Significant Efficacy and Safety of Low Level Intermittent Heat in Patients with Mild to Moderate Acne

S. Bruce[1], C. Conrad[2], R. D. Peterson[2], R. Conrad[2], L. S. Arambide[3], J. Thompson[3], and W. Klemp[2]

[1]Suzanne Bruce and Associates, [2]Tyrell Inc., [3]Synergos Inc.

Abstract

Patients with mild to moderate acne that do not require prescription medications are limited to over the counter (OTC) preparations. Unfortunately, many of these preparations are of limited value. We investigated and demonstrated that the application of low to moderate levels of sustained heat was effective in reducing colony counts of *P. acnes* bacteria in pre-clinical studies. This suggested that the application of low-level sustained heat could potentially be beneficial to individual acne lesions. A small hand-held device was constructed that allowed the delivery of a thermal dose of 121° F (49.4° C) for 2.5 min and a surface area of roughly 0.099 in^2. This device was used to evaluate this particular technique in a placebo controlled double-blind clinical trial. Patients (51) were enrolled in this trial, all of which met the protocol inclusion criteria and had mild to moderate acne and were not currently on systemic medications. The results demonstrated that the active treatment significantly shortened both the median time to improvement (12.8 versus 35.6 hours, $p < 0.0001$, Log-Rank test) as well as the median time to resolution (89.7 versus 140.1 hours, $p = 0.0020$, Log-Rank test) as reported by the subjects. The observations were made by blinded physicians and by subjective analysis by the patients undergoing this treatment. These objective and subjective evaluations had a high level of agreement, suggesting both internal consistency with both the treatment as well as the clinical design. Additionally, this treatment modality was without any adverse events either observed by the monitoring physicians or by the subjective diaries submitted by the treated subjects. These results suggest that this treatment is both effective and safe for patients that experience mild to moderate acne.

Background

Acne is a chronic inflammatory disorder of the pilocebaceous unit that has been associated with Propionibacterium acnes (*P. acnes*) and is estimated to affect approximately 85% of adolescents and young adults.[1, 2] Patients who suffer from mild to moderate acne are generally treated with over the counter (OTC) topical cleansers, astringents and benzoyl peroxide preparations with occasional prescription of topical or systemic antibiotics provided when more severe flares occur. These OTC preparations are minimally effective and patients that fall into this category often feel the psychological pain of few effective treatments and of chronic persistence of this disease process.[3, 4] Moreover, most of these patients do not qualify for more drastic treatment with agents such as *cis*-retinioc acid (Accutane). In addition, due to more stringent requirements placed on physicians able to prescribe Accutane, fewer patients are being offered this treatment modality. It is also becoming increasingly clear that *P. acnes* is developing increased resistance to antibiotics treatments making this modality less effective and desirable.[5] The longstanding recognition that hot compresses can be very effective in the treatment of some small localized abscess formations[6], prompted us to evaluate if low level heat could be effective in the treatment of *P. acnes* and therefore possibly acne lesions.

Although it is not precisely clear how the normal colonization within a hair follicle of *P. acnes* triggers the inflammatory reaction pathogonomic of an acne lesion, most researchers agree that these bacteria are at least partially if not fully responsible in the pathophysiology triggering acne lesions.[7] It was this rationale and the recognized emergence of antibiotic resistant strains of *P. acnes* that lead us to investigate the possibility of targeting these bacteria by an alternate method to control it, namely heat. We postulated that if *P. acnes* could be eliminated or reduced within a forming acne lesion, then this treatment may promote quicker resolution of this process.

We first evaluated the heat responses of *P. acnes* anaerobic cultures to various heat ranges and a variety of exposure times to determine if tolerable heat levels and exposure times could be found that kill the bacteria but would at the same time be

tolerable to patient's skin. These investigations were followed by producing a prototype device, which can maintain heat to a small treatment tip in a very controlled and accurate fashion. The device was then tested on the investigators to subjectively determine which heat ranges were tolerable. Interestingly, the heat ranges found to be tolerable and seemingly safe were in the range that was able to reduce colony counts from treated *P. acnes* anaerobic cultures. Finally, the chosen temperature level, exposure time and treatment frequency were tested in a double-blind placebo controlled clinical trial. Results of this trial are reported here and demonstrate significant clinical efficacy without any adverse reaction.

