Preclinical Anti-inflammatory Activity and Safety Assessment of SIG990, a Novel Topical Small Molecule for the Treatment of Rosacea

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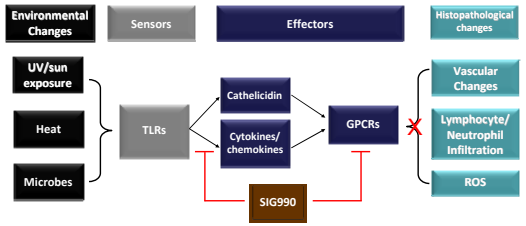
Abstract

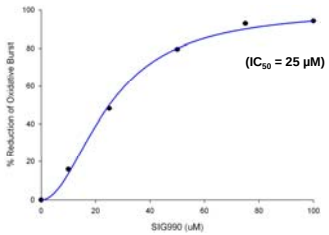
Isoprenylcysteine (IPC) analogs are structural mimics of the lipidated C-termini of the Gγ subunit of all heterotrimeric G-proteins and small G-proteins, which elicit many inflammatory responses such as the release of pro-inflammatory mediators, and the migration and activation of inflammatory cells. We have previously shown that a novel IPC analog SIG990, could inhibit TPA-induced erythema, edema, neutrophil infiltration and induction of several cytokines (TNF-α, IL-6, IL-8) *in vivo* (SID 2010 poster # 118) and that IPC analogs reduce toll-like receptor (TLR) inflammatory signaling *in vitro* (SID 2010 poster #761). Patients with rosacea over-express TLR-2, activating the cutaneous innate immune response thus acting as a key element in disease pathogenesis. Furthermore, activation of G-protein coupled receptor, formyl peptide receptor-like 1 (FPRL1) and reactive oxygen species (ROS) release have also been proposed to be involved in the pathogenesis of rosacea. Utilizing human keratinocytes, endothelial cells and differentiated neutrophils we show SIG990 inhibits both TLR2-induced cytokine production and FPRL1-induced ROS and cytokine release. *In vivo* experiments demonstrate SIG990 when applied topically inhibits LL-37 induced cutaneous inflammation and neutrophil infiltration. Our results demonstrate SIG990 is a potential therapeutic candidate possessing a novel multi-faceted anti-inflammatory activity towards TLR and GPCR signaling. Moreover, SIG990 has undergone a comprehensive preclinical safety evaluation and was found to have an excellent topical and systemic safety profile. This new chemical entity will be the subject of an Investigational New Drug application targeting rosacea.
(see also Poster # 567: IPC analogs inhibit Langerhans cell (LC) antigen presenting capability)

Fig 1. Molecular Pathogenesis of Rosacea: SIG990 Inhibits GPCR and TLR Signaling



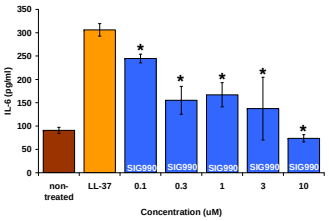
Several challenges including UV light, sun exposure, heat and microbes are sensed directly or indirectly by toll-like receptors (TLRs) in skin cells. TLR signaling induces cathelicidin (LL-37) and pro-inflammatory cytokine/chemokine expression. Once produced, LL-37 and several cytokines act as ligands for G-protein-coupled receptors (GPCRs) activating further inflammatory signaling. Together, these effector molecules elicit vascular changes, neutrophil recruitment, lymphocyte infiltration and ROS production which contribute to the pathogenesis of rosacea. *In vitro* and *in vivo* results demonstrate SIG990 blocks both GPCR, TLR inflammatory signaling and cytokine production.
(Figure above was adapted from K. Yamasaki et al., J. Derm Sci, 2009. 55(2): 77-81)

Fig 2. SIG990 Inhibits fMLP-GPCR Induced ROS Release in Neutrophils



Extracellular inflammatory agonists such as fMLP bind to GPCRs such as formyl peptide receptors (FPRs), to trigger the oxidative burst response (rapid release of ROS). Oxidative burst was measured as previously described (J. Ding *et al.*, JBC, 1994. 269 (24): 16837-44). 1 x 10^6 HL-60 cells were differentiated with 1.3% DMSO for 5 days resulting in differentiated neutrophils (dHL-60). Cells were pre-incubated with SIG990 at various concentrations (0 to 100 μM) for 10 min, then burst initiated with addition of fMLP (200 nM). Supernatant removed and absorbance measured at 550 and 556.5 nM.

