# CENTER FOR DRUG EVALUATION AND RESEARCH

*APPLICATION NUMBER:*

**22-436**

# MICROBIOLOGY REVIEW(S)

**MICROBIOLOGY REVIEW**
**DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**

**NDA 22-436 SN 000 (Original and 4 Amendments) REVIEW DATE: 12/22/2008**

**Reviewer:** N. Biswal

**Date Submitted:** 9/30/2008
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10/30/2008 (Amendment 2); 11/19/2008 (Amendment 3)
12/05/2008 (Amendment 4)

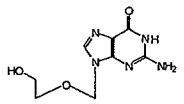
**Sponsor:** Medivir AB
PO Box 10868
SE-141 22 Huddinge
Sweden

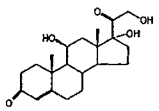
**Product Names:**

**Code Name:** Lipsovir®
ME-609 Cream, (Acyclovir, 5% and hydrocortisone, 1%)

**Chemical Names:** 2-amino-1.9- dihydro-9-[(2-hydroxy)methyl]6-*H*-purin-6-oneon;
Hydrocortisone

**STRUCTURAL FORMULA:**





| | Acyclovir | Hydrocortisone |
|---|---|---|
| **Empirical Formula:** | $C_8H_{11}N_5O_3$ | $C_{21}H_{30}O_5$ |
| **Molecular Weight:** | 225.21 | 362.47 |

**DRUG CATEGORY:** Antiviral Antiinflammatory

**INDICATION:** Recurrent herpes labialis

**DOSAGE Form/Route of Administration:** Cream (5% Acyclovir and 1% hydrocortisone)/ Topical

**SUPPORTING DOCUMENTS:** IND 58,500 – ME-609; DMF [redacted] b(4)
DMF [redacted]

**BACKGROUND AND SUMMARY:** In this New Drug Application the sponsor is seeking marketing approval of a new topical formulation of ME-609 cream (containing acyclovir 5% and hydrocortisone 1%) as a prescription drug product for the treatment of early signs and symptoms of recurrent herpes

labialis to prevent the development and reduce the duration of ulcerative cold sores in adults and adolescents (12 years and older). The sponsor has also submitted four amendments (BM) as noted earlier to change the name of ME-609 to Lipsovir® (amendment 1), to provide the final report on the time to next recurrence data collected in a one year follow-up completed in August 2008 for the clinical study 609-06 (see below) (amendment 2), and two administrative letters on the payment of fees appropriate for this NDA (amendments 3 and4). For simplicity, all these submissions except amendments 3 and 4 are reviewed together.

Both acyclovir (ACV) and hydrocortisone (now combined in a topical formation of ME-609 cream or Lipsovir®) are FDA approved drugs [redacted] respectively, the sponsor has provided copies of letters authorizing FDA to review [redacted] Drug Master File N0. [redacted] and [redacted] Type [redacted] DMF N0 [redacted] in Module 1.4.1 of this submission. b(4)

**ACV** is a synthetic guanosine analogue with inhibitory activity against a number of human herpesviruses. Without any intrinsic antiviral activity by itself, however, ACV must be phosphorylated as ACV-triphosphate (ACVTP) to exert its antiviral activity. Viral-encoded deoxypyrimidine kinase, or thymidine kinase (Tk) is the key enzyme which catalyzes the initial phosphorylation of ACV in virus infected cells to ACV-monophosphate (ACVMP), thus providing the basis for the selective antiherpetic action of ACV. The conversion of ACVMP to ACV diphosphate (ACVDP) in HSV-infected cells is catalyzed by cellular guanylate kinase which is present in equimolar amounts in both uninfected and virus-infected cells. After further phosphorylation of ACVDP by cellular enzymes, predominantly phosphoglycerate kinase, ACVTP is capable of inhibiting the viral DNA polymerase. Mammalian DNA polymerases (•, •, • and •) from uninfected cells are also inhibited by ACVTP but at a much higher concentration than needed for herpes viral DNA polymerases thus contributing to the overall selectivity of ACV in the inhibition of herpesvirus replication.

Sensitivity test results, expressed as the concentrations of ACV required to inhibit by 50 % the replication of herpesviruses in cell culture ($EC_{50}$) have ranged from 0.02-1.5 μg/ml (0,088-60.16 μM) against HSV-1, and 0.01-9.9 μg/ml (0,044-43.96 μM) against HSV-2, depending upon the virus strain, cell type and method(s) of virus assay used. In a clinical setting, ACV is effective as oral or intravenous therapy of primary genital HSV infection, and as oral prophylaxis of recurrent episodes of genital HSV infection. ACV has been less successful as topical therapy for recurrent labial or genital HSV infection. Any formulation of ACV is not effective against the latent state of herpesvirus infection.

HSV mutants resistant to ACV have been isolated from cultured cells *in vitro* as well as from patients undergoing treatment for HSV infections with ACV. Serial passage of various HSV strains in the presence of increasing concentrations of ACV has resulted in the emergence of mutants exhibiting $EC_{50}$ values of more than 10 fold greater than that of the wild type HSV. HSV resistance to ACV is usually defined as when ACV $EC_{50} \geq 3$ •g/ml (13.32 μM). Resistance of HSV to ACV are known to result from mutations in either of the two viral genes, *Tk* and/or DNA *pol*, involved in viral DNA replication. There are three different kinds of $ACV^r$ *Tk* mutations. The simplest and most common **Tk-deficient ($TK^-$)** mutants are completely devoid of any Tk activity and the viruses containing these

mutations are resistant to ACV and other TK-dependent antivirals. These mutations include missense point mutations in the enzyme active site, nonsense mutations and single base deletions or insertions that shift the translational reading frame. The second type of mutant has an impaired Tk; the virus is $ACV^r$ but still possess some Tk activity **(Tk partial).** They can not be easily distinguished from $Tk^-$ mutants. The third kind, **Tk altered ($Tk^a$) mutants,** can phosphorylate natural substrates such as thymidine and other TK-dependent antivirals but its phosphorylation of acyclovir is highly impaired. Some other mutants may be both $TK^a$ and $Tk^-$. **DNA *pol* mutants** are rare and result from single nucleotide changes in the polymerase gene regions associated with substrate interactions. Although many of these mutant phenotypes are variable in their pathogenic potential, quite often they demonstrate the functional attributes of wild-type virus. As noted earlier, FDA has already approved ACV (Zovirax®) for the treatment of herpes labialis, genital herpes as well as herpes zoster (shingles) and chicken pox (varicella).

**Hydrocortisone** is a glucocorticosteroid that is believed to be naturally produced by the adrenal cortex. While the exact molecular mode of action of hydrocortisone is complex and not fully elucidated, it is known to enter the cell by passive diffusion, and exerts its major activity by binding to cytoplasmic receptors. It is also known to modulate cellular inflammatory response and vasoconstriction. In infective skin diseases, hydrocortisone is often used together with an antibiotic to promote healing and reduce inflammation. There are conflicting reports on the effects of glucocorticoids upon the replication of HSV in cell culture, depending in part on the type of cells and strain of HSV used in the experiments. Treatment of such virus infected cells with dexamethasone or other glucocorticoids has shown both stimulation (Dreyer LL et al., 1989, J Clin Lab Anal 3:236-243) and inhibition of viral replication (Notter MF and Docherty JJ, 1978, J Med Virol 2:247-252: Hardwicke MA and Schaffer PA, 1997, J Virol, 71:3580-3587; see below). The molecular mechanisms for the stimulatory or inhibitory effects of glucocorticoids on HSV replication are not known.

Rationale for the proposed clinical indication for ME-609 cream is based on the following premise.

Topical treatment of recurrent HSV infection with antiviral drugs such as ACV alone has not been very successful. A combination of the antiviral ACV with an anti-inflammatory agent such as hydrocortisone, the sponsor believes, may represent a new approach to the treatment of recurrent HSV infection in which both the viral replication and the inflammatory process (e.g., herpes lesions, pain and healing) can be controlled more efficiently by ACV and hydrocortisone, respectively. To substantiate this claim, the sponsor has conducted a number of nonclinical (in cell culture and in animal models) and clinical studies to demonstrate effectiveness of ME-609 as an anti-HSV agent. Results of some of these studies (which have been reviewed earlier for IND 58,500) relevant to the current submission may be summarized as follows.

