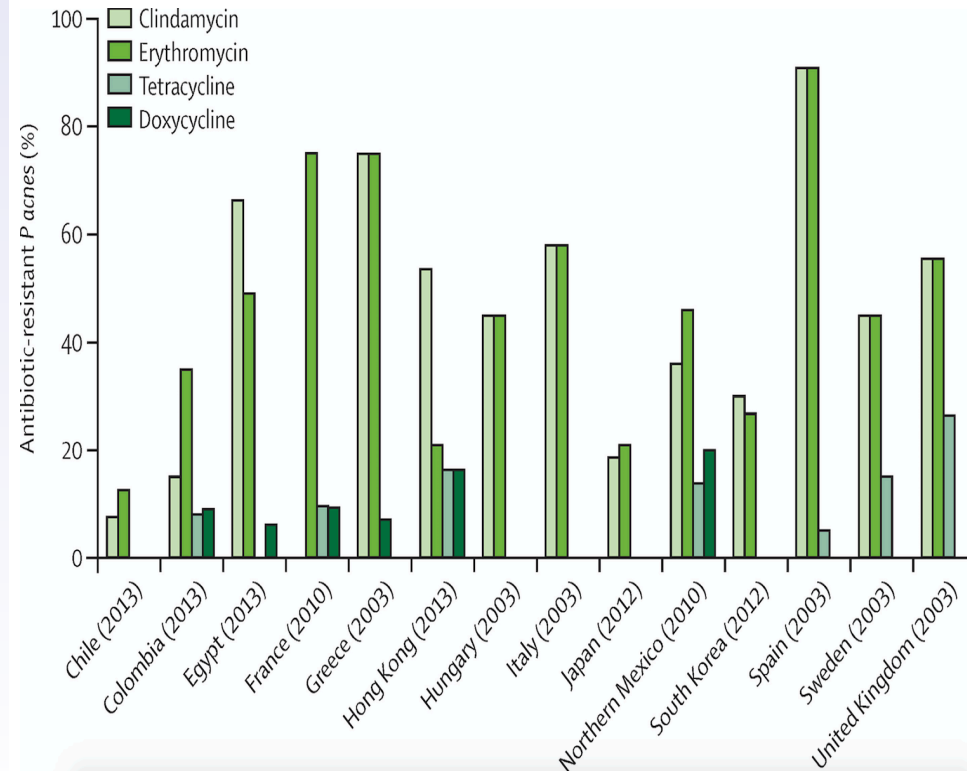


Building the Evidence in Microbiome-based therapies

Opportunity: Probiotics as alternatives to Antibiotics

Antibiotic Resistance

- **Selective pressure of antibiotics**
 - Enabled emergence of “superbugs”
- **Worldwide Problem-**
 - Cost US \$20 billion annually
 - Dermatologists-long course Abx
- **Current Solution**
 - race against time and bacterial evolution to develop new antibiotics
 - Antibiotic Stewardship-CDC/NIH/CARB-X initiatives to reduce Abx
 - **Development of antibiotic alternatives**

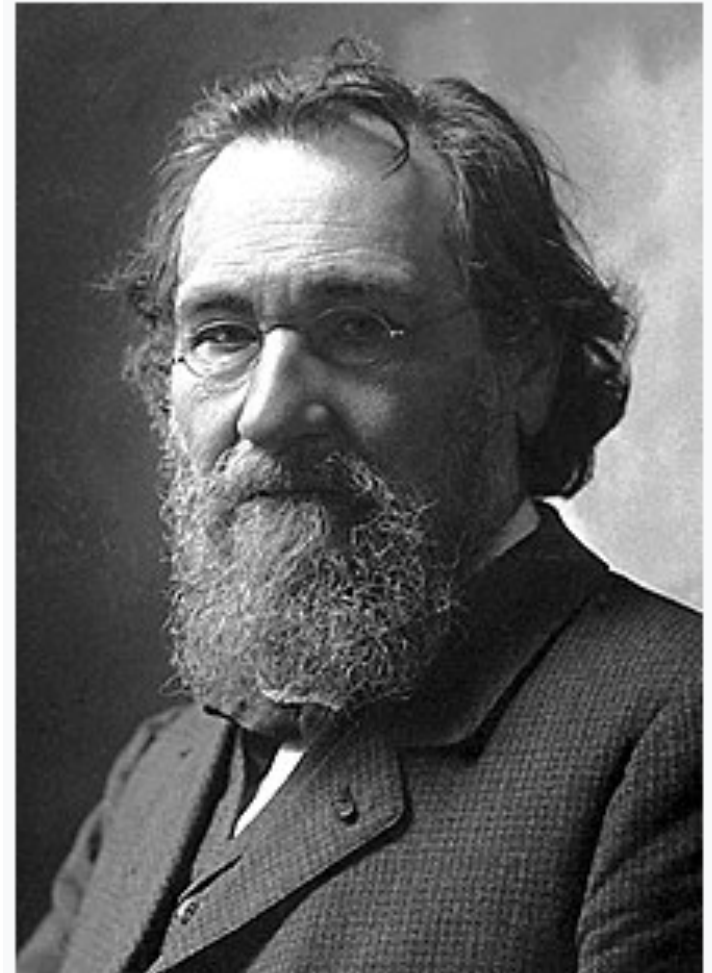


Building the Evidence in Microbiome-based therapies

- We know microbiome treatments are effective, as evidenced by successes with FMT, but overall evidence of efficacy across multiple diseases and disciplines is otherwise early in development, incomplete, & there's a continued dependence on antibiotics
 - Industry-Wide Challenges
 - Battling misinformation and “Alternative Facts”
 - Building the Evidence
 - Continued dependence on antibiotics in Medicine

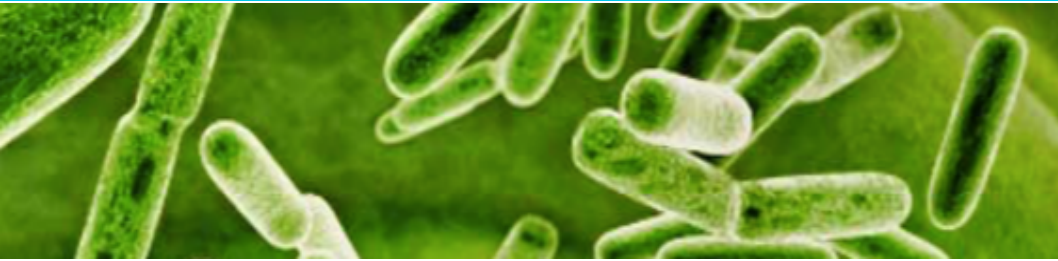
The Probiotic Revolution

- Elie Metchnikoff won the nobel prize in 1908 for his work in immunology
- Research interest in the gut lead to the concept of probiotics to promote health
- Tools to define and characterize the microbiome didn't happen until the genomics revolution
 - Cheaper
 - Faster
 - Strain level definition
- **Lack of regulation over the probiotic industry, vague terminology, and soft claims have enabled a blurring of valid science and pseudoscience**



Élie Metchnikoff, c. 1908

Important Regulatory Terms



Why your skin needs
topical probiotics

- **Probiotic:** “Live microorganisms, which, when administered in adequate amounts, confer a *health benefit* on the host.” - United Nations Food and Agricultural Organization (FAO)/ World Health Organization (WHO)
 - regulated as a dietary supplements, do not need FDA premarket-review
- **Live Biological Product:** (42 U.S.C. § 262(i)) Live microorganisms applicable to the prevention, treatment, or cure of a disease or condition
 - **includes probiotics for clinical applications**
 - safety, purity, potency, efficacy need to be reviewed by FDA
 - Regulated by CBER-Office of Vaccines Research and Review (OVR) via biologic license application BLA
- **Intended use determines how a substance is to be regulated**
- **If there is no medical claim, there is limited regulation**

Probiotics: What is real science and how do we filter out noise?



Consumer Health Reports: Probiotics

There are **5 factors** which impact our grade of each probiotic. They are listed and explained below.

1. Price – this one is pretty self-explanatory.
2. Potency – WAY too many companies leave their probiotics sitting around gathering dust, over **50% of the brands that we tested had ZERO live cultures left!** Potency is the % of live cultures we discovered upon testing the brand, you'd be surprised at how many companies store their probiotics for way too long.
3. CFU (Colony-Forming Units) – this is the total count of all of the bacteria in the probiotic, **VERY** important because probiotics are **ONLY EFFECTIVE IN ADEQUATE AMOUNTS.**
4. Strains – total number of different types of bacteria in each probiotic, varies greatly and, along with strain diversity, affects the types and levels of benefits that you will notice.
5. Strain Purity – a combination of the effective concentration of the strains within the probiotic and the functional quality of the active cultures (the ability of the bacteria to perform its specific job).

Know your Source of Information: World Wide Web of Alternative Facts

PALEO FOR WOMEN
STUNNINGLY HEALTHY WOMANHOOD

Topical probiotics: can applying bacteria to your face cure your acne?

by Stefani Ruper | Oct 7, 2014 | Acne, Blog, Body | 34 comments

The dermatologist's premise

Very few dermatologists believe that the food you eat is important for the quality of your skin. This is mad! I bet me if I'm wrong, but I'm pretty sure Dante reserved the fifth circle of hell in *Inferno* for medical professionals deliberately blurring the facts.

The standard response to the dermatologist's premise

Smart health practitioners (like me!) can't sit on dermatology, and they recommend that food is medicine.

Going one step further

[Researchers led by Dr. Huiying Li at UCLA lifted P. acnes bacteria from the pores of 49 acne-prone and 52 clear-complexioned volunteers. They found more than 1,000 strains of the bacteria, from which they were able to identify genes unique to each strain.](#)

C'mon... does that really work?

More importantly for me – I have tried them. **The first time I sprayed on my P. Acnes**, I noticed a difference within days. I simply got fewer breakouts....and I haven't had a new pimple since.



Almost truths and Little White Lies



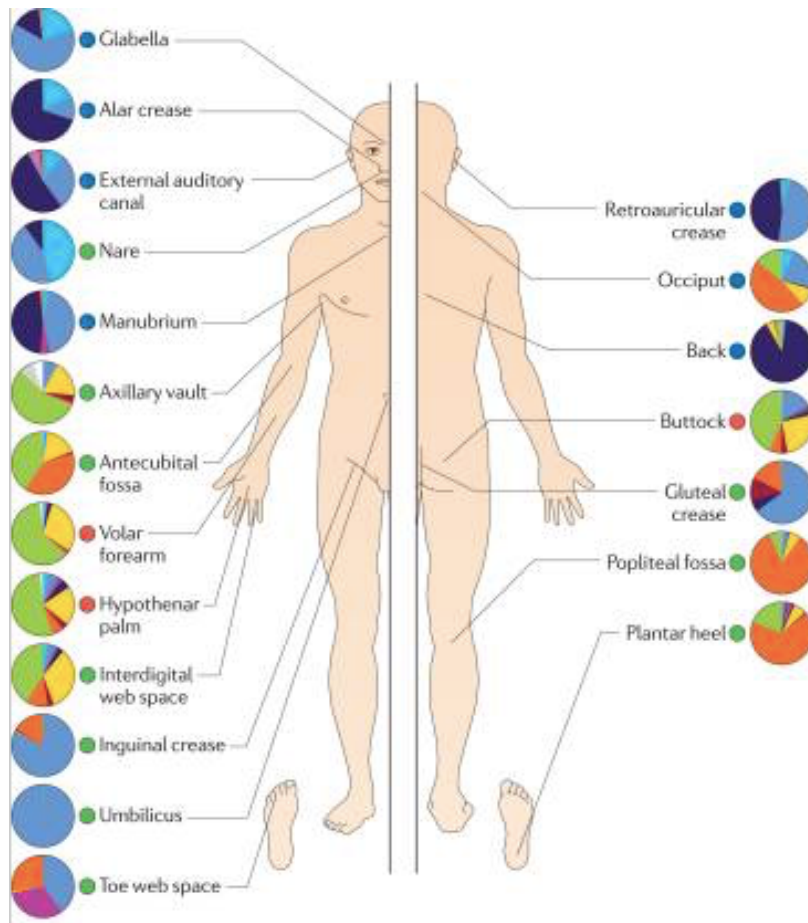
Q. What is Probiotic Action™?

Probiotic Action™ is a liquid topical that contains “good bacteria”, **healthy human skin.** **ing probiotic bacteria,** that makes up Probiotic Action's unique spore structure for healthy skin and clean skin. A natural alternative for skin.

