Efficacy and safety of highly selective novel IRAK4 inhibitors for treatment of ABC-DLBCL

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ABSTRACT

Interleukin-1 receptor associated kinases (IRAKs) are serine/threonine protein kinases belonging to the tyrosine-like kinase (TLK) family. IRAKs function as mediators of Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways and play an important role in innate immune signaling. TLR/IL-1R stimulation leads to recruitment of MYD88, an adaptor molecule, to the activated receptor complex, which then complexes with IRAK4 and activates IRAK1. TRAF6 is then activated by IRAK1 leading to NFkB activation. Recent studies have reported the occurrence of gain of function oncogenic mutation (L265P) in MYD88 in ~30% of activated B cell diffuse large B-cell lymphoma(ABC DLBCL) and ~90% of Waldenstrom's macroglobulinemia (WM) leading to constitutive activation of IRAK4 and NFkB pathway. Among the DLBCL subtypes (GCB, ABC DLBCL and PMBL), ABC DLBCL is the most refractory. Inhibition of constitutive IRAK4 signalling can be used as a therapeutic strategy to treat ABC DLBCL. Small molecule inhibitors of IRAK4 were synthesized based on hits originating from Aurigene's compound library. Structure-guided drug design approach was used to further improve the potency. Lead compounds demonstrated moderate to very high selectivity towards IRAK4 (S35 score of 0.03) when screened against a large panel of 329 kinases. Aurigene's lead compounds exhibited excellent PK profile and good oral bioavailability in mice, leading to good in-vivo activity in TLR4 induced cytokine release model. Selected lead compounds were tested in a OCI-Ly3 xenograft model, which has a MYD88(L265P) mutation leading to constitutive activation of IRAK4 signaling. An advanced lead compound demonstrated excellent efficacy in OCI-Ly3 and OCI-Ly10 models, with tumor stasis at low doses and tumor regression at higher doses. In summary, a selective IRAK4 inhibitor has been identified with excellent efficacy and good safety profile.

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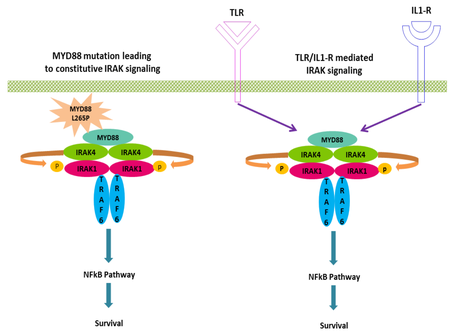
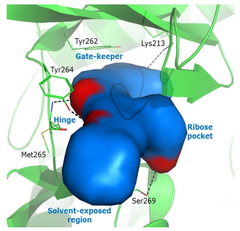
IRAK Inhibitors for treatment of ABC DLBCL



- MYD88 adaptor protein transduces receptor signaling to IRAK4
- ~30% ABC-DLBCL, ~90% WM and ~6% CLL patients have activating MYD88 mutations
- Activating MYD88 mutations lead to constitutive activation of IRAK signaling

Binding mode of Aurigene Compound

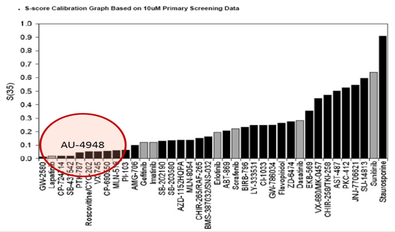


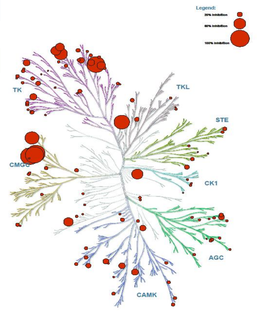
- Aurigene's lead compound docked with hIRAK4-kinase domain
- Compound anticipated to bind in ATP binding pocket close to gate-keeper residue (Tyr262)
- Polar interactions with hinge residues (Tyr264 & Met265) and back-pocket catalytic lysine (Lys213)

Potency and Selectivity Profile

Compound	hIRAK4 IC_{50} nM	Kinase Selectivity S35 Score	
		at 1µM	at 10µM
AU-4948	37	0.0343	0.0515

AU-4948 exhibits best-in-class selectivity, comparable to the most selective kinase inhibitors in market



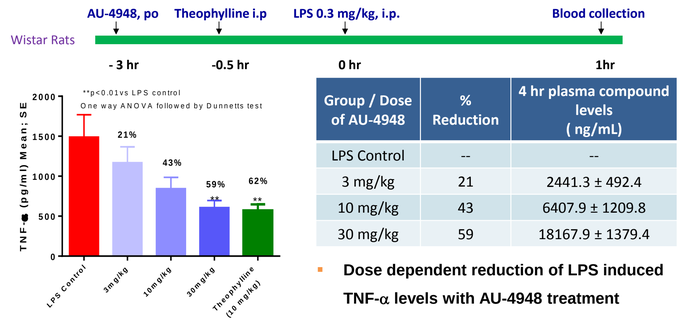


"Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)"

