IRAK4 Degrader GS-6791 Inhibits IL-1- and IL-36-Driven Responses in Skin Epithelial Cell Systems and Demonstrates Efficacy in a Mouse Model of Dermatitis

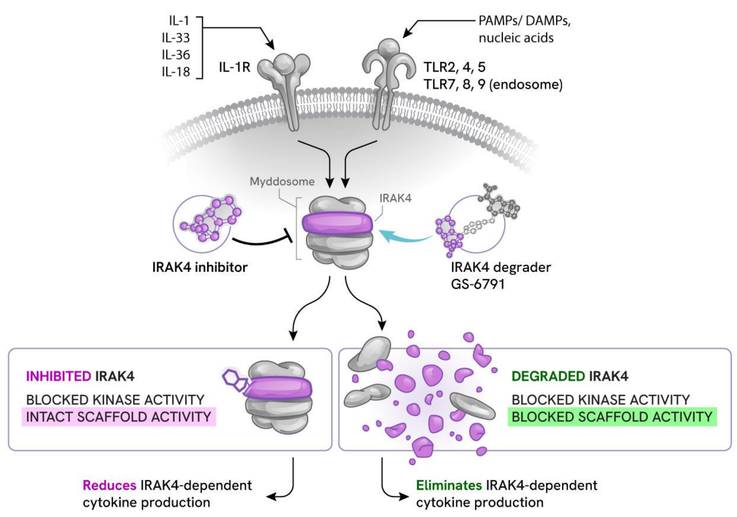
Ceyda Llapashtica[1], Grace Teng[1], Zhiyu Huang[1], Maria Mouchess[1], Julia Wu[1], Vivian Barry[1], Sunish Mohanan[1], Annamaria Mocciaro[2], Adam Schrier[1], Wylie S. Palmer[2], Alexandra Borodovsky[2], Gundula Min-Oo[1]

[1]Gilead Sciences, Inc., Foster City, CA, United States; [2]Nurix Therapeutics, Inc., San Francisco, CA, United States

Background

- IRAK4 plays a critical role in toll-like receptor (TLR) and interleukin-1 family receptor (IL-1R) signaling, to induce NFκB-, IRF-, and p38-driven inflammatory responses[1]
- IRAK4 performs both scaffold and kinase functions within the myddosome, and targeted protein degradation of IRAK4 provides a differentiated MOA from IRAK4 kinase inhibition
- The IRAK4 scaffold has been shown to be particularly critical in IL-1-mediated signaling across diverse cell types, but the mechanism has not been fully elucidated[2,3,4]
- GS-6791 is an IRAK4 protein degrader, discovered in joint research partnership with Nurix Therapeutics, that demonstrates deep and sustained inhibition of responses to TLR ligands and IL-1-family cytokines in immune and non-immune cells relevant to inflammatory diseases

IRAK4 is a Central Node Governing Inflammatory Responses



Methods

Cellular assays: Human PBMC or whole blood were treated with GS-6791 for 24 h and IRAK4 levels were evaluated via flow cytometry. PBMC were pre-treated with GS-6791 for 6 h, stimulated with TLR7/8 agonist, R848 (1 μg/mL) for 20 h, and cytokines were measured in supernatants by MSD. Human basal or differentiated keratinocytes were pre-treated with GS-6791 for 24 h, and IRAK4 levels were evaluated by Western blot. After stimulation (48 or 72 h) with 100 ng/mL IL-1β or IL-36α, cytokines were measured in culture supernatants by MSD, and gene expression was measured by qRT-PCR. For *in* vitro differentiation of primary human basal keratinocytes, the cells were plated on Transwell inserts. Once confluent, culture medium was supplemented with $CaCl_2$. EpiDerm RHE tissue models were purchased from MatTek. RHE were pre-incubated with compound for 24 hours, then stimulated with 100 ng/mL recombinant IL-1β or IL-36α for 48-72 hours. Cytokines and chemokines were measured in culture supernatants by MSD.

***In vivo* IL-1β challenge model**: Mice were dosed with GS-6791 in chow starting five days prior to intraperitoneal challenge with 200 ng of IL-1β. Splenocyte and skin tissue IRAK4 protein levels were assessed by Western blot. Plasma cytokines were assessed 2 h after IL-1β-challenge.

