PS991

24th EHA Congress
June 13-16, 2019

CA-4948, an IRAK4/FLT3 Inhibitor, Shows Antileukemic Activity in Mouse Models of *FLT3* Wild-Type and *FLT3* Mutated Acute Myeloid Leukemia (AML)

Robert N Booher, Maria Elena S. Samson, Mylissa Borek, Holly Modafferi, and Robert Martell
Curis, Inc., Lexington, MA, USA

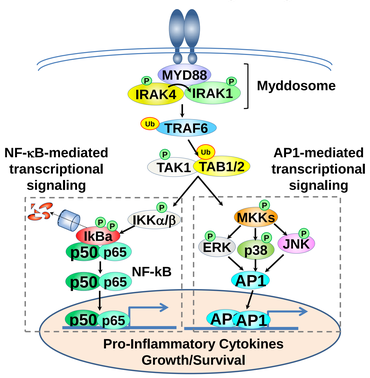
Abstract

Background: AML is an aggressive hematopoietic malignancy that arises from a population of aberrant hematopoietic stem cells in the bone marrow (BM). Advances in understanding the molecular basis of AML has led to the development of new targeted therapies. CA-4948 is a novel, oral IRAK4 kinase inhibitor with additional inhibitory activity against wild-type (wt) and mutated FLT3 kinase. IRAK4 (Interleukin-1 Receptor Associated Kinase 4) is required for interleukin 1 receptor (IL-1R) and toll-like receptor (TLR) innate immune pathway signaling, pathways that are frequently over activated in AML and myelodysplastic syndromes (MDS). For example, AML patients have increased IL-1R agonist (IL-1β) levels that promote the survival of AML cells and IL-1R KO represses AML cell growth in vitro and in vivo (Carey et al 2017). Dysregulation of the FLT3 signaling pathway is a well validated driver of AML. Constitutively activating mutations in FLT3 that comprise the ITD or the tyrosine kinase domain (KD) are frequently acquired late in AML disease and are poor prognostic factors with high relapse rates. FLT3 kinase inhibitors targeting FLT3-ITD or ITD/KD double mutations show high remission rates; however, multiple resistance mechanisms have been reported in both nonclinical models and AML patients. CA-4948 has both IRAK4 and FLT3 inhibitory activity, which may impart benefit to FLT3-wt and FLT3-mutant AML patients.

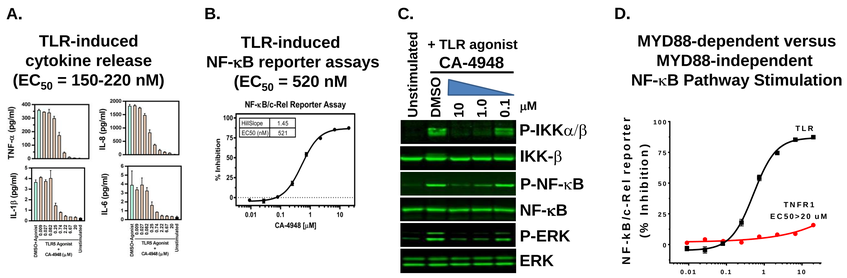
Aims: Evaluate the ability of a novel IRAK4/FLT3 inhibitor, CA-4948, to block IRAK4 and FLT3 in FLT3-wt and FLT3-mutant AML in vitro and in vivo.

Methods: CA-4948's kinome profile was assessed against 378 kinases and 9 FLT3 mutant variants (DiscoverX). CA-4948's ability to inhibit TLR/IL-1R or FLT3 signaling pathways was evaluated using NF-κB reporter, cytokine production, or western blot. The growth inhibitory and pro-apoptotic activity of CA-4948 was tested in vitro against AML cell lines by viability assay or flow cytometry. For AML FLT3-wt in vivo efficacy, THP-1 cells were tail-vein injected into mice and animal survival and degree of AML cell engraftment in BM were monitored in CA-4948, FLT3i, or vehicle treated mice. For AML FLT3 mutant in vivo efficacy, subcutaneous MV4-11 and MOLM-14 FLT3-ITD and MOLM-14 double FLT3-ITD/KD tumor models were treated with CA-4948 or other FLT3i.