We have experience in determining the survival of the bacterium *P. acnes* when exposed to various heat sources and to various lengths of time. This information is critical in determining the heat tolerance of human tissue versus the tolerability of *P. acnes* under thermal delivery. Based on research to evaluate these parameters, we have been able to endow Zeno™ with optimal heat and timing characteristics to effectively treat the underlying cause of acne and pustular-form eruptions through localized thermal delivery.

Materials and Methods

Cultures

The bacterial strain *P. acnes* was purchased from The American Type Culture Collection ATCC (No. 11827, Lot 419571, Manassas, VA). The cultures were stored in KWIK-STIK lyophilized preparations.

In Vitro Procedures

The lyophilized cells (*P.acnes*) were rehydrated according to the manufacturer's recommendations and initially grown on a streak plate to isolate individual colonies under anaerobic conditions and with sterile phosphate-buffered saline and then streaked using sterile technique with a wire loop on to TSA-plates. These plates were then incubated overnight at 37° C in an anaerobic chamber. Individual colonies were then isolated and inoculated into TSB-growth media with medium agitation overnight. From these aliquots of 0.1 ml of TSB, broth culture was added to the 0.9 ml of PBS sterile buffer. This mixture was then transferred to thin-walled Eppendorf 1.5 ml tubes and placed in a heating block at various times and temperatures. The cultures, after specific incubation times, were removed and 0.1 ml of the material was plated onto TSA plates. This mixture was then spread with a sterile hockey-stick and then allowed to incubate at 37° for five (5) days in anaerobic conditions. The plates were then removed and colonies were counted and recorded.

Prototype Device

The specific engineering details of the device construction are beyond the scope of this manuscript. Essentially the device was designed and constructed to maintain a precisely controlled temperature at a treatment tip size of approximately 3/8 inch in diameter. This was accomplished through regulating the power applied to a heating element using a closed loop feedback system. The device used Proportional, Integral and Differential (PID) calculations in determining the necessary power applied to the heating element to quickly ramp the temperature to the set point, while minimizing any overshoot effects of the target temperature. The PID system was also used to maintain temperature regulation of the device treatment tip to within 1° C from the set point, thereby allowing a constant precise temperature to be applied to the lesion throughout the treatment cycle.

Clinical Trial

The clinical trial was designed as a double-blind placebo-controlled study that enrolled patients with mild to moderate acne and who were not receiving concurrent prescription medications.

This randomized double-blind study was controlled within each subject. Subjects received treatments with both active and placebo devices. After a subject had been determined to meet the eligibility criteria and had signed Informed Consent, two (2) similar, clinically-matched single blemishes were selected for treatment, one (1) with the Zeno device and one (1) with the placebo device. The placebo device was identical in appearance to the Zeno device but did not deliver any heat. An unblinded administrator administered treatments with active and placebo devices in an identical fashion. Neither the subject nor the Physician performing the study related assessments knew to which treatment assignment each blemish was assigned.

Blinding

At each site initiation, a randomization list was provided to the unblinded study staff. This list designated which treatment, active or placebo, each blemish was to receive. The Investigator instructed the study staff where the selected

blemishes were located on the face. Both study blemishes to be treated were photographed and labeled as “Blemish 1” or “Blemish 2”, along with the date the photographs were taken, to ensure that the additional treatment administrations and assessments of the treated areas would be associated with the correct acne blemish and treatment. Each subject received three (3) study treatments to each blemish of 2.5 minutes each. The two (2) treatments on Day 1 were to be a minimum of at least one (1) hour and no longer than 12 hours apart. The third treatment was performed on the second day and occurred at a minimum of 18 hours and no later than 48 hours after the first treatment. Subjects had photographs of the actively treated and placebo treated blemishes taken for documentation purposes. Investigator evaluation of the actively treated and placebo treated blemishes was conducted prior to treatment on Days 1 and 2 and at the Day 5 follow-up visit. Subjects assessed their blemishes using a Subject Diary and VAS tenderness scale twice daily (am/pm) until resolution of both blemishes or Day 14, whichever occurred earliest.

