ABI-5366 and ABI-1179, Two Potent, Long-Acting Helicase Primase Inhibitors for the Treatment of Recurrent Genital Herpes

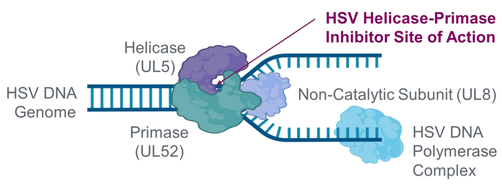
Heidi Contreras[1], Ran Yan[1], Michael Shen[1], Lewyn Li[1], Gene Schulze[1], Mark Bures[1], Xiaolin Zhang[2], Ting Wang[2], Qing Yao[2], Arabinda Nayak[2], Xinchao Yu[2], Ed Gane[3], Christian Schwabe[4], Grace Wang[1], Jieming Liu[1], Steven J. Knox[1], Kathryn M. Kitrinos[1], Katie Zomorodi[1], Anuj Gaggar[1], Michel Perron[1], William Delaney[1]

1. Assembly Biosciences, Inc., South San Francisco, CA, USA; 2. Gilead Sciences, Inc., Foster City, CA, USA; 3.University of Auckland, Auckland, New Zealand; 4. New Zealand Clinical Research, Auckland, New Zealand

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, an estimated >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 suppressive therapies for RGH, 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] (**Figure 1**)
  - 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 and ABI-1179 are promising investigational long-acting oral HPIs with potential anti-HSV activity

Figure 1. The HSV Helicase-Primase Enzyme Complex



Created with Biorender.com. HSV, herpes simplex virus.

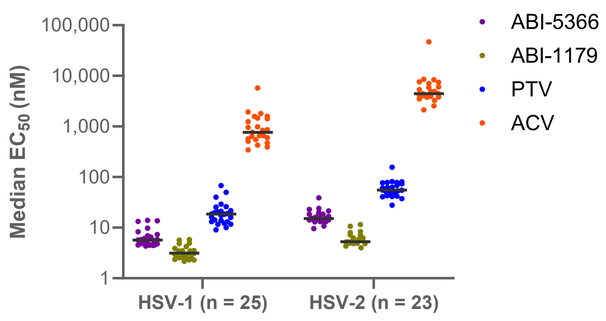
Methods

- **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)
- **Carbonic anhydrase assay:**
  - Compounds were incubated with recombinant carbonic anhydrases I (CAI) or II (CAII). For esterase activity, the reaction was initiated with the addition of equal volume of 4 mM of 4-nitrophenyl acetate for 1 hour incubation at room temperature, followed by measurement of absorbance at 405 nm. Data were normalized to a positive (AZA, 100% inhibition) and a negative (vehicle, 0% inhibition) control
- **Preclinical Pharmacokinetic (PK) studies:**
  - Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys were dosed intravenously (IV) with 0.1 to 1 mg/kg ABI-5366, and ABI-5366 plasma levels were monitored for up to 120 hours.
- **Sequence homology studies:**
  - Human PIF1 helicase domain (residues 203-618) amino acid sequence was aligned with HSV-1 UL5 sequence based on a structural alignment of PIF1 (PDB: 6HPT) and HSV-1 UL5 in PyMol.
- **Preclinical Toxicology studies:**
  - Sprague-Dawley or Wistar (Han) rats, beagle dogs or cynomolgus monkeys were dosed with ABI-5366 (0-300 mg/kg/day in rats and 0-30 mg/kg/day in dogs) or ABI-1179 (0-1000 mg/kg/day in rats and 0-300 mg/kg/day in monkeys) for 28-days in a standard GLP toxicity studies.
  - Standard ICH GLP *in vitro* and *in vivo* genetic toxicology and safety pharmacology studies were conducted with ABI-5366 and ABI-1179.
- **Phase 1a Clinical study:**
  - Single-dose, dose escalation studies in healthy subjects were conducted for ABI-5366 (NCT06385327) and ABI-1179 (NCT06698575). In each study, each dose level enrolled 8 subjects (6 active, 2 placebo). After single dose administration, subjects underwent routine safety follow-up evaluations with characterization of PK profiles (**Figure 5**).