TLR and IL-1R Signaling

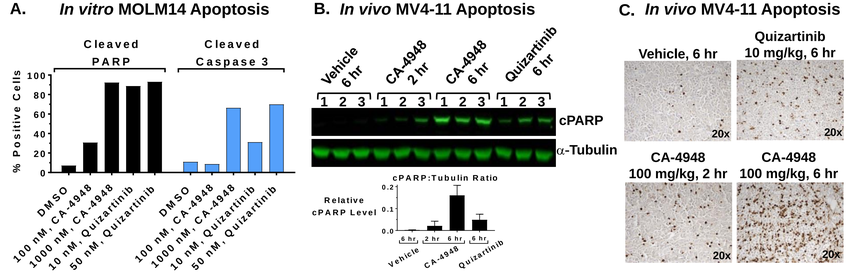


CA-4948 Blocks TLR/IL-1R Induced NF-κB Signaling



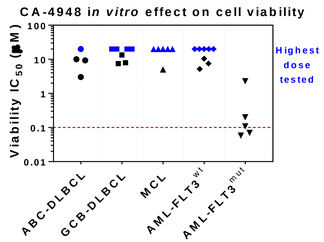
A. THP-1 monocytic cells stimulated with TLR5 agonist for 4 hour in presence of CA-4948. Percent cytokine released into media relative to DMSO was used to determine EC_{50}
B. CA-4948 dose response in THP-1 cells containing NF-kB driven SEAP reporter (InvivoGen) stimulated with TLR5 agonist, 4 hr
C. Western blots of THP-1 cells treated CA-4948 during 30 min TLR5 agonist stimulation
D. CA-4948 dose response in THP-1 cells stimulated with MYD88-dependent TLR5 agonist (flagella) and MYD88-independent TNFR1 pathway agonist (TNF-α)

CA-4948 Induced Apoptosis in AML *FLT3*-ITD Cells



A. Apoptosis induction measured by flow cytometry staining for cleaved PARP or cleaved Caspase 3 proteins after 48 hr treatment
B. Apoptosis induction measured by cleaved PARP western blot. MV4-11 AML s.c. tumor-bearing mice were administered a single dose of vehicle, 100 mg/kg CA-4948, or 10 mg/kg quizartinib. The level of cPARP relative to vehicle is shown below blots
C. Apoptosis induction measured by cleaved PARP immunohistochemistry of MV4-11 tumors in (B)

CA-4948 Effect on *FLT3*-wt and *FLT3*-mut Cell Lines

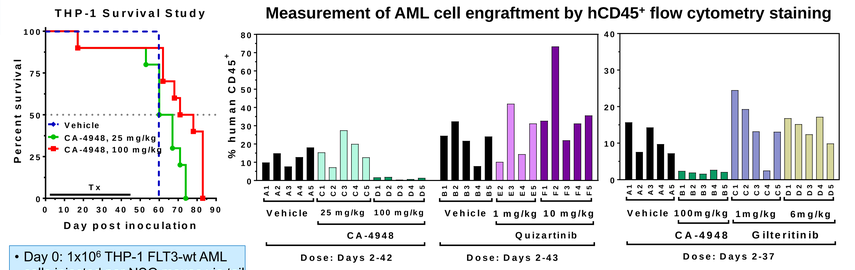


Target	Inhibitor	*In vitro* Viability Activity (EC50, nM)				
		MV4-11 *FLT3* ITD	MOLM13 *FLT3* ITD	MOLM-14 (fold-change relative to parental)		
				FLT3 ITD	*FLT3* ITD+D835Y	*FLT3* ITD+F691L
IRAK4	CA-4948	70	200	58	108 (2x)	2152 (37x)
	PF-06650833	nd	nd	>2500	>2500	>2500
	ND-2158	1590	>3000	>2500	>2500	>2500
FLT3	Quizartinib	2	3	0.5	85 (170x)	94 (188x)
	Midostaurin	nd	nd	9.6	53 (6x)	35 (4x)

nd = not determined

CA-4948 exhibits high potency against *FLT3*-ITD and *FLT3*-ITD/KD lines *in vitro* (CellTiter-Glo, 72 hour, n=3)

CA-4948 Inhibits AML *FLT3*-wt Bone Marrow Engraftment



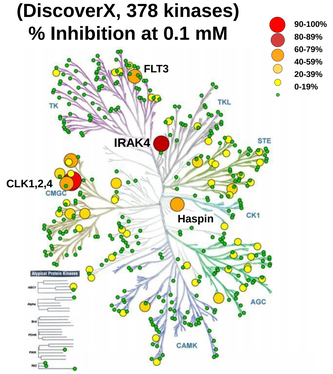
- Day 0: 1x10⁶ THP-1 FLT3-wt AML cells injected per NSG mouse via tail IV injection (n=10/group)
- QD dosing for 45 days

