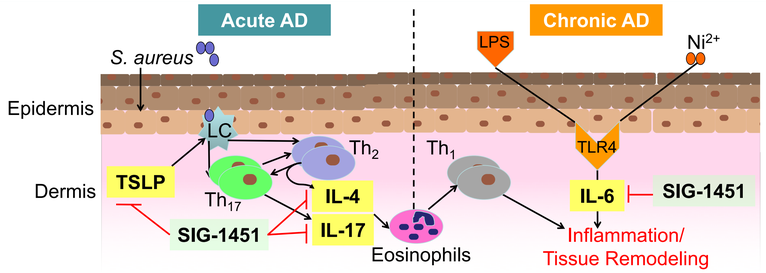
SIG-1451: A topical anti-inflammatory new chemical entity for atopic dermatitis (AD)

José R. Fernández[1], Karl Rouzard[1], Corey Webb[1], Michael Voronkov[1], Jason Healy[1], Kristen L. Huber[1], Jeffry B. Stock[1,2]
Maxwell Stock[1], Joel S. Gordon[1], Eduardo Pérez[1]
[1]Signum Dermalogix, 133 Wall Street, Princeton, NJ; [2]Princeton University, Department of Molecular Biology, Princeton, NJ

Abstract

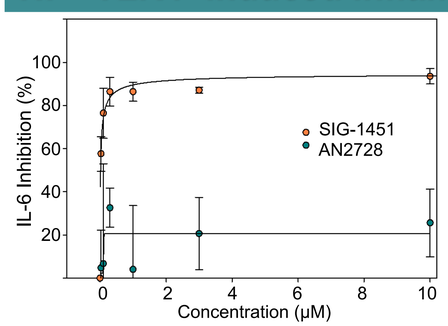
AD is a pruritic allergic inflammatory skin disease with increasing prevalence among pediatric and adolescent age groups. Current topical treatments are associated with a range of detrimental side effects. Isoprenylcysteine (IPC) small molecules represent a novel class of topically applied non-steroidal 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 CD3/CD28 (IC_{50} = 20 µM) and abrogates a Ni^{2+}-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, AN2728, with the exception of the inhibition of IL-4 induction by AN2728. Allergic responses are characterized by early and late phases, possibly representing different inflammatory pathways. Thus, we suggest SIG-1451's stronger inhibition of IL-6 production would predict a greater effect than AN2728 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 AN2728, 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 findings, SIG-1451 represents a drug development candidate and we have commenced preclinical safety evaluations towards IND submission in Q1/Q2 2018.

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 Th_2/Th_{17} immune response, while chronic AD lesions are primarily Th_1. 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. Ni^{2+}, *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 $Th_1/Th_{17}/Th_2$ cytokine signaling provides a novel therapeutic approach for topically treating atopic dermatitis.

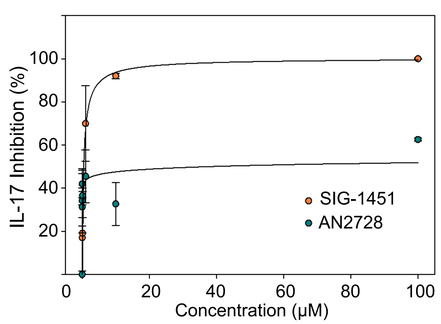
Fig 2. SIG-1451 inhibits Ni^{2+}-TLR4 -induced inflammation in HDMECs



Compound	IL-6 IC_{50} (µM)
SIG-1451	0.02
AN2728	>30

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 AN2728 at various concentrations simultaneous to addition of the stimulant nickel sulfate. Media supernatants were collected after 6 hours and analyzed by ELISA for Interleukin-6 (IL-6) levels. Data represents average ± StDev of a representative set from 3 independent experiments. IC_{50} values were determined by non-linear regression analysis using the four-parameter logistic equation.

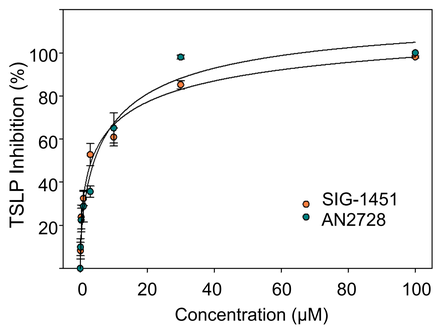
Fig 3. SIG-1451 inhibits Th_2 and Th_{17} activation in PBMCs



Compound	IL-4 IC_{50} (µM)	IL-17 IC_{50} (µM)
SIG-1451	20	0.5
AN2728	2	3

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 ± StDev of a representative set from 3 independent experiments. IC_{50} values were determined by non-linear regression analysis using the four-parameter logistic equation.