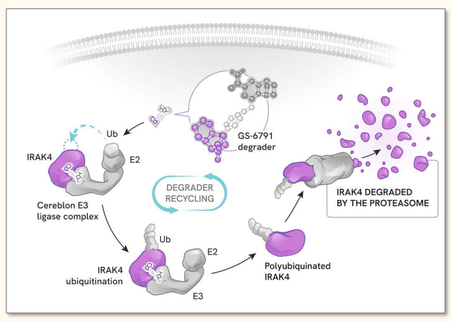
Mouse dermatitis study: Animals were dosed with GS-6791 PO QD starting two days prior to disease induction. Dermatitis was induced by repeated topical exposure to SDS and *S. aureus* Protein A (SpA)[5]. Skin clinical scores were evaluated at baseline, then daily starting on the third day after disease induction. Trans-epithelial water loss (TEWL) was measured every other day, starting one day prior to disease induction. Splenocyte IRAK4 protein levels were assessed by Western blot.

Results

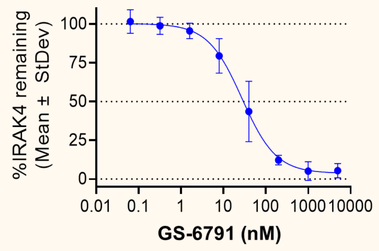
Figure 1: GS-6791 is a potent, bifunctional IRAK4 protein degrader

GS-6791 recruits IRAK4 to the Cereblon E3 ligase complex, which targets IRAK4 for proteasomal degradation

A



B IRAK4 Degradation in Human Blood



Tissue	DC_{50} (nM)	D_{max} (%)
PBMC	2.3	91
Whole Blood	34	95

C Functional Inhibition of TLR responses in human PBMC

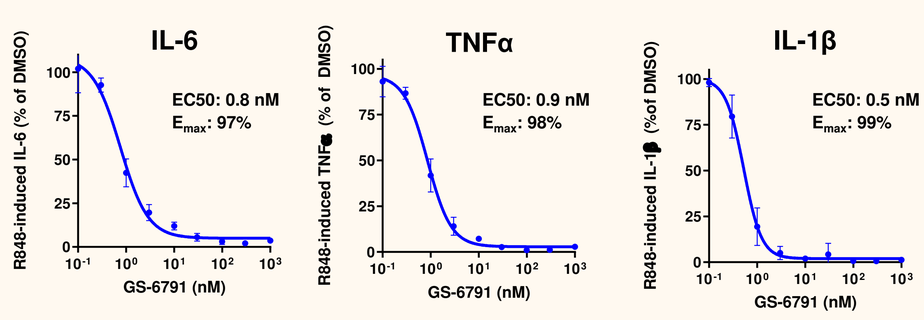
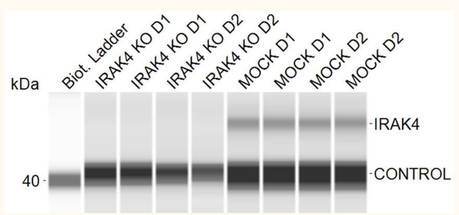


Figure 1. **(A)** Mechanism of IRAK4 protein degradation. **(B)** *In vitro* GS-6791-mediated IRAK4 degradation was assessed in human whole blood by flow cytometry after 24 h in n=11 donors. **(C)** Human PBMC were treated with GS-6791 and stimulated with the TLR7/8 agonist R848 (1 μg/mL). Cytokine production was measured using MSD, and EC_{50} (half-maximal inhibitory) potency values were calculated from n=3 donors. E_{max} = maximal percent inhibition.

Figure 2. IRAK4 Knockout in Primary Keratinocytes Reduces Responsiveness to IL-1 Family Cytokines