## A. Nonclinical Studies

### A.1. Anti-HSV-1 activity of the Combination of ACV and Glucocorticoids in Cell Culture

**Study No. 01-609-021** (summarized in Module 2.6.2.3.1) was conducted by Erlandsson and coworkers to investigate whether combination of glucocorticoid with ACV therapy of herpes simplex virus infections will up-regulate the HSV replication.
Briefly, the effects of two glucocorticoid preparations, hydrocortisone and dexamethasone, with or without ACV on HSV-1 replication were evaluated in three different cell lines, the primary human embryonic lung fibroblasts (HEL), the human epithelial (A101D) cell line and the primary human gingival fibroblasts (HGF) known to express the glucocorticoid receptor protein by the standard viral yield assays. Confluent monolayers of the cells were separately inoculated with HSV-1 (C42 p2 strain) at a low (0.001) multiplicity of infection to mimic the *in vivo* situation and thereafter treated for 48h with acyclovir in the range of 0-10 μM in combination with a weak or a strong glucocorticoid in the range 0-100μM. The cell supernatants were then assayed in a standard plaque assay using Vero cells.

Results presented in Fig. 1 show that while various concentrations of ACV inhibited the replication of HSV-1 in the two different host cells, various concentrations (ranging from 0-100 μM) of hydrocortisone (Fig. 1A) or dexamethasone (Fig.1B) alone (0 μM) did not influence the viral replication in the host HEL cells. More importantly, a combination of these two glucocorticoids (up to 100 μM) separately with various concentrations (ranging from 0-10 μM) of ACV, did not significantly alter the anti-HSV-1 activity, as measured by the plaque forming units of ACV in cultured host cells.

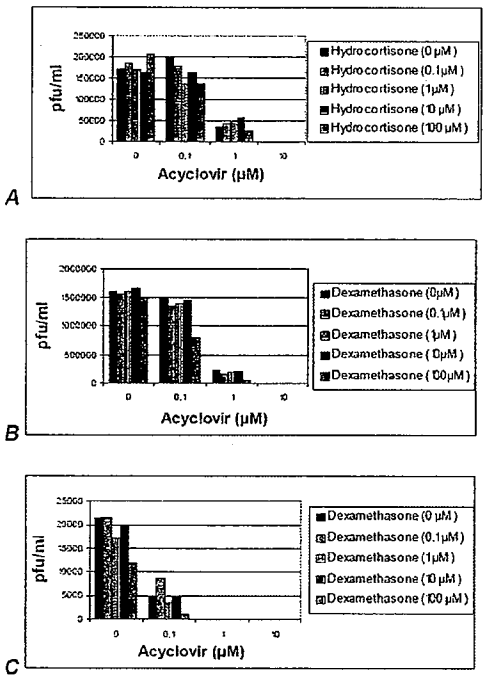



Figure 1. Viral multiplication in HSV-1 infected cells treated with a combination of increasing concentrations of ACV and either hydrocortisone or dexamethasone. HEL cells were infected at a multiplicity of infection of 0.001 and after 45 minutes of absorption fresh medium containing ACV and hydrocortisone (**A**) or dexamethasone (**B**) was added. After 48 hours of infection the viral supernatants were titrated in Vero cells. HSV-1 infected A101D cells treated with a combination of acyclovir and dexamethasone (**C**). Each point is an average of 8 values.

The sponsor has stated that the experiments involving HGF cells were conducted as described above for HEL and A101D cells to address the results from a previous report (Harrell A J and Sydiskis R H, 1982, J Baltimore Coll Dent Surgery 35:9-13) that HSV-1 yield in HGF cells was increased in response to glucocorticoid (Dexamethasone) treatment.

The results presented in Figure 2 show that the virus yield was not changed in HGF cultures when dexamethasone (1 μM) was added at the time of infection (0hDex, Fig. 2A). On the other hand, pre-treatment (24h prior to HSV-1 infection, -24hDex) with dexamethasone, and continuing treatment during the infection, caused viral exacerbation in the HGF cells (Fig. 2A). However, this increased virus replication was inhibited in the presence of ACV (1 μM) (Fig. 2B).

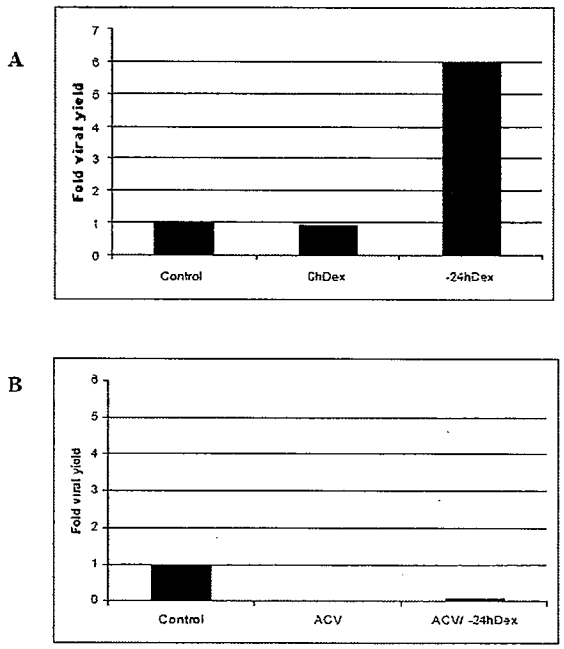



**Figure 2.** Viral multiplication in HSV-1 infected human gingival fibroblasts (HGF) co-treated (0hDex) or pre-treated (-24hDex) with dexamethasone alone **(A)** or a combination of ACV and dexamethasone 24h prior to HSV-1 infection **(B)**. HGF Cells were either pretreated with dexamethasone (-24hDex), or non-treated prior to infection at a multiplicity of infection of 0.001. After 45 minutes of virus absorption, fresh medium containing dexamethasone alone or a combination of ACV and dexamethasone was added. Forty eight hours of infection, the cell supernatants were titrated for infectious virus in Vero cells.

From these results the sponsor has concluded that

- There was no change in HSV replication in host cells treated with a glucocorticoid at the time of virus infection
- There was an increase in HSV replication when the host cells had been pretreated with hydrocortisone or dexamethasone prior to virus infection.

- This increase in viral yield was effectively inhibited by the addition of ACV. The addition of hydrocortisone or dexamethasone in the absence of ACV did not influence viral replication in primary human embryonic lung fibroblasts or human epithelial A101D cells.

A.2. **Anti-HSV-1 activity of ME-609 in Animal Models**

**A.2a. In a Mouse Model (Study Report No 02-609-030, along with a revised Report 01-609-017 by Awan AR and coworkers, summarized in Module 2.6.2.2.2)**

As reviewed earlier (for the IND 58,500), most of these experiments submitted in this NDA application were conducted Awan *et al.*, 1998 (Antiviral Res., 38:43-53) to test the effectiveness of ME-609 in a mouse model that is supposed to mimic recurrent HSV infection in the presence of a primed immune response.

In this model, a group of immune donor BALB/c mice (female) were prepared by inoculating HSV-1 ($10^5$ pfu of strain SC16) into the skin of both ears. Seven days later, immunocompetent cells from the cervical lymph nodes were removed and inoculated to a group of recipient mice through the coccygeal vein. Two days before the inoculation with the immunocompetent cells, both the ears of the recipient mice were also infected with HSV-1. This process of transferring immunocompetent cells from the donor mice to the recipient mice was termed adoptive transfer of immunity (ATI). Between day 0 and day 4 after infection, the virus was supposed to have reached the nerve endings in the skin of the ear, and symptoms of HSV-1 infection, including zosteriform lesions in the ears of the recipient mice appeared. The disease parameters, such as the extent of the lesions (the zoster score), the extent of inflammation and swelling (the thickness of the ear) were then measured daily and the amounts of virus shedding (as plaque forming units in cell culture) from the infected ear were also determined.

This model was used to examine the efficacy of ME-609 cream in comparison to 5% acyclovir (Zovirax cream), 1% hydrocortisone and an untreated group.

The ME-609 cream used in these experiments was composed as follows.

| | |
|---|---|
| Acyclovir | 5.0 % |
| Hydrocortisone | 1.0 % |
| Cetyl stearyl alcohol | |
| (mineral oil) | |
| Sodium lauryl sulphated | |
| Poloxamer 188 | |
| Propylene glycol | |
| Isopropyl myristate | |
| water to | |

b(4)

Each study group consisted of 10 animals for observation (zoster score and ear thickness). All of these animals were given ATI. The animals were treated topically with the creams every eight hours (three times daily) on days four to seven post-infection. A second control group of 6 animals were not given ATI and were left untreated for observation only.

Results presented in several tabular and graphic forms demonstrated that ME-609 was "as good as or slightly better" in reducing both the ear thickness and zoster score when compared to the other treatments with ACV cream or hydrocortisone alone. Hydrocortisone alone was effective in reducing the ear thickness, while the effect on zoster score was limited. ACV cream alone exhibited "surprisingly good effect" on zoster score while the effect on ear thickness was negligible. The sponsor has hypothesized that the better than expected results on zoster score shown by ACV cream may be due to experimental variations or to the number of live immune cells used for the ATI procedures.

