The origins of blood cancers

Benjamin Ebert

Meyenburg Cancer Research Symposium

November 7, 2019

Dana-Farber Cancer Institute

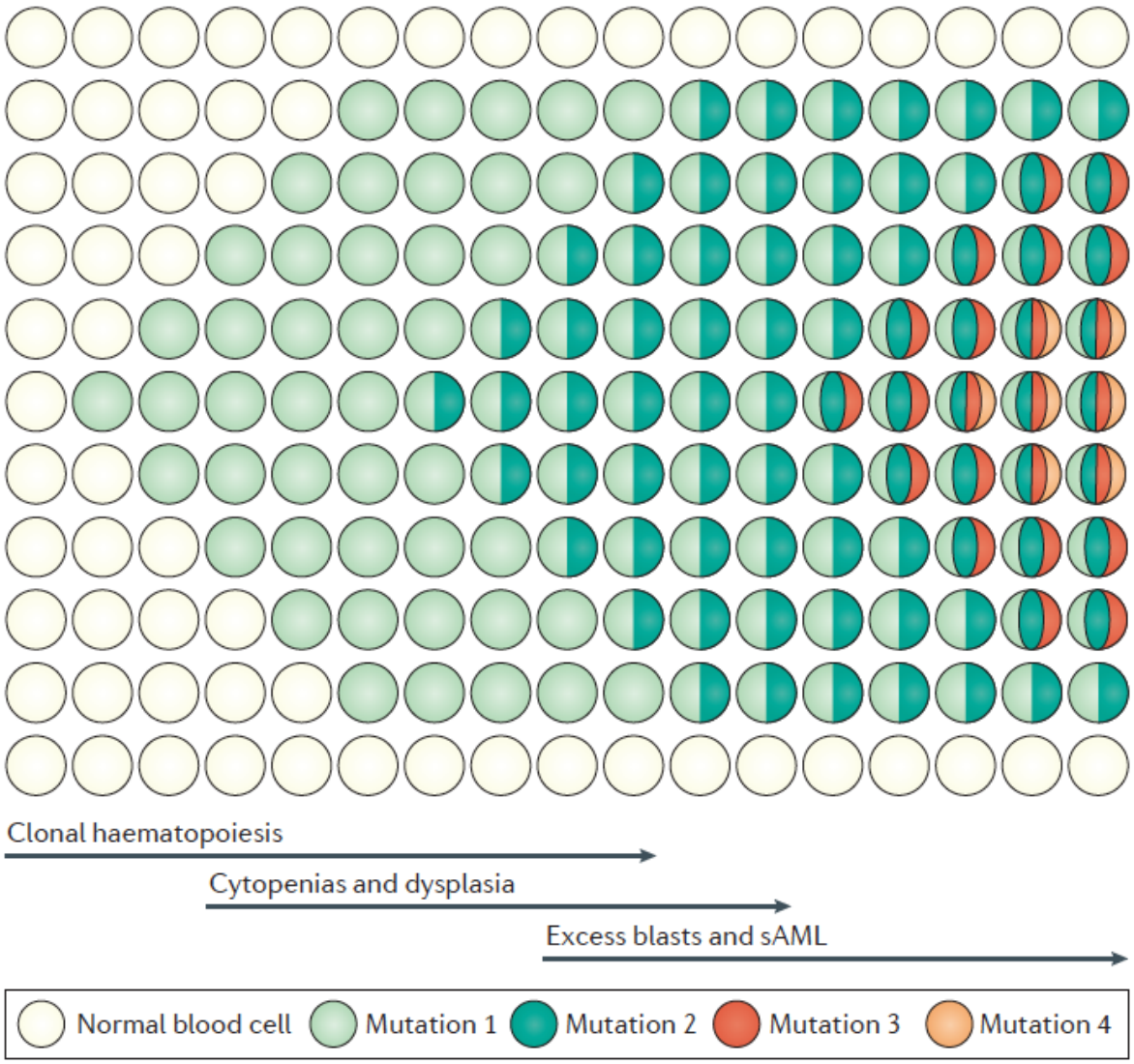
Howard Hughes Medical Institute

Disclosures

- **Faculty:** Benjamin Ebert, MD, PhD

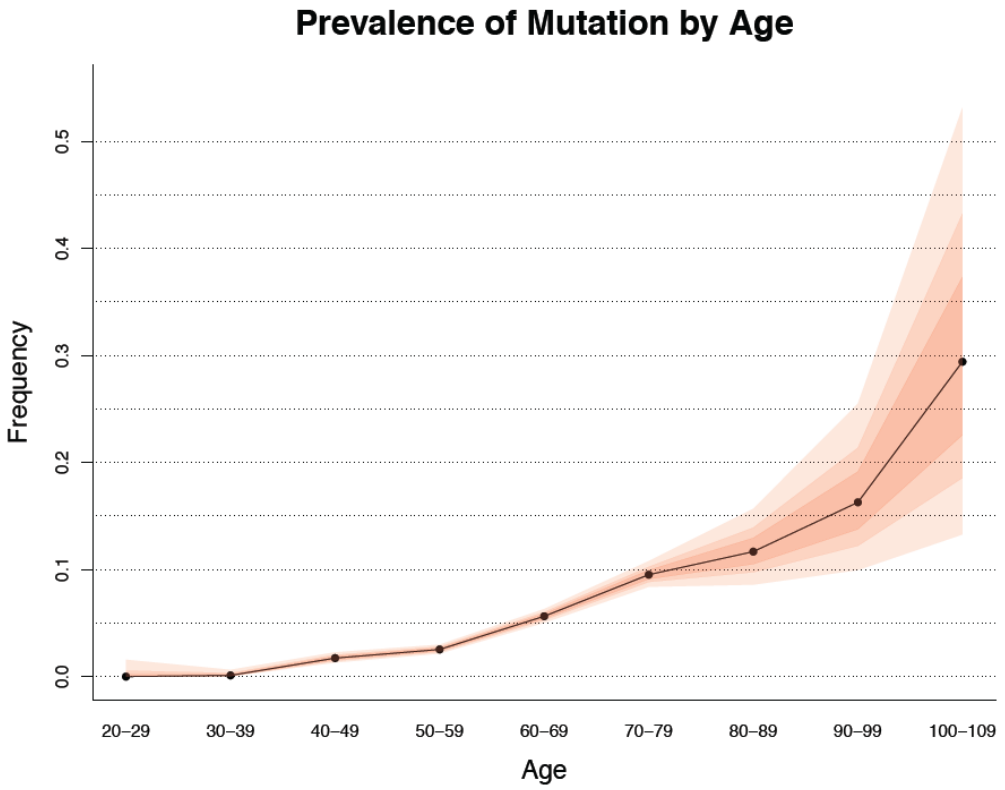
- **Relationships with commercial interests:**
  - **Consulting Fees:** Grail
  - **Scientific advisory board:** Skyhawk Therapeutics, Exo Therapeutics
  - **Research funding**: Celgene, Deerfield

Evolution of hematologic malignancies



Sperling and Ebert, *Nature Reviews Cancer* 2017

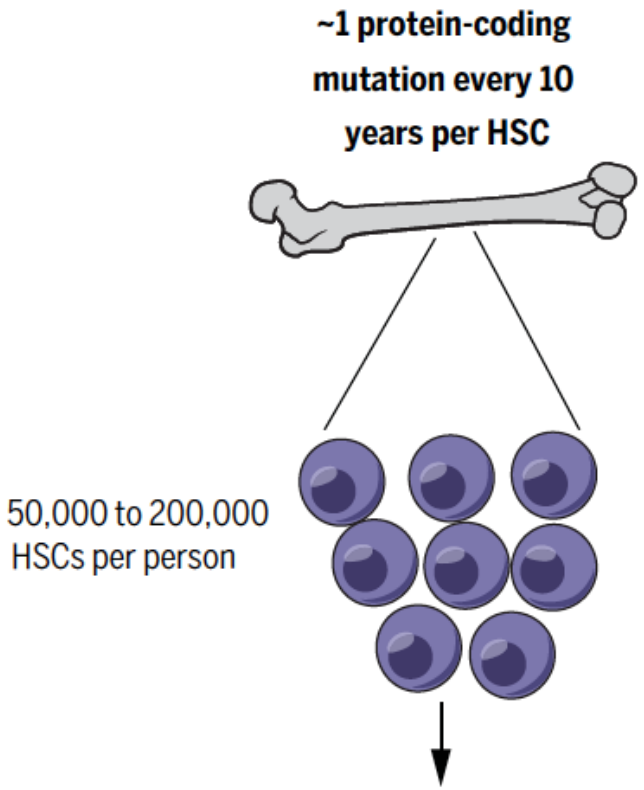
Clonal hematopoiesis



- Exome sequencing data from peripheral blood of >17,000 individuals
- Unselected for hematologic phenotype