Sample Size

The study was planned for a total of 50 subjects. Each subject was required to have had two (2) similar blemishes treated and assessed, one (1) treated with the Zeno (active) device and one (1) treated with a placebo device. The sample size was based on safety considerations and the probability of experiencing adverse events. A sample of 50 allowed adequate power to detect the incidence of rare safety events. A sample of 50 subjects yielded 95% probability that the study would reveal at least one (1) occurrence of all events that occur at a rate of 5.8% or greater. This sample size was adequate to demonstrate that the Zeno device was safe and effective.

Statistical Considerations

Standard statistical methods were employed to analyze all data. The following techniques were used: descriptive statistics, paired t-test, Fisher’s Exact test, Kaplan-Meier techniques, McNemar’s test, Bowker’s test and graphical displays. Assumptions of normality and homogeneity of variance were tested with the Shapiro-Wilks test. If the distributional assumptions were violated, non-parametric techniques, such as Wilcoxon’s rank-sum test were employed. All tests were declared statistically significant if the calculated p-value was less than or equal to 0.0500. All tests appeared as two-sided p-values.

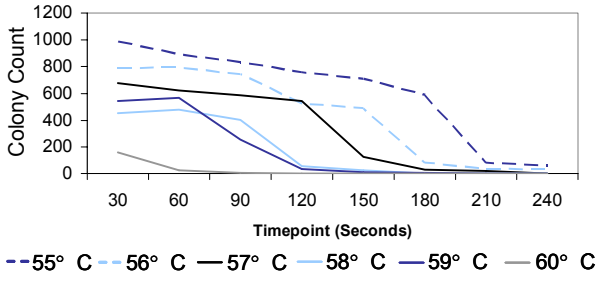
Summary statistics consisted of numbers and percentages of responses in each category for discrete measures, and of means, medians, standard deviations, 95% confidence intervals, minimum, and maximum values for continuous measures, and are presented for each treatment group, where applicable. Version 8.0 or higher of the SAS® statistical software package was used to provide all statistical analyses.

Results

Pre-clinical

The temperature kill assay performed clearly demonstrated that *P. acnes* is sensitive to increasing temperatures. There was a general trend of reduction of the required time needed to reduce the colony counts at higher temperature incubations. **Figure 1** demonstrates the rapid decline of *P. acnes* in response to various temperatures and duration of treatment. Also of note is what appears to be a temporal thermal threshold, whereas the number of viable colonies drops off in a very steep fashion. By using the curves generated by such experiments the optimal thermal output and the timing for each temperature can be extrapolated for a localized heating device. For some of the higher temperatures, or very long periods of time the number of colonies drops off below the detection

Figure 1. Temperature Death Curves for *P. acnes*



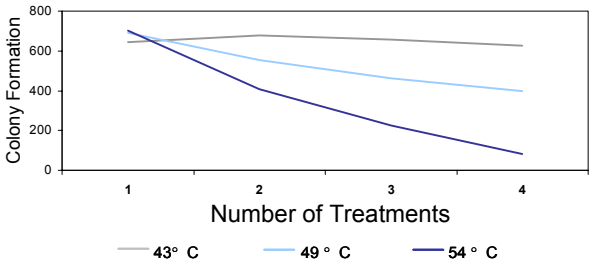
limits of this particular dilution assay. It should be noted that probable longer drop-offs are seen and multiple serial dilutions may be useful to further define the extent of these curves at the higher temperatures and longer incubation times.

Next, the effects of multiple treatments were then investigated. Cultures were established as before, however the treatment with heat was repeated for four (4) time points on separate occasions to evaluate if there was an additive effect. **Figure 2** demonstrates a clear difference in the viability of *P. acnes* colony formation with increasing number

of treatments provided. The data shown represent four (4) treatments given to the individual cultures. These results suggest strongly that pulsing treatment can be very effective in reducing colony counts of *P. acnes*.

In summary, the in vitro data shown demonstrates significant sensitivity of *P. acnes* bacterial cells to the effects of sustained low-level heat as well as pulsing heat treatments, these treatments provide

Figure 2. Multiple in Vitro Heat Treatments



the conceptual basis for the localized treatment with this modality. Due to these results, a clinical trial was initiated to confirm that this treatment strategy is able to translate into effective in vivo treatment.

