Spliceosome Mutations, IRAK4 and CA-4948 in MDS and AML

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Background Spliceosome Mutations

SF3B1 mutation

>80% of patients with MDS with ring sideroblasts (MDS-RS)[1]

11.5% of patients with MDS refractory anemia (MDS-RA)

U2AF1 mutation

Mutations: S34F, R156H, and Q157P/R[2]

6.3% RA/RARS

9.3% RAEB/RAEBT

5.6% CMML

Retention of mutation at progression of disease[2]

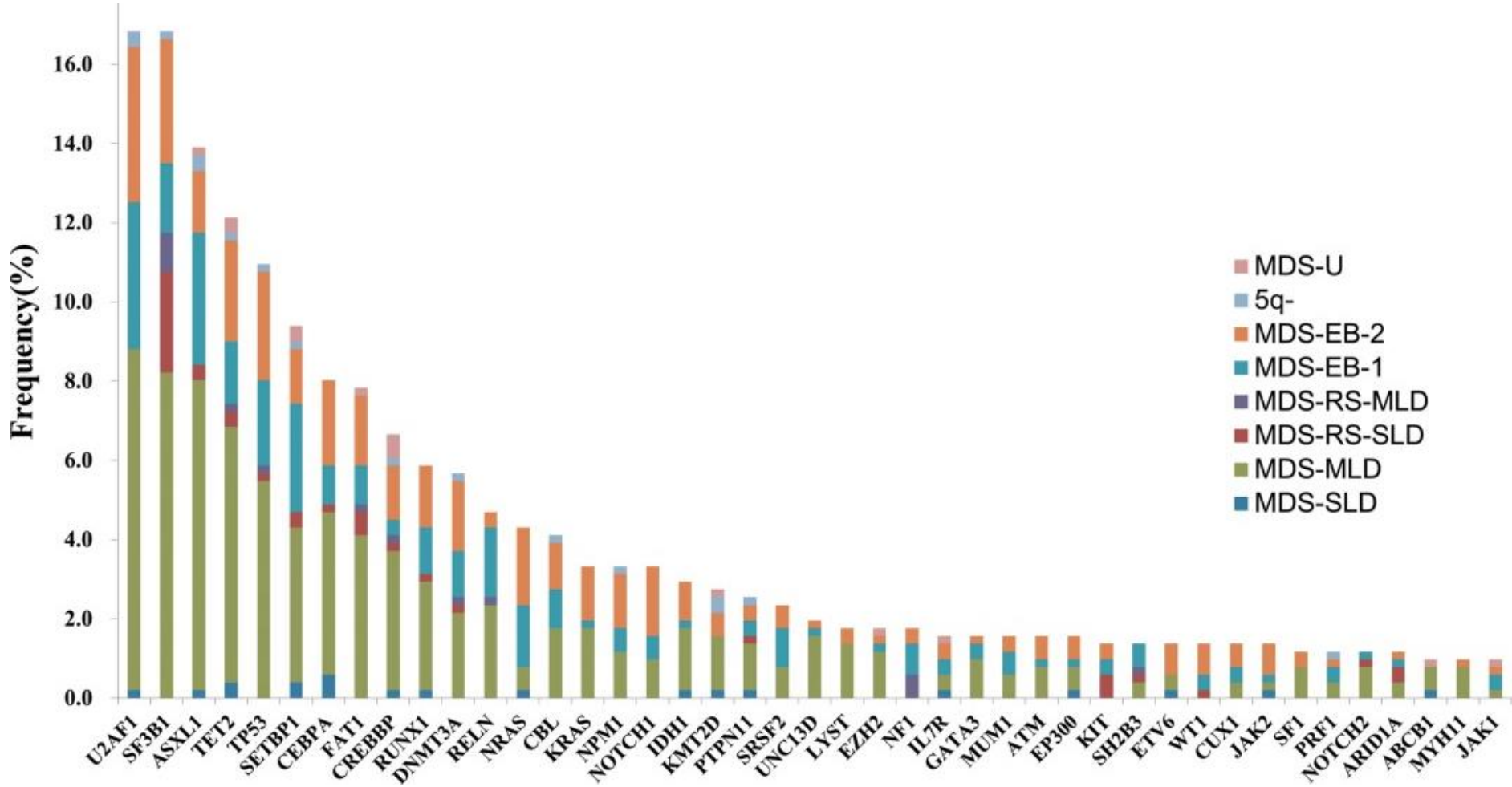
67% retained original mutation

33% had CR after allo transplant or intensive chemo and lost mutation in subsequent samples

0% of patients without mutation acquired one

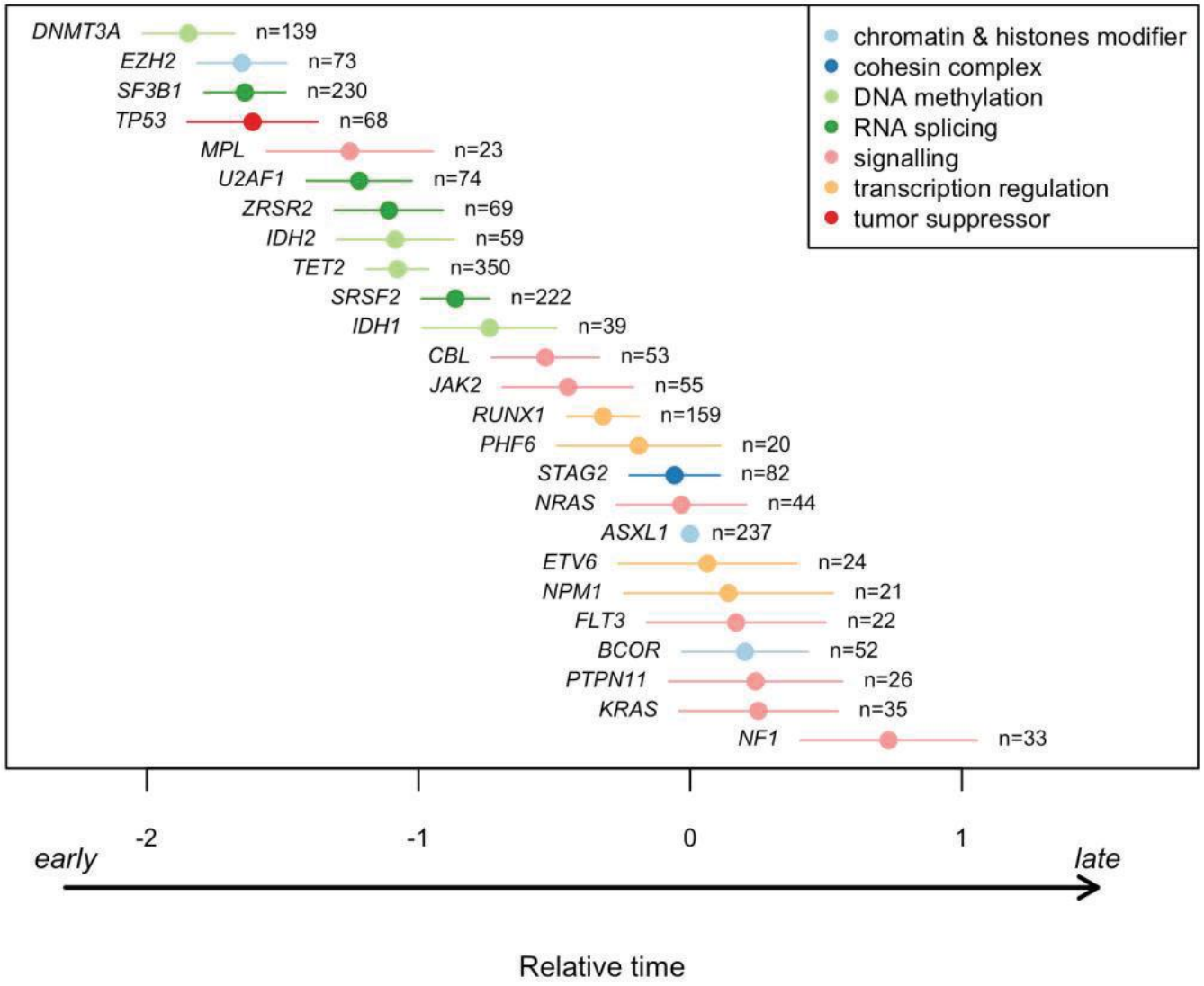
1) Malcovati Blood 2011; Mortera-Blanco Blood 2017
2) Wu AJH 2013

