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RESEARCH**

APPLICATION NUMBER:

203791Orig1s000

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 22; Review Completed: 04/09/13

Reviewer: LALJI MISHRA, Ph.D.

Date Submitted: 04/09/13

Date Received: 04/09/13

Date Assigned: 04/04/13 (email presubmission)

Sponsor: (b) (4)

For BioAlliance Pharma, Inc.

59 Boulevard du General Martial Vlain

75015 Paris, France

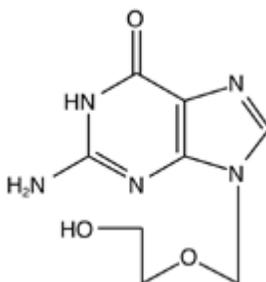
Product Name(s):

Proprietary: Sitavig

Non-proprietary: Acyclovir Lauriad[®]

Chemical: 2-amino-1,9-dihydro-9-[(2-hydroxy)methyl]6-*H*-purin-6-one

Structure:



ACYCLOVIR

Molecular formula: C₈H₁₁N₅O₃

Molecular mass: 225.2

Drug category: Antiviral

Indication: Treatment of recurrent herpes labialis (cold sores) in immunocompetent adults.

Dosage Form/Route of administration: mucoadhesive buccal tablet (MBT)/oral

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 22; Review Completed: 04/09/13

BACKGROUND

BioAlliance Pharma submitted on 3/12/12 a 505(b2) new drug application (NDA) for acyclovir Lauriad™ mucoadhesive buccal tablet for the treatment of recurrent orofacial herpes and (b) (4) which was previously review (see the Clinical Virology review of Lalji Mishra, Ph.D. dated 11/16/12). The sponsor submitted a major amendment on 1/03/13 extending the review deadline.

Acyclovir Lauriad™ mucoadhesive buccal tablet (ABT 50 mg) has been designed with the objective of delivering high (over the EC₅₀ value of acyclovir against HSV-1) and prolonged acyclovir concentrations in the oral cavity for an improved cutaneous and mucosal diffusion at the expression and infection sites of labial herpes virus.

The sponsor had previously submitted published data on the mechanism of action of acyclovir, antiviral activity of acyclovir in cell culture against laboratory and clinical isolates, antiviral activity in animal models, acyclovir-resistance associated substitutions and susceptibility of acyclovir resistant isolates in cell culture. These published data were reviewed and documented in the Virology Review of NDA 203791 (Virology review of NDA 203791 SDN 000, dated 10/22/12). In addition, the sponsor had submitted a virology report by D. Boutolleau, Pharm.D., Ph.D., Associate Professor, Department of Virology at Pitié-Salpêtrière University Hospital, Paris, France. In this report, Dr. Boutolleau determined the antiviral activity of acyclovir against 81 HSV clinical isolates obtained from immunocompromised patients (HIV-1 infected and transplant recipients) with various HSV-induced diseases. The report by Dr. D. Boutolleau was previously reviewed (Virology Review of NDA203791 SDN 000 dated 10/22/12).

The sponsor submitted a label in the original application which was reviewed and revised. The same label was submitted on 02/06/13 via E-mail. The revised label (Microbiology section) along with the changes made in the original label was sent to the sponsor on February 26, 2013. The sponsor accepted the revised version of the Virology label (dated 03/07/13, 03/18/13). The original and revised version of the Virology label was presented in the Virology review of NDA 203791 SDN 018 dated 03/20/13).

After the Virology review was entered in DARRTS, the sponsor proposed to add the following sentence (shown in red) to the Antiviral Activity section of the Virology label (see E-mail dated 04/04/13).

Antiviral Activity

The quantitative relationship between the cell culture susceptibility of herpes viruses to antivirals and the clinical response to therapy has not been established in humans, and virus sensitivity testing has not been standardized. Sensitivity testing results, expressed as the concentration of drug required to inhibit by 50% the growth of virus in cell culture (EC₅₀), vary greatly depending upon a number of factors. Using plaque-reduction assays on Vero cells, the median EC₅₀ value of

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NDA 203791 SDN 22; Review Completed: 04/09/13

acyclovir against clinical herpes virus isolates (subjects receiving placebo) was 1.3 μ M (range: <0.56 to 3.3 μ M).

[REDACTED] (b) (4)

Comment

[REDACTED] (b) (4)

Therefore, the sponsor was advised to remove the proposed sentence (shown above in red font). The sponsor agreed to DAVP recommendation and deleted the above sentence (NDA 203791 SDN 22 dated 04/09/13).

RECOMMENDATIONS

With respect to virology, this revised label is consistent with the previous version of the label documented in Virology review of NDA 203791 SDN 018 and current label dated 04/08/13 is acceptable.

Lalji Mishra, Ph.D.

Virologist

CONCURRENCES:

_____ Date _____

HFD-530/J. O'Rear /TL Micro

CC:

HFD-530/NDA 203791

HFD-530/ Division File

HFD-530/ Micro/ L. Mishra

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 22; Review Completed: 04/09/13

HFD-530/RPM/S. Mosaddegh.

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/s/

LALJI MISHRA
04/10/2013

JULIAN J O REAR
04/10/2013

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 018; Review Completed: 03/11/13

Reviewer: LALJI MISHRA, Ph.D.

Date Submitted: 01/03/13

Date Received: 01/04/13

Date Assigned: 01/04/13

Sponsor: (b) (4)

For BioAlliance Pharma, Inc.

59 Boulevard du General Martial Vlain

75015 Paris, France

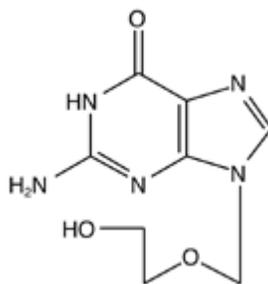
Product Name(s):

Proprietary: Sitavig

Non-proprietary: Acyclovir Lauriad[®]

Chemical: 2-amino-1,9-dihydro-9-[(2-hydroxy)methyl]6-*H*-purin-6-one

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Molecular formula: C₈H₁₁N₅O₃

Molecular mass: 225.2

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Indication: Treatment of recurrent herpes labialis (cold sores) in immunocompetent adults.

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VIROLOGY REVIEW
NDA 203791 SDN 018; Review Completed: 03/11/13

Dosage Form/Route of administration: mucoadhesive buccal tablet (MBT)/oral

Additional Submission Reviewed

Supplement #	Date of Correspondence	Date of Receipt
N203791 SDN 19	02/12/13	02/12/13
N203791 SDN20	03/06/13	03/07/13

BACKGROUND

BioAlliance Pharma submitted on 3/12/12 a 505(b2) new drug application (NDA) for acyclovir Lauriad™ mucoadhesive buccal tablet for the treatment of recurrent orofacial herpes and (b) (4) which was previously review (see the Clinical Virology review of Lalji Mishra, Ph.D. dated 11/16/12). The sponsor submitted a major amendment on 1/03/13 extending the review deadline.

Acyclovir Lauriad™ mucoadhesive buccal tablet (ABT 50 mg) has been designed with the objective of delivering high (over the EC₅₀ value of acyclovir against HSV-1) and prolonged acyclovir concentrations in the oral cavity for an improved cutaneous and mucosal diffusion at the expression and infection sites of labial herpes virus.