Jaiswal et al., *NEJM* 2014

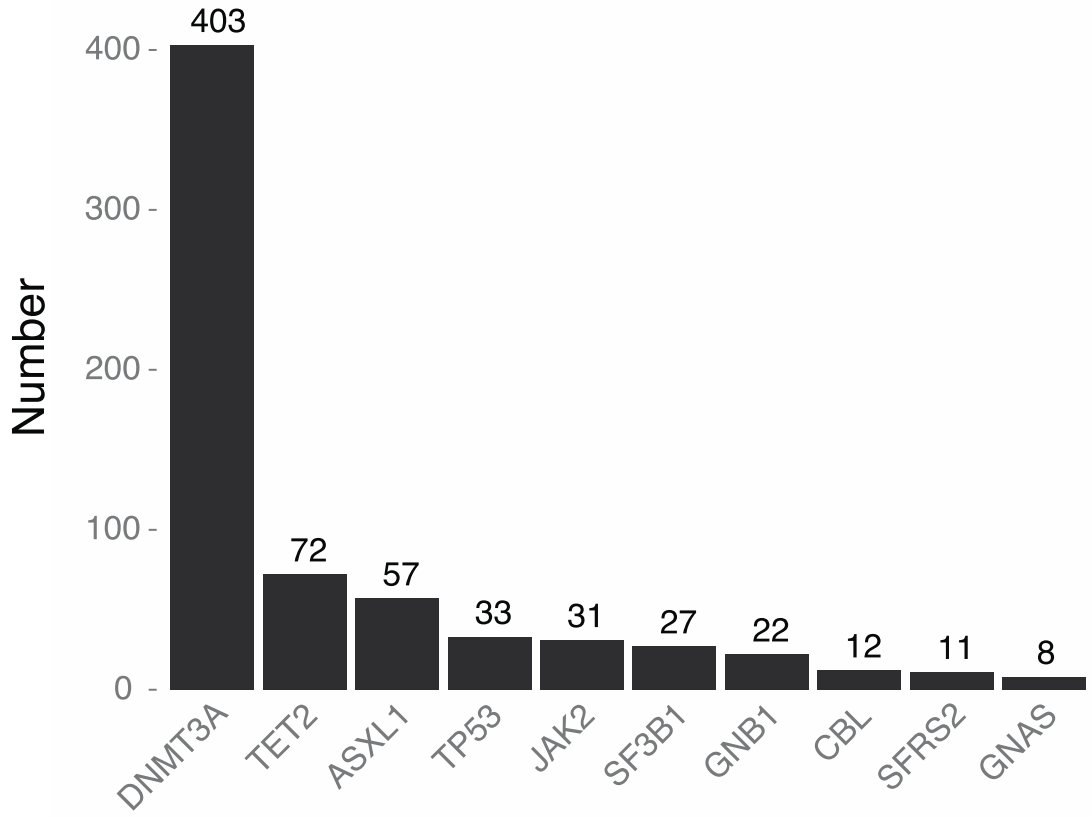
Somatic mutations and age



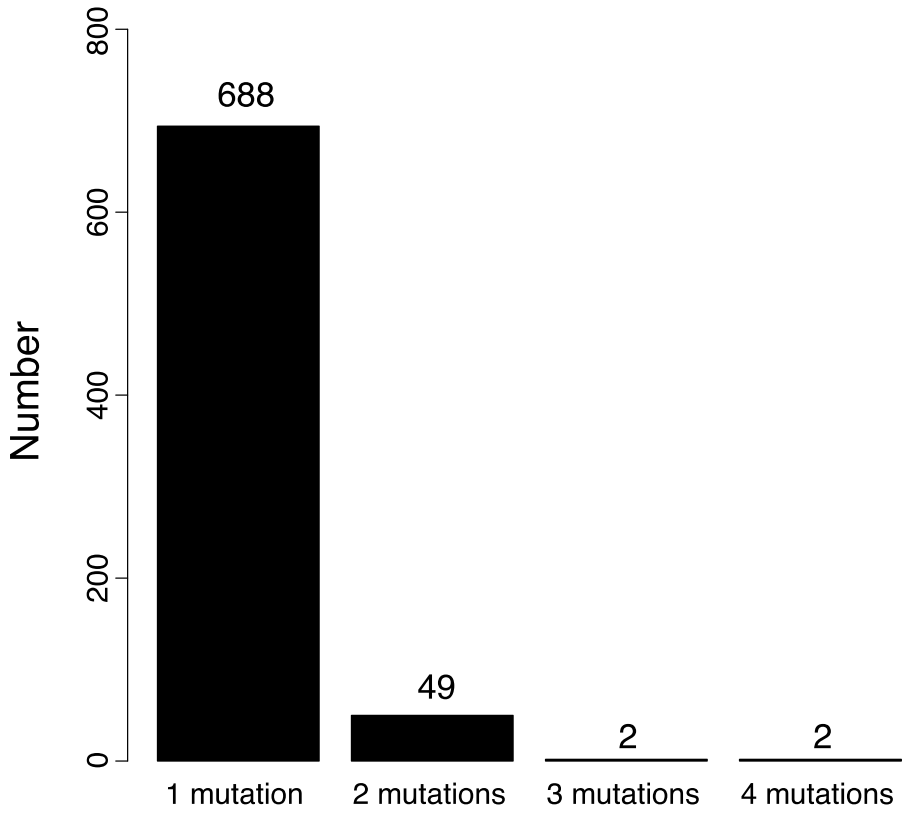
25 – 100 mutations per gene by age 70

Jaiswal and Ebert, *Science* 2019

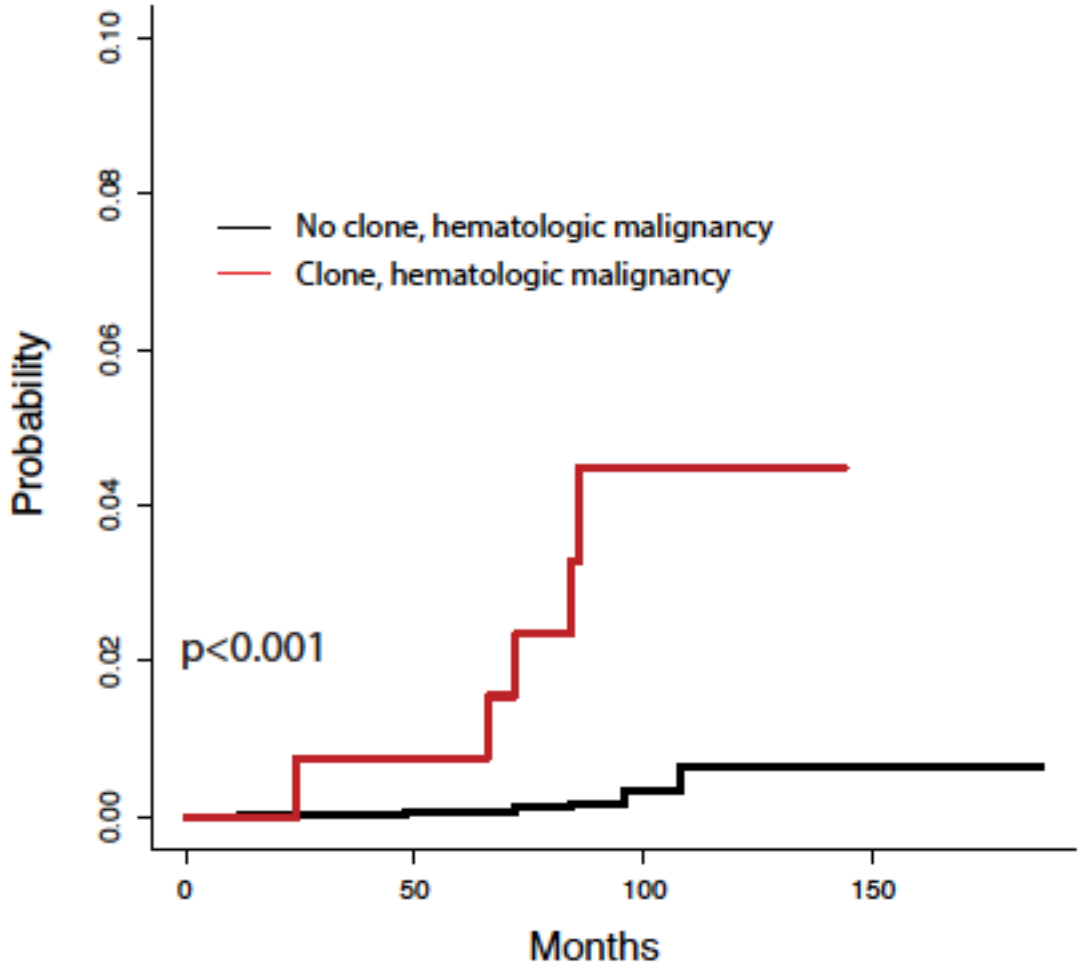
DNMT3A is frequently mutated



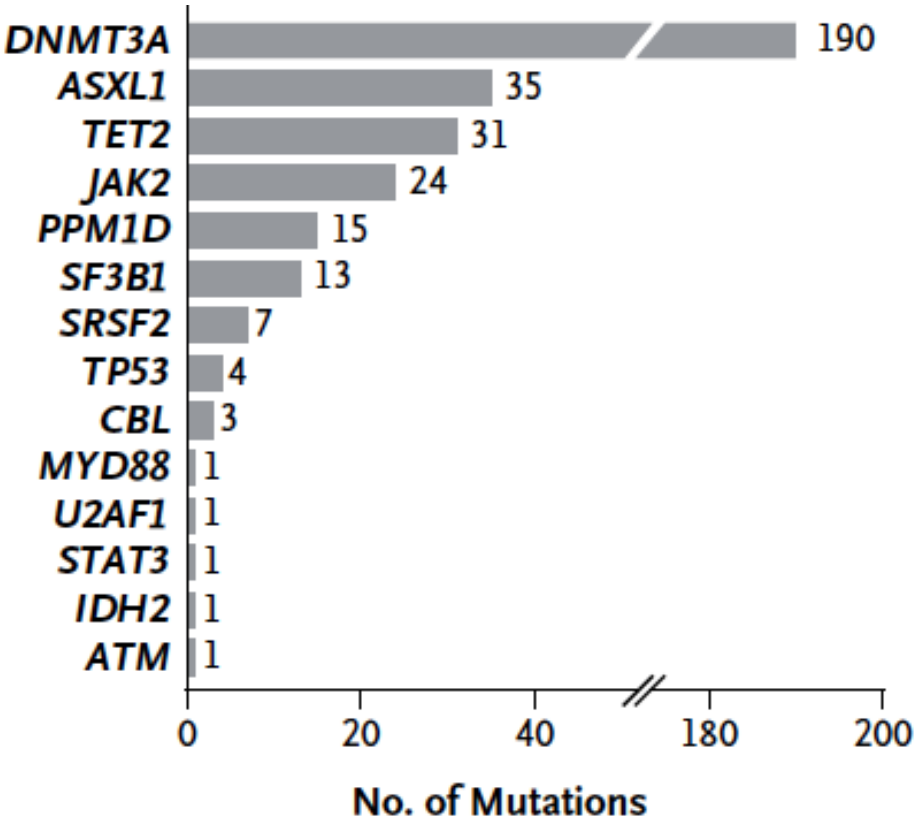
Most subjects had only one mutation

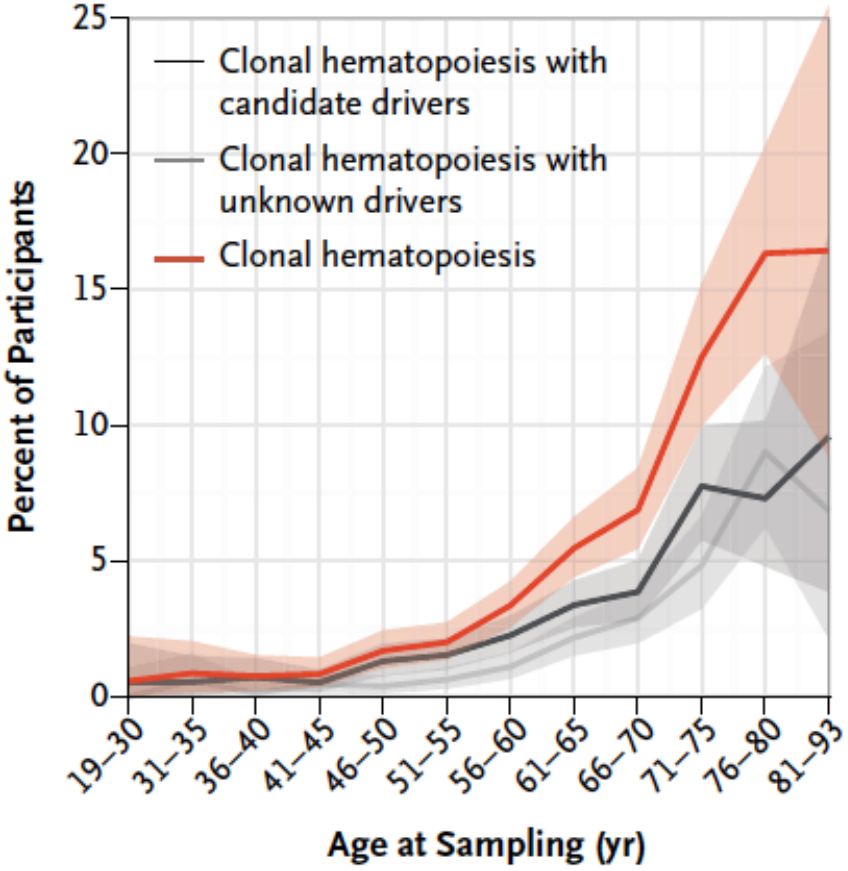


Clonal hematopoiesis increases the risk of hematologic malignancy



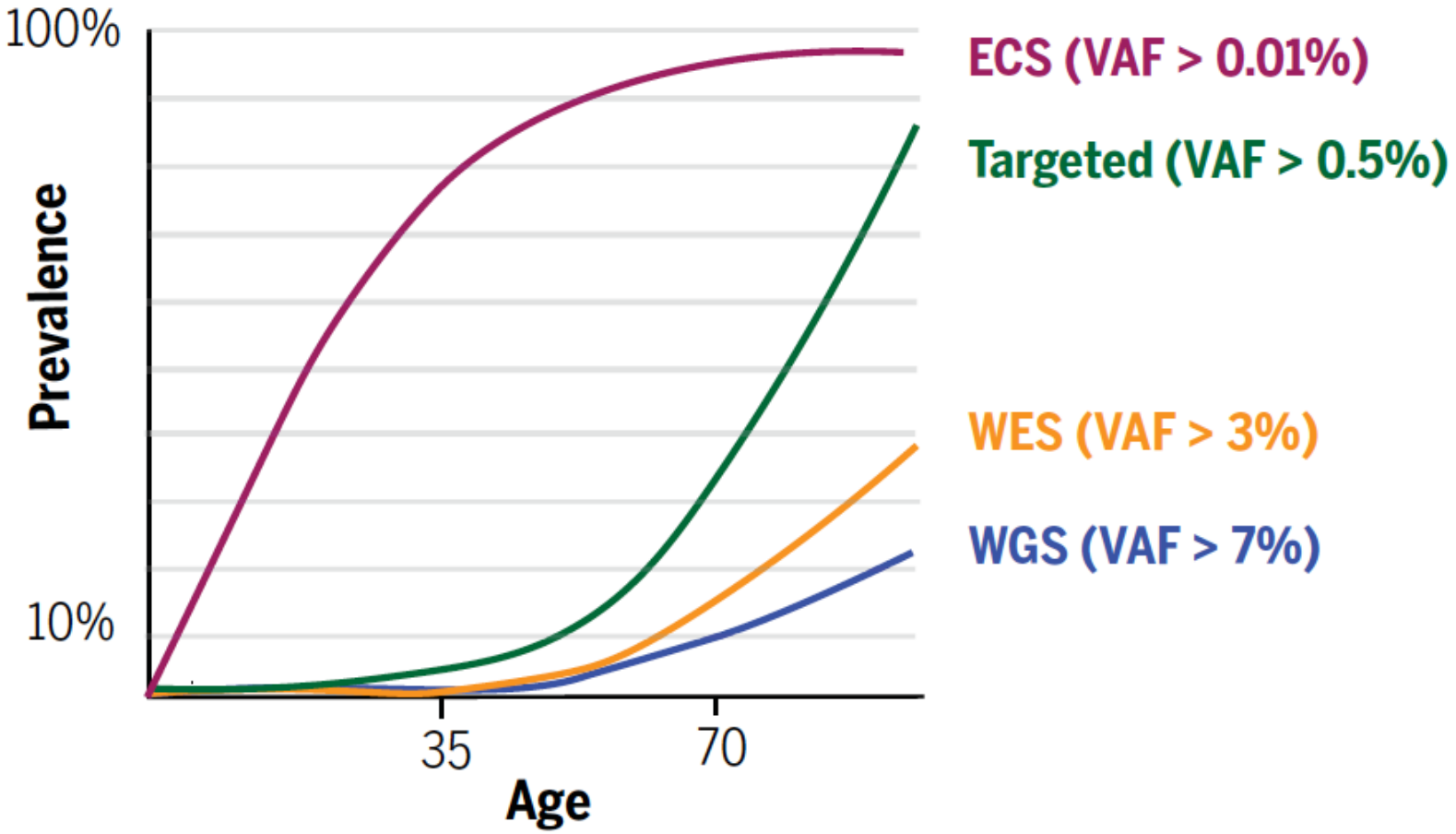
Clonal hematopoiesis: concordant findings from multiple studies





Genovese et al., NEJM 2014
Xie et al., Nat Med, 2014
McKerrell et al., Cell Rep 2015

Prevalence of clonal hematopoiesis



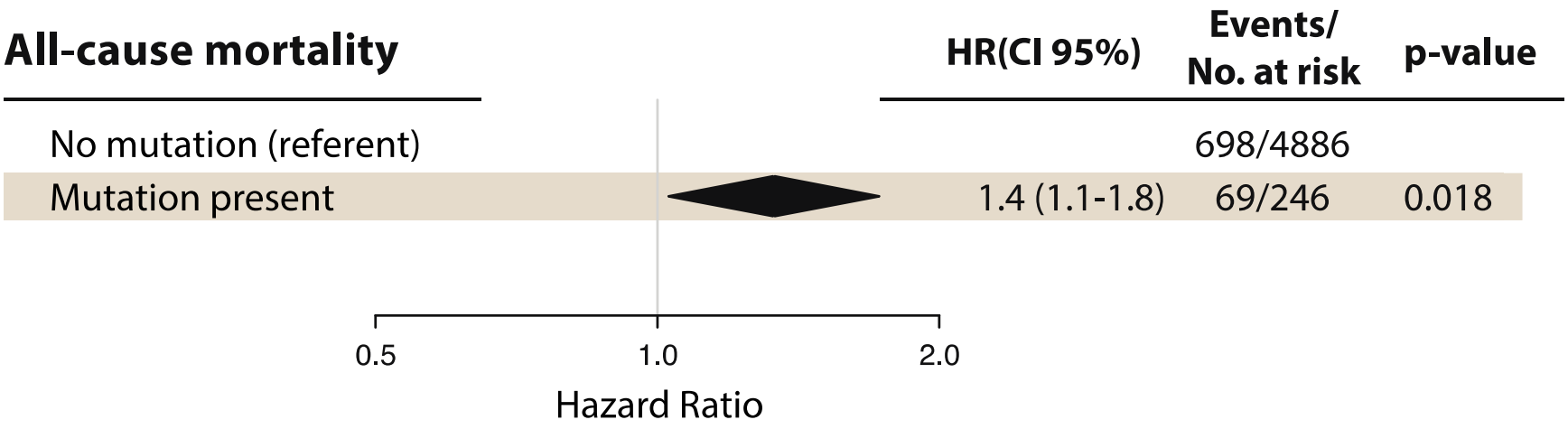
Jaiswal and Ebert, *Science* 2019