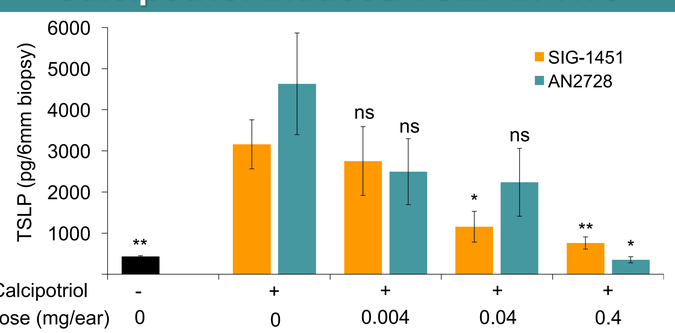
Fig 4. SIG-1451 inhibits *S. aureus* induced TSLP production in NHEKs



Compound	TSLP IC_{50} (µM)
SIG-1451	3
AN2728	5

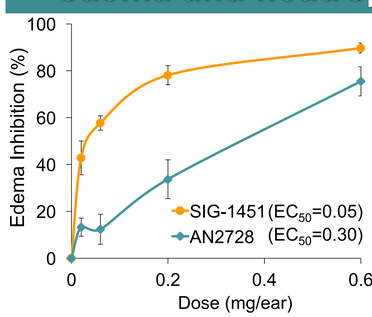
Normal Human Epidermal Keratinocytes (NHEKs) were cultured in the presence of each compound at various concentrations followed by addition of the stimulant *S. aureus* (ATCC® 33591™). Media supernatants were collected after 24 hours and analyzed by ELISA for Thymic stromal lymphopoietin (TSLP) levels. Data represents average ± StDev of a representative set from 3 independent experiments. IC_{50} values were determined by non-linear regression analysis using the four-parameter logistic equation.

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 EtOH:H_20 (SIG-1451) or 1:1 EtOH:Acetone (AN2728). Data represents average ± S.E. of a representative set from 2 independent experiments (n=6 mice per group). *p value < 0.05; **p value ≤ 0.01 by Student *t* test compared to Calcipotriol + vehicle-only treated animals. ns, not significant

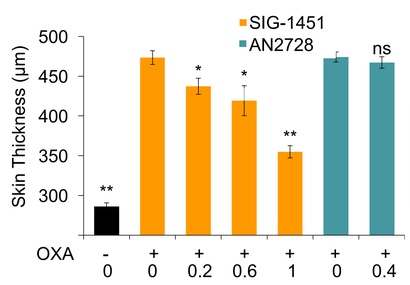
Fig 6. SIG-1451 reduces TPA-induced edema and neutrophil infiltration *in vivo*



Dose (mg/ear)	Inhibition (%) Edema SIG-1451	Edema AN2728	MPO Activity SIG-1451	MPO Activity AN2728
0.02	43 ± 7	13 ± 4		
0.06	58 ± 5	12 ± 6	48 ± 12	73 ± 3
0.2	78 ± 3	34 ± 8	88 ± 5	89 ± 2
0.6	90 ± 2	76 ± 6		

Swiss-Webster mice were topically exposed to 1.2 µg/ear TPA. Five minutes after TPA application, test compounds were applied. Twenty-four hours later, skin thickness (edema) was measured. Vehicle for compounds were 60:40 EtOH:H_20 (SIG-1451) or 1:1 EtOH:Acetone (AN2728). Protein samples were extracted from skin biopsies and analyzed for MPO activity as marker of neutrophil infiltration. Data represents average ± S.E. of cumulative data from 2-3 independent experiments (n=12-18 mice per group). EC_{50} values were determined from curve fitting using 4-parameter logistic equation. * p value < 0.05, ** p value < 0.01 by Student *t* test compared to TPA+vehicle treated animals.

Fig 7. SIG-1451 outperforms AN2728 in the oxazolone-DTH mouse model



Compound	Dose (mg/ear)	Inhibition (%) Edema	IL-4	MPO
SIG-1451	0.2	23 ± 2	33 ± 9	22 ± 9
	0.6	53 ± 5	41 ± 10	67 ± 23
	1	72 ± 4	62 ± 11	81 ± 5
AN2728	0.4	17 ± 5	76 ± 9	36 ± 12

Balb/c mice backs were shaved and 100 µl of 3% w/v oxazolone (OXA) in acetone/olive oil (4:1) was applied to dorsal skin for sensitization. 7 days after, ears were challenged with 0.5% w/v OXA and 30 minutes after challenge, compounds were applied. Vehicle for compounds were 60:40 EtOH:H_20 (SIG-1451) or 1:1 EtOH:Acetone (AN2728). 24 hours later, skin thickness (edema) was measured. Protein samples were extracted from skin biopsies and analyzed for IL-4 levels by ELISA method and MPO activity as marker for macrophage activity. Data represents average ± S.E. of cumulative data from three independent experiments (n=12-15 mice per group). * p value < 0.05, ** p value < 0.01 by Student *t* test compared to Oxazolone + vehicle-only treated animals.

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 AN2728 both target Th_2 responses, SIG-1451 is superior vs Th_{17} responses and is active in contact allergy models where AN2728 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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