#4168

ASH 2018

Preclinical Activity of IRAK4 Kinase Inhibitor CA-4948 Alone or in Combination with Targeted Therapies and Preliminary Phase 1 Clinical Results in Non-Hodgkin Lymphoma

Robert N Booher[1], Grzegorz S. Nowakowski[2], Krish Patel[3], Matthew A. Lunning[4], Maria Elena S. Samson[1], Ruzanna Atoyan[1], Anna W Ma[1], Guang-Xin Xu[1], Steven Dellarocca[1], Holly Modafferi[1], Mylissa Borek[1], Zhidong Zhang[1], Jefferson Parker[1], Duncan Whitney[1], Hongwei Wang[1], David P Tuck[1] and Anas Younes[5]

[1]Curis, Inc., Lexington, MA, [2]Division of Hematology, Mayo Clinic, Rochester, MN, [3]Center for Blood Disorders and Stem Cell Transplantation, Swedish Cancer Institute, Seattle, WA, [4]University of Nebraska Medical Center, Omaha, NE, [5]Memorial Sloan Kettering Cancer Center, New York, NY

Abstract

Background: 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. The MYD88-L265P activating mutation is prevalent in DLBCL (~30% in ABC subtype) and WM (>90%). MYD88- L265P leads to constitutive activation of NF-κB signaling that is associated with worse prognosis. In MCL, dysregulation of B-cell receptor (BCR) and TLR pathway components correlate with constitutive NF-κB signaling.

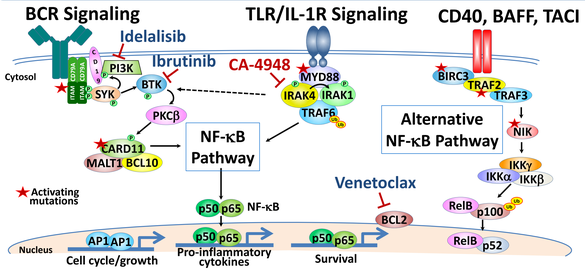
CA-4948 is a small molecule inhibitor of IRAK4 kinase that modulates the TLR and IL-1R signaling cascades. CA-4948 is being developed as a novel agent for the treatment of hematologic cancers with dysregulated IRAK4 signaling and is currently in a Ph1 trial for R/R NHL (clinicaltrials.gov NCT03328078). In preclinical studies, CA-4948 demonstrates pharmacodynamic and antitumor activity in *in vitro* and *in vivo* models with MYD88 alterations, and was previously shown to have a synergistic anti-tumor activity when combined with venetoclax in vivo. To further guide CA-4948's clinical development in NHL, we report here nonclinical studies exploring a twice-daily dosing schedule in DLBCL xenograft models. We also investigated the use of an *ex-vivo* whole-blood TLR-stimulation assay as a surrogate PD response biomarker. Additionally, we tested the efficacy of CA-4948 alone or in combination with the BTK inhibitor ibrutinib in DLBCL and MCL tumor models. Furthermore, preliminary PK and PD data from the first-in-human Ph1 trial are presented.

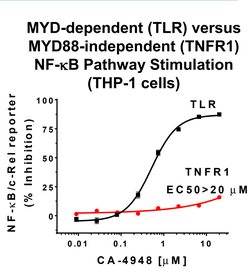
Methods: Mice bearing DLBCL PDX tumors were orally administered CA-4948 twice-daily (BID) with 37.5 or 75 mg/kg doses and once-daily (QD) with 75 or 150 mg/kg doses. The ex-vivo whole blood assay involved TLR-stimulation of blood isolated at various time-points after CA-4948 administration. For the drug combination studies, mice bearing subcutaneous tumors of a MYD88-L265P DLBCL cell line or six MCL cell lines were treated.

Results: (1) CA-4948 exhibited dose-dependent tumor growth inhibition in two DLBCL PDX xenograft tumor models with BID dosing showing equal or enhanced efficacy as compared to the equivalent total daily QD dose. The BID schedule was well tolerated with only a slight body weight loss as compared to the equivalent total QD dose schedule. (2) Overall, in mouse, the ex-vivo blood assay showed a time and exposure dependent relationship with the level of cytokine production after TLR-stimulation. A similar CA-4948 dose-dependent inhibition of TLR-stimulated cytokine production was observed in healthy human whole blood samples in which CA-4948 was spiked into the blood sample. Based on these findings, CA-4948 exposure levels capable of inhibiting TLR-stimulation are anticipated to be readily achievable in clinical studies. This was also supported by preliminary clinical PD data showing post treatment, on-target, reduced release of NF-κB-associated cytokines in 2 of 4 patients treated so far. (3) In xenograft efficacy studies using MCL models, single agent CA-4948 and ibrutinib (IBN) exhibited anti-tumor activity and showed an additive effect when combined in the majority of the models known to have BCR-driven constitutive canonical NF-κB signaling (REC-1, MINO, and JeKo-1). Interestingly, neither CA-4948, ibrutinib, nor the combination had anti-tumor activity in Z-138 and GRANTA-591 xenograft models, consistent with these cell lines having activated NF-κB through the alternative NIK signaling pathway. (4) The human QD PK data (n=4) demonstrated that CA-4948: was rapidly absorbed, T_{max} 1-3 hr, and $t_{1/2}$ of 3.6-6.8 hr. The bioavailability and exposure, as assessed by C_{max} and AUC, is within the expected range compared to non-clinical PK and did not show any evidence of accumulation after QD dosing for 15 consecutive days.