SF3B1 and U2AF1 Mutations are Among the Commonest Mutations in MDS[1]



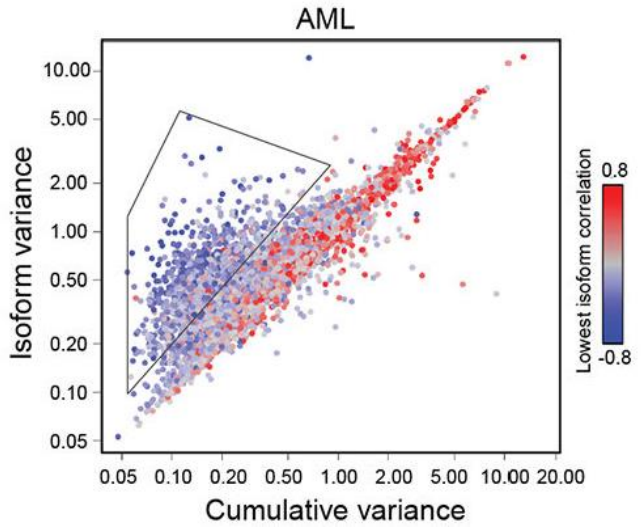
1) Li Genes Chrom 2017

SF3B1 and U2AF1 among earliest genes mutated[1]

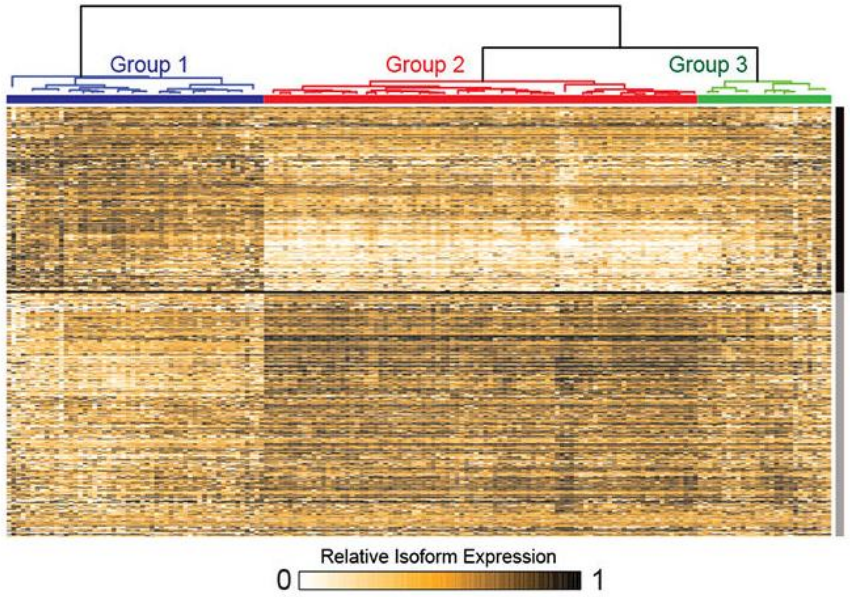


1) Bersanelli et al. J Clin Oncol 2021

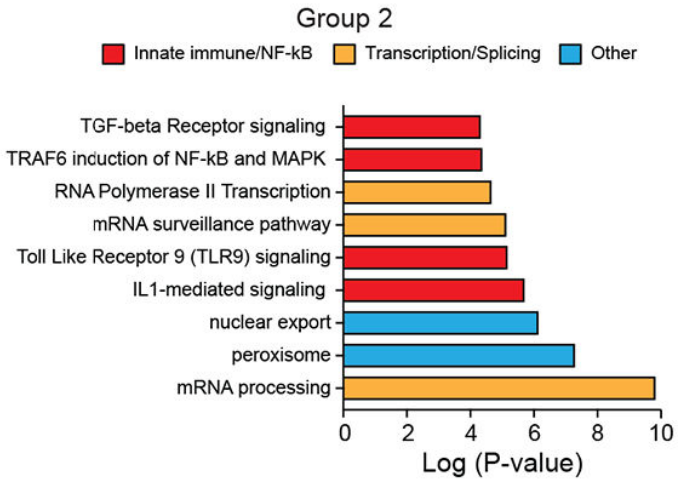
RNA isoforms define a subset of AML associated with innate immune pathway activation[1]



Scatterplot of cumulative and isoform variances of genes in AML samples from TCGA (n = 160). Blue-colored genes have at least one pair of isoforms with mutually exclusive expression pattern.



Hierarchical clustering analysis and relative expression of mRNA isoforms in AML samples

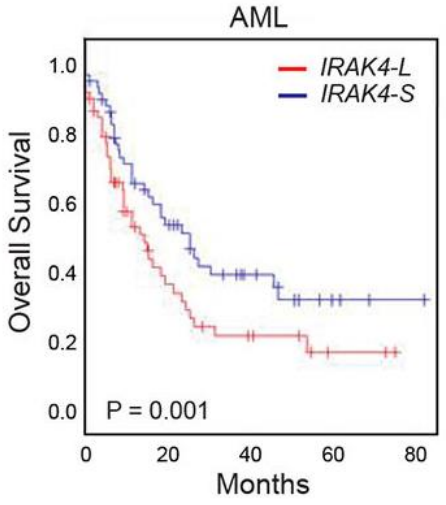


Pathway analysis of genes in Group 2 associated with worse clinical outcome (n = 347 genes) determined by hypergeometric distribution test.

