Combination of IRAK4 Inhibitor CA-4948 with BCL2 Inhibitor Venetoclax Induces Tumor Regression in an ABC-DLBCL Xenograft Model

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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 diffuse large B-cell lymphoma (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-κB signaling that is associated with worse prognosis. In Waldenstrom macroglobulinemia (WM), the *MYD88*-L265P activating mutation is present in >90% of cases. Thus, the development of small molecule inhibitors targeting IRAK4 is an attractive anticancer strategy for *MYD88* mutation-containing cancers such as DLBCL and WM.

We are developing a novel IRAK4 inhibitor, CA-4948, as a therapeutic agent for non-Hodgkin lymphoma (NHL) and hematological cancers with dysregulated TLR/MYD88/IRAK4 signaling. CA-4948 is a reversible kinase inhibitor that modulates IRAK4 function in both the toll-like receptor (TLR) and interleukin 1 receptor (IL-1R) signaling cascades, and demonstrates pharmacodynamic and antitumor activity in *in vitro* and *in vivo* nonclinical models. 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 and patient-derived tumors.

Activating mutations in the B-cell receptor (BCR) signaling pathway are frequently present in various NHL subtypes, leading to activation of NF-kB signaling, and growth and survival pathways. Dysregulation of both TLR and BCR pathways suggest that targeting both pathways will be required for improved therapeutic responses in NHL. Here, we report efficacy results from combined treatment of CA-4948 and the BCL2 inhibitor venetoclax in an ABC-DLBCL xenograft tumor model. We performed an *in vivo* study in female SCID Beige mice implanted with the ABC-DLBCL cell line OCI-Ly10, which harbors TLR (*MYD88*-L265P) and BCR (CD79A ITAM) pathway activating mutations. OCI-Ly10 tumor-bearing mice were treated with CA-4948 (50 mg/kg, qd, po), venetoclax (75 mg/kg, qd, po) or the combination for 21 continuous days. Single agent treatment of CA-4948 and venetoclax exhibited moderate tumor growth inhibition (TGI) of 63% and 71%, respectively, and the combination demonstrated tumor regression. The combination was tolerated at the end of treatment without body weight loss relative to predose weight. After the 21-day drug treatment period, the mice were given a 19-day dosing holiday. Within 5 days of the holiday rapid tumor growth occurred in the previously treated single drug-treated mice and this rapid growth continued for the remainder of the dosing holiday. In contrast, tumors from the combination drug-treated mice did not commence detectable regrowth until the 10th day of the drug holiday and proceeded with a slow growth rate for the subsequent 9 days.

After the 19-day drug holiday, dosing of the respective drug treatments was reinstated to each cohort. After 7 days of redosing, the tumors in each cohort exhibited antitumor responses to respective drug treatments with the CA-4948 plus venetoclax combination again driving the tumors into regression. In summary, CA-4948 plus venetoclax was a well-tolerated drug combination that exhibited a much greater *in vivo* antitumor response as compared to the single agent treatments. These results underscore the therapeutic potential of targeted IRAK4 kinase inhibition by CA-4948 in combination with other targeted agents for the treatment of NHL.

CA-4948 IRAK4 Inhibitor for Treatment of DLBCL

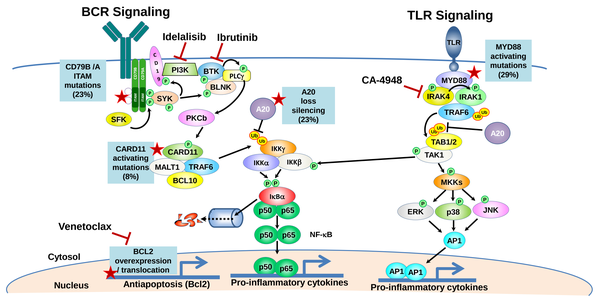

Illustration modified from L. Staudt & Kainn, 2010 CSH Perspect Biol. Mutation frequencies from Ngo et al. 2011 Nature 470:115

- **Potential to enhance efficacy of CA-4948 Toll-like receptor (TLR) pathway inhibition by combining with antiapoptosis Bcl2 or B-cell receptor (BCR) pathway inhibitors**

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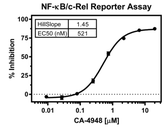
CA-4948 Blocks the TLR/IL-1R Induced Canonical NF-κB Signaling Pathway

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

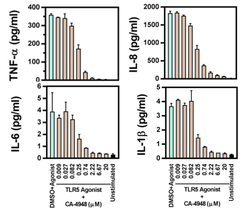
CA-4948 Binding Affinity Activity

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

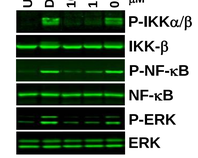
TLR-induced NF-κB reporter assays (IC_{50} = 520 nM, THP1)



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

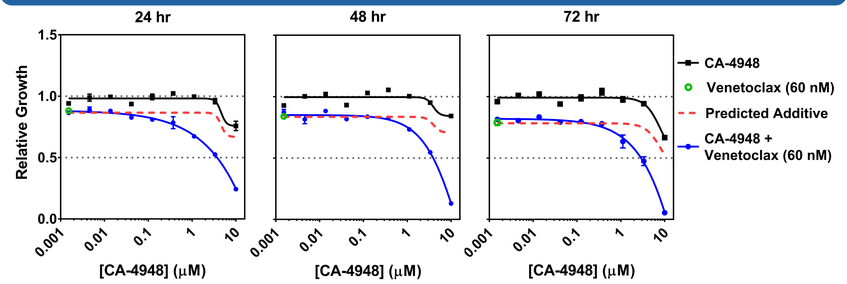


In vitro THP1 monocytic cell assays



- **TLR/IL-1R signaling pathway inhibition. Good correlation between inhibition of NF-kB reporter, secreted cytokine levels, and phospho-signals**