- Day 1: 1x10⁶ THP-1 cells injected per NSG mouse via tail IV injection (n=5/group)
- %hCD45⁺ is % relative to total cell count in bone marrow samples isolated day after last dose

CA-4948's FLT3 inhibitory activity is not responsible for its ability to block AML BM engraftment

CA-4948 Kinase Selectivity Profile

Active-site binding (DiscoverX, 378 kinases) % Inhibition at 0.1 mM



Kinome illustration reproduced courtesy of Cell Signaling Technology.

CA-4948 Binding Affinity Activity (DiscoverX)

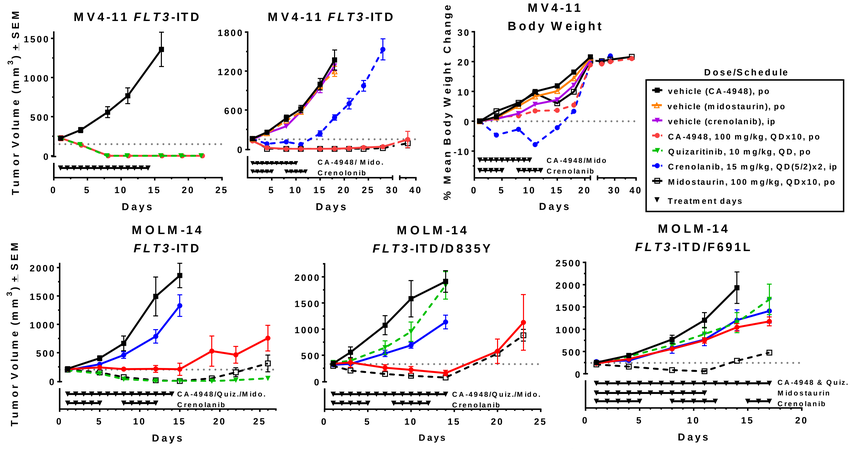
Kinase	K_d (nM)
IRAK4	23
IRAK1	12,000
IRAK2	>20,000
IRAK3	8,500

Other top hits:

CLK1	10
CLK4	14
CLK2	20
FLT3	31
DYRK1A	25
Haspin (GSG2)	32
TrkA	130

FLT3-variant	K_d (nM)
FLT3	31
FLT3 (D835H)	4.6
FLT3 (D835V)	44
FLT3 (D835Y)	2.5
FLT3 (ITD)	7.8
FLT3 (ITD, D835V)	31
FLT3 (ITD, F691L)	20
FLT3 (K663Q)	47
FLT3 (N841I)	16
FLT3 (R834Q)	160

CA-4948 Represses *FLT3*-ITD and *FLT3*-ITD/KD AML Tumor Growth



CA-4948 induced tumor regression in AML *FLT3*-ITD and *FLT3*-ITD/D835Y s.c. tumors (n=10/group)

Summary and Future Direction

- CA-4948 is a potent, oral inhibitor of IRAK4 kinase with >500-fold selectivity against IRAK1
- CA-4948 also exhibited high binding affinity for the FLT3 receptor tyrosine kinase and FLT3 variants containing ITD, KD, and double ITD/KD mutations
- CA-4948 showed *in vitro* and *in vivo* pro-apoptotic, antitumor activity against AML cell lines harboring *FLT3*-ITD and *FLT3*-ITD/D835Y mutations
- CA-4948 conferred a survival advantage in a disseminated mouse model of *FLT3*-wt AML, with nearly complete repression of bone marrow engraftment, in contrast to selective FLT3 inhibitors
- In addition to its current Phase 1 trial for non-Hodgkin lymphoma (ClinicalTrials.gov: NCT03328078), these results demonstrate that targeting the IL-1R/TLR signaling pathway with IRAK4 inhibitor CA-4948 may be an effective therapeutic strategy in *FLT3*-wt AML and MDS
- CA-4948's additional FLT3i activity may repress the emergence of *FLT3*-mutant AML clones

Correspondence

Curis, Inc.
4 Maguire Road
Lexington, MA 024221

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

Poster PDF Copy