Journal of Evidence-Based Integrative Medicine
Volume: 27: 1-10

DOI: 10.1177/2515690X221078004
journals.sagepub.com/home/cam
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Low-dose Oral Thimerosal for the Treatment of Oral Herpes: Clinical Trial Results and Improved Outcome After Post-hoc Analysis

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Abstract

Background: Thimerosal (TML) is an organomercury antimicrobial. Low doses (1/250th of the amount in a typical vaccine dose) may promote an antiviral immune response. Low-dose TML (BTL-TML) was evaluated for safety and efficacy against herpes labialis in two FDA-approved, randomized, double blind, placebo-controlled clinical trials.

Methods: BTL-TML was evaluated in a Phase IIa trial for its ability to block progression to lesion in subjects with recurrent oral herpes caused by dental trauma. Subjects were administered BTL-TML or a saline control over a 7-day period. In a Phase IIb trial, BTL-TML was evaluated for its ability to block progression to lesion over a 7-day period in subjects with herpes lip infections induced by exposure to ultraviolet (UV) radiation.

Results: Progression to lesion post-dental procedure was prevented in 54.5% (12/22) TML subjects versus 22.2% (2/9) control subjects ($p = 0.106$). Progression to lesion post-UV irradiation was blocked in 47.8% (11/23) BTL-TML treatment subjects and 42.8% (6/14) control subjects. A post-hoc analysis yielded 52.2% (12/23) BTL-TML subjects with no progression to lesion versus 28.6% (6/21) control subjects with no progression ($p = 0.099$). There were no significant differences in adverse effects between treatment and control groups in either trial.

Conclusions: Neither clinical trial showed a statistically significant effect of BTL-TML on progression to lesion. However, the post-hoc analysis suggested there is a 48-hour period following UV radiation exposure during which the anti-herpes activity of antivirals such as BTL-TML is reduced. Accordingly, BTL-TML may have promise in subsequent, properly designed and powered clinical trials.

Keywords

HSV1, herpes simplex labialis, cold sores, thimerosal, oral treatment, oral herpes

Received July 28, 2021. Received revised January 5, 2022. Accepted for publication January 15, 2022.

Herpes simplex virus (HSV) is the causative agent of herpes labialis, commonly known as cold sores or fever blisters. Most herpes labialis is caused by herpes simplex virus type 1 (HSV-1), and to a lesser extent, by HSV-2.[1–3] This virus establishes latency in the sensory ganglia and, when reactivated, causes the typical lesions of the infection.[4,5] Viral reactivation can be caused by any number of internal and external stimuli, including but not limited to, trauma to the oral cavity, exposure to ultraviolet (UV) light, fever, menstruation or other stresses.[1,6,7] After activation of the virus, the viral load increases until the first symptoms appear. There are six common stages of an episode of infection. The first of these is the prodrome stage, with symptoms that include burning, pain, itching, and tingling. This progresses to the macule stage (featuring erythema) and papule stage, followed by development of the main lesion or vesicle stage, involving

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pustulation, ulceration and, ultimately scabbing (the soft crust and hard crust stages).[1,8,9] Cold sores can be frequent and painful, can last up to 10–14 days and can stigmatize infected patients. Diagnosis rates are low, as many infected people remain asymptomatic. However, as of 2016, the number of persons age 14–49 infected and occasionally suffering from HSV-1 herpes labialis worldwide has been estimated to be almost 3.8 billion, for a prevalence of over 63%.[10] Most of these patients will typically suffer 1–3 cold sore episodes per year, but some patients can suffer six or more recurrences per year.[11,12] While there are no cures for a herpes infection, there are treatments with various efficacies. The most common antiviral agents used for the treatment of recurrent oral herpes episodes are acyclovir (Zovirax®), valacyclovir (Valtrex®), and famciclovir (Famvir®).[9,11,13,14] Their common mechanism of action is inhibition of herpes viral DNA polymerase, an enzyme involved in viral replication. There are also over-the-counter (OTC) drugs, such as docosanol cream (Abreva®), that are used to treat cold sores.[14,15] These drugs are capable of decreasing the duration of episodes and/or reducing the time to healing of herpes lesions. However, these antiviral agents have notable disadvantages in terms of efficacy. As examples, acyclovir cream is known to reduce the time required for a lesion to heal from 4.8 days to 4.3 days, while docosanol cream reduces healing time from 4.8 days to 4.1 days.[16,17] For such products, reducing time to healing does not address the stigma and embarrassment of having visible lesions in the first place. As an alternative to such products, an agent capable of blocking progression from prodrome to lesion may offer a clinically relevant benefit in treating herpes labialis episodes.

Thimerosal (TML; alternatively, Thiomersal) is an organomercury antimicrobial agent commonly utilized as a vaccine preservative. The typical TML concentration in vaccines is 0.01%; a 0.5 ml dose of vaccine thus contains 50 ug of TML. The presence of TML as a vaccine preservative has been controversial because of alleged links to neurological diseases, with autism being the most notable disorder of concern.[18–20] Consequently, the presence of TML in vaccines has been limited, particularly over the past two decades.[21] However, there has been interest in using low concentrations of TML as an antiviral agent. The idea that TML could act against herpes viruses originated with the study of Miller (1979), who reported that influenza vaccine could also be used to treat herpes virus infections.[22] This prompted further investigations regarding which component(s) of influenza vaccine were responsible for the anti-herpes activity. It was subsequently determined that this activity was related to TML rather than to any influenza virus fragment.[23] TML appeared to have little or no direct antiviral activity versus herpes, influenza or other viruses, though some antiviral activity has been observed in plaque assays (Beech Tree Labs, unpublished data). The working hypothesis is that at low doses (0.2 ug, or 1/250th of the amount that would be contained in a typical vaccine dose), TML may signal or promote a host immune response. In support of this, TML has been shown to inhibit the production of proinflammatory cytokines and chemokines, including IFN-gamma, IL-1 beta, IL-6, IL-12p70, and MCP-1.[24] This may modulate the inflammatory activities known to be associated with recurrent herpes labialis.[1,8,25] Although more research on the specific mechanism of action is needed, there was sufficient evidence of antiviral action to prompt clinical investigations of low-dose TML as a treatment for herpes labialis infections, based on its simple formulation and sublingual dosing regimen. Accordingly, low-dose TML was evaluated for safety and efficacy against oral herpes in two FDA-approved, randomized, double blind, placebo-controlled clinical trials. The first trial, a Phase IIa study, evaluated low-dose TML for its ability to block progression to lesion in patients with recurrent oral herpes caused by dental trauma. The subsequent Phase IIb study evaluated the ability of low-dose TML to block progression to lesion in patients with herpes caused by exposure to UV radiation.