Clonal **H**ematopoiesis of **I**ndeterminate **P**otential (CHIP)

- Features:
  - Absence of definitive morphological evidence of a hematological malignancy
  - Presence of a somatic mutation associated with hematological malignancy at a variant allele fraction of at least 2%
  - Odds of progression to overt neoplasia are approximately 0.5-1% per year, similar to MGUS

Steensma et al., *Blood* 2015

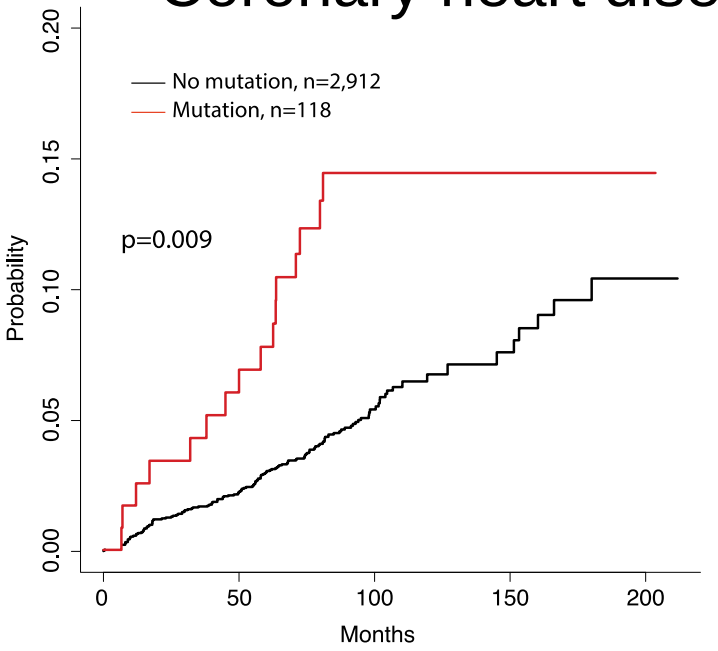
CHIP is associated with reduced overall survival



Cox proportional hazards models which included age, gender, and diabetes status as covariates, with results for cohorts analyzed as a fixed-effects meta-analysis

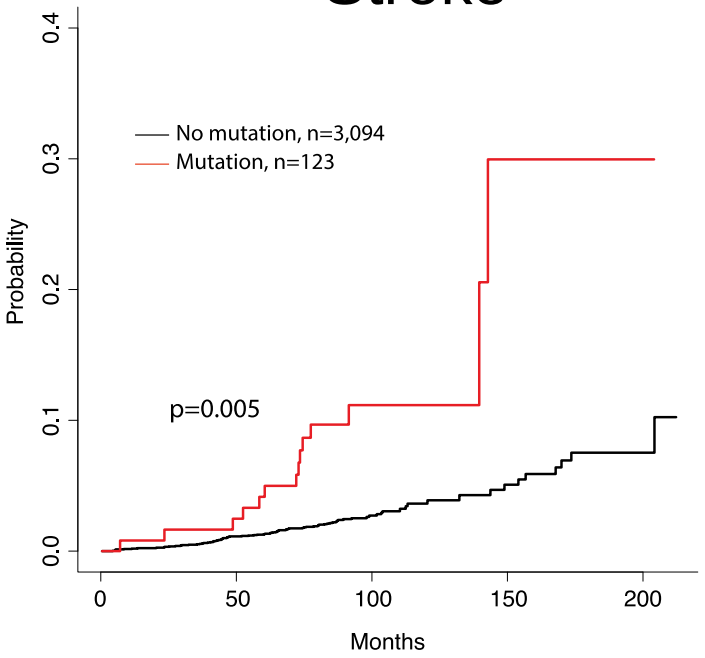
CHIP is associated with higher risk of heart attack and stroke

