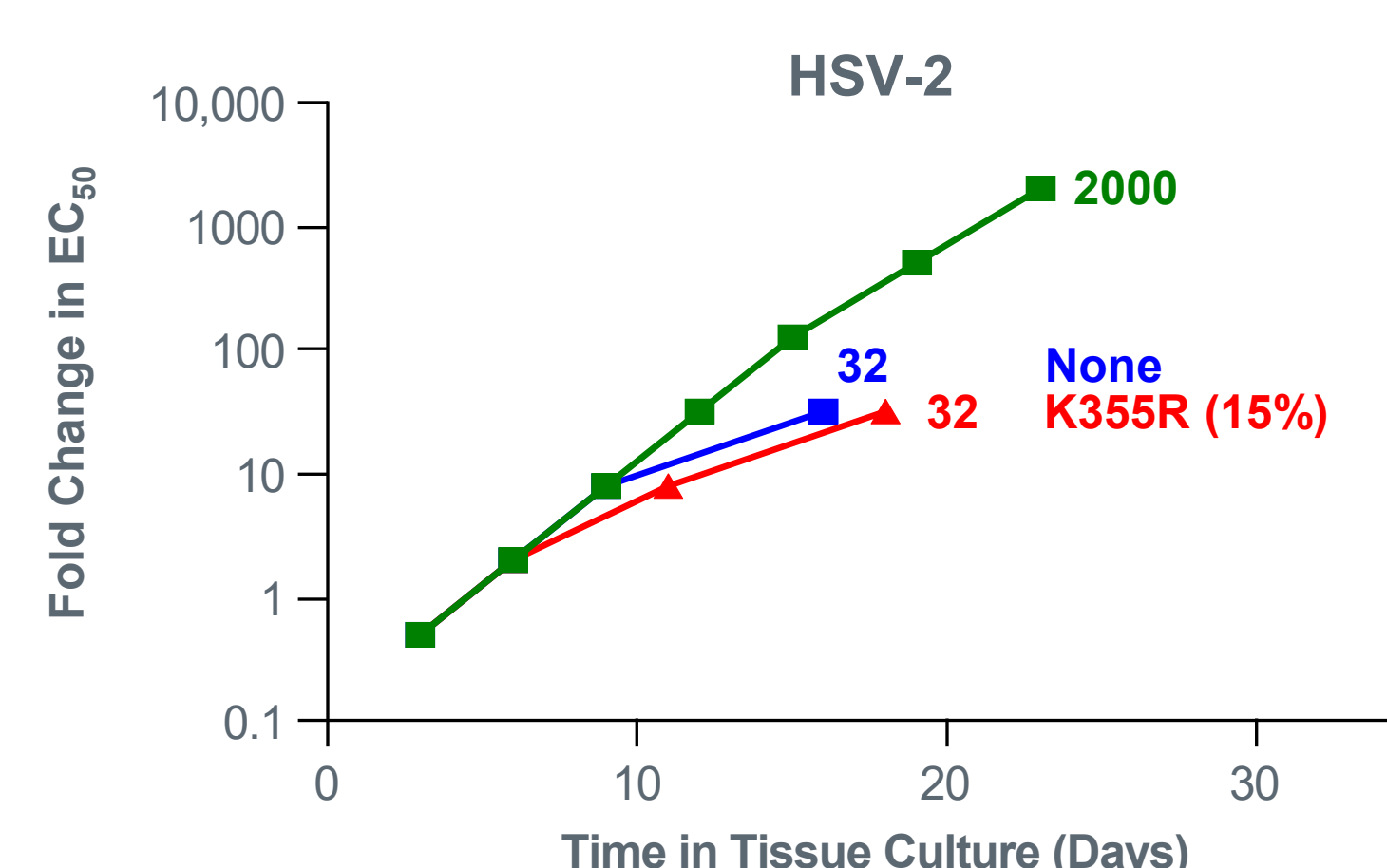
