The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 16, 2014 VOL. 370 NO. 3

Helicase–Primase Inhibitor Pritelivir for HSV-2 Infection

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ABSTRACT

BACKGROUND

Pritelivir, an inhibitor of the viral helicase–primase complex, exhibits antiviral activity in vitro and in animal models of herpes simplex virus (HSV) infection. We tested the efficacy and safety of pritelivir in otherwise healthy persons with genital HSV-2 infection.

METHODS

We randomly assigned 156 HSV-2–positive persons with a history of genital herpes to receive one of four doses of oral pritelivir (5, 25, or 75 mg daily, or 400 mg weekly) or placebo for 28 days. Participants obtained daily swabs from the genital area for HSV-2 testing, which was performed with a polymerase-chain-reaction assay. Participants also maintained a diary of genital signs and symptoms. The primary end point was the rate of genital HSV shedding.

RESULTS

HSV shedding among placebo recipients was detected on 16.6% of days; shedding among pritelivir recipients was detected on 18.2% of days among those receiving 5 mg daily, 9.3% of days among those receiving 25 mg daily, 2.1% of days among those receiving 75 mg daily, and 5.3% of days among those receiving 400 mg weekly. The relative risk of viral shedding with pritelivir, as compared with placebo, was 1.11 (95% confidence interval [CI], 0.65 to 1.87) with the 5-mg daily dose, 0.57 (95% CI, 0.31 to 1.03) with the 25-mg daily dose, 0.13 (95% CI, 0.04 to 0.38) with the 75-mg daily dose, and 0.32 (95% CI, 0.17 to 0.59) with the 400-mg weekly dose. The percentage of days with genital lesions was also significantly reduced, from 9.0% in the placebo group to 1.2% in both the group receiving 75 mg of pritelivir daily (relative risk, 0.13; 95% CI, 0.02 to 0.70) and the group receiving 400 mg weekly (relative risk, 0.13; 95% CI, 0.03 to 0.52). The rate of adverse events was similar in all groups.

CONCLUSIONS

Pritelivir reduced the rates of genital HSV shedding and days with lesions in a dose-dependent manner in otherwise healthy men and women with genital herpes. (Funded by AiCuris; ClinicalTrials.gov number, NCT01047540.)

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N Engl J Med 2014;370:201-10.
DOI: 10.1056/NEJMoa1301150

Treatment of genital infections with the herpes simplex virus (HSV) relies on nucleoside analogues that inhibit the HSV DNA polymerase after phosphorylation by the viral thymidine kinase.[1] These compounds, which were developed three decades ago and are in widespread use today, ameliorate clinical disease. However, they do not abrogate viral shedding and only partially reduce the risk of transmission to sexual partners.[2-4] In immunocompromised persons, resistance to nucleoside analogues develops occasionally, and treatment options for acyclovir-resistant HSV are limited.[5,6]

The thiazolylamide pritelivir (BAY 57-1293; AIC316) is the first in a new class of antiviral agents that inhibit HSV replication by targeting the viral helicase–primase enzyme complex. Pritelivir binds to the complex composed of the gene products of UL5, UL8 and UL52.[7] Unlike the nucleoside analogues, pritelivir does not require activation by phosphorylation, and it is active in uninfected cells. Pritelivir exhibits potent in vitro activity against HSV-1 and HSV-2 isolates, including strains resistant to nucleoside analogues, and has shown efficacy in studies in animals, including a study of genital infection in guinea pigs.[8-10] In phase 1 studies of pritelivir, no safety concerns were identified, and the terminal half-life reported was up to 80 hours.[11]

To investigate the efficacy and safety of pritelivir in the treatment of genital infection with HSV-2 and to determine the most effective, safe dose, we conducted a proof-of-concept study to evaluate the effect of four different doses of oral pritelivir on mucocutaneous viral shedding in adults with genital HSV-2 infection.

Methods

Study Participants

We enrolled otherwise healthy adults (≥18 years of age) who were seropositive for HSV-2 and had a history of genital herpes. Eligible participants had a history of one to nine recurrences of genital herpes per year. Other inclusion criteria were a body-mass index (the weight in kilograms divided by the square of the height in meters) between 18 and 35, the use of effective contraception, and normal results on laboratory tests, including electrocardiography and urinalysis. Infection with the human immunodeficiency virus or with hepatitis B or C virus, pregnancy, immunosuppression, treatment with medications that induce drug-metabolizing enzymes or transporters (e.g., carbamazepine or phenytoin) were exclusion criteria. Receipt of other anti-HSV therapy, consumption of grapefruit, and intake of quinine or products containing quinidine were not permitted during the study. All participants provided written informed consent.