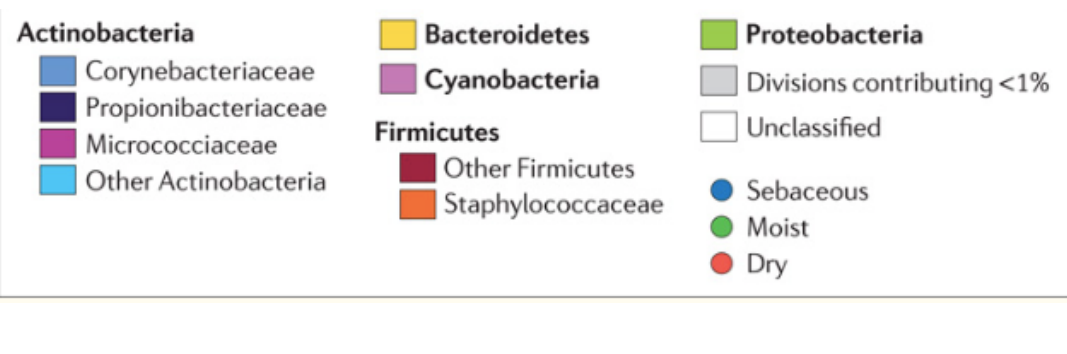
Q. What is the main ingredient?

The main ingredient is spore-forming **species***

Data for Bacillus and the Skin



- *Bacillus* belongs to phylum firmicutes
- Firmicutes are found in very low levels on the skin.
- *Bacillus oleronius* has been associated with rosacea
- *Bacillus subtilis* has been associated with foot odor
- *Bacillus anthracis* causes anthrax

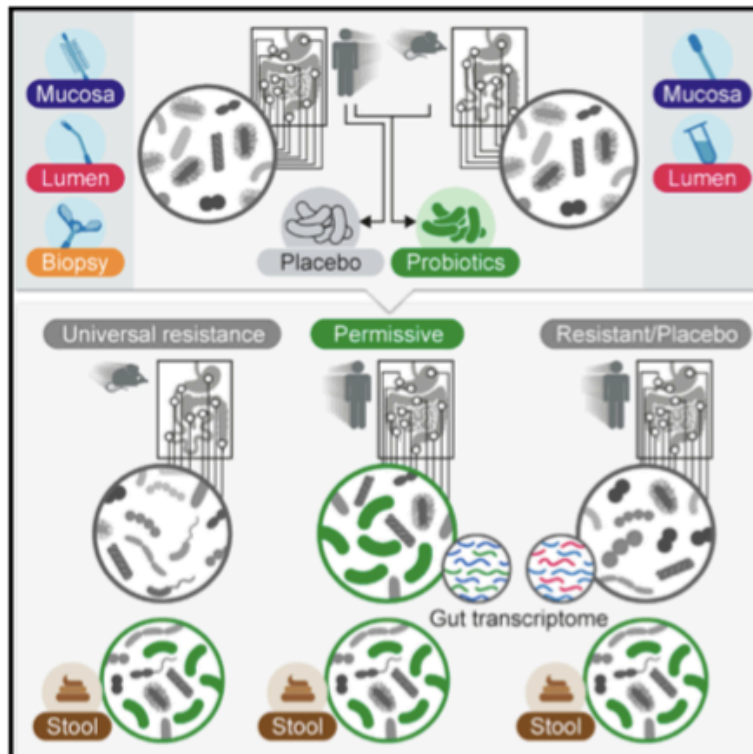


Grice EA, et al. Topographical and temporal diversity of the human skin microbiome. Science. 2009;324:1190–1192. [PubMed] A comprehensive analysis of skin microbiota across 20 sites. [Ref list]

Cell

Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features

Graphical Abstract



Authors

Niv Zmora, Gili Zilberman-Schapira, Jotham Suez, ..., Zamir Halpern, Eran Segal, Eran Elinav

Correspondence

zamir@tlvmc.gov.il (Z.H.),
eran.segal@weizmann.ac.il (E.S.),
eran.elinav@weizmann.ac.il (E.E.)

In Brief

Probiotics transiently colonize the human gut mucosa in highly individualized patterns, thereby differentially impacting the indigenous microbiome and host gene-expression profile, a trait which is predictable by baseline host and microbiome features, but not by stool shedding.

What the media says about what the Science Says

Probiotics labelled 'quite useless'

By James Gallagher Health and science correspondent, BBC News



<https://www.bbc.com/news/health-45434753>

Consumers are a vulnerable population

The Truth

Exposing The Truth One Story At A Time...

Economy Conspiracy Earth Changes Police State Christian Politics Preppers Soci

10 Health Conspiracy Theories That Turned Out To Be Real

March 23, 2014 by Michael Snyder



- Consumer beliefs and purchasing habits are influenced greatly by the media, celebrities, popular blogs, advertising, and often less so by the scientific and medical communities
- The same audience that reads this conspiracy theory blog “The Truth” and is swayed by Jenny McCarthy and Gweneth Paltrow about the “dangers of vaccination”, the magical powers of jade eggs, also ascribes to the notion that antibiotics give rise to super bugs and big pharma is evil
- These individuals have lower vaccination rates, tend to avoid antibiotics and prescriptions, but will avidly buy “natural alternatives”- vitamins, health supplements and probiotics
- **How do we in the microbiome field break free from pseudoscience and convince the public of it’s validity and steer the public to legitimate sources of information?**

<http://thetruthwins.com/archives/10-health-conspiracy-theories-that-turned-out-to-be-real>

Gwyneth Paltrow's Goop to pay \$145,000 over claims its vaginal eggs have health perks

[f Share](#) / [🐦 Tweet](#) / [👤 Reddit](#) / [📌 Flipboard](#) / [@ Email](#)

Actress Gwyneth Paltrow's lifestyle company, Goop, agreed to a \$145,000 settlement after being accused of making unfounded claims about products designed to aid women's sexual and emotional health. A lawsuit filed by prosecutors from 10 California counties said the company did not have scientific backing for health claims it made for three products sold online: two vaginal eggs and a mix of essential oils.



Notify FDA of false/misleading claims

Autism: Beware of Potentially Dangerous Therapies and Products

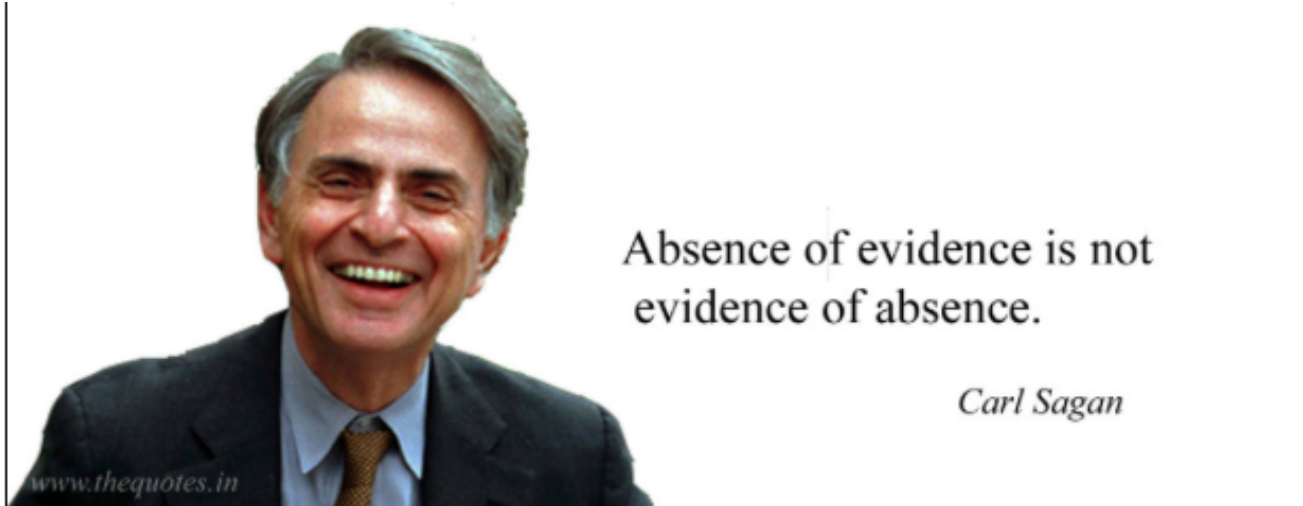
FDA Cracks Down on False Claims

According to Commander Jason Humbert, M.H.S., R.N., a regulatory operations officer in the FDA's Office of Regulatory Affairs, the agency has warned and/or taken action against a number of companies that have made improper claims about their products' intended use as a treatment or cure for autism or autism-related symptoms. Some of these so-called therapies carry significant health risks and include:

- **“Chelation Therapies.”** These products claim to cleanse the body of toxic chemicals and heavy metals by binding to them and “removing” them from circulation. They come in a number of forms, including sprays, suppositories, capsules, liquid drops and clay baths. FDA-approved chelating agents are approved for specific uses that do not include the treatment or cure of autism, such as the treatment of lead poisoning and iron overload, and are available by prescription only. FDA-approved prescription chelation therapy products should only be used under professional supervision. Chelating important minerals needed by the body can lead to serious and life-threatening outcomes.
- **Hyperbaric Oxygen Therapy.** This involves breathing oxygen in a pressurized chamber and has been cleared by FDA only for certain medical uses, such as treating decompression sickness suffered by divers.
- **Detoxifying Clay Baths.** Added to bath water, these products claim to draw out chemical toxins, pollutants and heavy metals from the body. They are improperly advertised as offering “dramatic improvement” for autism symptoms.
- Various products, including raw camel milk and essential oils. These products have been marketed as a treatment for autism or autism-related symptoms, but have not been proven safe and effective for these advertised uses.

<https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm394757.htm>

Build the evidence



- But **without the evidence** doctors won't prescribe and insurance companies won't reimburse
- Build the evidence
 - government and industry funded research and development opportunities
 - rigorous scientific studies/well designed clinical trials
 - peer reviewed publications
 - Influencing the medical community with sound science
 - Interfacing directly with the public to educate

Evidence-Based Medicine

- To build the case for microbiome-based therapeutics within the medical establishment, we need to build the evidence

Levels of Evidence:

- IA Evidence from meta-analysis of randomized controlled trials
- IB Evidence from at least one randomized controlled trial
- IIA Evidence from at least one controlled study without randomization
- IIB Evidence from at least one other type of quasi-experimental study
- III Evidence from non-experimental descriptive studies, such as comparative studies, correlation studies, and case-control studies
- IV Evidence from expert committee reports or opinions or clinical experience of respected authorities, or both

Grades of Recommendations:

- A Directly based on Level I evidence
- B Directly based on Level II evidence or extrapolated recommendations from Level I evidence
- C Directly based on Level III evidence or extrapolated recommendations from Level I or II evidence
- D Directly based on Level IV evidence or extrapolated recommendations from Level I, II, or III evidence

(from Shekelle PG, Woolf SH, Eccles M, Grimshaw J. [Developing clinical guidelines](#). West J Med. 170(6):348-51, 1999 June)

Evidence based-Physician Guidelines on Acne

CLINICAL RATIONALE

The 2007 guidelines from the American Academy of Dermatology on treatment of acne vulgaris¹⁶ include the following recommendations:

1. Topical therapy is a standard of care in acne treatment
 - Topical retinoids, benzoyl peroxide, and antibiotics are strongly recommended.
 - Topical antibiotics used alone can be associated with the development of bacterial resistance.
 - Azelaic acid is effective but some experts consider its efficacy limited.
 - Employing multiple topical agents that affect different aspects of acne pathogenesis can be useful.
 2. Systemic antibiotics are a standard of care in moderate and severe acne and treatment-resistant forms of inflammatory acne
 - Doxycycline and minocycline are more effective than tetracycline, and there is evidence that minocycline is superior to doxycycline in reducing *P. acnes*.
 - Although erythromycin is effective, use should be limited to those who cannot use the tetracyclines
 - Trimethoprim-sulfamethoxazole and trimethoprim alone are also effective in instances where other antibiotics cannot be used.
 3. Other Therapies
 - Estrogen-containing oral contraceptives can be useful in treatment of acne in some women.
 - Spironolactone and cyproterone can be useful, but the strength of recommendation is less
 - Oral isotretinoin is useful for severe recalcitrant nodular acne and also lesser degrees of acne that are treatment-resistant or for acne that is scarring
 - Intralesional corticosteroid injections are effective in the treatment of individual acne nodules¹⁶
- 2016 Acne Guidance-nearly a decade later-still have antibiotics as standard first line therapy-limited to 3 months or less