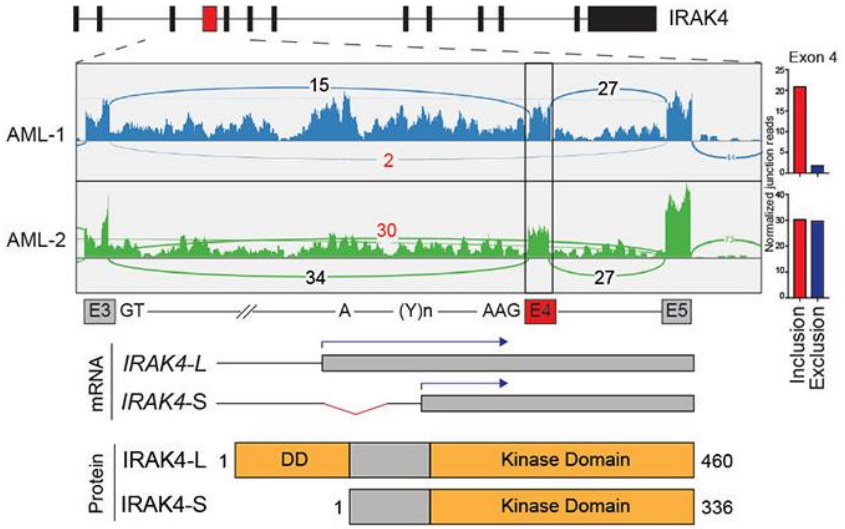
1) Smith et al. Nat Cell Biol. 2019

IRAK4 emerged as the leading candidate that drives innate immune signaling in MDS or AML[1]

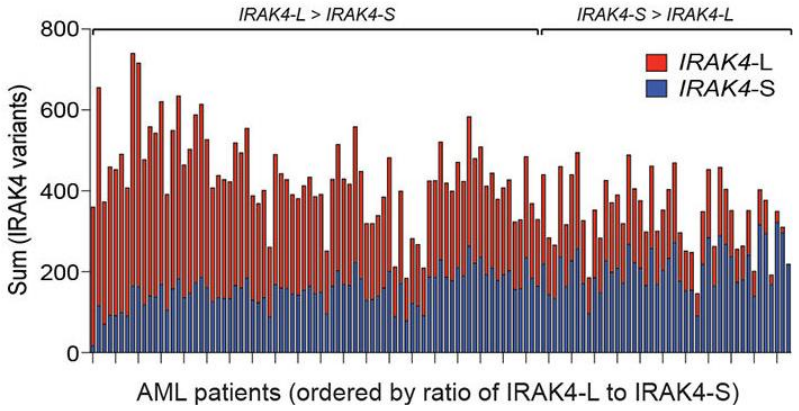
The magnitude of IRAK4 isoform switching was highly significant among AML samples and the inclusion of exon 4 alone correlated with worse outcome



Kaplan-meier analysis of AML patients stratified on IRAK4-L (exon 4 included) or IRAK4-S (exon 4 excluded) expression



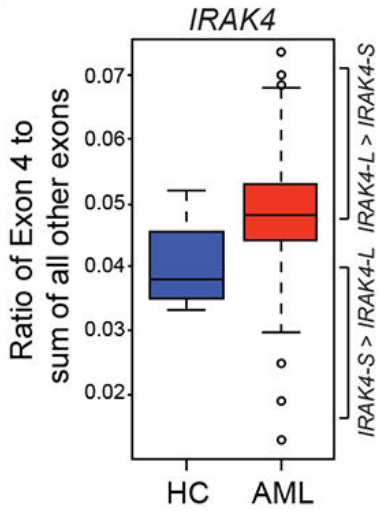
Exon architecture of IRAK4 and protein domains. Sashimi plots represent junction reads in representative AML samples.



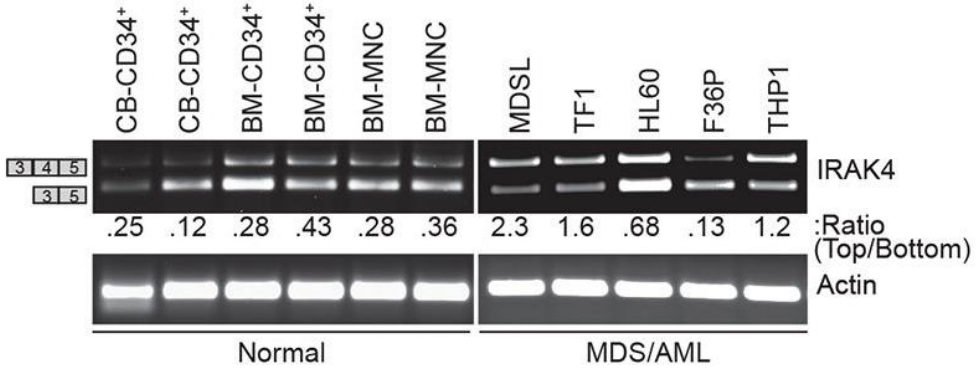
Cumulative expression of IRAK4-L and IRAK4-S in individual AML patients (TCGA). Patients are ordered according the relative expression of IRAK4-L versus IRAK4-S.

1) Smith et al. Nat Cell Biol. 2019

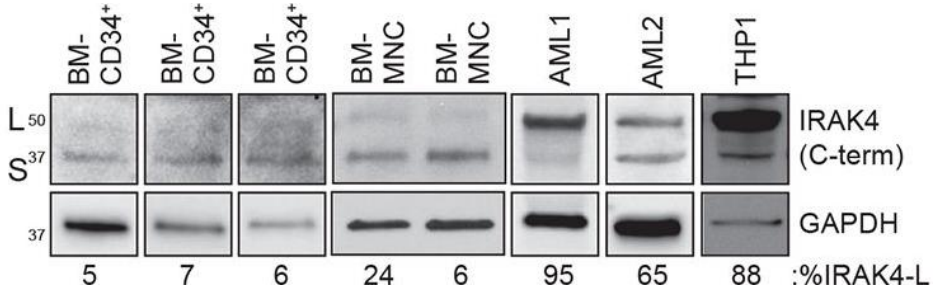
Significant proportion of AML patients primarily express the longer IRAK4 RNA and protein isoforms[1]



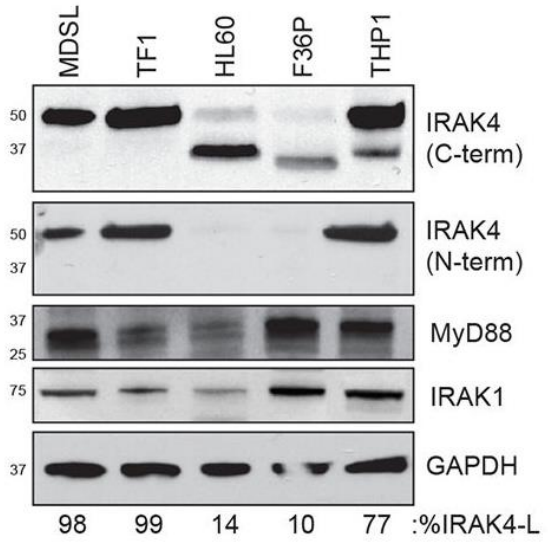
Relative expression of IRAK4-L to IRAK4-S in normal BM (n = 4) and AML (TCGA; n = 160)
P = 0.07



RT-PCR of IRAK4-L/S using primers flanking exon 4
(CB=cord blood; BM=bone marrow; MNC=mononuclear cells)



