Original Contribution

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SIG1273: a new cosmetic functional ingredient to reduce blemishes and *Propionibacterium acnes* in acne prone skin

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Summary

Background Propionibacterium acnes is a major contributing factor to the inflammatory component of acne. The interaction of *P. acnes* with keratinocytes leads to an innate immune response via activation of toll-like receptors (TLR2, TLR4) resulting in the production and secretion of pro-inflammatory mediators. SIG1273, an isoprenylcysteine small molecule modulates inflammatory signaling pathways and kills *P. acnes*. SIG1273 represents a novel cosmetic functional ingredient that provides relief from blemishes in acne prone skin.

Objective To assess the keratinocyte response and microbial growth of SIG1273 *in vitro* and evaluate the tolerability of SIG1273 gel applied topically in acne prone subjects.

Methods For *in vitro* studies, human keratinocytes were exposed in culture to live *P. acnes* and peptidoglycan (PGN) to induce IL-8 production. *P. acnes* were cultured to determine minimal inhibitory concentration and minimal bactericidal concentration values. A total of 30 subjects were randomized in a double-blind controlled trial receiving 3% SIG1273 gel or vehicle for 6 weeks. Evaluation included inflammatory lesions, noninflammatory lesions, microcomedones, Sebutape scores, and *P. acnes* counts.

Results In vitro studies demonstrate SIG1273 inhibits *P. acnes*-induced IL-8 production and inhibits *P. acnes* growth. SIG1273 gel was well tolerated with no signs of stinging, redness, or itching. Furthermore, improvement in some aspects of acne was observed in subjects applying SIG1273 gel, including inflammatory lesions, microcomedone counts and Sebutape scores. Facial scrubs taken to measure *P. acnes* colony-forming units showed those applying SIG1273 gel had ~1.0 Log 10 colony reduction over the length of the study, a statistically significantly improvement when compared with vehicle. No significant effects above vehicle were observed for noninflammatory lesions.

Conclusions SIG1273 represents a novel cosmetic functional ingredient that provides a safe dual modulating benefit to individuals with acne prone skin by reducing *P. acnes* counts and reducing inflammation.

Keywords: *Propionibacterium acnes*, acne, sebum flow, *P. acnes* control, isoprenylcysteine analog, inflammatory acne

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Introduction

Acne is a prevalent condition affecting millions of individuals worldwide. *Propionibacterium acnes*, a major component of the normal cutaneous microflora, plays a critical role in the development of inflammatory acne when it overgrows and colonizes the pilosebaceous unit.[1,2] The inflammation is driven in part by bacterial products directly activating host tissue resident inflammatory cells,[3] but recent evidence indicates a major role for the direct interaction of *P. acnes* with follicular cells, leading to production of inflammatory mediators.[4–8] These cells are activated upon recognizing pathogen-associated molecular patterns of the bacterial cell wall via toll-like receptors (TLRs) initiating an innate immune response.[9] For instance, TLR4 mediates host responses to bacterial lipopolysaccharide from Gram-negative bacteria, and TLR2 mediates responses to PGN from Gram-positive bacteria.[10] Recent work indicates that *P. acnes*, a Gram-positive anaerobe, induces an increase in IL-8 and other pro-inflammatory cytokines in keratinocytes and monocytes through activation of TLR2 and TLR4.[5–7]

Current treatments for acne prone skin include topical and oral retinoids, topical and oral antibiotics, and topical antimicrobials, all of which have potential side effects. In addition, long-term exposure to antibiotics results in antibiotic-resistant bacteria and the oxidizing agent benzyl peroxide for which resistance does not occur but can result in skin bleaching, dryness, and stinging. Thus, the development of safe, new compounds that provide relief to individuals with skin prone to acne is desired.

Isoprenylcysteine (IPC) analogs possessing growth control of *P. acnes* represent a novel topical approach to improve facial irritation. IPC analogs contain a 15- or 20-carbon side chain linked to the amino acid cysteine, thereby mimicking the C-terminus of processed CAAX proteins.[11] *In vitro* studies have shown IPC compounds to be effective modulators of inflammatory responses in neutrophils, macrophages, and platelets.[12–14] Furthermore, IPC analogs have been shown to inhibit LPS-TLR4-induced IL-8 production in human dermal endothelial cells (unpublished results). We reported earlier that certain IPC analogs when topically applied demonstrate activity *in vivo* inhibiting TPA-induced edema and myeloperoxidase activity using a TPA-induced dermatitis model.[15,16] In this study, we report IPC analog SIG1273 possesses *in vitro* and *in vivo* properties that should result in a benefit to individuals with irritated facial appearance.

