IRAK4 Degrader GS-6791 Inhibits TLR and IL-1R-Driven Inflammatory Signaling, and Ameliorates Disease in a Preclinical Arthritis Model

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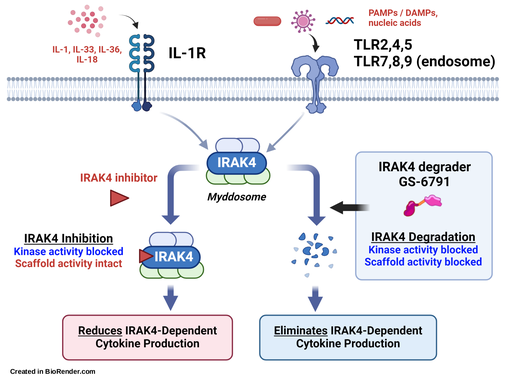
Background

- 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 has 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 and TLR9-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 rheumatological diseases

Rationale

IRAK4 is a Central Node Governing Inflammatory Responses



Methods

Cellular assays: Human PBMC and 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 B cells were pre-treated with compound for 6 h and stimulated with 10 μg/mL CpG-C (TLR9 agonist) for 20 h. Surface expression of CD80 and CD86 was assessed by flow cytometry. Human rheumatoid-arthritis fibroblast-like synoviocytes (RA-FLS) were pre-treated with compound for 24 h and stimulated with 1 ng/mL IL-1β for 20 h. Cytokine levels were assessed in culture supernatants by MSD.

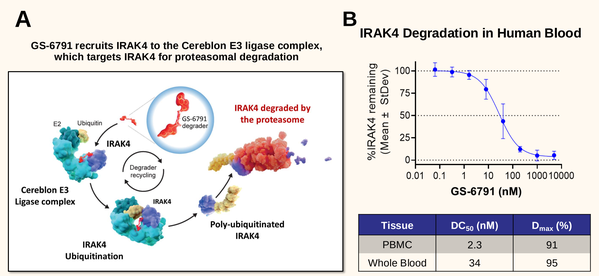
***In Vivo* non-human primate (NHP) study:** Cynomolgus monkeys (n=3) were dosed once daily with GS-6791 at 6 mg/kg for 3 consecutive days (QDx3) and blood was collected at indicated time points. IRAK4 levels were measured by flow cytometry and Western blot analysis in isolated PBMC.

***In Vivo* challenge models**: Mice were dosed orally with GS-6791 for two days prior to challenge with 200 μg of CpG-c (i.v.) or 200 μg of IL-1β (i.p.). Splenocyte IRAK4 protein levels were assessed by Western blot. Plasma cytokines were assessed 2 h after CpG challenge or 1.5 h after IL-1β-challenge.

Rat CIA study: Disease was induced by type II collagen injection on Days 0 and 7. Animals were dosed orally starting on d11 with GS-6791 or Dexamethasone. Ankle diameters were measured daily from d10-d18. IRAK4 levels were assessed in spleen and synovial tissue using Western Blot. Cytokine levels were evaluated in synovial tissue non-denatured lysates using MSD.

Results

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



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

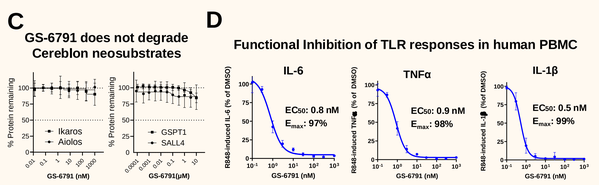


Figure 1. **(A)** Mechanism of IRAK4 protein degradation. **(B)** *In vitro* GS-6791-mediated IRAK4 degradation was assessed in human whole blood by flow cytometry after 24 h in n=11 donors. **(C)** Human PBMCs (n=3 donors) were treated with GS-6791 and Ikaros and Aiolos were measured by flow cytometry in n=4 donors. SALL4 and GSPT1 degradation was assessed in HiBiT tagged cell lines. **(D)** Human PBMC were treated with GS-6791 and stimulated with the TLR7/8 agonist R848 (1 μg/mL). Cytokine production was measured using MSD ELISA, and EC50 (half-maximal inhibitory) potency values were calculated from n=3 donors. E_{max} = maximal percent inhibition.