Methods

Description of Study Medication

Beech Tree Labs' low-dose TML formulation, hereafter abbreviated BTL-TML, was composed of 4 ug/ml of TML in physiological saline (Beech Tree Labs, Providence, RI). Clinical trial supplies were provided in dropper bottles for sublingual administration. Each sublingual dose (50 ul drop) thus contained 0.2 ug BTL-TML. The placebo was physiological saline in matching dropper bottles. Dosing was achieved by each subject self-administering a drop of BTL-TML (or saline placebo) sublingually, maintaining sublingual contact for 30 seconds, and then swallowing their oral saliva. The specific dosing regimen (number of drops per day) was as follows: On the first day of cold sore symptoms, subjects self-administered one sublingual drop of BTL-TML (or placebo) every 15 minutes for the first hour, followed by one drop sublingually every hour for eight hours or until bedtime; on the second and third days of symptoms, subjects took one drop sublingually every four hours (waking time); and on days 4–7 after experiencing symptoms, subjects took one sublingual drop every 12 hours. All clinical trial subjects had to demonstrate their ability to self-administer the sublingual drops as one of the clinical trial inclusion criteria. Assuming a given subject followed the dosing regimen to completion, it was estimated that the cumulative amount of BTL-TML would be about 7 ug. This compares to 50 ug of TML in a typical 0.5 ml injection of influenza vaccine.

Clinical Trial Registrations and Ethical Considerations

Both clinical trials (Phase IIa and Phase IIb) were approved by the trial sites' respective Institutional Review Boards and are listed in the clinicaltrials.gov database as identifiers NCT01308424 and NCT01902303, respectively. These studies were performed under applicable Food and Drug Administration (FDA) regulations, notably, 21 Code of Federal Regulations (CFR) Parts 50, 56 and 312, along with Good Clinical Practice (E6) International Conference of Harmonization (ICH) Guidelines. Informed consent was obtained from each prospective trial subject prior to their participation in their respective clinical trial. Confidentiality of trial subjects was maintained at all times, with identifications by either initials and or/study number. Records were available only to study personnel, the Institutional Review Boards and FDA representatives.

Safety Evaluations

Information regarding adverse events (AEs) was obtained during each clinical visit, either by visual examination of the oral mucosa, from descriptions by the subjects or from laboratory data. AEs were classified in accordance with the MedDRA dictionary, version 13.0 (see https://www.meddra.org). Any adverse event (AE) deemed a major concern was immediately evaluated by a medical monitor and, if necessary, reported to the FDA in a timely manner.

Subject Withdrawals

Any subject could withdraw from the clinical trial at any time. Withdrawals could have been because of drug-related AEs as well as being non-drug-related.

Phase IIa Clinical Trial Objective

The objective of the randomized, double-blind, placebo-controlled Phase IIa clinical trial was to determine the safety and efficacy of sublingually administered low-dose BTL-TML on the recurrence of orofacial mucocutaneous HSV infections within seven days of each trial subject's dental procedures. Trial subjects were persons with a history of recurrent HSV that were to undergo routine dental procedures, either preventive (eg, examination and cleaning) or interventional (eg, filling a cavity).

Phase IIa Trial Experimental Design

The design of this trial was based on a study of the effects of valacyclovir on recurrent HSV infections in dental patients.[26] About 250 prospective clinical trial subjects with a history of symptomatic, recurrent HSV infections and about to undergo routine dental procedures were recruited from several locations, specifically, three dental schools in the continental United States. Subjects were to be randomized into either a treatment (BTL-TML) group or placebo control (solvent vehicle) group of 105 subjects each. Prospective subjects were screened within 14 days of their respective dental procedures for clinical trial eligibility (Visit 1). The primary criterion was a clinical diagnosis of symptomatic, recurrent HSV infections (due to either HSV-1 or HSV-2), with a history of two or more cold sore episodes per year exhibiting classical lesions. Serum samples collected from each prospective subject were tested for HSV antibodies, with the expectation that the clinical trial subjects were HSV seropositive. Other inclusion criteria included age 18 or over and immunocompetence. Exclusion criteria included HSV seronegativity, active HSV lesions at Visit 1, being immunosuppressed or currently taking immunosuppressive drugs, having other types of oral lesions, eg, oral ulcers, and having used antiviral therapy within a week of Visit 1.

On the day of each prospective subject's dental procedure (Visit 2/Day 0), there was a reassessment of inclusion criteria, notably, confirmation of HSV antibodies and the demonstrated ability to self-administer sublingual drops. Individuals meeting these criteria at each clinical trial location were randomized into treatment and control groups. There were to be 105 test subjects in the treatment group and the same number of control group subjects. After an initial assessment of the oral mucosa, subjects were issued vials of either BTL-TML or saline control and instructed when to self-administer the medication (or control), as well as how to fill out a herpes symptom diary card twice daily (morning and evening with a 12-hour interval). Visits 3, 4 and 5 corresponded to Days 3, 7 and 14 after each subject's dental procedure. Subjects without cold sore symptoms by Day 7 were terminated from the clinical trial. It was expected that about 30 of the 210 prospective subjects would experience recurrent herpes cold sores with a prodromal stage by Day 7 post-dental procedure. Each subject with lesions at Visits 3 and 4 had a clinical assessment (including questions regarding AEs) and a review of their diary card. Visit 5 (Day 14 post-dental procedure) was for collection of diary cards and BTL-TML (or saline control) vials. Subjects without lesions by Day 7 had their cards and vials collected at Visit 4/Day 7. In addition to the diary cards, there was a case report form (CRF) maintained for each subject. Entries to these forms were made after each subject's visit to their respective clinical trial location.

