

# Novel IRAK-4 inhibitors exhibit highly potent anti-proliferative activity in DLBCL cell lines with activating MYD88 L265P mutation

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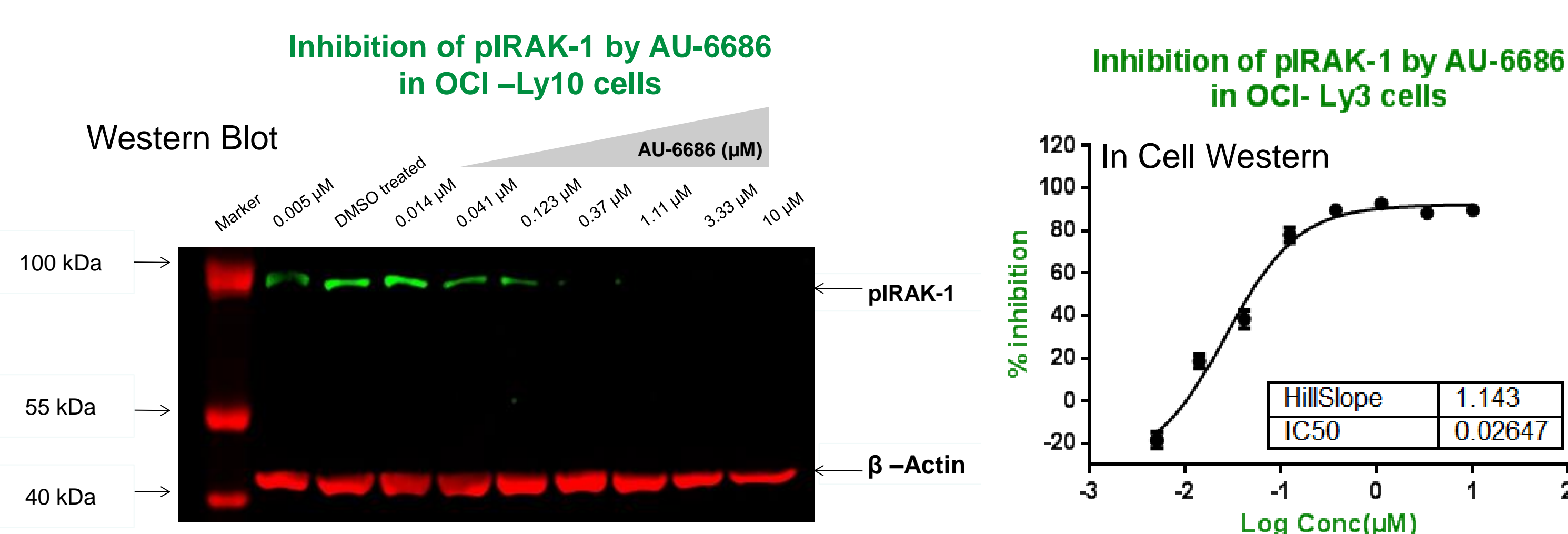
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## Abstract

Interleukin-1 Receptor Associated Kinase-4 (IRAK-4) is a serine/threonine protein kinase belonging to tyrosine like kinase (TLK) family. IRAK-4 is one of the important signalling components downstream of IL-1/Toll family of receptors (IL-1R, IL-18R, IL-33R, Toll-like receptors). Recent studies have reported occurrence of oncogenic mutations in MYD88 in 30% of activated B cell diffuse large B cell lymphomas (ABC DLBCL) and 90% of Waldenstrom's macroglobulinemia (WM). A significant proportion of ABC DLBCLs have a single amino acid substitution of proline for leucine at position 265 (L265P) in the TIR domain of MYD88 protein resulting in constitutive activation of MYD88 and enhanced activity of IRAK-4. Thus, IRAK-4 is an attractive therapeutic target for the treatment of B-cell lymphomas with activating MYD88 L265P mutation. We have recently designed, synthesized and characterized a series of ATP-competitive, bicyclic heterocycle small molecule compounds as IRAK-4 inhibitors. These novel compounds were profiled for their potency as IRAK-4 kinase inhibitors, kinase selectivity, and drug-like properties. Furthermore, selected compounds were tested in proliferation and pIRAK-1-based target inhibition assays using ABC-DLBCL cell lines with activating MYD88 L265P mutation, OCI-Ly10 and OCI-Ly3. Lead compounds exhibited potent inhibitory activity against IRAK-4 with single-digit nM IC<sub>50</sub>s in biochemical assays and decreased pIRAK-1 levels in MYD88 mutant DLBCL cell lines, and potently inhibited the proliferation of DLBCL cell lines in culture. Lead compounds demonstrated potent *in vivo* antitumor activity in OCI-Ly10 DLBCL murine xenograft model, had excellent pharmacodynamic effect in an *in vivo* LPS induced inflammation model, and resulted in potent activity in the rat Collagen-induced arthritis (CIA) model. In summary, a series of potent IRAK-4 inhibitors have been discovered and are being initially evaluated for treatment of B-cell lymphomas.