Materials and methods

Reagents

All reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA). Organic solvents were purchased from Fisher Scientific (Hampton, NH, USA). SIG1273 was synthesized according to methods as described in US patent application US 12/616 781. All chemicals were analyzed by LC/MS (Agilent 1100, Santa Clara, CA, USA), ^{1}H and ^{13}C NMR (500 and 125 MHz; Bruker, Billerica, MA, USA) for structural identity and confirmed to be >95% purity by analytical HPLC (Agilent 1200).

Culture of *Propionibacterium acnes*

Propionibacterium acnes (ATCC, ATCC 6919; Manassas, VA, USA) were cultured as previously described[17] under anaerobic conditions at 37 °C.

Antimicrobial assays

To determine minimal inhibitory concentration (MIC), reagents were dissolved in DMSO (5% v/v) and then added to bacteria suspension. *P. acnes* (10^6 CFU/mL) was incubated with SIG1273 or doxycycline at the concentrations of twofold serial dilution (0.25–500 μg/mL) in reinforced clostridium medium under anaerobic conditions for 72 h. To determine minimal bactericidal concentration (MBC) against *P. acnes*, bacteria (10^7 CFU/mL) was incubated with reagents at various concentrations (12.5–100 μg/mL in PBS) under anaerobic conditions. Cultures were diluted 1:10–1:10^6 with PBS, and MBC was determined by spotting the dilution (5 mL) on a Brucella broth agar plate to count colony-forming units (CFUs).

Cell treatments

Normal primary adult human keratinocytes (NHEKs) were obtained from pooled donors and purchased from Cascade Biologics (Gibco, Carlsbad, CA, USA) or ScienCell Research Laboratories (Carlsbad, CA, USA). Cell treatments were performed as previously described[18] with live bacteria cultures for 24 h. Cells were pre-incubated with SIG1273 (0.1–100 μM; 1% v/v ethanol vehicle) in fresh media in triplicates. Media supernatants were harvested for cytokine measurements, and cells were subjected to viability tests by MTS assay (Promega, Madison, WI, USA).

Cytokine ELISA

The levels of human IL-8 were measured from NHEK media supernatants by sandwich ELISA using appropriate standards and following the manufacture's protocols (BD Biosciences, San Jose, CA, USA).

Clinical study

This study was conducted at Springhouse Skin Research, Inc. (Philadelphia, PA, USA) in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the U.S. Code of Federal Regulations (CFR), the Declaration of Helsinki, and/or Springhouse Skin Research Standard Operating Procedures. This was a double-blind vehicle-controlled randomized study, with 31 subjects enrolled for 6 weeks. All subjects gave informed consent. Inclusion and exclusion criteria are listed in Table 3. Sixteen subjects received gel containing 3% SIG1273 gel for application to the face and 14 subjects, vehicle control gel. There were four study visits at enrollment (baseline), then at Week 1, Week 3, and Week 6. The test formulations were applied *bid*, morning and evening and subjects were told to use no other skin-care products on their faces during study participation, except for water washable eye makeup and lipstick. The subjects washed their faces with Cetaphil® (Galderma, Forth Worth, TX, USA) upon arrival at the study site, to ensure photographs and other assessments were made on clean skin. Subjects received a written explanation of the nature of the study and the possibility of being in the vehicle control group.

Statistical analysis

Statistical significance was determined by ANOVA followed by a Dunnett multiple comparisons test using *P*-values less than 0.05 as a significant difference. For all antibacterial measurements and cytokine levels, samples were assayed in triplicate. Cytokine dose–response curves were generated by fitting data with the Hill, three-parameter equation using the Sigma Plot software, Systat Software, Inc. (Chicago, IL, USA) from which the IC_{50} and maximum inhibition were determined.

Results and discussion

SIG1273 inhibits *Propionibacterium acnes* and PGN/TLR2/4-induced IL-8 release from human keratinocytes

The inflammatory phase of acne is exacerbated by host innate immune response to *P. acnes*. The bacterium stimulates the production of pro-inflammatory cytokines via TLR2 and TLR4.[4,7,8] Our results show SIG1273 strongly inhibits *P. acnes*-TLR2/4-induced interleukin-8 (IL-8) release from NHEKs (Table 1). Furthermore, using TLR2 ligand and bacterial cell wall product PGN, results demonstrate SIG1273 reduces PGN-induced IL-8 production (IC_{50} = 0.3 μM) (Table 1).