Any subjects who started to experience cold sores (with or without prodromal symptoms) within 14 days of their dental procedure had to fill out their diary cards four times per day. The diary questions were about the time they became aware of a lesion, time they started using the medication (or control), stage of lesion development (prodromal through healing) and any pain they were aware of. The dosing of medication (or control) drops was initiated within six hours of experiencing prodromal or other symptoms typical of cold sores in accordance with the dosing regimen described above.

Phase IIa Efficacy Endpoints

The primary efficacy endpoint was the effect of early treatment at the prodromal stage of herpes lesions triggered by (or related to) a dental procedure. This was based on comparing the rate of progression from the prodromal to the vesicle stage in treatment versus control subjects. A treatment success was the blocking or diminishing of prodromal lesions progressing to the vesicle stage. There were two secondary endpoints. The first was the time to healing of lesions that were not blocked from progressing to the vesicle stage in treatment versus control subjects. The other secondary endpoint was the rate of viral shedding between treatment and control subjects, based on viral swabs of lesions taken at Visits 2–5. Separate from efficacy, any pain subjects experienced from their herpes lesions was assessed over all days lesions were present, using an 11-point Likert pain scoring scale (0 = no pain, 1-3 = mild pain, 4-7 = moderate pain, 8-9 = severe pain, 10 = pain as bad as it could be).

Phase IIa Data Analysis

A formal statistical analysis plan was written prior to data lock. Data entry and analysis methods employed a combination of both statistical analysis software tools proprietary to the evaluators and SAS® version 9.x (SAS Institute Inc., Cary, NC, USA). Tests of two independent binomial proportions (both the normal approximation and the exact) were used to compare the rates of progression from the prodromal to vesicle stage in the treatment and control groups. It was expected that samples sizes of both treatment and control groups would be small, ie, about 15 subjects per group. Accordingly, random assignment of subjects to the two groups did not ensure equality with respect to potential confounders, notably, age, gender and the number of HSV attacks per person per year. The issues of age and attacks per year was solved in part by comparing treatment versus control groups using the independent samples t-test or (if necessary) the nonparametric Wilcoxon Rank Sum test. Gender was compared using the Chi-square test. The semi-parametric Cox Proportional Hazards regression model was the primary statistical method used to

compare time to healing in treatment versus control subjects who developed vesicles. The independent samples t-test was used to compare the rates of viral shedding between treatment and control groups.

Phase IIb Clinical Trial Objective

Trial subjects for the randomized, double-blind, placebo-controlled Phase IIb clinical trial were persons with a history of recurrent HSV that were to undergo experimental UV irradiation of the lips. The objective was to determine the efficacy of sublingually administered low-dose BTL-TML in blocking the progression of cold sore lesions beyond the prodromal stage.

Phase IIb Trial Experimental Design

The design of this trial was based on previously developed models for UV induction of herpes labialis.[7,27,28] About 300 prospective clinical trial subjects were recruited at several clinical trial locations in the continental United States. Prospective subjects were screened within 21 days of UV irradiation procedures for clinical trial eligibility (Visit 1). The primary inclusion criterion was a history of recurrent cold sores. Other inclusion criteria included a physical examination of the lips and mouth area and a variety of laboratory tests performed on blood and urine samples. Subjects who met the inclusion criteria were randomized into either a treatment (BTL-TML) group or placebo control (saline) group of about 60 subjects per group. These subjects were scheduled for Visit 2, which consisted of an initial determination of a minimal erythema dose induced by topical UV exposure (MED-UV). Each subject had six small areas of their ventral forearms exposed to the UV light from a DermaPal UV phototherapy lamp (Daavlin, Bryan, OH, USA). Subjects were instructed not to receive any additional UV exposure, eg, via sunbathing or tanning booth use. Visit 3, the MED-UV assessment, occurred 16–24 hours after Visit 2. The MED-UV was defined as the minimal amount of exposure time resulting in a distinct margin of erythema relative to the surrounding unexposed skin. Subjects who did not develop a MED-UV on Visit 3 underwent a second UV exposure on the opposite forearm, followed by a second MED-UV assessment 16–24 hours later (Visit 3b).

Subjects who had developed a valid MED-UV were then exposed to the appropriate UV dose on the lips (Visit 4). Subjects first covered their face, excluding their lips (plus a 1-cm border), with a non-para-aminobenzoic acid sunscreen with a skin protection factor of 30. The phototherapy lamp was placed next to the lip area, and the lips were exposed to a UV intensity four times the length of time established for the MED-UV. Subjects were instructed to not use any topical products (eg, lip balm or lipstick) and avoid any additional (non-trial-related) UV exposure for the remainder of the study. After UV lamp exposure, subjects were then randomized either to the BTL-TML treatment group or to the saline control group. There were to be 60 test subjects in the treatment group and a like number in the control group. Subjects were issued vials of either BTL-TML or saline control and instructed when to self-administer the treatment (or control). Subjects that experienced the prodrome stage of cold sore lesions (defined as tingling, itching, burning, pain, etc) within seven days (168 hours) post-UV exposure initiated self-administration of BTL-TML or saline control drops (Treatment Day Zero) and scheduled a clinic visit within 24 hours of lesion onset (Visit 5/Treatment Day 1). During this visit, subjects' lesions were evaluated by a trained grader, who took a digital photograph of the lesion, and observed the oral mucosa under the tongue to check for signs of irritation resulting from sublingual dosing. Additionally, subjects were queried regarding any AEs, and had their diaries reviewed. Vital signs, notably temperature, blood pressure, and heart and respiration parameters also were determined. The same clinical evaluations were performed for each subject at Visits 6–8, corresponding to Treatment Days 1–3. If for any reason treatment was not initiated, the subject was withdrawn from the study. Subjects remaining in the clinical trial continued with self-administration of drops and entering self-assessment information into their diaries. Follow-up Visit 9 occurred on Treatment Day 7, at which time subjects returned their BTL-TML or saline control vials, as well as returning all diaries/self-assessment forms for review. Visit 9 was the exit visit both for subjects whose lesions had healed by Treatment Day 7, as well as for those who did not develop a lesion within seven days of UV exposure. Subjects had their lesions (if present) graded and lips were photographed, vital signs were recorded, and subjects were queried about their compliance with dosing and any AEs encountered. Additionally, blood and urine samples were collected for routine hematology, blood chemistry analysis and urinalysis. Subjects who developed a cold sore lesion that was not healed by Visit 9 had the analogous evaluations and clinical laboratory tests performed at Visit 10 (Treatment Day 14). As these subjects stopped self-administration of BTL-TML or saline drops on Treatment Day 7, they were permitted to use a typical standard of care (topical or otherwise) between Visits 9 and 10.