A IRAK4 protein measurement



Functional Responses to IL-1β and IL-36α in human keratinocytes

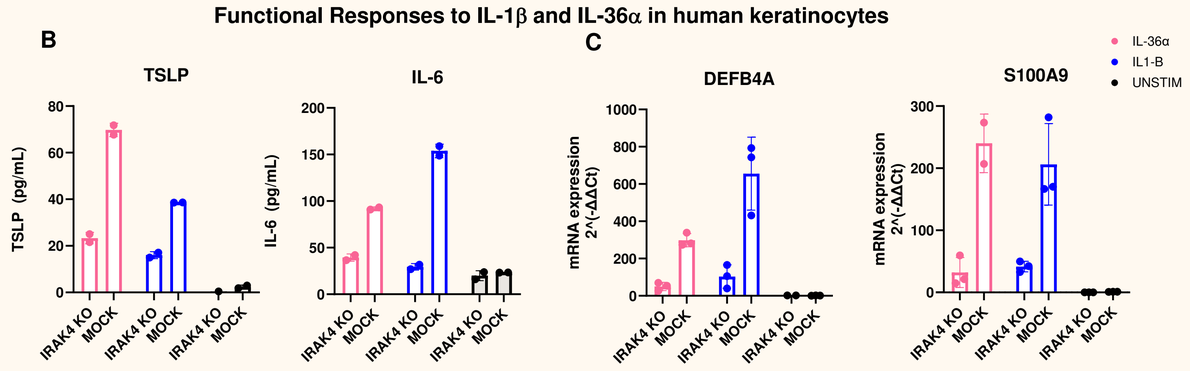


Figure 2. ***IRAK4* KO keratinocytes show reduced functional responses to IL-1β and IL-36α (A)** IRAK4 protein levels were assessed by Western blot in basal keratinocytes from two donors (D1, D2) after *IRAK4* or mock CRISPR KO. **(B)** IL-1-dependent cytokine secretion or **(C)** expression of IL-1 induced antimicrobial peptides associated with inflammatory skin disease (*DEFB4A, S100A9*) was measured after 72 h stimulation with IL-1β or IL-36α

Figure 3. GS-6791 Inhibits IL-1/36 Responses in Basal Human Keratinocytes

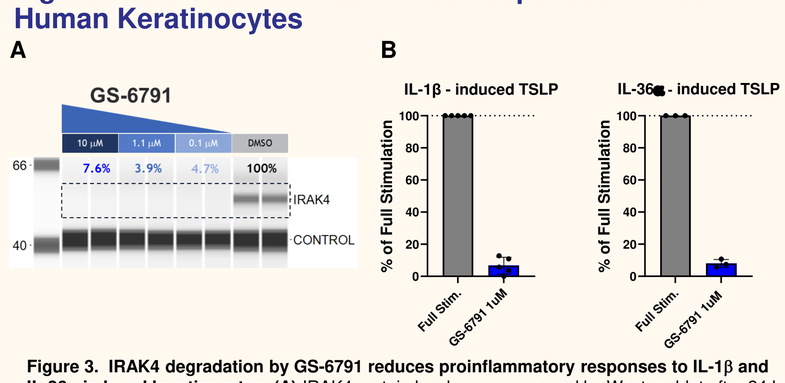


Figure 3. IRAK4 degradation by GS-6791 reduces proinflammatory responses to IL-1β and IL-36α in basal keratinocytes. (A) IRAK4 protein levels were assessed by Western blot after 24 h pre-treatment with IRAK4 degrader **(B)** IL-1β or IL-36α-induced TSLP was measured by MSD (range of TSLP production was 1-340 pg/mL). Data were derived from 5 donors.

Figure 4. GS-6791 Inhibits IL-1 and IL-36-Induced Functional Responses in Differentiated Human Keratinocytes

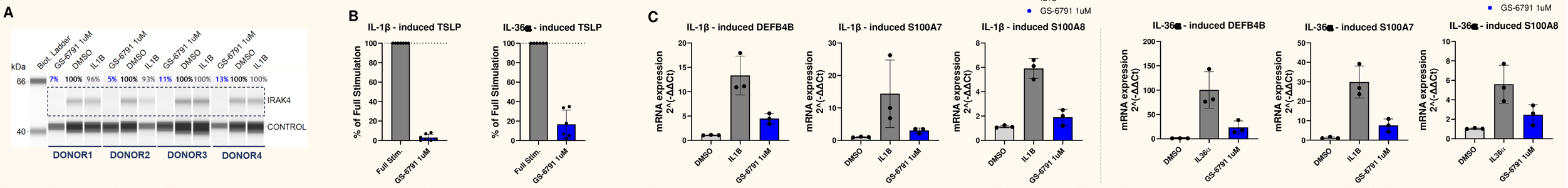


Figure 4. IRAK4 degradation by GS-6791 reduces functional responses to IL-1β and IL-36α in differentiated keratinocytes. (A) IRAK4 protein levels were assessed by Western blot after treatment with IRAK4 degrader. Effect of IRAK4 degradation on IL-1β or IL-36α-induced **(B)** TSLP secretion (range 0.5-2387 pg/mL) or **(C)** *DEFB4B*, *S100A7, S100A8* expression was measured by qRT-PCR. Data were derived from 3-5 donors.