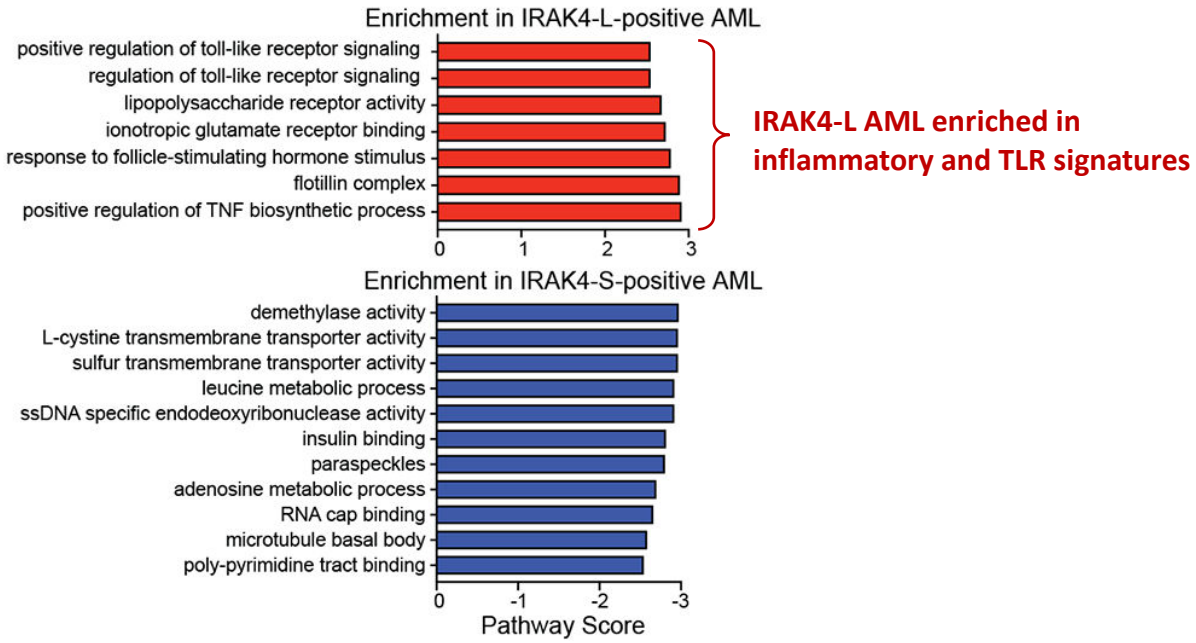
Immunoblot of IRAK4 in AML patient samples and healthy samples
(CB=cord blood; BM=bone marrow; MNC=mononuclear cells)



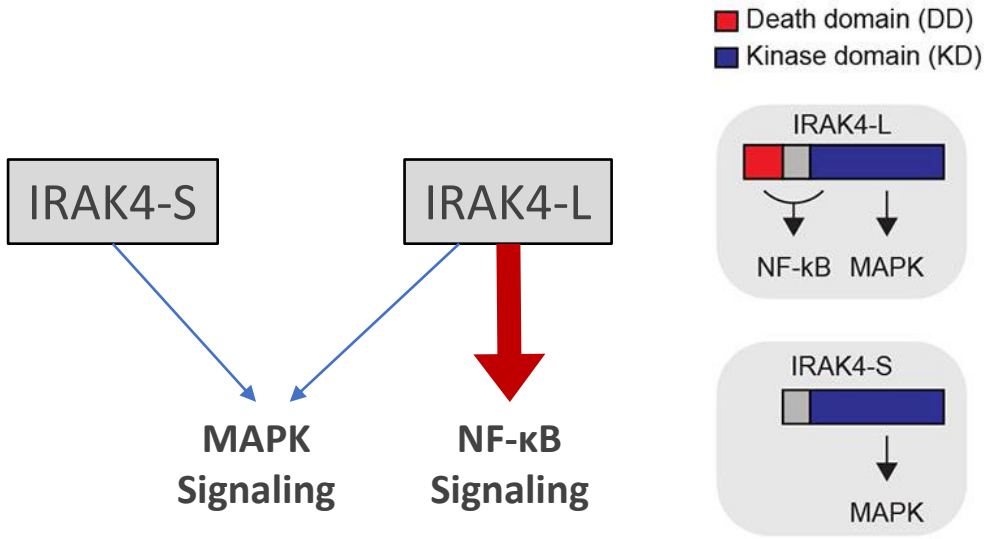
Immunoblot of IRAK4 using an
N-terminal antibody that recognizes IRAK4-L and a
C-terminal antibody that recognizes IRAK4-L and IRAK4-S
(MYD88 and IRAK1 comparable across lines)

1) Smith et al. Nat Cell Biol. 2019

IRAK4-L results in maximal activation of innate immune signaling[1]



Pathway analysis of enriched genes in AML patients preferentially expressing IRAK4-L (top) or IRAK4-S (bottom)

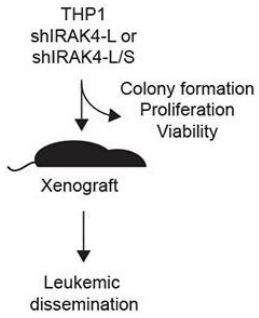


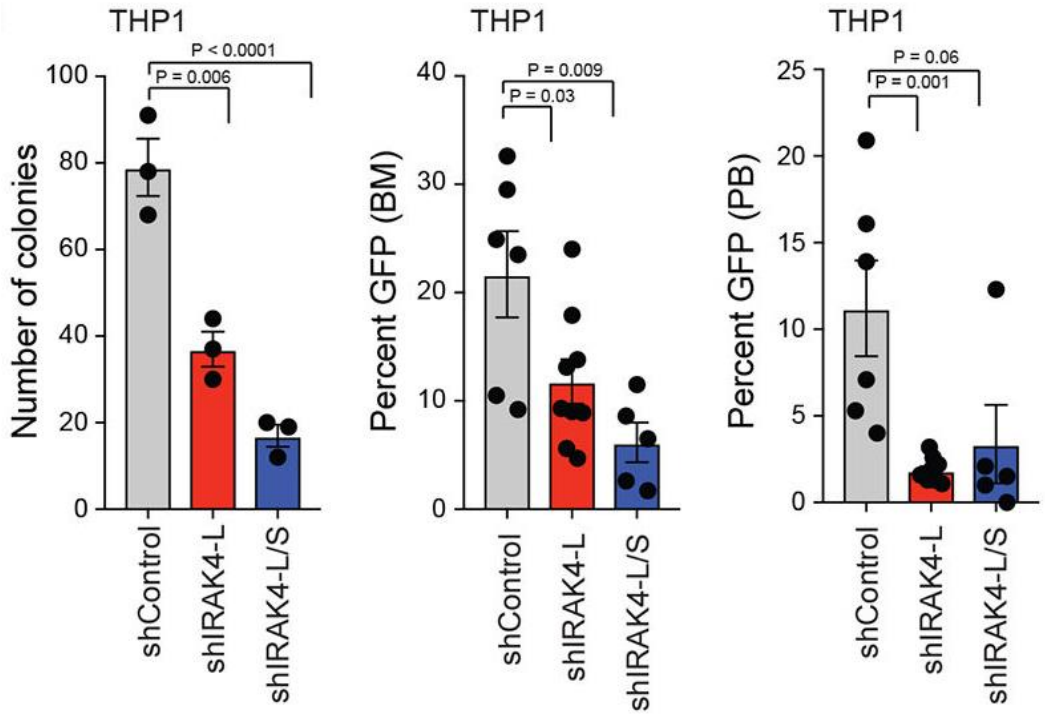
IRAK4-L is necessary and sufficient for NF-κB activation in AML