Effect of ME-609 on the viral shedding from the infected ears was also determined. Groups of four mice were sacrificed on day 5, 7, 9 and 13 after infection and the virus titers of the ears were assessed by a plaque reduction assay. The results showed that both ME-609 and ACV cream reduced the virus titers compared with untreated control mice. However, the effect of ACV cream on viral shedding was better than ME-609 treatment in that ACV cream inhibited virus shedding on day 9 post infection, while a low titer (average of 6 pfu/ear) of virus continued to be present in the ears treated with ME-609 alone. Hydrocortisone treatment increased the viral shedding period compared with the other groups and measurable virus titers were found also 13 days after infection. The sponsor has suggested that this prolonged viral shedding may be the cause for the rebound of ear thickness seen after the end of treatment in mice treated with hydrocortisone alone. Under similar conditions of experimentation, shedding of virus from the ears of untreated control mice given ATI was cleared between day 9 to day 13 post infection.

**Comment:** While the mouse model may offer the possibility of representing the immunological as well as the virological environment associated with recurrent HSV infections in man, the principal proponents of this model have several precautionary notes as follows.

1. The model is very complex with many different steps that can contribute to experimental variations, making interpretations of the results difficult. As pointed out above, the experimental protocols are quite dependent upon the extent of virus infection, the number and physiologic state of the donor immunocompetent cells and the degree of virus infection of the recipient host.
2. The mice may lick their ears and oral exposure to topical treatment can not be excluded even though the oral uptake of acyclovir and hydrocortisone is limited.
3. The mice with infected ears will scratch their ears, which could worsen the symptoms and increase the experimental variation.
4. The model has been developed with the view to inoculate the animals with maximally tolerated virus titers in order to obtain a clearly visible and measurable zosteriform virus infection. However, some mice will die at the peak of infection (day 9 p.i.) or after the peak of infection as HSV is known to cause encephalitis and morbidity in mice.
5. The model is very labor intensive and cumbersome to use.

b(4)

b(4)

**A.2b. In a Guinea pig Model (Study Report No 03-609-031, along with a revised Report 01-609-018 by Alenius S and Coworkers. Summarized in Module 2.6.2.2.1)**

This study was conducted to confirm and compare the anti-HSV-1 activities of the cream formulations of ME-609, Zovirax (ACV) and corresponding placebos by using a standard guinea pig model for cutaneous primary HSV infection.

The stated objective of this study was to determine which formulation of ACV in combination with hydrocortisone was most successful in reducing the lesion score in the HSV guinea pig model. The sponsor has also stated that the study was performed in 1998-1999 under non-GLP conditions and should be seen as supportive in the development of a cream containing acyclovir and hydrocortisone. In addition, no statistical analysis of the data was performed.

Briefly, Dunkin-Harley guinea pigs, weighing about 300-400g, of both sexes, were used in these experiments. The backs of the guinea pigs (2-9 animals in each experiment) were shaved and inoculated on four sites with HSV-1 (through a vaccination gun) with 20 μl of HSV-1 strain C42 (titer of $10^6$ PFU/ml). Two areas on each animal were treated with topical placebo and served as controls. The other two areas were treated with different topical formulations containing the antiviral compounds. Treatment was started at 24 or 48 hours after infection depending on experimental design and continued for 3 or 4 days. Approximately 30 μl of each formulation was applied gently over each infected area 2 to 4 times daily. Lesions were scored daily from day one post-infection until day twelve, when untreated lesions were healed. All scoring was performed blinded. A group of guinea pigs were also sacrificed at different stages of infection, individual areas were excised and samples were assayed for infectious virus in confluent monolayers of Vero cells.

Results presented in various tabular and graphic forms demonstrated that topical formulations containing a combination of 5% ACV and 1% hydrocortisone (ME609 and a modified aqueous cream (MAC) formulation) were superior to I% hydrocortisone alone and placebo in terms of lesion scores. ME-609 was found to be at least as good as the commercially available Zovirax cream containing 5% ACV. The lesion score profile of 1% hydrocortisone was similar to the placebo lesion score profile. More specifically, when tested simultaneously (head to head, as the sponsor has stated) on the back of the same animal, ME-609 Cream and Zovirax Cream, ME-609 Cream and Zovirax Cream reduced the average cumulative lesion score by 70% and 57%, respectively, and the average time-to-healing by 46% and 32%, respectively, compared with the Zovirax-like vehicle (Table 1, Experiment 1).

**Table 1: Average Cumulative Lesion Score and Time to Healing (Table 2.4-2, Study No. 03-609-031)**

| | Formulation | Average Cumulative Lesion Score (± SD) | Reduction Compared to Vehicle | Average Time to Healing (Days ± SD) | Reduction Compared to Vehicle |
|---|---|---|---|---|---|
| Experiment 1[a] | ME-609 | 7.7 ± 4.1 (n = 8) | 70% | 6.3 ± 2.1 (n = 9) | 46% |
| | Zovirax® Cream | 11.0 ± 4.4 (n = 8) | 57% | 7.9 ± 2.4 (n = 9) | 32% |
| | Zovirax® Vehicle | 25.6 ± 3.5 (n = 16) | | 11.6 ± 0.5 (n = 16) | |
| Experiment 2[b] | ME-609 | 7.8 ± 3.8 (n = 16) | 67%[c] | 5.5 ± 2.1 (n = 18) | 48%[c] |
| | Zovirax® Cream | 9.7 ± 4.2 (n = 16) | 45%[c] | 7.0 ± 1.9 (n = 18) | 29%[c] |
| | ME-609 Vehicle | 23.3 ± 2.5 (n = 16) | | 10.6 ± 0.8 (n = 16) | |
| | Zovirax® Vehicle | 17.6 ± 5.3 (n = 16) | | 9.9 ± 1.3 (n = 16) | |

[a] In Experiment 1, 1 animal had to be sacrificed on Day 7.
[b] In Experiment 2, 4 animals had to be sacrificed on Day 7.
[c] Compared to corresponding vehicle.
Source: Study Report No. 03-609-031; page 15-16(25)

Similarly, when compared to the ME-609 vehicle, ME-609 Cream reduced the lesion score and healing time by 67% and 48%, respectively, and Zovirax® Cream reduced these parameters by 45% and 29%, respectively, compared to the Zovirax vehicle (Table 1, Experiment 2). Animals treated with the MAC placebo formulation (cream 411) developed herpes lesions within two days of infection, which remained until around day 10-12 post-infection. In this primary infection model, ME-609 demonstrated similar effect in reducing viral shedding on day 5 as Zovirax cream containing 5% ACV only. No virus was found in any of the samples obtained on day 8 suggesting that no rebound or prolonged viral shedding was caused by ME-609 (Table 2).

**Table 2.** Virus titers in skin samples from guinea pigs (Table 3 in Study Report No 03-609-031)

| Treatment | DAY 5 | | | DAY 8 |
|---|---|---|---|---|
| | Average lesion score n=24 | % lesions with score 0 n=24 | Median virus titers PFU/sample n=9 (range) | Virus isolation n=9 |
| ME-609 | 0.12 | 88% | 300 (0 – 3500) | No virus found |
| Zovirax cream | 0.29 | 75% | 100 (0 – 600 000) | No virus found |
| Untreated | 3.00 | 0% | 220 000 (70 000 - 1 100 000) | No virus found |

From these results, the sponsor has concluded that the experiments in the guinea pig model of primary HSV infection, demonstrated that the combination of ACV and hydrocortisone in ME-609 resulted in larger reductions in lesion score and healing time than ACV alone when the drugs were compared to the corresponding vehicles. Virus replication was inhibited when hydrocortisone was combined with ACV.

**Comment:** Although the guinea pig model for cutaneous HSV infection has been used for the assessment of the effectiveness of antiviral compounds, interpretation of the results is usually limited due to two major factors.

1. Since the model is involved with a primary infection, generalization of the results to recurrent infections may not be entirely correct.

2. About a 10-fold higher level of deoxythymidine (dT) present in guinea pig skin cells (compared to human skin cells) may influence the antiviral activity of ACV, since both ACV and dT are capable of utilizing the viral specific thymidine kinase for initial phosphorylation in the virus infected cells.

## B. CLINICAL STUDIES

### B.1. Clinical Study 98-609-013

**Title: Efficacy of Acyclovir in Combination with a Glucocorticosteroid on UV Induced Herpes Labialis**

**Study Objectives:**

**The primary objective:** To compare the efficacy of ME-609 cream *vs* placebo cream on the time to healing of delayed classical herpes labialis (HSV) lesions experimentally induced after exposure to UV radiation.