Antimicrobial effects of SIG1273 against *Propionibacterium acnes in vitro*

To investigate the antibacterial properties of SIG1273, co-cultures with *P. acnes* for 72 h were prepared. Both SIG1273 and reference compound doxycycline were tested at concentrations of twofold serial dilutions from 1 to 500 μg/mL and were determined to each have a MIC of 2 μg/mL (Table 2). To determine the minimal bactericidal concentration (MBC), *P. acnes* was incubated with several concentrations of SIG1273 and doxycycline for 5 h and then spotted on agar plates to count for colony-forming units (CFU). Results showed SIG1273 at 5 μg mL and higher to kill *P. acnes*. Interestingly, doxycycline under the same conditions did not exhibit killing until 25–50 μg/mL (Table 2).

Table 1 *Propionibacterium acnes*-PGN-NHEK treatments*

Treatment	*P. acnes*-IL-8 IC_{50} (μM)	PGN-IL-8 IC_{50} (μM)
SIG1273	3	0.3

NHEK, normal primary adult human keratinocytes; PGN, peptidoglycan.
*NHEKs were pretreated with compound for 30 min and then applied with live *P. acnes* (1×10^7 CFU/mL) for 24 h or PGN (10 μg/mL) for 72 h. IL-8 production was measured by ELISA. No cell cytotoxicity was observed at concentrations tested.

Table 2 MIC and MBC values against *Propionibacterium acnes*

Compound	MIC* (μg/mL)	MBC† (μg/mL)
Doxycycline	2	~25–50
SIG1273	2	~1–5

MBC, minimal bactericidal concentration; MIC, minimal inhibitory concentration.
**P. acnes* (1×10^6 CFU/mL) were incubated with compound in 5% DMSO under anaerobic conditions at 37 °C for 72 h. After incubation, OD_{600} of each sample was measured to determine bacterial growth. MIC values were determined from dose-dependent curves using Hill parameter equation (Sigma Plot software).
†MBC range is defined as the minimal concentration of compound that causes >99.9% decrease in colony-forming units (CFUs) of *P. acnes* as compared to the control after 5 h of incubation.

Safety and tolerability in human subjects

In view of SIG1273 in vitro profile, we sought to determine its safety and tolerability in human subjects. SIG1273 at 3% was first tested clinically in a Human Repeated Insult Patch Test and was found to cause no skin sensitization or irritation (Product Investigations, data not shown). Given this result, 3% SIG1273 in a gel formulation was tested in a randomized double-blind vehicle-controlled study, to determine the safety and tolerability in subjects with prone acne skin. During this 6-week study, the signs and symptoms of acne on the face were clinically assessed and lesion counts, the density of microcomedones, numbers of *P. acnes* and casual sebum levels were measured.

Thirty of 31 enrolled subjects (Table 3) completed the 6 weeks of the study. One subject was dropped for noncompliance before the Week 1 assessment visit. Sixteen subjects received 3% SIG1273 gel and 14 subjects received vehicle gel only (Table 3). Investigator clinically assessed signs and symptoms of irritation on the face using a 0–3 ordinal severity scale (0-none, 1-slight, 2-moderate, and 3-severe) for erythema (Fig. 1), dryness, burning/stinging, and peeling. No signs of irritation on the face could be attributed to application of SIG1273 gel or vehicle (dryness, burning/stinging, and peeling were all scored 0 at baseline and remained as such through Week 6 (data not shown)). Importantly, there was no increase in the signs and symptoms of acne. In fact, subjects using the SIG1273 gel demonstrated marked visual improvement in the signs and symptoms of acne from baseline to Week 6 (Fig. 2).