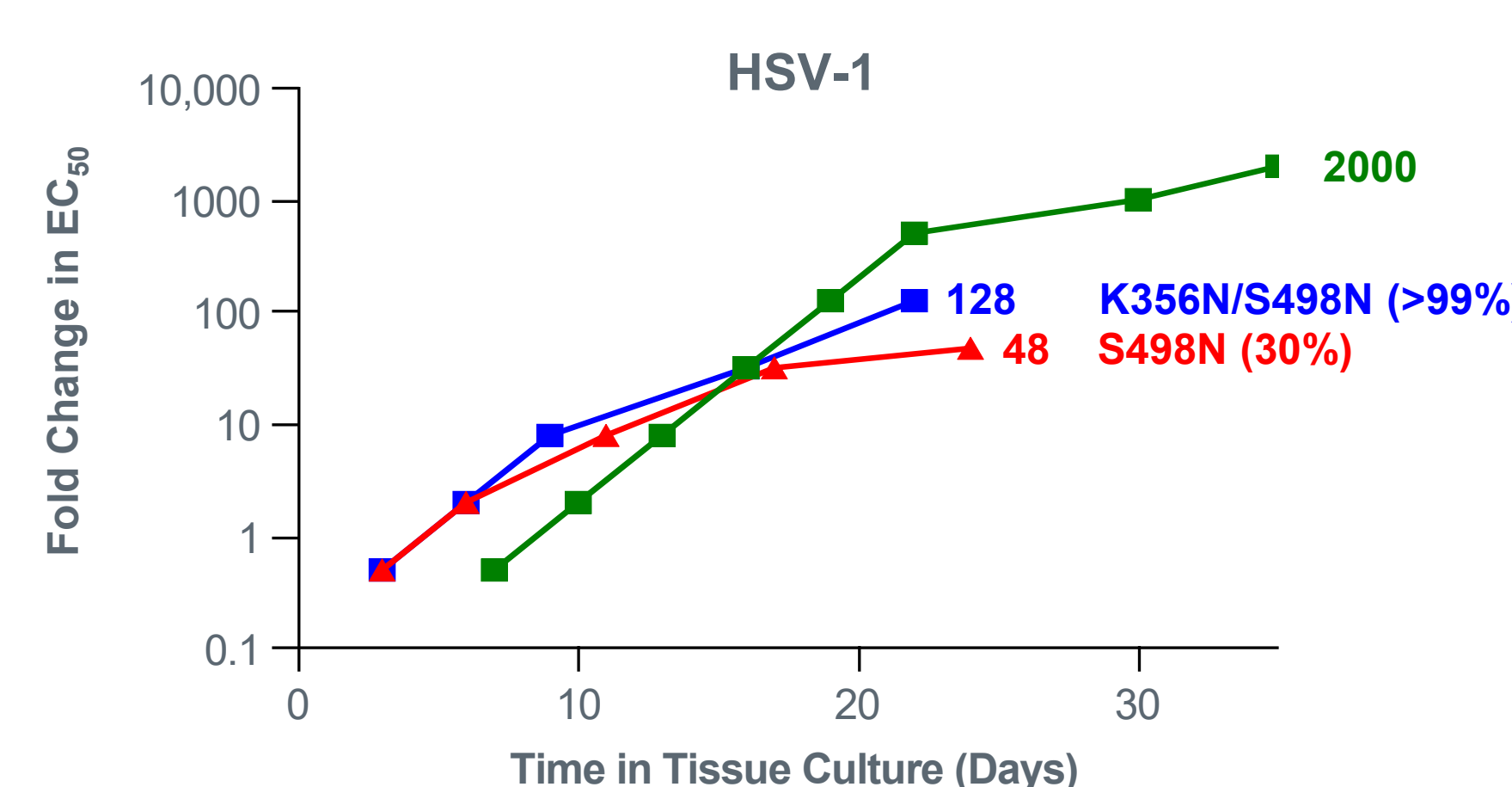