The sponsor had previously submitted published data on the mechanism of action of acyclovir, antiviral activity of acyclovir in cell culture against laboratory and clinical isolates, antiviral activity in animal models, acyclovir-resistance associated substitutions and susceptibility of acyclovir resistant isolates in cell culture. These published data were reviewed and documented in this Virology Review of the NDA 203791 (Virology review of NDA 203791 SDN 000, dated 10/22/12). In addition, the sponsor had submitted a virology report by D. Boutolleau, Pharm.D., Ph.D., Associate Professor, Department of Virology at Pitié-Salpêtrière University Hospital, Paris, France. In this report, Dr. Boutolleau determined the antiviral activity of acyclovir against 81 HSV clinical isolates obtained from immunocompromised patients (HIV-1 infected and transplant recipients), suffering from various HSV-induced diseases. This report by Dr. D. Boutolleau was reviewed (Virology Review of NDA203791 SDN 000 dated 10/22/12).

The sponsor submitted a clinical report for assessing the correlation of saliva acyclovir concentration with HSV-1 DNA copies/mL. However, the HSV-1 DNA levels for baseline

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samples were not available. A comparison of baseline saliva viral load for samples after ABT treatment for a given subject could not be made since data on baseline saliva viral load were not determined due to unavailability of the samples and study design. This report was reviewed previously (see Virology review of NDA 203791 dated 10/22/12). A comparison of saliva HSV-1 DNA copies/mL from subjects treated with ABT and placebo indicated that ABT treatment exhibited antiviral activity. Based on the published data on acyclovir and limited data submitted in the original application, Virology review team recommended for the approval of NDA 203791. However, clinical and statistical reviewer had concerns about the claimed efficacy of acyclovir buccal tablet and issued a complete response (CR) letter dated 12/20/2012. The sponsor submitted its response to CR letter on 01/03/13.

The sponsor had submitted label in the original application which was reviewed and revised. The same original label was submitted on 02/06/13 via E-mail. The revised label (Microbiology section) along with the changes made in the original label was sent to the sponsor on February 26, 2013. The sponsor has accepted the revised version of the Microbiology label (dated 03/07/13, 03/18/13). The original and revised version of the microbiology label is presented here.

CLINICAL PHARMACOLOGY

(b) (4)

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(b) (4)

Comment

The sponsor accepted all changes in the Microbiology label as proposed by DAVP.

12.4 Microbiology (03/18/13 version)

Mechanism of Action

Acyclovir is a synthetic purine nucleoside analogue that is phosphorylated intracellularly by the viral encoded thymidine kinase (TK) of HSV into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. In a biochemical reaction, acyclovir triphosphate inhibits replication of herpes viral DNA by competing with nucleotides for binding to the viral DNA polymerase and by incorporation into and termination of the growing viral DNA chain. The cellular thymidine kinase of normal, uninfected cells does not use acyclovir effectively as a substrate, hence toxicity to mammalian host cells is low.

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Antiviral activity

The quantitative relationship between the cell culture susceptibility of herpes viruses to antivirals and the clinical response to therapy has not been established in humans, and virus sensitivity testing has not been standardized. Sensitivity testing results, expressed as the concentration of drug required to inhibit by 50% the growth of virus in cell culture (EC_{50}), vary greatly depending upon a number of factors. Using plaque-reduction assays on Vero cells, the median EC_{50} value of acyclovir against clinical herpes virus isolates (subjects receiving placebo) was 1.3 μ M (range: <0.56 to 3.3 μ M).

Drug Resistance

Resistance of HSV to acyclovir can result from qualitative and quantitative changes in the viral TK and/or DNA polymerase. Clinical isolates of HSV with reduced susceptibility to acyclovir have been recovered from immunocompromised subjects, especially with advanced HIV infection. While most of the acyclovir-resistant mutant isolates from immunocompromised subjects thus far have been found to be TK-deficient, other mutant isolates involving the viral TK gene (TK partial and TK altered) or DNA polymerase have been identified. TK-negative mutants may cause severe disease in infants and immunocompromised adults.

The possibility of viral resistance to acyclovir should be considered in immunocompromised subjects who show poor clinical response during therapy.

RECOMMENDATIONS

With respect to virology, this revised label is acceptable.

Lalji Mishra, Ph.D.

Microbiologist

CONCURRENCES:

_____ Date _____

HFD-530/J. O'Rear /TL Micro

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 018; Review Completed: 03/11/13

CC:

HFD-530/NDA 203791

HFD-530/ Division File

HFD-530/ Micro/ L. Mishra

HFD-530/RPM/S. Mosaddegh.

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/s/

LALJI MISHRA
03/20/2013

JULIAN J O REAR
03/20/2013

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 000; Review Completed: 10/22/12

Reviewer: LALJI MISHRA, Ph.D.

Date Submitted: 03/12/12

Date Received: 03/13/12

Date Assigned: 03/13/12

Sponsor:

(b) (4)
For BioAlliance Pharma, Inc.
59 Boulevard du General Martial Vlain
75015 Paris, France

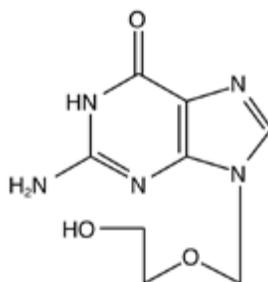
Product Name(s):

Proprietary: Sitavig

Non-proprietary: Acyclovir Lauriad[®]

Chemical: 2-amino-1,9-dihydro-9-[(2-hydroxy)methyl]6-*H*-purin-6-one

Structure:



ACYCLOVIR

Molecular formula: C₈H₁₁N₅O₃

Molecular mass: 225.2

Drug category: Antiviral

Indication: Treatment of recurrent oro-facial herpes and

(b) (4)

Dosage Form/Route of administration: mucoadhesive buccal tablet (MBT)/oral

Additional Submission Reviewed

Supplement #	Date of Correspondence	Date of Receipt
N203791 SDN 03	06/06/12	06/06/12
N203791 SDN 04	06/15/12	06/15/12
N203791 SDN 07	07/09/12	07/11/12
N203791 SDN 09	07/16/12	07/16/12
N203791 SDN 12	08/07/12	08/08/12
N203791 SDN 14	10/19/12	10/24/12
N203791 SDN 15	11/13/12	11/13/12

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA 203791 SDN 000; Review Completed: 10/22/12

EXECUTIVE SUMMARY

BioAlliance Pharma has submitted a 505(b2) new drug application (NDA) for acyclovir Lauriad™ mucoadhesive buccal tablet for the treatment of recurrent orofacial herpes and (b) (4). Acyclovir Lauriad™ mucoadhesive buccal tablet (ABT 50 mg) has been designed with the objective of delivering high (over the EC₅₀ value of acyclovir against HSV-1) and prolonged acyclovir concentrations in the oral cavity for an improved cutaneous and mucosal diffusion at the expression and infection sites of labial herpes virus.

The sponsor has submitted a review of the published literature for both the nonclinical and clinical sections of the NDA. The sponsor has submitted published data on the mechanism of action of acyclovir, antiviral activity of acyclovir in cell culture against laboratory and clinical isolates, antiviral activity in animal models, acyclovir-resistance associated substitutions and susceptibility of acyclovir resistant isolates in cell culture. These published data are reviewed and documented in this Virology Review of the NDA 203791. In addition, the sponsor has submitted a virology report by D. Boutolleau, Pharm.D., Ph.D., Associate Professor, Department of Virology at Pitié-Salpêtrière University Hospital, Paris, France. In this report, Dr. Boutolleau determined the antiviral activity of acyclovir against 81 HSV clinical isolates obtained from immunocompromised patients (HIV-1 infected and transplant recipients), suffering from various HSV-induced diseases.

This report showed that the EC₅₀ values of acyclovir for 19 sensitive HSV-1 isolates ranged from <1 to 2.4 μM whereas the EC₅₀ values of acyclovir for 16 resistant HSV-1 isolates ranged from >10 to 50 μM. Similarly, the EC₅₀ values of acyclovir for 19 HSV-2 sensitive isolates ranged from <1 to 6 μM whereas the EC₅₀ values of acyclovir for 27 resistant HSV-2 isolates ranged from 11 to >50 μM.