DMPK Profile of Lead Compound

Parameters		AU-4948
Equilibrium Solubility pH 7.4/5.8 (µM)		39/38
Metabolic Stability (MLM, RLM, HLM, DLM, MoLM) t ½ (min); Clint (µl/min/mg)		>60; <38 (all species)
Caco2 Permeability (A-B)		1.8E-05 cm/s, Medium
Caco2 Permeability Efflux Ratio (B-A/A-B) with / without P-gp inhibitor		2.44 / 2.17 (not a P-gp substrate)
CYP Inhibition IC50 1A2, 2B6, 2C9, 2C19, 2C8, 2D6, 3A4		>50 µM
PPB (%bound) Rat/Mice/Human/Dog/Monkey		99.8 / 92.8 / 77.6 / 85.4 / 76.4
Plasma Stability t ½ (hr) Rat/Mice/Human/Dog/Monkey		>5
Mouse IV PK Dose: 3 mg/kg	$t_{1/2}$ (hr)	2.58
	$AUC_{(0\text{-inf})}$ (ng.hr/mL)	5393
	Cl(mL/min/kg)	9.27
	Vd_{ss} (L/kg)	0.66
Mouse Oral PK Dose: 10 mg/kg	C_{max} (ng/mL)	6618
	$AUC_{(0\text{-inf})}$ (ng.hr/mL)	12741
	F(%)	71

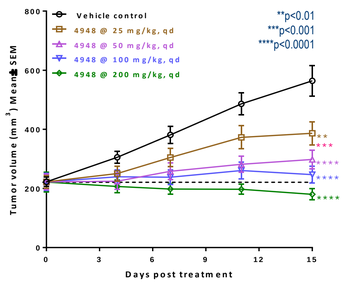
In-vivo activity of AU-4948 in TLR4 induced TNFα release



Group / Dose of AU-4948	% Reduction	4 hr plasma compound levels (ng/mL)
LPS Control	--	--
3 mg/kg	21	2441.3 ± 492.4
10 mg/kg	43	6407.9 ± 1209.8
30 mg/kg	59	18167.9 ± 1379.4

- Dose dependent reduction of LPS induced TNF-α levels with AU-4948 treatment

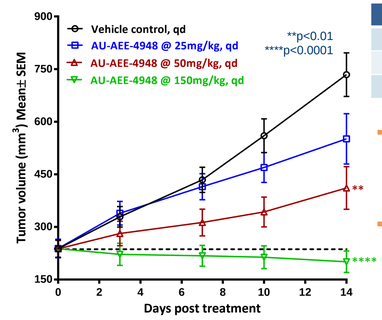
Efficacy of AU-4948 in OCI-Ly3 Xenograft Model



Treatment groups (n=9)	%TGI
AU-4948 @ 25 mg/kg, po,qd	52**
AU-4948 @ 50 mg/kg, po,qd	78****
AU-4948 @ 100 mg/kg, po,qd	93****
AU-4948 @ 200 mg/kg, po,qd	119****

- AU-4948 treatment led to dose dependent inhibition of tumor growth with MED of 50 mg/kg
- No body weight reduction in treatment groups
- Well tolerated with no clinical signs or gross pathological changes

Efficacy of AU-4948 in OCI-Ly10 Xenograft Model



AU- 4948	% TGI
25 mg/kg,qd	37%
50 mg/kg,qd	65%**
150 mg/kg,qd	Partial tumor regression****

- AU-4948 treatment resulted in dose dependent tumor growth inhibition in OCI-Ly10 xenograft model
- AU-4948 was well tolerated at all the tested doses without treatment related body weight changes & clinical signs

PD modulation in OCI-Ly3 tumors by AU-4948 in Single Dose PK/PD Study

AU-4948 treated (200 mg/kg)	% Inhibition of pIRAK1	% inhibition of IL-6
4 hr	No inhibition	No inhibition
8 hr	No inhibition	No inhibition
12 hr	78	46
24 hr	56	No inhibition

- Maximal inhibition of p-IRAK1 observed 12 hours after dosing
- Inhibition of IL-6 correlates well with the pIRAK1 inhibition

In-Vitro Toxicity Profile of AU-4948

Parameter	Profile
hERG (patch clamp)	<10% inhibition at 30µM
Ames test	Non-mutagenic in five strains of Salmonella typhimurium (@5 mg/plate)
CYP inhibition (8 isoforms)	IC_{50} >50µM
CYP induction (3 major isoforms)	No CYP induction (tested at 10 µM)
CEREP-44 panel	No Significant inhibition at 10 µM

Clean in-vitro toxicity profile

- No hERG inhibition / Negative in Ames test / No CYP inhibition/ No CYP induction

Conclusion

- Potent IRAK-4 inhibitors from multiple chemically distinct series identified
- Dose dependent inhibition of TLR4 induced TNFα release demonstrated in-vivo
- Dose dependent inhibition of tumor growth demonstrated in OCI-Ly3/OCI-Ly10 model with MED of 50 mg/kg
- PD modulation demonstrated in OCI-Ly3 model in a single dose PK/PD study
- AU-4948 was well tolerated at all tested doses in OCI-Ly3 and OCI-Ly10 models