n denotes the number of clinical isolates tested. Each point represents an individual isolate and the horizontal line depicts the median EC₅₀ across all clinical isolates tested. EC₅₀, half-maximal effective inhibitory concentration.

- 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	HSV-1		DMSO 20 Days
	ABI-1179 22 Days (128× EC ₅₀)	Pritelivir 24 Days (48× EC ₅₀)	
UL5/helicase	K356N (>99%)	S498N (29.5%)	-
	S498N (>99%)	V662I (72.8%)	V662I (25.1%)
	-	-	-
Gene/Protein	HSV-2		DMSO 20 Days
	ABI-1179 16 Days (32× EC ₅₀)	Pritelivir 18 Days (32× EC ₅₀)	
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; EC₅₀, 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 EC₅₀; **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 EC₅₀) 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

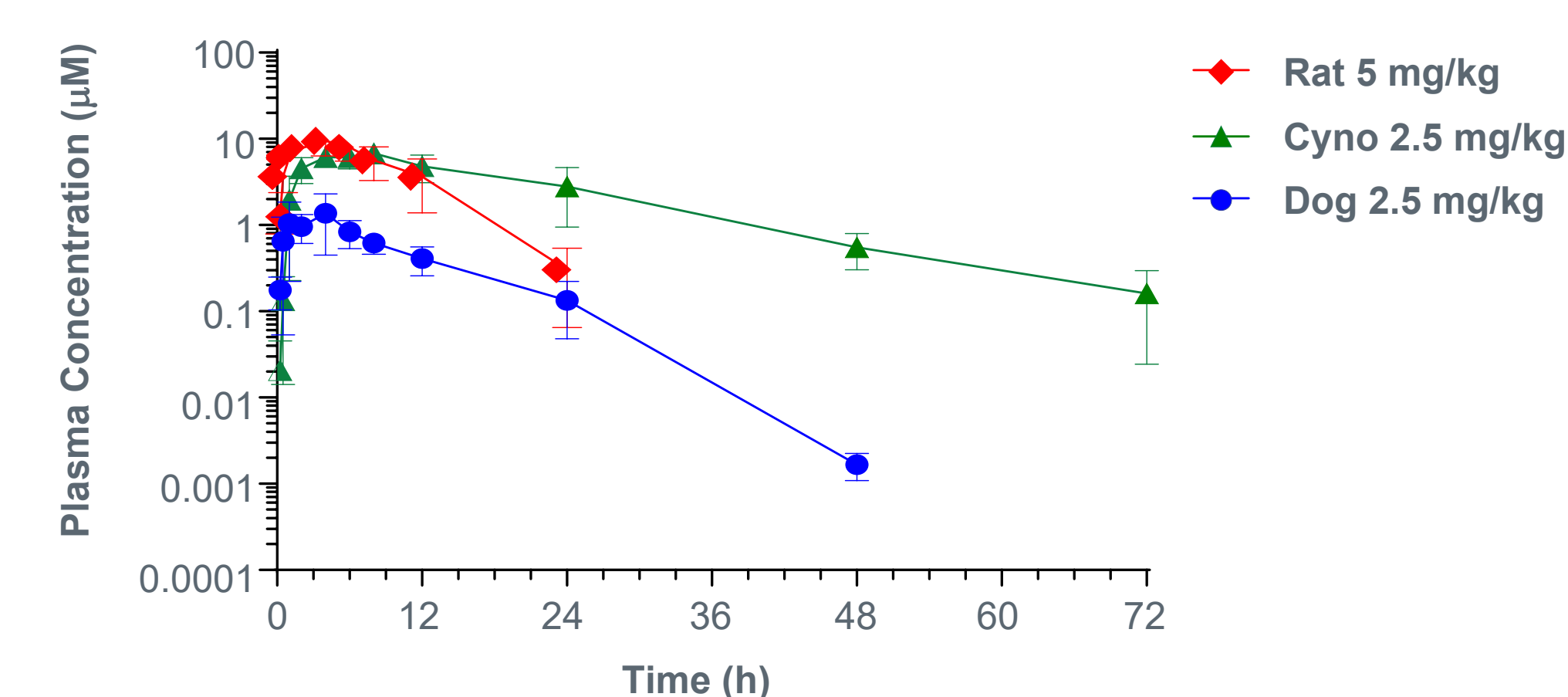
Constructs	EC ₅₀ (nM) [Fold Change From Wild Type]	
	ABI-1179	Pritelivir
Wild type	0.9	8.2
UL52 A906V	2.3 [3]	377 [46]
UL5 K355N	268 [306]	>2000 [243]
UL5 K355T	10.7 [12]	562 [68]
UL5 K355R	2.2 [3]	319 [39]
UL5 L805I	1.4 [2]	22.2 [3]
UL5 S497N	2.4 [3]	22.5 [3]
UL5 K355R + UL5 L805I	>1000 [1111]	>122,000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