Clinical trial results

Efficacy Results

There were fifty-one (51) patients enrolled in this study. The patient demographics are given in **Table 1** and represent a slight female to male predominance (30 versus 21) and a median age of 20.0. Zeno treated blemishes resolved faster than placebo treated blemishes. The differences in time

Table 1. Demographic Information

Attribute	Mean ± SD or N (%) (N=51)
Age (years)	20.0 ± 6.3
Gender	
Male	21 (41.2%)
Female	30 (58.8%)
Ethnicity	
White	33 (64.7%)
Black or African American	12 (23.5%)
Hispanic or Latino	4 (7.8%)
Asian	1 (2.0%)
Persian (Asian)	1 (2.0%)

to improvement between active and placebo treatments are easily seen by graphing the time to improvement and/or resolution of lesions by Kaplan-Meier curves (**Figures 3 and 4**). In

Figure 3. Hours to Resolution in the Subject Diary

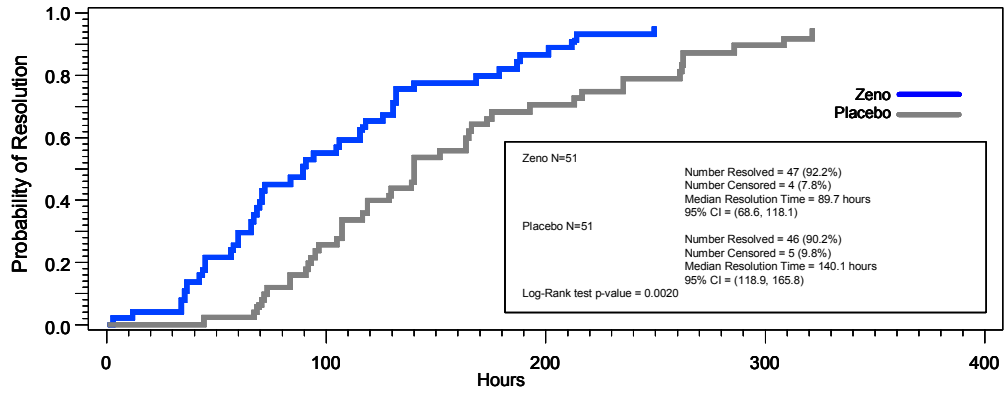
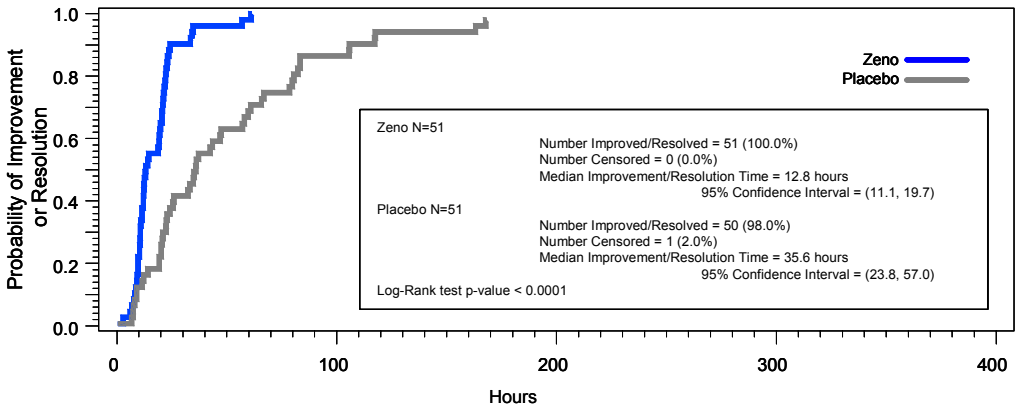


Figure 4. Hours to Improvement or Resolution as Reported in the Subject Diary



instances where blemishes were not fully resolved within 14 days, the data was censored.

The median resolution time for the blemishes treated with the Zeno device was 96.7 hours,

Table 2. Summary of Time to Resolution for Resolved Blemishes as Reported in the Subject Diary

Time	Zeno (N*=47) Mean ± SD	Placebo (N*=46) Mean ± SD	Paired Difference+ (N*=46) Mean ± SD
Days	3.9 ± 2.5	6.3 ± 3.0	2.3 ± 2.8
Hours	96.7 ± 58.5	151.8 ± 71.5	53.7 ± 65.6

* N corresponds with the Number of Blemishes Resolved

+ Paired difference was calculated as Zeno subtracted from Placebo, thus a positive difference indicates that the Zeno treated blemish resolved faster than the Placebo treated blemish.

