#C31

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Advances in Malignant Lymphoma

Efficacy of IRAK4 Kinase Inhibitor CA-4948 in Mantle Cell Lymphoma

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Abstract

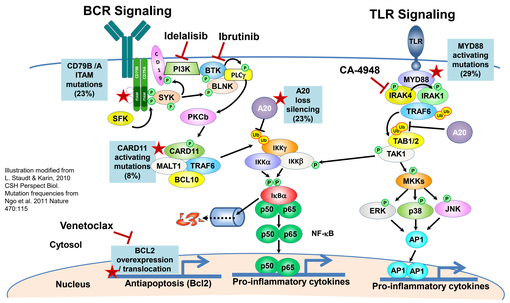
Background: NF-κB signaling plays a critical role in MCL as evidenced by the high response rate observed in refractory/relapsed MCL patients treated with BTK inhibitor ibrutinib. However, the majority of these patients relapse with a dismal prognosis. Thus, identifying additional targeted agents that affect the eventual activation of NF-κB mediated through the B-cell receptor (BCR) or other signaling pathways is needed to address primary or relapsed ibrutinib-resistance. As an essential component in the IL1R/toll-like receptor (TLR) mediated NF-κB signaling pathway, IRAK4 is one such target. For these studies, we tested the novel oral IRAK4 kinase inhibitor CA-4948, which is currently in a Phase I trial for R/R non-Hodgkin lymphoma (clinicaltrials.gov NCT03328078).

Experimental procedures: Six MCL cell lines (JeKo-1, MAVER-1, Mino, GRANTA-519, REC-1, and Z-138) were exposed to escalating doses of CA-4948 either alone or in combination with other targeted agents, and changes in viability were evaluated after 24-96 hr. These same six cell lines were also treated with CA-4948 to assess its effect on TLR- agonist-induced NF-κB signaling by evaluating cytokine production and western blot analysis of intracellular signaling pathway components. Finally, the *in vivo* efficacy of CA-4948 was evaluated in mouse xenograft subcutaneous tumor models of these six cell lines.

Results: Similar to previously observed results in CA-4948 treated DLBCL cell lines, blocking IRAK4 kinase function was neither cytostatic nor cytotoxic in these six MCL lines under standard *in vitro* growth conditions (EC50 > 10 μM). In contrast, CA-4948 blocked TLR- agonist-induced pro-inflammatory cytokine production and TLR pathway activation markers (e.g. p-IKK) in MCL lines, including Mino and REC-1. Interestingly, GRANTA-519 and Z-138 cells exhibited constitutive production of a subset of cytokines in the absence of TLR stimulation, consistent with reports that these lines have deregulated alternative NF-κB signaling. *In vivo,* CA-4948 exhibited anti-tumor activity in the Mino and REC-1 xenograft models. Consistent with constitutive NF-κB activation independent of BCR and TLR signaling, CA-4948 demonstrated no activity against the GRANTA-591 and Z-138 xenograft tumor models.

Conclusion: Our findings reveal a requirement for IRAK4 kinase function in TLR- agonist-induced NF-κB signaling and cytokine production in MCL cell lines. Oral administration of CA-4948 demonstrated an essential *in vivo* role for IRAK4 function in certain MCL cells grown as xenograft tumors. These results provide the rationale for continued testing of CA-4948 in combination with canonical and alternative NF-κB pathway-targeted agents.

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

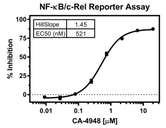


CA-4948:
- **Small molecule inhibitor**
- **ATP-competitive, reversible**
- **Oral bioavailable**

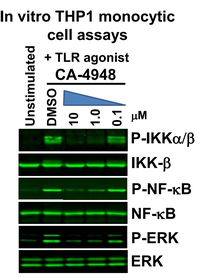
Kinase	DiscoverX Kd (nM)
IRAK4	23
IRAK1	12,000

CA-4948 Binding Affinity Activity

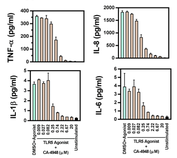
TLR-induced NF-κB reporter assays (IC_{50} = 520 nM, THP1)



In vitro THP1 monocytic cell assays



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



CA-4948 inhibition of NF-kB reporter, secreted cytokine levels, and phospho-signals in THP1 monocytic cells