Coronary heart disease



HR 2.0, 95% CI 1.2-3.4, p=0.018

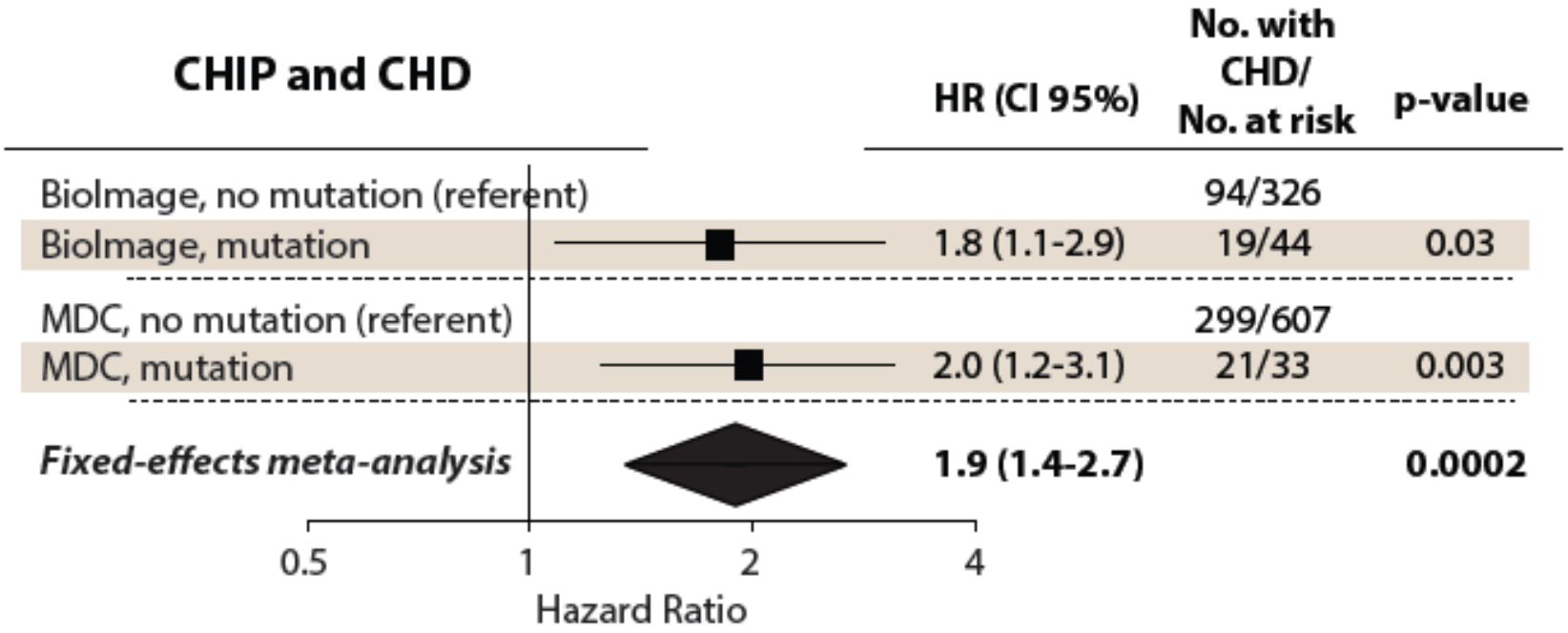
Stroke



HR 2.6, 95% CI 1.4 to 4.8, p=0.003

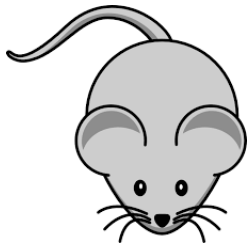
Regression models were adjusted for age, sex, BMI, lipids, blood pressure, and smoking

Replication in additional cohorts

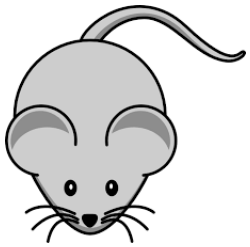


Jaiswal et al., *NEJM* 2017

Experimental examination of CHIP and cardiovascular disease



BMT



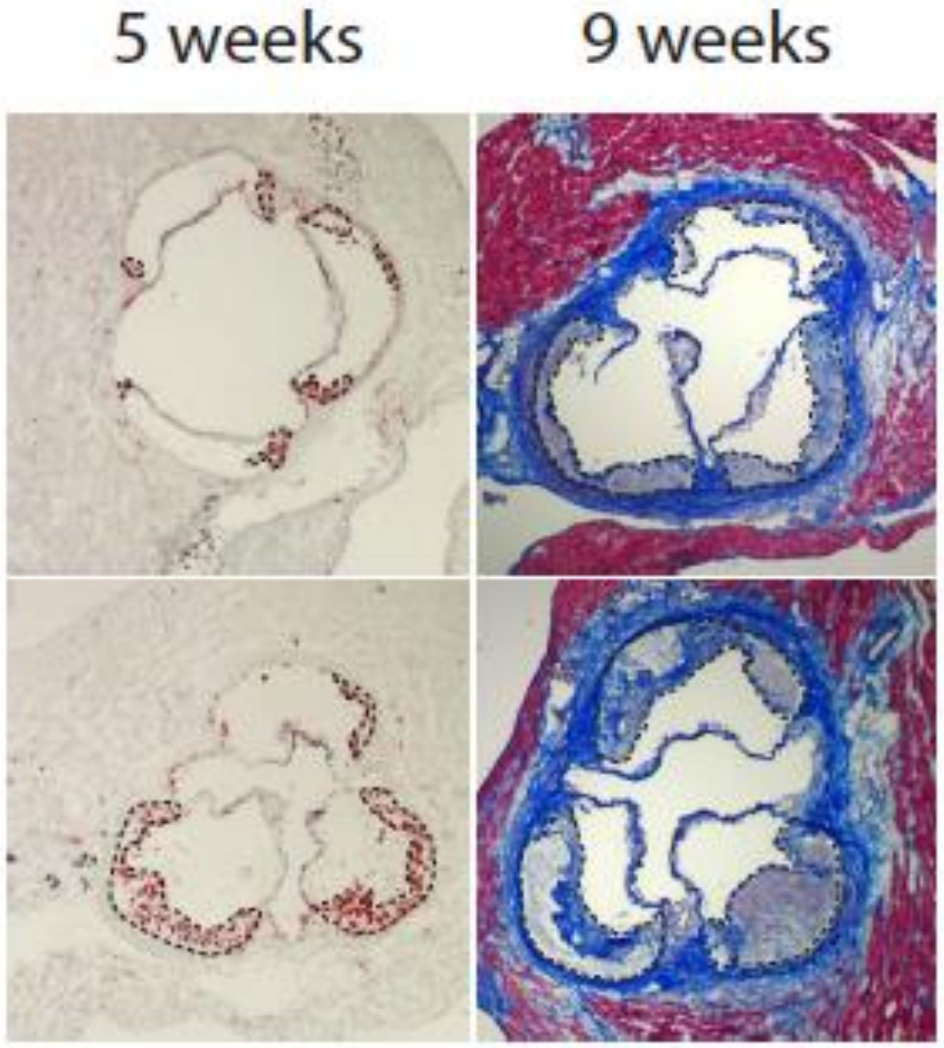
WT
Ldlr$^{-/-}$

Vav1-Cre, *Tet2*$^{fl/fl}$
Vav1-Cre (control)