Continued Dependence on Antibiotics in Medicine

- Despite pressure for antibiotic stewardship, prescribing habits of dermatologists/non-dermatologists between 2004 and 2013 haven't changed
- Skin Diseases dependent on Abx
 - Acne-long courses over years
 - Eczema-*Staph aureus* colonization
 - Rosacea-chronic low dose tetracyclines

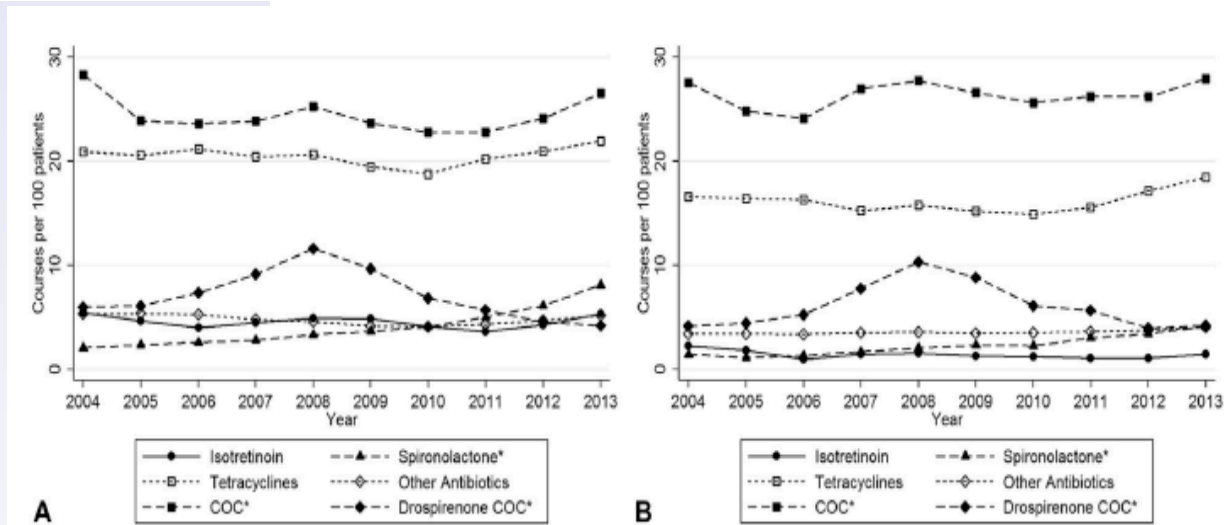


Fig 1. Change in the number of courses of therapy per 100 patients seen by a dermatologist (A) and managed by nondermatologists (B) between 2004 and 2013. *Tetracyclines* include doxycycline and minocycline. *Other antibiotics* includes trimethoprim-sulfamethoxazole, amoxicillin, cephalexin, and azithromycin. *COC*, Combined oral contraceptive. *Combined oral contraceptives, drospirenone, and spironolactone were evaluated among only female patients with acne.

Prescription Habits-Insurance Influence

Prior Authorization Criteria for Approval Initial and Renewal Evaluation

1. Is the patient 8 years of age or older?
If yes, continue to 2. If no, deny.
2. Does the patient have a diagnosis of moderate to severe acne vulgaris?
If yes, continue to 3. If no, continue to 5.
3. Has the patient previously tried and failed therapy with a prescription topical acne treatment?
If yes, continue to 5. If no, continue to 4.
4. Does the patient have an allergy, contraindication, or intolerance to prescription topical acne treatment?
If yes, continue to 5. If no, deny.
5. Has the patient previously tried and failed therapy with an oral prerequisite generic minocycline product or generic doxycycline product?
If yes, approve for 12 weeks. If no, continue to 6.
(Note: PA approvals will be limited to one approval per 12 months.)
6. Does the patient have an allergy, contraindication, or intolerance to an oral prerequisite generic minocycline product or generic doxycycline product?
If yes, approve for 12 weeks. If no, deny.
(Note: PA approvals will be limited to one approval per 12 months.)

- Sample BlueCross/BlueShield prior authorization form

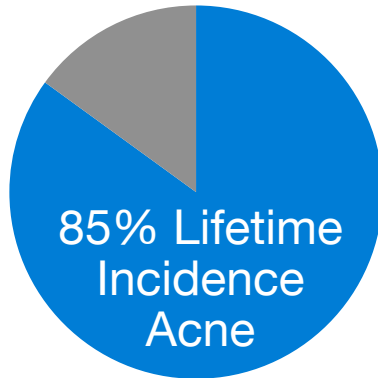
Insurance Guidance come from Physician Guidelines

CLINICAL RATIONALE

The 2007 guidelines from the American Academy of Dermatology on treatment of acne vulgaris¹⁶ include the following recommendations:

1. Topical therapy is a standard of care in acne treatment
 - Topical retinoids, benzoyl peroxide, and antibiotics are strongly recommended.
 - Topical antibiotics used alone can be associated with the development of bacterial resistance.
 - Azelaic acid is effective but some experts consider its efficacy limited.
 - Employing multiple topical agents that affect different aspects of acne pathogenesis can be useful.
 2. Systemic antibiotics are a standard of care in moderate and severe acne and treatment-resistant forms of inflammatory acne
 - Doxycycline and minocycline are more effective than tetracycline, and there is evidence that minocycline is superior to doxycycline in reducing *P. acnes*.
 - Although erythromycin is effective, use should be limited to those who cannot use the tetracyclines
 - Trimethoprim-sulfamethoxazole and trimethoprim alone are also effective in instances where other antibiotics cannot be used.
 3. Other Therapies
 - Estrogen-containing oral contraceptives can be useful in treatment of acne in some women.
 - Spironolactone and cyproterone can be useful, but the strength of recommendation is less
 - Oral isotretinoin is useful for severe recalcitrant nodular acne and also lesser degrees of acne that are treatment-resistant or for acne that is scarring
 - Intralesional corticosteroid injections are effective in the treatment of individual acne nodules¹⁶
- 2016 Acne Guidance-nearly a decade later-still have antibiotics as standard first line therapy-limited to 3 months or less

Acne – First Skin Probiotic Indication



- \$4.5 Billion market
- 40–50 million US
- There is in NEED for novel therapeutics
 - 55–75% resistant to antibiotics
 - ◆ Heavy dependence on antibiotics still
 - High side effect profile of existing therapies
 - No maintenance therapy outside Accutane
 - No natural acne solutions
 - Saturated with generics/expired patents recycled into new formulations/combinations
- All of these problems can be addressed with microbiome-based solutions



Evidence for PO probiotic therapy Acne

Benef Microbes. 2016 Sep 6:1-6. [Epub ahead of print]

Supplementation with *Lactobacillus rhamnosus* SP1 normalises skin expression of genes implicated in insulin signalling and improves adult acne.

Fabbrocini G¹, Bertona M², Picazo Ó³, Pareja-Galeano H⁴, Monfrecola G¹, Emanuele E².

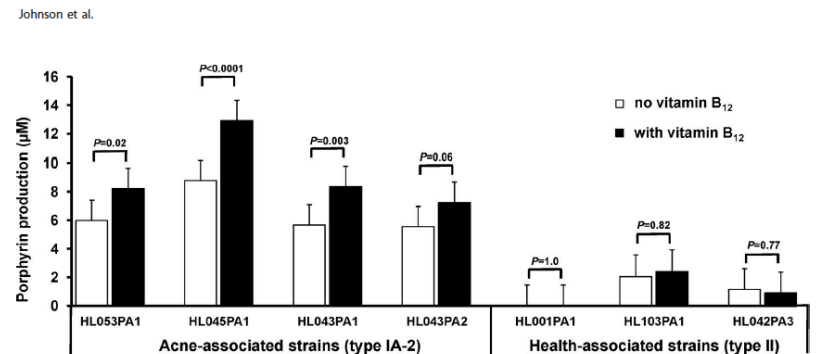
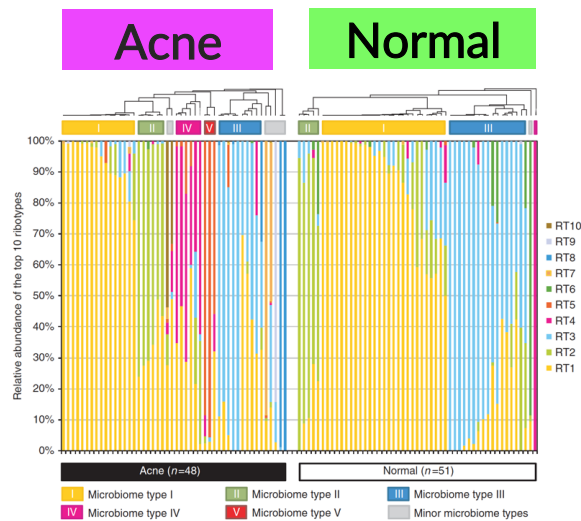
+ Author information



- Probiotic (*Lactobacillus rhamnosus* SP1) showed a 32% ($P<0.001$) reduction, and a 65% increase ($P<0.001$) in *IGF1* and *FOXO1* gene expression respectively.
- Probiotic odds ratio of 28.4 (95% confidence interval = 2.2-411.1, $P<0.05$) to be rated by physicians as improved compared with placebo
- **Conclusion-supplementation with the probiotic strain LSP1 normalizes skin expression of genes involved in insulin signaling and improves the appearance of adult acne.**

Scientific Foundation of the Acne Microbiome

- Human microbiome project discovered diseased subjects have different *P.acnes* than healthy subjects.
- Enabled the discovery of genotypic and phenotypic differences between healthy and pathogenic *P. acnes* (ex.Porphyrin is a known inflammatory metabolite in acne and is produced by *P. acnes* bacteria)
- Healthy strains repress porphyrin production
- **Hypothesis: Replacement of disease-associated high porphyrin producing strains with health-associated low porphyrin producing strains should decrease porphyrin levels and reduce acne**



Johnson T, et al. 2016 Strain Level Differences in Porphyrin Production and Regulation in *Propionibacterium acnes* Elucidate Disease Associations. mSphere 1(1):e00023-15. doi:10.1128/mSphere.00023-15.



