IRAK4 Degradation Abrogates Cytokine Release and Improves Disease Endpoints in Murine Models of IL-33/36- as well as Th17-driven Inflammation

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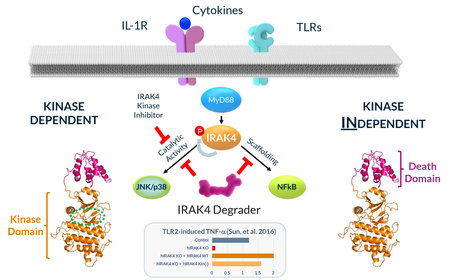
INTRODUCTION

Interleukin-1 receptor associated kinase 4 (IRAK4) plays a central role in myddosome signaling via kinase and scaffolding functions, making it an attractive target for the treatment of TLR- and IL-1R-driven inflammatory diseases. IL-1 family cytokines, Th17 cells and TLRs, are central to the pathophysiology of several chronic inflammatory diseases. IRAK4 exhibits both kinase dependent and independent activities, making degradation a more attractive modality than kinase inhibition alone. Kymera has developed orally administered hetero-bifunctional molecules that selectively target IRAK4 for degradation and elimination by the ubiquitin proteasome pathway. These degraders have broad and potent activity *in vitro* against IL-6, TNF-α and other proinflammatory cytokines and chemokines induced by TLR agonists and IL-1 family cytokines that is superior to IRAK4 kinase inhibitors. Kymera's most advanced IRAK4 degrader is in a Phase 1 trial in healthy volunteers and patients with hidradenitis suppurativa (HS) or atopic dermatitis (AD).



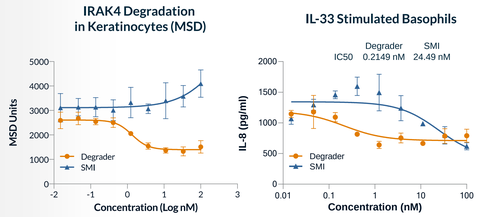
AIM

We sought to evaluate the efficacy of oral IRAK4 degraders *in vivo* compared to IRAK4 kinase small molecule inhibitors (SMI), in mechanistic and disease models of IL-33-, IL-36-, and Th17-stimulated inflammation. We explored skin and CNS-focused murine models, where these pathways are central drivers of disease, and we established parallels with human *in vitro* systems involving the IL-33-, IL-36-, and Th-17 signaling cascades as well.

RESULTS

Figure 1: IL-33 *in vitro* Assay

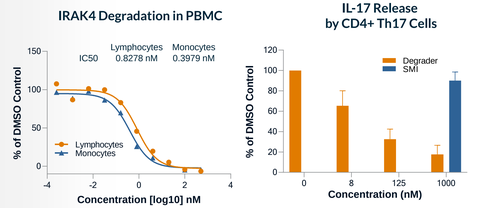
Understanding that IRAK4 is key to IL-1R signaling, we evaluated the effects of IRAK4 degraders vs. IRAK4 SMI on cell activation via the IL-1 family member, IL-33, in *in vitro* systems relevant to skin inflammation, e.g. keratinocytes and basophils (as a surrogate of mast cells).



In human keratinocytes, dose dependent IRAK4 degradation was observed following treatment with our compound; however, SMI tended to increase IRAK4 expression at higher doses, as previously reported. In human basophils stimulated with rhIL-33, IRAK4 degraders inhibited IL-8 production more potently than IRAK4 SMI.

Figure 2: CD4+ Th17 *In vitro* Assay

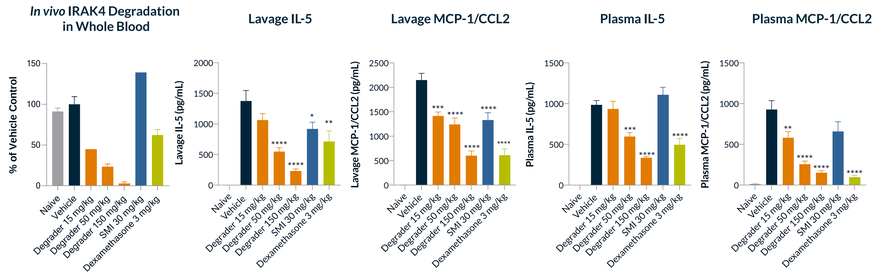
IL-1 family members and TLR are critical to Th17-driven inflammation in autoimmune responses. We evaluated the effects of IRAK4 degraders vs. IRAK4 SMI on the reactivation of established CD4+ Th17 cells.



