Early Comparison of the *in vitro* to *in vivo* Translation of Different IRAK4 Inhibitor Modalities

Rebecca Dowey, Ellen Olden, David Laughton, Roland Hjerpe, Karl Deacon, Falguni Vyas, Hannah Neal, Philip MacFaul, Zara Turnbull, Wioletta Pijacka, Gisele Lincevicius, Princess Pearl Marcelo, Alex Roberts, John Unitt*

*Sygnature Discovery, BioCity, Pennyfoot Street, Nottingham, NG1 1GR, UK *(D.Laughton@sygnaturediscovery.com)*

1 PROTACs are a novel therapeutic strategy to target IRAK4

Interleukin-1 Receptor-Associated Kinase 4 (IRAK4) is a master regulator of innate immunity, playing a central role in both toll-like receptor (TLR) and interleukin-1 (IL-1) mediated inflammation[1]. Proteolysis targeting chimeras (PROTACs) are hetero-bifunctional molecules that degrade target proteins using the ubiquitin–proteasome system. PROTACs are a novel therapeutic strategy to target IRAK4[2]. Recently, an IRAK4 PROTAC, KT-474, has entered Phase 2 clinical trials for the treatment of hidradenitis suppurativa and atopic dermatitis[2]. This provided an opportunity to compare the *in vitro* to *in vivo* (IVIV) translation of an IRAK4 degrader with the activity inhibitor PF-06650833.

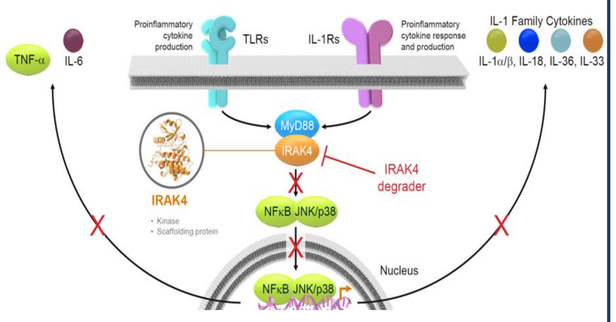


Figure 1: Mechanistic Rationale for IRAK4 Degrader in Immunology. Myddosome Targeting Blocks Cytokine Production. PROTACs have the efficacy advantage of targeting both the kinase & scaffolding function of IRAK4. Image: Veronica T. Campbell et al. (2019) Oral Poster Presentation. Kymera Therapeutics. ACR 2019. Available here: https://www.kymeratx.com/wp-content/uploads/2020/07/Kymera-Therapeutics-ACR-2019-FINAL.pdf