Results

Figure 2. ABI-5366 and ABI-1179 Exhibit Broad Activity Against HSV-1 and HSV-2 Clinical Isolates

A. Clinical Isolate Sensitivity



Horizontal lines indicate the mean; ACV, acyclovir; PTV, pritelivir; EC_{50}, half-maximal effective concentration

Table 1. Mean Antiviral Activity

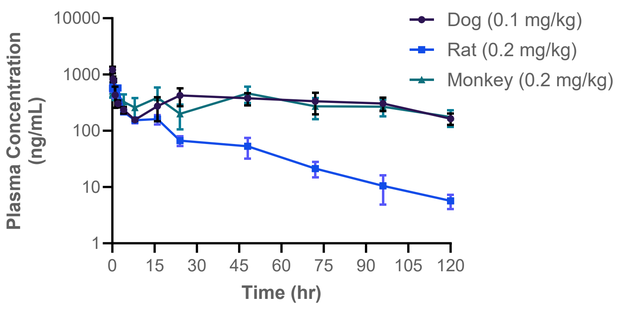
Virus	Strain	EC_{50} (nM)			
		ABI-5366	ABI-1179	Pritelivir	Acyclovir
HSV-1	Laboratory strain (HF)	18 ± 5 (n=44)	6.3 ± 2.7 (n=3)	66 ± 23 (n=35)	3380 ± 1070 (n=7)
	Clinical isolates (n=25)	7 ± 3	3.3 ± 1.1	21 ± 13	1174 ± 1211
HSV-2	Laboratory strain (G)	10 ± 3 (n=86)	4.6 ± 1.2 (n=4)	38 ± 12 (n=62)	1080 (n=1)
	Clinical isolates (n=23)	17 ± 6	6.2 ± 1.9	62 ± 26	6606 ± 7173

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

- ABI-5366 AND ABI-1179 exhibit 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 **(Table 1)**
- ABI-1179 is ~10-fold more potent than pritelivir and ~1000 fold more potent than acyclovir against HSV-2 clinical isolates **(Table 1)**

Figure 3. ABI-5366 and ABI-1179 Have Favorable IV PK Profiles in Preclinical Species

A. PK Profile of ABI-5366 After IV Administration



B. PK Profile of ABI-1179 After IV Administration

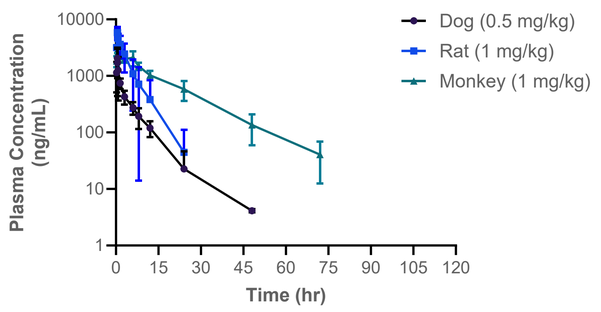


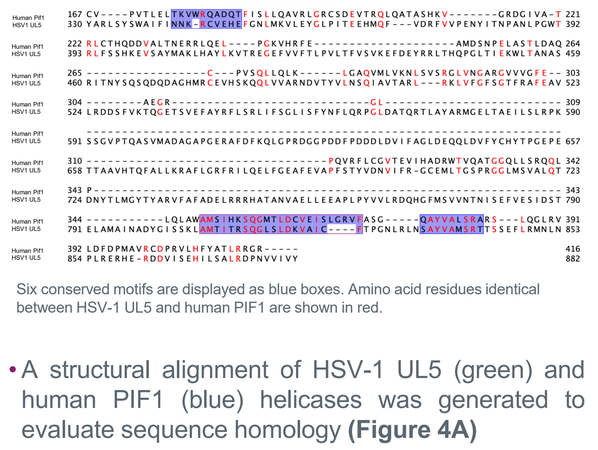
Table 2. ABI-5366 and ABI-1179 PK Profiles in Preclinical Species

Preclinical species	ABI-5366		ABI-1179	
	CL (L/hr/kg)	Half-life (hr)	CL (L/hr/kg)	Half-life (hr)
Rat	0.02	20	0.123	3.31
Dog	0.0023	55	0.197	5.89
Monkey	0.004	71	NA	12.1