Study Design and Oversight

The study was designed as a randomized, parallel, double-blind, placebo-controlled study by investigators at the University of Washington and by the sponsor, AiCuris, and was managed by FHI 360. The statistical analyses were performed by authors at the University of Washington, according to a statistical plan previously developed by the academic and industry investigators. Both the academic and industry authors contributed to all drafts of the manuscript and approved the submission of the final manuscript for publication. All authors had full access to all data, verified the accuracy of the data, and vouch for the fidelity of the study to the protocol and for the completeness of the data presented. The study was approved by the institutional review board at each study center. For full details of the design and conduct of the study, see the protocol, available with the full text of this article at NEJM.org.

Study Drug

The four dosing regimens for pritelivir were a loading dose of 20 mg followed by a daily dose of 5 mg, a loading dose of 100 mg followed by a daily dose of 25 mg, a loading dose of 300 mg followed by a daily dose of 75 mg, and a weekly dose of 400 mg. The regimen for the administration of placebo was the same as it was for the administration of pritelivir. The study drug was taken for a period of 28 days.

Study Procedures

Eligible participants were randomly assigned to one of the five study groups in a 1:1:1:1:1 ratio. A statistician who was not involved in the conduct of the study generated the randomization schema using the permuted-blocks method, with stratification according to sex and study site.

Throughout the study period, participants obtained daily swabs of genital skin and mucosa and delivered them to the study site weekly for the purpose of HSV detection.[3,4,12] Participants

also maintained a diary of genital signs and symptoms. If a lesion developed that was consistent with genital herpes, an additional swab was obtained from the lesion. Participants who had a recurrence (i.e., papules, vesicles or pustules, ulcers, or crusts) during the study were asked to return to the clinic for an examination within 24 hours.

LABORATORY STUDIES

Western blot analysis was used to confirm HSV-2 serostatus.[13] Samples of genital secretions were analyzed for HSV DNA with a real-time, quantitative, fluorescent polymerase-chain-reaction (PCR) assay (TaqMan) at the University of Washington Virology Laboratory.[14] Test results were considered to be positive when 150 HSV DNA copies per milliliter or more were detected.[15]

Samples with an HSV DNA copy number of more than 5000 per milliliter were sequenced to detect mutations in *UL5* and *UL52*. Primers (Table S2 in the Supplementary Appendix, available at NEJM.org) flanking the regions known to mediate resistance to helicase–primase inhibitors[16] and Platinum TaqDNA Polymerase High Fidelity reagents (Invitrogen) were used to generate PCR products. The PCR products were sequenced according to inner primers with the use of BigDye Terminator (version 3.1) cycle-sequencing technology.[17,18] Sequencing data were analyzed with the Sequencher 4.10 program (HSV-2 reference strain, HG52; NCBI accession number, NC_001798.1)[19] and were uploaded to GenBank under accession numbers KF008267-KF008349 and KF008350-KF008432.

SAFETY EVALUATION

Safety was evaluated by examining the rate, nature, duration, and severity of adverse events in each study group; events were graded with the use of the Division of Acquired Immunodeficiency Syndrome (DAIDS) table of adverse events (http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf). Additional safety assessments included physical examination, laboratory measurements, and electrocardiography.

STATISTICAL ANALYSIS

The frequency of HSV-2 detection (the shedding rate) was defined as the number of days with a genital swab that was positive for HSV divided by the total number of days on which genital swabs were obtained. The primary end point was the reduction in the HSV shedding rate among participants receiving pritelivir relative to the shedding rate among participants receiving placebo. Secondary end points included the rate of genital lesions, the reduction of HSV DNA copies, the frequency of herpes recurrence, and the rate of subclinical shedding.

The intention-to-treat analysis included all patients who took at least one dose of study medication and obtained at least one swab of genital secretions. Poisson regression with overdispersion was used to estimate the effect of treatment on the rates of viral shedding and lesion recurrence, with terms included for treatment and sex and a random term for investigational site. On days when HSV was detected, the $\log_{10}$ number of HSV DNA copies was compared between each of the pritelivir groups and the placebo group with the use of generalized estimating equations that accounted for the correlation between repeated measures per participant. The concordance between the detection of HSV from swabs obtained by a participant and detection from those obtained by an investigator was assessed with the kappa statistic and the Spearman coefficient.