Conclusion: These results provide a rationale for CA-4948 BID dosing and incorporating the use of an ex-vivo whole-blood TLR-stimulation assay as a surrogate PD response biomarker, the former of which will be evaluated in the current Ph1 dose escalation soon and the latter of which is currently being implemented in the Ph1 trial for patients with advanced NHL. The murine xenograft results further support exploration of CA-4948 as monotherapy and in combination with canonical and alternative NF-κB pathway-targeted agents in DLBCL and MCL.

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

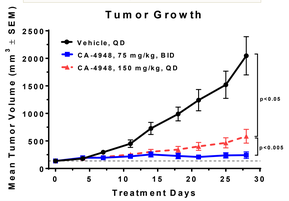
	DiscoverX
Kinase	Kd (nM)
IRAK4	23
IRAK1	12,000
IRAK2	>20,000
IRAK3	8,500

TLR-Stimulated THP-1 Cells

	CA-4948
Cytokine Release	EC_{50} (nM)
TNF-α	242
IL-8	201
IL-6	202
IL-1β	220

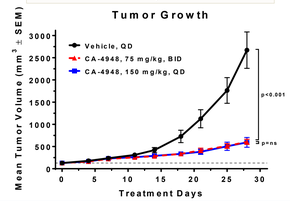
Efficacy of CA-4948 in Two DLBCL PDX Models: QD versus BID Dosing Schedule

LY2345 PDX: ABC DLBCL *MYD88*-wt, *TNFIAP3*-mt



Drug	Dosage (mg/kg)	Schedule	%TGI
CA-4948	75	BID	94
CA-4948	150	QD	77

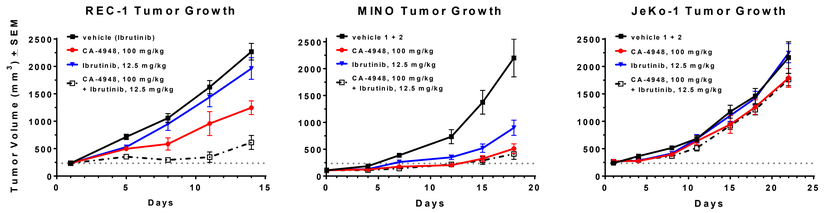
LY2298 PDX: ABC DLBCL *MYD88*-L265P, CD79B-mut



Drug	Dosage (mg/kg)	Schedule	%TGI
CA-4948	75	BID	82
CA-4948	150	QD	81

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

(A) MCL cell lines with chronic activation of BCR-driven canonical NF-κB signaling pathways

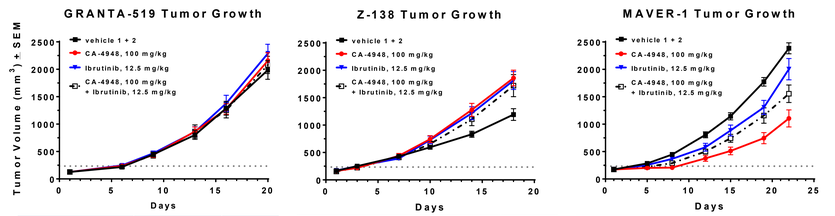


Drug (po, qd)	Dose (mg/kg)	%TGI
Vehicles	-	na
CA-4948	100	50**
Ibrutinib	12.5	15*
CA-4948 + IBN	100 + 12.5	81**

Drug (po, qd)	Dose (mg/kg)	%TGI
Vehicles	-	na
CA-4948	100	81**
Ibrutinib	12.5	62**
CA-4948 + IBN	100 + 12.5	85**

Drug (po, qd)	Dose (mg/kg)	%TGI
Vehicles	-	na
CA-4948	100	20*
Ibrutinib	12.5	3*
CA-4948 + IBN	100 + 12.5	21**

(B) MCL cell lines with chronic activation of alternative NF-κB signaling pathways



Drug (po, qd)	Dose (mg/kg)	%TGI
Vehicles	-	na
CA-4948	100	na
Ibrutinib	12.5	na
CA-4948 + IBN	100 + 12.5	na

Drug (po, qd)	Dose (mg/kg)	%TGI
Vehicles	-	na
CA-4948	100	na
Ibrutinib	12.5	na
CA-4948 + IBN	100 + 12.5	na

Drug (po, qd)	Dose (mg/kg)	%TGI
Vehicles	-	na
CA-4948	100	58**
Ibrutinib	12.5	17*
CA-4948 + IBN	100 + 12.5	38**

