

J. Pannu, A. McCarthy, A. Martin, S. Ciotti, J. Sutcliffe and J.R. Baker Jr.
 NanoBio Corporation, Ann Arbor, MI, USA

For additional information contact:
 John Coffey, Jr.
 Phone: (734) 302-9107
 E-mail: john.coffey@nanobio.com

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ABSTRACT

Background: NB-003 is an antimicrobial oil-in-water emulsion in development for the topical treatment of acne. NB-003 is formulated from materials included on the U.S.FDA list of inactive ingredients of Approved Drug Products. NB-003 contains nanometer-sized droplets that kill bacteria through lysis of lipid membranes. NB-003 concentrates in the pilosebaceous unit and has potent *in vitro* bactericidal activity against multidrug-resistant clinical isolates of *P. acnes*. NB-003 was evaluated in a pig skin model designed to mimic clinical studies that measures the reduction of *P. acnes* in human volunteers.

Methods: Full thickness pig skin was obtained from a commercial source (Sinclair Research Center, Inc.) and stored at -70°C until it was manually defatted prior to usage. The surface was cleaned and a glass ring (2 cm in diameter) was attached to the skin surface. The pig skin surface was inoculated with 10⁶-10⁷ cfu/cm² multidrug-resistant *P. acnes* and incubated under anaerobic conditions. One hour later 50 µl of varying NB-003 concentrations, vehicle or different commercial anti-acne products containing 2.5-5% benzoyl peroxide (BPO) were applied to the pig skin. After one or 24 hours exposure to the test article, *P. acnes* was collected from the swine skin using two combined washes, and colony-forming units were then determined.

Results: A dose response in the reduction of surface *P. acnes* on pig skin was observed over the range of NB-003 concentrations tested (0.001 – 0.5% NB-003). In 1 hour, 0.01% NB-003 reduced the *P. acnes* colony counts by 2 logs and concentrations ≥ 0.1% NB-003 resulted in ≥ 3.8-log reduction. BPO at 2.5% (Proactiv® repairing lotion) or 5% BPO (Clearasil® cream) gave 2.5 – 2.6 log reductions in *P. acnes* (consistent with published data). Vehicle and 0.001% NB-003 had less than a one log reduction. For all treatment arms, no additional reduction was noted in *P. acnes* counts at 24 hours post-application.

Conclusions: NB-003 is more effective at reducing surface *P. acnes* on pig skin than commercial products containing BPO. NB-003 is currently being studied in a Phase 1 clinical trial to measure the reduction of facial *P. acnes*.

BACKGROUND

- Acne is a common skin disorder caused by multiple factors, including an inflammatory response to the gram negative bacillus, *Propionibacterium acnes*, that selectively grows in the pilosebaceous glands of humans.
- Topical and oral antibiotics have been used to treat acne, but their effectiveness has been diminished due to the increasing incidence of drug resistant *P. acnes* isolates.
- NB-003 is a novel microbicidal nanoemulsion with low resistance potential that has potent *in vitro* bactericidal activity against resistant strain of *P. acnes* (Figure 2 and Table 1).
- We hypothesized that due to the unique surface activity of the nanoemulsion formulation, it would preferentially distribute to hair follicles and sebaceous glands (Figure 1).
- We tested this hypothesis by determining the *in vitro* activity of NB-003 and comparators against *P. acnes* using swine skin model as a surrogate of *P. acnes* reduction in human skin (Figures 3 and 4).