RESULTS

STUDY POPULATION

The trial was conducted between April and December 2010 at seven sites in the United States. Among 261 persons screened, 156 underwent randomization; 1 person who underwent randomization was deemed ineligible and did not receive the study drug or participate in the study. The predominant reason for ineligibility was an absence of serologically confirmed HSV-2 infection. The median age of the participants was 41 years; 104 (67%) were women, and most were white (Table 1). The median duration of genital herpes before study entry was 11 years; 21% of the participants were receiving suppressive therapy before study entry. A total of 147 participants (94%) completed the study. Participants discontinued treatment prematurely for the following reasons: withdrawal of consent (4 participants), adverse events (3 participants), loss to follow-up (2 participants), pregnancy (1 participant), and noncompliance with the protocol (1 participant). The median percentage of days on which swabs were obtained was 100% (range, 11 to 100%);

Table 1. Demographic and Clinical Characteristics of the Study Participants.*

Characteristic	Placebo (N=30)	Pritelivir			
		5 mg Daily (N=33)	25 mg Daily (N=32)	75 mg Daily (N=29)	400 mg Weekly (N=31)
Age — yr					
Median	42	36	36	43	44
Range	24–64	19–71	22–63	20–67	19–67
Female sex — no. (%)	21 (70)	22 (67)	21 (66)	20 (69)	20 (65)
White race — no. (%)	21 (70)	25 (76)	27 (84)	20 (69)	23 (74)
Seropositivity for HSV-1 and HSV-2 — no. (%)	14 (47)	20 (61)	14 (44)	10 (34)	16 (52)
Duration of genital herpes — yr					
Median	15	10	10	15	9
Range	1–35	1–31	1–52	1–40	1–30
Recurrences of genital herpes before study entry — no./yr†‡					
Median	4	3	3	4	4
Range	1–9	1–9	1–9	1–9	1–9
Treatment with suppressive agent before study entry — no. (%)	7 (23)	7 (21)	7 (22)	7 (24)	4 (13)
Total no. of days swabs obtained by participants and investigators	833	846	883	766	852

* HSV-1 denotes herpes simplex virus type 1, and HSV-2 type 2.
† Data for this variable were not available at one of the study sites, where there were 18 participants.
‡ For patients receiving suppressive therapy, the number of recurrences is the number in the period before the initiation of that therapy.

80% of the participants obtained more than 96% of the swabs. Mean adherence to the study medication, as calculated according to the pill count, was 99% (median, 100%; range, 72 to 100) with 94% of participants taking more than 95% of the study drug; adherence was similar in all study groups. There was a high concordance in the frequency with which HSV was detected and the quantity of HSV DNA detected in the swabs obtained by participants and the swabs obtained by the investigators ($r=0.87$, $P<0.001$) (Fig. S1 in the Supplementary Appendix).

EFFECT OF PRITELIVIR ON VIRAL SHEDDING

A total of 4180 daily genital swabs were obtained, 435 of which (10.4%) were positive for HSV. Genital HSV was detected at least once in 87 of the 155 participants (56%). The rate of HSV shedding in the placebo group was 16.6%; among the groups receiving pritelivir daily, the rate was 18.2% with 5 mg, 9.3% with 25 mg, and 2.1% with 75 mg; in the group receiving 400 mg weekly, the rate was 5.3% (Table 2 and Fig. 1A). The relative risk of viral shedding with pritelivir as compared with placebo was 1.11 (95% confidence interval [CI], 0.65 to 1.87; $P=0.70$) at a daily dose of 5 mg, 0.57 (95% CI, 0.31 to 1.03; $P=0.06$) at a daily dose of 25 mg, 0.13 (95% CI, 0.04 to 0.38; $P<0.001$) at a daily dose of 75 mg, and 0.32 (95% CI, 0.17 to 0.59; $P<0.001$) at a weekly dose of 400 mg (Table 2).

When HSV was detected, the median $\log_{10}$ number of HSV DNA copies was 5.1 with placebo, 4.5 with 5 mg of pritelivir daily, 3.6 with 25 mg daily, 2.4 with 75 mg daily, and 3.6 with 400 mg weekly ($P<0.001$ for the comparisons of placebo with doses of pritelivir of 25 mg or more) (Fig. 1B). Thus, as compared with placebo, pritelivir significantly reduced both the frequency of HSV shedding on the genital mucosa and the quantity of the virus detected when breakthrough shedding occurred. A similar dose response was observed for subclinical viral shedding. The overall rate of subclinical shedding

was 9.8% with placebo, 11.9% with 5 mg of pritelivir daily, 7.4% with 25 mg daily, 1.6% with 75 mg daily, and 4.8% with 400 mg weekly (Fig. 1C). The relative risks of subclinical shedding at pritelivir doses of 5 mg daily, 25 mg daily, 75 mg daily, and 400 mg weekly were 1.26 (95% CI, 0.67 to 2.35; P=0.48), 0.73 (95% CI, 0.37 to 1.47; P=0.38) , 0.16 (95% CI, 0.05 to 0.49; P=0.001), and 0.47 (95% CI, 0.24 to 0.91; P=0.02), respectively.

Pritelivir reduced viral shedding in both men and women. For example, among men in the 75-mg group, HSV shedding occurred on 3.2% of days as compared with 12.5% of days among men in the placebo group; among women in the 75-mg group, HSV shedding occurred on 1.6% of days as compared with 18.3% of days in the placebo group. No significant interaction was detected between treatment with 75 mg of pritelivir daily and sex, nor was there a significant interaction between treatment and site.