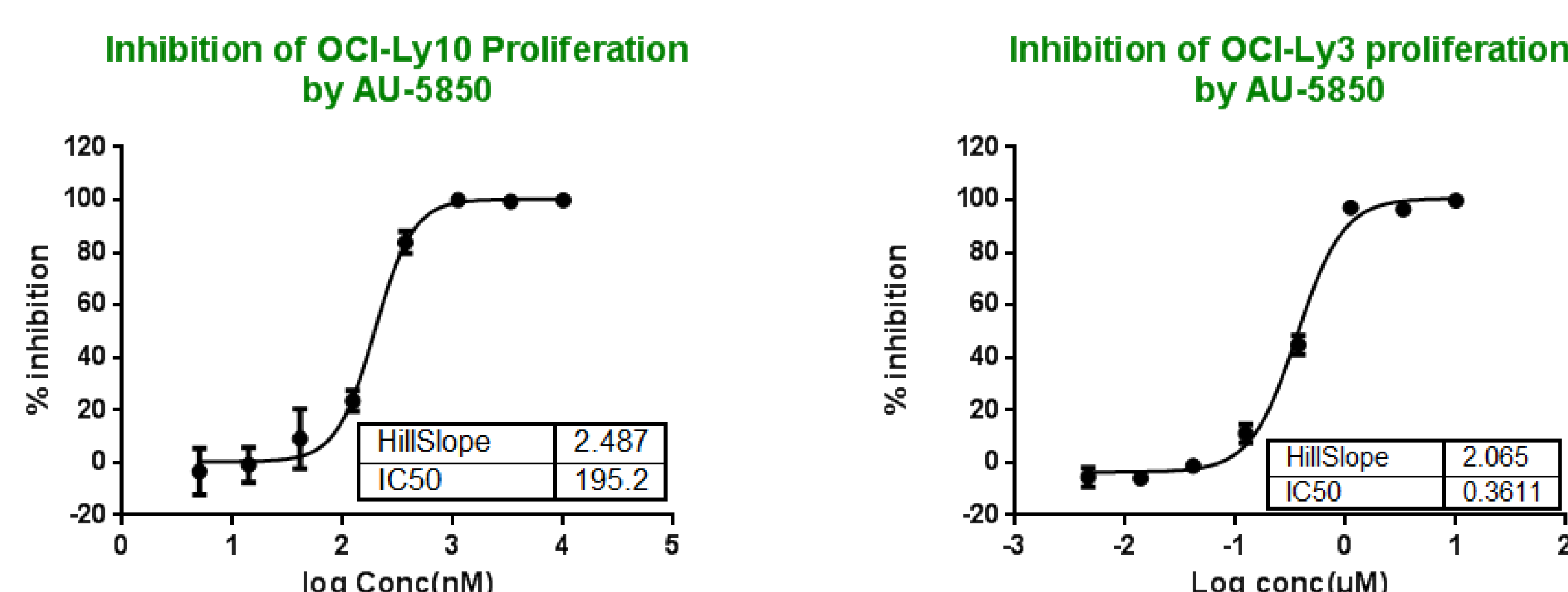
## Cell Based Activity

**Mechanistic Assay - p-IRAK-1 Inhibition in OCI-Ly10/3 cells:** OCI-Ly10 cells were treated with compounds for 12 hr followed by lysis preparation. 50µg of total protein was loaded on gel for western blot analysis. Compound inhibition was normalized to DMSO control. OCI-Ly3 cells were seeded in Poly-D-Lysine coated plates and allowed to attach. Cells were treated with compounds for 12 hrs. followed by In cell Western (ICW) assay to measure pIRAK-1 levels. A representative compound AU-6686 inhibited phosphorylation of IRAK-1 in a dose dependent manner demonstrating potent target engagement.