Genotypic analyses of the TK gene (UL23) were performed for two acyclovir resistant HSV-1 and two HSV-2 strains. Amino acid substitution C336Y and nonsense mutation D228stop were identified in the acyclovir resistant HSV-1 strains and amino acid substitution G201D and nonsense mutation L263stop in the acyclovir resistant HSV-2 strains.

The sponsor submitted a clinical report for assessing the correlation of saliva acyclovir concentration with HSV-1 DNA copies/mL. However, the HSV-1 DNA levels for baseline samples were not available. A comparison of baseline saliva viral load for samples after ABT treatment for a given subject could not be made since data on baseline saliva viral load were not determined due to unavailability of the samples and study design. The duration after ABT administration when saliva samples were collected for determination of acyclovir concentration and HSV-1 viral load varied from 2.25 to 49 hours. Similarly, acyclovir concentration in saliva varied from 5.95 nM (1.34 ng/mL) to 3.795 mM (854,000 ng/mL).

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A comparison of saliva HSV-1 DNA copies/mL from subjects treated with ABT and placebo indicated that ABT treatment exhibited antiviral activity. Median and mean HSV DNA levels were determined for subjects with HSV DNA >1,000 copies/mL. Median HSV DNA copies/mL of saliva for subjects treated with placebo (n=17) were 353,713 copies/mL and for ABT treated subjects (n=16), 46,350 copies/mL. Similarly, mean HSV-1 DNA copies/mL of saliva for placebo treated subjects were 1,290,955 copies/mL and for ABT treated subjects, 173,198 copies/mL. There was approximately a 87% reduction in saliva HSV DNA copies/mL in ABT treated subjects compared to placebo. These results indicate ABT exhibited anti-HSV activity in subjects who received a single dose of 50 mg ABT

Recommendations

Recommendation and Conclusion on Approvability

With respect to virology, this NDA is approvable.

Administrative

Reviewer's Signature(s)

Lalji Mishra, Ph.D.
Microbiologist, HFD-530

Concurrence

Julian O'Rear, Ph.D. Clinical Microbiology Team Leader

BACKGROUND

BioAlliance Pharma has submitted a new drug application (NDA) for acyclovir Lauriad™ mucoadhesive buccal tablet for the treatment of recurrent orofacial herpes and _____^{(b) (4)}. Acyclovir is an acyclic nucleoside analog of guanine approved for the treatment of both acute and recurrent herpes simplex virus infections and acute varicella zoster infections.

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Acyclovir is marketed in various prescription and over-the-counter products worldwide and has been approved in the United States since 1982 under various trade names, Zovirax[®] being the most common or referenced. Approved formulations in the United States include 200 mg capsules, 400 and 800 mg tablets, 200 mg/5 mL oral suspension, 5% topical cream, 5% topical ointment and solution for injection. Acyclovir has been marketed in the European Union since the 1980's under the trade name Zovirax[®] (oral 200, 400 and 800 mg tablets, oral suspension, and topical formulations including creams and ointments and solution for injection).

Acyclovir Lauriad[™] mucoadhesive buccal tablet is a new propriety delivery system that allows for a rapid and prolonged release of an active substance in the patient's buccal cavity with a once daily application. Acyclovir Lauriad[™] mucoadhesive buccal tablet (ABT 50 mg) has been designed with the objective of delivering high (over the EC₅₀ value of acyclovir against HSV-1) and prolonged acyclovir concentrations in the oral cavity for an improved cutaneous and mucosal diffusion at the expression and infection sites of labial herpes.

The sponsor stated (NDA 203791 SDN 03 dated 06/06/12) that the 505(b2) NDA application is only based on a thorough review of the literature for both the nonclinical and clinical Sections. No NDAs were referenced. However, the sponsor stated that for specific sections of the label, NDA 018828-Zovirax, and NDA 021478-Zovirax Cream 5%, were referred.

In support of this NDA, the sponsor has submitted two studies: a pharmacokinetic trial (BA2004/21/01), and one Phase 3 clinical trial (BA2005/21/02). For the virology section of the NDA, the sponsor has summarized data from published literature, submitted a virology report by D. Boutolleau, Pharm.D., Ph.D. However, the sponsor stated that no resistance data were available. Non-clinical virology data from published literature, and virology report by D. Boutolleau, Pharm.D., Ph.D. and viral load data in saliva samples and correlation with acyclovir concentration in salivary samples following lauriad tablet administration are reviewed here.

I. Mechanism of Action

Acyclovir is an acyclic nucleoside analogue of guanine. Acyclovir is selectively phosphorylated to acyclovir monophosphate in infected cells by the HSV or VZV encoded thymidine kinase (TK) ([Furman et al., 1981](#); [Elion, G.B., 1982](#)). Acyclovir monophosphate is further phosphorylated to acyclovir diphosphate by cellular guanylate kinase and diphosphate to triphosphate by cellular enzymes. Acyclovir triphosphate inhibits DNA synthesis by competing with deoxyguanosine triphosphate (dGTP) for viral DNA polymerase. Viral DNA polymerases exhibit a 10- to 30-fold greater affinity for acyclovir triphosphate than does cellular DNA polymerase ([Gnann et al., 1983](#); [Bacon et al., 2003](#)). Incorporation of the acyclovir triphosphate into the growing DNA chain prevents further extension of the DNA chain and causes DNA chain termination. Therefore, acyclovir

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inhibits herpes simplex virus replication by inhibiting viral DNA polymerase as a competitive inhibitor and blocks viral DNA synthesis by causing chain termination ([Gnann et al., 1983](#)).

Acyclovir triphosphate has a short intracellular half-life, about 0.7-1 h, after which, it is rapidly metabolized to the acyclonucleoside which diffuses out of the cell ([Weinberg et al., 1992](#)).

II. Antiviral Activity

The anti-HSV activity of acyclovir was determined by plaque reduction assay or inhibition of cytopathic effect. The concentration of acyclovir required to inhibit 50% the growth of virus in cell culture (EC_{50}) varies greatly depending upon cell types employed and assay used. EC_{50} values ranged from 0.08 μ M to 0.53 μ M against HSV type 1 and 0.12 μ M to 1.62 μ M against HSV type 2 ([Schaeffer, 1982](#); [Collins, 1983](#); [Fiddian et al., 1984](#)).

In human embryonic lung fibroblast, HEL cells, the mean EC_{50} values of acyclovir against HSV-1 isolates (n=6) were $2.22 \pm 1.77 \mu$ M ($0.5 \pm 0.4 \mu$ g/mL) as determined by plaque reduction assay and $3.11 \pm 2.22 \mu$ M ($0.7 \pm 0.5 \mu$ g/mL) by enzyme-linked immunoassay (EIA). Under similar assay conditions, EC_{50} values of acyclovir against HSV-2 isolates were $5.77 \pm 12.44 \mu$ M ($1.3 \pm 2.8 \mu$ g/mL) by plaque reduction assay and $6.6 \pm 12.00 \mu$ M ($1.5 \pm 2.7 \mu$ g/mL) by EIA ([Weinberg et al., 1992](#)).

As reported by Palmer et al. (2000), immunocompetent hairless SKH-1 mice were exposed to HSV-1 applied to the oral mucous membrane as a model for herpes labialis. Topical treatment with 5% acyclovir, 5% acyclovir monophosphate (ACV-MP), or 1% penciclovir (Denavir) was initiated 24, 48, or 72 h after infection and continued 3 times daily for 7 days. Treatment with acyclovir or ACV-MP significantly reduced viral replication and lesion formation when applied as late as 72 h post infection. In contrast, penciclovir was not effective even when treatment was begun 24 h after infection. These results indicate that the orofacial HSV-1 infection of mice shares many clinical and virological features with human herpes labialis, that the infection can be ameliorated with an effective drug such as acyclovir, and should be a good model for evaluating new therapies for oral and cutaneous HSV infections.