**The secondary objectives**: To compare ME-609 cream with placebo cream with regards to a number of clinical and safety parameters involving the herpes lesions (incidence, size and time), pain, and tenderness and safety. Virologic parameters were to determine the frequency of virus positive lesions, mean titer of virus excretion (optional), and the time to cessation of viral shedding (by viral culture),

To achieve the objectives of this randomized, placebo-controlled, double-blind clinical study in immunocompetent patients with experimentally induced (by UV irradiation of the lips) herpes labialis, about 420 adult patients at four clinical centers were enrolled. Eligibility for enrollment for this study was determined by a number of clinical and laboratory tests. Virologic parameters required that the patients must have a history of recurrent herpes labialis, must be in apparent good health without any other infectious diseases and must not be taking any steroids.

After screening, the minimal erythema dose (MED) was determined for each patient to calculate the UVR exposure to the lips. Patients started treatment with ME-609 cream or placebo cream in the morning of the second day after UVR exposure and continued until healing of the lesion or up to a maximum of 5 days after the start of the lesion. If a lesion developed, the investigator performed a lesion evaluation (number of lesions, size of each lesion) and a viral culture was taken daily from the first appearance of the lesion (papule or later stage) until development of a hard crust. Patients who

did not develop a lesion continued treatment for 5 days. Lesions were assessed regularly by the investigator at clinic visits, and also by the patient by means of a diary card. If more than one lesion developed, the investigator identified a primary lesion in each patient. The primary lesion was treated and evaluated at each assessment. Treatment of lesions other than the primary lesion was optional.

**Study Efficacy:** Most of the results of this study relate, as noted earlier, to the clinical and safety parameters. The efficacy results, as they relate to the clinical virology, may be summarized as follows.

ME-609 cream prevented the development of the UV induced delayed lesions in 26.8% of patients through a decreased incidence of this condition in comparison with placebo. The median time to healing (loss of hard crust) in such patients was reduced from 6.8 days in the placebo group to 5.4 days in the ME-609 group. In the ME-609 treatment group 39/65 patients (60.0%) had one or more virus positive cultures, compared to 55/76 patients (72.4%) in the placebo group. For delayed classical lesions, 54/70 patients (77.1%) and 34/49 patients (69.4%) treated with placebo and ME-609, respectively, had a virus positive culture for the intent-to-treat population (Table 3).

Table 3. Frequency of Virus Positive Lesions and Type of Lesion by Treatment Group (Table 15.1.1 in the Study Report).

| Lesion Type / Treatment | | Patient Had Virus Positive Lesion | | | | | |
|---|---|---|---|---|---|---|---|
| | | NO | | YES | | ALL | |
| | | N | % | N | % | N | % |
| DELAYED CLASSICAL | PLACEBO | 16 | 22.9 | 54 | 77.1 | 70 | 100.0 |
| | ME-609 | 15 | 30.6 | 34 | 69.4 | 49 | 100.0 |
| IMMEDIATE CLASSICAL | PLACEBO | 2 | 66.7 | 1 | 33.3 | 3 | 100.0 |
| | ME-609 | 5 | 50.0 | 5 | 50.0 | 10 | 100.0 |
| DELAYED ABORTED | PLACEBO | 1 | 100.0 | 0 | 0 | 1 | 100.0 |
| | ME-609 | 3 | 100.0 | 0 | 0 | 3 | 100.0 |
| IMMEDIATE ABORTED | PLACEBO | 2 | 100.0 | 0 | 0 | 2 | 100.0 |
| | ME-609 | 3 | 100.0 | 0 | 0 | 3 | 100.0 |
| ALL | PLACEBO | 21 | 27.6 | 55 | 72.4 | 76 | 100.0 |
| | ME-609 | 26 | 40.0 | 39 | 60.0 | 65 | 100.0 |

The mean time to cessation of viral shedding was longer in patients treated with placebo compared with patients treated with ME-609 for all types of lesion combined. There was a similar pattern for delayed classical lesions, where the mean time was 1.6 ± 1.1 days for placebo and 1.3 ±1.2 days for ME-609 (Table 4).

Table 4. Time to Cessation of Viral Shedding by Treatment Group and Type of Lesion: Intent-to-Treat Population (Table 15.1.2 of this Study Report)

| Lesion Type / Treatment | | Time to Cessation Viral Shedding (Days) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mean | Median | SD | Min | Max | N |
| DELAYED CLASSICAL | PLACEBO | 1.6 | 2.0 | 1.1 | 0 | 4 | 60 |
| | ME-609 | 1.3 | 1.0 | 1.2 | 0 | 6 | 46 |
| IMMEDIATE CLASSICAL | PLACEBO | 0.7 | 0.0 | 1.2 | 0 | 2 | 3 |
| | ME-609 | 1.3 | 1.0 | 1.4 | 0 | 3 | 7 |
| DELAYED ABORTED | PLACEBO | 0 | 0 | 0 | 0 | 0 | 0 |
| | ME-609 | 0.0 | 0.0 | 0.0 | 0 | 0 | 2 |
| IMMEDIATE ABORTED | PLACEBO | 0.0 | 0.0 | 0 | 0 | 0 | 1 |
| | ME-609 | 0.0 | 0.0 | 0.0 | 0 | 0 | 3 |
| ALL | PLACEBO | 1.6 | 1.5 | 1.1 | 0 | 4 | 64 |
| | ME-609 | 1.2 | 1.0 | 1.2 | 0 | 5 | 58 |

## B.2. Clinical Study 609-04

**Study Title:** A Randomized, Double-Blind, Active Controlled, Vehicle Controlled, Subject Initiated Study Comparing Efficacy and Safety of ME-609 versus Acyclovir Cream for Treatment of Recurrent Herpes Simplex Labialis

**Objectives of the Study:** To evaluate the efficacy and safety of ME-609 for the treatment of herpes labialis recurrences compared with ACV and vehicle in immunocompetent adults (18 years or older).

To achieve the objectives of this randomized, double-blind, vehicle-controlled study, about 2437 patients was enrolled in this study. Eligibility for enrollment to this study was based on a number of clinical, laboratory and virologic parameters specified in Section 9.3 of this Clinical protocol. The following virologic parameters were evaluated for eligibility.

All patients must be in general good health with a history of recurrent herpes labialis with at least 3 recurrences during the prior 12 months. The history of herpes labialis recurrences (as defined by the first physical sign of a lesion such as a vesicle or papule) must have been typically (50% of episodes) associated with prodromal symptoms and at least 75% of herpes recurrences producing ulcerative lesions. Eligible patients must not have a previous infection with HSV-1 isolates known to be resistant to ACV, valaciclovir, famciclovir or ganciclovir and must not be in an immunosuppressed state due to underlying disease (e. g., HIV infection) or concomitant treatment (e.g., cancer chemotherapy). These patients must not be participating another clinical trial or treatment with an investigational drug within four weeks prior to screening.

A total of 2437 eligible patients were randomized into the study: 1018, 1033, and 386 in the ME-609, acyclovir, and vehicle treatment groups, respectively. Treatment was patient-initiated within 1 hour of experiencing the first signs or symptoms of a herpes recurrence. The subject visited a study clinic as soon as possible after treatment initiation, but no later than midnight of the following day, for evaluation. Duration of the treatment was for a total of 5 days. A number of clinical, laboratory and virologic tests and assessments were performed to evaluate the efficacy and safety of the treatment regimens as listed in Table 2 of the Study Report.

The efficacy assessments were mostly of clinical nature requiring daily observations and recordings of about eight stages of herpes lesions such as the prodrome, erythema, papule, vesicle, ulcer/soft crust, hard crust, residual abnormalities and normal skin. It was also necessary to measure the lesion area, assess the tenderness (pain) and record the time for start of treatment, each application of study drug, loss of hard crust, and normal skin/no signs or symptoms over a period of approximately 10 days during the herpes labialis recurrence,

For virologic assessments, clinical samples (swabs) from patients with ulcerative recurrences were collected at ulcer/soft crust stage only (no sampling was performed at vesicle or hard crust stage). The samples in cell culture medium were refrigerated, sent to a central laboratory where one part of the sample was put on cell culture and the other part of the sample was frozen at -70°C. Following the virus culture and qualitative analysis, the cultured sample was stored at -70°C to be tested for ACV susceptibility as follows.

The samples collected from all patients of various treatment groups who had a positive virus culture obtained at a later time point than the median healing time (time to loss of hard crust) in the ACV control group were assessed for ACV susceptibility (in the presence of 50 µM thymidine in the cell growth medium to deactivate any residual ACV) according to the standard US antiviral susceptibility testing procedure for HSV M33-A, National Committee for Clinical Laboratory Standards. The genotypic nature of any ACV resistant HSV isolates was then characterized, and ACV resistant HSV strains were tested with regards to ACV susceptibility in the presence of hydrocortisone. The frequency of virus positive lesions was calculated as the proportion of subjects with positive viral cultures in any sample at any time point.