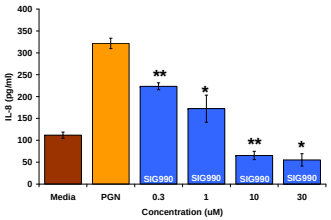
Fig 3. SIG990 Inhibits LL-37-GPCR Induced Cytokine Release in Endothelial Cells



Compound	LL-37 induced IL-6 release IC_{50} (μM)
SIG990	2
Doxycycline	6
Metronidazole	14
Oxymetazoline	14
Azelaic acid	100
Tacrolimus	1
Clobetasol	0.008

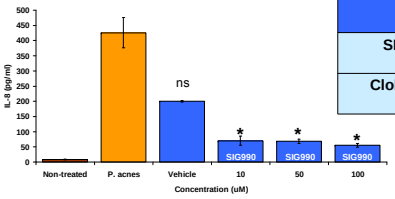
Primary Human Dermal Microvascular Endothelial Cells (HDMECs) were co-treated with cathelicidin (LL-37, 10 μg/ml) and compounds for 24 hours. IL-6 cytokine production was measured by ELISA. Compounds including SIG990, several other rosacea therapeutics (doxycycline, metronidazole, azelaic acid, oxymetazoline) as well as tacrolimus primarily used for atopic dermatitis and clobetasol, were tested at several concentrations (0 to 100 μM). The IC_{50} is the concentration of compound producing half maximal inhibition. Vehicle had no effect on IL-6 levels. (*p value < 0.05)

Fig 4. SIG990 Inhibits TLR2-Induced Cytokine Release in Keratinocytes



Compound	PGN induced IL-8 release IC_{50} (μM)
SIG990	0.8
Clobetasol	1.0

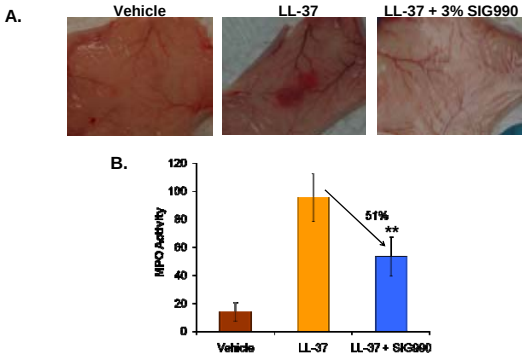
Sub-confluent Primary Human Epidermal Keratinocytes (NHEKs) were pre-treated with SIG990 or clobetasol for 30 min, then co-treated with *S. aureus*-derived peptidoglycan (PGN, 10 μg/ml) for 72 hours. IL-8 cytokine production was measured by ELISA. Compounds were tested at several concentrations (0 to 30 μM). The IC_{50} is the concentration of compound producing half maximal inhibition. Vehicle had no effect on IL-8 levels. Asterisk indicates statistical significance (**p< 0.01; *p< 0.05).



Compound	*P. acnes* induced IL-8 release IC_{50} (μM)
SIG990	3.0
Clobetasol	1.0

Sub-confluent NHEKs were pre-treated with SIG990 or clobetasol for 30 min, then co-treated with live *P. acnes* culture (1x10^7 CFU/ml) for 24 hours. IL-8 cytokine production was measured by ELISA. The IC_{50} is the concentration of compound producing half maximal inhibition. Asterisk indicates statistical significance (*p< 0.05; ns = not significant).

Fig 5. SIG990 inhibits LL-37 Induced Inflammation in a Murine Model of Rosacea



A previously described mouse model of rosacea was used to investigate the effect of SIG990 *in vivo* (*Yamasaki et al., Nat Med, 2007. 13(8): 975-80; Zhang et al., PLoS One, 2011. 6(2): e* 16658). Intradermal injection of LL-37 into Balb/c mice at 12 hour intervals for 48 hours (5 mice per group) produced cutaneous erythema, prominent intradermal neutrophil infiltration and myeloperoxidase (MPO) activity. Topical treatment of SIG990 in a 3% gel formulation after LL-37 injection decreased (a) macroscopic signs of inflammation and erythema in inverted dorsal skins (B) and myeloperoxidase (MPO) activity, a marker for neutrophil infiltration. (**p value < 0.01)

Fig 6. Summary of SIG990 Toxicity Studies

Study Name	Species	Dose/Strength	Result
28 Day Topical Safety	Minipig	1, 3, 5, 10%	No significant dermal irritation at all doses
28 Day Systemic Safety	Rat	5, 20 and 70 mpk	NOAEL = 5 mpk
Mouse Ear Swelling Test (MEST)	Mouse	3%	No skin sensitization activity
Ames test	*in vitro*	10 uM	No mutagenic activity
7 Day Dermal Tolerability	Mini pig	1, 3, 5, 10%	No tolerability issues
Primary Dermal Irritation Study	Rabbits	3%	No irritation
Oral Gavage PK Study	Rat	20 mpk	Cmax = 492 ng/mL; AUC_{INF} =1507.1 ng•hr/mL; F = 5.3%
Organ Distribution Study	Mouse	3 mpk	After 24 hrs >99.5% of the dosed radio-labeled material was cleared

Summary/Conclusions

- **SIG990 inhibits key sensors and effectors that contribute to the molecular pathogenesis of rosacea:**
  - **inhibits TLR-NHEK induced cytokine production**
  - **Inhibits fMLP-FPRL1 induced ROS production in neutrophils**
  - **inhibits LL-37 activation of FPRL1 induced pro-inflammatory cytokine production *in vitro***
- **Topically applied SIG990 inhibits LL-37 induced neutrophil infiltration and erythema in a mouse model, suggesting a potential effect in LL-37 mediated inflammation in rosacea**
- **SIG990 is a safe and novel^ topical small molecule in development for the treatment of rosacea with a target IND filing date of Q2/Q3 2011**

^ Patent pending

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