## Functional Assay – Inhibition of Proliferation of OCI-Ly10 and OCI-Ly3 Cells

Cells were treated with compounds for 72 hrs. followed by addition of XTT to measure inhibition of proliferation

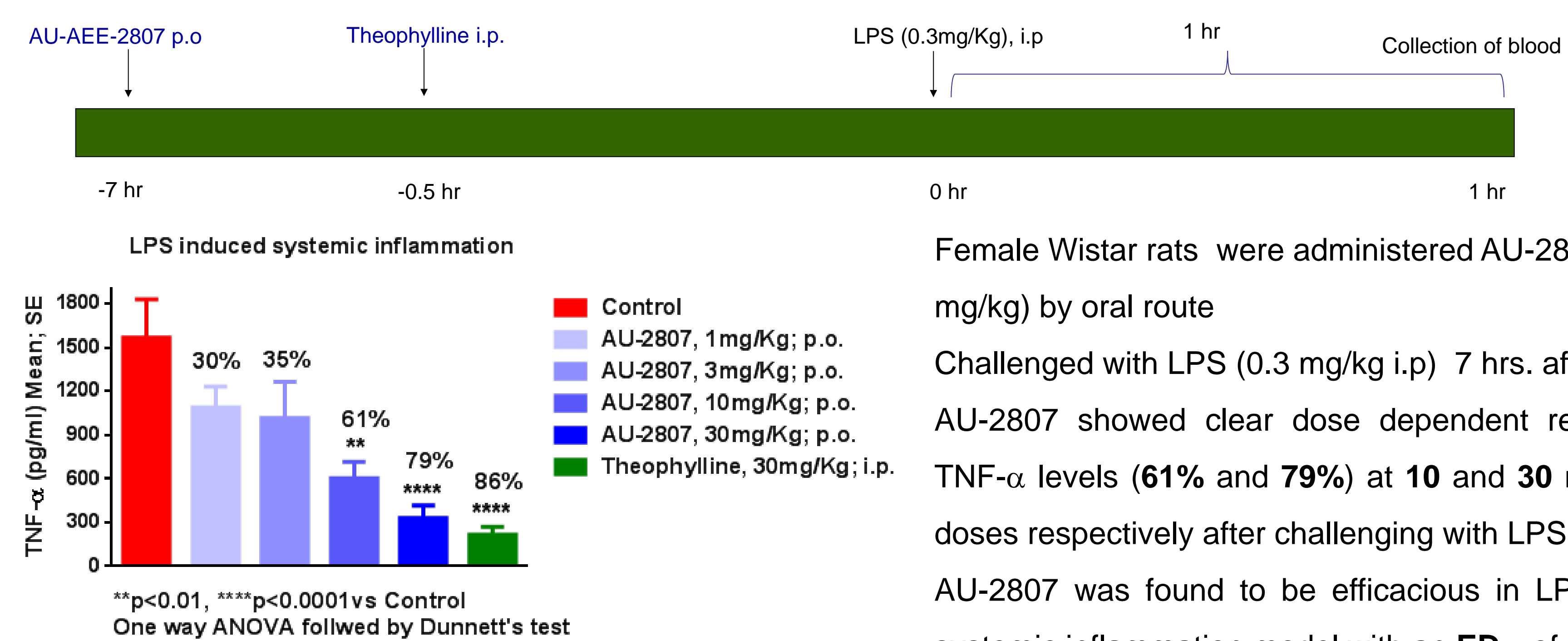


## Profile of Representative Compounds

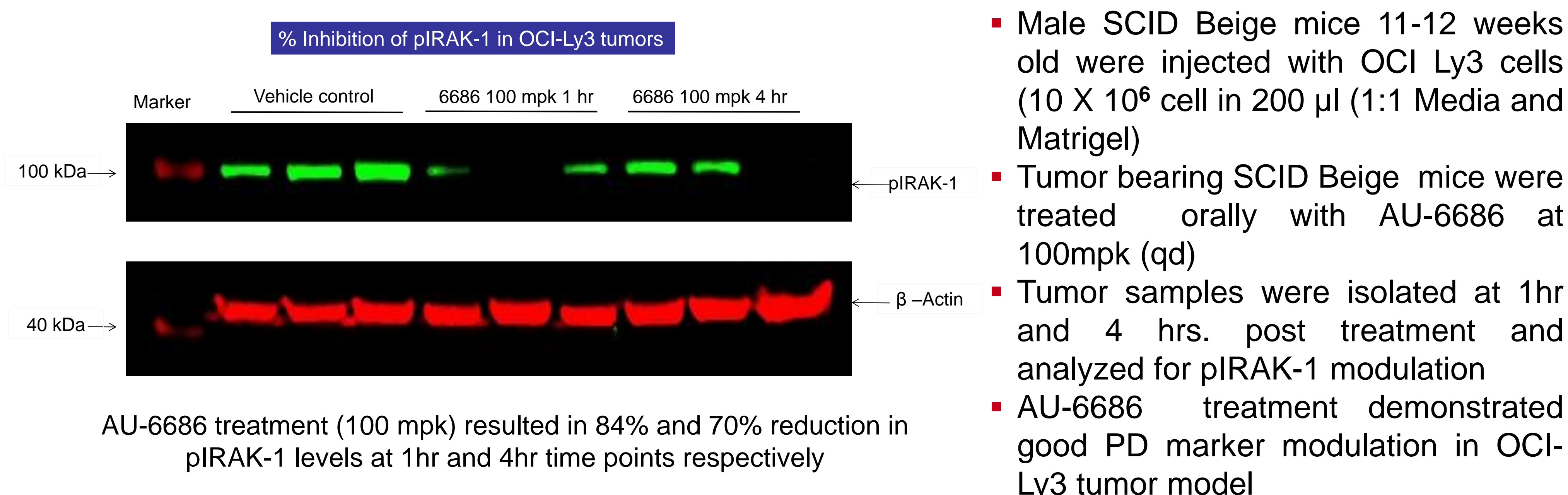
Parameters	AU-5850	AU-2807	AU-6686	AU-5792
hIRAK-4 cell free IC <sub>50</sub> (nM)	6	7.5	3.5	3
LPS induced TNFα in hPBMC IC <sub>50</sub> (nM)	ND	23.2	ND	12.5
OCI-Ly10 Proliferation IC <sub>50</sub> (nM)	195	387 / 298	9	52
OCI-Ly3 Proliferation IC <sub>50</sub> (nM)	361	53% @ 0.3 µM	31	132
pIRAK-1 inhibition in OCI-Ly10 IC <sub>50</sub> (nM)	795	123/72.8	100	1510
Karpas 422 Proliferation IC <sub>50</sub> (nM)	2096	4% @ 10µM	61	307
Ramos Proliferation IC <sub>50</sub> (nM)	419	37% @ 1µM	14,16	347
MLM, HLM, RLM t1/2(min); Cl <sub>int</sub> (µl/min/mg)	>90, <26, >90, <26, >90, <26	>90, <26, >90, <26, >90, <26	76, 30, 218.8, 10.6, 122, 18.9	>90, <26, >90, <26, >90, <26
PK	Mice PK	Rat PK	Mice PK	Mice PK
(IV) dose (mpk)	3	3	3	3
(IV) T ½ (hr)	3	5.4	5.97	1.4
(IV) AUC (ng.hr/mL)	1387	10631	2516	376
(IV) Cl (mL/hr/kg)	2181	286	1352	2667
(IV)Vd (mL/kg)	4276	1551	12942	4931
(PO) dose (mpk)	10	10	10	10
(PO) C max (ng/mL)	1245	427	1274	1598
(PO) AUC (ng.hr/mL)	3409	7306	6170	3054
%F	73.7	21	73.6	81.4

