Preclinical Evidence for Nitric Oxide-Releasing SB414 In a Psoriasis Animal Model

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ABSTRACT

Psoriasis is an immune-mediated inflammatory skin disease attributed to dysregulated interactions between keratinocytes and immune cells. IL-1β is a central cytokine responsible for enhancing the infiltration of activated immune cells, triggering the release of additional pro-inflammatory cytokines, and is also a key signal responsible for the maturation of naïve T cells into Th17 cells. In several inflammatory skin disorders the aberrant activation and dysregulation of this helper T-cell phenotype results in the production and release of IL-17, which binds to its cognate receptor and serves to prompt inflammasome activation and initiate a positive inflammatory feedback loop. Nitric oxide has the potential to disrupt IL-17 propagation locally in the skin via its ability to disrupt the assembly and activity of the NLRP3 inflammasome.

Topical application of imiquimod (IMQ) produces a cutaneous inflammatory phenotype on the back of BALB/c mice that is frequently utilized as a model of acute psoriasis. In this study, IMQ was applied daily to the backs of mice. The mice were then treated with SB414, a topical nitric oxide-releasing cream, BID for 10 days. Compared to untreated controls the highest dose used in this experiment, 6% SB414, demonstrated a statistically significant reduction in the psoriasis back pathology starting at day 3 and was maintained until the end of the experiment showing a 31%, 40%, and 28% decrease in composite psoriasis score, erythema and plaque score, respectively. In this model, pro-inflammatory cytokines associated with the IL-23/IL-17 axis are significantly upregulated in ear tissue after IMQ application with peak levels observed on Day 4. Compared to untreated controls, 6% SB414 treatment reduced tissue levels of pro-inflammatory cytokines: IL-1β, IL-6, IL-22, IL-17A, IL-17F and IL-33 by 84%, 55%, 64%, 87%, 71% and 71%, respectively (Study B).

These data support the potential for a nitric-oxide releasing topical treatment to reduce the pro-inflammatory cytokines associated with perturbation of the IL-23/IL-17 axis and result in a decrease of psoriasis pathology.

MATERIALS & METHODS

SB414: SB414 Cream is a two-component investigational drug product formulation comprised of an active ointment phase, containing the NVN1000 nitric oxide-releasing drug substance, and an inactive acetate buffered hydrogel phase that are mixed together at time of application to form a self-emulsifying cream. The aqueous hydrogel phase provides the proton source that initiates nitric oxide release from the active ointment phase.

IMQ-induced Psoriasis Model: Female BALB/c mice aged 7-8 weeks were assigned to treatment groups. On Study Day -1 the backs (~10% BSA) of all animals were shaved to create a dose application site. Beginning on Day 0 all animals were examined for clinical signs of disease. Topical application of IMQ cream to the backs and ears of animals results in the development of psoriasis-like lesions that are underpinned by an influx of immune cells as well as epidermal hyperplasia and abnormal epithelial differentiation. Topical IMQ began on Study day 0 and was applied to animals each morning. Each day prior to IMQ application the backs and ears of all animals were cleansed of residual IMQ or test article by gently wiping the area with moistened kimwipes. Beginning on Study Day 0 animals treated with topical test articles were dosed 2 hours following the IMQ application. A 2nd topical dose of test article was applied 4 hours after the first test article treatment. Animal body weights and clinical observations were recorded daily.

Composite Clinical Psoriasis Score: A composite clinical psoriasis score was determined on a scale of 0-11 by summing the individual plaque (0-7) and erythema (0-4) scores observed on each study day. **Ear thickness:** Right ear thickness was measured via calipers on Study Days 0, 3, 5, 8, and 10.

Study termination: On Study Day 10, animals were sacrificed and spleens were collected and weighed.

Cytokine Assessments: Punch biopsies were either obtained from the left ear (Study Day 4) or back (Study Day 2) 4 hours post-IMQ application for multiple proinflammatory cytokine analyses via Luminex. Tissue samples were homogenized and total protein concentration from each sample was determined via BCA assay. Relevant cytokines of the Th17-IL-23 axis were assessed from all treatment groups.

Statistical Analysis: Where appropriate, statistical analysis was performed using ANOVE followed by Dunnett's test to compare scores between treatment groups. P-values of less than 0.05 were considered statistically significant.

BACKGROUND

Figure 1. Nitric Oxide Affects Multiple Targets of the IL-17 Axis Inflammatory Loop in Psoriasis.