**Efficacy Endpoints:**

**The primary efficacy endpoint** was the proportion of patients with non-ulcerative recurrences measured as the proportion of patients in whom the study recurrence did not progress beyond the papule stage.

**The secondary efficacy endpoints** were episode duration and episode duration to normal skin.
**The tertiary efficacy endpoints** were cumulative lesion area, lesion healing time to normal skin, lesion healing time to loss of hard crust, maximum lesion area, duration and severity of tenderness, and subject preference.

**Virology efficacy endpoint** included determination of the ACV susceptibility and genotyping of virus positive samples collected from patients with a prolonged healing time.

**EFFICACY RESULTS:**

**The primary efficacy endpoint** was the prevention of recurrences progressing beyond the papule stage as measured as the proportion of patients with non-ulcerative recurrences. Results presented in Table 12 of this study report shows that the proportion of patents with non-ulcerative recurrences in the ME-609 group (42.3%) was higher compared to the acyclovir (35.4%, $p = 0.0144$) and the vehicle group (25.9%, $p < 0.0001$). However, the sponsor has stated that the predefined level of significance ($p < 0.001$) for the comparison between ME-609 and acyclovir was not achieved.

**The secondary efficacy endpoints** for episode duration and episode duration to normal skin in the ME-609 group were significantly shorter than in the vehicle group. The average episode duration in the ME-609 group was 5.4 days compared with 5.9 days in the vehicle group ($p = 0.0455$). The average episode duration to normal skin in the ME-609 group was 7.6 days compared with 9.3 days in the vehicle group ($p = 0.0001$). The average episode durations in the ME-609 and acyclovir groups were not statistically different (5.4 vs. 5.5 days). The episode duration and episode duration to normal skin for ulcerative recurrences were significantly shorter in the ME-609 group compared with the vehicle group (5.7 vs. 6.5 days; $p = 0.0076$ and 9.6 vs. 11.0 days; $p = 0.024$, respectively). The reviews of the clinical and statistical reviewers should be referred to for the adequacy of these clinical observations and statistical results.

**Virology efficacy results** are presented in several tabular forms (Tables 14.3.4.1 through 14.3.4.4) in Section 14 of this study report. These results may be summarized as follows.

1. Number of patients with ulcerative recurrences had positive virus samples collected in various groups of the ITT population:

| | |
|---|---|
| ME-609 group | 32 (22.4%) |
| ACV group | 44 (24.2%) |
| Vehicle group | 31 (39.7%). |

2. Number of patients with a positive viral culture and a healing time longer than 5.5 days:

| | |
|---|---|
| ME-609 group | 18 |
| ACV group | 29 |
| Vehicle group | 24 |

Thus the number of patients with a positive viral culture and a healing time longer than 5.5 days were 18, 29, and 24 in the ME-609, acyclovir and vehicle groups, respectively, in the ITT population. When tested for ACV susceptibility in the plaque reduction assay, clinical samples from two patients (ID C20-CC02 and C59-CC10) in the ACV group showed reduced ACV sensitivity with $EC_{50}$ values >8.9 μM (Table 6A).

The Tk gene of these cell cultured virus samples (along with the original non-cultured samples from the two patients noted above (ID C20-CC02 and C59-CC10) were sequenced to verify the mutations leading to ACV resistance. Results presented in tabular form (Table 6B) show that there were amino acid substitutions (in red at position 51 and 185) in the viral TK possibly due to a frame shift mutation in the Tk gene. However, the sponsor has suggested that these mutations are not indicative of the known ACV resistance; rather the ACV resistance noted in the two patients could be due to the "cell culturing" of the clinical (viral) sample with remaining (residual) ACV collected from the lip of the patients that had applied the cream during treatment. Therefore, the sponsor has summarized that no ACV resistance was identified in any of the patients that had a prolonged healing time. The sponsor has also stated to have sequenced original samples from 4, 6 and 5 patients in the ME-609, acyclovir and vehicle groups (2 of the acyclovir subjects are referenced above) as a complementary analysis,. No mutations leading to ACV resistance were identified in these complementary tests.

Table 6. Summary of Results from Plaque Reduction Assay (A) and Nucleotide Sequence Analysis of Thymidine Kinase Gene (B) in patients with healing time > 5.5 days (Table 14.3.4.5 of the study report).

A.

*Plaque Reduction Assay results*

| SMIno. | Site-Subject (Subj. ID) | Requisition number | Date of collection | Results PRA (µM) | Comments |
|---|---|---|---|---|---|
| | ME-609 | | | | |
| 101B | 005-0018 | 42014298 | 20 Sep 2006 | < 8.9 uM | Sensitive |
| 102 | 009-0018 | 42028685 | 14 Jun 2007 | < 8.9 uM | Sensitive |
| 103 | 011-0020 | 42017633 | 28 Sep 2006 | < 8.9 uM | Sensitive |
| 104 | 015-0005 | 42015848 | 05 Sep 2006 | < 8.9 uM | Sensitive |
| 105 | 018-0024 | 42015987 | 20 Sep 2006 | < 8.9 uM | Sensitive |
| 106 | 021-0032 | 42017383 | 22 Nov 2006 | < 8.9 uM | Sensitive |
| 107 | 022-0021 | 42012372 | 03 Oct 2006 | < 8.9 uM | Sensitive |
| 108 | 024-0039 | 42026839 | 08 Mar 2007 | < 8.9 uM | Sensitive |
| 109 | 028-0005 | 42017310 | 02 Mar 2007 | < 8.9 uM | Sensitive |
| 110 | 033-0055 | 42012762 | 27 Sep 2006 | | Negative for virusisolation. See sequencing. |
| 111 | 038-0005 | 42024890 | 06 Dec 2006 | < 8.9 uM | Sensitive |
| 112 | 045-0036 | 42013530 | 18 Nov 2006 | < 8.9 uM | Sensitive |
| 113 | 046-0011 | 42032356 | 30 Nov 2006 | < 8.9 uM | Sensitive |
| 114 | 046-0030 | 42035100 | 13 Dec 2006 | < 8.9 uM | Sensitive |
| 115 | 057-0006 | 42065116 | 11 May 2007 | < 8.9 uM | Sensitive |
| 116 | 059-0024 | 42034838 | 07 Aug 2007 | < 8.9 uM | Sensitive |
| 117B | 059-0025 | 42036140 | 03 Aug 2007 | | Negative for virusisolation. See sequencing. |
| 118 | 060-0004 | 42036154 | 14 Jan 2007 | < 8.9 uM | Sensitive |
| 119 | 064-0021 | 42103730 | 08 Nov 2007 | < 8.9 uM | Sensitive |
| | Acyclovir | | | | |
| 120 | 005-0057 | 42034122 | 14 Sep 2007 | < 8.9 uM | Sensitive |
| 121 | 012-0002 | 42029740 | 07 Nov 2006 | < 8.9 uM | Sensitive |
| 122 | 012-0038 | 42056480 | 28 Sep 2007 | < 8.9 uM | Sensitive |
| 123 | 018-0029 | 42026623 | 27 Oct 2006 | < 8.9 uM | Sensitive |
| 124 | 018-0030 | 42026628 | 03 Dec 2006 | < 8.9 uM | Sensitive |
| 125 | 020-0002 | 42016349 | 27 Oct 2006 | > 8.9 µM | Resistant. See sequencing. |
| 126 | 021-0017 | 42022321 | 05 Sep 2006 | < 8.9 uM | Sensitive |
| 127 | 021-0049 | 42019365 | 27 Dec 2006 | < 8.9 uM | Sensitive |
| 128 | 025-0002 | 42013574 | 26 Sep 2006 | < 8.9 uM | Sensitive |
| 129 | 028-0026 | 42017307 | 16 Feb 2007 | < 8.9 uM | Sensitive |
| 130 | 029-0004 | 42030513 | 01 Apr 2007 | < 8.9 uM | Sensitive |
| 131C | 031-0004 | 42015542 | 16 Sep 2006 | | Negative for virusisolation. See sequencing. |
| 132B | 031-0044 | 42026287 | 08 Nov 2006 | < 8.9 uM | Sensitive |
| 133 | 033-0036 | 42013386 | 14 Dec 2006 | < 8.9 uM | Sensitive |
| 134 | 037-0020 | 42015550 | 17 Sep 2006 | < 8.9 uM | Sensitive |
| 135 | 038-0003 | 42046275 | 14 Jun 2007 | | Negative for virusisolation. See sequencing. |
| 136 | 041-0004 | 42012455 | 07 Mar 2007 | < 8.9 uM | Sensitive |