ND-not determined

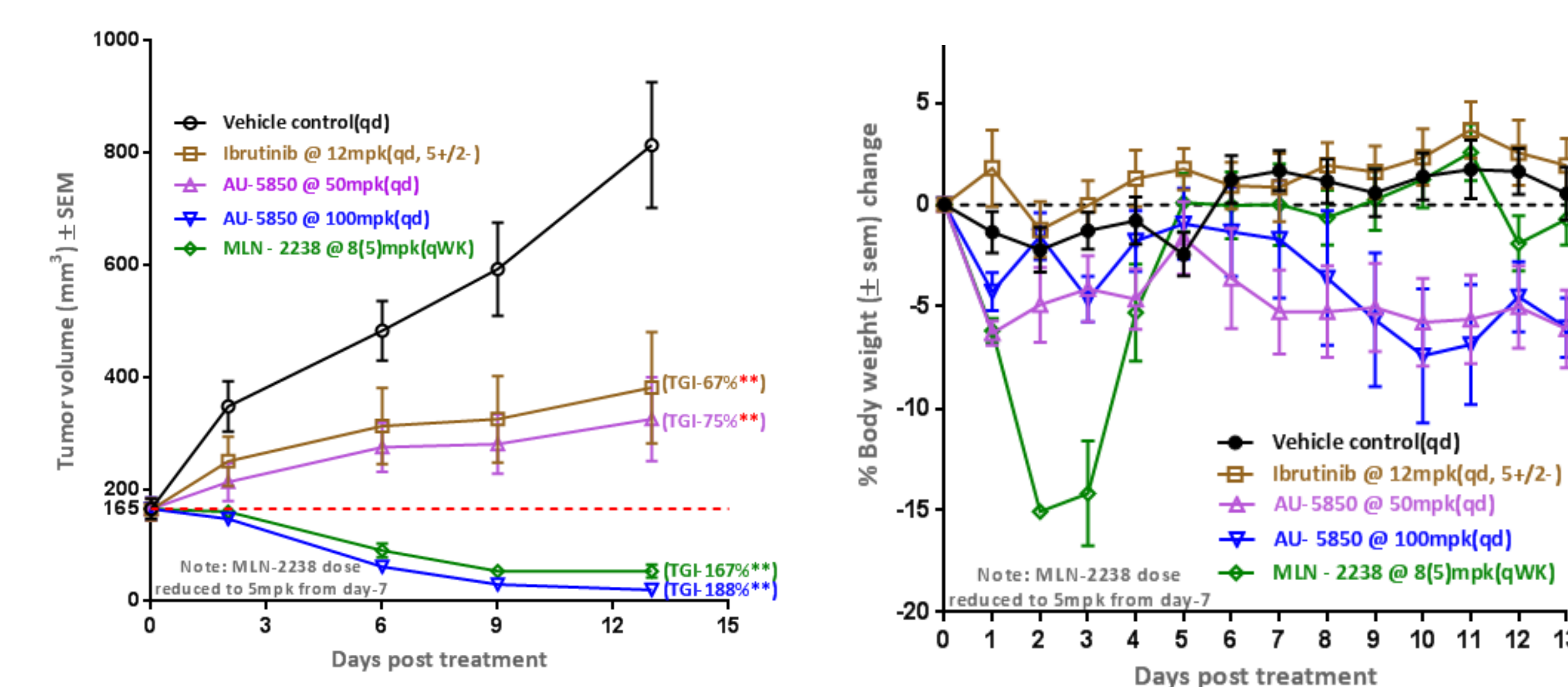
## In-vivo PK/PD Analysis in Inflammation Models