Overview Naked Biome



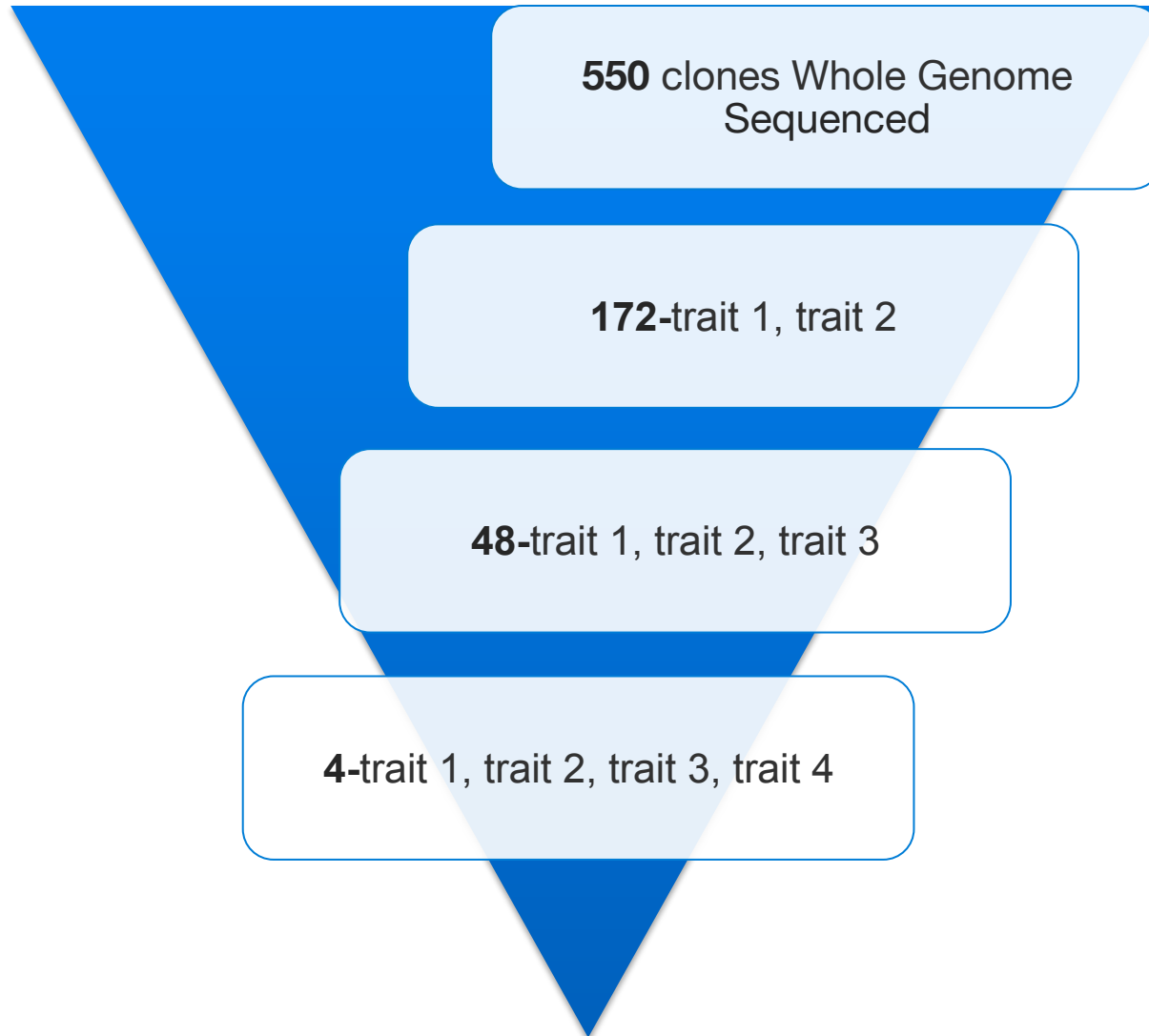
Naked Biome

- We are a translational microbiome-therapeutics company.
- Lead product is a **topical Live Biologic Therapeutic (LBT)** for treatment/prevention of acne.
- LBT is derived from healthy individuals that have been extensively genotyped and phenotyped
- Completed Phase IB single application and about to initiate Phase IB/IIA multiple application

Illustration: Charis Tsevis

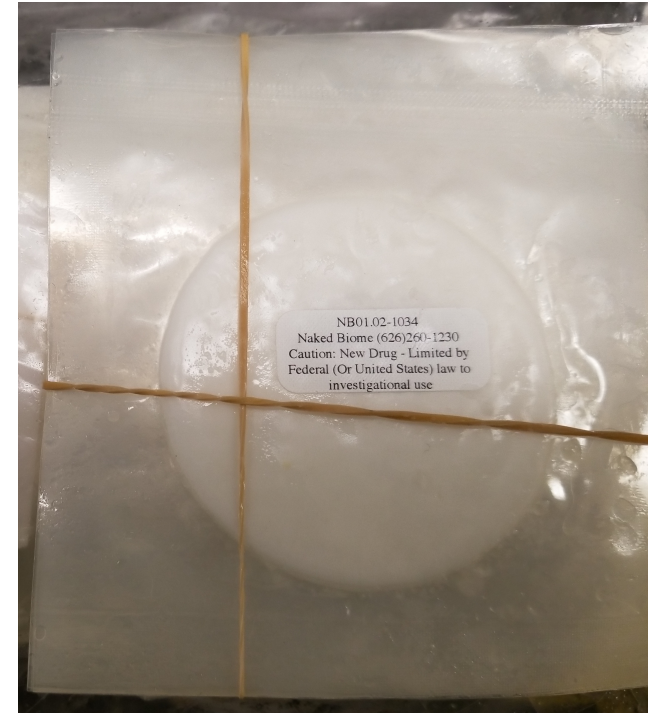


Genotype Specification



GMP Manufacturing

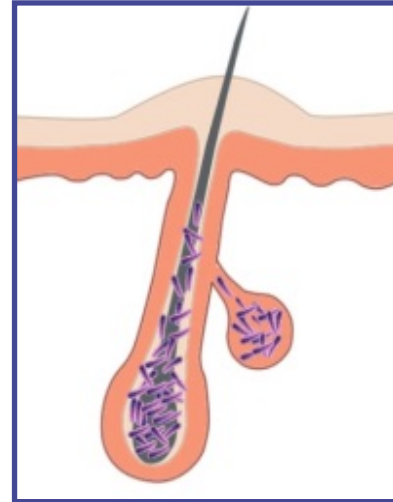
- Contract executed with world leading probiotic manufacturers
 - Experience with US FDA
 - Experience with Pharma GMP
 - Experience with Propionibacterium
- GMP process established
- Formulation
 - All GRAS materials
 - Non-comedogenic materials
 - Aesthetic textural quality
- Delivery-Vacuum-sealed Cotton applicator pads



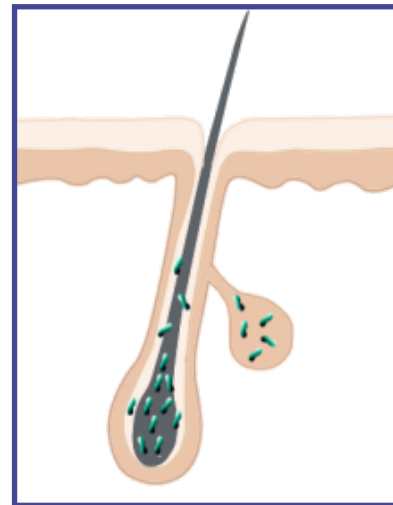
Naked Biome De-risking-Clinical Trial

Probiotic Trial Phase IB

- Trial in acne subjects to determine safety and possible early efficacy measures
- Outcome measures
 - Safety
 - Engraftment
 - Efficacy
- Hypothesis:
 - healthy bacteria lack virulence factors
 - continuous supply will prevent regrowth of disease-associated strains and therefore acne flares



Colonization by
“Bad” Bacteria
(Antibiotic
Resistant)



Colonization by
“Good” Bacteria

Objectives-Develop Genotype Assay

- To develop an assay to profile subjects and monitor engraftment of our drug candidate
- Assay requirements:
 - **PanBac**-Find a loci common to facial skin bacteria (*P.acnes*, *P.avidum*, *P. acidipropionici*, *S.epidermidis*, *S.Aureus*)
 - **Gene1**--Loci specific to health-associated strains) and in low abundance in diseased subjects
 - **Gene2**--Loci specific to health-associated strains and low abundance in diseased subjects
 - **Gene3**—-Loci specific to pathogenic strains

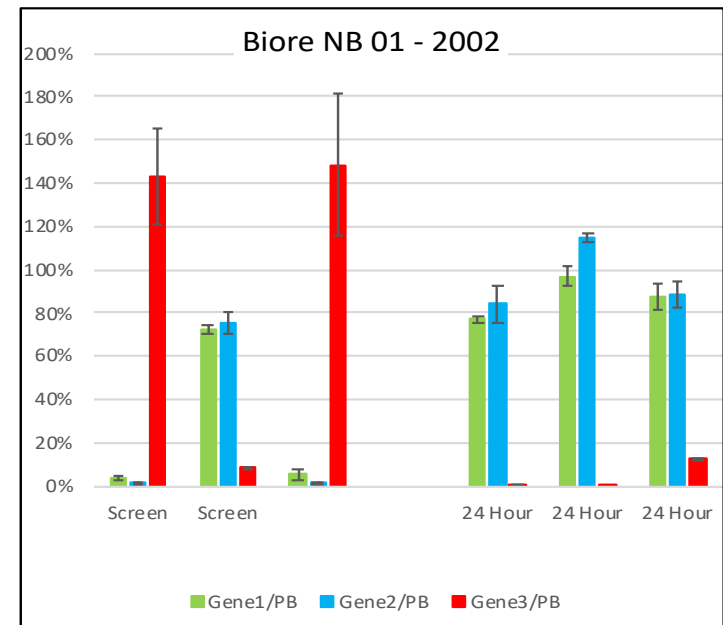
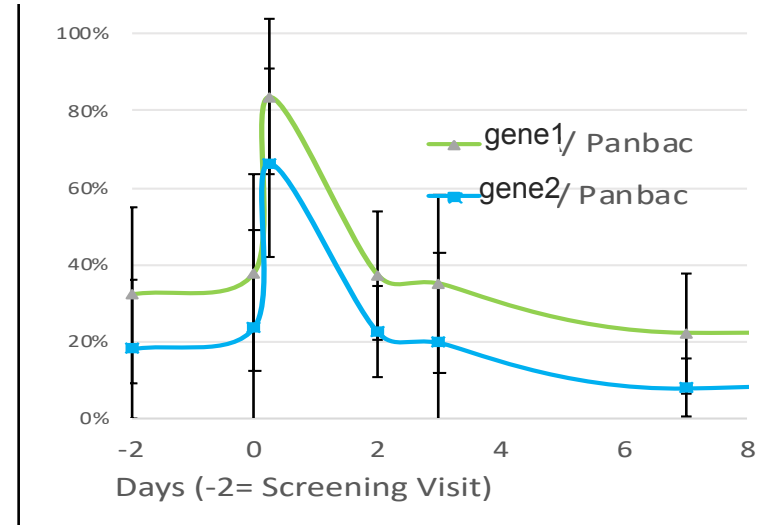
Gene2/PanBac=%Healthy

Gene3/PanBac=%Pathogenic

	All	NB_1	NB_2	Pathogenic
PANBAC	X	X	X	X
Gene 1		X	X	
Gene 2			X	
Gene 3				X

Preliminary Clinical Data-Primary Endpoints

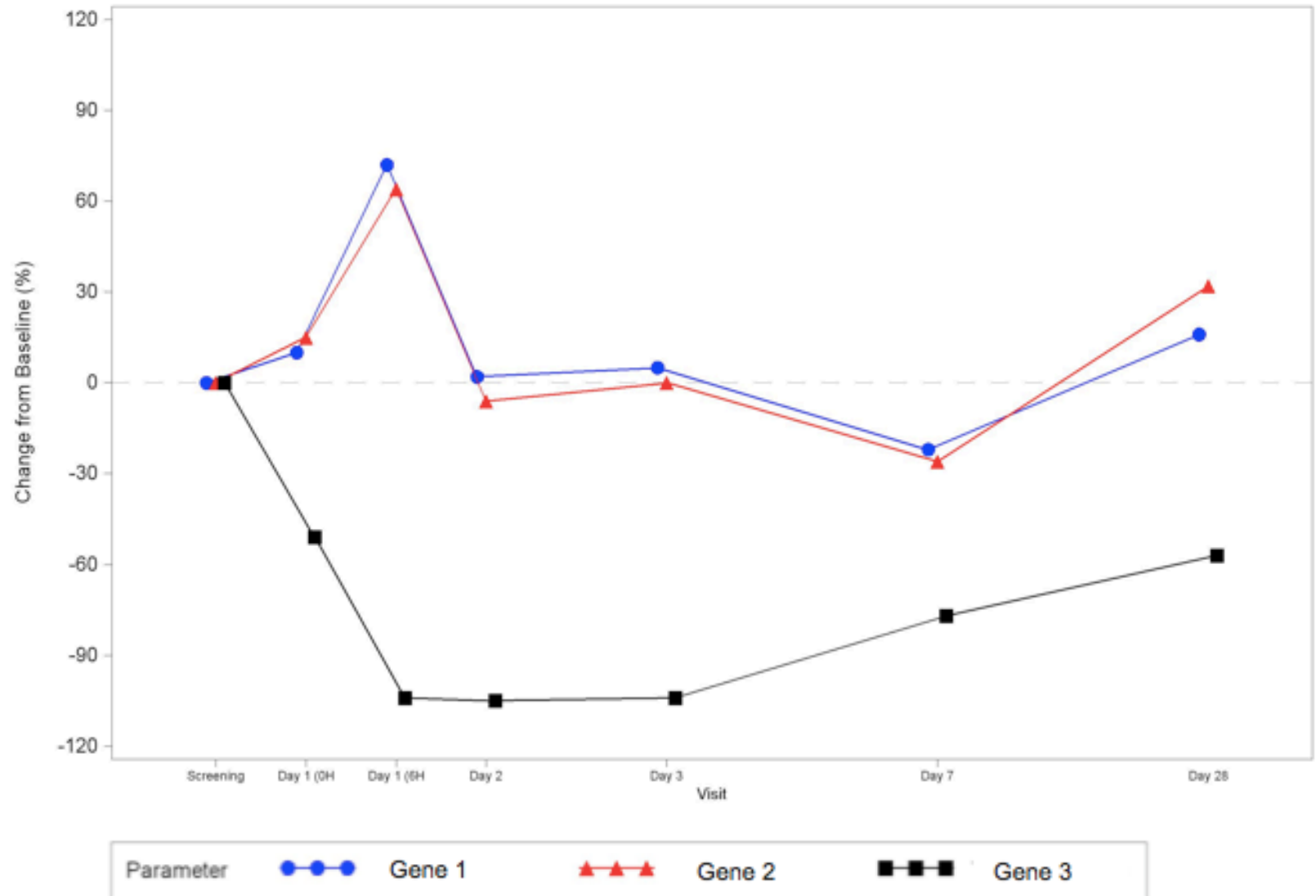
- Safety
 - Findings-There were no drug related adverse events in all 10 subjects
- Engraftment
 - Genotype Assay Swab
 - ◆ Drug appeared to be present for at least 6H in our upper bound subjects
 - Genotype assay Biore
 - ◆ Findings-We have possible suggestion of follicular engraftment in 2 subjects
 - ◆ **Note:** For our subsequent study we are confirming engraftment by recovery of live NB01 from follicles and Sanger Sequencing specific to our strain