***Table 6A. Plaque Reduction Assay Results (Continued)***

| | | | | | |
|---|---|---|---|---|---|
| 137 | 042-0003 | 42015561 | 01 Aug 2006 | < 3.9 µM | Sensitive |
| 138B | 042-0034 | 42022714 | 02 Nov 2006 | < 3.9 µM | Sensitive |
| 139 | 044-0014 | 42012818 | 13 Nov 2006 | < 3.9 µM | Sensitive |
| 140 | 046-0003 | 42012837 | 31 Jul 2006 | < 3.9 µM | Sensitive |
| 141 | 046-0016 | 42029693 | 09 Nov 2006 | | Negative for virusisolation. See sequencing. |
| 142 | 046-0017 | 42021493 | 31 Aug 2006 | < 3.9 µM | Sensitive |
| 143 | 047-0017 | 42024544 | 15 Nov 2006 | | Negative for virusisolation. See sequencing. |
| 144 | 047-0102 | 42036622 | 15 Feb 2007 | < 3.9 µM | Sensitive |
| 145 | 047-0131 | 42087886 | 06 Sep 2007 | < 3.9 µM | Sensitive |
| 146 | 048-0016 | 42016346 | 04 Jan 2007 | < 3.9 µM | Sensitive |
| 147 | 059-0010 | 42036148 | 19 Mar 2007 | IC50 not evaluable | Growth of virus despite high concentration of Acyclovir (35.5 µM). The reduction in plaque number seen with increasing concentration of Acyclovir might be due to mixed population of virus. See sequencing. |
| 148 | 064-0012 | 42101729 | 14 Nov 2007 | < 3.9 µM | Sensitive |
| | **Vehicle** | | | | |
| 149 | 003-0014 | 42017677 | 19 Oct 2006 | < 3.9 µM | Sensitive |
| 150 | 005-0023 | 42094283 | 11 Oct 2007 | < 3.9 µM | Sensitive |
| 151 | 005-0066 | 42084123 | 06 Sep 2007 | < 3.9 µM | Sensitive |
| 152 | 009-0008 | 42016331 | 03 Oct 2006 | | Negative for virusisolation. See sequencing. |
| 153 | 011-0001 | 42017634 | 26 Sep 2006 | | Negative for virusisolation. See sequencing. |
| 154 | 013-0036 | 42017644 | 13 Jan 2007 | < 3.9 µM | Sensitive |
| 155 | 018-0003 | 42015996 | 11 Aug 2006 | < 3.9 µM | Sensitive |
| 156 | 022-0008 | 42012370 | 08 Nov 2006 | < 3.9 µM | Sensitive |
| 157 | 024-0005 | 42026841 | 05 Feb 2007 | < 3.9 µM | Sensitive |
| 158E | 026-0055 | 42013561 | 06 Oct 2006 | < 3.9 µM | Sensitive |
| 159 | 027-0007 | 42012737 | 15 Aug 2006 | < 3.9 µM | Sensitive |
| 160 | 042-0004 | 42032280 | 16 Feb 2007 | | Negative for virusisolation. See sequencing. |
| 161 | 042-0008 | 42012708 | 18 Oct 2006 | < 3.9 µM | Sensitive |
| 162 | 044-0024 | 42042287 | 03 May 2007 | < 3.9 µM | Sensitive |
| 163 | 045-0038 | 42013524 | 15 Oct 2006 | | Negative for virusisolation. See sequencing. |
| 164 | 046-0018 | 42012838 | 18 Aug 2006 | < 3.9 µM | Sensitive |
| 165 | 046-0029 | 42035097 | 01 Jan 2007 | | Negative for virusisolation. See sequencing. |
| 166 | 047-0004 | 42024547 | 31 Oct 2006 | < 3.9 µM | Sensitive |
| 167 | 047-0021 | 42024545 | 08 Nov 2006 | < 3.9 µM | Sensitive |
| 168 | 047-0027 | 42036632 | 03 Aug 2007 | < 3.9 µM | Sensitive |
| 169 | 047-0049 | 42032855 | 04 Dec 2006 | < 3.9 µM | Sensitive |
| 170 | 047-0061 | 42036637 | 09 Jan 2007 | < 3.9 µM | Sensitive |
| 171 | 049-0030 | 42016367 | 30 Oct 2006 | < 3.9 µM | Sensitive |
| 172B | 059-0003 | 42034837 | 19 Jan 2007 | < 3.9 µM | Sensitive |

***B.***

*Amino acid alterations in thymidine kinase (TK) in comparison to HSV-1 MacIntyre (ATCC:VR-539)*

| | | | | 5 | 23 | 36 | 42 | 51 | 76* | 89 | 155 | 192 | 240 | 251 | 267 | 283 | 373* | 391 | 486 | 522* | 545 | 574 | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SMNo. | Site-Subject (Subj. ID) | Requisition no. | Sample type | C | S | E | P | R | D | R | Q | A | G | C | L | T | E | R | E | M | V | H | |
| 105 | 018-0024 | 42055987 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms |
| 110 | 033-0055 | 42012762 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 117B | 050-0025 | 42026140 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 118 | 050-0004 | 42026154 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms |
| 125 | 020-0002 | 42016349 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (W51 was not identified in this sample) |
| 125 | 020-0002 | 42016349 | isolate 0080512 | | | | | | | | | | | | | | | | | | | | [illegible] (50-90) of resistant and sensitive strain |
| 132C | 031-0004 | 42015542 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 135 | 038-0003 | 42046275 | direct | | | | | | | | | | | | | | | | | | | | *reported variation in non-conserved region (only sequenced) |
| 141 | 046-0016 | 42029693 | direct | | | | | | | | | | | | | | | | | | | | *reported variation in non-conserved region (only sequenced) |
| 143 | 047-0017 | 42024544 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 147 | 059-0010 | 42036148 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (553insC was not identified in this sample) |
| 147 | 059-0010 | 42036148 | isolate W080611 | | | | | | | | | | | | | | | | | | | | [illegible] mutant strain (553insC) resulting in frame shift |
| 152 | 009-0008 | 42016331 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 153 | 011-0001 | 42017634 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 160 | 042-0004 | 42032280 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 163 | 045-0038 | 42013524 | direct | | | | | | | | | | | | | | | | | | | | [illegible] published polymorphisms (only sequenced) |
| 165 | 046-0029 | 42035097 | direct | | | | | | | | | | | | | | | | | | | | *reported variation in non-conserved region (only sequenced) |

*Unreported variation in non-conserved region.

b(4)

## B.3. Clinical Study 609-06

**Study Title:** A randomized, double-blind, active controlled, and subject-initiated study comparing ME-609 to ACV cream for treatment of recurrent herpes simplex labialis in immunocompromised patients

## MICROBIOLOGY REVIEW
## DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

**NDA 22-436 SN 000 (Original and 4 Amendments) REVIEW DATE: 12/22/2008**

**Study Objective:** To evaluate the episode duration of a herpes labialis recurrence, following a 5-day treatment with 5-time daily topical administration of ME-609 or acyclovir cream, in immunocompromised adults (18 years and older).

Composition of ME-609 and acyclovir cream used in this study is presented in Table 7.

Table 7. Compositions of acyclovir cream and ME-609 (Table 9.1 of the Study Report 609-06)

| Component, % w/w | acyclovir cream | ME-609 | |
|---|---|---|---|
| Acyclovir | 5.0 | 5.0 | |
| Hydrocortisone | | | |
| Mineral oil | | | b(4) |
| Cetostearyl alcohol | | | |
| White Petrolatum | | | |
| Isopropyl myristate | | | |
| Sodium lauryl sulphate | | | |
| Poloxamer 188 | | | b(4) |
| Propylene glycol | | | |
| Citric acid | | | |
| Sodium hydroxide | | | |
| Sodium hydroxide/ hydrochloric acid | q.s. | q.s. | |
| Water | | | b(4) |

To achieve the objectives of this randomized, double-blind, active controlled multi center (19 in Russia and 6 in Ukraine) study comparing the efficacy and safety of ME-609 and ACV cream in about 201 immunocompromised patients were enrolled. A number of clinical and pharmacologic parameters (enlisted in section 9.3 of this protocol) were evaluated for the study participation. The virologic parameters did not include diagnosis (or confirmation of the diagnosis) of either recurrent herpes labialis or immunocompromized HIV infection by appropriate virologic markers. Thus the virology related parameters used to evaluate eligibility for this study were as follows.