EFFECT OF PRITELIVIR ON GENITAL LESIONS

To assess the effect of pritelivir on the clinical manifestations of genital infection with HSV-2, we calculated the percentage of days on which participants had genital lesions according to study group. Genital lesions were noted on 9.0% of days with placebo, 12.5% with 5 mg of pritelivir, 3.5% with 25 mg, 1.2% with 75 mg, and 1.2% with 400 mg (Fig. 1D). The risk of genital lesions with pritelivir as compared with placebo was 1.39 (95% CI, 0.59 to 3.30; P=0.46) with 5 mg of pritelivir daily, 0.41 (95% CI, 0.16 to 1.09; P=0.07) with 25 mg daily, 0.13 (95% CI, 0.02 to 0.70; P=0.02) with 75 mg daily, and 0.13 (95% CI, 0.03 to 0.52; P=0.004) with 400 mg weekly. In addition, we observed 12 recurrences in participants receiving placebo, 18 in participants receiving 5 mg of pritelivir daily, 12 in those receiving 25 mg daily, 3 in those receiving 75 mg daily, and 6 in those receiving 400 mg weekly (P=0.04 for the comparison of placebo with 75 mg of pritelivir daily).

Viral shedding at the time of lesion recurrence was also reduced in a dose-related manner among participants receiving pritelivir (Table 2). In the placebo group, HSV was detected on 85.3% of days on which participants had lesions, as compared with 60.4% of days in the group receiving 5 mg of pritelivir daily, 50.0% in the group receiving 25 mg daily, 44.4% in the group receiving 75 mg daily, and 50.0% in the group receiving 400 mg weekly. The median HSV DNA $\log_{10}$ copy number was 6.1 among participants with lesions who were receiving placebo. The $\log_{10}$ copy number was decreased somewhat among participants receiving lower doses of pritelivir, reaching 2.9 at a dose of 75 mg daily (P<0.001 for the comparison of 75 mg of pritelivir daily with placebo) (Table 2).

VIRAL RESISTANCE

Analysis of the resistance-associated regions of *UL5* and *UL52* revealed variations in DNA in all study groups as compared with the HSV-2 reference sequence. Four changes in *UL5* were identified in five participants. Among these four changes, three were synonymous (G879A [in a recipient of 5 mg of pritelivir daily], C996T [in a placebo recipient], and G1053A [in a recipient of 400 mg of pritelivir weekly]) and one was nonsynonymous (A1001G [in a recipient of 5 mg of pritelivir daily]); the corresponding amino acid change is H334R, which is not within or downstream of the fourth functional motif of *UL5* and is therefore not considered to confer resistance to pritelivir.[16] In *UL52*, two synonymous changes were identified in two participants (G2649A [in a placebo recipient] and C2676T [in a recipient of 25 mg of pritelivir daily]). All sequences within participants were identical.

ADVERSE EVENTS

No pattern of adverse events or laboratory abnormalities emerged during the study (Table 3, and Table S1 in the Supplementary Appendix). One serious adverse event occurred in a participant receiving 25 mg of pritelivir daily, who was hospitalized for pancreatitis 2 weeks after completing treatment; a review of the participant's medical history revealed prior alcohol-related pancreatitis and probable continued alcohol intake. The number of days with nausea was highest in the group receiving 400 mg of pritelivir per week (33 days of nausea) and the group receiving 5 mg per day (44 days), although a single participant accounted for 75% of the days of nausea in the group receiving 400 mg per week. Three participants receiving pritelivir discontinued treatment because of adverse events: one participant receiving 5 mg daily discontinued treatment because of moderate headache, nausea, chest pain, and dyspnea; one receiving 25 mg daily discontinued treatment be-

Table 2. Virologic and Clinical End Points.*

End Point	Placebo (N=30)	Pritelivir, 5 mg Daily (N=33)	Relative Risk or Change in Copy Number (95% CI)	P Value	Pritelivir, 25 mg Daily (N=32)
Virologic					
Rate of genital HSV shedding — no. of days/total no. (%)	138/833 (16.6)	154/846 (18.2)	1.11 (0.65 to 1.87)	0.70	82/883 (9.3)
Rate of subclinical shedding — no. of days/total no. (%)	74/758 (9.8)	88/737 (11.9)	1.26 (0.67 to 2.35)	0.48	61/825 (7.4)
Rate of lesional shedding — no. of days/total no. (%)	64/75 (85.3)	64/106 (60.4)	0.85 (0.59 to 1.24)	0.39	15/30 (50.0)
HSV DNA — $\log_{10}$ copy no.					
Overall					
Median	5.1	4.5	–0.36 (–0.83 to 0.09)	0.12	3.6
Range	2.2 to 8.7	2.2 to 8.0			2.2 to 7.8
On days with no lesions					
Median	4.1	3.8	–0.27 (–0.80 to 0.26)	0.31	3.3
Range	2.2 to 7.9	2.2 to 6.9			2.2 to 7.5
On days with lesions					
Median	6.1	5.7	–0.47 (–1.19 to 0.24)	0.19	5.4
Range	2.2 to 8.7	2.2 to 8.0			2.7 to 7.8
Clinical					
Days with lesions — no./total no. (%)	75/836 (9.0)	108/863 (12.5)	1.39 (0.59 to 3.30)	0.46	30/864 (3.5)
No. of recurrences	12	18			12
Recurrence rate (annualized)	5.2	7.6	1.39 (0.70 to 2.78)	0.36	5.1