Swab Engraftment-01-2001

Figure 14.2.1.1 Cheek Swab Engraftment – Lab Results – Chg. from Baseline

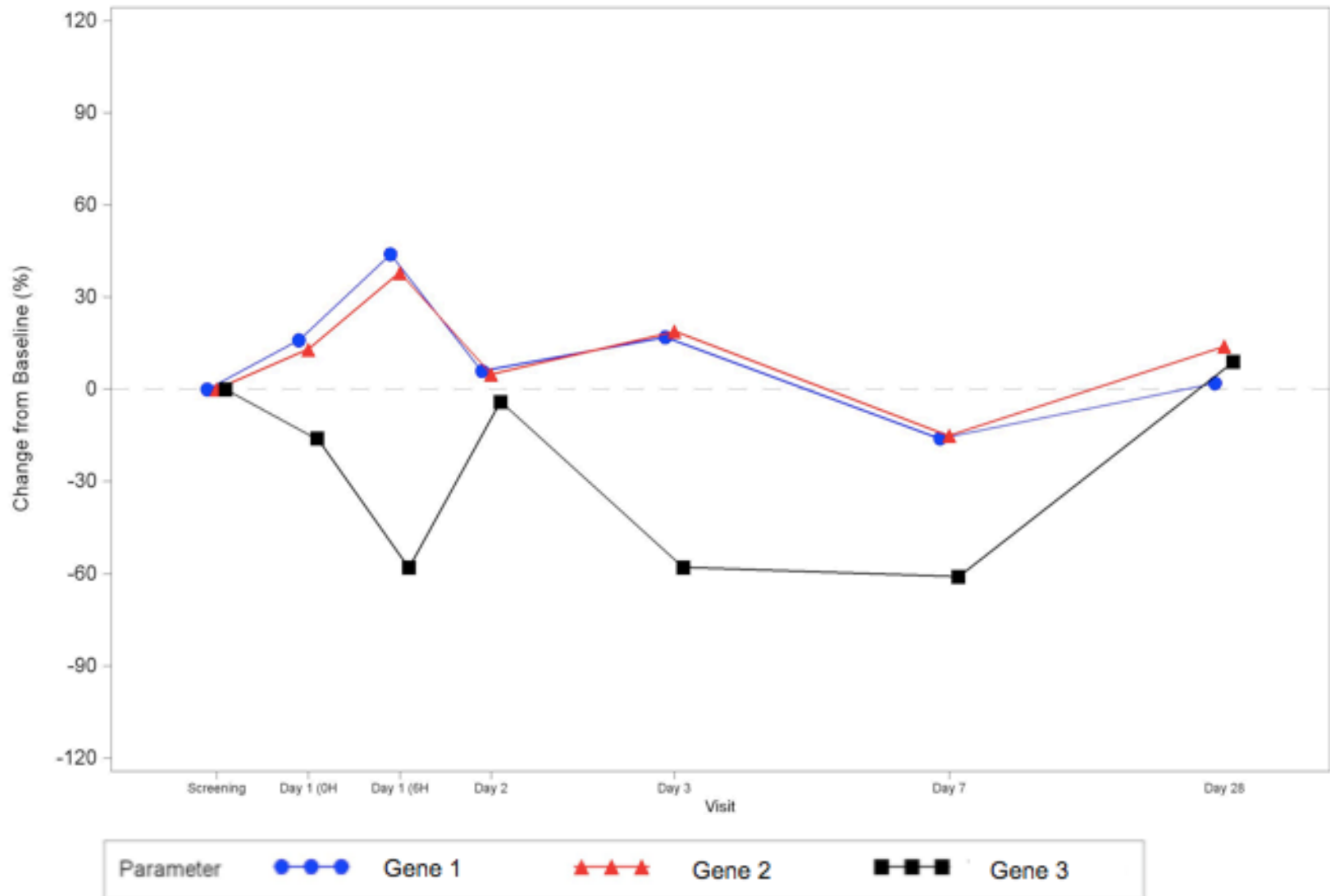
Subject 01-2001



Swab Engraftment-01-2002

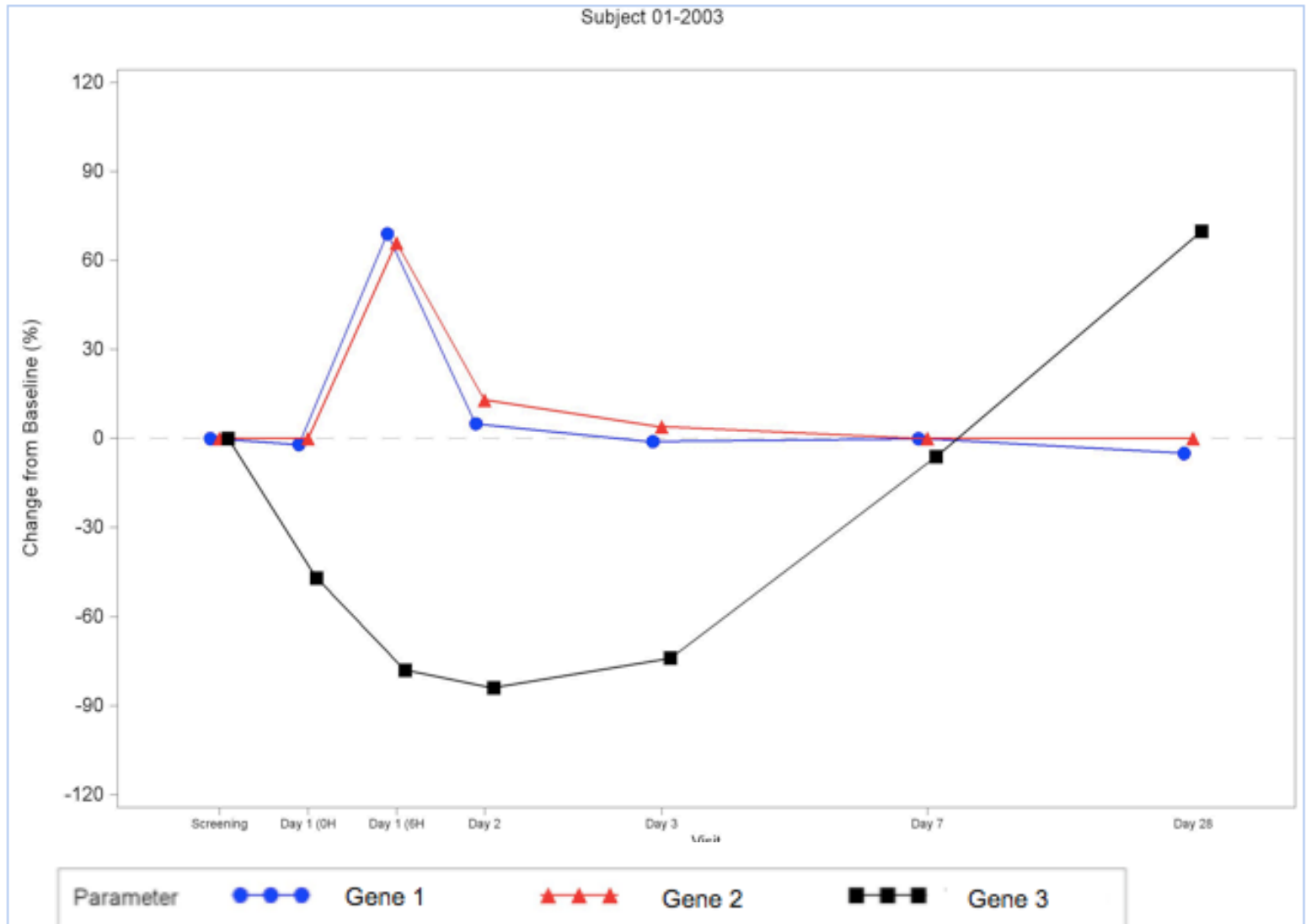
Figure 14.2.1.1 Cheek Swab Engraftment – Lab Results – Chg. from Baseline

Subject 01-2002



Swab Engraftment-01-2003

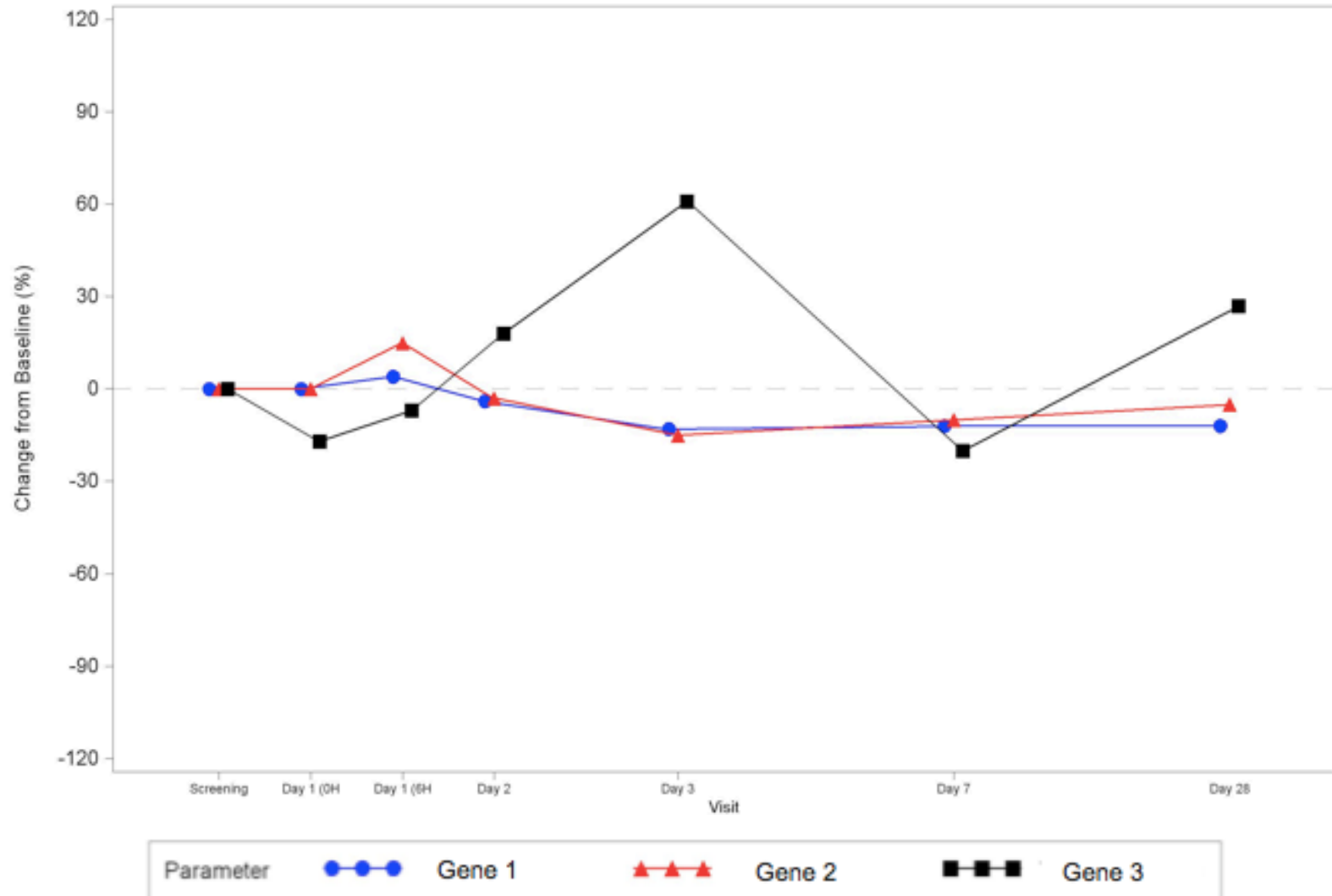
Figure 14.2.1.1 Cheek Swab Engraftment – Lab Results – Chg. from Baseline