## In-vivo PK/PD Analysis in DLBCL Xenograft Models

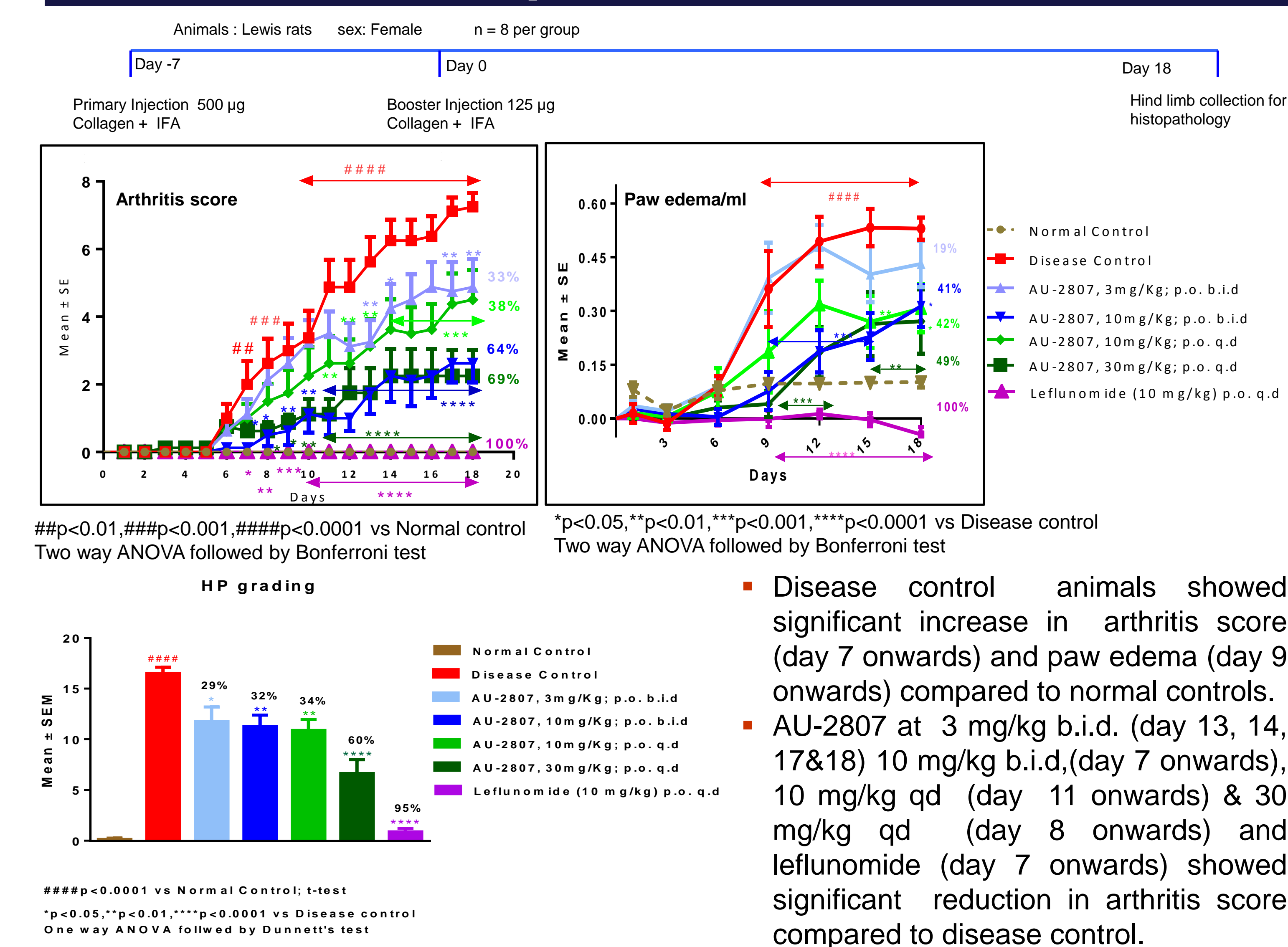


## Efficacy in OCI-Ly10 Xenograft Model



NOD-SCID mice bearing OCI-Ly10 tumors were treated orally once daily with AU-5850 at the indicated doses for two weeks. AU-5850 treatment resulted in 75% tumor growth inhibition at 50 mg/kg dose & tumor regression at 100 mg/kg dose. AU-5850 was well tolerated at both the doses with no major changes in body weight. MLN-2238, a proteasome inhibitor, was used as positive control.

## Efficacy in Rat CIA Model



AU-2807 treatment shows superior efficacy (oral dosing, 69% inhibition) compared to reference compound (bid IP dosing, 39% inhibition) at same dose of 30 mpk.

## In Vitro Tox Profile of AU-2807

- No rat hepatocyte cytotoxicity (EC<sub>50</sub> >5 µM)
- Negative in Ames test (tested with TA98, TA100, TA102, TA1535 & TA1537)
- No significant hERG activity

## Conclusion

- Potent IRAK-4 inhibitors from multiple chemically distinct series identified
- Excellent potency in both biochemical and cell based assays
- Good PK profile with oral administration
- Good PK/PD correlation established in LPS-induced rat systemic inflammation model.
- Efficacy demonstrated with oral dosing in disease models of inflammation (CIA) and DLBCL (OCI-Ly3 xenograft model)
- In-vivo* 14 day tox study in progress for select compounds