Table 3 Demographic and baseline data*

Characteristic	SIG1273 gel (n = 16)	Vehicle (n = 14)
Age (±SD)	20.8 (7.1)	18.7 (3.2)
Sex (Male/Female)	7/9	10/4
Mean baseline inflammatory lesion counts (±SD)	8.8 (4.2)	10.7 (5.4)
Mean baseline noninflammatory lesion counts (±SD)	19.9 (10.8)	21.4 (12.6)
Mean baseline total lesions (±SD)	28.7 (13.6)	32.1 (16.2)
Mean baseline Log_{10} *Propionibacterium acnes* counts (±SD)	5.6 (0.7)	5.0 (0.8)
Mean baseline microcomedone counts		
Cheek (±SD)	37.7 (19.8)	31.4 (16.6)
Forehead (±SD)	17.2 (16.8)	12.2 (9.6)
Mean baseline Sebutape score (±SD)	3.1 (1.3)	3.3 (1.1)

*Inclusion criteria were subjects in good health, with mild-to-moderate acne and 16 years or older. The following exclusion criteria were used in this study: (i) moderate-to-severe acne, and/or with excessive acne scarring; (ii) history of skin reactions to topical medications, cosmetics, or soaps; (iii) currently or within 6 months prior to Study entry have been under treatment with systemic retinoids, or 30 days prior to entry with systemic antibiotics, antihistamines, or systemic steroids; (iv) currently or within 2 weeks have been under treatment with topical retinoids, steroids, keratolytics (e.g., salicylic acid), antimicrobials (e.g., benzoyl peroxide), or other acne products; (v) received an experimental drug and/or used an experimental device within 30 days prior to admission to this study; (vi) pregnant or nursing a child; (vii) subjects planning on taking vacation where they will have high amounts of sun exposure.

In vivo antimicrobial assessment

Facial scrub samples were taken from subject foreheads, and aliquots from serial dilutions were inoculated onto growth media as previously described.[19] Baseline measurements showed 11 of 16 subjects on SIG1273 gel and 8 of 14 subjects on vehicle had at least 10^4 colony-forming units (CFUs) per cm^2, the cut-off for inclusion in measuring for *P. acnes* counts reduction.[20,21] Results demonstrate the group using SIG1273 gel to have a statistically significant decrease (P = 0.01) of almost 1.0 logarithmic colony reduction (-0.9 Log_{10}) from baseline to Week 6, as compared to the vehicle group, which showed no effect on *P. acnes* colony formation (Table 4). As reported by Cunliffe *et al.*, treatment in acne subjects with 1% clindamycin for 16 weeks resulted in a similar -0.9 Log_{10} reduction. Converting CFU counts to percentage of

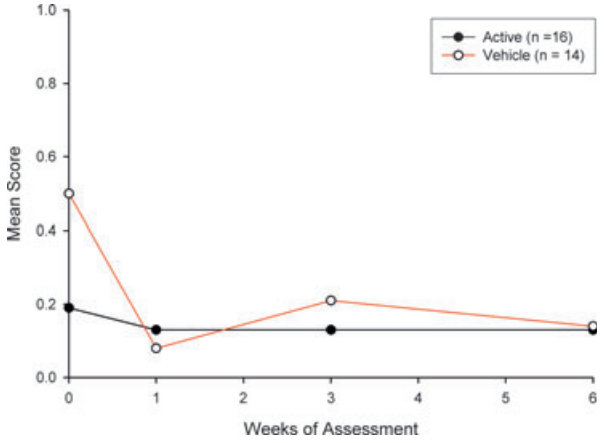

Figure 1 Erythema score. Investigator clinically assessed signs and symptoms of irritation on the face using a 0–3 ordinal severity scale (0 = none, 1 = slight, 2 = moderate, and 3 = severe) for erythema. SIG1273 gel and vehicle are represented by black and red lines, respectively. Assessments were measured at baseline, weeks 1, 3, and 6.