In vitro CA-4948 Viability Assays in MCL Cell Lines

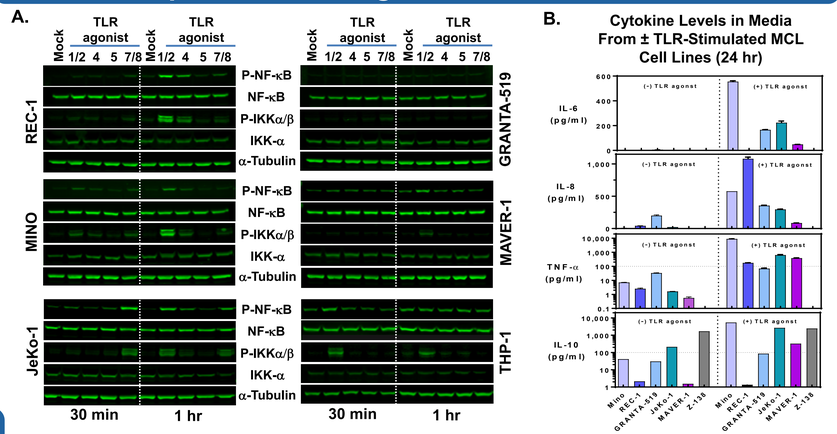
Cell Line	CA-4948 EC50 (μM)* 3 days	5 days	7 days
REC-1	>10	4.7	2.9
Mino	>10	>10	>10
JeKo-1	>10	>10	>10
GRANTA-519	>10	>10	>10
Z-138	>10	>10	>10
MAVER-1	>10	>10	>10

*: average of 2 or more CellTiter Glo viability assays

Cell Line	CA-4948 Combination* Palbociclib (CDK4/6i)	Ibrutinib (BTKi)	Venetoclax (BCL2i)	Bortezomib (Proteasome)
REC-1	no synergy	mild synergy	mild synergy	mild synergy
Mino	no synergy	no synergy	no synergy	no synergy
JeKo-1	no synergy	no synergy	no synergy	mild synergy
GRANTA-519	no synergy	no synergy	no synergy	no synergy
Z-138	no synergy	no synergy	no synergy	mild synergy
MAVER-1	no synergy	no synergy	no synergy	no synergy

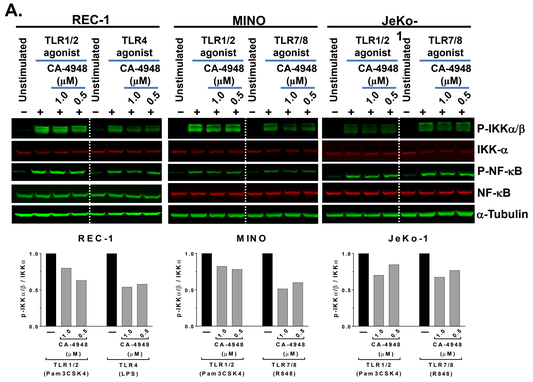
*: summary results of CellTiter Glo viability assays after 24, 48, 72 and 96 hr treatments

Activation of NF-κB pathway and Cytokine Production in Response to TLR Agonists in MCL Cell Lines



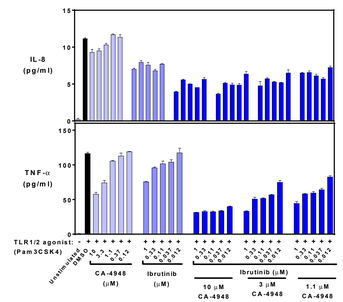
A. MCL cell lines were stimulated with agonists for TLR1/2 (Pam3CSK4), TLR4 (LPS), TLR5 (FLA-ST), or TLR7/8 (R878). NF-κB p65 blots are shown
B. MCL cells were stimulated with TLR cocktail (Pam3CSK4, LPS, FLA-ST, and R848)

CA-4948 ± Ibrutinib Inhibition of NF-κB p65 Signaling and Cytokine Production



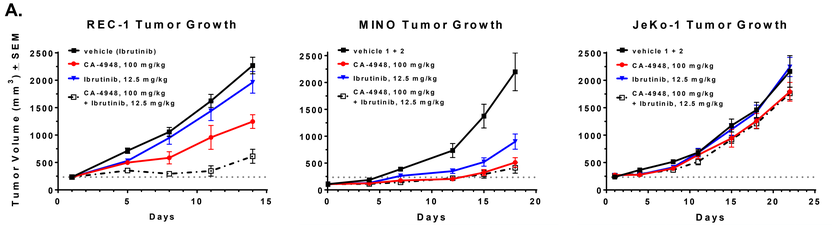
A. CA-4948 inhibition of TLR signaling pathway components after 1 hr stimulation
B. Combination effect of CA-4948 + ibrutinib on TLR-induced cytokine production