* P values are for the comparison of each pritelivir dose group with the placebo group. A P value of less than 0.0125 was considered to indicate statistical significance after Bonferroni correction for multiple comparisons. Relative risks are shown for viral shedding and clinical end points; changes in the $\log_{10}$ copy number are shown for HSV DNA.

cause of suspected systemic lupus erythematosus; and one receiving 400 mg per week discontinued treatment because of mild anxiety.

DISCUSSION

Our study showed that pritelivir, an HSV-specific helicase–primase inhibitor, significantly reduces the frequency of genital HSV shedding and lesions in otherwise healthy men and women with genital HSV-2 infection. The effect was dose-related, with a daily dose of 75 mg having the greatest antiviral effect. Furthermore, at this dose, pritelivir reduced the quantity of HSV in breakthrough shedding by more than 98%. Although our study was not powered to evaluate the effect of pritelivir on recurrences of symptomatic genital herpes, we did observe significant reductions in the number of days of genital lesions at doses of 75 mg daily and 400 mg weekly that paralleled the trend toward fewer recurrences at these doses.

In our small study, pritelivir, when administered daily for 4 weeks, was not associated with any clinical, laboratory, or cardiac abnormalities that emerged during treatment. Reports of nausea did not appear to be related to the dose of pritelivir. (Nausea was not observed in phase 1 studies at higher doses.[11]) Sequencing of samples obtained before and after treatment suggests that drug resistance did not emerge, even

Relative Risk or Change in Copy Number (95% CI)	P Value	Pritelivir, 75 mg Daily (N=29)	Relative Risk or Change in Copy Number (95% CI)	P Value	Pritelivir, 400 mg Weekly (N=31)	Relative Risk or Change in Copy Number (95% CI)	P Value
0.57 (0.31 to 1.03)	0.06	16/766 (2.1)	0.13 (0.04 to 0.38)	<0.001	45/852 (5.3)	0.32 (0.17 to 0.59)	<0.001
0.73 (0.36 to 1.47)	0.38	12/757 (1.6)	0.16 (0.05 to 0.49)	0.001	40/842 (4.8)	0.47 (0.24 to 0.91)	0.02
0.56 (0.33 to 0.95)	0.03	4/9 (44.4)	0.57 (0.16 to 2.03)	0.38	5/10 (50.0)	0.58 (0.31 to 1.09)	0.09
–0.85 (–1.31 to –0.39)	<0.001	2.4	–1.63 (–2.57 to –0.69)	<0.001	3.6	–1.09 (–1.68 to –0.50)	<0.001
		2.2–4.8			2.2 to 6.9		
–0.45 (–0.96 to 0.06)	0.08	2.4	–1.33 (–2.45 to –0.21)	0.02	3.4	–0.57 (–1.32 to 0.17)	0.13
		2.2 to 4.8			2.2 to 6.8		
–1.02 (–1.63 to –0.40)	0.001	2.9	–1.75 (–2.09 to –1.41)	<0.001	5.2	–0.83 (–1.11 to –0.54)	<0.001
		2.4 to 3.5			4.3 to 6.9		
0.41 (0.16 to 1.09)	0.07	9/775 (1.2)	0.13 (0.02 to 0.70)	0.02	10/861 (1.2)	0.13 (0.03 to 0.52)	0.004
		3			6		
0.86 (0.35 to 2.11)	0.75	1.4	0.28 (0.08 to 0.95)	0.04	2.5	0.48 (0.17 to 1.38)	0.17

in participants treated with the lowest doses of pritelivir.

Our study design, in which the number of days of viral shedding was the primary outcome, provided an approach toward defining in vivo antiviral efficacy and an effective, safe dose for subsequent evaluation. Studies of the natural history of HSV-2 have shown that shedding frequency, the quantity of HSV during shedding, and the occurrence and duration of genital lesions are all correlated.[20] Early studies in the development of nucleoside analogues for the treatment of genital herpes measured the reduction in the duration of symptomatic recurrences. However, lesions reflect mucosal damage as well as viral replication, and even effective antiviral agents have marginal benefits when started after the formation of lesions. Evaluations of viral shedding have shown that nearly all persons with HSV-2 shed virus.[21] We selected a patient population with clinically symptomatic disease to obtain a preliminary assessment of both the effects of extended antiviral therapy on shedding and the potential clinical benefit of pritelivir. Our data indicate that pritelivir may have the potential to reduce clinical recurrences of HSV.