1) Smith et al. Nat Cell Biol. 2019

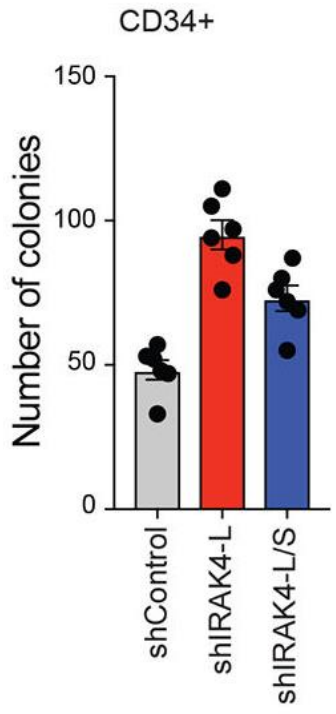
IRAK4-L is Required for Leukemic Cell Function[1]

IRAK4-L is the dominant isoform in leukemic cells





Similar findings when IRAK4 isoforms deleted using CRISPR/Cas9 gene editing



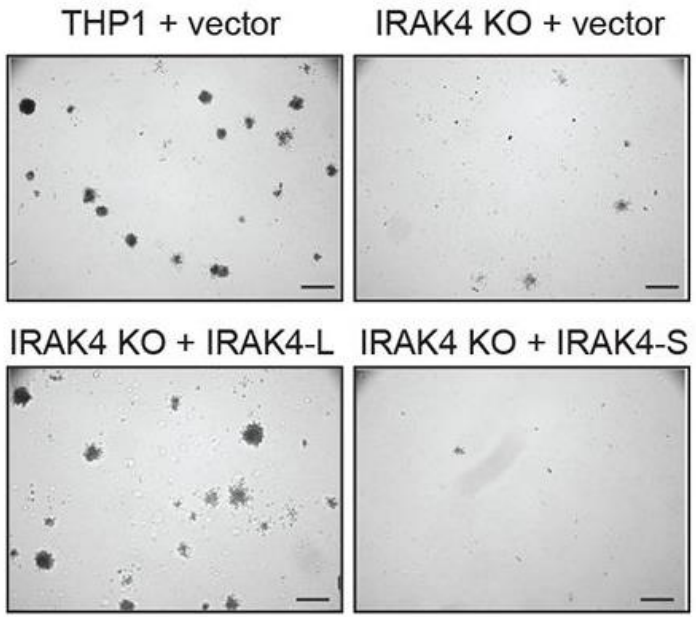
Reduced IRAK4-L did not impair progenitor function of normal cord-blood CD34+ cells, rather increased myeloid and erythroid differentiation

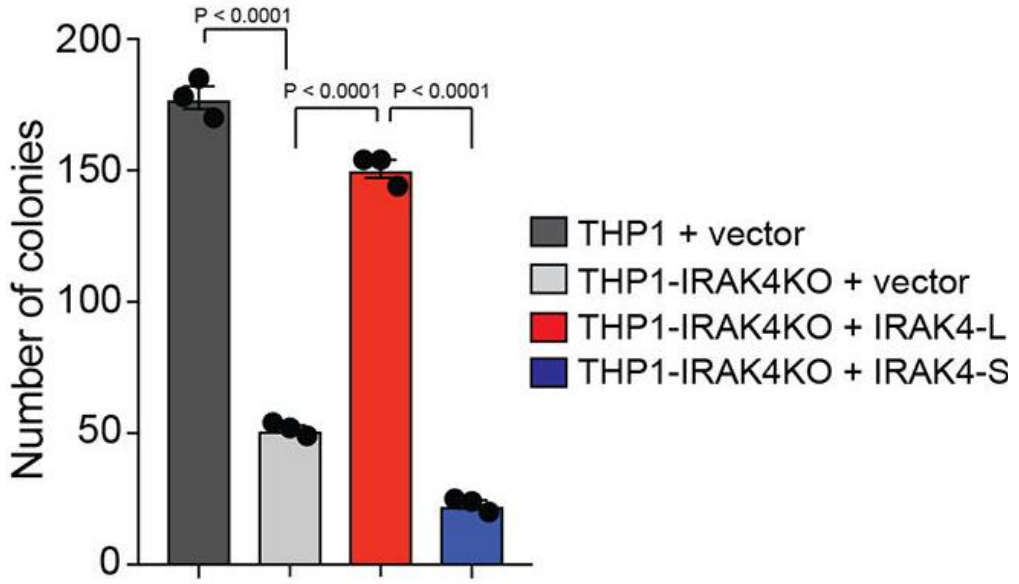
1) Smith et al. Nat Cell Biol. 2019

IRAK4-L is Required for Leukemic Cell Function[1]

IRAK4 KO ablates progenitor activity

IRAK4-L is sufficient to reconstitute progenitor function





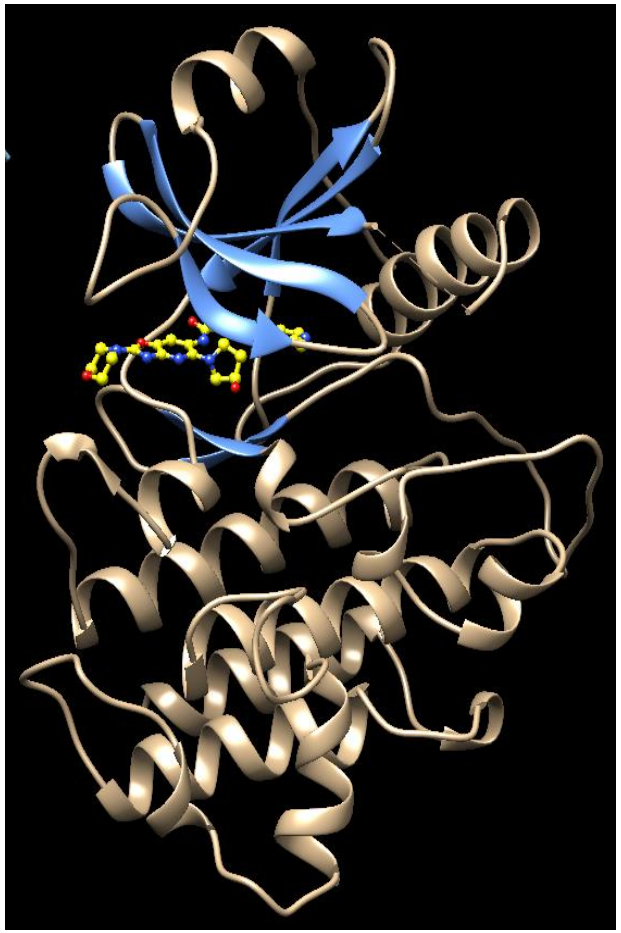
Parental and IRAK4-KO THP1 cells examined for leukemic progenitor function in methylcellulose.
IRAK4-L, but not IRAK4-S, is required for leukemia-propagating cells.