In addition, BioAlliance Pharma has submitted a study ([Boutolleau Report](#)) on cell culture activity of acyclovir against 81 HSV clinical isolates. The report is reviewed and described in the Results section.

III. Resistance

Isolation of drug-resistant HSV from immunocompetent patients remains infrequent (0.1 to 0.7%). However, the isolation of resistant HSV from immunocompromised patients is more common (4 to 7%) ([Bacon et al., 2003](#); [Coen, 1994](#)). Susceptibility based on a plaque reduction assay using Vero cells defined a break point for resistance to acyclovir of $\geq 9 \mu$ M ($\geq 2 \mu$ g/mL) ([Field 2001](#); [Sarisky et al., 2002](#); [Bacon et al., 2003](#)).

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When an HSV-1 isolate initially sensitive to acyclovir (EC_{50} value = 0.16 μM) was passaged serially in the presence of 10 μM acyclovir in Vero cells, resistant virus was selected ([Crumpacker et al., 1982](#)). By the third passage in 10 μM acyclovir, resistant virus exhibited an EC_{50} value of 40 μM and had diminished TK activity.

In 18 out of 20 acyclovir resistant viral isolates, EC_{50} values of >35.55 μM (>8.0 $\mu\text{g/mL}$) acyclovir could be calculated ([Sauerbrei et al., 2010](#)). Two strains, one HSV-1 and one HSV-2 strain had EC_{50} values of 23.2 and 16.4 μM (5.8 and 4.1 $\mu\text{g/mL}$) acyclovir, respectively. For penciclovir, 17 strains had an EC_{50} value of >35.55 μM (>8.0 $\mu\text{g/mL}$) and in 3 strains, 1 HSV-1 and 2 HSV-2 isolates, the EC_{50} value ranged between 10.22 and 16.00 μM (2.3 and 3.6 $\mu\text{g/mL}$). These results illustrate that all acyclovir resistant strains were cross resistant to and penciclovir ([Sauerbrei et al., 2010](#)). In comparison, the four acyclovir sensitive HSV-1 isolates had EC_{50} values between 0.44 and 1.33 μM (0.1 and 0.3 $\mu\text{g/mL}$) acyclovir, and 0.89 μM and 1.77 μM (0.2 and 0.4 $\mu\text{g/mL}$) penciclovir. ([Sauerbrei et al., 2010](#)).

Two viral-encoded proteins, the viral thymidine kinase (TK) and DNA polymerase (pol) are the only targets for acyclovir, and, resistance mutations in the genes for these two proteins account for all of the resistance to acyclovir observed in cell culture or in a clinical use of acyclovir ([Crumpacker et al., 1982](#); [Coen et al., 1982](#); [Crumpacker, 1988](#); [Crumpacker, 2009](#)).

Resistance to acyclovir, which is mediated by the viral TK, occurs by three mechanisms: (1) selection of a TK-deficient mutant; (2) selection of a TK-low producer mutant of herpes simplex; (3) selection of a mutant that produces an altered TK which is capable of phosphorylation of thymidine but no longer phosphorylates acyclovir ([Crumpacker, 2009](#); [Coen, 1994](#)).



Figure 1: Mechanism of action of acyclovir against herpes simplex virus and mechanism of resistance of HSV towards acyclovir; ACV-P, acyclovir monophosphate; ACV-P3, acyclovir triphosphate (Source: [Coen, D.M.](#) Trends in Microbiology, 1994; Vol 2, Page 482).

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TK mutations associated with resistance are either null due to an insertion or deletion (codons 92 and 146 of TK gene) or amino acid substitutions (codon 176_177, 336 of TK gene). DNA polymerase substitutions are mainly located at conserved positions of the enzyme. ([Morfin and Thouvenot, 2003](#)).

Table 1 shows that the substitution K62N, located in the ATP binding site of the TK, conferred the highest level of resistance to acyclovir with the exception of the TK-deleted virus and the dual TK-DNA pol mutant ([Sergeie and Boivin, 2006](#)). This substitution, also described in an acyclovir-resistant varicella-zoster virus isolate has been associated with a TK-low-producer phenotype. The substitution C336Y, implicated in the conformation of the TK protein, also resulted in a phenotype highly resistant to acyclovir. The substitution P131S, located in a non-conserved region of the TK gene, also conferred an acyclovir resistant phenotype. A single TK mutation (deletion of a C at position 467 leading to a truncated protein) resulted in a high level of acyclovir resistance whereas the single DNA pol substitution D907V, within a non-conserved gene region, resulted in a low level of resistance to acyclovir. The recombinant virus containing the C467 TK deletion and D907V substitution had the highest acyclovir EC₅₀ value among all recombinant viruses, confirming the synergistic effect of dual TK-DNA pol mutations ([Sergeie and Boivin, 2006](#)).

Table 1: Phenotypic and genotypic analyses of thymidine kinase and/or DNA polymerase HSV-1 recombinant mutants^a (Source: [Sergeie and Boivin, 2006](#).)

Mutations/Substitution				Gene Location	acyclovir EC ₅₀ (μM) (fold change vs. WT)
Tk		DNA pol			
Nt	aa	nt	aa		
Wild-type (WT)					0.18 (1)
Deleted C467	Stop 25 aa ds			String of C's (nt 463-467)	7.15 (40)
		A3735T	D907V	Between regions I and VII of DNA pol	0.66 (4)
Deleted C467	Stop 25 aa ds	A3735T	D907V		10.93 (61)
G1007A	C336Y			C-terminal active region of TK	5.38 (30)
A186C	K62N			ATP-binding site of TK	7.46 (41)
C391T	P131S			Non-conserved	3.73 (21)

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				regions of TK	
TK deleted	TK deleted				8.4 (47)

^a abbreviations: ds, downstream; aa, amino acid

Acyclovir EC₅₀ values were obtained from two independently generated recombinant viruses, each tested induplicate using a plaque reduction assay.

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Zovirax[®] labelling (2002)

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Boutolleau Report

Title: In vitro evaluation of the antiviral activity towards herpes simplex viruses of a mucoadhesive gingival tablet delivering acyclovir

Objective

The aim of this work was to evaluate the antiviral activity of a mucoadhesive gingival tablets delivering acyclovir against herpes simplex virus (HSV) in cell culture. Mucoadhesive gingival tablets were developed by BioAlliance Pharma. This study was conducted by Dr David Boutolleau, Pharm.D., Ph.D., Associate Professor, Department of Virology at Pitié-Salpêtrière University Hospital, Paris, France. This evaluation was done on the basis of:

1. The determination of acyclovir susceptibility, by plaque reduction assay, of two HSV ATCC strains and eight HSV clinical isolates.
2. A review of the data previously obtained in the Virology Department regarding HSV susceptibility and resistance to acyclovir

Comment:

The sponsor has determined antiviral activity of acyclovir against 81 HSV clinical isolates obtained from immunocompromised patients (HIV-1 infected and transplant recipients), suffering from various HSV-induced diseases. These patients had not received LauriadTM tablets.

