# Antibacterial Activity of Clindamycin/BPO in Combination With Adapalene

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# SYNOPSIS AND OBJECTIVE

- Acne treatment guidelines recommend the addition of the antimicrobial benzoyl peroxide (BPO) when long-term topical antibiotic use is necessary, to reduce the risk of antibiotic resistance in Cutibacterium acnes (C. acnes)<sup>1</sup>
- Pairing the antibiotic/BPO combination with a retinoid, such as adapalene, may further increase treatment efficacy<sup>2,3</sup>
- Adapalene targets acne pathogenesis by modulating cellular proliferation, differentiation, and keratinization<sup>4,5</sup>
- However, as research on adapalene's antibacterial activity is limited,<sup>6</sup> it is not known if adapalene can improve the antimicrobial activity of antibiotics and BPO
- To determine if adapalene improves antimicrobial activity, this in vitro study evaluated the susceptibility of *C. acnes* isolates to clindamycin, adapalene, and BPO alone or in combination (Figure 1)

# METHODS AND RESULTS

# Part 1a: C. acnes Susceptibility to Clindamycin, Adapalene, and BPO

The susceptibility of 6 acne-associated C. acnes strains was assessed via minimum inhibitory concentration (MIC) values obtained from the broth microdilution method (Figure 2, left)

# FIGURE 1. Study Overview

Clindamycin demonstrated low MIC values against some strains and high values against others; BPO and adapalene demonstrated high MIC values (**Figure 2**, right)

### Part 1b: Effect of Adapalene + Clindamycin or BPO on C. acnes Inhibition

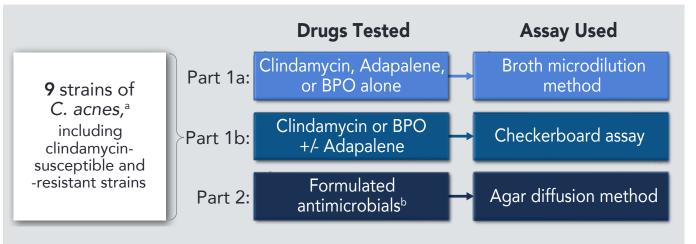
- The effect of combining adapalene with clindamycin or BPO on C. acnes inhibition was evaluated using a checkerboard assay, wherein 2 test compounds are combined in varying concentrations (Figure 3, left)
- The combination of adapalene and clindamycin had an additive effect for 3 out of 4 acne-associated strains tested and no interaction for 1 strain (**Figure 3**, *right*)
- This additive effect was maintained when the experiment was repeated in the presence of sebum (data not shown)

# Part 2: C. acnes Susceptibility to Antibiotic Formulations

- *C. acnes* susceptibility to single or combination formulations was determined by measuring the antibacterial zone of inhibition using agar diffusion method (**Figure 4**, *left*)
- Activity of single formulations varied against the 8 acne-associated C. acnes strains tested, whereas fixed-dose combination formulations had generally similar activity against the strains (Figure 4, right)

### **Study Limitations**

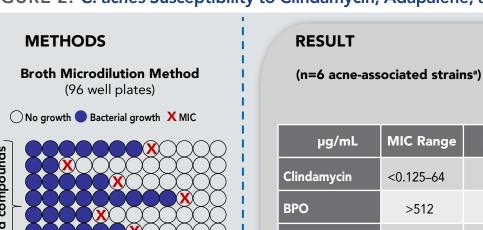
Limitations of this in vitro study are detailed in Figure 5



<sup>a</sup>lncluding 8 acne-associated strains and 1 neutral strain that is sometimes classified as acne-associated. Classification based on Fitz-Gibbon S, et al.

<sup>b</sup>CLIN 1.2%/adapalene 0.15%/BPO 3.1% gel (Ortho Dermatologics), Clindamycin 1% gel (Ortho Dermatologics), CLIN 1.2%/BPO 3.75% gel (Ortho Dermatologics), BPO 5%/erythromycin 3% gel (Ortho Dermatologics), BPO 3% gel (Ichthyol-Gesellschaft), adapalene 0.1% gel (Galderma laboratories)

BPO, benzoyl peroxide; CLIN, clindamycin phosphate.



### Compound concentration gradient

 $MIC_{v}$  = concentration needed to inhibit X% of strains tested I MIC, = **†** *C. acnes* susceptibility

# Clindamycin demonstrated strain-dependent activity, whereas BPO and adapalene<sup>b</sup> had no/low activity against C. acnes strains in vitro

alncluding 1 neutral strain that is sometimes classified as acne-associated. Classification based on Fitz-Gibbon S, et al.<sup>7</sup> <sup>b</sup>Assessment of MIC for adapalene was likely limited by challenges with dissolving adapalene in the testing medium. BPO, benzoyl peroxide; MIC, minimum inhibitory concentration.