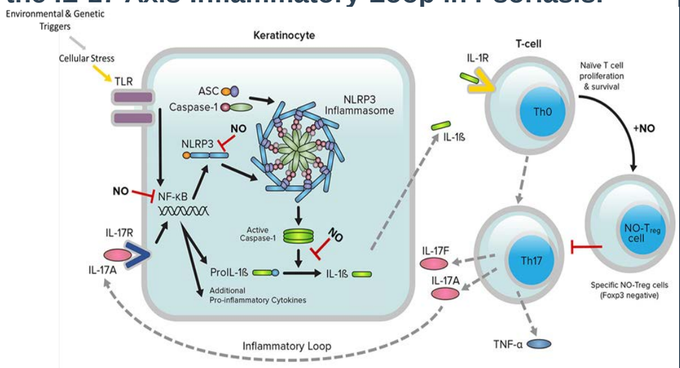


Figure 2. Nitricil Technology and Indication-specific Formulation Science.

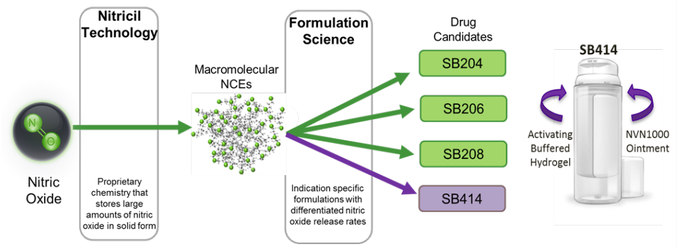


Figure 3. Imiquimod-induced Psoriasis Model Experimental Design.

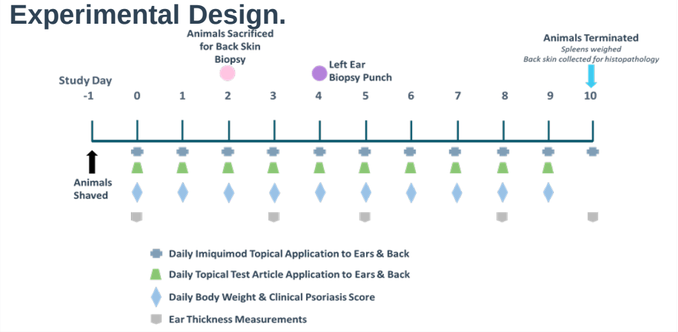


Table 1. Comparison of Psoriasis Drug Candidates in the IMQ-induced Psoriasis Model.

Treatment	Composite Clinical Psoriasis Score (% Reduction)	Epidermal Thickness (% Reduction)	Effect on Pro-inflammatory Cytokines
Etanercept[1] (SC, 10mg/kg, D1+D3)	10.2%	28.3%	Not Determined
Dexamethasone[1] (PO, 0.3mg/kg, QD)	13.5%	41.6%	Not Determined
ROR-γ[2] (GSK, Top, 1% Oint, pre-treat 3 days)	-	31.0%	Not Determined
Clobetasol[1] (Top, 0.05% Cream, QD)	60.0%	65.0%	*significantly reduced vs. disease *(ear biopsy assessment)*

Literature References:

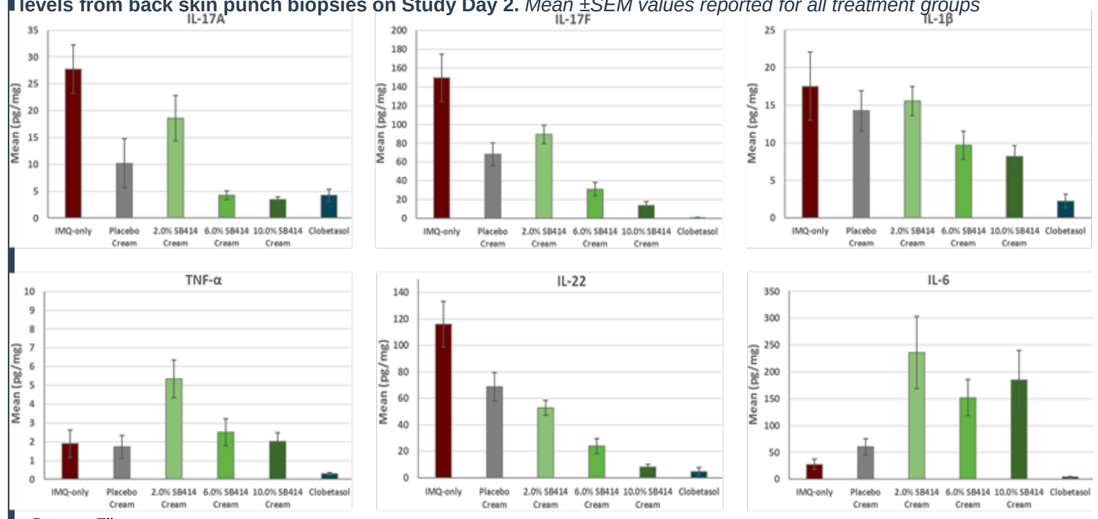
1. **Rashid**, H et al. *World Congress on Inflammation.* 2015. Charles River.
   * *Study Duration = 9 days, Trx Days 1-8.*
2. **Smith**, S et al. *PLoS One.* 2016;11(2):e0147979.
   * *Study Duration = 9 days, pre-treatment*