Materials and Methods

(b) (4)

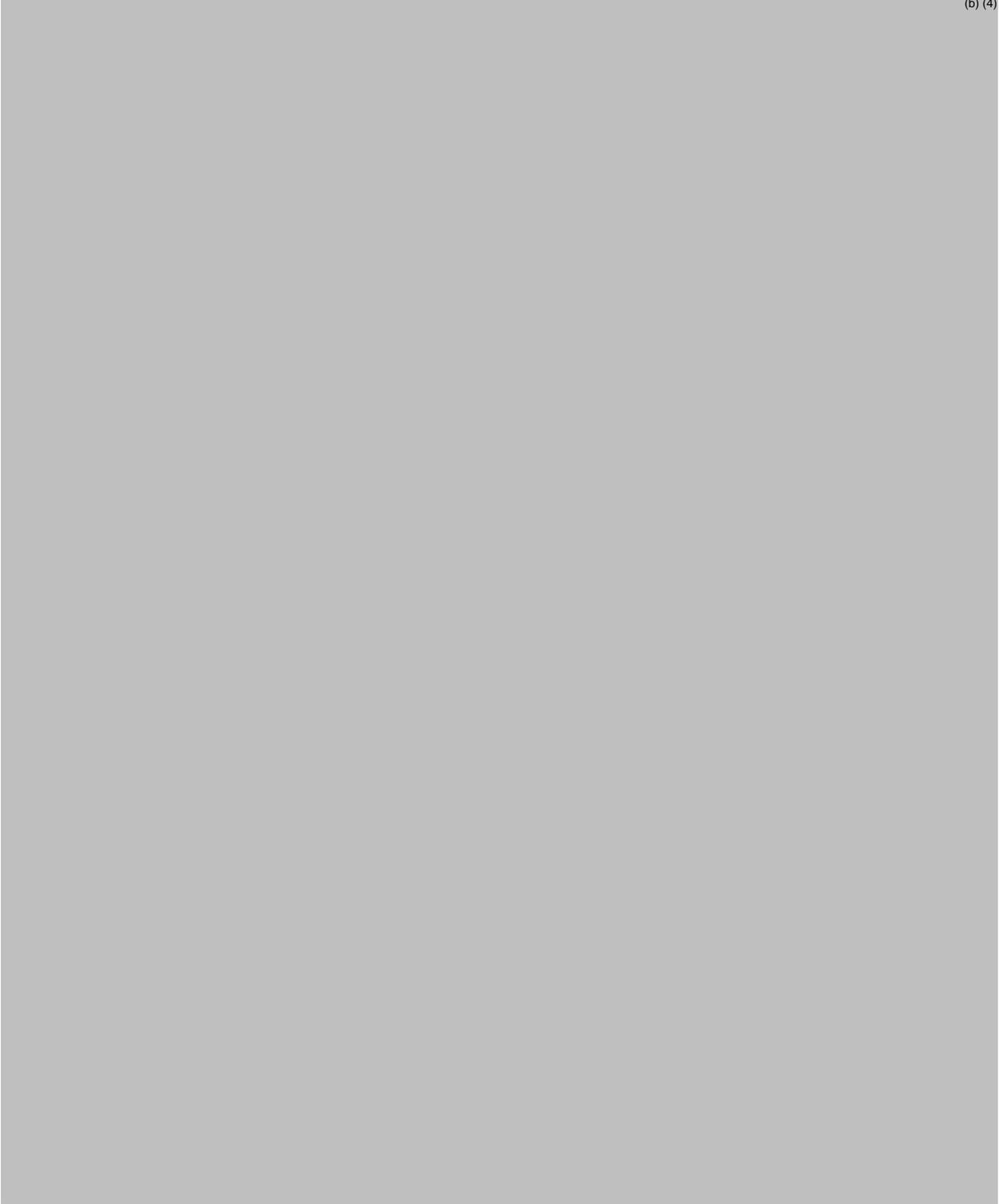
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(b) (4)

CONCLUSIONS

Following conclusions are drawn from this report entitled “In vitro evaluation of the antiviral activity towards herpes simplex viruses of a mucoadhesive gingival tablet delivering acyclovir.”

- EC₅₀ values of acyclovir for 19 sensitive HSV-1 isolates ranged from <1 to 2.4 μM whereas EC₅₀ values of acyclovir for 16 resistant HSV-1 isolates ranged from 10 to >50 μM.
- EC₅₀ values of acyclovir for 19 HSV-2 sensitive isolates ranged from <1 to 6 μM whereas EC₅₀ values of acyclovir for 27 resistant HSV-2 isolates ranged from 11 to >50 μM
 - The sponsor stated that results obtained in the modified plaque reduction assay performed with two HSV ATCC reference strains and eight clinical isolates are in agreement with the results previously obtained by both phenotypic and genotypic assays conducted in the Virology Department at Pitié-Salpêtrière University Hospital, Paris, France.

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Appendix 1

Table 3: Susceptibility of 35 HSV-1 clinical isolates determined by PRA.

(b) (4)



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Table 4: Susceptibility of 46 HSV-2 clinical isolates determined by PRA

(b) (4)



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Appendix 2

(b) (4)



Figure 1: Alignment of UL23 gene nucleotide sequences of the 2 acyclovir-resistant HSV-1 clinical isolates in comparison of that of the HSV-1 reference strain 17.
Nucleotide substitution (strain n°1) and insertion (strain n°2) are indicated in red.

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Figure 2: Alignment of TK amino acid sequences of the 2 acyclovir-resistant HSV-1 clinical isolates in comparison of that of the HSV-1 reference strain 17. Frameshift is indicated in grey and amino acid substitutions associated with acyclovir resistance are indicated in red. *: stop codon.

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Figure 3: Alignment of UL23 gene nucleotide sequences of the 2 acyclovir-resistant HSV-2 clinical isolates in comparison of that of the HSV-2 reference strain HG52. Nucleotide deletion (strain n°1) and substitution (strain n°2) are indicated in red.

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(b) (4)

Figure 4: Alignment of TK amino acid sequences of the 2 acyclovir-resistant HSV-2 clinical isolates in comparison of that of the HSV-1 reference strain HG52. Frameshift is indicated in grey and amino acid substitutions associated with acyclovir resistance are indicated in red. *: stop codon.

Clinical study: BA 2005/21/02

Title: A Randomized, Double-Blind, Single dose, One-Day Early Administration, Multicentre Study Comparing the Efficacy and Safety of Acyclovir LauriadTM 50 mg Mco-Adhesive Buccal Tablet to Matching Placebo in the Treatment of Herpes Labialis in Immunocompetent Patients

Objectives

Primary

To demonstrate the efficacy of a single dose of ABT 50 mg versus a single dose of matching placebo on the primary vesicular lesion of labial herpes.

Secondary

- To compare the efficacy of ABT 50 mg versus placebo on:
 - The evolution of prodromal symptoms to aborted lesions (herpes lesions that did not progress beyond the papule stage, preceded by recorded prodromal symptoms);
 - The healing of non primary lesions;
 - The duration of herpes episode;

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- The duration of symptoms;
- The healing of aborted primary lesions;
- The healing of intra-oral and mucosal non primary lesions;
- The incidence of and time to recurrence during 9 months following treatment (ancillary study in selected centers);
- To compare the local tolerability and general safety of ABT 50 mg to those of placebo;
- To evaluate the concentration of acyclovir in saliva (ancillary study in selected centers) and to assess its relationship with viral load in saliva and efficacy criteria;
- To evaluate the adhesion time of ABT 50 mg, the incidence of detachment and/or swallow within 6 hours post-dosing and the number of tablets replaced.

Study Design

This was a randomized, double-blind, patient-initiated, single dose multicentre study comparing ABT 50 mg with matching placebo (randomization in a 1:1 ratio) in immunocompetent patients suffering from recurrent labial herpes.

The primary objective of the trial was to compare (two-sided log-rank test) the time to healing of the primary vesicular lesion considered as time-to-event data in the ABT 50 mg group versus the placebo group.