compared to 151.8 hours for the blemishes treated with the placebo device (**Table 2**), which is a statistically significant difference (p-value = 0.0001, Log-Rank test). Blemishes treated with the Zeno device also showed improvement sooner than placebo treated blemishes. The median time to resolution or improvement for the blemishes treated with the Zeno device was 12.8 hours,

Table 3. Kaplan-Meier Time to Resolution as Reported in the Subject Diary

Treatment	Blemishes Resolved (%)	Blemishes Censored (%)	Median Resolution Time (Hours) (95% CI)	Log-Rank p-value
Zeno (N=51)	47 (92.2%)	4 (7.8%)	89.7 (68.6, 118.1)	< 0.0001
Placebo (N=51)	46 (90.2%)	5 (9.8%)	140.1 (118.9, 165.8)	

Table 4. Summary of Time to Improvement or Resolution for Improved Blemishes as Reported in the Subject Diary

Time	Zeno (N*=51) Mean ± SD	Placebo (N*=50) Mean ± SD	Difference* (N*=50) Mean ± SD
Days	0.5 ± 0.6	1.8 ± 1.7	1.3 ± 1.6
Hours	17.2 ± 11.2	48.0 ± 39.1	30.8 ± 36.5

* N corresponds with the Number of Blemishes Resolved

+ Difference was calculated as Zeno subtracted from Placebo, thus a positive difference indicates that the Zeno treated blemish resolved faster than the Placebo treated blemish.

compared to 35.6 hours (**Table 3 and 4**) for the blemishes treated with the placebo device, also a statistically significant difference (p-value < 0.0001, Log-Rank test). Also shown are composite bar graphs (light color showing lesion improvement and dark color showing lesion resolution) that are based on the blinded Investigator's evaluation in **Figure 5** and subjects' evaluations as reported in the daily diary in **Figure 6**. As shown in **Figure 5**, 29.4% of the Zeno treated blemishes resolved or improved with only

Figure 5. Percentage of Blemishes Resolved or Improved as Reported by the Investigator

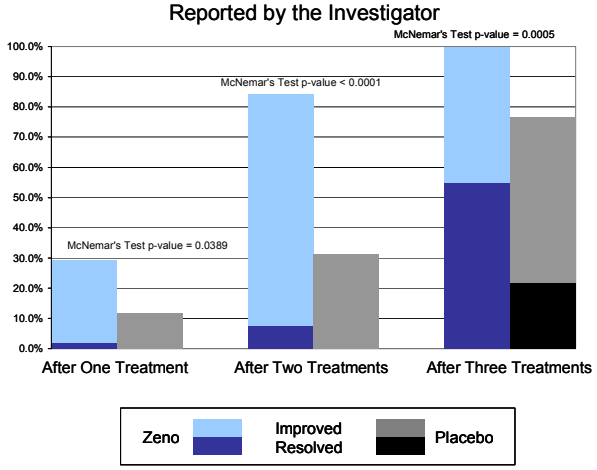
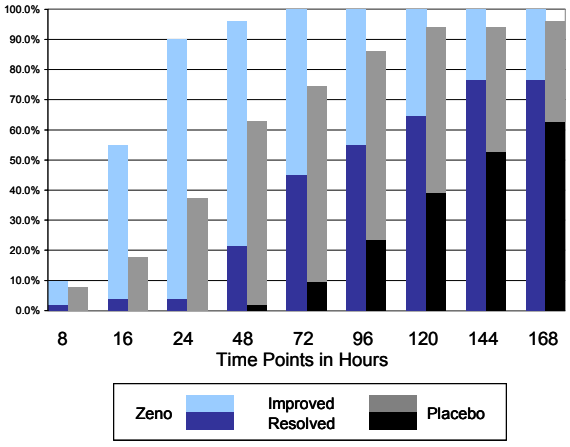


Figure 6. Percentage of Blemishes Resolved or Improved at Each Time Point As Reported in the Subject Diary



one (1) treatment versus 11.8% of the placebo treated blemishes. With only two (2) treatments, 84.3% of Zeno treated blemishes resolved or improved versus 31.4% of the placebo treated blemishes. At the Day 5 follow-up, 100.0% of the Zeno treated blemishes resolved or improved compared to only 76.5% of the placebo treated blemishes. The proportion of blemishes that resolved or improved was statistically significant at each of these time points (each p-value < 0.05, McNemar's test). A panel of photographs (**Figure 7 and Figure 8**) is also shown to demonstrate the