The study participants must have a history of recurrent herpes labialis with at least two recurrences during the twelve months prior to the study. The patients must be immunocompromised, defined as diagnosis of HIV infection with

- Stable HIV-infection, as judged by the Investigator
- CD4+ T-cell count 100 to 500/mm$^3$ (laboratory parameter at screening)

The eligible patients must not be taking any antiviral agents (except for antiretroviral treatment in HIV infected patients), corticosteroids, cancer chemotherapy or investigational drugs within two weeks prior to and during the treatment period until normal skin/no signs or symptoms. Such patients must not have suffered from previous infections with HSV-1 or HSV-2 isolates known to be resistant to ACV, valaciclovir, famciclovir or ganciclovir.
**Note:** In addition to current HIV infection (an inclusion criterion, as judged by the clinician), prior

history of ongoing other infections were often reported, with hepatitis C virus [44 patients (41%)], hepatitis B virus [13 (11%)] and oropharyngeal candidiasis [10 (9%)] being the most frequent.

At screening, all subjects had CD4+ T-cell counts in the range of 100 to 500/mm$^3$ with a median value of 344/mm$^3$. The mean CD4+ T-cell count at screening was 328 ±111/mm$^3$ for the ME-609 group and 320 ± 116/mm$^3$ for the ACV group, and the median was 344/mm$^3$ and 346/mm$^3$, respectively. The CD4+ T-cell count was <200/mm$^3$ for 14 (18%) ME-609 patients and 5 (17%) ACV patients.

The eligible patients were randomized approximately in a 2:1 ratio (136 patients to ME-609 cream group and 65 patients to ACV cream group) to initiate treatment as soon as possible after experiencing signs or symptoms of a herpes recurrence. The subject visited the study clinic soon after the treatment initiation, but no later than midnight of the following day, for assessment of the recurrence by the Investigator. The study medications were applied 5 times daily for a total of 5 days, and the patients were followed up for up to 1 year after treatment to record the next recurrence. A number of clinical, laboratory and virologic tests were performed for the conduct of the clinical study 609-06 as presented in Table 8.

**Table 8.** Flow Sheet of Study Procedures (Schedule of events: screening, treatment, observation and follow-up periods (Table 9.2 of the Study Report 609-06).

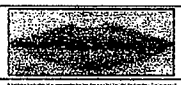
| | Screening | Treatment period, 5 days for all subjects<br>Visits every day | Observation period (for ulcerative recurrences)<br>Visits every day until "loss of hard crust", thereafter every other day[3] until "normal skin" | Observation period (for non-ulcerative recurrences)<br>Visits every other day[3] until "no signs or symptoms". | Follow-up period<br>Follow-up by phone 3 weeks (≅ 1 week) after healing to normal skin/no signs or symptoms. | Time-to-next-recurrence period |
|---|---|---|---|---|---|---|
| Informed consent | X | | | | | |
| Eligibility criteria[1] | X | | | | | |
| Symptoms-driven physical examination | X (if applicable) | | | | | |
| Medical & herpes hist. | X | | | | | |
| Demographics recorded | X | | | | | |
| Laboratory parameters | X | X (one visit) | | | | |
| Pregnancy test | X (if applicable) | X (first visit) | | | | |
| Randomization | X | | | | | |
| Study & diary instr. | X | | | | | |
| Dispense of study medication | X | | | | | |
| Admin. of study medication | | X | | | | |
| Recurrence stage assess. | | X | X | X | | |
| Lesion size assessment[2] | | X | X | | | |
| Concomitant med. | X | X | X | X | X | |
| Use & check of diary | | X | X | X | | |
| Adverse events | | X | X | X | X | |
| Viral isolation[2] | | X | X | | | |
| Drug accountability | | | X (first visit) | X (first visit) | | |
| Time of next recurrence | | | | | | X |

[1]Applicable eligibility criteria were checked at regular contacts with subjects.
[2]For ulcerative recurrences only
[3]Excluding weekends

Thus the daily visits were scheduled during the 5-day treatment period for all patients. For patients with ulcerative recurrences, daily visits were required until the stage "loss of hard crust" was reached. Thereafter, visits were scheduled every other day (excluding weekends) until the stage "normal skin" was observed. For patients with non-ulcerative recurrences, visits were scheduled every other day after the initial 5-day treatment period (excluding weekends) until the stage "no signs or symptoms". All patients had a follow up interview by telephone 3 weeks after their recurrence had healed completely ("normal skin" or "no signs or symptoms"), and were asked to report to the clinic when they experienced their next recurrence (time-to-next recurrence). The clinic also contacted patients monthly to ask for this information and will continue to do so for 1 year after the subject completed the acute phase of the study. If no new recurrence is experienced within 1 year, the time-to-next recurrence will be recorded as > 1 year.

In addition to the clinical assessment for the presence, size and stage of the herpes lesions (prodrome, erythema, papule, vesicle, ulcer/soft crust, hard crust, loss of hard crust, or normal skins or symptoms of the recurrence) (Table 9), viral samples (swabs) were also collected at clinic visits from patients with ulcerative recurrences in the ulcer/soft crust stages.

Table 9. Herpes Lesion Staging (Study 609-06, Appendix 16.1.1, page 78)

| | | |
|---|---|---|
| Prodome (or tingling, itching, tenderness/pain) |  |  |
| Erythema (or redness) |  |  |
| Papule/Edema (raised bump) |  |  |
| Vesicle (or blister) |  |  |
| Ulcer/soft crust |  |  |
| Hard crust |  |  |
| Normal skin (healed with some residual redness) |  |  |



All viral samples were tested at a central laboratory for ACV susceptibility by a conventional plaque reduction assay (PRA) using host MRC-5 cells and HSV-1 strain MacIntyre as control in the presence of 50 μM dT (Appendix 16.1.9.2, page 536). Viral samples were also genotyped for ACV resistance

sequences in the viral Tk (UL 23) and DNA pol (UL 30) genes after quantitative polymerase chain reaction (PCR) (Appendix 16.1.9.2, page 532). Both of these methods are adequately described in Appendix 16.1.9.2.

**Efficacy Endpoints:**

**The primary efficacy endpoint** was investigator-assessed episode duration, measured from the start of treatment until loss of hard crust for an ulcerative recurrence and from the start of treatment to time of no signs or symptoms for a non-ulcerative recurrence.

**The secondary** efficacy endpoint was the time to next recurrence measured from the start of the study recurrence until the start of the next recurrence.

**Tertiary endpoints** were proportion of subjects with non-ulcerative recurrences, maximum lesion area, and episode duration to normal skin.

**Virology endpoint** was the evaluation of ACV susceptibility of viral samples by plaque reduction assay (PRA) and by nucleotide sequence analysis of the Tk and DNA polymerase genes.

**Efficacy Results**

As noted earlier, the initial (original, SDN 000) submission of this NDA included the results for short-term observations during the initial study recurrence in an "**interim report.**" A final study report was submitted (in SDN 000 BM, amendment 2) after the "long-term follow up" (up to 1 year after treatment completion) was completed. Results from these two reports are essentially clinical and statistical in nature involving the state of the herpes lesions, lesion size, duration of the lesions, and stature of the infected skin (lip). Therefore, while these results are summarized below, the reviews of the clinical and statistical reviewers should be referred to for a more detailed critical review of these results.

Briefly, the mean episode duration for the ITT population, as described in the original interim report, was 6.7 days in both treatment groups, ranging from 2 to 12.9 days in the ME-609 group and from 2 to 14.8 days in the ACV group. The median episode duration was 6.4 days in the ME-609 group and 6.6 days in the ACV group. The median ratio (ME-609/ACV) was 0.97 (95% CI: 0.79, 1.25). These results with the ITT population were also comparable to the PP and evaluable populations.

For ulcerative recurrences, the mean episode duration was 6.6 days and 6.9 days in the ME-609 and ACV groups, respectively. The median (minimum, maximum) values were 6.4 (3, 10.2) days in the ME-609 group and 6.7 (2, 9.8) days for the ACV group. The ratio (95% CI) of the median values was 0.96 (0.78, 1.34).

For non-ulcerative recurrences, the mean episode duration was 6.7 days and 6.6 days in the ME-609 and ACV groups, respectively. The median (minimum, maximum) values were 6.1 (2, 12.9) days in the ME-609 group and 5.7 (3, 14.8) days for the ACV group. The ratio (95% CI) of the median values was

1.06 (0.69, 1.41). The proportion of subjects with non-ulcerative recurrences was larger for ME-609 subjects (42%) than for ACV patients (38%).

For ulcerative recurrences, the mean maximum lesion area was 37 ± 61 $mm^2$ for ME-609 and 24 ± 22 $mm^2$ for ACV. The median (minimum, maximum) value was 22 (1, 375) $mm^2$ and 20 (2, 80) $mm^2$, respectively.