Phase IIb Efficacy Endpoints

The primary efficacy endpoint was the effect of early treatment at the prodromal stage of herpes lesions triggered by UV irradiation. This was based on comparing the progression from the prodromal to the vesicle stage in BTL-TML treatment versus saline control subjects by Treatment Day 7. A treatment success was the blocking or diminishing of prodromal lesions progressing to the vesicle stage, based on both Treatment Day 7 (Visit 9) evaluator assessments and subject self-assessments. Conversely, a treatment failure was any lesion that reached the vesicle stage by Treatment Day 7. In addition to efficacy, any pain experienced by the subjects from the herpes lesions was self-assessed over all days lesions were present, using the 11-point Likert pain scoring scale (0 = no pain… 10 = pain as bad as it could be).

Phase IIb Data Analysis

All Phase IIb data entry, code programming and statistical analysis was done using SAS® version 9.3. Test article weights and diaries were used to track treatment compliance.

Data from the BTL-TML treatment and saline control groups were both descriptively summarized using evaluator assessments, and subject self-assessments. The numeric scores (semiquantitative where 1 = prodrome, 2 = erythema/macule, 3 = papule, 4 = vesicle, 5 = ulcer, 6 = hard crust and 7 = healing) were summarized by treatment versus control groups using descriptive statistics such as mean, standard deviation, etc. The percentages of treatment and control subjects for which cold sores did not progress to the lesion stage by Day 7 were evaluated using Fisher's exact test, the Chi-square test or the log rank test. Other statistical comparisons of treatment and control data were made using the Chi-square or Binomial Proportion tests.

Results

Phase IIa Clinical Trial

Phase IIa demographics for randomized subjects. The final numbers of randomized subjects were 84 in the treatment group and 87 in the control group (171 total subjects). There were 34 subjects with recurrent cold sores, with 23 in the treatment group self-administering the BTL-TML, and 11 in the control group, self-administering the saline placebo, for a 2:1 randomization ratio of BTL-TML to saline control subjects. (The randomization ratio was expected to be 1:1). One subject withdrew from the BTL-TML group and two subjects withdrew from the saline group before finishing dosing. Thus, 22 BTL-TML subjects and nine saline placebo subjects completed the study. The demographic details of the randomized subjects are shown in Table 1. There were no statistically significant differences in the demographics of the randomized treatment and control groups, whether for type of dental procedure (almost all of which were preventive), age, sex, weight (body mass index), race or the average number of lesions per year. However, there was a significant difference between the number of subjects who completed self-administering BTL-TML and those taking the saline control ($p = 0.0078$). This difference (2:1 randomization ratio of BTL-TML and control subjects who completed the trial) was an unintended outcome.

Table 1. Demographic Data for Randomized Phase IIa Clinical Trial Subjects.

	BTL-TML Treatment Group	Saline Control Group	Total
# Randomized Subjects	84	87	171
Age, average (years)	45	42	43
Age Range	20-77	19-71	19-77
Gender, n (%)			
Male	23 (27%)	32 (37%)	55 (32%)
Female	61 (73%)	55 (63%)	116 (68%)
Race, n (%)			
African American	12 (14%)	18 (21%)	30
Caucasian	67 (80%)	63 (72%)	130
Hispanic	2 (2%)	4 (5%)	6
Asian	3 (4%)	2 (2%)	5
Body Mass Index (BMI), average	28	30	
BMI Range	19-58	19-55	
# Subjects with Recurrent Cold Sores (% of Randomized)	23 (27%)	11 (13%)	34
# Lesions per Year (Average, Self-reported)	5	5	
Type of Dental Procedure (%)	99% Preventive, 1% Interventional	96% Preventive, 4% Interventional	
# Withdrawals	1	2	3
# Subjects Completing Clinical Trial	22	9	31

Phase IIa efficacy endpoints. The primary endpoint in the subjects experiencing prodrome was the percentage not proceeding to lesion (vesicle, ulcer or crust). This percentage was 54.5% (12/22) in the BTL-TML treatment group and 22.2% (2/9) in the saline control group. The difference in the two percentages (treatment minus control) was 32.3%. The data showed a trend toward efficacy, but the results were not statistically significant at alpha = 0.05 ($p = 0.106$ by Fisher's exact test, $p = 0.075$ by the log rank test). With respect to the secondary endpoint (time to healing in subjects with a vesicle, ulcer or crust), there was no indication of treatment effect ($p = 0.83$). These data suggest that BTL-TML can help prevent the development of herpes vesicles, ulcers and crusts from prodrome, but does not improve time to healing from the formed lesions. There also were no statistically significant differences in viral shedding in BTL-TML and saline groups. Shedding ranged from about 10% to over 20% of subjects in Visits 2–5.

Phase IIa pain scores in subjects with herpes lesions. An 11-point Likert Scale (range, 0-10) was used to assess pain in treatment and control subjects experiencing cold sore lesions, and the results were averaged over all days lesions were present. The mean Likert Scale values for the BTL-TML and saline control groups were 1.04 and 1.21, respectively. This was not statistically significant ($p = 0.55$).

Phase IIa adverse events. AEs in randomized subjects who were administered either BTL-TML or the saline control are shown in Supplemental Table 1. The mean numbers of AEs were 1.52 per subject in the BTL-TML group (23 total subjects, with one withdrawal during the study period) and 2.42 per subject in the control group (11 total subjects, with two withdrawals during the study). Most of these AEs were mild, with the only serious AEs in three subjects randomized to the control group. There were no statistically significant differences at alpha = 0.05, but oral lesions (classified under gastrointestinal AEs) were markedly higher in the saline control group (5 vs 2; $p < 0.1$).