- Preclinical IV PK studies in dogs, rats, and monkeys demonstrate that both ABI-5366 and ABI-1179 have a very low clearance and an extended half-life **(Figure 3 and Table 2)**

Figure 4. Limited Sequence Homology Observed Between HSV and Human Helicases

A. Superimposition of HSV-1 UL5 and Human PIF1 Helicase



HSV-1 UL5 (green); PIF1 (PDB:6HPT, blue); RMSD:3.79 Å. ~ 200 residues from the HSV-1 UL5 do not align with PIF1 and are not shown

B. Amino Acid Sequence Alignment of HSV-1 UL5 and human PIF1

Six conserved motifs are displayed as blue boxes. Amino acid residues identical between HSV-1 UL5 and human PIF1 are shown in red.

- A structural alignment of HSV-1 UL5 (green) and human PIF1 (blue) helicases was generated to evaluate sequence homology **(Figure 4A)**
- Sequence identity between human helicases (PIF1) and HSV-1 or HSV-2 UL5 was limited (<12%), suggesting minimal potential for off target effects **(Figure 4B)**

Table 3. ABI-5366 and ABI-1179 Are Highly Selective for HSV Helicase vs Carbonic Anhydrases

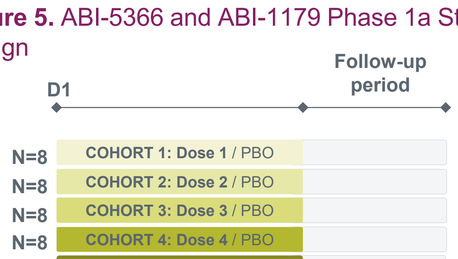
CA Isoform	ABI-5366		Acetazolamide [a]	ABI-1179		Acetazolamide [a]
	IC_{50} (μM)	Selectivity index (HSV-1/HSV-2) [b]	IC_{50} (μM)	IC_{50} (μM)	Selectivity index (HSV-1/HSV-2) [c]	IC_{50} (μM)
CA-I	2.6 ± 0.6	2015/1092	0.03 ± 0.012	>50	> 600	1.2 ± 2
CA-II	1.4 ± 0.2	1077/583	0.02 ± 0.002	3.8 ±2.3	> 40	0.046 ± 0.013

Abbreviations: IC_{50}=half-maximal inhibitory concentration; CAI=carbonic anhydrase I; CAII=carbonic anhydrase II; SD=standard deviation. The data represent the mean ±SD of at least 3 independent experiments.

a. Assay positive control; acetazolamide is a well-known CA inhibitor and contains sulfonamide pharmacophore.
b. Fold selectivity was calculated using an average EC_{50} against HSV-1 and HSV-2 antiviral activities.
c. Fold selectivity was calculated using an average IC_{50} against HSV-1 and HSV-2 HP in DNA unwinding of 0.165 nM.

- Preclinical toxicology studies for ABI-5366 and ABI-1179 demonstrated:
  - Neither is genotoxic
  - No off-target CNS or CV effects were noted nor effects against human carbonic anhydrases I and II, with selectivity indexes for HSV noted in **Table 3**
  - Favorable safety profiles in 28-day oral toxicity studies (ABI-5366: rats and dogs; ABI-1179: rats and monkeys), with high safety margins relative to the predicted human equivalent dose (data not shown)

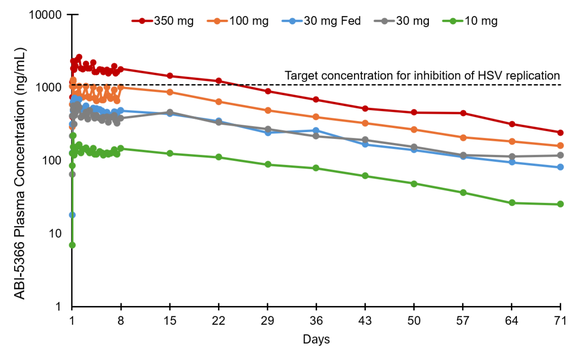
Figure 5. ABI-5366 and ABI-1179 Phase 1a Study Design