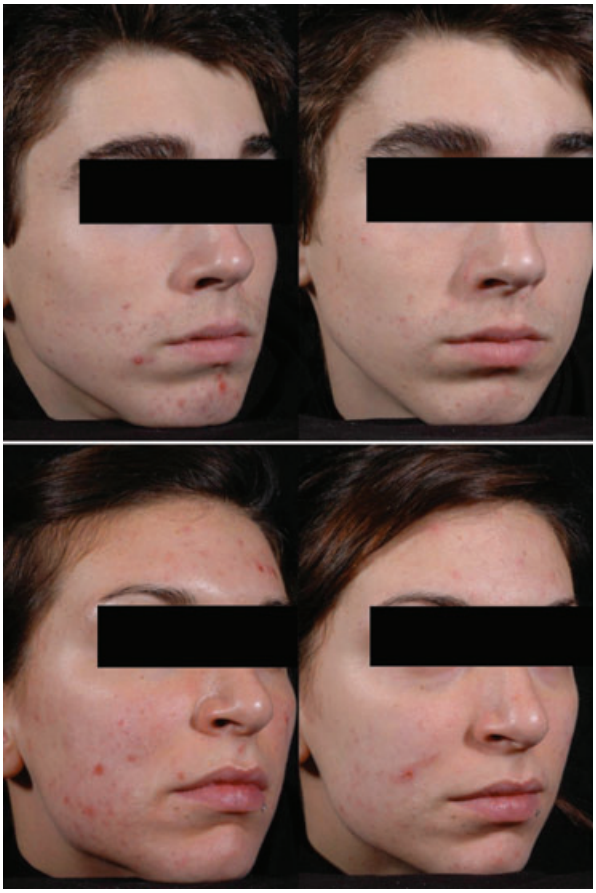

Figure 2 SIG1273 gel subjects: before and after. Subjects applied SIG1273 gel twice daily. Photograph on left represents subjects at baseline (week 0) and at the end of the study (week 6).

Table 4 Log_{10} total *Propionibacterium acnes* counts (CFU/cm^2)*

Assessment time	SIG1273 gel (n = 11)	Vehicle (n = 8)	P value†
Baseline	5.6 ± 0.2	5.0 ± 0.3	
1	5.2 ± 0.2 (−0.4)‡	5.1 ± 0.3 (+0.1)‡	0.009
3	4.8 ± 0.2 (−0.8)‡	4.5 ± 0.3 (−0.5)‡	0.360
6	4.7 ± 0.2 (−0.9)‡	5.0 ± 0.2 (0.0)‡	0.01

*Values are given as mean ± SE.
†P values are between group differences from Log_{10} reduction values from baseline.
‡Values in parenthesis are Log_{10} total counts relative to baseline.

baseline total *P. acnes* counts, results demonstrate the SIG1273 gel group inhibits *P. acnes* counts by ~85% over 6 weeks, while the vehicle group exhibits a modest reduction at 3 weeks, but returns to baseline levels (0%) at Week 6.

Table 5 Effect on inflammatory and noninflammatory lesions*

Assessment time	SIG1273 gel† (n = 16)	Vehicle† (n = 14)
Inflammatory lesion count baseline	8.8 ± 1.0	10.7 ± 1.5
Noninflammatory lesion count baseline	19.9 ± 2.7	21.4 ± 3.4
Inflammatory lesion count Week 1	8.3 ± 1.0	12.5 ± 2.1
Noninflammatory lesion count Week 1	18.0 ± 2.3	19.4 ± 3.3
Inflammatory lesion count Week 3	5.6 ± 0.8	6.3 ± 0.8
Noninflammatory lesion count Week 3	18.0 ± 1.8	17.6 ± 2.5
Inflammatory lesion count Week 6	5.5 ± 1.0	6.6 ± 0.8
Noninflammatory lesion count Week 6	17.4 ± 1.8	17.1 ± 1.9

*Inflammatory lesions include papules and pustules; noninflammatory lesions include open and closed comedones.
†Values are given as mean ± SE.

Inflammatory, noninflammatory lesion counts and microcomedone formation

Inflammatory lesions (pustules and papules) and noninflammatory lesions (open and closed comedones) on the face were counted from hairline to chin, using a 2× illuminated magnifier. Results demonstrate that SIG1273 gel and vehicle groups had a statistically significant reduction in inflammatory lesions at Week 3 and Week 6 when compared with baseline (Table 5). For example, at Week 6, the active group exhibited a 36% reduction in inflammatory lesions. No significant effects were observed for noninflammatory lesions (Table 5).

The density (number per cm^2) of microcomedones on the cheek and forehead was determined by microscopic examination of skin surface biopsies taken from these facial sites, as described in Table 6. SIG1273 gel and vehicle groups both showed a reduction in microcomedone formation in the cheek and forehead (Table 6). However, only the SIG1273 gel group demonstrated a statistically significant reduction from baseline to Week 6 measurements taken from the cheek (45% reduction) (Table 6). Moreover, despite a decrease in microcomedone formation from forehead biopsies, no significant reduction for either SIG1273 gel or vehicle groups was observed (Table 6).

