Efficacy of the IRAK4 Inhibitor CA-4948 in Patient-Derived Xenograft Models of Diffuse Large B Cell Lymphoma

#1168

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Abstract

IRAK4 kinase activity is required for toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling in a variety of myeloid and lymphoid cell types. Recruitment of IRAK4 to these receptors and its subsequent activation is facilitated by the MYD88 adaptor protein, which is mutated in ~22% of DLBCL cases. The MYD88 L265P activating mutation is found in ~30% of the activated B-cell (ABC) and ~6% of germinal center B-cell (GCB) subtypes of DLBCL and leads to constitutive activation of NF-kB signaling that is associated with worse prognosis. Thus, the development of small molecule inhibitors targeting IRAK4 is an attractive anticancer strategy for MYD88 mutation-containing cancers such as DLBCL.

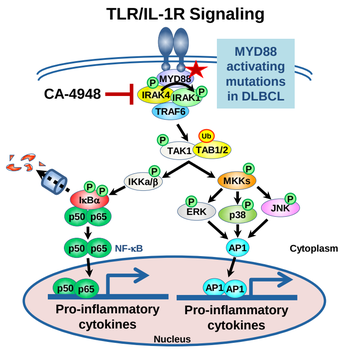
We are developing an IRAK4 inhibitor, CA-4948, as a therapeutic agent for hematological cancers with dysregulated TLR/MYD88/IRAK4 signaling. CA-4948 (previously AU-4948) is a selective and potent IRAK4 kinase inhibitor with in vivo activity in a TLR4-induced cytokine release model. CA-4948 exhibits favorable DMPK properties, oral bioavailability, and is well tolerated in mice. Furthermore, CA-4948 was previously shown to exhibit dose-dependent efficacy in ABC-DLBCL MYD88-L265P xenograft tumor models using cell lines OCI-LY3 and OCI-LY10.

Here, we report the efficacy results from testing CA-4948 in a panel of well characterized, patient-derived DLBCL tumor xenograft (PDX) mouse models. CA-4948 exhibited the greatest efficacy in four of the five ABC-DLBCL PDX models tested as compared to GCB-DLBCL and ABC/GCB DLBCL PDX models. Furthermore, CA-4948 was efficacious in ABC-DLBCL PDX tumors containing activating mutations in both TLR/IL-1R and BCR signaling pathways (MYD88 and CD79B double mutants). Interestingly, the one ABC-DLBCL PDX model that failed to respond to CA-4948 treatment contained a MYD88 L265P mutation as well as a BCL6 translocation. While this particular PDX model was resistant to CA-4948, and showed a weak anti-tumor response to single-agent ibrutinib, the combination treatment of ibrutinib and CA-4948 exhibited a synergistic tumor growth inhibition effect. In summary, CA-4948 exhibited anti-tumor activity in ABC-type DLCBL PDX tumor models including those containing combinations of activating mutations in the TLR/IL-1R and BCR signaling pathways. These results underscore the therapeutic potential of IRAK4 kinase inhibition by CA-4948, as a single-agent or in combination with BCR inhibitors, for the treatment of DLBCL.

CA-4948 IRAK4 Inhibitor for Treatment of DLBCL

CA-4948 Kinase Inhibitory Activity

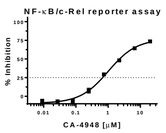
Kinase	DiscoverX Kd (nM)
IRAK4	23
IRAK1	>100



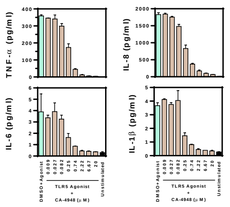
CA-4948 Blocks the TLR/IL-1R Induced Canonical NF-κB Signaling Pathway

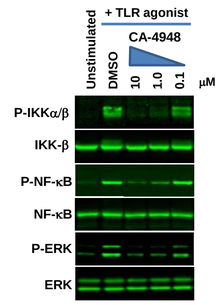
In vitro THP1 monocytic cell assays

TLR-induced NF-κB reporter assays (IC_{50} = 520 nM, THP1)



TLR-induced cytokine release assays (IC_{50} = 150-220 nM, THP1)



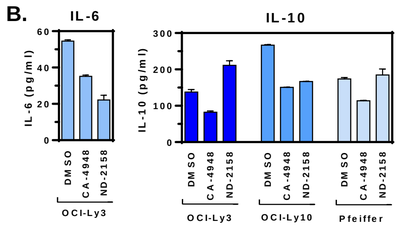


CA-4948 In Vitro Effect on DLBCL Cell Lines

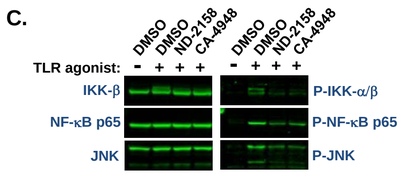
A.

DLBCL Cell Line	Cell-of-origin	MYD88	Cell Viability IC_{50} (μM) CA-4948	ND-2158
OCI-Ly3	ABC	L265P	>20	>10
OCI-Ly10	ABC	L265P	3.58	1.09
SUDHL-2	ABC	S222R	7.86	>10
U2932	ABC	-	10.42	>10
DOHH-2	GCB	wt	>20	>10
OCI-Ly1	GCB	-	>20	-
Pfeiffer	GCB	wt	6.35	>10
SUDHL-4	GCB	wt	>20	>10
Toledo	GCB	wt	6.08	>10
SUDHL-6	GCB	S219C	>20	>10
SUDHL-10	GCB	-	>20	-
WSU-DLCL2	GCB	wt	>20	>10
SUDHL-5	GCB	wt	>20	-
JM1	GCB	wt	15.2	-

B.



C.