RESULTS

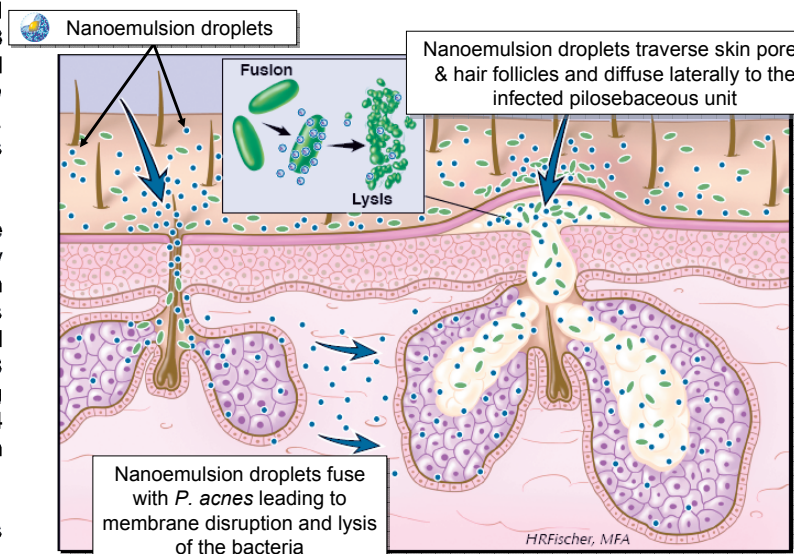


Figure 1. Mechanism of action of NB-003

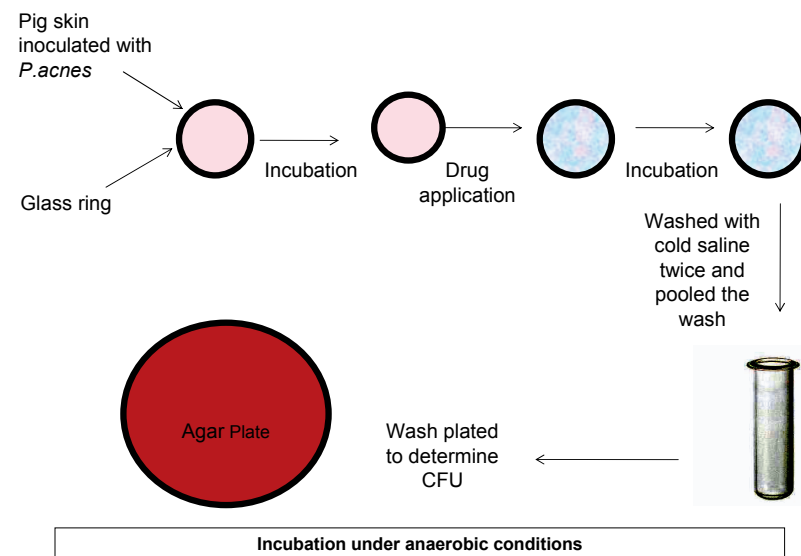


Figure 3. Experimental design of Pig skin model used in this study

Table 1. MIC/MBC values of NB-003 and comparators against Erythromycin, Clindamycin and Tetracycline resistant isolate of *P. acnes* (SW101T*)

Compound	MIC (µg/ml)	MBC (µg/ml)
NB-003	1	2
BPO (Clearasil®)	100	200
BPO (Proactiv®)	98	391

*Heller, S., et al. 2007. Antipropionibacterial activity of BAL19403, a novel macrolide antibiotic. J. Antimicrob. Chemother. 51:1956-1961

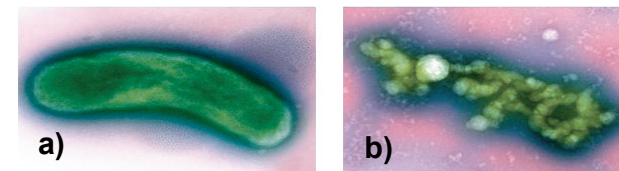


Figure 2. Scanning Electron micrographs of *P. acnes* before (a) and after treatment (b) with NB-003 for 10 minutes at room temperature.

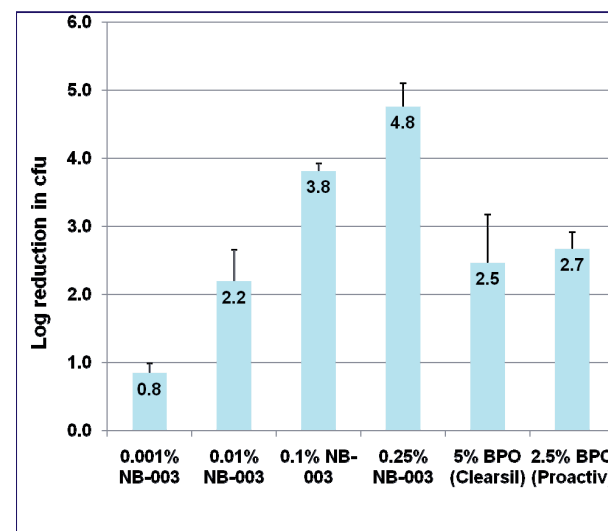
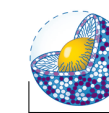