Swab Engraftment-01-2004

Figure 14.2.1.1 Cheek Swab Engraftment – Lab Results – Chg. from Baseline

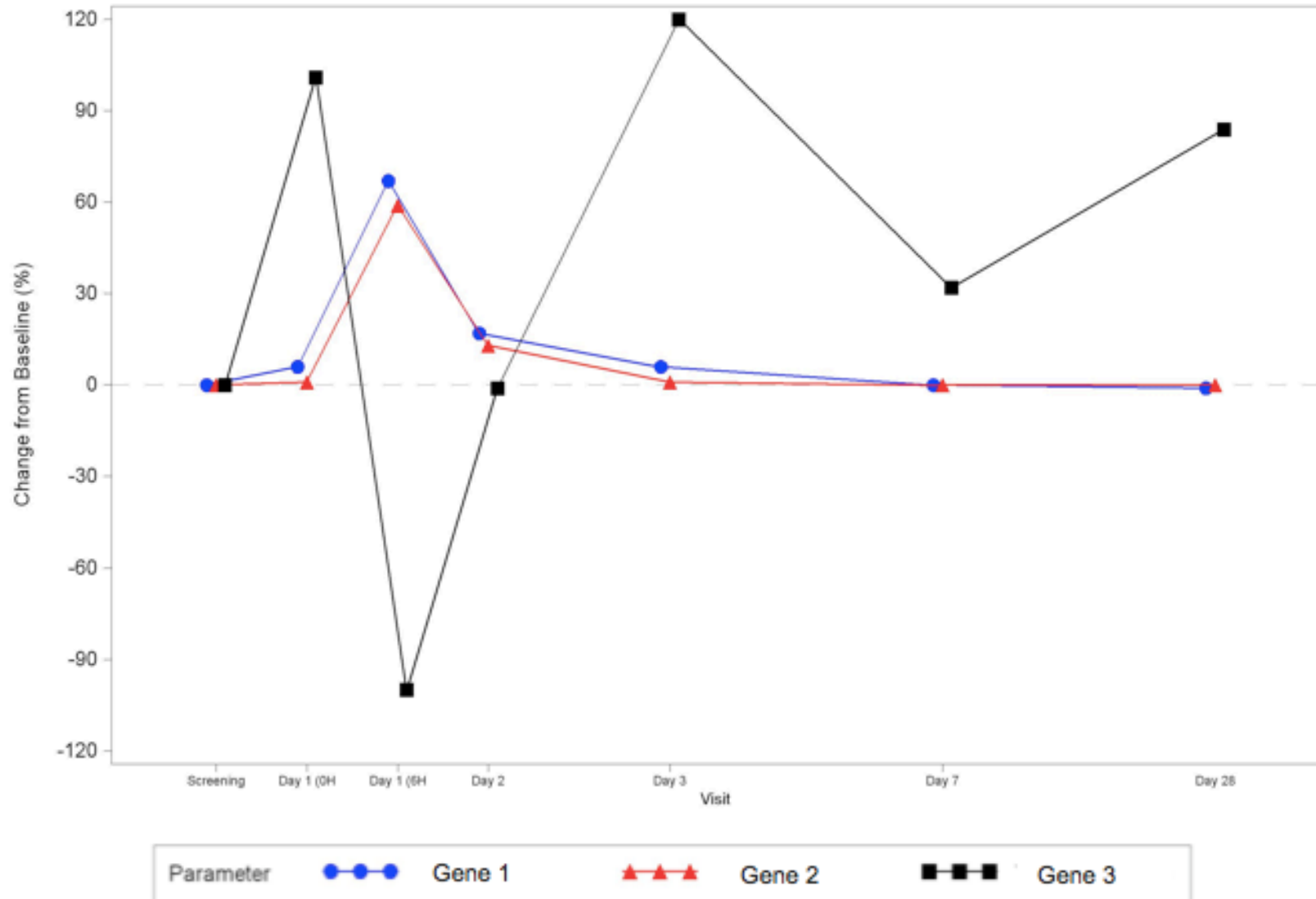
Subject 01-2004



Swab Engraftment-01-2005

Figure 14.2.1.1 Cheek Swab Engraftment – Lab Results – Chg. from Baseline

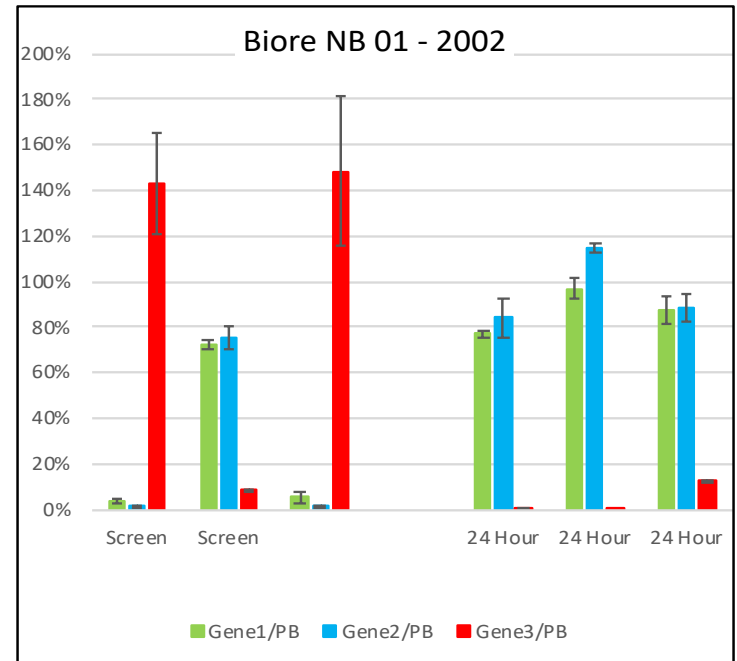
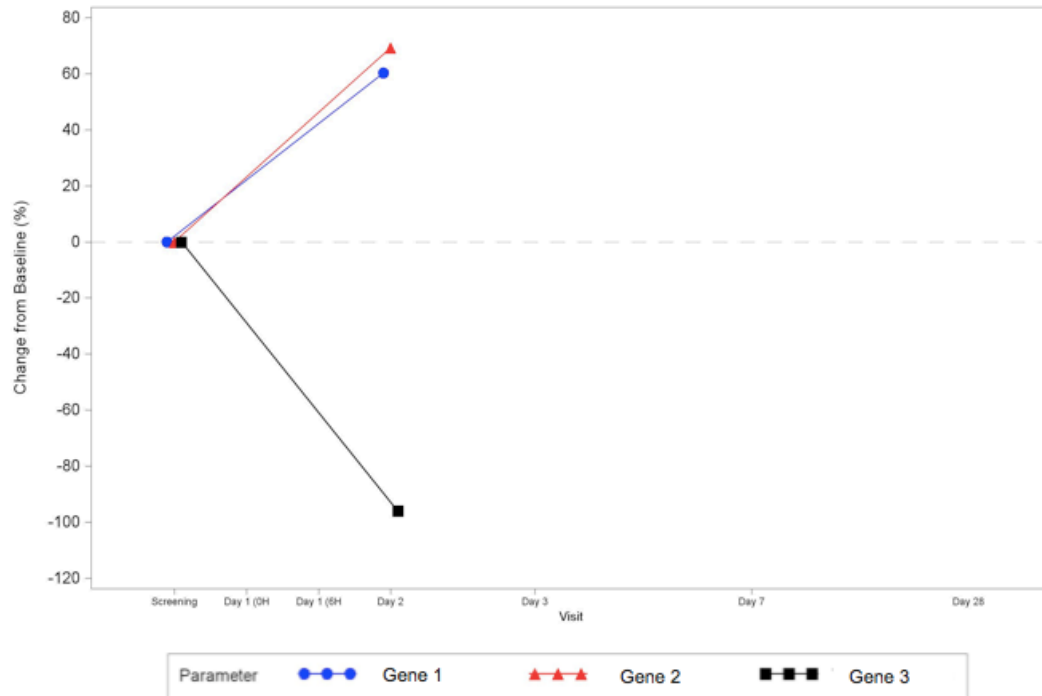
Subject 01-2005