In human PBMC, IRAK4 degraders showed comparable potency in lymphoid and myeloid cells. IRAK4 degraders potently inhibited IL-17A/F production, whereas a top dose of SMI used as reference (1 μM) showed no apparent effect on Th17 release

Figure 3: rmIL-33 Intraperitoneal Challenge Model

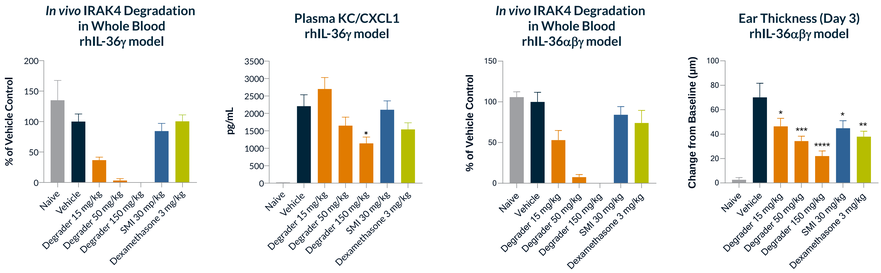
At mucosal barriers such as the skin, IL-33 is thought to play a critical role as a danger signal upstream of the Th2 activation cascade. We used a mechanistic model of rmIL-33 injection i.p. to induce cytokine production locally and systemically 6h post-injection.



IRAK4 levels were dose-dependently reduced by degraders in blood cells. Local (lavages) and systemic (plasma) levels of IL-5 and CCL2 were both dose-dependently reduced in IRAK4 degrader-treated mice, more potently than with IRAK4 SMI treatment (* p<0.05, ** p<0.01, *** p<0.001, and **** p<0.0001).

Figure 4: rhIL-36γ Subcutaneous and rhIL-36αβγ Intradermal Challenge Models

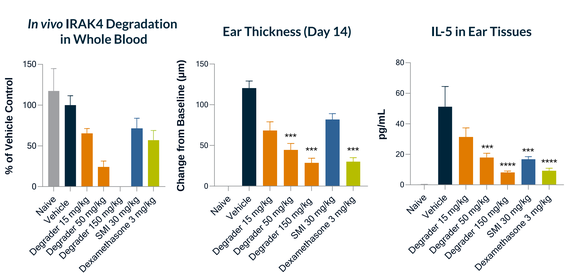
IL-36 is another IL-1 family member that has been implicated in Th1/Th17-driven inflammatory processes at mucosal barriers such as the skin and gut. We used mechanistic models of rhIL-36 injection s.c. (flank) or i.d. (ear) to induce cytokine production and tissue changes few hours post-challenge.



IRAK4 levels were dose-dependently reduced by degraders in blood cells of mice challenged with rhIL-36γ (s.c., flank) or rhIL-36αβγ (i.d., ear). Systemic levels of CXCL1 were dose-dependently decreased in IRAK4 degrader-treated mice but not in IRAK4 SMI-treated mice. Ear swelling was also dose-dependently reduced in IRAK4 degrader-treated mice, more robustly than with IRAK4 SMI or dexamethasone (* p<0.05, ** p<0.01, *** p<0.001, and **** p<0.0001).

Figure 5: rmIL-33 Intradermal Challenge Model

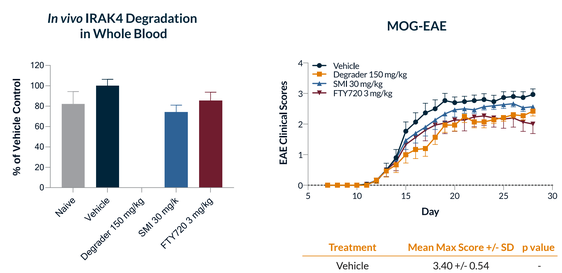
We further explored the modulation of mucosal inflammation with this ear swelling model triggered by repeated injections of rmIL-33 in the ear pinna over 2 weeks.