Although the HSV shedding rate was reduced by more than 85% at the highest doses of pritelivir, as compared with placebo, some breakthrough shedding remained. Persistent, low-level shedding during nucleoside therapy has been documented previously.[3] The pathogenesis of breakthrough viral shedding during adequate antiviral therapy with nucleoside analogues is poorly un-

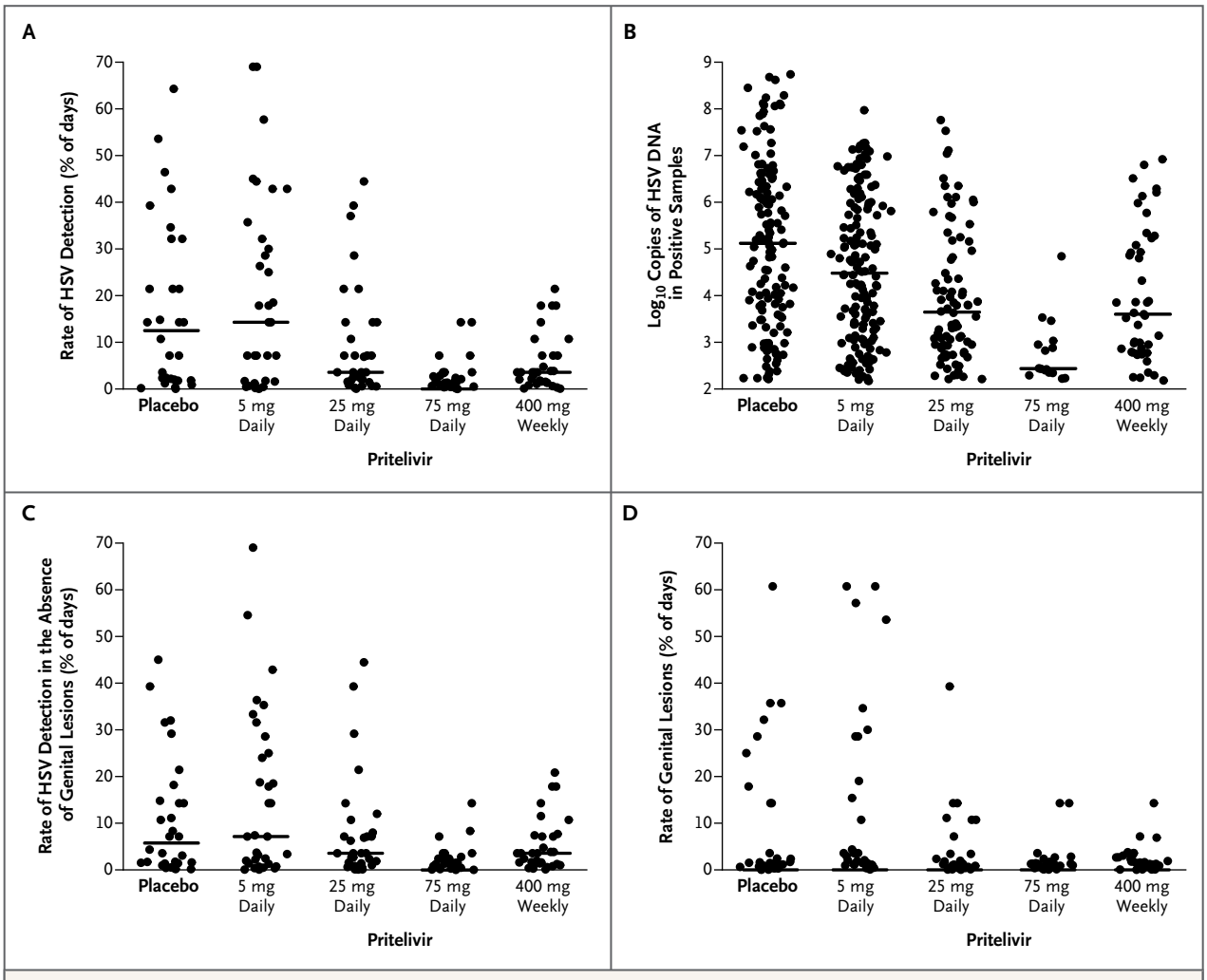

Figure 1. Rates of Herpes Simplex Virus (HSV) Detection, Viral Load, and Recurrence of Lesions in Patients with Genital Lesions, According to Study Group.