The study duration for each patient included a screening period of 10 days maximum (Screening; Visit 1) before randomization (Day 0; Visit 2). The patient then had to wait for a new labial herpes episode to occur. If the patient did not experience an episode of labial herpes within the 6 months after randomization, he/she was excluded from the study. As soon as the patient experienced prodromal symptoms, he/she self-initiated his/her treatment by positioning the tablet with a finger on the side of the lesion on the upper gum, in the slight depression known as the canine fossa. Treatment was to be applied within one hour after the onset of prodromal symptoms and before the appearance of any signs of labial herpes lesions.

After initiation of treatment, the patients were under evaluation up to Day 14, or up to the healing of primary lesions, whichever came first. Patients were to complete a patient diary composed of a self-questionnaire and visual analogue scale (VAS) daily in the evening to record their symptoms and the stage of their herpes lesions (normal lip, erythema, papule, vesicle, crust)

Patients were requested to return to the clinic within 24 hours following treatment application. In selected centers, saliva samples were taken within 24 hours following treatment application to measure viral HSV-1 load and acyclovir concentration. Evaluation visits took place on Days 1, 3, 5, 7 and 14 (or when healing was reached).

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Microbiologic Specific Inclusion Criteria

- History of recurrent herpes labialis lesions where:
 - Recurrence is defined as at least 4 episodes in the preceding 12 months.
 - Herpes labialis lesions are characterized by their localization on the cutaneous and/or mucosal surfaces of the lips.
- At least 50% of previous episodes produced classical lesions progressing to the vesicular stage (i.e. episodes that progressed through macula, papule, vesicle, crust and healing).
- Prodromal symptoms (itching, tingling, pain etc.) should precede herpes labialis lesions in at least 50% of the recurrent episodes.

Microbiologic Specific Exclusion Criteria

Patients with any of the following criteria were not to participate in the study:

- More than 50% of recurrences that aborted spontaneously in the past 12 months.
- Primary herpes lesion outside the lips (e.g. nose, chin, etc.).
- Abnormal peri-oral skin condition that might affect the normal course of cold sores (e.g. eczema, psoriasis, etc.).
- Oral diseases whose prodromal symptoms may mimic those of herpes labialis, including recurrent oral aphthous disease.
- Oral diseases that might interfere with the evaluation of the efficacy or safety of the treatments, including gingivitis, parodontitis, mucositis, oropharyngeal candidiasis, etc.
- History of infection known to be resistant to acyclovir family agents.
- Previous vaccination against herpes.
- Immunocompromised condition including human immunodeficiency virus (HIV-1) positive.
- Allergy to any acyclovir containing agents.
- Treatment with topical steroids in the oral area within 4 weeks prior to study drug administration.

Treatments

Patients were randomized to receive either ABT 50 mg, or matching placebo. Treatments were administered only once, within 1 hour following the occurrence of prodromal symptoms. MBTs were to be applied by positioning the tablet with a finger on the side of the lesion on the upper gum, in the slight depression known as the canine fossa. The tablet could be held in place for about 30 seconds to facilitate adhesion. The tablet was not to be sucked or chewed.

The gingival tablet was to be applied within one hour after the onset of prodromal symptoms and before the appearance of any signs of herpes labialis lesions. After application, the tablet should have remained in the mouth until it had totally disappeared.

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The duration of tablet adhesion to the gum was shown in the PK/PD study on 12 healthy volunteers to be a minimum of 6 hours with a mean of 14 hours and a maximum of 18 hours.

If the first signs of herpes labialis were the appearance of vesicular lesions, then the patient was not to apply the tablet and was to wait for the next episode with prodromal symptoms.

Patients were to refrain from tooth brushing during the treatment day.

Selection of Doses in the Study

A single dose of ABT 50 mg or matching placebo was used. The dose used was based on the results of a PK/PD study in 12 healthy volunteers which showed that a single application of ABT 50 mg or 100 mg provided rapid (<30 min), high 1.78 mM (400 µg/mL) and prolonged (over 24 hours) acyclovir concentrations in saliva. Likewise, high and prolonged acyclovir concentrations were obtained in labial mucosa. These concentrations were markedly over the EC₅₀ value 0.1 µM (22.5 ng/mL) for HSV-1 ([Schaeffer, 1982](#)) (11,700-fold and 25,500-fold higher for saliva and at least 4- and 70-fold higher for labial mucosa, respectively). With ABT 50 mg, acyclovir plasma concentrations were low and only transiently reached the EC₅₀ values for HSV-1 with a relative bioavailability corrected by the dose of 49%. With ABT 100 mg, acyclovir plasma concentrations were over the EC₅₀ values of HSV-1 for several hours with a relative bioavailability corrected by the dose of 70%. These high and prolonged acyclovir concentrations in saliva and in labial mucosa and low plasma concentrations supported the choice of the 50 mg strength. The sponsor stated that based on the natural history of recurrent orofacial herpes (early, high and transient viral proliferation), it was considered that the higher and more prolonged acyclovir concentrations obtained with ABT 100 mg did not provide advantages over ABT 50 mg.

Endpoints

Primary Endpoint

The primary end-point was Time to Healing (TTH) of primary vesicular lesion.

1. Healing was defined as the loss of crust. Erythema may have been present. This was to be assessed by the investigator.
2. The TTH was the time from the treatment initiation (date and hour recorded) to the healing as defined above.
3. The primary vesicular lesion was the first developed lesion. It should have been located on the lip and should not have extended more than 1 cm outside the lip. Pure intra-oral lesions were not considered to be primary lesions.

Secondary Endpoint

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Aborted lesions were defined as herpetic lesions preceded by prodromal symptoms that did not progress beyond the papule stage. The evolution of prodromal symptoms to aborted lesions was a secondary endpoint.

Comment: For efficacy and safety analyses, please see the reviews of Statistician Zeng Wen, Ph.D. and Medical Officer Regina Alivisatos, M.D.

Relationship between saliva viral titer, acyclovir saliva concentration and efficacy parameters

The relationship between saliva viral titer and acyclovir saliva concentration (measured in selected centers) and efficacy parameters was to be investigated as an exploratory analysis. Saliva samples were taken on Day 1 (Visit 3: within 24 hours of study drug application). The saliva viral titers and acyclovir saliva concentrations (in patients from selected centers) were summarized after logarithmic transformation of the data if appropriate.

Drug Dose, Drug Concentration, and Viral load data in saliva samples

The sponsor stated that mean viral load (HSV-1/mL of saliva) was considerably lower in the ABT 50 mg group than in placebo group. However the difference was not statistically significant because of the low number of patients and the large expected variability. Due to limited data, the correlation between viral load, acyclovir concentration in saliva and efficacy could not be done.

The sponsor stated that data on viral load and acyclovir salivary concentrations in the ITT population are available in a separate report.

Comment:

The above mentioned virology report on viral load and acyclovir saliva concentration for subjects enrolled in study BA2005/21/02 could not be located.

In a fax dated June 29, 2012, the sponsor was asked to provide the virology report on viral load and acyclovir saliva concentration or identify its location in the NDA submission.

The sponsor provided Viral Load Report –Study BA2005/21/02 on July 16, 2012 (NDA 203791 SDN 09 dated 07/16/12). However, this report comprised mostly Excel spreadsheets with column headings which were not clear. The following comments were communicated to the sponsor on July 25, 2012. The sponsor provided a response to the virology comments on August 7, 2012 (NDA 203791 SDN 12 dated 08/07/12).

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Response to Virology Comments communicated on July 25, 2012.

DAVP Comment 1

Please provide baseline (prior to acyclovir Lauriad tablet application) saliva HSV-1 viral load (DNA copies/mL and titer) for patients listed in the Excel spreadsheet (Page 10).