Diet:
1.25% cholesterol

harvest

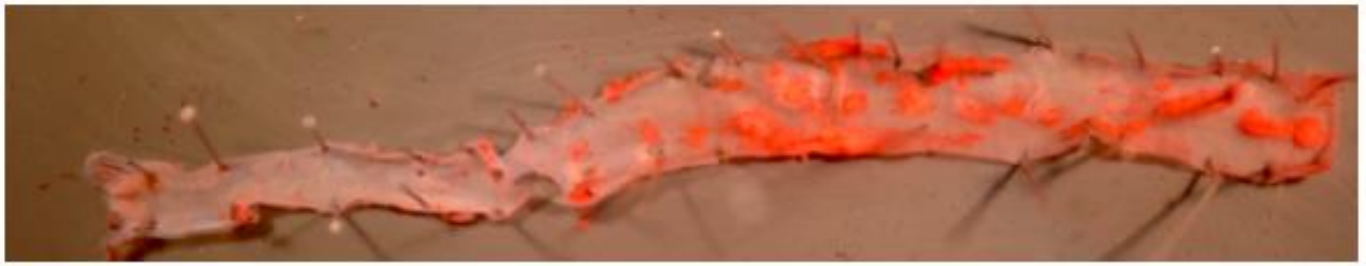
Aortic root



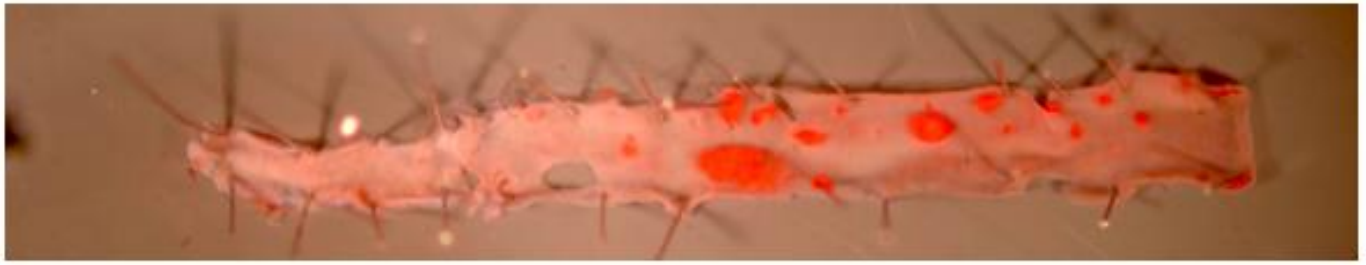
Jaiswal et al., *NEJM* 2017

Descending aorta lesion area is larger in *Tet2*$^{-/-}$ recipients

Tet2 fl/fl, Vav1-Cre

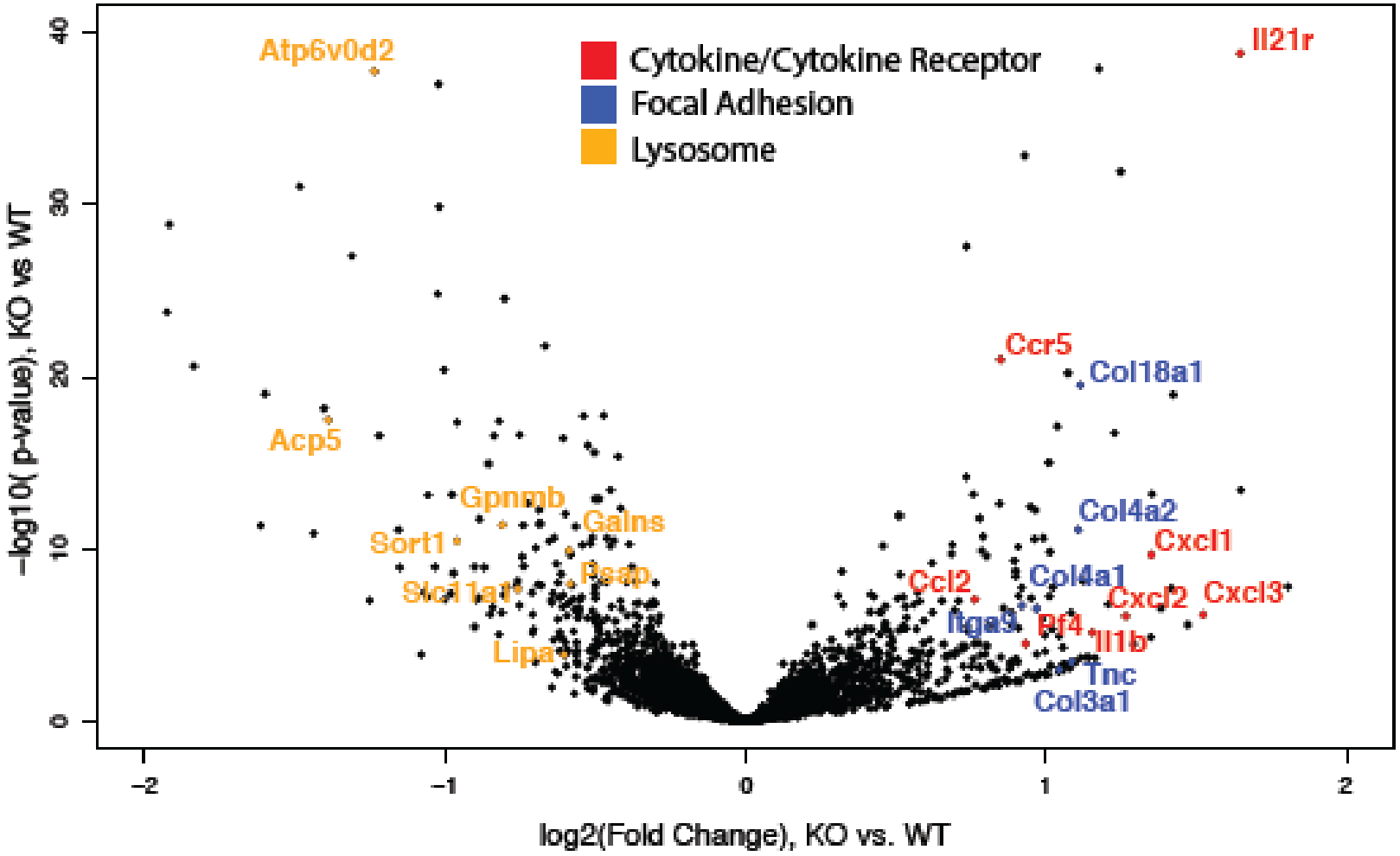


Tet2 +/fl, Vav1-Cre



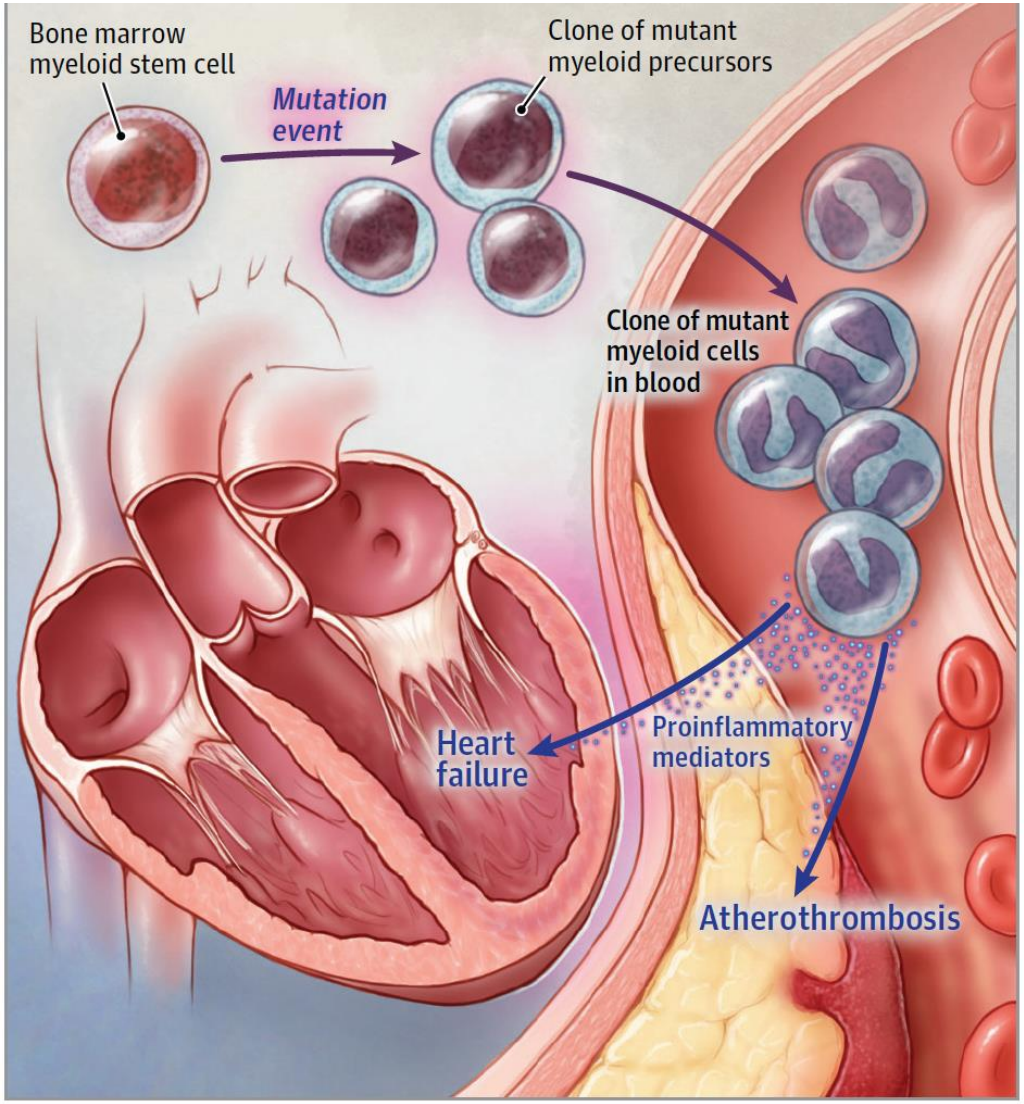
Vav1-Cre

Gene expression in *Tet2* knockout macrophages



Jaiswal et al., *NEJM* 2017

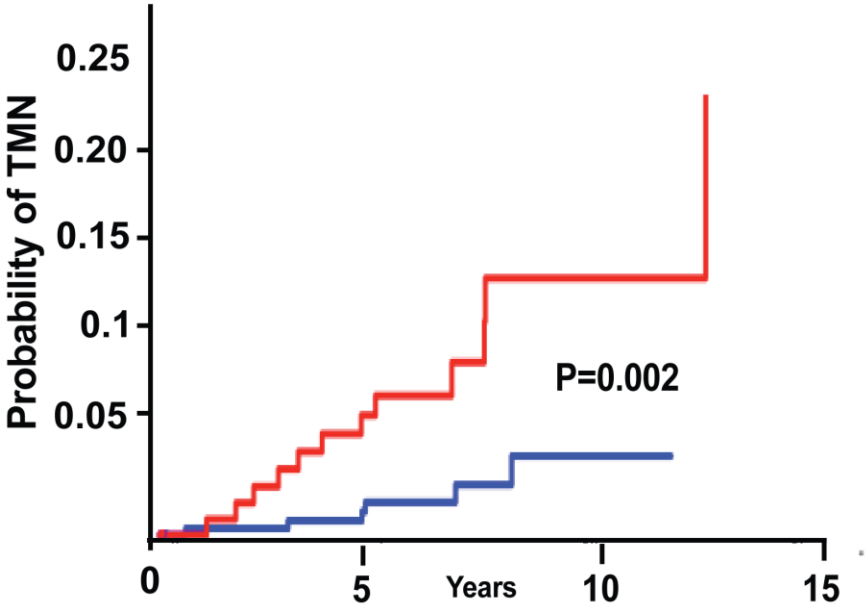
Effect of CHIP on cardiovascular disease

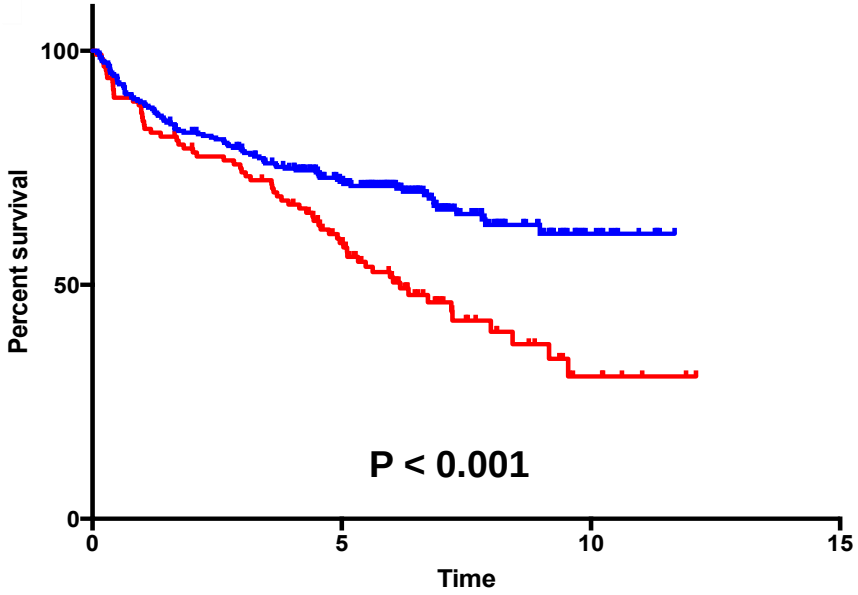


Can CHIP predict the development of therapy-related myeloid malignancies?