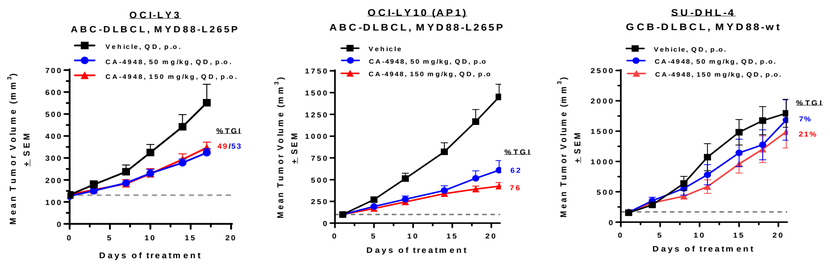


A. Viability IC_{50} values determined after 72 hr treatment (CellTiter-Glo) with IRAK4 inhibitors CA-4948 and ND-2158

B. Level of cytokines present in media after 21 hr treatment with 10 μM of IRAK4 inhibitors

C. Western blot analysis of a DLBCL cell line during a 20 min TLR-agonist stimulation with 10 μM IRAK4 inhibitors ND-2158 and CA-4948

CA-4948 Exhibits Enhanced In Vivo Efficacy in MYD88-L265P Mutant Versus MYD88-wt DLBCL Tumors



CA-4948	%TGI	n
50 mg/kg	53%	8/8
150 mg/kg	49%	7/8

*p<0.05

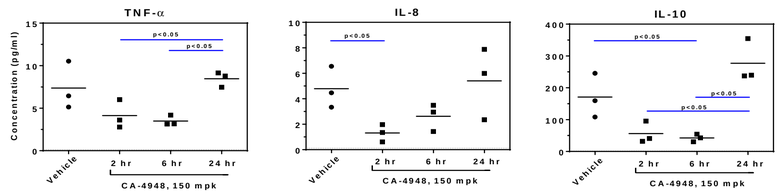
CA-4948	%TGI	n
50 mg/kg	62*	10/10
150 mg/kg	76*	10/10

*p ≤0.002

CA-4948	%TGI	n
50 mg/kg	7*	7/8
150 mg/kg	21*	8/8

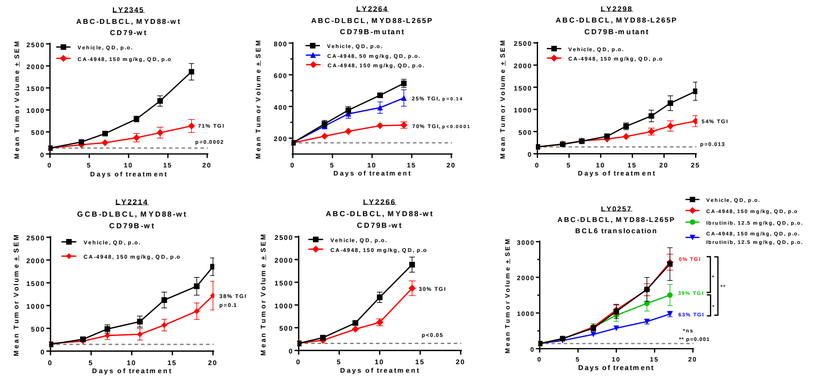
*p = ns

CA-4948 Pharmacodynamic Response in DLBCL PDX Tumor-Bearing Mice



➢ **Tumor-derived human TNF-α, IL-8 and IL-10 cytokine levels in mouse plasma 2, 6 and 24 hr after administering a single oral dose of 150 mg/kg CA-4948**

CA-4948 Efficacy in 6 DLBCL PDX Tumor Models



DLBCL PDX Model	COO Subtype Classification: CrownBio Gene Signature	COO Subtype Classification: PhenoPath Hans IHC	Mutations/Expression: MYD88 Mutation	Mutations/Expression: B-Cell Receptor Mutation	Mutations/Expression: BCL6 Expression (IHC)	CA-4948 Efficacy: 150 mg/kg po, QD %TGI
LY2345	ABC	non-GCB	WT	-	2%	71**
LY2264	ABC	non-GCB	L265P	CD79B	60%	70**
LY2298	ABC	non-GCB	L265P	CD79B	20%	54*
LY2214	GCB	non-GCB	WT	WT	95%	38
LY2266	ABC/GCB	non-GCB	WT	WT	0%	30*
LY0257	ABC	non-GCB	L265P	WT	95%	0

*p<0.05
**p≤0.001

Summary

- **CA-4948 is a potent IRAK4 Ser/Thr kinase inhibitor with >100-fold less activity against IRAK1**
- **CA-4948 exhibited weak to no anti-proliferative effect against DLBCL cell lines grown in 10% FBS. However, in ABC-DLCBL cells, CA-4948 repressed constitutive IL6 and IL10 cytokine secretion and TLR-agonist stimulation of the TLR/NF-κB signaling pathway**
- **CA-4948 exhibited greater efficacy in ABC-DLBCL, MYD88-L265P cell line xenograft models (OCI-LY3 and OCI-LY10) compared to a GCB-DLBCL, MYD88-WT model (SU-DHL-4)**
- **A single oral dose of 150 mg/kg CA-4948 repressed tumor-derived cytokine levels in the plasma of a DLBCL PDX model**
- **CA-4948 exhibited efficacy (>50% TGI) in 3-of-5 ABC-DLBCL PDX models, independent of MYD88 or B-cell receptor (BCR) mutations**
- **High BCL6 expression in the ABC-DLBCL, MYD88-L265P PDX tumor model LY0257 may be the cause of the tumor's resistance to CA-4948 treatment**
- **In conclusion, these results support the continued investigation of CA-4948, as a single-agent or in combination with BCR inhibitors, for the treatment of DLBCL**

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