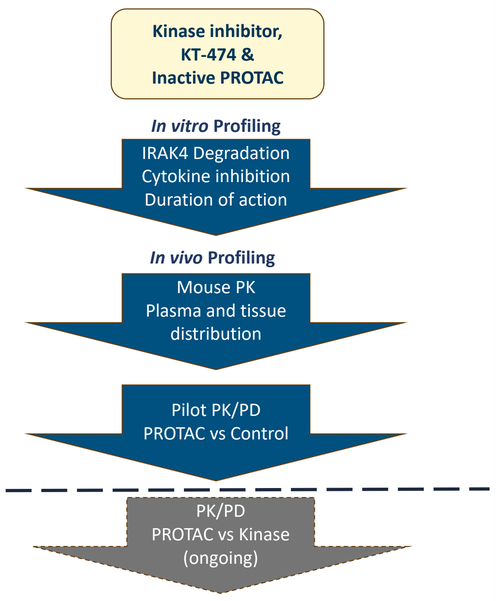
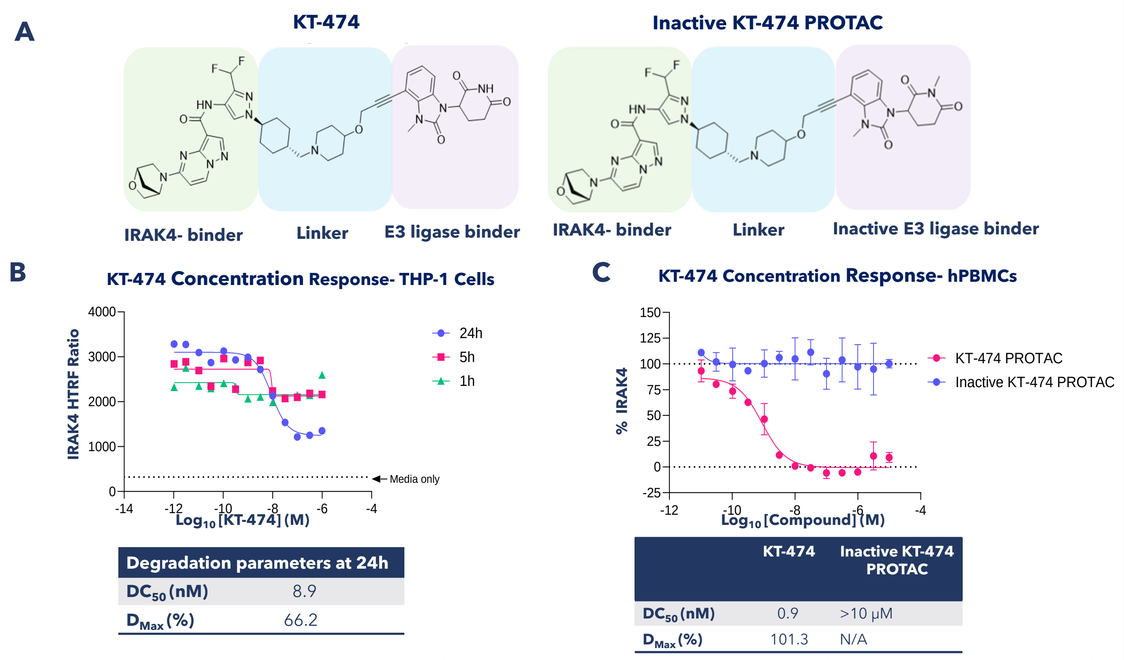


Figure 2: Strategy outline of the IVIV translation of KT-474.

2 KT-474 is a potent IRAK4 degrader *in vitro*

To establish optimal assay conditions, IRAK4 levels were first detected in the monocytic THP-1 cell line. A 24-hour KT-474 treatment demonstrated optimum IRAK4 degradation parameters (DC_{50} of 8.9 nM & D_{max} 66.2 %). The assay was repeated using human PBMCs (hPBMCs) and again KT-474 potently degraded IRAK4 (DC_{50} 0.9 nM & D_{max} 101.3 %). The inactive KT-474 PROTAC, lacking a functional E3 ligase binder, did not affect IRAK4.



Degradation parameters at 24h	
DC_{50} (nM)	8.9
D_{Max} (%)	66.2

	KT-474	Inactive KT-474 PROTAC
DC_{50} (nM)	0.9	>10 µM
D_{Max} (%)	101.3	N/A

Figure 3: KT-474 degrades IRAK4 in THP-1 cells and hPBMCs. A) KT-474 is a heterobifunctional PROTAC molecule. The inactive KT-474 PROTAC lacks a functional E3 ligase binder. B) IRAK4 levels were detected by HTRF following treatment of THP-1 cells (100k/well) with a range of KT-474 concentrations for 1h (green), 5h (pink), or 24h (blue). C) hPBMCs (200k/well) were treated with KT-474 (pink) or the inactive PROTAC (blue) for 24h. Data is representative of n2. Error bars represent mean +/- SEM.

3 KT-474 inhibits IL-6 production *in vitro*

KT-474 and PF-06650833 inhibited IL-6 production following stimulation with LPS & R848. The inactive KT-474 PROTAC also inhibited IL-6 production, likely due to inhibition of IRAK4 kinase activity via the IRAK4 binding moiety of the degrader. Importantly, inhibition of IL-6 was maintained following removal of KT-474 in a wash-out experiment after 4h, whereas the activity of PF-06650833 was lost, thus highlighting the longevity of the PROTAC effects on inflammation.