B. IL-8 and TNF-α levels in tissue culture medium of REC-1 cells after 5 hr TLR1/2 agonist stimulation



CA-4948 Exhibits Anti-Tumor Efficacy in Xenograft MCL Models with Canonical NF-κB Activation

A.

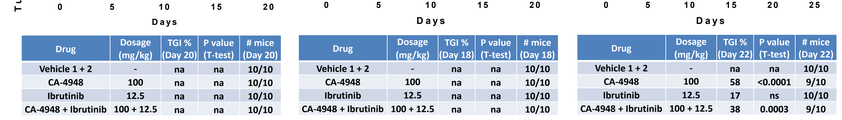


Drug	Dosage (mg/kg)	TGI % (Day 14)	P value (T-test)	# mice (Day 14)
Vehicle (Ibrutinib)	-	na	na	10/10
CA-4948	100	50	< 0.0001	9/10
Ibrutinib	12.5	15	ns	9/10
CA-4948 + Ibrutinib	100 + 12.5	81	< 0.0001	9/10

Drug	Dosage (mg/kg)	TGI % (Day 18)	P value (T-test)	# mice (Day 18)
Vehicle 1 + 2	-	na	na	11/12
CA-4948	100	81	< 0.001	12/12
Ibrutinib	12.5	62	0.002	12/12
CA-4948 + Ibrutinib	100 + 12.5	85	< 0.001	12/12

Drug	Dosage (mg/kg)	TGI % (Day 22)	P value (T-test)	# mice (Day 22)
Vehicle 1 + 2	-	na	na	8/10
CA-4948	100	20	0.26	10/10
Ibrutinib	12.5	3	0.81	9/10
CA-4948 + Ibrutinib	100 + 12.5	21	0.18	10/10

B.



Drug	Dosage (mg/kg)	TGI % (Day 20)	P value (T-test)	# mice (Day 20)
Vehicle 1 + 2	-	na	na	10/10
CA-4948	100	na	na	10/10
Ibrutinib	12.5	na	na	10/10
CA-4948 + Ibrutinib	100 + 12.5	na	na	10/10

Drug	Dosage (mg/kg)	TGI % (Day 18)	P value (T-test)	# mice (Day 18)
Vehicle 1 + 2	-	na	na	10/10
CA-4948	100	na	na	10/10
Ibrutinib	12.5	na	na	10/10
CA-4948 + Ibrutinib	100 + 12.5	na	na	10/10

Drug	Dosage (mg/kg)	TGI % (Day 22)	P value (T-test)	# mice (Day 22)
Vehicle 1 + 2	-	na	na	10/10
CA-4948	100	58	<0.0001	9/10
Ibrutinib	12.5	17	ns	10/10
CA-4948 + Ibrutinib	100 + 12.5	38	0.0003	9/10

In vivo efficacy studies of xenograft subcutaneous MCL tumor models. MCL cell lines with chronic activation of (A) BCR-driven classical and (B) alternative NF-κB pathways (Rahal R. et al., (2014) Nature Medicine 20(1) 87-94)

Summary

- CA-4948 is a potent, oral IRAK4 Ser/Thr kinase inhibitor with >500-fold against IRAK1
- CA-4948 treatment in culture did not result in antiproliferative/cytotoxicity activity in the 6 MCL cell lines tested (>10 μM at 3 days)
- CA-4948 inhibited TLR-induced signaling and cytokine production in MCL cell lines with an intact BCR-driven canonical NF-κB pathway
- CA-4948 resulted in potent *in vivo* anti-tumor activity in MCL models with intact canonical NF-κB signaling, which was enhanced in combination with ibrutinib treatment
- CA-4948 exhibited weakest *in vivo* activity in models with alternative NF-κB signaling
- These results underscore the therapeutic potential of targeted IRAK4 kinase inhibition by CA-4948 in combination with BTK inhibitors for the treatment of MCL

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