Figure 2: GS-6791 achieves robust degradation of IRAK4 *in vivo* across cell types, in non-human primates

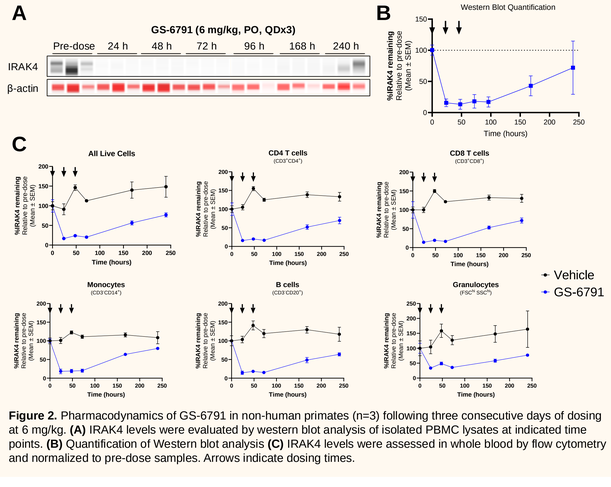


Figure 2. Pharmacodynamics of GS-6791 in non-human primates (n=3) following three consecutive days of dosing at 6 mg/kg. **(A)** IRAK4 levels were evaluated by western blot analysis of isolated PBMC lysates at indicated time points. **(B)** Quantification of Western blot analysis **(C)** IRAK4 levels were assessed in whole blood by flow cytometry and normalized to pre-dose samples. Arrows indicate dosing times.

Figure 3: GS-6791 inhibits CpG/TLR9 and IL-1β dependent cytokine induction *in vivo*

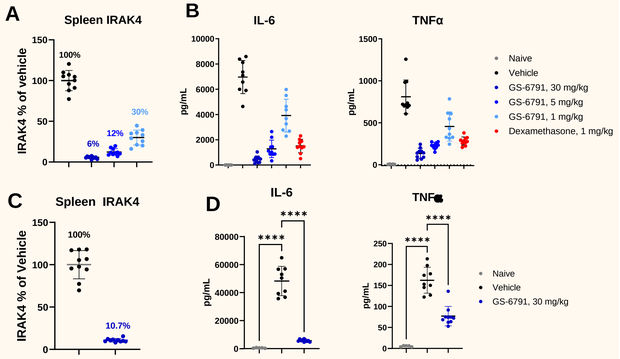


Figure 3. **GS-6791 reduces CpG- and IL-1β-induced plasma cytokines.** **(A-B)** Mice were dosed orally with GS-6791 (1, 5, or 30 mg/kg) or Dexamethasone for two days prior to i.v. challenge with 200 μg CpG-c (TLR9 ligand). **(A)** Splenocyte IRAK4 protein levels (corrected against Vinculin loading control) were measured by Western blot and **(B)** plasma cytokines were assessed 2 hours after CpG-challenge. **(C-D)** Mice were dosed orally with 30 mg/kg GS-6791 for two days prior to intraperitoneal challenge with 200 μg IL-1β. **(C)** Splenocyte IRAK4 protein levels were measured by Western blot and **(D)** plasma cytokines were assessed 90 minutes after IL-1β –challenge.