Response

The LIP study was a patient initiated trial. Patients were randomized on the basis of their herpes history and were not suffering from herpes symptoms or lesions at the time they were included in the trial. Once randomized, patients were given study treatment to be applied as soon as prodromal symptoms occurred. It was thus considered that patients would not be able to come to investigational centers once symptoms occur and before applying treatment as they were requested to apply the treatment as soon as possible after first symptoms. Consequently, no saliva samples were drawn before treatment application. Therefore no baseline saliva viral load is available.

Comment

Adequate response

DAVP Comment 2

Please provide acyclovir concentrations for saliva samples for which HSV DNA copies/mL and titers were determined.

Response

See spreadsheet in Appendix 1 of this response document.

Comment

See response to Comment 2 and 3 are reviewed together.

DAVP Comment 3

Please provide time in hours after acyclovir Lauriad tablet application when saliva samples were collected for the HSV viral load determination for patients listed in the Excel spreadsheet (Page 10).

Response

See spreadsheet in Appendix 1 of this response document. A single Excel spreadsheet is provided with all information requested in questions 2 and 3. Two columns with

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acyclovir concentrations are included. As specified in the NDA documents, all saliva samples were retested for acyclovir using a more sensitive method.

Table 1: Saliva acyclovir concentration after Lauriad 50 mg treatment and HSV-1 copies/mL in saliva

Subject Identifier	Anti-viral TRT	Actual Treatment	Relative time (hours)	HSV-1 copies/mL saliva	Acyclovir Concentration (nM)
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(b) (4)

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(b) (4)



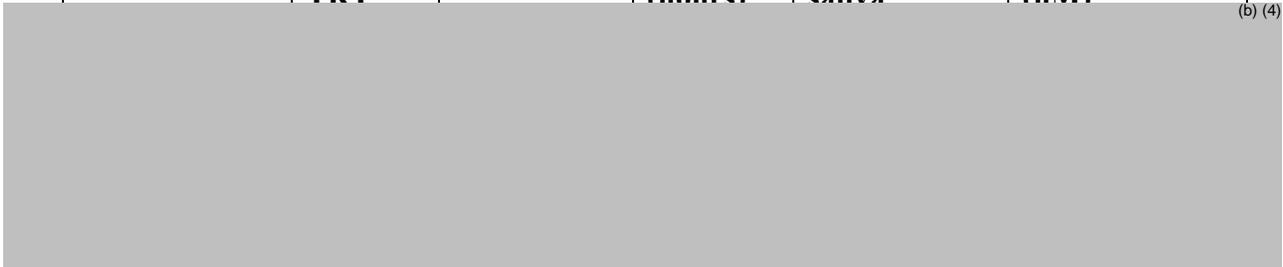
Summary

- Total number of lauriad treated subjects for whom saliva samples were used for HSV-1 quantification= 48
- Number of subjects positive for saliva HSV-1 DNA= 16.
- HSV DNA copies/mL range for these 16 samples ranged from 1,350 to 1.35x10⁶ copies/mL saliva. Only subjects above 1,000 copies/mL were counted as HSV-1 positive.
- Percentage HSV-1 positive = 33% (16/48)
- Six of the 16 HSV positive samples had higher concentration of saliva acyclovir concentration after lauriad 50 mg administration yet these samples contained HSV-1 DNA ranging from 0.2x10⁵ to 1.58x10⁶ copies/mL
- Median HSV-1 DNA = 46,350 copies/mL
- Mean HSV-1 DNA = 173,198 copies/mL

Table 2: Saliva acyclovir concentration after placebo treatment and HSV-1 copies/mL in saliva

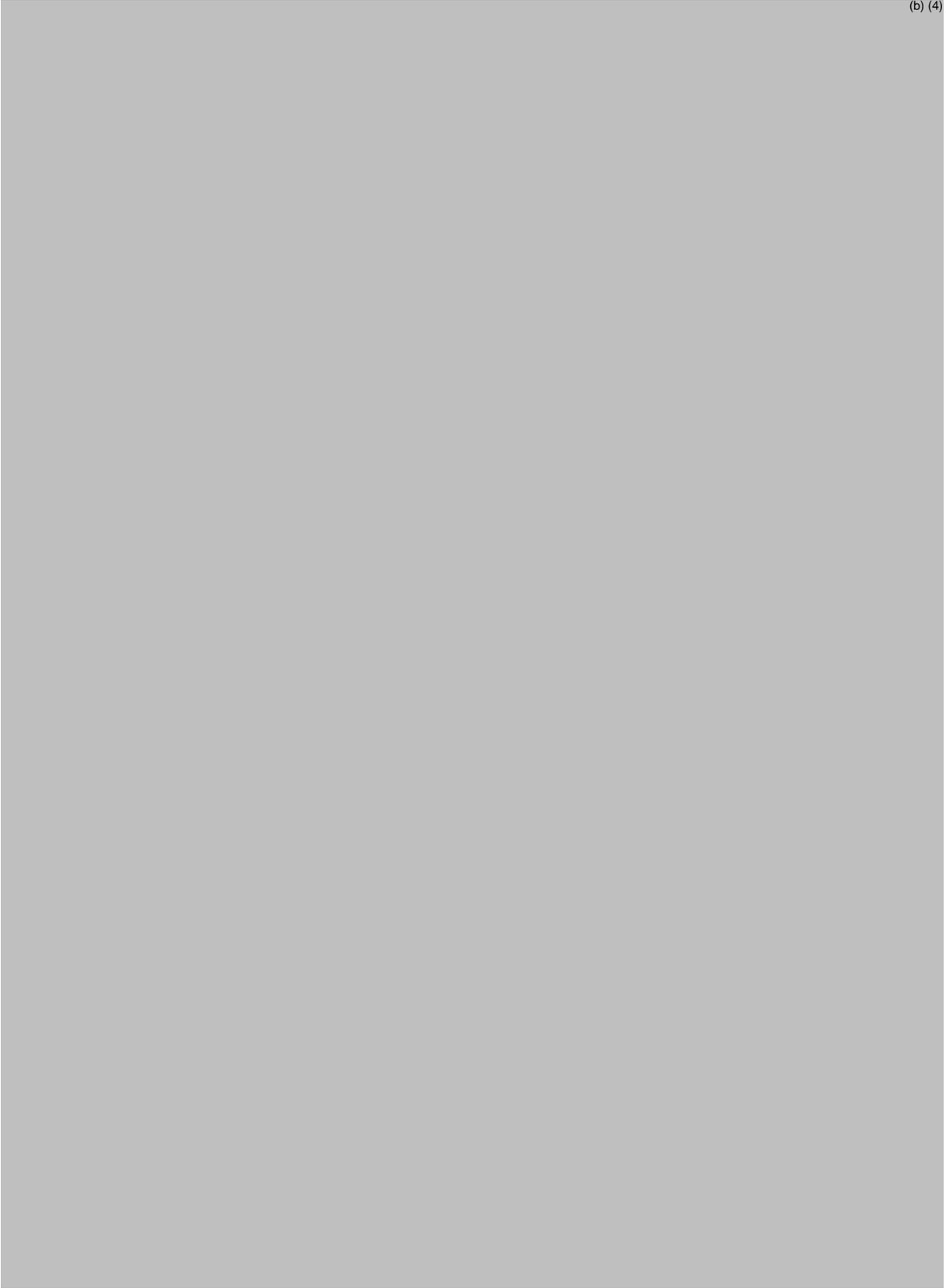
Subject Identifier	Anti-viral TRT	Actual Treatment	Relative time (hours)	HSV-1 copies/mL saliva	Acyclovir Concentration (nM)
---------------------------	-----------------------	-------------------------	------------------------------	-------------------------------	-------------------------------------

(b) (4)



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(b) (4)

* subject excluded for analysis since exposure was for 1 year?