RESULTS

Table 2. Study A - Mean Composite Clinical Psoriasis Score. The composite clinical psoriasis score is the result of summing the individual erythema (0-4) and plaque (0-7) score on each assessment day. *Mean ±SEM values reported for all treatment groups.*

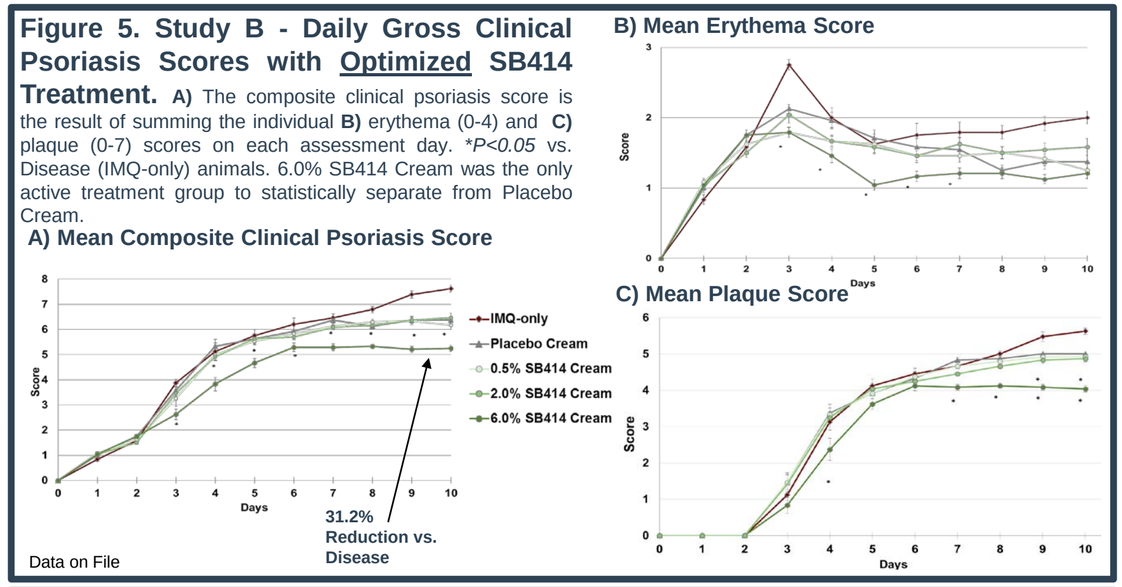
	IMQ-only	Placebo Cream	2.0% SB414 Cream	6.0% SB414 Cream	10.0% SB414 Cream	Clobetasol
Composite Clinical Psoriasis Score	8.38%	6.58%	6.96%	6.96%	7.50%	2.00%
Percent Reduction Over Disease Only	--	21%	17%	17%	10%	76%

Figure 4. Study A - Mean Pro-Inflammatory Cytokine Levels. **Pro-inflammatory cytokine levels from back skin punch biopsies on Study Day 2.** *Mean ±SEM values reported for all treatment groups*



Data on File

Figure 5. Study B - Daily Gross Clinical Psoriasis Scores with Optimized SB414 Treatment. **A)** The composite clinical psoriasis score is the result of summing the individual **B)** erythema (0-4) and **C)** plaque (0-7) scores on each assessment day. **P<0.05* vs. Disease (IMQ-only) animals. 6.0% SB414 Cream was the only active treatment group to statistically separate from Placebo Cream.



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CONCLUSIONS

- SB414 Cream topical treatment reduced pro-inflammatory cytokine levels in the backs of IMQ-treated animals.
  - IL-17a, IL-17f, and IL-1β demonstrated the greatest reduction following nitric oxide treatment
- A statistically significant (31%) reduction in the mean composite clinical psoriasis score from Study Days 3-10 vs. IMQ-only disease controls was observed with an optimized formulation (6.0% SB414 Cream).
- Based upon these promising preclinical results, and reported efficacy for approved psoriasis treatments, SB414 Cream should be explored in clinical studies for safety, tolerability, and efficacy in Psoriasis patients.