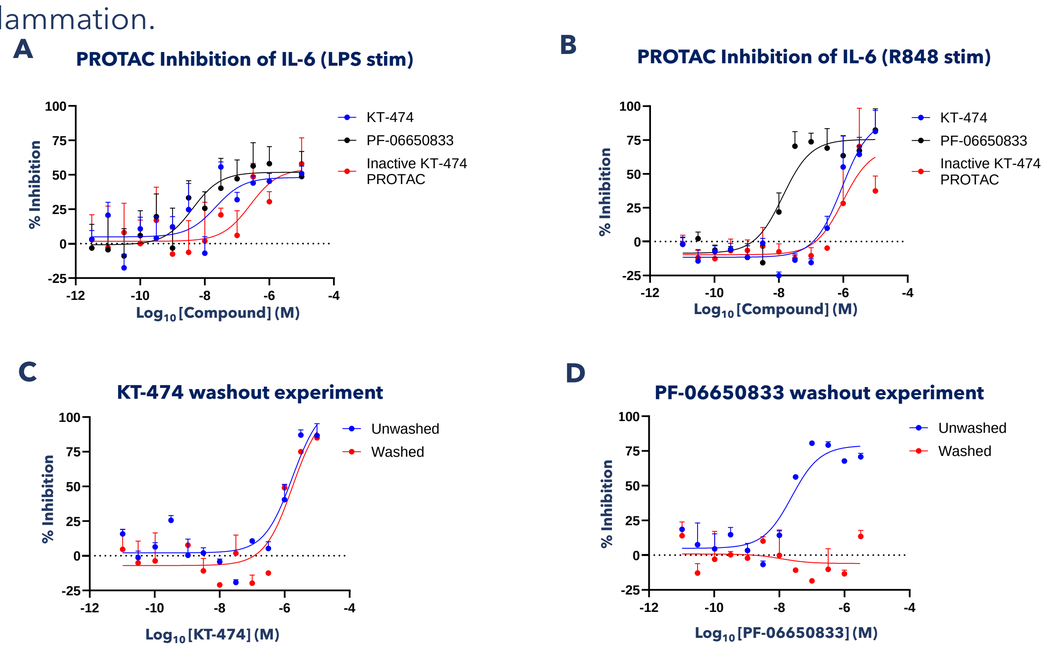


Figure 4: KT-474 inhibition of IL-6. IL-6 levels were detected by HTRF following treatment of hPBMCs with KT-474 (blue), PF-06650833 (black) or the inactive KT-474 PROTAC (red) for 24h and stimulated with A) LPS or B) R848 for 4h. The IC_{50s} for the compounds were: KT-474 21 nM (A) & 902 nM (B), PF-06650833 4.2 nM (A) & 13.3 nM (B) and inactive KT-474 PROTAC 256 nM (A) & 979 nM (B). (C-D) Compounds were removed by washing (W) or cells were left unwashed (UW) following a 24h treatment and prior to stimulation with R848 as before. The IC_{50s} for the compounds were: KT-474 1.7 µM (UW) & 1.7 µM (W) and PF-06650833 23.8 nM (UW) & >10 µM (W). Data is representative of n2. Error bars represent mean +/- SEM.

4 PK/PD of KT-474

Following oral administration, KT-474 was observed to be rapidly absorbed, reaching a C_{max} at 2 hours. Plasma levels were still measurable up to 28 hours post dose. Total plasma concentrations were observed to be above the DC_{50} for 24 hours, and free concentrations above the DC_{50} for around 18 hours. Higher levels of KT-474 were observed in liver, kidney and spleen tissues compared to plasma at 28 hours post dose, whereas no compound levels were detected in brain tissue. Subsequently, an acute model of systemic inflammation was induced in mice by LPS administration. The effect of KT-474 on inhibiting IL-6 production was evaluated 24 hours after oral treatment with KT-474 or a KT-474 negative control, followed by LPS administration 4 hours prior to evaluation. IRAK4 expression levels were evaluated using western blotting in spleen tissue.