Summary

- Total number of placebo treated subjects for whom saliva samples were used for HSV-1 quantification = 49
- Number of subjects positive for saliva HSV-1 DNA= 17
- HSV DNA copies/mL range for these 17 samples ranged from 1,963 to 7.7×10^6 copies/mL saliva. Only subjects above 1,000 copies/mL were counted as HSV-1 positive.
- Percentage HSV-1 positive= 35% (17/49).
- Median HSV-1 DNA = 353,713 copies/mL.
- Mean HSV-1 DNA = 1,290,955.765 copies/mL

Comments:

1. A comparison of baseline saliva viral load for samples after ABT treatment for a given subject cannot be made since data on baseline saliva viral load was not determined due to unavailability of the samples and study design.
2. Duration of ABT administration after which saliva samples were taken and saliva acyclovir concentration also varied. The duration after ABT administration when saliva samples were collected for determination of acyclovir concentration and HSV-1 viral load varied from 2.25 to 49 hours. Similarly, acyclovir concentration in saliva varied from 5.95 nM (1.34 ng/mL) to 3.795 mM (854,000 ng/mL).
3. For some subjects, both saliva HSV-1 viral load and saliva acyclovir concentrations after ABT administration were very high. These subjects were: 3050017, 4010048, 4010054, 4010065, 4020031, and 4020043 (Table 2 above). Higher saliva viral load (copies/mL and a very high acyclovir saliva concentration for these subjects indicates that ABT was not effective in these subjects. The duration of hours after lauriad administration when saliva samples were collected for these subjects ranged from 4.25 to 27.25 hours. It's also likely that if saliva samples for these subjects were available at a later time for determination of viral load (HSV DNA copies/mL), ABT might have exhibited significant anti-HSV activity in these subjects.

METHODOLOGY

HSV DNA quantification by real-time PCR

(b) (4)

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(b) (4)



Product description

(b) (4)



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(b) (4)



CONCLUSIONS

A comparison of saliva HSV-1 DNA copies/mL from subjects treated with ABT and placebo showed that ABT treatment exhibited antiviral activity. Median HSV DNA copies/mL of saliva for subjects treated with placebo were 353,713 copies/mL and for ABT treated subjects, median HSV DNA copies/mL of saliva were 46,350 copies/mL. Similarly, mean HSV-1 DNA copies/mL of saliva for placebo treated subjects were 1,290,955 copies/mL and for ABT treated subjects 173,198 copies/mL. There was approximately a 87% reduction in saliva HSV DNA copies/mL in ABT treated subjects compared to placebo. These results suggest that ABT showed anti-HSV activity in subjects who were received a single dose of 50 mg ABT

Appendix: Excel Sheet

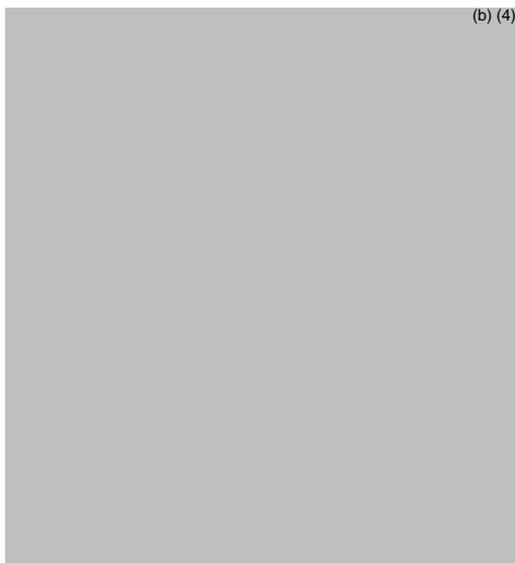
Placebo

Lauriad

(b) (4)



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Draft Labeling test

The draft labelling for Acyclovir Lauriad™ 50 mg buccal tablets is provided herein and is based on the approved labelling for the reference listed drugs Zovirax®, Zovirax® cream 5% and Xerese®-package insert.

The package Zovirax and Zovirax Cream 5% have been obtained from the DailyMed website at the following addresses respectively:

<http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=53854> and
<http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=38016>.

The sponsor proposed version of Microbiology label with the DAVP corrections (strike-through) are shown in red font and the proposed changes in the blue font.

Comment

In response to E-mail correspondence dated November 1, 2012, the sponsor stated that all HSV-1 and HSV-2 isolates tested were recovered from immunocompromised patients (i.e., HIV-1 infected patients and transplant recipients) treated with acyclovir. HSV isolates listed in Table 2 and Table 3 of the Boutolleau's report were all obtained from patients receiving acyclovir. Therefore, no isolate obtained from patients who had not previously received acyclovir was investigated (NDA 203791 SDN 15 dated 11/13/12). The range, median EC₅₀ value and the number of isolates tested for HSV-1 and HSV-2 can only be provided for isolates obtained from patients previously treated with acyclovir and are as follows:

- Number of isolates tested for HSV-1: 19
 - Median EC₅₀ value: 0.2 μM
 - Range: 0.2 – 2.4 μM
- Number of isolates tested for HSV-2: 19

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- Median EC₅₀ value: 2.2 μM
- Range: 0.2 – 5.9 μM

12 CLINICAL PHARMACOLOGY

(b) (4)



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(b) (4)



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RECOMMENDATIONS

With respect to virology, this NDA is approvable.

Lalji Mishra, Ph.D.
Microbiologist

CONCURRENCES:

HFD-530/J. O'Rear /TL Micro

Date _____

CC:
HFD-530/NDA 203791
HFD-530/ Division File
HFD-530/ Micro/ L. Mishra
HFD-530/CSO/ Mosaddegh, S.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LALJI MISHRA
11/16/2012

JULIAN J O REAR
11/16/2012

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 203791

Applicant: BioAlliance Pharma, **Stamp Date:** 03/12/12
 Inc.
 59 Boulevard du General Martial
 Vlain
 75015 Paris, France

Drug Name: Sitavig **NDA Type:**000

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	√		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	√		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	√		
4	On its face, has the applicant <u>submitted</u> cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	√		Applicant has cited EC ₅₀ values form published papers
5	Has the applicant <u>submitted</u> any required animal model studies necessary for approvability of the product based on the submitted draft labeling?		√	Animal model study is not required
6	Has the applicant <u>submitted</u> all special/critical studies/data requested by the Division during pre-submission discussions?	√		Applicant has cited Zovirax NDA and published papers.
7	Has the applicant <u>submitted</u> the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?		√	Applicant has not submitted HSV resistance data in DAVP Resistance Template Format. Applicant has not identified amino acid substitutions and not provided sequences of HSV resistant isolates.
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the		√	Applicant has referred to publications which describe methods used for genotypic and

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?			phenotypic analysis.
9	Has the applicant <u>submitted</u> draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	√		
10	Has the applicant <u>submitted</u> annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	√		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	√		
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		√	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE?

_____ **Yes** _____

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

The sponsor is requested to submit HSV resistance data in DAVP Resistance Template Format.

The sponsor should provide a complete description of assays used for genotypic and phenotypic analyses (Boutolleau report) in addition to the gene sequences and amino acid substitutions identified in HSV resistant clinical isolates.

Lalji Mishra, Ph.D.	04/23/12
Reviewing Microbiologist	Date
Jules O'Rear, Ph.D.	04/23/12
Microbiology Team Leader	Date

File name: 5_Microbiology Filing Checklist for a NDA or Supplement 010908

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/s/

LALJI MISHRA
04/27/2012

JULIAN J O REAR
04/27/2012