At the end of the study, IRAK4 levels were dose-dependently reduced by degraders in blood cells. Ear thickness and levels of IL-5 in skin tissues were dose-dependently decreased in IRAK4 degrader-treated mice, whereas IRAK4 SMI reduced IL-5 levels but not ear thickness (*** p<0.001 and **** p<0.0001).

Figure 6: Myelin Oligodendrocyte Glycoprotein-induced Experimental Autoimmune Encephalomyelitis

We evaluated the therapeutic modulation of clinical scores (paralysis) in this classic model of CD4+ Th17 cell inflammation.



IRAK4 degraders administered therapeutically (d13-d28) proved as efficacious as FTY720, whereas IRAK4 SMI did not reduce disease scores significantly.

Treatment	Mean Max Score +/- SD	p value
Vehicle	3.40 +/- 0.54	-
Degrader 150 mg/kg	**2.69 +/- 0.52**	0.0018
SMI 30 mg/kg	3.07 +/- 0.42	0.0822
FTY720 3 mg/kg	**2.70 +/- 1.28**	0.0271

METHODS

***In vitro* Assays:** All cells were incubated overnight with IRAK4 degraders or IRAK4 SMI prior to stimulation.

Basophil Assay: Freshly isolated human basophils were stimulated for 8h with IL-3/IL-33 before collecting supernatants for cytokine analysis (MSD).

Keratinocyte Assay: IRAK4 levels in primary human keratinocytes evaluated by MSD were normalized vs. DMSO controls.

Th17 Assay: CD4+ T cells from huPBMC were differentiated into Th17 cells using anti-CD3/CD28 beads and a cocktail of cytokines/antibodies. IL-17 production was evaluated after restimulation with IL-2/IL-23/IL-1β for 4 days.

Murine Models: Compounds were administered orally, either BID (IRAK4 degraders) or QD (IRAK4 SMI).

IL-33 i.p. and Ear Injection: C57BL/6 mice were injected with rmIL-33 either as a single dose (i.p.) or BIW (i.d. in the ear pinna). Peritoneal lavages and plasma samples were collected 6h post-injection (i.p. model). Ear thickness and tissue cytokines were assessed over a 2-week period (i.d. model).

IL-36 s.c. and Ear Injection: C57BL/6 mice were injected with huIL-36γ once (s.c. model) or twice with a cocktail of huIL-36α/β/γ (i.d. model in the ear pinna). Plasma samples were collected 2h post-injection (s.c. model). Ear thickness was measured 24h post-2nd IL-36 injection (i.d. model).

MOG-EAE Study: C57BL/6 mice were immunized with MOG emulsified in CFA on day 0. They also received injections of Pertussis toxin on day 0 and 1. Therapeutic dosing started on day 13, after 20% of the mice developed clinical signs of EAE. Clinical scores were recorded up to day 28 of the study.

See literature references for additional materials and methods.

IRAK4 SMI inhibitor was PF-06650833, IRAK4 degrader *in vivo* was KT-474.

CONCLUSIONS

- The data presented here highlight the anti-inflammatory effects of IRAK4 degradation vs. IRAK4 kinase inhibition.
- *In vitro* assays with human leukocytes and keratinocytes show that IRAK4 degraders reduced the release of cytokines more efficiently than IRAK4 SMI.
- Data from *in vitro* mechanistic assays were confirmed by *in vivo* mechanistic and disease-like models.
- In these studies, IRAK4 degraders inhibited cytokine production and skin inflammation upon IL-33 or IL-36 injection more potently than IRAK4 SMI.
- In fact, IRAK4 degraders provided anti-inflammatory effects comparable or better than dexamethasone in these murine models.
- Moreover, in a classic model of antigen-induced, Th17-driven neuroinflammation (MOG-EAE), IRAK4 degraders reduced clinical scores similarly to FTY720 (a standard of care for MS), and more robustly than IRAK4 SMI.
- In conclusion, we showed that targeted IRAK4 degradation is an effective approach to decreasing inflammatory cytokine production that is superior to IRAK4 SMI, with relevance to treatment of inflammatory skin diseases as well as other IL-1R/TLR-driven autoimmune indications.
- Kymera's most advanced IRAK4 degrader is in Phase 1 trial in healthy volunteers and patients with hidradenitis suppurativa (HS) or atopic dermatitis (AD).

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