A

Total plasma and tissue concentrations for KT-474

Compound	Time post dose (h)	Plasma concentration (ng/mL)	Tissue concentration (ng/g)			
			Brain	Kidney	Liver	Lung
KT-474	1	369	N/A	N/A	N/A	N/A
	28	3.0	<LLOQ	24.2	45.7	6.5
Inactive KT-474 PROTAC	1	384	N/A	N/A	N/A	N/A
	28	<LLOQ	<LLOQ	16.0	28.8	8.2

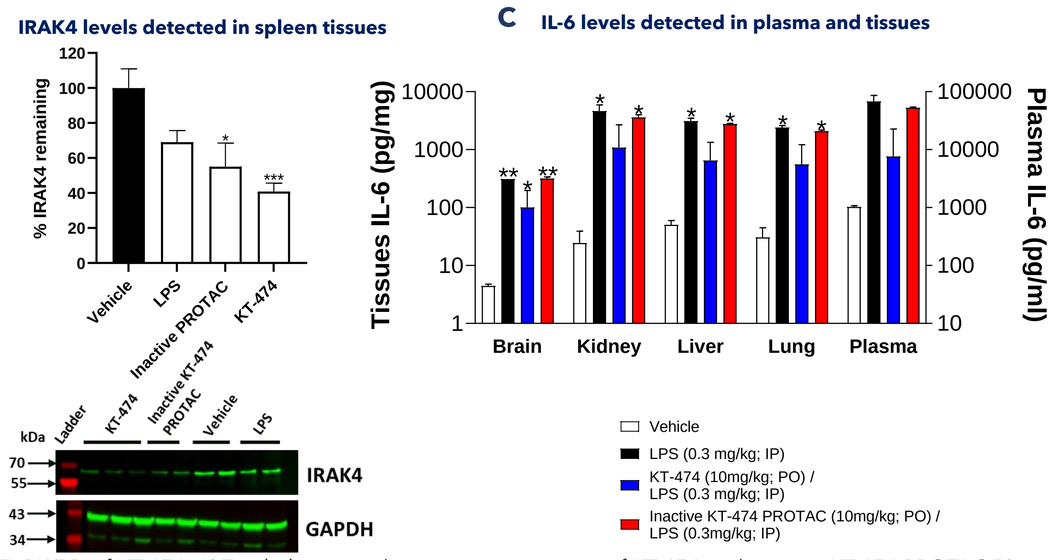


Figure 5: PK/PD of KT-474. A) Total plasma and tissue concentrations of KT-474 and inactive KT-474 PROTAC B) IRAK4 detection. Data are normalised to GAPDH and represented as percentage of vehicle (mean +/- SEM, n=2-3). C) IL-6 levels detected in plasma and tissues in the LPS-induced inflammation model (Data was log transformed and analysed by one-way ANOVA, mean +/-SEM, N=2-3). Significant differences vs. vehicle group: *p<0.05, **p<0.01, ***p<0.001. There were no significant differences vs. LPS group.

5 Conclusion

- KT-474 and kinase inhibitors of IRAK4 potently inhibited LPS stimulated inflammatory responses *in vitro*. KT-474 dosed orally provided good exposure levels, with modest inhibition of IL-6 in a mouse LPS model.
- Studies are ongoing to quantify tissue IRAK4 levels for further evaluation of PK/PD relationships

References:
1. De Nardo D, Balka KR, Cardona Gloria Y, Rao VR, Latz E, Masters SL. Interleukin-1 receptor-associated kinase 4 (IRAK4) plays a dual role in myddosome formation and Toll-like receptor signaling. J Biol Chem. 2018 Sep 28;293(39):15195-15207. doi: 10.1074/jbc.RA118.003314. Epub 2018 Aug 3. PMID: 30076215; PMCID: PMC6166714
2.Ackerman, L., Acloque, G., Bacchelli, S. et al. IRAK4 degrader in hidradenitis suppurativa and atopic dermatitis: a phase 1 trial. Nat Med 29, 3127–3136 (2023). https://doi.org/10.1038/s41591-023-02635-7

SYGNATURE DISCOVERY