Panel A shows the percentage of days on which HSV was detected in genital samples, Panel B shows the level of HSV DNA in positive samples, Panel C shows the rate of subclinical shedding (i.e., detection of HSV in the absence of lesions), and Panel D shows the percentage of days with genital lesions. Genital samples with 150 $\log_{10}$ HSV DNA copies per milliliter or more were considered to be positive. Horizontal bars represent medians.

derstood; it is not related to lack of adherence to the treatment regimen or viral resistance.[12] The question of whether further increases in the daily dose of pritelivir would completely abrogate viral shedding will have to be addressed in additional studies. As shown in Table S3 in the Supplementary Appendix, pritelivir had a stronger effect on the reduction of viral DNA than the nucleoside analogues studied in earlier trials. Although it is appealing to compare the efficacy of pritelivir with that of currently licensed products, the frequency of viral shedding and the magnitude of the reduction in the quantity of virus shed with antiviral medication depend heavily on the characteristics of the participants enrolled; thus, a direct comparison in a clinical trial is warranted to properly determine comparative efficacy.

This study shows that pritelivir, the first in a new class of antiviral agents developed for the treatment of herpes simplex infections, is effective in suppressing viral shedding and lesion development in patients with genital herpes. Further studies will be needed to assess the magnitude of clinical benefit in the treatment of HSV infections and to establish the usefulness

Table 3. Adverse Events.

Event	Placebo (N=30)	Pritelivir				
		5 mg Daily (N=33)	25 mg Daily (N=32)	75 mg Daily (N=29)	400 mg Weekly (N=31)	Total (N=155)
		no. of participants (%)				
Any adverse event	23 (77)	27 (82)	26 (81)	27 (93)	23 (74)	103 (82)
Serious adverse events	0	0	1 (3)*	0	0	1 (1)
Adverse events leading to study discontinuation	0	1 (3)†	1 (3)‡	0	1 (3)§	3 (2)
Moderate adverse events	12 (40)	13 (39)	11 (34)	12 (41)	8 (26)	44 (35)
Severe adverse events	3 (10)	1 (3)	1 (3)	2 (7)	1 (3)	5 (4)
Common adverse events						
Headache, total	12 (40)	12 (36)	14 (44)	11 (38)	14 (45)	51 (41)
Moderate	4 (13)	6 (18)	3 (9)	4 (14)	6 (19)	19 (15)
Severe	0	0	0	0	0	0
Nausea, total	4 (13)	9 (27)	5 (16)	5 (17)	9 (29)	28 (22)
Moderate	0	4 (12)	0	0	1 (3)	5 (4)
Severe	0	0	0	0	0	0
Fatigue, total	5 (17)	4 (12)	2 (6)	5 (17)	3 (10)	14 (11)
Moderate	3 (10)	1 (3)	0	3 (10)	1 (3)	5 (4)
Severe	0	0	0	1 (3)	0	1 (<1)
Upper abdominal pain, total	3 (10)	2 (6)	4 (13)	4 (14)	2 (6)	12 (10)
Moderate	0	0	1 (3)	0	0	1 (<1)
Severe	1 (3)	0	0	0	0	1 (<1)

* Pancreatitis was reported 2 weeks after administration of the last dose in one participant, who may have had a history of alcohol abuse and who had a history of pancreatitis.
† Moderate headache, nausea, chest pain, and dyspnea led to discontinuation in one patient.
‡ Suspected systemic lupus erythematosus led to discontinuation in one patient.
§ Mild anxiety led to discontinuation in one patient.

of pritelivir in treating severe HSV disease and in reducing sexual transmission.

In May 2013, the clinical development of pritelivir was placed on hold by the Food and Drug Administration because of unexplained dermal and hematologic findings in a toxicology study of monkeys treated with daily doses ranging from 75 mg per kilogram of body weight to 1000 mg per kilogram (these doses were 70 to more than 900 times as high as a dose of 75 mg in humans). The reason for the findings in monkeys is currently under investigation; such findings were not observed in the current trial.

Presented in part at the 19th Meeting of the International Society for Sexually Transmitted Diseases Research, Quebec, Canada, July 10–13, 2011; and at the 36th International Herpesvirus Workshop, Gdansk, Poland, July 24–28, 2011.

Supported by AiCuris.

Dr. Wald reports receiving consulting fees from Amgen, travel support from Vical, and grant support through her institution from Agenus, Genentech, Genocea, Gilead, and Vical; Dr. Corey reports holding stock in Immune Design and being a coinventor on several patents associated with the development of an HSV-2 vaccine; Drs. Timmler, Stoelben, Stobernack, Ruebsamen-Schaeff, and Birkmann are employees of AiCuris; Drs. Timmler, Ruebsamen-Schaeff, and Birkmann report holding stock options in AiCuris; Dr. Birkmann reports contributing to patents related to galenics and the synthesis of drug substances through AiCuris; Dr. Magaret reports receiving consulting fees from Immune Design; Dr. Warren reports receiving travel support from AiCuris and Vical and grant support through her institution from Agenus, Genocea, AiCuris, and Vical; Dr. Fife reports receiving travel support from Vical and grant support through his institution from Genocea, Vical, and AiCuris; Dr. Johnston reports receiving grant support through her institution from AiCuris; and Dr. Tyring reports receiving grant support through his institution from Agenus, Genocea, AiCuris, and Vical. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES

1. Elion GB, Furman PA, Fyfe JA, de Miranda P, Beauchamp L, Schaeffer HJ. Selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc Natl Acad Sci U S A 1977;74:5716-20.
2. Douglas JM, Critchlow C, Benedetti J, et al. A double-blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. N Engl J Med 1984;310:1551-6.
3. Johnston C, Saracino M, Kuntz S, et al. Standard-dose and high-dose daily antiviral therapy for short episodes of genital HSV-2 reactivation: three randomised, open-label, cross-over trials. Lancet 2012; 379:641-7. [Erratum, Lancet 2012;379:616.]
4. Corey L, Wald A, Patel R, et al. Once-daily valacyclovir to reduce the risk of transmission of genital herpes. N Engl J Med 2004;350:11-20.
5. Crumpacker CS, Schnipper LE, Marlowe SI, Kowalsky PN, Hershey BJ, Levin MJ. Resistance to antiviral drugs of herpes simplex virus isolated from a patient treated with acyclovir. N Engl J Med 1982;306:343-6.
6. Reyes M, Shaik NS, Graber JM, et al. Acyclovir-resistant genital herpes among persons attending sexually transmitted disease and human immunodeficiency virus clinics. Arch Intern Med 2003;163: 76-80.
7. Kleymann G, Fischer R, Betz UA, et al. New helicase-primase inhibitors as drug candidates for the treatment of herpes simplex disease. Nat Med 2002;8:392-8.
8. Betz UA, Fischer R, Kleymann G, Hendrix M, Rübsamen-Waigmann H. Potent in vivo antiviral activity of the herpes simplex virus primase-helicase inhibitor BAY 57-1293. Antimicrob Agents Chemother 2002;46:1766-72.
9. Biswas S, Jennens L, Field HJ. The helicase primase inhibitor, BAY 57-1293 shows potent therapeutic antiviral activity superior to famciclovir in BALB/c mice infected with herpes simplex virus type 1. Antiviral Res 2007;75:30-5.
10. Baumeister J, Fischer R, Eckenberg P, Henninger K, Ruebsamen-Waigmann H, Kleymann G. Superior efficacy of helicase-primase inhibitor BAY 57-1293 for herpes infection and latency in the guinea pig model of human genital herpes disease. Antivir Chem Chemother 2007;18: 35-48.
11. Birkmann A, Kropeit D, McCormick D, Zimmerman H, Ruebsamen-Schaeff H. Safety and human pharmacokinetics of AIC316, a potent helicase-primase inhibitor of herpes simplex virus. Presented at the 24th International Conference on Antiviral Research Sofia, Bulgaria, May 8–11, 2011. abstract.
12. Wald A, Zeh JE, Barnum G, Davis LG, Corey L. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. Ann Intern Med 1996;124:8-15.
13. Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 1988;26:662-7.
14. Wald A, Huang ML, Carrell D, Selke S, Corey L. Polymerase chain reaction for detection of herpes simplex virus (HSV) DNA on mucosal surfaces: comparison with HSV isolation in cell culture. J Infect Dis 2003;188:1345-51.
15. Magaret AS, Wald A, Huang ML, Selke S, Corey L. Optimizing PCR positivity criterion for detection of herpes simplex virus DNA on skin and mucosa. J Clin Microbiol 2007;45:1618-20.
16. Field HJ, Biswas S. Antiviral drug resistance and helicase-primase inhibitors of herpes simplex virus. Drug Resist Update 2011;14:45-51.
17. Rosenblum BB, Lee LG, Spurgeon SL, et al. New dye-labeled terminators for improved DNA sequencing patterns. Nucleic Acids Res 1997;25:4500-4.
18. Heiner CR, Hunkapiller KL, Chen SM, Glass JI, Chen EY. Sequencing multimegabase-template DNA with BigDye terminator chemistry. Genome Res 1998;8: 557-61.
19. Dolan A, Jamieson FE, Cunningham C, Barnett BC, McGeoch DJ. The genome sequence of herpes simplex virus type 2. J Virol 1998;72:2010-21.
20. Schiffer JT, Wald A, Selke S, Corey L, Magaret A. The kinetics of mucosal herpes simplex virus-2 infection in humans: evidence for rapid viral-host interactions. J Infect Dis 2011;204:554-61.
21. Magaret AS, Johnston C, Wald A. Use of the designation "shedder" in mucosal detection of herpes simplex virus DNA involving repeated sampling. Sex Transm Infect 2009;85:270-5.