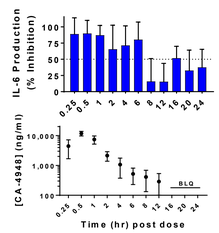
*p=ns; **p<0.005

Reference for (A) and (B) NF-κB status: Rahal R. et al., (2014) Nature Medicine 20(1) 87-94

Disclosures: Booher*, Samson*. Atoyan*, Ma*, Dellarocca*, Modafferi*, Borek*, Zhang*, Parker*, Whitney*, Wang*, Tuck: *Curis, Inc.: Employment, Equity Ownership; Nowakowski, no disclosures; Patel: Juno Therapeutics, Celgene, and Sunesis Pharmaceuticals: Consultancy; Pharmacyclics/Janssen: Speakers Bureau; Genentech: Consultancy, Speakers Bureau; AstraZeneca: Consultancy, Research Funding, Speakers Bureau; Lunning: Consultancy: Celgene, AbbVie, Astra-Zeneca, Bayer, Genentech, Genzyme, Gilead, Janssen, Juno, Kite, Portola, Seattle Genetics, Spectrum, TG Therapeutics, Verastem; Younes: Honoraria: Merck, Celgene, Abbvie, Seattle Genetics, Sanofi, Takeda, Incyte, Bayer; Honoraria, Research Funding: Roche, Janssen, BMS; Research Funding: Pharmacyclics, J&J, Novartis, Genentech, Astra Zeneca, Curis

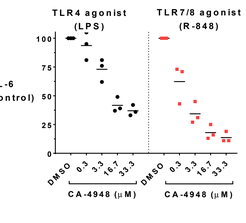
CA-4948 PK/PD Relationship Determined using an Ex-Vivo Whole Blood Assay (Mouse & Human)

Mouse *Ex vivo*, TLR-stimulation whole-blood assay (BALB/c, n=3-6):

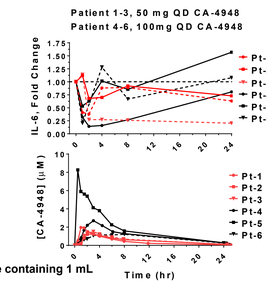


- Dose mice with 50 mg/kg CA-4948
- Collect blood, dilute 1:1 with media
- Stimulate with TLR agonist, hr, 37°C
- Measure plasma cytokine levels

IL-6 production in TLR-stimulated whole blood from healthy volunteers (n=3) (CA-4948 spike-in)



IL-6 production in TLR-stimulated whole blood from CA-4948 Ph1 trial patients (TLR7/8 agonist stimulation)



Human *ex vivo* whole blood assay

- Collect 1 mL blood in TruCulture tube containing 1 mL media with TLR agonist
- Incubate tubes 24 hr, 37°C, measure plasma cytokine levels

Pharmacokinetic Parameters of CA-4948 Starting Dose 50 mg QD on Day 1 and Day 15 from NHL Patients

		T_{max} (hr)		C_{max} (ng/ml)		$AUC_{0\text{-}24h}$ (ng*hr/ml)		$T_{1/2}$ (hr)	
Day	N	Median	Range	Mean	SD	Mean	SD	Mean	SD
1	4	2.0	1.0-3.0	766	146	5783	875	5.4	1.5
15*	2	1.5	1.0-2.0	987	702-1272	5950	4920-6981	5.8	4.9-6.7

*SD is not reported when the sample size is n=2. Therefore, a range is provided for Day 15 C_{max}, $AUC_{0\text{-}24h}$r, and $T_{1/2}$ parameters

The preliminary clinical PK data demonstrated that, CA-4948 as an oral drug, has rapid absorption, good bio-availability, rapid clearance and no accumulation following consecutive daily dosing for 15 days

Summary and Future Direction

- CA-4948 is a potent, oral inhibitor of IRAK4 Ser/Thr kinase with >500-fold selectivity against IRAK1
- CA-4948 BID dosing exhibits improved efficacy compared to QD dosing in DLBCL PDX xenograft models. Along with preliminary human PK, these results support BID during Ph 1 testing
- CA-4948 exhibited *in vivo* anti-tumor activity in NHL models with 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
- An *ex-vivo* TLR-stimulated whole blood assay was developed for *in vivo* or clinical PK/PD analysis
- A preliminary PK/PD relationship was observed with patients treated with 50 and 100 mg CA-4948
- These results underscore the therapeutic potential of targeting IRAK4 kinase with CA-4948 alone and in combination with targeted agents for the treatment of NHL
- The Ph1 dose escalation is ongoing and evaluating both QD and BID dosing schedules. Expansions cohorts in selected hematological malignancies such as DLBCL with MYD88 mutation and AML are being planned

Correspondence

Curis, Inc.
4 Maguire Road
Lexington, MA 024221

Contact Information:
Robert Booher, Ph.D.
rbooher@curis.com

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