Figure 7. Patient Study Photographs

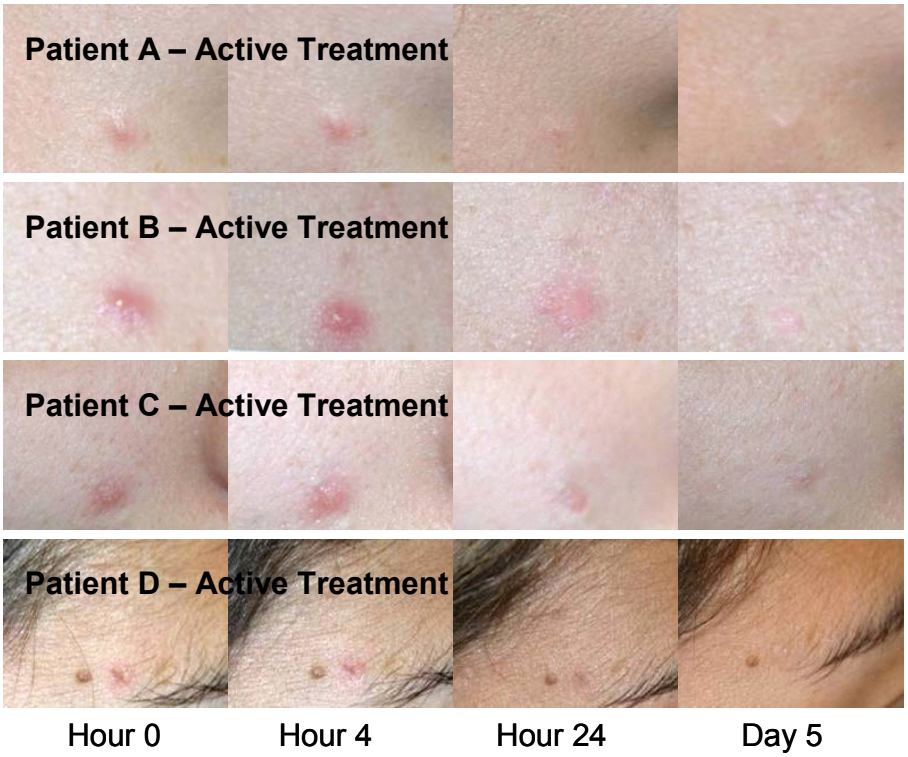
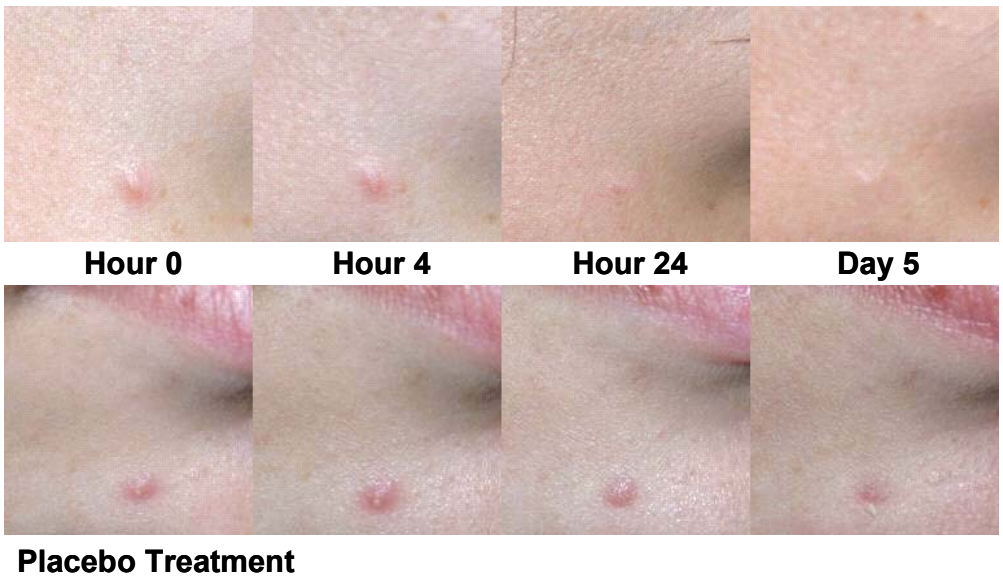


Figure 8. Patient A Comparison of Active and Placebo Treatments



effect on four (4) of the subjects during the treatments.