Sebum production measurements (Sebutape)

Casual sebum levels on the forehead were assessed by the application of Sebutape strips between the eyebrows. The Sebum Level scores (1–5) were based upon the photographic standards provided by the CuDerm Corporation (Dallas, TX, USA). The vehicle group exhibited a + 0.22 in Sebutape scoring from baseline to Week 6, Indicating a slight increase in sebum production, while

Table 6 Effect on microcomedones using cyanoacrylate follicular biopsy technique (per cm^2)*

Assessment time	SIG1273 gel† (n = 16)	Vehicle† (n = 14)
Baseline count right cheek	38.0 ± 7.2	31.4 ± 5.9
Baseline count left forehead	17.4 ± 6.1	12.1 ± 3.4
Week 3 count left cheek	34.3 ± 7.2	32.6 ± 7.4
Week 3 count right forehead	17.2 ± 6.0	14.3 ± 2.6
Week 6 count right cheek	21.1 ± 1.8*	22.7 ± 4.4
Week 6 count left forehead	10.8 ± 2.0	8.1 ± 1.9

*Statistically significant reduction (P = 0.01) within SIG1273 gel group from baseline to Week 6. Subjects briefly wash their faces with Cetaphil and water, and after drying, put on a pair of safety goggles. A drop (about 0.05 mL) of cyanoacrylate glue (Krazy Glue®) is applied to one end of a plastic slide (1 × 3-inch Rinzl Plastic Slides), and spread out to a uniform thickness, using the nozzle of the Krazy Glue® bottle. The slide is then pressed against the medial cheek (or other skin site), causing the glue to spread to a thin film, the width of the slide (1 inch) and approximately half its length (1.5 inch). The slide is left in place for 5 min, while the cyanoacrylate hardens as it polymerizes. Finally, the slide is gently peeled from the skin surface and retained for examination under a dissecting microscope, to determine the density of microcomedones.
†Values are given as mean ± SE.

Table 7 Sebutape scoring*

Assessment time	Active† (n = 16)	Vehicle† (n = 14)
Baseline	3.12 ± 0.31‡	3.28 ± 0.28
Week 1	2.87 ± 0.31	3.35 ± 0.27
Week 3	2.81 ± 0.34‡	3.42 ± 0.27
Week 6	3.0 ± 0.30	3.5 ± 0.27

*Sebutape after removed from forehead is placed on the black background of a scorecard. Sebum on the tape becomes clearly visible as black spots. These spots are scored by on a scale of 1–5, where 1 indicates dry skin and no oil and 5 represents very oily skin.
†Values are given as mean ± SE.
‡Significant reduction from baseline to Week 3 for the active group (P = 0.03).

the SIG1273 gel showed a slight reduction (−0.18) (Table 7). Furthermore, a statistically significant reduction (P = 0.03) from baseline to Week 3 was observed within the SIG1273 gel group (Table 7). All together these results suggest SIG1273 may decrease sebum levels, a key contributing factor of acne.

Summary

Results show in cultured keratinocytes that SIG1273 inhibits *P. acnes*- and PGN-induced IL-8 secretion. Anti-microbial studies demonstrate SIG1273 to possess antibacterial properties against *P. acnes*. In a randomized, double-blind, vehicle-controlled 6-week study in 30 subjects with mild-to-moderate facial acne SIG1273 gel was well tolerated with no signs of burning, stinging, redness, or itching. Facial scrub samples taken from foreheads demonstrate subjects applying SIG1273 gel had almost a 1.0 logarithmic *P. acnes* colony reduction (-0.9 Log_{10}) measured from baseline to Week 6, as compared to subjects using the vehicle only which showed no statistical reduction over the same time period. The instrumentally measured reductions in microcomedone counts, Sebutape scores, and *P. acnes* counts suggest SIG1273 suppresses the bacteria that can cause spots and blemishes and in addition can control excess oil to produce a cleansing and soothing effect on skin.

Conflict of interest

JSG is a paid consultant for Signum Dermalogix/ Signum Biosciences, while JSF, KR, MV, XF, MS, BS, and EP are employees. JBS serves on the board of directors. All authors have stock and/or stock options in the company, except for MSC who was the lead PI on the clinical study conducted by Springhouse Skin Research, Inc.