EC₅₀, 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

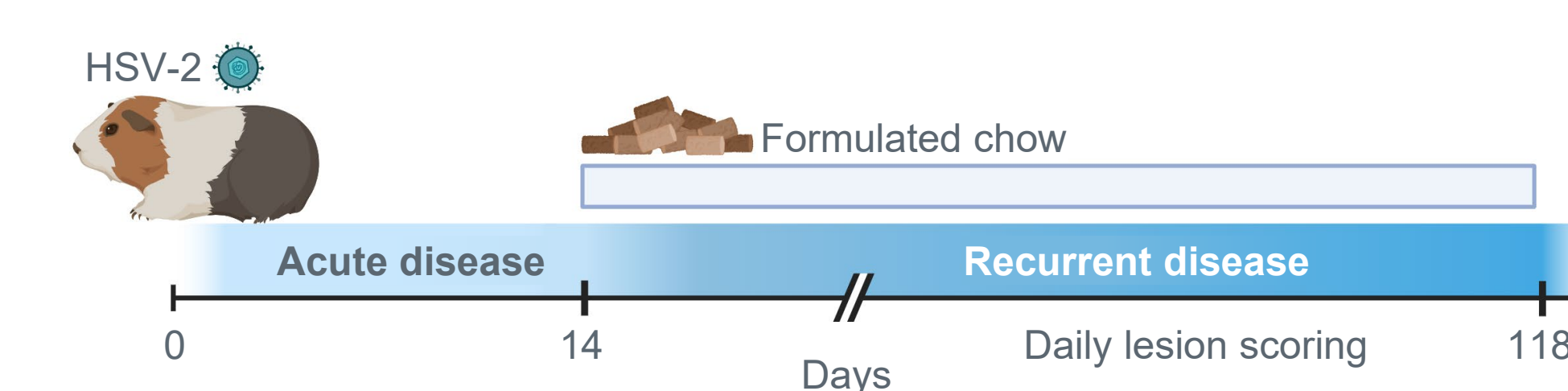
Species	%Free in Plasma (n=3)	In Vitro ³ H Hepatocyte Clearance (L/h/kg)	In Vivo Blood Clearance (L/h/kg)	Restriction Factor
Rat	0.34 ± 0.07	0.950 ± 0.087	0.139	6.8
Human	0.24 ± 0.04	0.051 ± 0.005	(0.0053)	(9.6)

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.

