**SIG-1451: A topical anti-inflammatory new chemical entity for atopic dermatitis (AD)**

José R. Fernández, Karl Rouzard, Corey Webb, Michael Voronkov, Jason Healy, Kristen L. Huber, Jeffry B. Stock, Maxwell Stock, Joel S. Gordon, Eduardo Pérez

*Signum Dermalogix, 133 Wall Street, Princeton, NJ; Princeton University, Department of Molecular Biology, Princeton, NJ*

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### Abstract

AD is a pruritic allergic inflammatory skin disease with increasing prevalence among pediatric and adolescent age groups. Current topical treatments are associated with a range of detrimental side effects. Isopropylcysteine (IPC) small molecules represent a novel class of topically applied nonsteroidal anti-inflammatory drugs, whose action is locally restricted. We demonstrate here the use of a novel IPC analog, SIG-1451 for AD in multiple cell-based assays targeting key pro-inflammatory cytokines that drive AD allergic pathogenesis. In human PBMCs, SIG-1451 inhibits IL-4 cytokine release elicited by OX3/DC28 (≤ 20 µM) and abrogates a N\(^\text{p}\)-TLR4 response in endothelial cells by reducing IL-6 (IC\(50\) = 0.02 µM). In NHEKs, SIG-1451 inhibits S. aureus-induced release of TSLP (IC\(50\) = 3 µM). SIG-1451 activity in vitro is equal to or more potent than topical AD therapies, ANZ2728, with the exception of the inhibition of IL-4 induction by ANZ2728. Allergic responses are characterized by early and late phases, possibly representing different inflammatory pathways. Thus, we suggest SIG-1451’s stronger inhibition of IL-4 production would predict a greater effect than ANZ2728 on the early inflammatory phase, while it would be less effective in targeting the late phase characterized by IL-4 production. Utilizing in vivo models, SIG-1451 exhibits anti-inflammatory activity in the TPA acute inflammation ear model. Moreover, in the delayed type hypersensitivity (DTH) oxazolone mouse model, which involves both early and late phases, SIG-1451 has higher potency than ANZ2728, reducing edema and has similar effect on blocking IL-4 production, possibly due to SIG-1451’s greater effect on early phase pathways. Based on these data, SIG-1451 represents a novel therapeutic approach for topical treatment of atopic dermatitis.

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### Fig. 1. SIG-1451: Multiple targets in atopic dermatitis pathogenesis

Histopathology of AD skin lesions reveals an intense mononuclear cell infiltrate in the dermis with T cells playing a critical role in inducing and maintaining inflammatory cutaneous conditions. In the acute stage of AD, the predominant phenotype is a Th2/Th17 immune response, while chronic AD lesions are primarily Th2. The cytokines produced in these skewed immune responses have received great attention as potential targets for therapeutic intervention. Activation of Toll-like receptor-4 (TLR4) signaling via several ligands (e.g. N\(^\text{p}\), S. aureus, and LPS) in endothelial cells, keratinocytes and monocytes also contributes to the developing inflammatory response resulting in AD. Thus, effectively targeting both TLR and Th2/Th17 cytokine signaling provides a novel therapeutic approach for topically treating atopic dermatitis.

### Fig. 2. SIG-1451 inhibits N\(^\text{p}\)-TLR4-induced inflammation in HDMECs

Human dermal microvascular endothelial cells (HDMECs) were pre-treated with compounds for 2 hours and later cultured in the presence of either SIG-1451 or new AD therapeutic ANZ2728. SIG-1451 represents a small molecule to the agonist candidate and the endogenous nickel sulfate. Media supernatants were collected after 6 hours and analyzed by ELISA for Interleukin-6 (IL-6) levels. Data represents average ± SEM of a representative set from 3 independent experiments. IC\(50\) values were determined by non-linear regression analysis using the four-parameter logistic equation.

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### Fig. 3. SIG-1451 inhibits Th2 and Th17 activation in PBMCs

Peripheral blood mononuclear cells (PBMCs) were cultured in the presence of each compound at various concentrations followed by addition of the stimulant anti-CD3/CD28 or 20µg/mL Concanavalin A. Media supernatants were collected after 24-48 hours and analyzed by ELISA for IL-4 and IL-17 levels. Data represents average ± SEM of a representative set from 3 independent experiments. IC\(50\) values were determined by non-linear regression analysis using the four-parameter logistic equation.

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### Fig. 4. SIG-1451 Inhibits S. aureus induced TSLP production in NHEKs

Normal Human Epidermal Keratinocytes (NHEKs) were cultured in the presence of each compound at various concentrations followed by addition of the stimulant S. aureus (ATCC\(\text{C}\) 33581\%). Media supernatants were collected after 24 hours and analyzed by ELISA for Thymic stromal lymphopoietin (TSLP) levels. Data represents average ± SEM of a representative set from 3 independent experiments. IC\(50\) values were determined by non-linear regression analysis using the four-parameter logistic equation.

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### Fig. 5. SIG-1451 inhibits Calcipotriol-induced TSLP in vivo

CD1 mice were topically exposed to 4 nmol/ear Calcipotriol. Five minutes after challenge compounds were applied. Twenty-four hours later, protein samples were extracted from ear skin biopsies and analyzed for TSLP levels by ELISA method. Vehicle for compounds were 60.40 EIOH:H\(2\)O (SIG-1451) or 1:1 EIOH:Acetone (ANZ2728). Data represents average ± S.E. of a representative set from 2 independent experiments (n=6 mice per group). \(p<0.05\), \(p<0.01\) by Student t test compared to Calcipotriol + vehicle-only treated animals, ns, not significant.

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### Summary/Conclusions

- **SIG-1451 inhibits the induction of several pro-inflammatory mediators of AD inflammation in cell culture models.**
- **Topical applications of SIG-1451 reduces the full range of acute inflammation by chemical-induced skin inflammation evidenced by edema.**
- **SIG-1451 inhibits the contact allergic inflammatory response in animal models, and in vitro models suggesting application of IPC analogs on skin can inhibit adaptive and innate immune responses.**
- **SIG-1451 and ANZ2728 both target Th\(\text{1}\) responses, SIG-1451 is superior vs Th\(\text{1}7\) responses and is active in contact allergy models where ANZ2728 is ineffective.**
- **SIG-1451 is a safe and novel topical IPC small molecule for the treatment of atopic dermatitis and potentially other dermatological disorders.**
- **SIG-1451 was nominated for clinical development and we have commenced preclinical safety/toxicological evaluations towards IND submission in Q1/Q2 2018.**

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