Figure 5. GS-6791 Inhibits IL-1 and IL-36-Induced Cytokine Production in Reconstructed Human Epidermis (RHE)

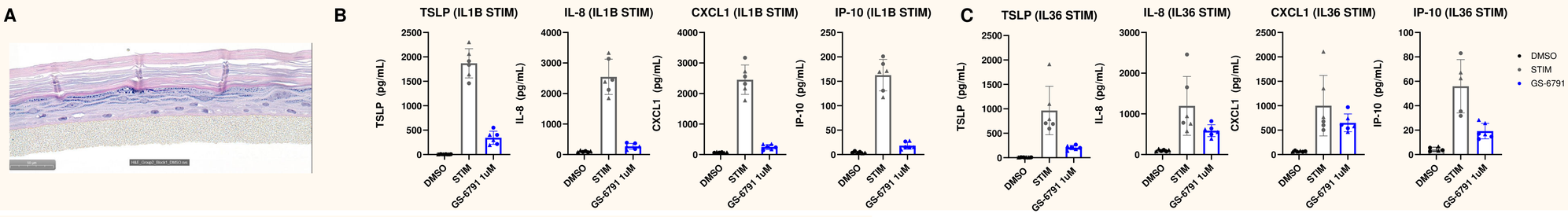


Table 1. Selected keratinocyte response genes

Gene Symbol	Gene Name	Assay ID
DEFB4A	Defensin Beta 4A	Hs00175474_m1
DEFB4B	Defensin Beta 4B	Hs00823638_m1
S100A7	S100 Calcium Binding Protein A7	Hs01923188_u1
S100A8	S100 Calcium Binding Protein A8	Hs00374264_g1
S100A9	S100 Calcium Binding Protein A9	Hs00610058_m1

Figure 5. GS-6791 reduces proinflammatory responses to IL-1β and IL-36α in 3D reconstructed human epidermis (A) Representative histological section of H&E-stained RHE **(B)** IL-1β or **(C)** IL-36α-induced cytokines and chemokines were measured by MSD. Replicate values derived from 2 donors are shown (Donor 1 ●, Donor 2 ▲)

Figure 6: GS-6791 inhibits IL-1β dependent cytokine induction *in vivo*

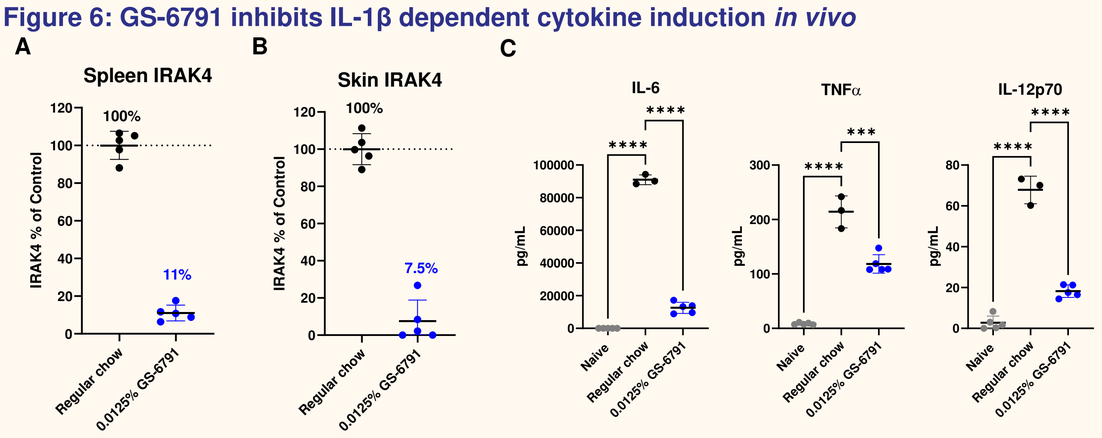


Figure 6. GS-6791 reduces *in vivo* responses to IL-1β. Mice were dosed with 0.0125% GS-6791 in chow starting five days prior to IP challenge with IL-1β. IRAK4 protein levels were measured by Western blot in **(A)** splenocytes and **(B)** skin tissue. **(C)** Plasma cytokines were assessed by MSD. Statistics: Ordinary one-way ANOVA, ***p≤0.001, ****p≤ 0.0001 relative to Regular Chow group