All of the analyses of the blinded Investigator blemish assessments and subject diary blemish assessments support that the Zeno treated blemishes improve and resolve faster than the clinically matched placebo treated blemishes.

Safety Results

Subjects did not experience any adverse events or serious adverse events for the duration of this study, monitored from the time of the first treatment with study device through Day 5. There were no device complications reported during this study. Both questions from the investigators as well as subjective evaluations provided by the individual diaries are in complete agreement with each other, specifically no adverse events were reported.

Discussion

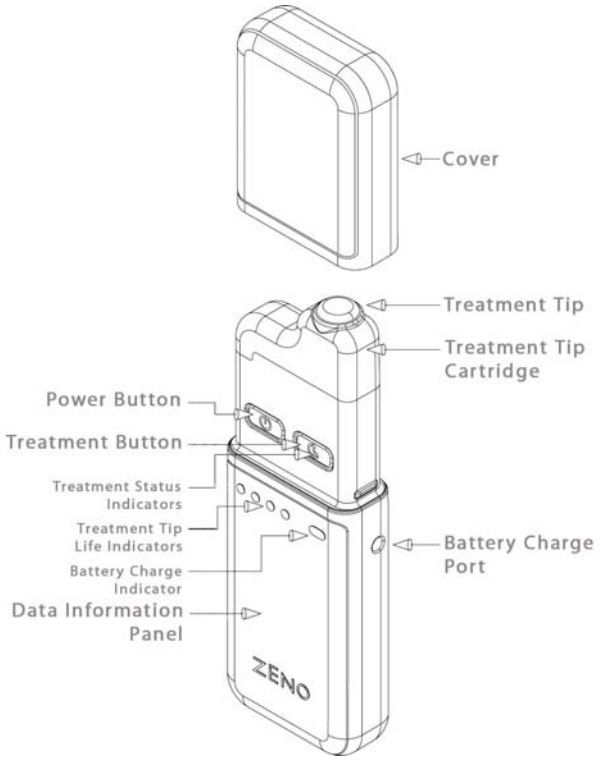
Provided herein is an alternative to currently available strategies to treat mild to moderate acne outbursts in patients with a simple handheld device that delivers a very controlled temperature at predefined times.

The Zeno device (shown in **Figure** 9) was evaluated in a double-blinded controlled clinical trial and demonstrated significant improvement in the resolution time of individual acne lesions from active versus placebo devices. Both objective and subjective measures of lesion improvement or resolution were significantly observed in both hours and days. Importantly, there were no adverse reactions to this treatment nor were there any complications with the device.

Although, *P. acnes* is quite sensitive to heat treatments, the exact mechanism that triggers this death response is not clearly defined. It is well known that various "heat-shock" proteins increase in cells after exposure to stress events as well as heat.[8-12] These "heat-shock" proteins are increased and the cells then follow a well ordered death cascade. It is unclear whether these responses are causative in the death occurring after exposure to heat or whether these are merely adaptive responses that were ineffective in preventing the bacterial cell death. It suffices that these molecular changes are associated with bacterial cell death after heat treatment is applied.

This treatment modality is simple, non-toxic and effective against individual acne lesions and represents an additional tool for patients who suffer from mild to moderate outbreaks of acne.

Figure 9. Zeno Device



Legends

Figure 1. This represents the reduction in colony counts of *P. acnes* after subjected to temperature at varying time points. The temperatures were given in Celsius from 55° up to 60° for various points of time at 30 second intervals. As can be seen there appears to be a time and temperature dependant threshold where the number of colonies can not be accurately counted at this particular dilutional scheme.

Figure 2. This represents the number of colony counts obtained at the three different temperatures of 43° C, 49° C and 54° C with multiple (4) X 2 ½ minute treatments being applied. The number of treatments is seen on the X-axis up to four 2 ½ minute treatments. As can be seen, the number of treatments at various temperature profiles significantly reduces the colony count and viability.