1) Smith et al. Nat Cell Biol. 2019

CA-4948 Background

- CA-4948 is a novel small molecule oral inhibitor of interleukin-1 receptor associated kinase 4 (IRAK4)
- IRAK4 kinase plays an essential role in toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways; these pathways are frequently dysregulated in Non-Hodgkin Lymphomas (NHL) and AML/MDS
- Oncogenic IRAK4- L, which is driven by spliceosome mutations (including SF3B1 and U2AF1), is preferentially expressed in >50% of AML/MDS patients[1]
- Orally bioavailable; moderate plasma binding (77% human)
  - Stable in plasma, liver microsomes, hepatocytes
  - No inhibition of 7 major CYP450s
  - No significant metabolism *in vitro*
- CA-4948 inhibits a defined subset of malignancies driven by over-activity of the TLR pathway, which is dependent on IRAK4
- Activated IRAK4 has been identified as a driver of adaptive resistance in AML and other tumors[2]
- CA-4948 strongly inhibits IRAK4 and FLT3 in vitro and in-vivo models. It has demonstrated safety and activity in patients with high-risk MDS and AML

IRAK4/CA-4948 Co-crystal Structure

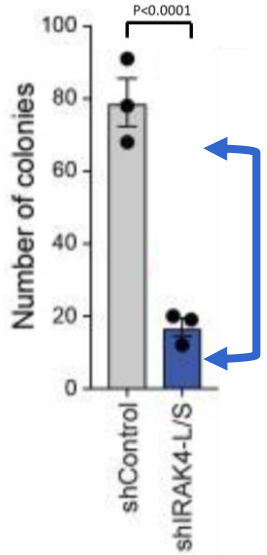


1) Smith et al. Nat Cell Biol. 2019
2) Melgar et al. Sci Transl Med. 2019

CA-4948 or IRAK4-L Knockdown is Sufficient to Reduce Leukemic Colonies and Blasts

IRAK4-L is Oncogenic

IRAK4-L provides a genetic link
to oncogenic immune signaling in AML/MDS

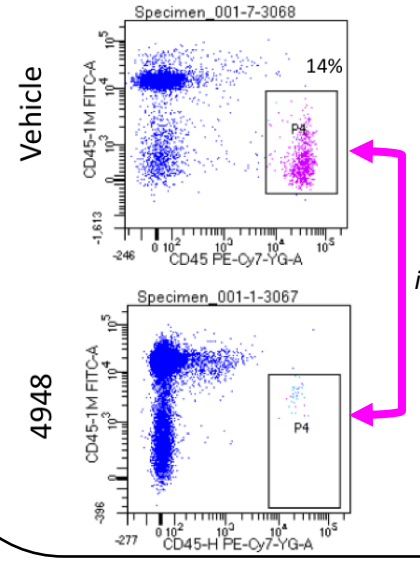


*Blocking IRAK4-L reduces the formation of **leukemia colonies** in preclinical studies*

Smith et al. Nat Cell Biol. 2019

CA-4948 Directly Targets IRAK4

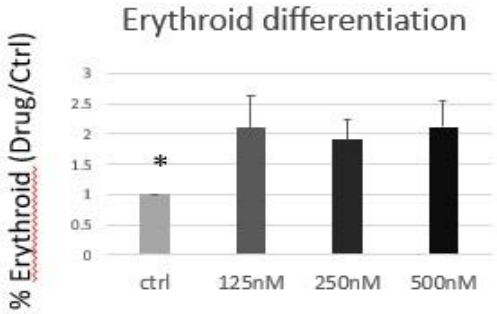
Direct inhibition of IRAK4
is associated with disease

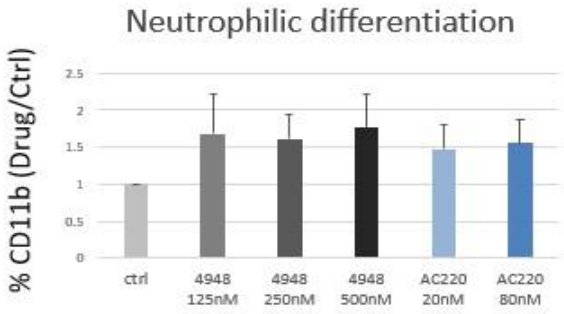


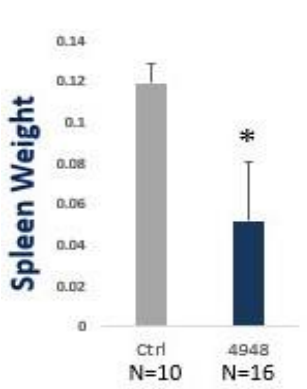
*CA-4948 treatment reduces **leukemic blasts** in patient-derived xenografts*

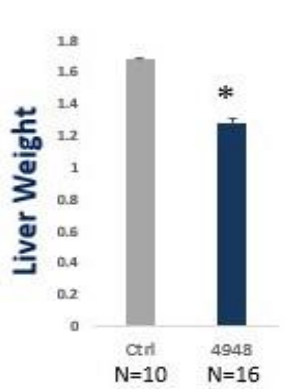
Choudhary et al. AACR 2017

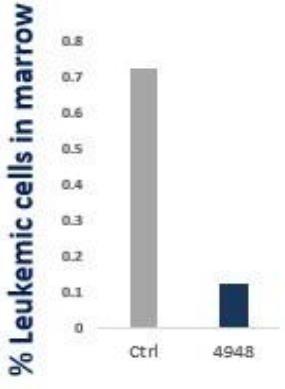
CA-4948 Increases Cell Differentiation from Primary MDS/AML HSPCs