Phase IIb Clinical Trial

Phase IIb demographics for randomized subjects. There were 303 prospective subjects initially screened for the Phase IIb clinical trial during the recruiting process. The final numbers of randomized subjects were 81 in the BTL-TML treatment group and 77 in the saline control group (158 total subjects). Initially, there were 62 subjects self-administering BTL-TML and 56 subjects self-administering the saline control (118 total subjects), but there were five and two withdrawals from the treatment and control groups, respectively. Thus, there were 111 subjects with UV-induced cold sores that completed

Table 2. Demographic Data for Randomized Phase IIb Clinical Trial Subjects.

	BTL-TML Treatment Group	Saline Control Group	Total
# Randomized Subjects	62	56	118
Age, average (years)	47	48	47
Age Range	20-73	18-72	18-73
Gender, n (%)			
Male	11 (18%)	19 (34%)	30 (25%)
Female	51 (82%)	37 (66%)	88 (75%)
Race, n (%)			
African American	1 (2%)		1
Caucasian	58 (93%)	55 (98%)	113
Hispanic	2 (3%)	1 (2%)	3
Asian	1 (2%)		1
# Lesions per Year (Average, Self-reported)	3.5	3.5	
# Lesions per Year (Range)	2-10	2-10	
# Withdrawals	5	2	7
# Subjects Completing Clinical Trial	57	54	111

the clinical trial, with 57 in the treatment group self-administering the BTL-TML and 54 in the control group, self-administering the saline placebo. The main demographic characteristics were age, sex, race/ethnicity and the average number of herpes labialis episodes per year (Table 2). All 118 randomized subjects were between ages 18 and 72, with an overall average age of 47 years. The percentages of female and male subjects were 66% (88) and 34% (40), respectively. About 96% of the subjects were white. Subjects had an average of 3.5 herpes episodes per year. There were no statistically significant differences in the demographics of the treatment and control groups for any demographic parameter.

Phase IIb efficacy endpoints. The primary endpoint in the UV-exposed subjects experiencing prodrome was the percentage not proceeding to lesion (vesicle, ulcer or crust) by Treatment Day 7. Although there were 57 subjects in the BTL-TML treatment group and 54 in the saline control group, 34 of the subjects randomized to the treatment group and 40 of the subjects randomized to the control group did not develop cold sore prodromes that could be evaluated on Treatment Days 1–3. Consequently, there were only 37 total subjects (23 treatment, 14 control) that were assessed by the clinical evaluators on Treatment Day 7. The evaluator assessment was the 47.8% of the BTL-TML treatment subjects (11/23) and 42.8% of the saline control subjects (6/14) did not proceed to the lesion stage ($p > 0.5$ by Fisher's exact test). The secondary endpoint relied on patient self-assessments of the ability to block progression to lesion on Treatment Day 7. However, progression from prodrome to lesion was reported as blocked in only 32% of the BTL-TML subjects (8/25) versus 21.4% of the control subjects (6/28; $p = 0.38$). The difference in the two percentages (treatment minus control) was only 5% by evaluator assessment, but differences based on self-assessment were twice as high at 10.6%. Regardless, the data indicate that, under the conditions of the Phase IIb clinical trial, BTL-TML was unable to block the progression of herpes labialis lesions in persons whose lips were exposed to UV irradiation.

Phase IIb pain scores in subjects with herpes lesions. Based on self-assessments of pain (11-point Likert scale) on Treatment Days 0–7, pain scores for the BTL-TML group averaged 1.84 (range, 0.52-2.83). The average pain score for the saline control group was slightly higher at 2.03 (range, 0.61-3.18). This was not statistically significant ($p = 0.26$). Pain scores were higher on Treatment Days 0–4 than on Days 5–7 (Figure 1).

Phase IIb adverse events. AEs in subjects that were randomized and self-administering either BTL-TML (62 subjects) or saline (56 subjects) are shown in Supplemental Table 2. The mean numbers of AEs were 0.37 per subject in the BTL-TML group and 0.27 per subject in the saline control group. All of these AEs were mild or moderate, with no serious AEs in either group. There were no statistically significant differences at alpha = 0.05.

Phase IIb Post-hoc data analysis. As noted above, the primary endpoint of the Phase IIb study was to determine if BTL-TML was capable of blocking progression from prodrome to lesion. However, under the Phase IIb trial protocols, only 37 of the clinically evaluated and 53 of the self-assessed subjects reported symptoms of prodrome, although other subjects may have experienced initial symptoms of some later stage of a herpes labialis episode (macule, papule, vesicle, etc) during the 7-day treatment period. A subsequent review of the literature led to consideration of the concept that herpes patients developing cold sores within the first 48 hours after UV radiation exposure do not respond to any type of antiviral drug.[7,27,29] Consequently, a post-hoc data analysis was conducted to analyze the self-assessment data from 44 subjects (23 BTL-TML, 21 saline control) whose first symptoms did not appear until after the suggested 48-hour cut-off point (2-7 days after UV exposure; Supplemental Table 3). As shown in Figure 2, the percentage of these BTL-TML subjects with no progression to lesion was 52.2% (12/23), whereas the percentage of saline control subjects with no progression was 28.6% (6/21). While this still was not statistically significant ($p = 0.099$), the results were markedly improved relative to that seen in the reported Phase IIb results. The difference in the two percentages (treatment minus control) was 23.6%.

Discussion

The hypothesis being tested in the Phase IIa trial was that BTL-TML could prevent progression to lesion in patients experiencing prodrome following trauma induced by a (mainly routine) dental procedure. In this study, 54.5% (12 of 22 subjects) in the BTL-TML treatment group and 22.2% (two of nine subjects) in