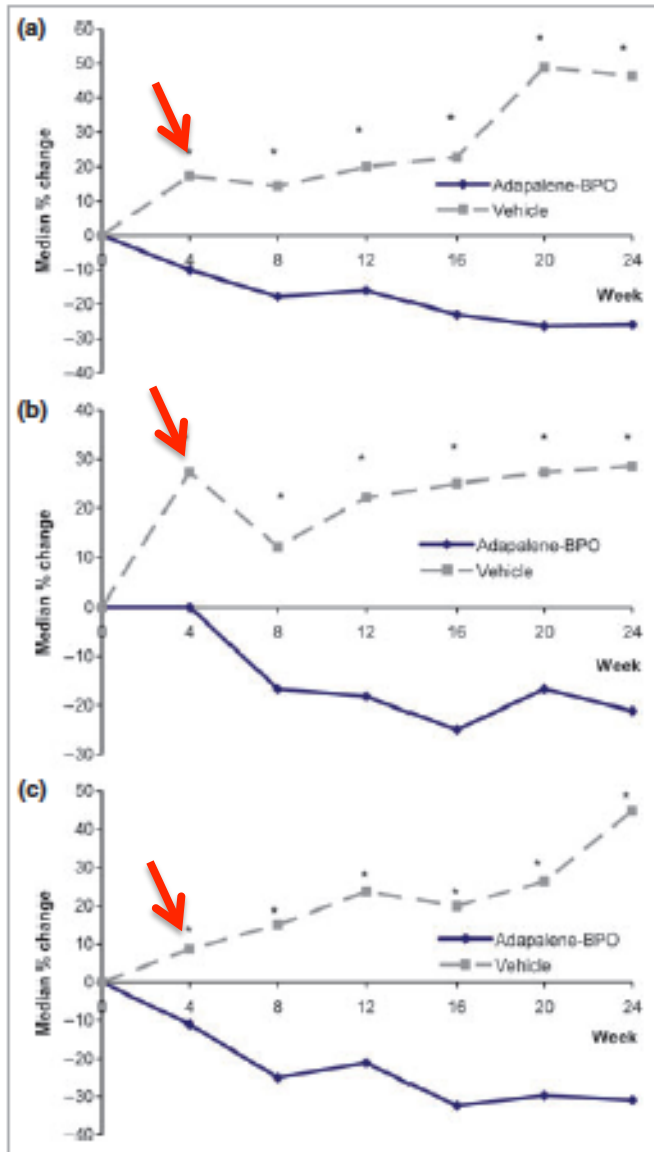
Biore-Follicular Engraftment

Figure 14.2.1.2 Biore Strip Assessment – Lab Results – Chg. from Baseline

Subject 01-2002



Recurrence Rate Acne with Vehicle



A-total lesion count ~20% increase in 4 weeks

B-total inflammatory count ~30% increase in 4 weeks

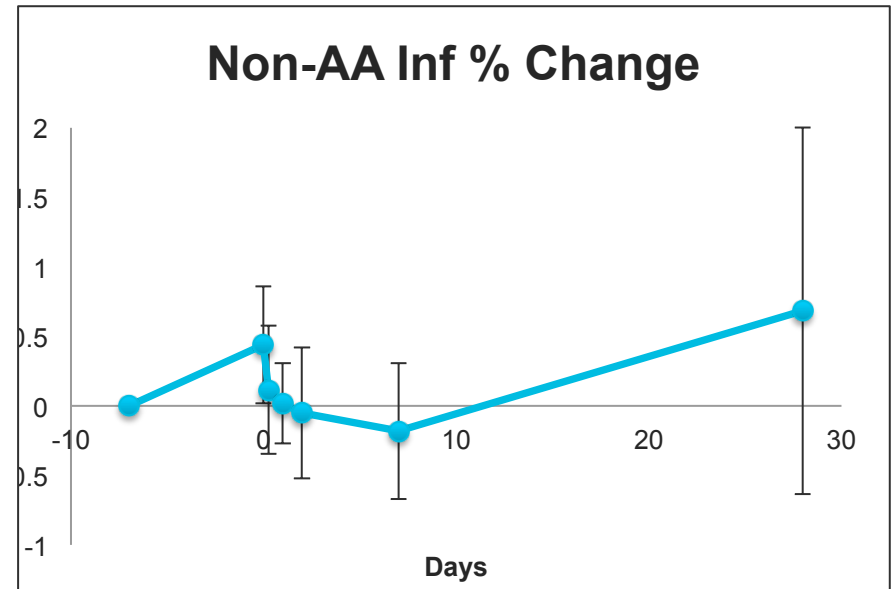
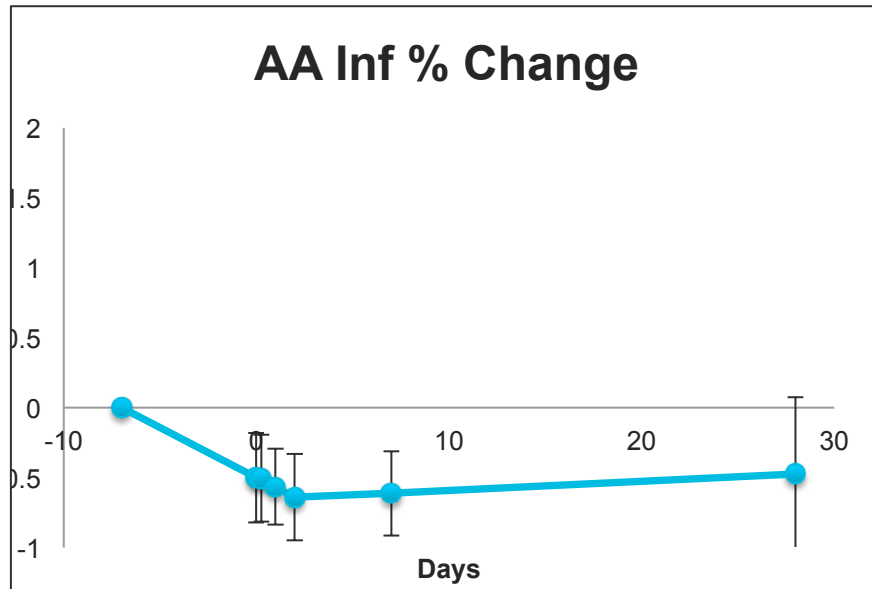
C-total non-inflammatory count ~10% increase in 4 weeks

Fig 2. Median percentage change from baseline in total, inflammatory and noninflammatory lesions (intent-to-treat-observed population). (a) Total lesions † $P < 0.001$; (b) inflammatory lesions * $P < 0.05$; (c) noninflammatory lesions # $P \leq 0.001$.

Preliminary Clinical Data-Secondary Endpoints

- Inflammatory Lesion Count
 - Findings-We saw an expected drop in inflammatory lesion count post BPO. Surprisingly, this drop was maintained through day 28
- Investigator Global Assessment of Acne
 - Findings-We saw an expected drop in IGA post BPO, but an unexpected retention of a lower IGA score through day 28

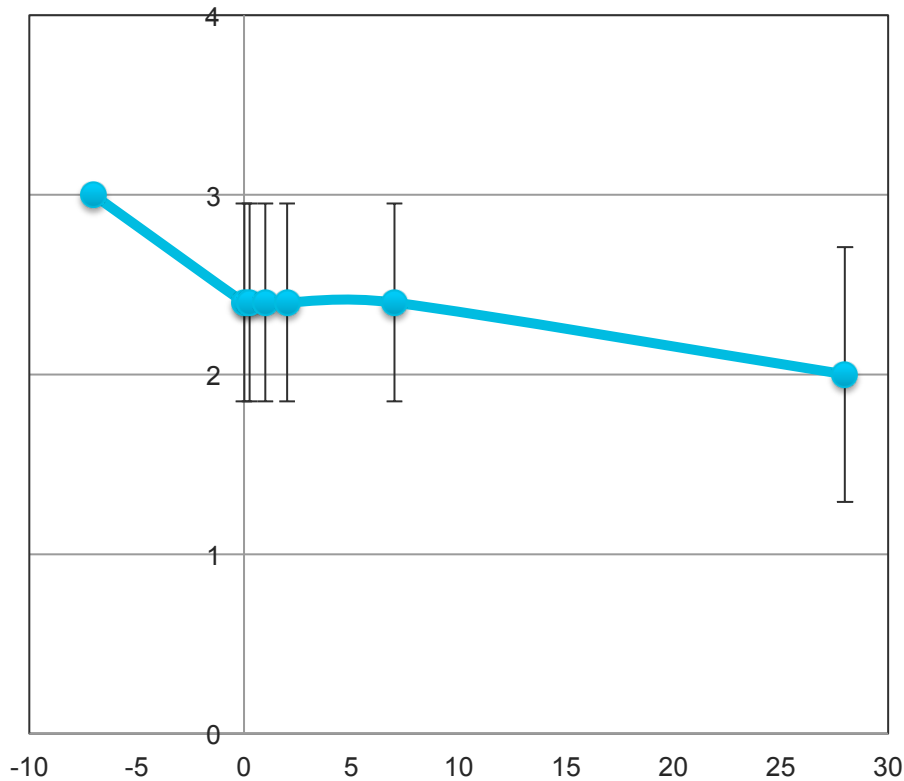
Upper Bound-Inflammatory Lesion % Change Application Area (AA) vs. Non-Application Area (Non-AA)



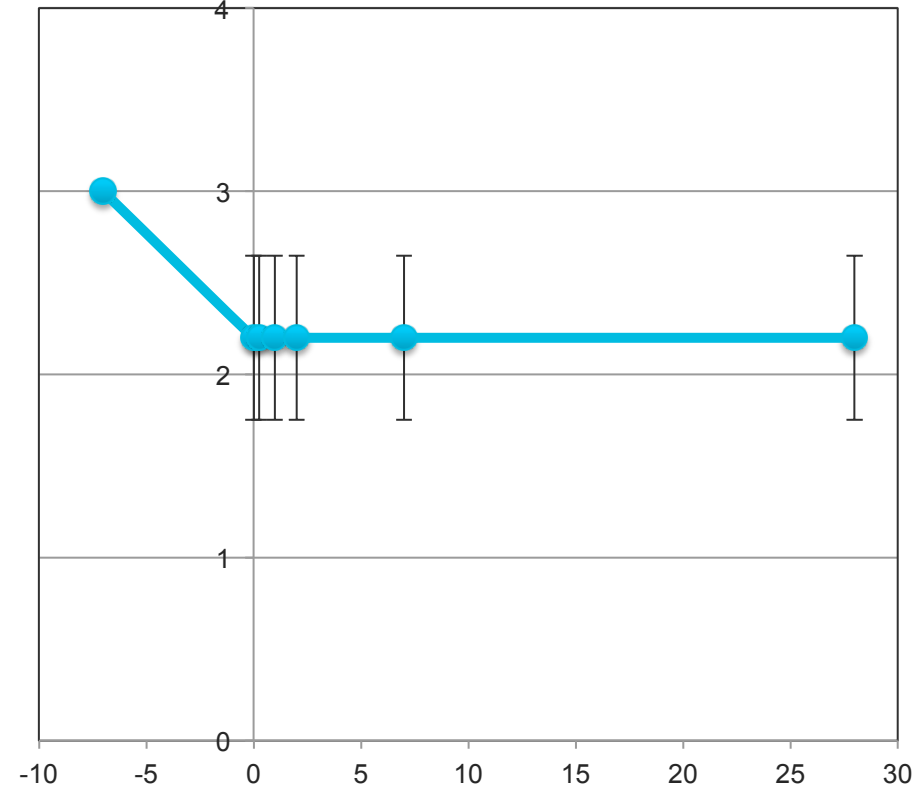
- Y axis is % change with -1 being 100% improvement and 1 being %100 worsening
- Area of application improved with BPO and non-application area did not improve post BPO (no clinically consistent trend with BPO)
- Inflammatory lesion counts was 48% improved 28 days out
- Non-application area 68% worse than baseline 28 days post application

Investigator Global Assessment (IGA)

IGA Lower Bound



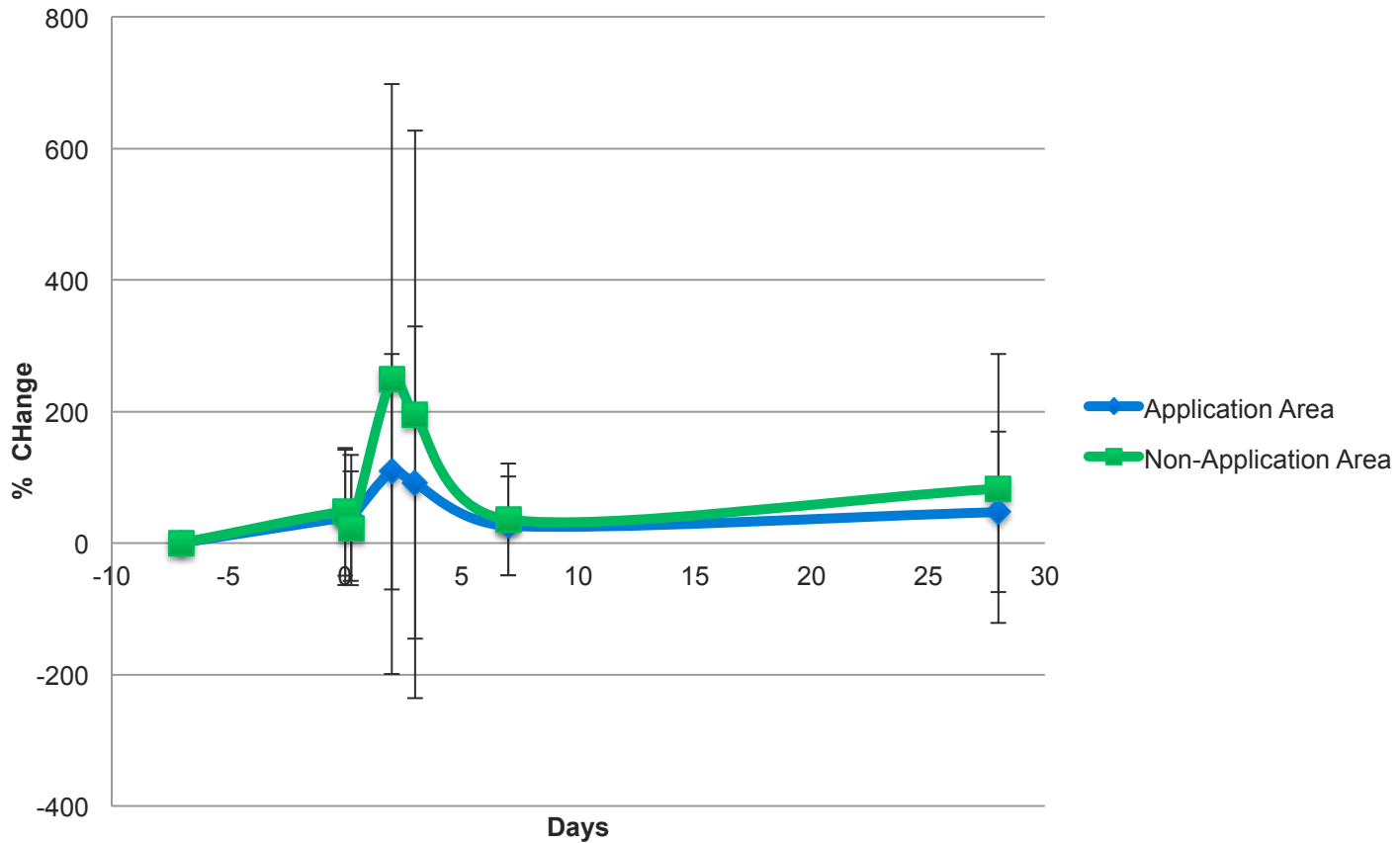
IGA-Upper Bound



A 1 point drop in IGA in lower bound and a 0.8 point drop in IGA in upper bound was noted 28 days post BPO and single application of drug compared to baseline, and was statistically significant to baseline IGA