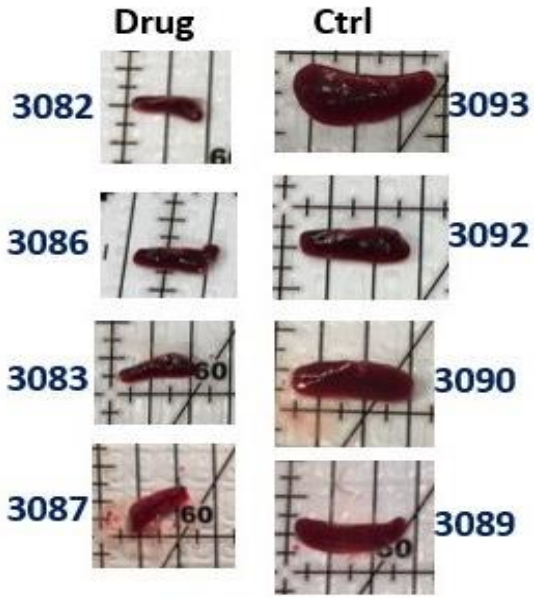






6 weeks of treatment at 12.5mg/kg

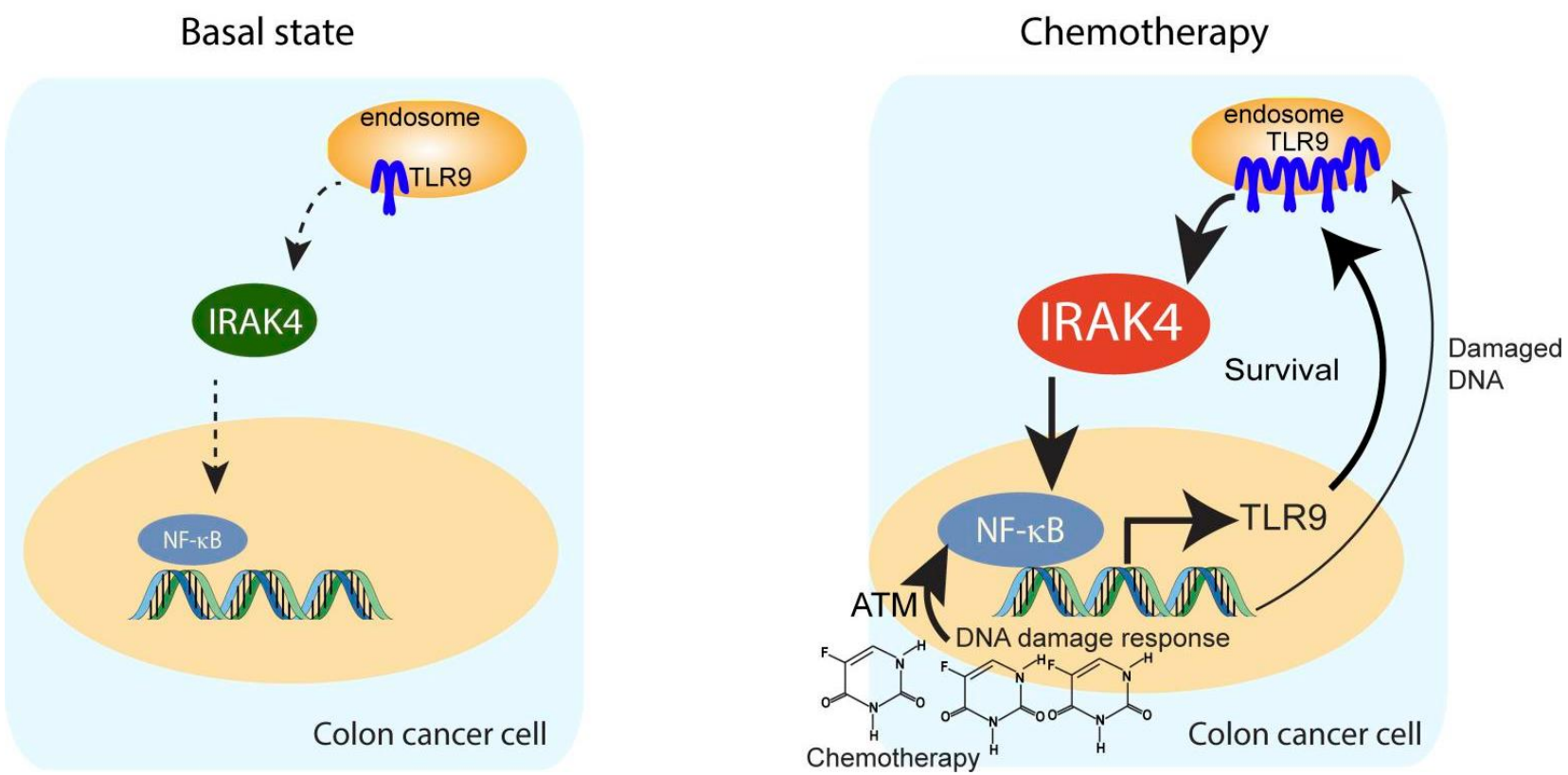
Decreases Disease Burden in THP-1 Xenografts



Ulrich Steidl Lab (Albert Einstein) collaboration

IRAK4 Inhibition May Inhibit Adaptive Malignancy Resistance

DNA damage induces TLR9-IRAK4-NF-κB survival mechanism[1]



1) Li et al. JCI Insight 2019

Landscape of Disease Targets in AML/MDS

Disease Driver	% of Patient Population
IRAK4-L	> 50%[1]
FLT3	25-30%[2]
TET2	10-20%[3]
IDH2	9-13%[4]
IDH1	6-10%[4]
CEBPA	~10%[3]

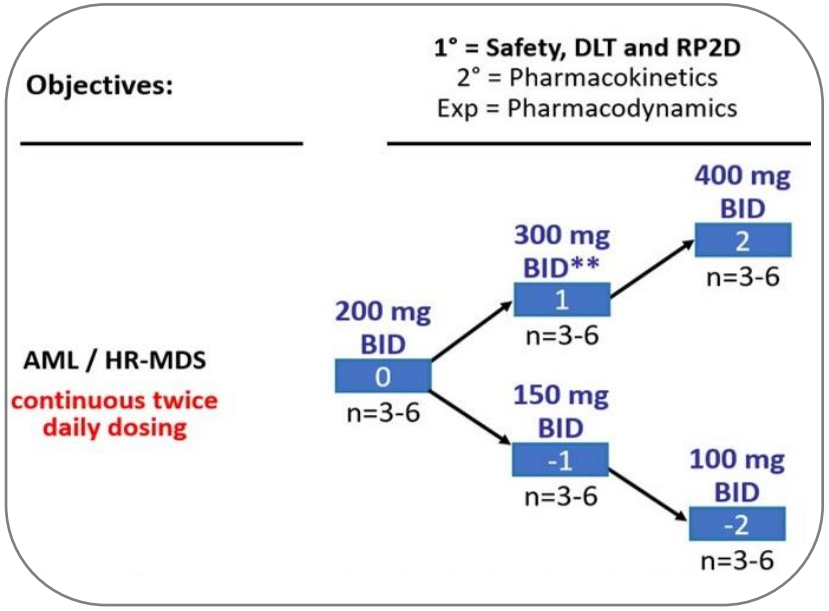
- Non-targeted therapies administered in monotherapy have historically provided limited clinical benefit, especially in relapsed/refractory patients
- Targeted therapies (e.g., FLT3, IDH) have been limited by the size of their respective target patient populations
- IRAK4-L is a novel target in AML/MDS and has been shown to be preferentially expressed in >50% of the AML/MDS patient population
  (>50% of patients have an IRAK4 long-to-short ratio > 1.25)

1) Smith et al. Nat Cell Biol 2019
2) Saygin, et al. J Hematol Oncol. 2017 Apr 18
3) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6142505/
4) DiNardo et al. N Engl J Med 2018

Study Design and Methods

- This is a multicenter, open-label, single arm Phase 1 dose escalation study of orally administered CA-4948 monotherapy in adult patients with AML or high risk MDS *(NCT04278768)*
- Each treatment cycle of CA-4948 will be 28 days in length and repeated in the absence of unacceptable toxicity or disease progression

Dose Escalation



Dose reduction to an intermediate dose level may be explored following review from the CSC

Primary Objective:

- Determine the maximum tolerated dose (MTD) and Recommended Phase 2 Dose (RP2D) for CA-4948 based on the safety and tolerability, DLTs and PK/PD findings.