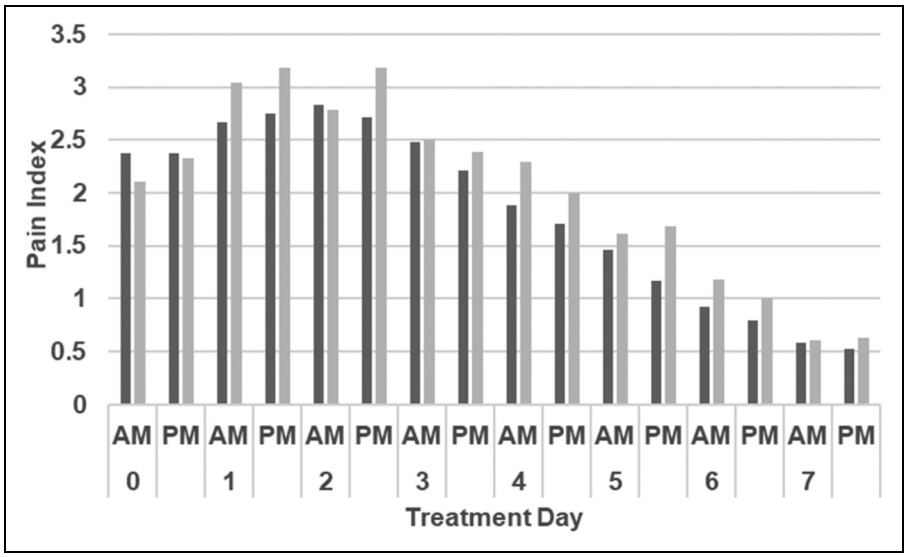

Figure 1. Average pain scores for the treatment and control groups, treatment days 0-7. Black = BTL-TML, grey = saline control.

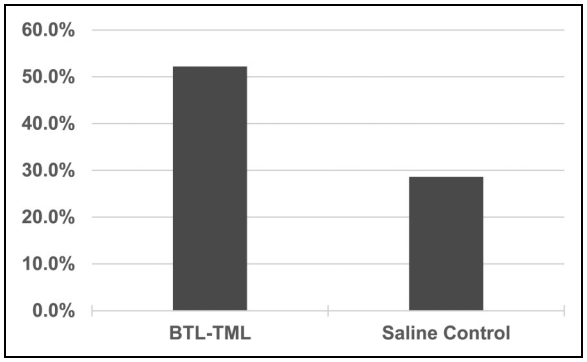

Figure 2. Prevention of progression to lesion from post-hoc analysis of self-assessment data (>48 hours through 7 days post-UV exposure).

the saline control group had progression to lesion blocked. This showed a trend toward efficacy but missed being statistically significant ($p = 0.106$), probably because of small numbers of evaluable subjects. Difficulty enrolling patients was an expected limitation of the dental trauma model. In a previous study, only about 20% of patients that had undergone a dental procedure developed symptoms of a cold sore shortly thereafter.[26] Similarly, the 31 subjects in the present study represented 18% of the 171 prospective subjects enrolled at the start of the trial. It was concluded that this was an inefficient model in which to evaluate the effectiveness of BTL-TML. Subsequently, it was decided that a planned Phase IIb clinical trial should employ a different herpes model, that of UV radiation-induced herpes labialis.[7,27,28] The main attraction of this model was the expectation that 40–50% of subjects could progress from prodrome to lesion within 7 days of UV exposure. Enrollment of 200–250 subjects and exposure of each subject's lips to UV was expected to yield about 100 evaluable subjects, adequate for demonstrating a statistically significant effect. A total of 111 patients were eventually enrolled and evaluated in this Phase IIb study. The primary endpoint was to determine the effectiveness of BTL-TML in blocking progression to lesion following symptoms of prodrome. Unfortunately, just 37 patients (23 treatment, 14 control) reported prodrome symptoms, while the remaining 74 experienced initial symptoms of some later stage of a herpes labialis episode. BTL-TML had essentially no efficacy in blocking progression from prodrome to lesion (47.8% vs 42% in the BTL-TML and control groups, respectively). The secondary endpoint, analysis of the self-assessment data with respect to blocking progression, yielded a minor effect in a total of 53 subjects, again with no statistical significance ($p = 0.38$). One further analysis, using an entry criterion for analysis broadened to include any pre-lesion symptoms (prodrome/macule/papule), the number of evaluable subjects improved from 37 (primary endpoint) or 53 (secondary endpoint) to over 100, but a statistically significant difference between the BTL-TML and control groups still was not achieved ($p = 0.308$). The lack of statistically significant responses in Phase IIb data analyses with respect to BTL-TML's potential to block progression from prodrome or pre-vesicle to the vesicle stage in the UV model led to a re-examination of the model described by Spruance et al.[7,27,29] Those researchers postulated that patients developing cold sores within the first 48 hours after UV radiation exposure did not respond to antiviral drugs of any sort. Such patients may develop what was termed "immediate lesions" within 6–48 hours post-UV exposure, or what was termed "delayed" lesions 3–7 days after UV exposure. In prior studies, the immediate lesions did not respond to antiviral drugs. In contrast, the delayed lesions were hypothesized to develop from virus reactivation in the trigeminal nerve, centrifugal axonal virus transport, and seeding of the epidermis in the UV-irradiated area.[29] As BTL-TML was not a conventional antiviral agent, it was thought that BTL-TML could in fact work against immediate lesions (<= 48 hr). The apparent non-responsiveness of early lesions to BTL-TML provided a plausible

explanation for the poor efficacy reported in the Phase IIb trial, and was the primary reason for the post-hoc analysis. This analysis, composed of 109 subjects that experienced pre-lesion symptoms, identified 44 subjects (23 BTL-TML, 21 saline control) whose first symptoms did not appear until over 48 hours after UV irradiation. There were 12 of 23 (52%) BTL-TML subjects with blocked progression to lesion, versus only six of 21 (28%) saline control subjects. This was in direct contrast to the Phase IIb clinical trial percentages for both the primary and secondary endpoints. These data appear to confirm that BTL-TML had efficacy in blocking progression to lesion only after 48 hours post-UV irradiation, thus validating further the concept that there is a period following exposure to UV radiation during which antiviral activity may be reduced or absent.

That the Phase IIa or post-hoc Phase IIb trials did not yield statistically significant results at alpha = 0.05 may be more related to being underpowered from a statistical standpoint than from an actual failure of BTL-TML to block lesion progression. Assuming that the oral herpes lesions recur regardless of the trigger (dental procedure or UV exposure), the data from the Phase IIa and post-hoc Phase IIb clinical trials can then be pooled. The resulting pooled sample sizes would be 45 subjects in the BTL-TML group and 30 subjects in the saline control group. As 53% (24/45) did not progress to lesion in the BTL-TML group but only 27% (8/30) did not progress to lesion in the control group, the resulting p-value (by Fisher's Exact one-tailed test) would have been statistically significant at $p = 0.019$. It is thus hypothesized that statistical significance could be achieved in any future Phase IIb clinical trial using BTL-TML in the UV-induced herpes labialis model in which a) the evaluation period should begin 48-plus hours post-exposure and up to 7 days, and b) the number of evaluable subjects approaches or exceeds a target of 75–100.