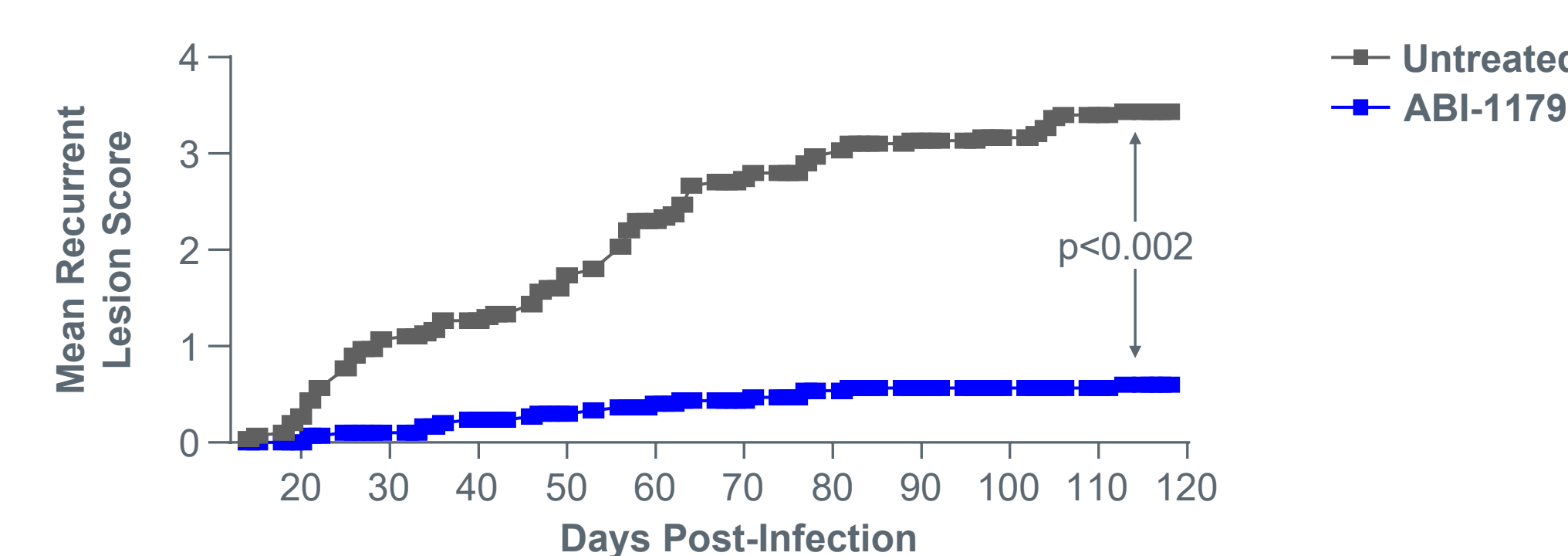
- In vivo* systemic clearance is lower than *in vitro* predicted clearance in nonclinical species (**Figure 7A**)
- ABI-1179 shows a similar unbound fraction between rat and human plasma; therefore, the restriction factor observed in rats is used for human PK projections (**Figure 7B**)
- A once-weekly 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 EC₉₅ (133 nM). EC₉₅, 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 in preclinical species
- In a Phase 1a study in healthy volunteers, a plasma half-life for ABI-1179 of approximately 4 days was observed, which supports the potential for a once weekly oral dosing regimen
- Preparation for a Phase 1b study in participants with RGH is underway

REFERENCES

- WHO herpes simplex virus detailed fact sheet. Last revised April 5, 2023. <https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus>.
- Gupta R, et al. *Lancet*. 2007;370(9605):2127-37.
- Reitano M, et al. *J Infect Dis*. 1998;178(3):603-10.
- Valtrex (valacyclovir). US package insert. GlaxoSmithKline; revised 2021.
- Shiraki K, et al. *Viruses*. 2021;13(8):1547.
- Wald A, et al. *JAMA*. 2016;316(23):2495-503.
- Kropp KA, et al. *J Virol*. 2020;94(20):e01370-20.
- Carta F, et al. *J Med Chem*. 2017;60(7):3154-64.

ACKNOWLEDGEMENTS

Writing and editorial assistance were provided by Sylvia Stankov, PhD, of Red Nucleus, and were funded by Assembly Biosciences, Inc. This study was sponsored by Gilead Sciences, Inc. Contact: mperron@assemblybio.com

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, RY and MP are employees and stockholders of Assembly Biosciences, Inc. FJB has nothing to disclose. DIB is a consultant for Inovavio Therapeutics.