The mean episode duration to normal skin/no signs and symptoms was 9.0 days in the ME-609 group and 8.8 days in the acyclovir group. The median (95% CI) episode duration to normal skin was 8.1 (7.5, 9.6) days for ME-609 and 9.0 (6.9, 10.6) days for ACV. The hazard ratio (95% CI) was 0.93 (0.60, 1.44).

For ulcerative recurrences, the mean episode duration to normal skin was 10.8 days in the ME-609 group and 10.2 days in the ACV group. The median (95% CI) episode duration to normal skin was 10.0 (9.1, 12.0) days for ME-609 group and 10.5 (9.2, 11.7) days for ACV group. The hazard ratio (95% CI) was 0.81 (0.46, 1.42).

The results of the long-term follow-up period (1 year) was submitted recently in an amended version (#2, as noted above) are presented in tabular forms (Tables 14.2.5.1 through 14.2.5.3) and a listing (16.2.6.6) for the individual time to recurrence data without a description of the results. The data presented in these tables are related to the secondary efficacy variable on the time to next herpes recurrences. These data, as presented in Table 10 below are also of clinical nature and does not contain any information related to the virology ME-609.

Table 10. Secondary Efficacy Variable: Time to Next Herpes Recurrence (days) - ITT Population (Table 14.2.5.1 in amendment 2 of this NDA)

| Time to next herpes recurrence (days) | ME-609 (N=77) | Acyclovir (N=30) |
|---|---|---|
| n | 44 | 14 |
| Mean (SD) | 148.0 (86.88) | 123.4 (68.04) |
| Median | 150.5 | 121.0 |
| Minimum/Maximum | 17.0 / 382.0 | 30.0 / 249.0 |
| Number of subjects with next herpes recurrence | 44 (57.1%) | 14 (46.7%) |
| Number of censored subjects | 33 (42.9%) | 16 (53.3%) |
| 25% time to next recurrence with 95% CI | 111.0 (92.0,178.0) | 135.0 (55.0,230.0) |
| 50% time to next recurrence with 95% CI | 251.0 (181.0,NE) | NE |
| 75% time to next recurrence with 95% CI | NE | NE |

Data missing for time to the next herpes recurrence is the number of censored subjects

From all of these results of this study the sponsor has concluded that ME-609 and ACV provided comparable therapeutic clinical benefits such as episode duration, time to next recurrence, and episode duration to normal skin were comparable for both the treatment groups. The clinical reviewer may wish to comment on these conclusions.

### Virology Results

As noted earlier, the clinical samples (swabs) collected during the treatment period were limited to isolation of HSV during the ulcer/soft crust stages of ulcerative recurrences. Samples were analyzed

using standard ACV susceptibility testing (PRA), quantitative PCR, and nucleotide sequence analysis of TK- and DNA-polymerase genes. Results of some of these experiments summarized in tabular forms (Tables 14.3.8.1.1 through 14.3.8.1.3 of this study report, and a Listing 16.2.8. Laboratory Assessments) for various treatment populations. Since the number of samples collected and tested for the virologic assays were woefully low for any of these populations, and only one sample was tested to be positive for reduced ACV sensitivity, the results from one of the tables representing the ITT population is reproduced below in Table 11.

Table 11. Virology Assessments - ITT Population (Table 14.3.8.1.1 of original Study Report 609-06)

| Viral assessments | ME-609 X (%) | Y | Acyclovir X (%) | Y |
|---|---|---|---|---|
| Number of subjects with at least one sample | 29 (100.0%) | 58 | 12 (100.0%) | 18 |
| Number of subjects with Q-PCR positive sample(s) | 22 ( 75.9%) | 37 | 8 ( 66.7%) | 14 |
| Number of subjects with culture positive sample(s) | 13 ( 44.8%) | 23 | 7 ( 58.3%) | 9 |
| Number of subjects with result for TK-gene sequencing | 20 ( 69.0%) | 23 | 8 ( 66.7%) | 8 |
| Number of subjects with reduced acyclovir sensitivity due to mutation in TK gene | 0 ( 0.0%) | 0 | 0 ( 0.0%) | 0 |
| Number of subjects with result for DNA polymerase-gene sequencing | 17 ( 58.6%) | 19 | 8 ( 66.7%) | 8 |
| Number of subjects with reduced acyclovir sensitivity due to mutation in DNA pol gene | 0 ( 0.0%) | 0 | 0 ( 0.0%) | 0 |
| Number of subjects with PRA result | 13 ( 44.8%) | 16 | 7 ( 58.3%) | 7 |
| Number of subjects with reduced acyclovir sensitivity in PRA | 1 ( 3.4%) | 1 | 0 ( 0.0%) | 0 |
| Data missing [1] | 15 ( 34.1%) | | 6 ( 33.3%) | |

n = Number of subjects with at least one sample taken
% = Percent of subjects with at least one sample taken
Y = Total number of samples per analysis
[1] Number of subjects with ulcerative recurrences for which viral swabs were not obtained. Percents are based on the total number of subjects in each treatment group with ulcerative recurrence

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From the tabular presentations of the virology data, the sponsor has summarized the results as follows.

- All patients who initiated treatment, quantitative PCR (Q-PCR) positive samples were obtained from 29% and 27% of patients in the ME-609 and ACV groups, respectively.
- In patients who had at least one clinical sample collected, 22 (76%) ME-609 patients and 8 (67%) ACV patients were positive by Q-PCR.
- About 45% (13/29) in the ME-609 group and 58% (7/12) in the ACV group of patients were found to be positive for HSV in the cell culture assay.
- One patient was HSV-2 positive.
- None of the patients had reduced ACV susceptibility due to a mutation in the Tk or DNA-*pol* genes or as identified in the plaque reduction assay.
- One sample from 1 ME-609 patient (No 3804T003, Appendix 16.2.8) showed reduced ACV sensitivity in the plaque reduction assay. When this sample was further cultured with 100 μM

dT (instead of 50 µM dT) or the medium was changed 1 hr after adsorption, no resistance was observed. Nucleotide sequence analysis of Tk and DNA *pol* genes from the original (non-cultured sample) from this subject showed no mutations indicating ACV resistance (Appendix 16.2.8 listing the virology assessments for Study 609-06). Thus, the reduced ACV sensitivity had most likely developed during sample culturing due to a remaining high concentration of ACV in the sample. From these results the sponsor has concluded that topical treatment with hydrocortisone in addition to ACV did not promote the emergence of resistant virus strains.

**Comment:** The conclusion that topical treatment of recurrent herpes labialis with ACV in combination with hydrocortisone did not promote the emergence of resistant HSV strains is based on the results obtained from an extremely limited number of patients.

**B.4. Clinical Study 609-07**

**Study Title:** An open label, multi-center, phase III, subject initiated safety study of ME-609 in treatment of recurrent herpes simplex labialis in adolescents

**Study Objective:** To evaluate the safety of ME-609 for the treatment of herpes labialis recurrences in immunocompetent adolescents, 12-17 years of age, following a 5-day treatment with 5-time daily topical administration.

This study was not designed to evaluate any aspect of the virology of ME-609.

**C. Label (Package Insert)**

Sections 12.4 (Antiviral Activities) [redacted], of the Label for LIPSOVIR® should be revised a follows. b(4)

**12.4. Antiviral Activities**

The revision to this section is needed to conform to the basic standardization of writing the label dealing with the virology section as follows.

- Sensitivity test results [redacted] should be revised to $EC_{50}$
- The $EC_{50}$ values should be presented as µg/mL as well as µM in bracket b(4)

[redacted]

b(4)

**RECOMMENDATIONS:** With respect to microbiology. the NDA for LIPSOVIR® intended for the

**MICROBIOLOGY REVIEW**
**DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)**

**NDA 22-436 SN 000 (Original and 4 Amendments) REVIEW DATE: 12/22/2008**

[redacted] is b(4)
approved under the following conditions.

Sections 12.4 (Antiviral Activities) and [redacted] of the Label for LIPSOVIR® should be b(4)
revised a follows.

**12.4. Antiviral Activities**

The revision to this section is needed to conform to the basic standardization of writing the label dealing with the virology section as follows.

- Sensitivity test results [redacted] should be revised to $EC_{50}$ b(4)
- The $EC_{50}$ values should be presented as µg/mL as well as µM in bracket

[redacted] b(4)

[redacted] b(4)

**Nilambar Biswal, Ph.D.**
**Microbiologist**

**CONCURRENCES:**

**HFD-530/Tlmicro/J. O'Rear** **Date**

**CC:**
**HFD-530/Original NDA 22-436**
**HFD-530/Division File**
**HFD-530/Araojo, RPM**

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

/s/

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