Secondary Objective

- Characterize the pharmacokinetic (PK) parameters and preliminary efficacy.

Exploratory Objectives:

- Assess the potential association between target-related biomarkers (including IRAK4-L and downstream signaling parameters), selected genetic mutations (including spliceosome mutations), gene expression signatures, cell of origin, or other molecular classification subtypes and anti-leukemic activity (including central morphology review).
- Assess the pharmacodynamic effects of CA-4948 on selected biomarkers in peripheral blood and bone marrow.

Inclusion and Exclusion Criteria

Inclusion:

- Males and females ≥18 years of age
- Cytomorphology based confirmed diagnosis of MDS or AML per the World Health Organization 2016 classification:
  a) Relapsed or refractory AML (primary or secondary, including treatment-related); OR
  b) High/very high risk relapsed/refractory MDS (IPSS-R criteria), following at least 6 cycles of hypomethylating agents (HMA) or evidence of early progression

Exclusion:

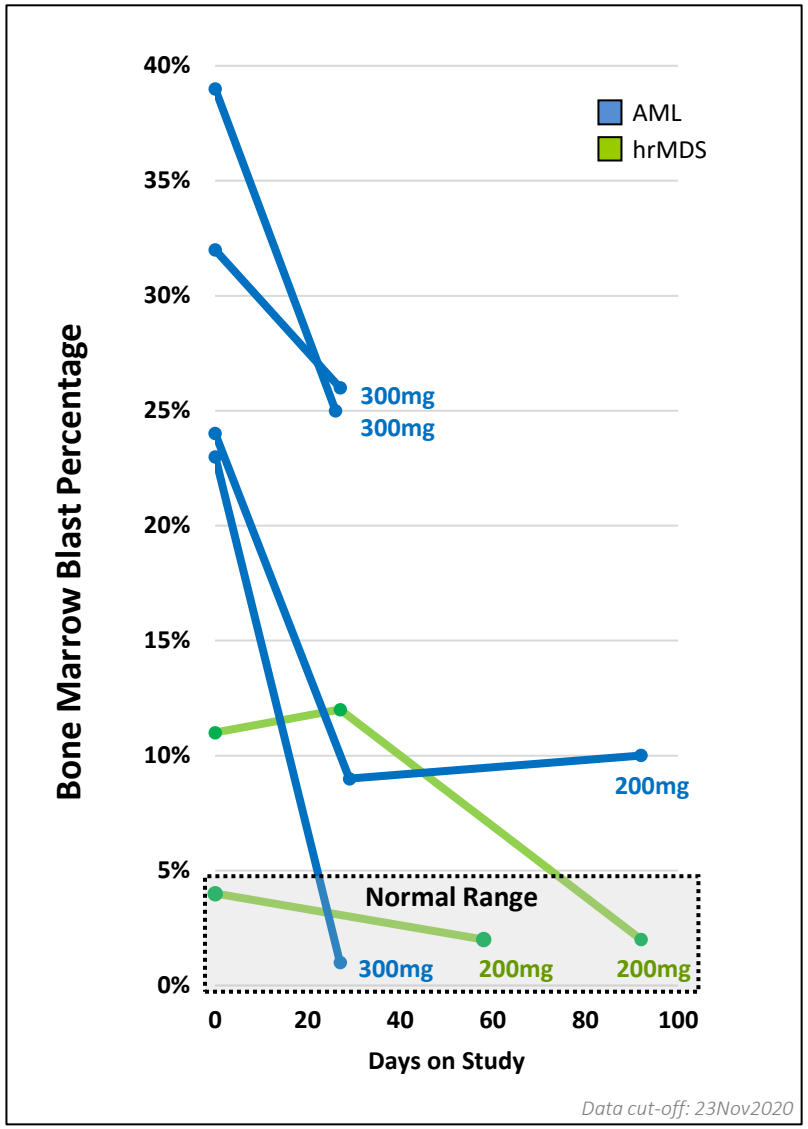
- Patients diagnosed with Acute Promyelocytic Leukemia (APL, M3), blast phase of CML, Allogeneic Hematopoietic Stem Cell Transplant (Allo-HSCT) within 60 days of the first dose of CA-4948, or clinically significant Graft-Versus-Host Disease (GVHD) requiring ongoing up-titration of immunosuppressive medications prior to start of CA-4948

Patient Characteristics

Baseline Characteristics of Ph1 Patients	Overall (N=6)
Male (%)	5 (83%)
Female (%)	1 (17%)
Median Age (range)	72 (32-84)
Median Prior Therapies (range)	3 (1-4)
Histology	
Acute Myelogenous Leukemia (AML)	4 (67%)
Myelodysplastic Syndrome (MDS)	2 (33%)

Data cut-off: 23Nov2020

Monotherapy Anti-Cancer Activity Observed in Early Ph1 Data



- **1st patient dosed in Q3 2020**
- **Consistent reduction of Marrow Blasts across population (6 patients)**
- **2 patients have achieved Marrow CR**

		Blasts Baseline	Blasts Best Resp	Change	
AML	005-2003	32%	26%	-19%	
AML	005-2002	39%	25%	-36%	
AML	003-1002	24%	9%	-63%	
hrMDS	003-1003	4%	2%	-50%	
hrMDS	003-1001	11%	2%	-82%	Marrow CR
AML	005-2001	23%	1%	-96%	Marrow CR

Note: To achieve Marrow CR, a patient's blast count must be elevated at baseline (>5%) and, after treatment, decrease by ≥ 50% from baseline into the normal range (≤5%)

CA-4948-102 Trial in Progress: Monotherapy in R/R High-Risk MDS and AML

UPDATED

Cohort 1: 200 mg BID
3 patients (2 hrMDS and 1 AML)
No DLT in first 3 patients during DLT period *(1st four-week cycle)*

Cohort 2: 300 mg BID
4 patients (1 hrMDS and 3 AML)
No DLT in first 3 patients during DLT period

Cohort 3: 400 mg BID
3 patients (3 hrMDS)
No DLT in first 3 patients during DLT period

Cohort 4: 500 mg BID
open for enrollment

Conclusion and References

- All initial patients in the first two cohorts completing cycle 1 had marrow blast reduction, including several Marrow CRs, clinical updates to be later reported
- Dose escalation cohorts have subsequently included 200, 300, 400 and 500mg twice daily
- After the RP2D has been determined, it may be subsequently amended including patient selection and combination therapy in a controlled design

References:

- Rhyasen, GW, Starczynowski DT, 2015. Brit J Cancer 112, 232–237
- Smith MA. et al., 2019 Nat Cell Biol. 21(5): 640–650
- Choudhary G et al.2019. Blood 134 (Suppl 1): 4224.
- Booher R. et al.: 2019: EHA Annual Meeting, Abstr. PS991
- Melgar, K. et al., 2019. Sci Transl Med, 11: eaaw8828

Thank You

Thank you to the participating trial investigators, clinical staff, patients and their families

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