# FIGURE 3. Effect of Adapalene + Clindamycin or BPO on C. acnes Inhibition (Part 1b)

METHODS	RES	ULT			
<b>Checkerboard analysis</b> (96 well plates)	(n=4	acne-associated	d strainsª)		
	C. acnes strain	MIC Combo ADAP / Individual ADAP	MIC Combo CLIN / Individual CLIN	FICI	
	HL053PA2	0.063 / 256	0.063 / 0.063	1.00	7
	HL086PA1	0.063 / 256	0.063 / 0.063	1.00	CLIN + ADAP
	HL007PA1	0.063 / 256	0.063 / 0.063	1.00	
	HL045PA1	64 / 256	64 / 64	1.25	No interaction
Clindamycin concentration	C. acnes strain	MIC Combo ADAP / Individual ADAP	MIC Combo BPO / Individual BPO	FICI	
Fractional inhibitory concentration index	HL053PA2	256 / 256	512 / 512	2.00	
calculation: used to assign numerical	HL086PA1	256 / 256	512 / 512	2.00	BPO + ADAP
value to the interaction of two compounds	HL007PA1	256 / 256	512 / 512	2.00	No interaction
MIC drug A in combination MIC drug B in combination	HL045PA1	256 / 256	512 / 512	2.00	
+					
MIC drug A aloneMIC drug B alone• Synergism: $\leq 0.5$ • Additive action: $>0.5$ to $\leq 1.0$ • Indifferent: $>1$ to $\leq 2$ • Antagonistic: $>2.0$	resu whe	ombination of a ulted in an addi ereas adapalen ny interaction a	tive effect aga e with BPO dic	inst 3 I not re	strains, esult in

<sup>a</sup>Classification based on Fitz-Gibbon S, et al.<sup>7</sup>

ADAP, adapalene; BPO, benzoyl peroxide; CLIN, clindamycin phosphate; FICI, fractional inhibitory concentration index; MIC. minimum inhibitory concentration.

# FIGURE 2. C. acnes Susceptibility to Clindamycin, Adapalene, and BPO (Part 1a)

Range	MIC <sub>50</sub>	MIC <sub>90</sub>
5–64	64	64
512	>512	>512
64	>64	>64

# FIGURE 4. C. acnes Susceptibility to Antibiotic Formulations (Part 2)

# **METHODS Agar Diffusion Method** C. acnes Brucella blood agar culture Zone of Antimicrobial inhibition

Zone of inhibition (cm) was determined using agar diffusion method diameter = C. acnes susceptibility

Drug <sup>b</sup>	Range (cm)
BPO 3.1% + CLIN 1.2% + ADAP 0.15%	1–3
<b>BPO</b> 5% + <b>ERY</b> 3%	1.2–5°
BPO 3.75% + CLIN 1.2%	0.9–3
Clindamycin 1%	0–5
BPO 3%	1–1.2
Adapalene 0.1%	0 (no activity)
	— More potent activit

adapalene 0.1% having no activity in vitro

ncluding 1 neutral strain sometimes classified as acne-associated. Classification based on Fitz-Gibbon S, et al.7  $\,^{
m b}$ All drugs are branded formulations. Activity may be influenced by the higher concentration of BPO (5%) compared with other formulations (3.0-3.75%) or the presence of 70% ethyl alcohol in the formulation, which may impact the ability of active ingredients to diffuse in the agar. ADAP, adapalene; BPO, benzoyl peroxide; CLIN, clindamycin phosphate; ERY, erythromy

# **FIGURE 5. Study Limitations**

### In vitro analyses: antimicrobial activity of compounds may not fully reflect in vivo activity

• For example, the relatively high MIC of BPO in this study (>512 µg/mL) is not unexpected and aligns with previous studies in which the in vitro activity of BPO was low compared to its high in vivo antimicrobial activity<sup>8-12</sup>



Adapalene: challenging to dissolve or tended to precipitate at high concentrations in the broth dilution and checkerboard assays, respectively

• Likely caused by interaction between adapalene and ions in the medium



Activity of formulated antimicrobials: may be impacted by differences in formulation, including inactive ingredients, which may enhance or reduce ability of the formulation to diffuse in agar

Overall, while these in vitro data provide valuable mechanistic insights, they must be placed in the context of in vivo and clinical data where additional factors can impact drug efficacy

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# CONCLUSIONS

- Clindamycin demonstrated strain-dependent activity against C. acnes in vitro, as expected for an antibiotic
- Adapalene had an additive effect on the antimicrobial activity of clindamycin against 3 out of 4 *C. acnes* strains tested, but no effect on BPO activity in vitro
- These data suggest that when combined with clindamycin/BPO, adapalene may enhance clindamycin's antimicrobial activity, while also bringing its own, unique retinoid mechanism of action to the triple combination
- This triple combination may further benefit from the ability of BPO to enhance clindamycin's antimicrobial activity<sup>13</sup>
- This is corroborated by a meta-analysis in which the combination of an antibiotic. retinoid, and BPO was among the top 2 most efficacious of all treatments for acne<sup>14</sup>

# AUTHOR DISCLOSURES

Mahmoud Ghannoum has acted as a consultant or received contracts from Scynexis, Inc, Bausch & Lomb, Pfizer, and Mycovia. James Q. Del Dermatologics, AbbVie, Almirall, Amgen, Arcutis, Biofrontera, Cassiopea, Cutera, Dermavant, EPI Heath, Evommune, Galderma, Incyte, JEM Health, Journey, La Roche-Posay, LEO Pharma, Lilly, L'Oreal, MC2 Therapeutics, Novan, Nutrafol, Pfizer, Sente, Strata, Sun Pharma, UCB, and Vyne, Linda Stein Gold has served as investigator/consultant or speaker for Ortho Dermatologics, LEO Pharma, Dermavant, Incyte, Novartis, AbbVie, Pfizer, Sun Pharma, UCB, Arcutis, and Lilly. Leon H. Kircik has served as either a consultant, speaker, advisor, or investigator for Allergan, Almirall, Epi Health, Galderma, Novartis, Ortho Dermatologics, and Sun Pharma. Julie C. Harper has received honoraria from Almirall, Cutera, Galderma, LaRoche-Posay, Ortho Dermatologics, and Sun Pharma. Other authors have nothing to disclose.