How did BTL-TML compare to standard treatments in blocking progression to lesion in other clinical trials? The difference in the treatment percentage minus the control percentage can be used as an objective comparator between studies. Based on the results of the post-hoc analysis of the Phase IIb data, the difference (BTL-TML treatment minus saline control) was 23.6%. In a clinical trial of oral valacyclovir plus topical clobetasol for blocking progression to lesion, the difference (treatment minus control) was 34.2%.[30] However, high-dose oral valacyclovir alone only showed a difference of 8% in blocking progression.[31] A buccal adhesive formulation of acyclovir (Aciclovir Lauriad®) had an even smaller difference in blocking progression to lesion relative to the placebo control (6.8%).[32] Another combination treatment, topical acyclovir plus hydrocortisone (also known as ME-609; Xerese or Xerclear) showed a difference in blocking progression of 16%.[33] Taken together, these data suggest that BTL-TML may be superior to single chemotherapeutic agents, and comparable to combination therapies, used in other clinical trials of similar experimental design. This raises an intriguing possibility that BTL-TML may be even better at blocking progression to lesion if used in combination with a standard herpes labialis therapeutic agent.

One other noteworthy observation is that while the number of AEs per subject in both the BTL-TML and saline control groups in both clinical trials were low, they were substantially higher in the Phase IIa clinical trial. This suggests that several of the AEs in that trial resulted from the dental procedures. This observation is supported by the one statistically significant difference, more oral lesions in the saline control group than in the BTL-TML group.

In closing, BTL-TML may have potential as a safe and efficacious treatment for herpes labialis. However, another properly designed Phase IIb clinical trial would be necessary both to confirm these observations and to achieve a statistically significant effect of BTL-TML in blocking progression of herpes labialis from the pre-lesion to the vesicle or lesion stage after UV irradiation.

Author Contributions

Mamber - Drafted paper with strong contributions to results and conclusions section.
Hatch - Helped design study and contributed to analysis and interpretation of data.
Miller - Principal Investigator of P2a dental trauma study. Organized and managed the study, including analysis and interpretation of study-specific data.
Murray - Principal Investigator of P2b UV herpes study. Helped design and manage the study, including analysis and interpretation of study-specific data.
Strout - Investigator at an additional site for the P2b UV herpes study. Oversaw patient activities and data collection efforts.
McMichael - Helped design study and contributed to the analysis and interpretation of data.

Ethical Approval

Both studies were approved by respective Institutional Review Boards and are listed in the clinicaltrials.gov database under identifiers NCT01308424 and NCT01902303.

Declaration of Conflicting Interests

Hatch and McMichael are employees of Beech Tree Labs, the sponsor of both the P2a and P2b studies.

Funding

Both the P2a dental trauma study and the P2b UV exposure study were funded by Beech Tree Labs.

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Supplemental material

Supplemental material for this article is available online.

References

1. Spruance SL. The natural history of recurrent oral-facial herpes simplex virus infection. *Semin Dermatol*. 1992 Sep;11(3):200-206. PMID: 1390034.