Figure 7: GS-6791 Reduces Disease in a Preclinical Model of Dermatitis

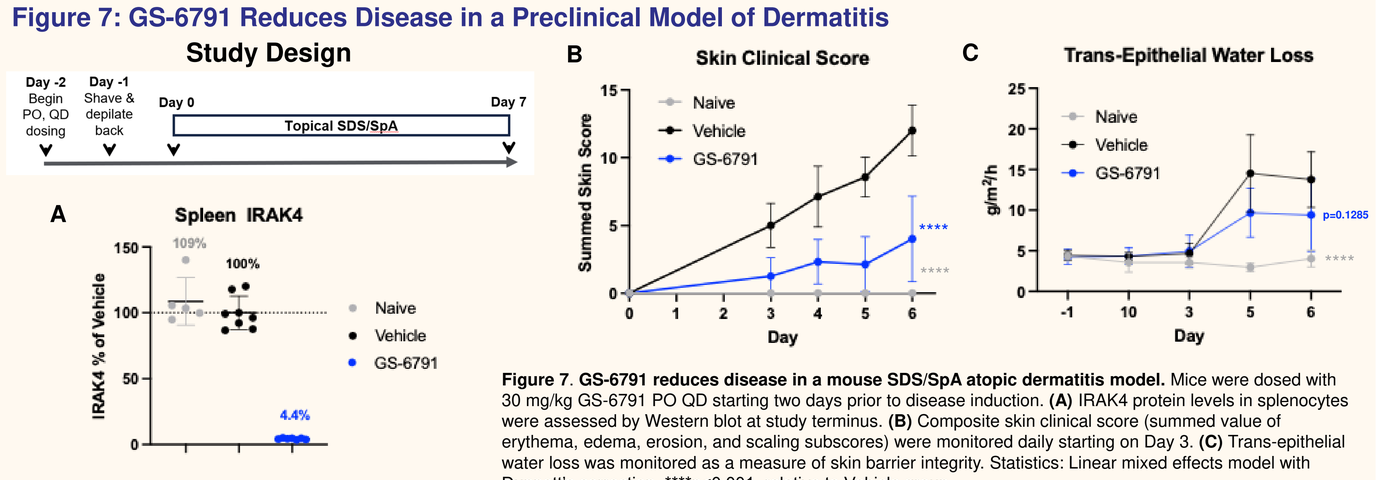


Figure 7. GS-6791 reduces disease in a mouse SDS/SpA atopic dermatitis model. Mice were dosed with 30 mg/kg GS-6791 PO QD starting two days prior to disease induction. **(A)** IRAK4 protein levels in splenocytes were assessed by Western blot at study terminus. **(B)** Composite skin clinical score (summed value of erythema, edema, erosion, and scaling subscores) were monitored daily starting on Day 3. **(C)** Trans-epithelial water loss was monitored as a measure of skin barrier integrity. Statistics: Linear mixed effects model with Dunnett's correction. ****p<0.001, relative to Vehicle group

Conclusions

- GS-6791 is a selective, orally bioavailable IRAK4 heterobifunctional protein degrader
- GS-6791 mediates IRAK4 protein degradation in keratinocytes and potently inhibits *in vitro* IL-1 and IL-36-mediated functional responses, including genes upregulated in lesional skin in inflammatory conditions[7]
- GS-6791 inhibits IL-1-induced cytokine release in mice and demonstrates efficacy in a preclinical model of atopic dermatitis, with comparable effect size as α-IL-4R blockade (not shown)
- GS-6791 provides a differentiated pharmacological profile from IRAK4 kinase inhibitors[6] with potential to deliver efficacy in multiple inflammatory indications, including dermatological diseases

References

1. Suzuki *et al*, *Nature,* (2002) 416(6882):750-6
2. Chiang *et al*, *J Immunol*, (2011) 186:1279-1288
3. De Nardo *et al*, *JBC*, (2018) 293(39) 15195–15207
4. De *et al*, *JBC*, 2018, (2018) 293(39) 15208–15220
5. Terada *et al*, *PNAS*, (2006) 103(23):8816-8821
6. Teng *et al, ACR Convergence* (2024)
7. Morgner *et al. Nature,* (2006) 13(1):1807

Disclosures

[1]CL, GT, ZH, MM, JW, VB, SM, AS, and GM are current or former employees of Gilead Sciences. [2]AM, WSP, and AB are employees of Nurix Therapeutics.

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