Figure 3. Represents a Kaplan-Meier curve showing the time to resolution as recorded in the subject diary for both the active treatment and placebo treatment. The number of patients resolved with the active treatment is 47 (92%) with the number of censored cases at 4 (7.8%) with a median time of resolution of 89.7 hours. For the placebo group, 46% report resolved (90.2%) with the number of censored cases being 5 (9.8%) with a median resolution time of 140.1 hours.

Figure 4. Represents the time to either improvement or resolution of the lesions as reported in the subject diaries as represented by Kaplan-Meier curves. The number of subjects that reported improved or resolved lesions with the active treatment was 51 (100%). The number of patients reporting improvement or resolution with the placebo was 50 (98%). Median time of resolution or improvement for the active treatment arm was 12.8 hours whereas the median time for improvement or resolution for the placebo group was 35.6 hours.

Figure 5. Represents the percentage of lesions either improved or resolved after either one treatment, two treatments or at 5 day follow-up evaluation. The light colors represent the portion of lesions that had improved and the dark colors represent the portion of lesions that had resolved for either the active treatment or the placebo treatment.

Figure 6. Represents the percentage of lesions either improved or resolved at each observation time point as reported in the subject diaries. Again, the lighter colors represent the portion of lesions that had improved and the dark colors represent the portion that had resolved for both the active treatment and placebo groups.

Figure 7. Photographs of actual patients (4) within the clinical trial demonstrating the improvement of lesions from base line at 4 hours, 24 hours and at day 5.

Table 1. Shows the demographic features of the patients involved in the study. The median age was 20.0 years. There was a female predominance of 58.8% compared to males at 41.2%. The table also shows a mix of ethnicities with a Caucasian predominance.

Table 2. Represents the median time for resolution between the active treatment, placebo treatment and the difference between the mean resolution time given in both days and hours. These results gave a significant p-value of less than 0.0001 in paired T tests.

Table 3. Represents the time to improvement or to resolution as reported in the subject diaries and given in hours. The median time in hours for either improvement or resolution was 12.8 hours for the active treatment group as compared to 35.6 hours for the placebo group.

Table 4. Represents the time to improvement or resolution for those blemishes that did improve in the subject diary for the active and placebo treatments and the difference as reported in days and hours. The median difference in hours between the two treatment groups was 1.3 days and 30.8 hours, respectively.

References

1. Krowchuk, D.P., Managing acne in adolescents. Pediatric Clinics of North America, 2000. 47(4): p. 841-57.
2. Jansen, T. and G. Plewig, Advances and perspectives in acne therapy. Eur J Med Res, 1997. 2(8): p. 321-34.
3. Hanna, S., J. Sharma, and J. Klotz, Acne vulgaris: more than skin deep. Dermatology Online Journal, 2003. 9(3): p. 8.
4. Aktan, S., E. Ozmen, and B. Sanli, Anxiety, depression, and nature of acne vulgaris in adolescents. International Journal of Dermatology, 2000. 39(5): p. 354-7.
5. Cunliffe, W.J., et al., Comedogenesis: some new aetiological, clinical and therapeutic strategies. Br J Dermatol, 2000. 142(6): p. 1084-91.
6. Shamblin, W.R., The diagnosis and treatment of acute infections of the hand. Southern Medical Journal, 1969. 62(2): p. 209-12.
7. Chen, Q., et al., Propionibacterium acnes-induced IL-8 production may be mediated by NF-kappaB activation in human monocytes. Journal of Dermatological Science, 2002. 29(2): p. 97-103.
8. Segal, G. and E.Z. Ron, Regulation of heat-shock response in bacteria. Annals of the New York Academy of Sciences, 1998. 851: p. 147-51.
9. Bugl, H., et al., RNA methylation under heat shock control. Molecular Cell, 2000. 6(2): p. 349-60.
10. Narberhaus, F., et al., A novel DNA element that controls bacterial heat shock gene expression. Molecular Microbiology, 1998. 28(2): p. 315-23.
11. Tomoyasu, T., et al., Levels of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in Escherichia coli. Molecular Microbiology, 1998. 30(3): p. 567-81.
12. Watanabe, Y.H., et al., Heat-inactivated proteins managed by DnaKJ-GrpE-ClpB chaperones are released as a chaperonin-recognizable non-native form. Journal of Biological Chemistry, 2000. 275(17): p. 12388-92.