Figure 4. *P. acnes* log reduction on pig skin after one hour of a single topical application of 18 µl/cm²

METHODS



- 180 nm
- Cetylpyridinium chloride
- Polysorbate
- Oil

Emulsion manufacturing. NB-003 is an oil-in-water emulsion manufactured from ingredients that are Generally Recognized As Safe (GRAS) with a cationic detergent (cetylpyridinium chloride, CPC) that has proven safe for oral human use. The emulsion is formed from highly purified oil, ethanol, polysorbate, CPC and water. The average droplet size was 180 nm as measured by dynamic light scattering. The relative activity of NB-003 can be expressed in terms of the concentration of cationic surfactant present. The antibacterial activity of NB-003 is expressed in microgram CPC/ml.

Source of *P. acnes* isolates. Drug resistant clinical isolate of *P. acnes* was obtained from Stuart Shapiro, Basilea Pharmaceutica, AG, Basel, Switzerland.

MIC/MBC determinations. Minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC) to *P. acnes* was determined using standard methodology (Clinical and Laboratory Standards Institute). *P. acnes* strain grown on sheep blood agar for 24-48 hrs at 35°C was used as the sources of inocula for susceptibility study. A bacterial suspension with turbidity equivalent to a 0.5 McFarland standard was diluted to 1:75 in saline or Wilkins-Chalgren broth (GLP Corporation) to give >10⁶ cfu/ml in each well after inoculation. Because of the opacity of benzoyl peroxide, 20 µl of Cell Titer Blue (Alamar blue from Promega G8080) was added after 48 hrs; the plates were incubated for an additional hour prior to reading. Colony-forming units were counted after 72 h of incubation to ensure that the initial inocula were between 2.5 x 10⁶ cfu/ml. The minimal bactericidal concentrations (MBC) for *P. acnes* were determined by plating 10 µl from the well determined to be the MIC plus 4 wells above the MIC on blood-supplemented Mueller-Hinton agar plate. Inoculated petri plates were incubated for 72 h at 35°C under anaerobic conditions. The MBC was calculated as the concentration of drug that gave ≥3-log reduction from the initial inoculum concentration.

Time kill study. Log reduction in *P. acnes* was determined by using a pig skin model; parameters are given in table 2. Evaluation of viable colony count in saline wash after treatment was determined by plating 100µL of wash on to blood agar plates. All the plates were incubated under anaerobic conditions for 72hrs. Colony forming units were counted and reduction in bacterial count was calculated based on CFU recovered in saline control

Table 2. Parameters for pig skin model	
Apparatus	Six well titer plate and glass rings of 2cm diameter
Skin	Full thickness of pig abdominal skin shaved, defatted and glued to glass ring
Inoculum size	10 ⁶ to 10 ⁷ /cm ²
Formulations	0.001% NB-003 (10 µg CPC/ml), 0.01% NB-003 (100 µg/ml CPC), 0.1% NB-003 (1 mg CPC/ml), 0.25% NB-003 (2.5 mg CPC/ml), 5% BPO (50 mg BPO/ml), 2.5% BPO (25mg BPO/ml)
Dose volume	18 µl/cm ² was applied once and spreaded using sterile loop
Treatment duration	1- hour
Wash solution	0.85% Sterile saline solution refrigerated overnight
Wash volume	One ml each of wash solution us used to rinse skin twice
Colony count method	Pooled wash , serially diluted and plated instantaneously
Incubation conditions	Anaerobic at 35°C

CONCLUSIONS

- NB-003 is bactericidal against *P. acnes*, and kills more effectively than BPO on the surface of pig skin
- The combination of bactericidal activity, selective permeation and low resistance potential make NB-003 an interesting candidate for clinical trials in acne
- A Phase I clinical study is currently underway examining the *in vivo* bactericidal activity of NB-003