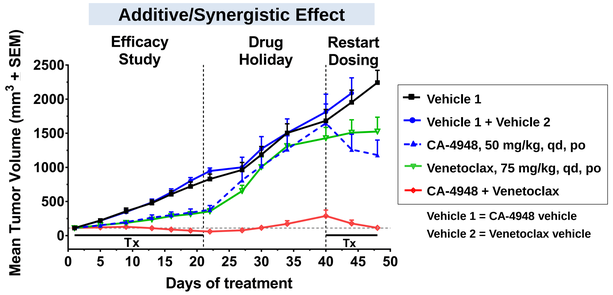
In vitro Cell Viability Effect of CA-4948 + Venetoclax Combination in MYD88-L265P ABC DLBCL Cell Line



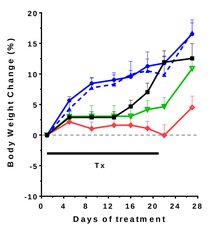
- **OCI-Ly10 (ap1) cells: ABC-DLBCL, *MYD88*-L265P, *CD79A*-G208del**
- **OCI-Ly10 (ap1) cells were derived from a mouse-grown OCI-Ly10 subcutaneous tumor**

CA-4948 Plus Venetoclax Combination Exhibits Enhanced Efficacy in a OCI-Ly10 (ap1) DLBCL Xenograft Tumor Model

Additive/Synergistic Effect



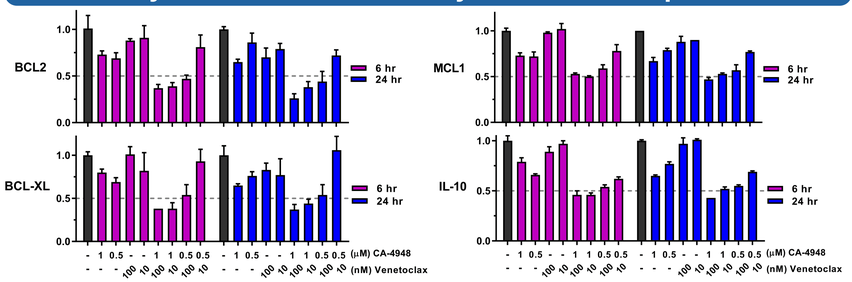
Combination was tolerated



Groups	%TGI (Day 22)
CA-4948, 50 mg/kg, po, qd	63* (n=9)
Venetoclax, 75 mg/kg, po, qd	71* (n=10)
CA-4948, 50 mg/kg + Venetoclax, 75 mg/kg	Regression* (n=10)

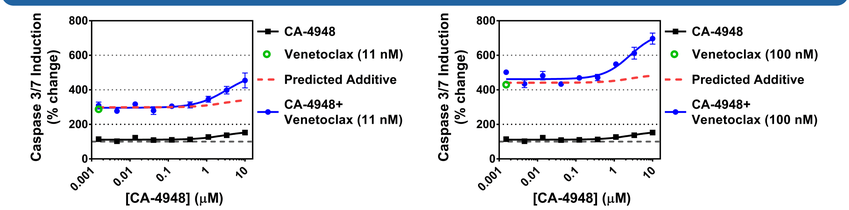
*p<0.0002

CA-4948 Plus Venetoclax Combination Downregulates BCL2 Family Members and IL-10 Cytokine RNA Expression



- **OCI-Ly10 (ap1) cells: ABC-DLBCL, *MYD88*-L265P, *CD79A*-G208del**
- **RNA expression levels were normalized to respective DMSO control samples (black bars)**
- **Bars represent the average of two independently-treated samples, each assayed in duplicate**

Synergistic Caspase-3/7 (Apoptosis) Induction by CA-4948 Plus Venetoclax Combination



- **Caspase-3/7 induction after 5 hr treatment of CA-4948 ± venetoclax in OCI-Ly10 (ap1) cells**

Summary

- CA-4948 is a potent, oral IRAK4 Ser/Thr kinase inhibitor with >500-fold less activity against IRAK1
- *In vitro* cell viability assays showed synergy with the CA-4948 + venetoclax combination in the *MYD88*-L265P mutant ABC DLBCL cell line OCI-Ly10 (ap1)
- Additive/synergistic anti-tumor effect was observed for the CA-4948 + venetoclax combination in mice bearing OCI-Ly10 (ap1) xenograft tumors, and the combination was well tolerated *in vivo* with no BWL
- *In vitro*, the CA-4948 + venetoclax combination enhanced downregulation of BCL2 antiapoptosis family members and IL-10 mRNA expression. Synergistic caspase-3/7 induction was also observed
- These results underscore the therapeutic potential of targeted IRAK4 kinase inhibition by CA-4948 in combination with other targeted agents for the treatment of NHL

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Disclosures: R. Booher*, S. Dellarocca*, R. Atoyan*, M. Borek*, M Samson*, and D. Tuck*
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