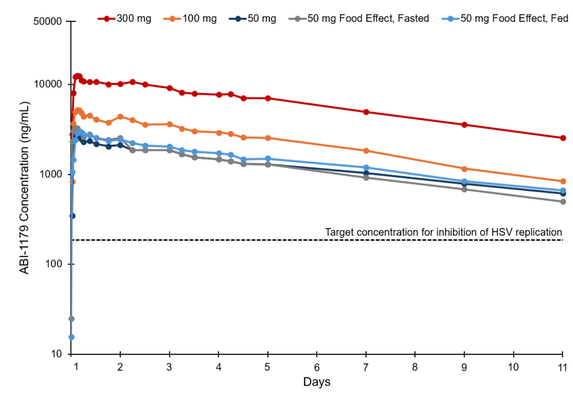
- Key parameters measured:
  - Safety: ECGs, physical examinations, adverse events and laboratory abnormalities
  - PK: $T_{1/2}$ and C_{min}

Figure 6. ABI-5366 and ABI-1179 have Extended Half-Lives in Humans

A. Mean Plasma Concentrations vs Time Following Single Doses of ABI-5366 (Semi-Log Scale)



B. Mean Plasma Concentrations vs Time Following Single Doses of ABI-1179 (Semi-Log Scale)



- Both molecules were safe and well tolerated at single doses (up to 350 mg for ABI-5366 and 300 mg for ABI-1179)
- The PK profile of ABI-5366 suggests intestinal reabsorption with a mean elimination half-life estimate of 20 days, supportive of once-weekly and potentially once-monthly dosing regimens
- Consumption of a high-fat meal does not appear to affect the PK profile of ABI-5366 or ABI-1179
- The PK profile of ABI-1179 has a mean elimination half-life estimate of 4 days, supportive of once-weekly dosing regimens

Conclusions

- ABI-5366 and ABI-1179 are small-molecule inhibitors of HSV helicase-primase enzyme complex
- Both potently inhibit HSV-1 and HSV-2 replication and exhibit broad activity against HSV clinical isolates
- Preclinical PK studies with ABI-5366 and ABI-1179 demonstrate their long-acting potentials
- Both have favorable safety profiles (clinical and preclinical) with minimal potential for off-target effects
- Phase 1a studies demonstrated both molecules have extended half-lives, supporting weekly (and potentially monthly for ABI-5366) oral dosing
- Phase 1b studies in individuals with recurrent genital herpes are underway for both ABI-5366 and ABI-1179

REFERENCES

1)WHO herpes simplex virus detailed fact sheet. Last revised December 11, 2024. https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus; **2)** Gupta R, et al. *Lancet*. 2007;370(9605):2127-37; **3)** James C, et al. *Bull World Health Organ*. 2020;98(5):315-29; **4)** McQuillan G, et al. NCHS Data Brief. 2018;304:1-8; **5)** Alareeki A, et al. *Lancet Reg Health Eur*. 2022;25:100558; **6)** Fanfair RN, et al. *Sex Transm Dis*. 2013;40(11):860-4; **7)** Benedetti J, et al. *Ann Intern Med*. 1994;121(11):847-54; **8)** Benedetti JK, et al. *Ann Intern Med*. 1999;131(1):14-20; **9)** Valtrex (valacyclovir). US package insert. GSK; revised 2022; **10)** Shiraki K, et al. *Viruses*. 2021;13(8):1547; **11)** Wald A, et al. *JAMA*. 2016;316(23):2495-503.

ACKNOWLEDGEMENTS

These studies were sponsored by Assembly Biosciences, Inc. We express our gratitude to all the patients, investigators, and site staff who participated in the clinical studies.

DISCLOSURES

HC, RY, MS, LL, GS, MB, GW, JL, SK, KK, KZ, AG, MP, and **WD** are employees and stockholders of Assembly Biosciences, Inc. **XZ, TW, QY, AN,** and **XY** are employees and stockholders of Gilead Sciences, EJG is a member of scientific advisory boards for Aligos, Assembly Biosciences, AusperBio, Gilead Sciences, GSK, Janssen, Roche, Surrozen, Tune Therapeutics, Vir Biotechnology, Virion Therapeutics, and Precision BioSciences and has given sponsored lectures for AbbVie and Roche Diagnostics. CS is a stockholder in New Zealand Clinical Research.