2. Fatahzadeh M, Schwartz RA. Human herpes simplex labialis. *Clin Exp Dermatol*. 2007 Nov;32(6):625-630. doi: 10.1111/j.1365-2230.2007.02473.x. PMID: 17953630.
3. Patel AR, Romanelli P, Roberts B, Kirsner RS. Treatment of herpes simplex virus infection: rationale for occlusion. *Adv Skin Wound Care*. 2007 Jul;20(7):408-412. doi: 10.1097/01.ASW.0000280199.58260.62. PMID: 17620742.
4. Petti S, Lodi G. The controversial natural history of oral herpes simplex virus type 1 infection. *Oral Dis*. 2019 Nov;25(8):1850-1865. doi: 10.1111/odi.13234. Epub 2019 Nov 27. PMID: 31733122.
5. Gilbert S, Corey L, Cunningham A, et al. An update on short-course intermittent and prevention therapies for herpes labialis. *Herpes*. 2007 Jun;14(Suppl 1):13A-18A. PMID: 17877887.
6. Marques-Silva L, Castro WH, Gomez EL, Guimarães AL, Silva MS, Gomez RS. The impact of dental surgery on HSV-1 reactivation in the oral mucosa of seropositive patients. *J Oral Maxillofac Surg*. 2007 Nov;65(11):2269-2272. doi: 10.1016/j.joms.2007.05.029. PMID: 17954324.
7. Spruance SL, Kriesel JD, Evans TG, McKeough MB. Susceptibility to herpes labialis following multiple experimental exposures to ultraviolet radiation. *Antiviral Res*. 1995 Sep;28(1):57-67. doi: 10.1016/0166-3542(95)00038-n. PMID: 8585760.
8. Hull CM, Levin MJ, Tyring SK, Spruance SL. Novel composite efficacy measure to demonstrate the rationale and efficacy of combination antiviral-anti-inflammatory treatment for recurrent herpes simplex labialis. *Antimicrob Agents Chemother*. 2014;58(3):1273-1278. doi: 10.1128/AAC.02150-13. Epub 2013 Dec 16. PMID: 24342632; PMCID: PMC3957859.
9. Cunningham A, Griffiths P, Leone P, et al. Current management and recommendations for access to antiviral therapy of herpes labialis. *J Clin Virol*. 2012 Jan;53(1):6-11. doi: 10.1016/j.jcv.2011.08.003. Epub 2011 Sep 1. PMID: 21889905; PMCID: PMC3423903.
10. James C, Harfouche M, Welton NJ, et al. Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bull World Health Organ*. 2020 May 1;98(5):315-329. doi: 10.2471/BLT.19.237149. Epub 2020 Mar 25. PMID: 32514197; PMCID: PMC7265941.
11. Raborn GW, Grace MG. Recurrent herpes simplex labialis: selected therapeutic options. *J Can Dent Assoc*. 2003 Sep;69(8):498-503. PMID: 12954137.
12. Harmenberg J, Oberg B, Spruance S. Prevention of ulcerative lesions by episodic treatment of recurrent herpes labialis: a literature review. *Acta Derm Venereol*. 2010 Mar;90(2):122-130. doi: 10.2340/00015555-0806. PMID: 20169294.
13. Spruance SL, Kriesel JD. Treatment of herpes simplex labialis. *Herpes*. 2002 Dec;9(3):64-69. PMID: 12470603.
14. Usatine RP, Tinitigan R. Nongenital herpes simplex virus. *Am Fam Physician*. 2010 Nov 1;82(9):1075-1082. PMID: 21121552.
15. Leung AKC, Barankin B. Herpes labialis: an update. *Recent Pat Inflamm Allergy Drug Discov*. 2017;11(2):107-113. doi: 10.2174/1872213X11666171003151717. PMID: 28971780.
16. Sacks SL, Thisted RA, Jones TM, et al. Docosanol 10% Cream Study Group. Clinical efficacy of topical docosanol 10% cream for herpes simplex labialis: a multicenter, randomized, placebo-controlled trial. *J Am Acad Dermatol*. 2001 Aug;45(2):222-230. doi: 10.1067/mjd.2001.116215. PMID: 11464183.
17. Opstelten W, Neven AK, Eekhof J. Treatment and prevention of herpes labialis. *Can Fam Physician*. 2008 Dec;54(12):1683-1687. PMID: 19074705; PMCID: PMC2602638.
18. Pless R, Risher JF. Mercury, infant neurodevelopment, and vaccination. *J Pediatr*. 2000 May;136(5):571-573. doi: 10.1067/mpd.2000.106797. PMID: 10802484.
19. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics*. 2001 May;107(5):1147-1154. doi: 10.1542/peds.107.5.1147. PMID: 11331700.
20. Clarkson TW. The three modern faces of mercury. *Environ Health Perspect*. 2002 Feb;110 Suppl 1(Suppl 1):11-23. doi: 10.1289/ehp.02110s111. PMID: 11834460; PMCID: PMC1241144.
21. FDA Thimerosal in vaccines. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/thimerosal-and-vaccines.
22. Miller JB. Treatment of active herpes virus infections with influenza virus vaccine. *Ann Allergy*. 1979 May;42(5):295-305. PMID: 453646.
23. McMichael J. 2001, Jan 16. Methods for treating herpes virus infections. US patent 6,174,916.
24. Loison E, Poirier-Beaudouin B, Seffer V, et al. Suppression by thimerosal of ex-vivo CD4+ T cell response to influenza vaccine and induction of apoptosis in primary memory T cells. *PLoS One*. 2014 Apr 1;9(4):e92705.
25. Mei XX, Lei SS, Xu L, et al. Herpes simplex virus type I-infected disorders alter the balance between treg and Th17 cells in recurrent herpes labialis patients. *Int J Immunopathol Pharmacol*. 2020 Jan-Dec;34:2058738420933099. doi: 10.1177/2058738420933099. PMID: 32735468; PMCID: PMC7401041.
26. Miller CS, Cunningham LL, Lindroth JE, Avdiushko SA. The efficacy of valacyclovir in preventing recurrent herpes simplex virus infections associated with dental procedures. *J Am Dent Assoc*. 2004 Sep;135(9):1311-1318. doi: 10.14219/jada.archive.2004.0407. PMID: 15493396.
27. Spruance SL. Pathogenesis of herpes simplex labialis: experimental induction of lesions with UV light. *J Clin Microbiol*. 1985 Sep;22(3):366-368. doi: 10.1128/jcm.22.3.366-368.1985. PMID: 2995436; PMCID: PMC268411.
28. Sayre RM, Dowdy JC, Harris KA, Berg JE, Trimble MW. A practical UV source to induce herpes simplex labialis lesions in the clinic. *Photodermatol Photoimmunol Photomed*. 2007 Feb;23(1):20-23. doi: 10.1111/j.1600-0781.2007.00266.x. PMID: 17254031.
29. Spruance SL, Rowe NH, Raborn GW, Thibodeau EA, D'Ambrosio JA, Bernstein DI. Peroral famciclovir in the treatment of experimental ultraviolet radiation-induced herpes simplex labialis: a double-blind, dose-ranging, placebo-controlled, multicenter trial. *J Infect Dis*. 1999 Feb;179(2):303-310. doi: 10.1086/314605. PMID: 9878012.
30. Hull C, McKeough M, Sebastian K, Kriesel J, Spruance S. Valacyclovir and topical clobetasol gel for the episodic treatment of herpes labialis: a patient-initiated, double-blind, placebo-controlled pilot trial. *J Eur Acad Dermatol Venereol*. 2009 Mar;23(3):263-267. doi: 10.1111/j.1468-3083.2008.03047.x. Epub 2009 Jan 8. PMID: 19143902.

31. Spruance SL, Jones TM, Blatter MM, et al. High-dose, short-duration, early valacyclovir therapy for episodic treatment of cold sores: results of two randomized, placebo-controlled, multicenter studies. *Antimicrob Agents Chemother*. 2003 Mar;47(3):1072-1080. doi: 10.1128/AAC.47.3.1072-1080.2003. PMID: 12604544; PMCID: PMC149313.
32. Bieber T, Chosidow O, Bodsworth N, et al. LIP Study Group. Efficacy and safety of aciclovir mucoadhesive buccal tablet in immunocompetent patients with labial herpes (LIP Trial): a double-blind, placebo-controlled, self-initiated trial. *J Drugs Dermatol*. 2014 Jul;13(7):791-798. PMID: 25007361.
33. Hull CM, Harmenberg J, Arlander E, et al. ME-609 Study Group. Early treatment of cold sores with topical ME-609 decreases the frequency of ulcerative lesions: a randomized, double-blind, placebo-controlled, patient-initiated clinical trial. *J Am Acad Dermatol*. 2011 Apr;64(4):696.e1-11. doi: 10.1016/j.jaad.2010.08.012. Epub 2010 Sep 20. PMID: 20851499.