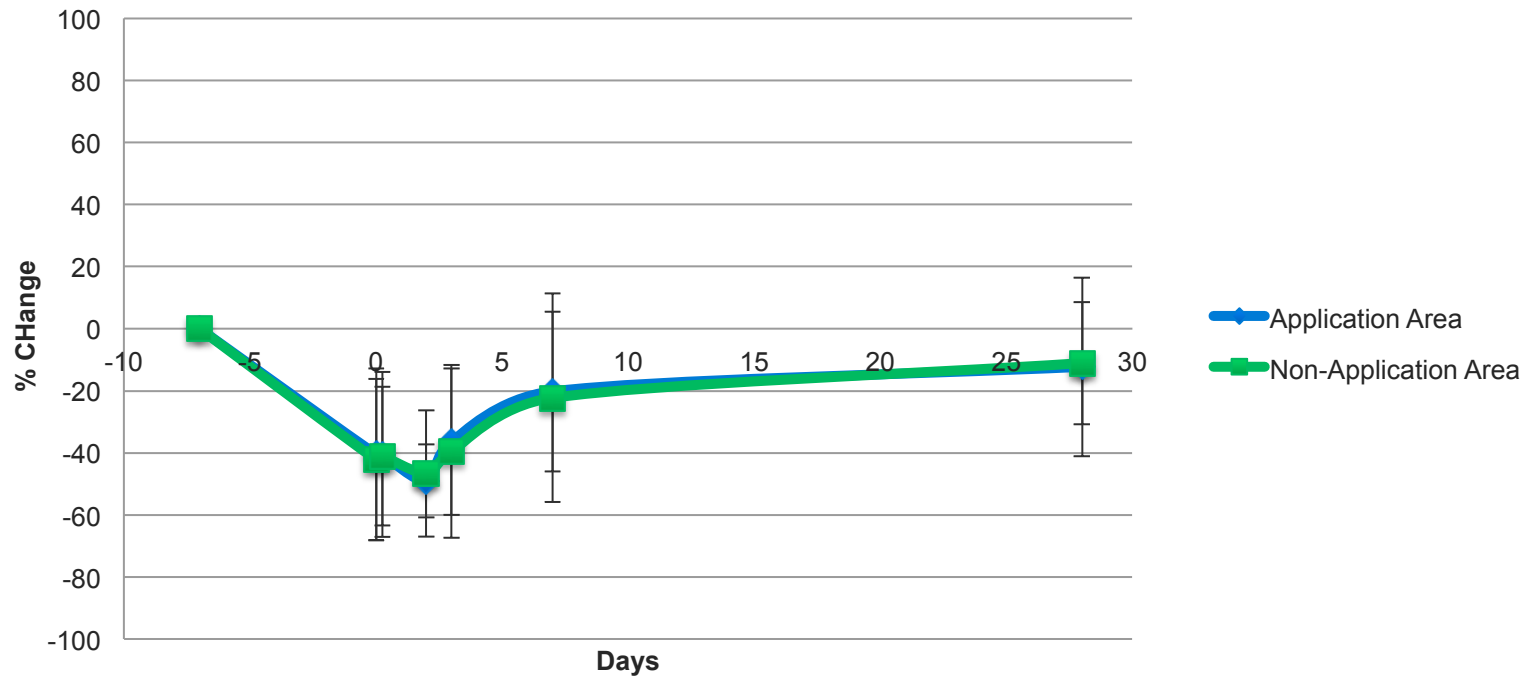
Sebum

Sebum Upper Bound



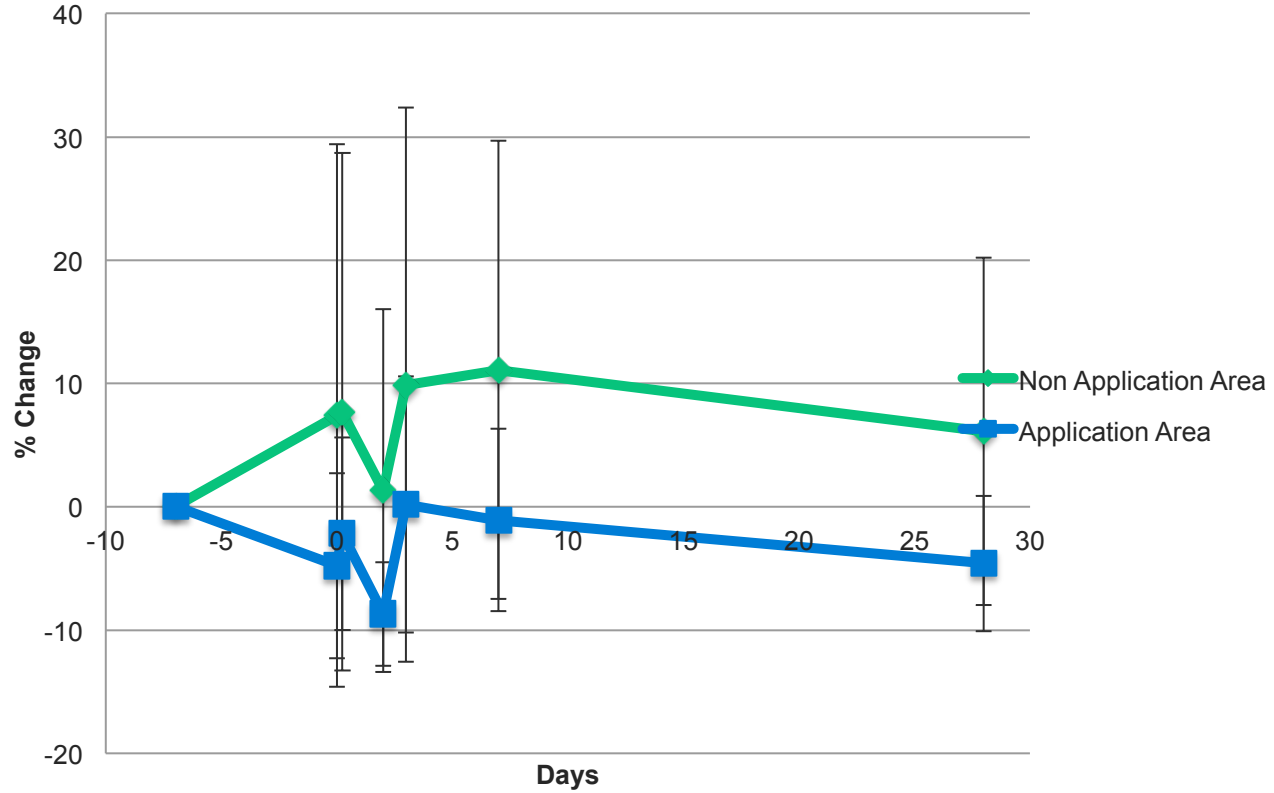
Porphyrin

Porphyrin Upper Bound

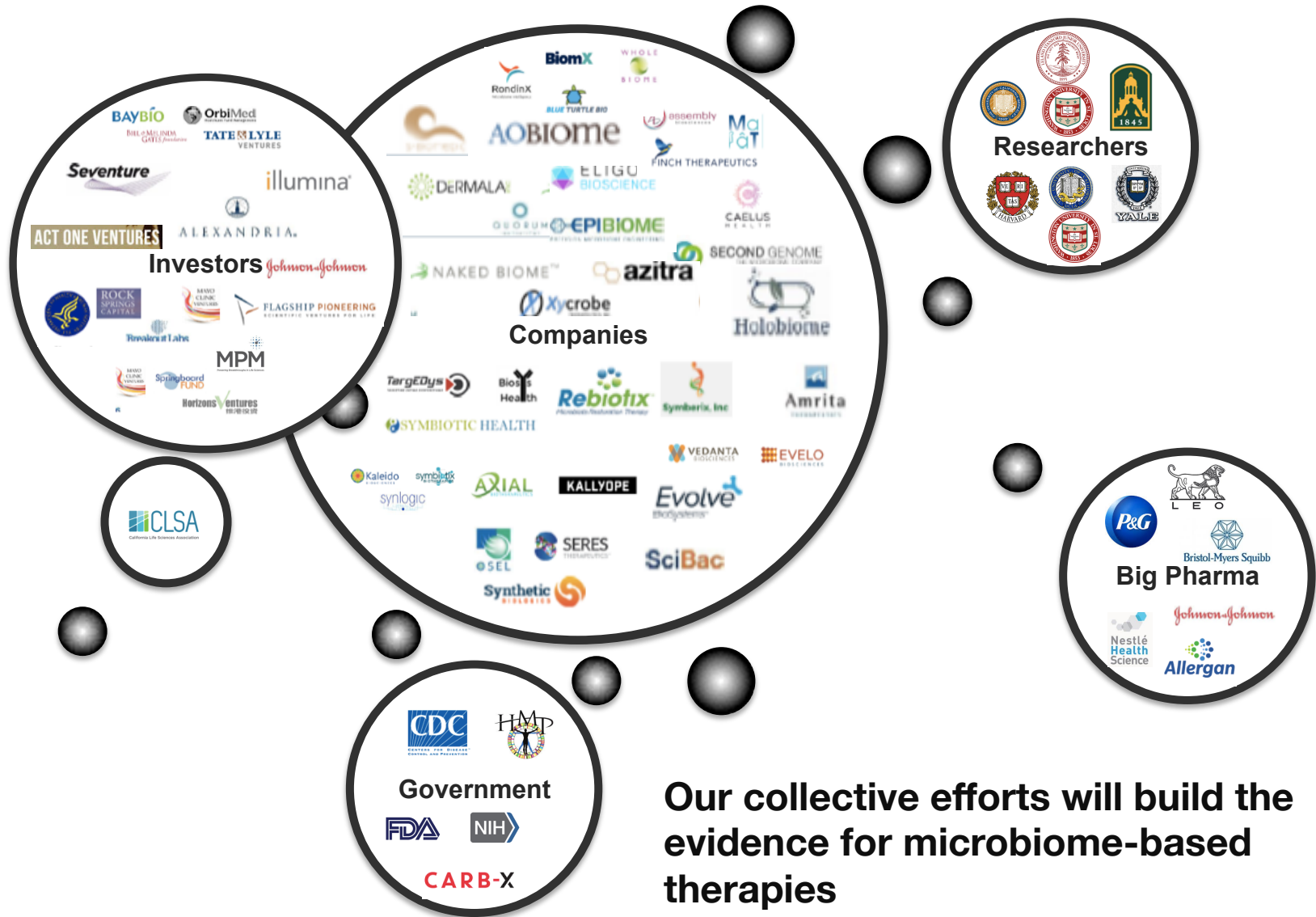


PH

PH Upper Bound

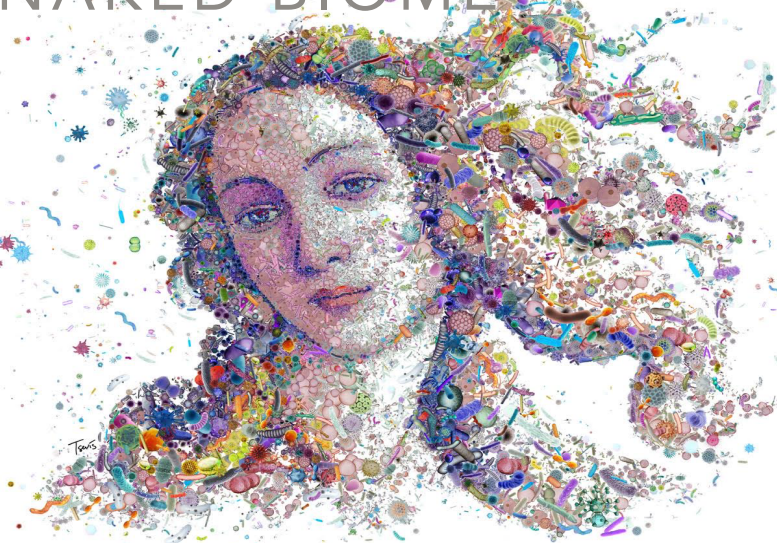


Building the Evidence



Our collective efforts will build the evidence for microbiome-based therapies

 NAKED BIOME



Presented by: Emma Taylor, MD | CEO & Co-Founder
626-260-1230 | emma.t@NakedBiome.com

Is it a Drug?

	Drug	DTC Probiotic
Time to Market	Long	Short
FDA IND/BLA	Yes	No
Cost	High	Low
Claims-treat, prevent, mitigate disease, impact structure/function of the body	Yes-clear indications for Physicians and patients, clear and validated clinical evidence	No-consumer confusion, different strains for different disease #alternativefacts
Standardization	Yes-stringent	Uncertain
Viability/stability	Well-defined	Uncertain
Safety	FDA oversight	Onus is on manufacturer to not willingly produce something harmful
Engraftment	Defined	Uncertain