Figure 4: GS-6791 demonstrates dose-dependent efficacy in rat arthritis model

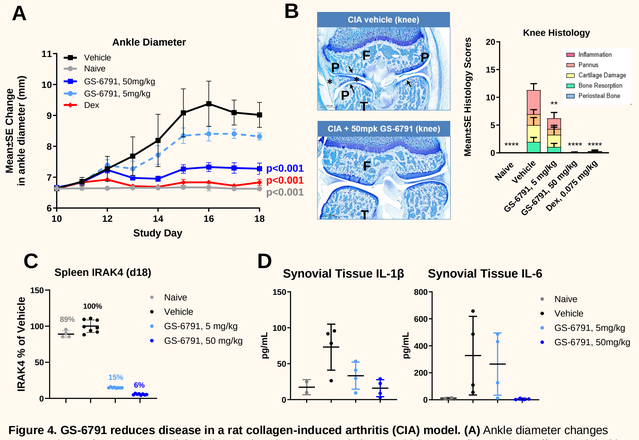
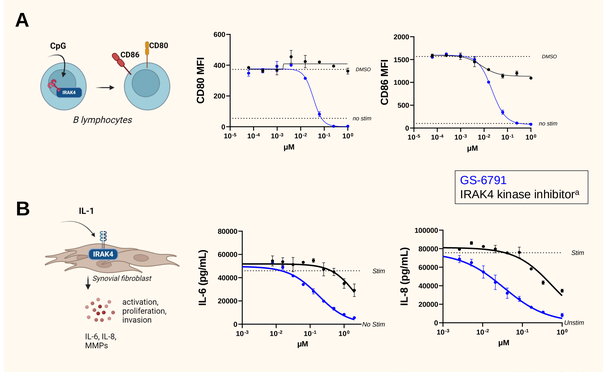


Figure 4. GS-6791 reduces disease in a rat collagen-induced arthritis (CIA) model. (A) Ankle diameter changes measured over time represent clinical disease (p-values shown relative to vehicle group, linear mixed-effect model with Dunnett's correction). **(B)** Histological examination of knees (ankles not shown), with areas of tissue damage and inflammation indicated (*Inflammation, P=pannus, Arrow=Cartilage damage, T=Tibia, F=Femur). Mean composite histology scores for knees represented as a histogram (p-values shown for total histology scores relative to vehicle group, ANOVA with Dunnett's correction). **(C)** At study terminus (Day 18), IRAK4 protein levels in splenocytes were measured by Western blot. **(D)** Cytokine levels in synovial tissue were measured using MSD in tissue lysates.

Figure 5: IRAK4 Degradation Provides Deeper Inhibition of Human B Cell and Fibroblast Responses Compared to IRAK Kinase Inhibition



[a]IRAK4 kinase inhibitor IC50 = 0.5 nM

Figure 5: GS-6791 fully blocks TLR9-driven activation in B cells and IL-1 driven responses in synovial fibroblasts. (A) B cells were pre-treated with GS-6791 or an IRAK4 kinase inhibitor and stimulated using CpG. Costimulatory marker expression (CD80 and CD86) was measured on the cell surface by flow cytometry **(B)** Primary RA-patient derived synovial fibroblasts were pre-treated with GS-6791 or an IRAK4 kinase inhibitor for 24 h and stimulated with 1 ng/mL of IL-1. IL-6, and IL-8 were measured in the supernatant by MSD at 24 h post stimulation.

Conclusions

- GS-6791 is a selective, orally bioavailable IRAK4 protein degrader with good potency *in vitro* and demonstrates degradation *in vivo*, in NHP at a low dose (6 mg/kg)
- GS-6791 mediates rapid IRAK4 protein degradation and after 6 h potently inhibits *in vitro* TLR and IL-1R-mediated cellular responses
- GS-6791 inhibits IL-1- and TLR-induced cytokine release in PK/PD models and demonstrates robust dose-dependent efficacy in a preclinical model of arthritis
- GS-6791 provides a differentiated pharmacological profile and distinct biological activities from an IRAK4 kinase inhibitor with potential to deliver superior efficacy in rheumatological disease

References

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Acknowledgments

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Disclosures

[1]GT, CL, AH, JG, ZH, MM, VG, WM, SM, AS, GM are current or former employees of Gilead Sciences. [2]TF, AM, WSP, AB are employees of Nurix Therapeutics

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