CHIP increases the risk of therapy-related myeloid neoplasms

Evaluation of CHIP in lymphoma patients undergoing autologous stem cell transplant





No CHIP

CHIP

Gibson et al., *JCO* 2017

Summary

CHIP is associated with increased overall mortality

- Increased risk of hematologic malignancy
- Increased risk of cardiovascular disease
- Increased risk of thrombosis (*JAK2* mutations)
- Increased risk of therapy-related malignancy

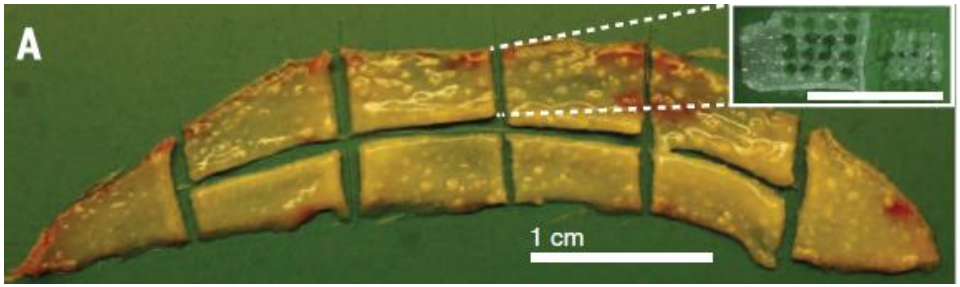
JAK2 CHIP mutations are associated with thrombosis

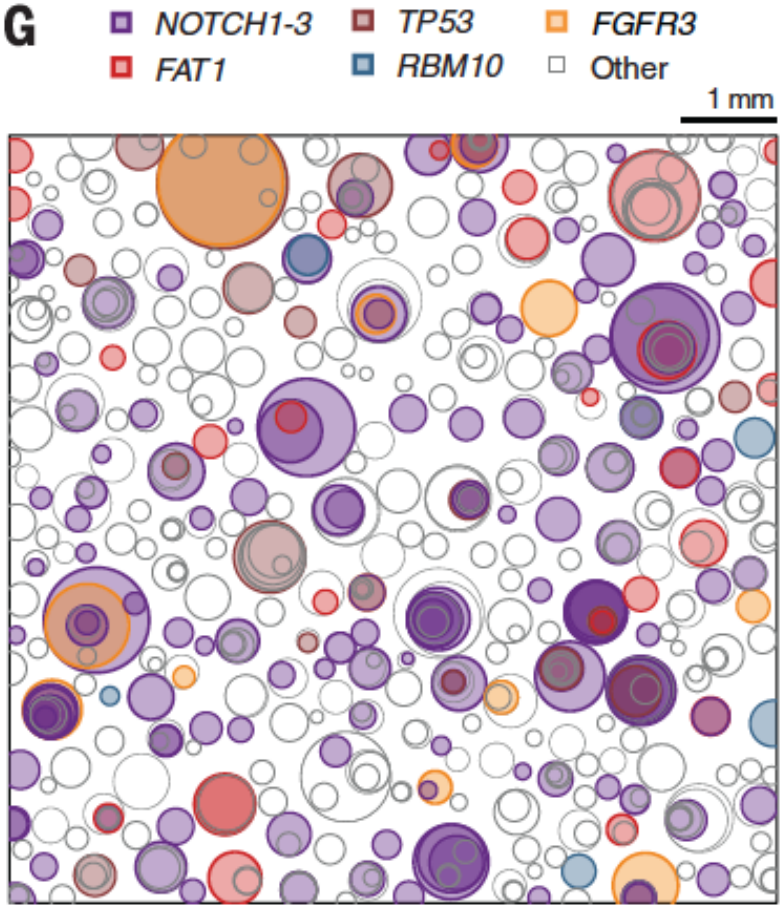
- JAK2 mutations sensitize neutrophils to NETosis

Tet2 mutations accelerate atherosclerosis *in vivo*

- Development of xanthomas
- Altered expression of inflammatory cytokines

Clonal expansions in skin





Mortincorena et al., *Science* 2015

Immunomodulatory Drugs (IMiDs)

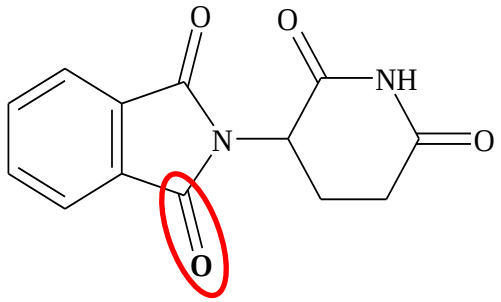
Thalidomide

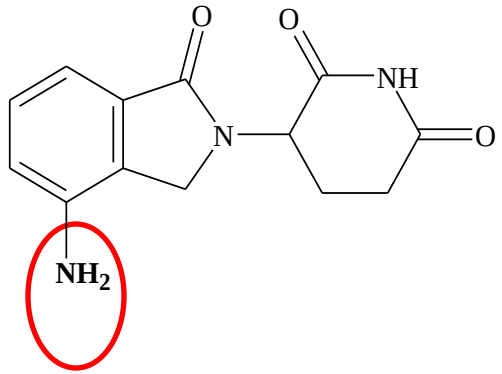
-1957 to 61: Treatment of morning sickness during pregnancy
>10,000 children born with phocomelia
-1991: TNF inhibitor
-1994: Antiangiogenesis: first trials in cancer
-2006: Approved for multiple myeloma

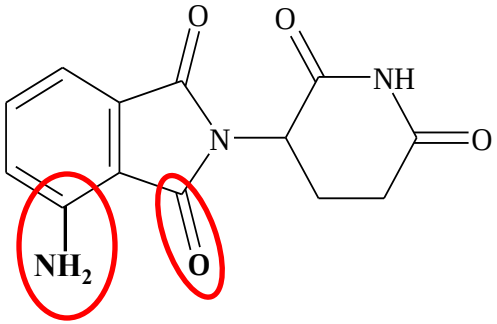
q)

Pomalidomide

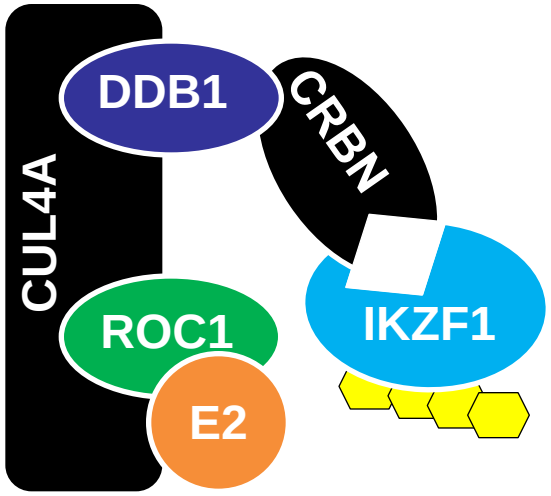
FDA approved for multiple myeloma



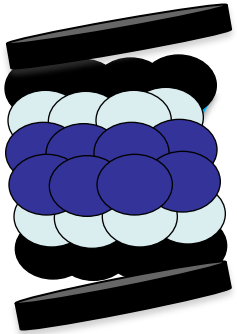




Lenalidomide modulates the activity of the CRL4-CRBN ubiquitin ligase



CRBN-CRL4



Proteasome

Lenalidomide targets:

Multiple Myeloma: IKZF1, IKZF3

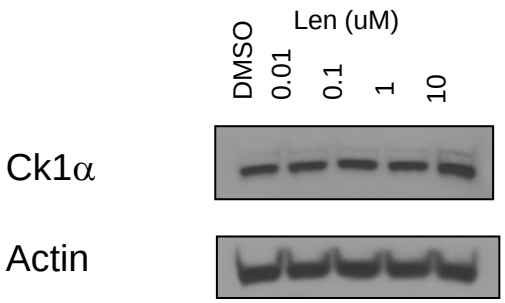
Del(5q) MDS: CK1α

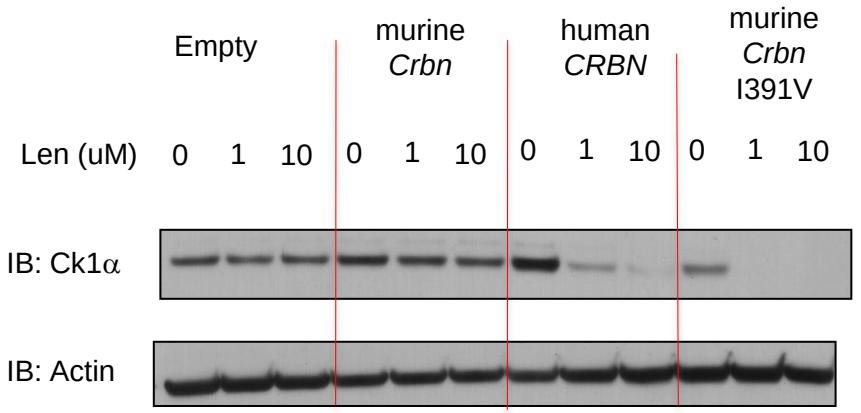
Krönke et al., Science, 2014; Lu et al., Science, 2014; Krönke, Fink et al., Nature, 2015

Lenalidomide activity in mouse cells

Generated *Ck1α* conditional knockout mouse (Schneider et al., *Cancer Cell* 2014)
Does lenalidomide target *Ck1α* haploinsufficient cells? … No effect.

murine Baf3 cells



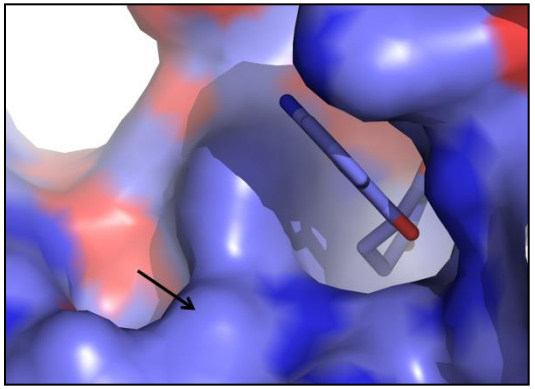


- No toxicity in mice

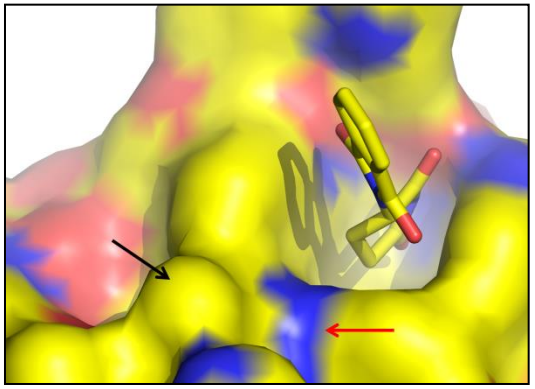
Kronke, Fink et al., *Nature* 2015

Structural basis for human vs mouse lenalidomide activity

Steric bump of mouse isoleucine prevents access of substrates to H bonds on CRBN surface