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References

1 Leeming JP, Holland KT, Cuncliffe WJ. The microbial colonization of inflamed acne vulgaris lesions. *Br J Dermatol* 1988; **118**: 203–8.
2 Bojar RA, Holland KT. Acne and *Propionibacterium acnes*. *Clin Dermatol* 2004; **22**: 375–9.
3 Trinchieri G, Sher A. Cooperation of toll-like receptor signals in innate immune defence. *Nat Rev Immunol* 2007; **7**: 179–90.
4 Kim J. Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. *Dermatology* 2005; **211**: 193–8.
5 Kim J, Ochoa MT, Krutzik SR *et al.* Activation of toll-like receptor 2 in acne triggers inflammatory cytokine responses. *J Immunol* 2002; **169**: 1535–41.
6 Lee YJ, Choi HJ, Kang TW *et al.* CBT-SL5, a bacteriocin from *Enterococcus faecalis*, suppresses the expression of interleukin-8 induced by *Propionibacterium acnes* in

cultured human keratinocytes. *J Microbiol Biotechnol* 2008; **18**: 1308–16.
7 Jugeau S, Tenaud I, Knol AC *et al.* Induction of toll-like receptors by *Propionibacterium acnes*. *Br J Dermatol* 2005; **153**: 1105–13.
8 Nagy I, Pivarcsi A, Kis K *et al. Propionibacterium acnes* and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. *Microbes Infect* 2006; **8**: 2195–205.
9 Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila toll protein signals activation of adaptive immunity. *Nature* 1997; **388**: 394–7.
10 Kamisango K, Saiki I, Tanio Y *et al.* Structures and biological activities of peptidoglycans of Listeria monocytogenes and *Propionibacterium acnes*. *J Biochem* 1982; **92**: 23–33.
11 Winter-Vann AM, Casey PJ. Post-prenylation-processing enzymes as new targets in oncogenesis. *Nat Rev Cancer* 2005; **5**: 405–12.
12 Volker C, Miller RA, McCleary WR *et al.* Effects of farnesylcysteine analogs on protein carboxyl methylation and signal transduction. *J Biol Chem* 1991; **266**: 21515–22.
13 Huzoor-Akbar, Wang W, Kornhauser R *et al.* Protein prenylcysteine analog inhibits agonist-receptor-mediated signal transduction in human platelets. *Proc Natl Acad Sci USA* 1993; **90**: 868–72.
14 Philips MR, Pillinger MH, Staud R *et al.* Carboxyl methylation of Ras-related proteins during signal transduction in neutrophils. *Science* 1993; **259**: 977–80.
15 Gordon JS, Wolanin PM, Gonzalez AV *et al.* Topical *N*-acetyl-*S*-farnesyl-L-cysteine inhibits mouse skin inflammation, and unlike dexamethasone, its effects are restricted to the application site. *J Invest Dermatol* 2008; **128**: 643–54.
16 Fernandez JR, Feng X, Rouzard K *et al.* Isoprenylcysteine (IPC) analogs suppresses 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cytokine production in primary human keratinocytes and mouse skin in vivo. *J Invest Dermatol* 2010; **130**: S20.
17 Nakatsuji T, Kao MC, Fang JY *et al.* Antimicrobial property of lauric acid against *Propionibacterium acnes*: its therapeutic potential for inflammatory acne vulgaris. *J Invest Dermatol* 2009; **129**: 2480–8.
18 Jarrousse V, Castex-Rizzi N, Khammari A *et al.* Zinc salts inhibit in vitro Toll-like receptor 2 surface expression by keratinocytes. *Eur J Dermatol* 2007; **17**: 492–6.
19 McGinley KJ, Webster GF, Ruggieri MR *et al.* Regional variations in density of cutaneous propionibacteria: correlation of *Propionibacterium acnes* populations with sebaceous secretion. *J Clin Microbiol* 1980; **12**: 672–5.
20 Leyden J, Kaidbey K, Levy SF. The combination formulation of clindamycin 1% plus benzoyl peroxide 5% versus 3 different formulations of topical clindamycin alone in the reduction of *Propionibacterium acnes*. An in vivo comparative study. *Am J Clin Dermatol* 2001; **2**: 263–6.
21 Cunliffe WJ, Holland KT, Bojar R *et al.* A randomized, double-blind comparison of a clindamycin phosphate/benzoyl peroxide gel formulation and a matching clindamycin gel with respect to microbiologic activity and clinical efficacy in the topical treatment of acne vulgaris. *Clin Ther* 2002; **24**: 1117–33.