Human CRBN

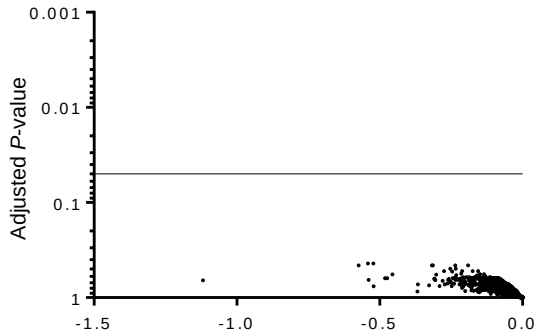


Mouse Crbn

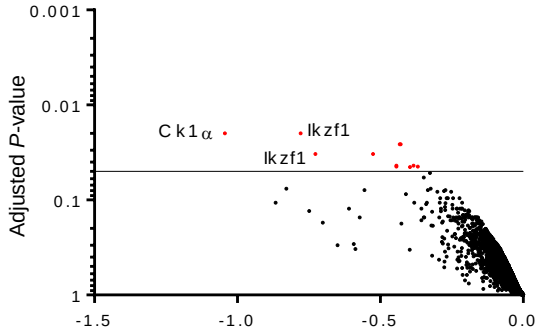
Kronke, Fink et al., *Nature* 2015

Crbn I391V knock-in mouse

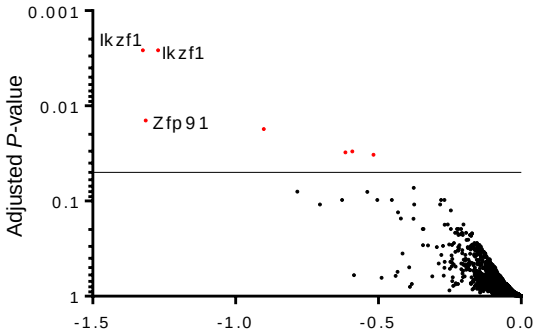
WT Len 1 μM vs. DMSO



$Crbn^{I391V/I391V}$ Len 1 μM vs. DMSO

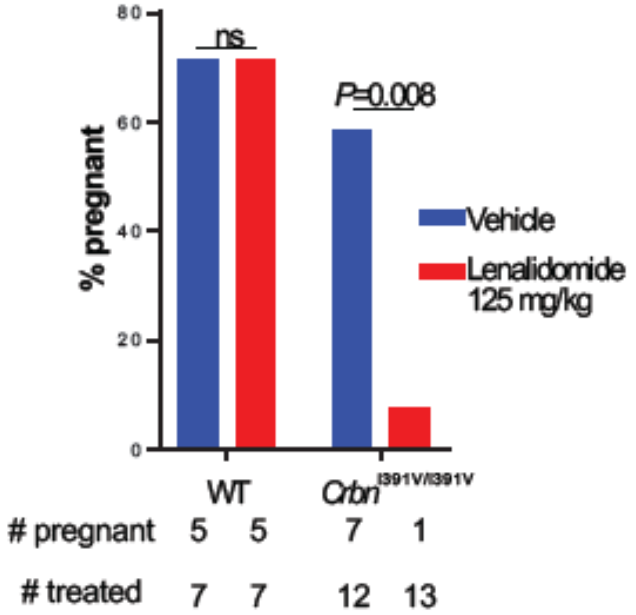


$Crbn^{I391V/I391V}$ Pom 1 μM vs. DMSO



Fink et al., *Blood* 2018

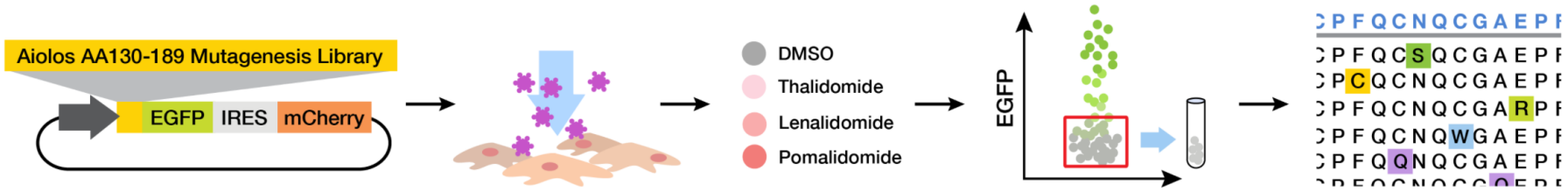
Crbn I391V knock-in mouse: effect on pregnancy



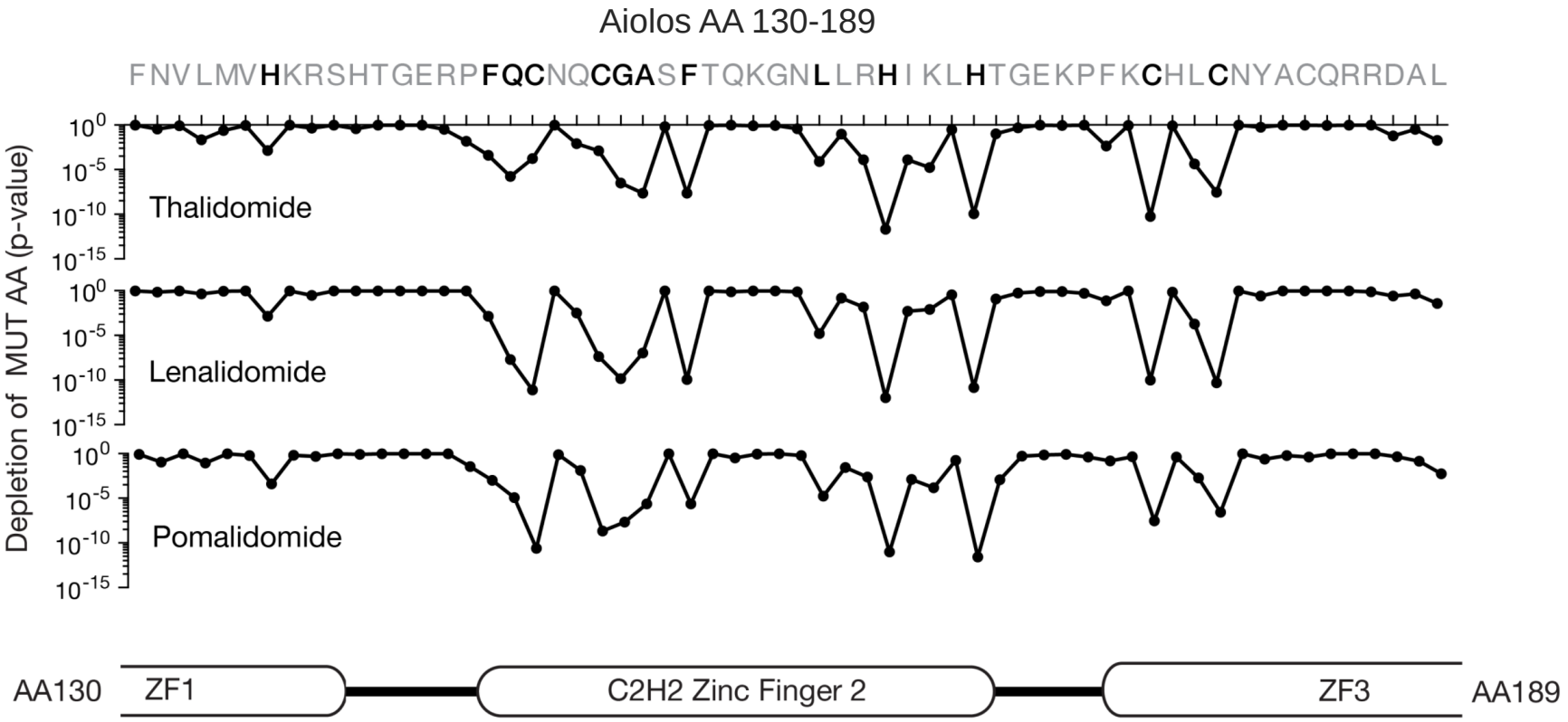
Fink et al., *Blood* 2018

Defining a lenalidomide-dependent degron sequence

Saturation mutagenesis of IKZF3 degron

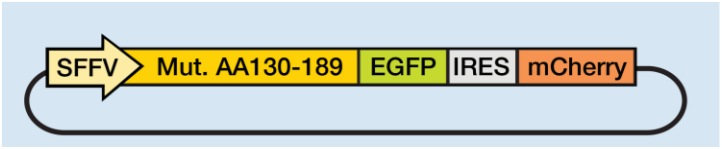


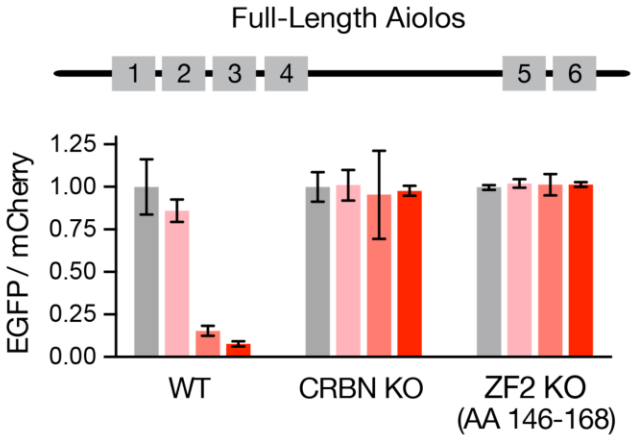
IKZF3 zinc finger 2 is critical for degradation

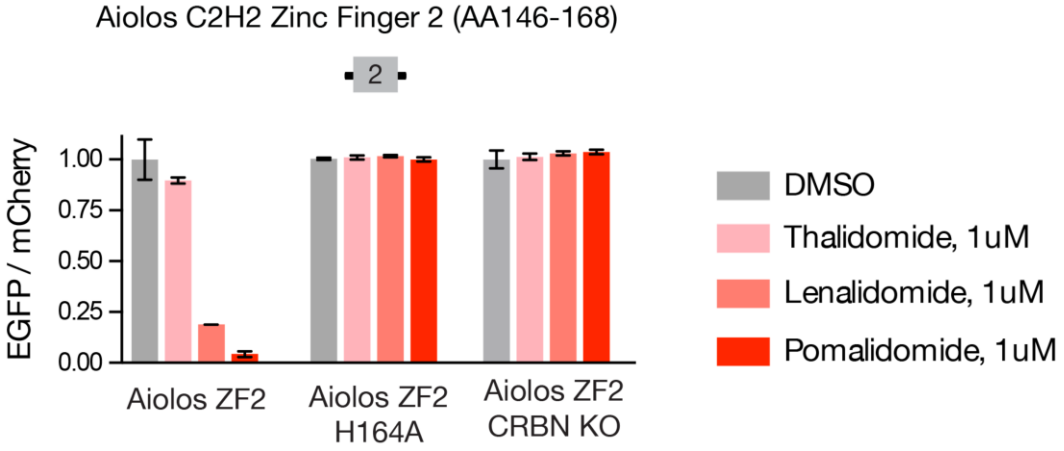


Sievers, Petzold et al., *Science* 2018

IKZF3 zinc finger 2 is necessary and sufficient for lenalidomide-dependent degradation

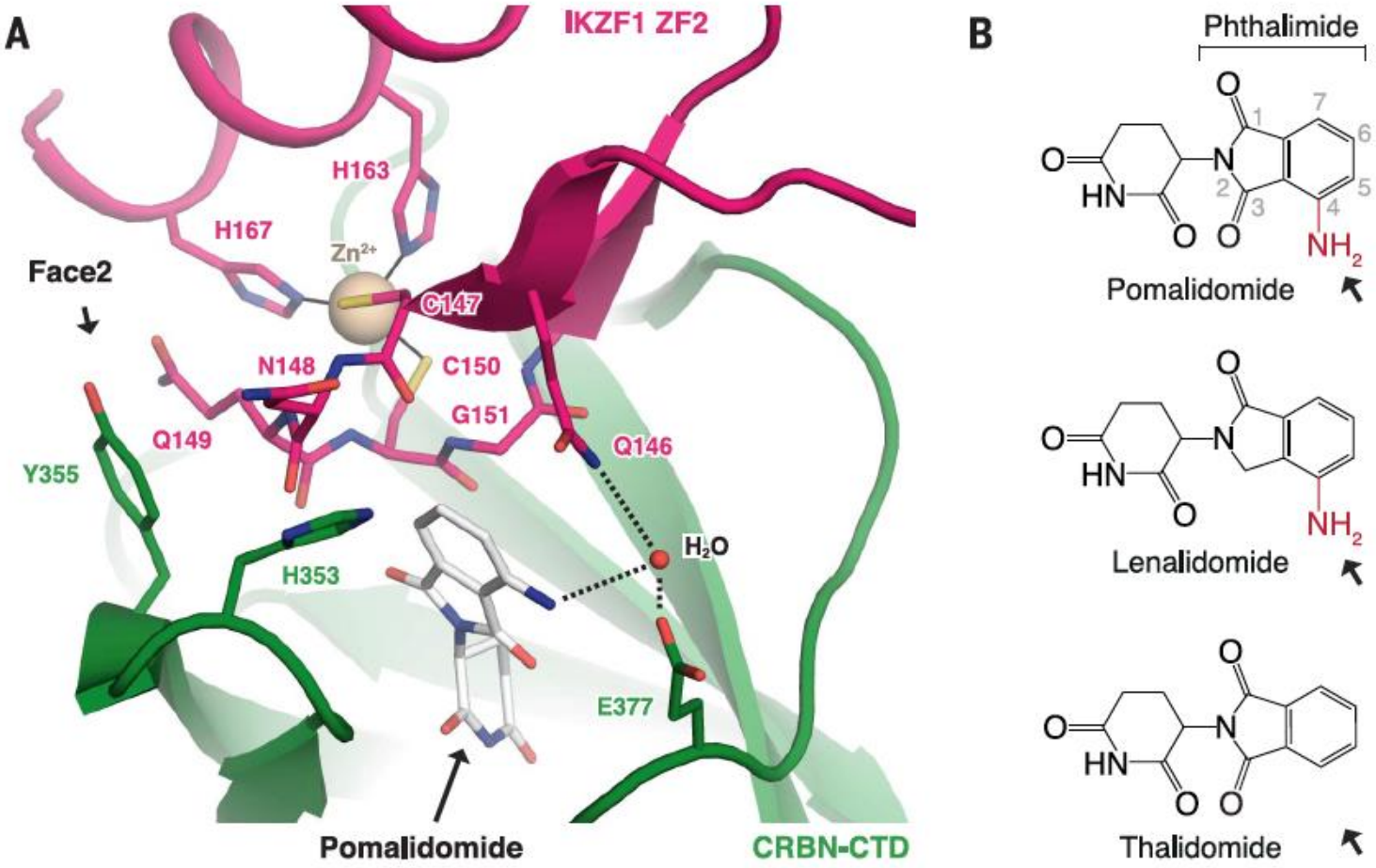






Sievers, Petzold et al., *Science* 2018

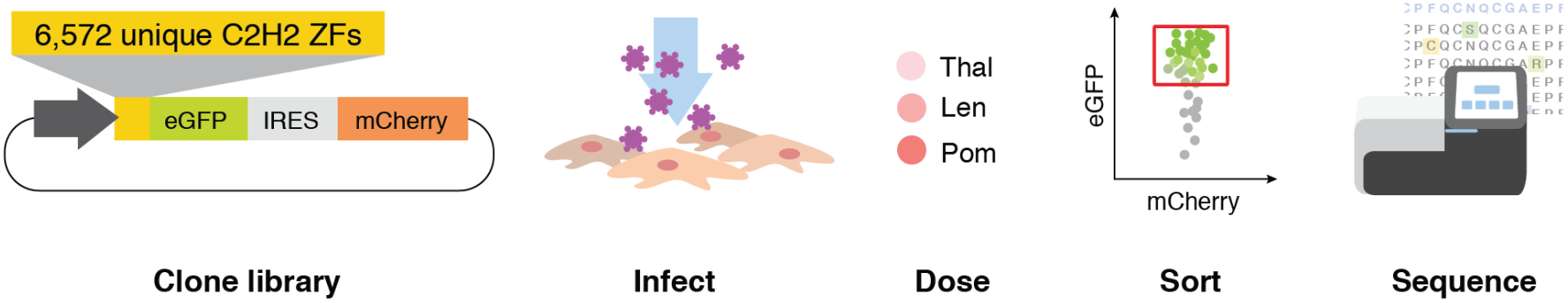
Structure of zinc finger – pomalidomide - CRBN



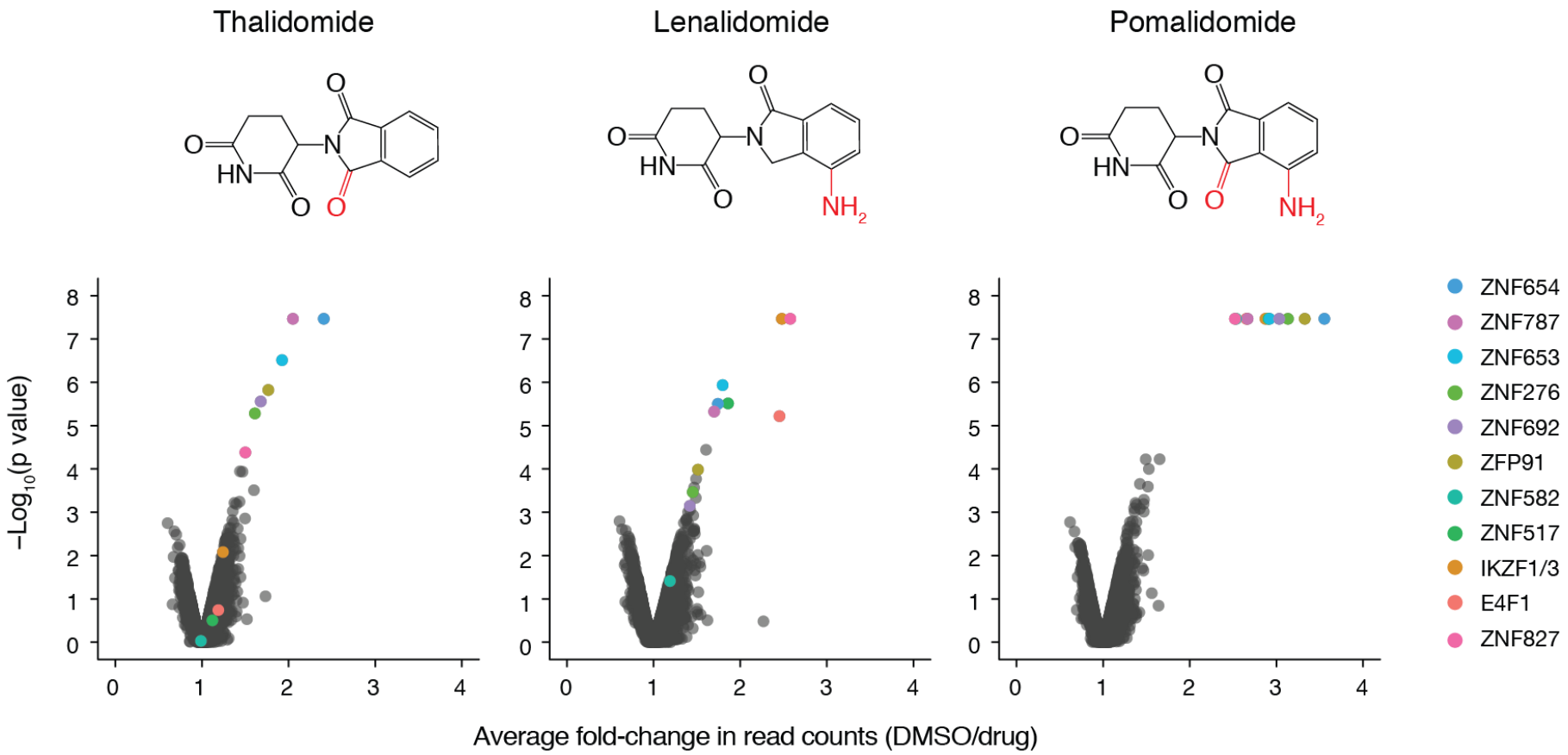
Sievers, Petzold et al., *Science* 2018

Can we target other zinc fingers in the genome?

Proteome-wide C2H2 zinc finger screen

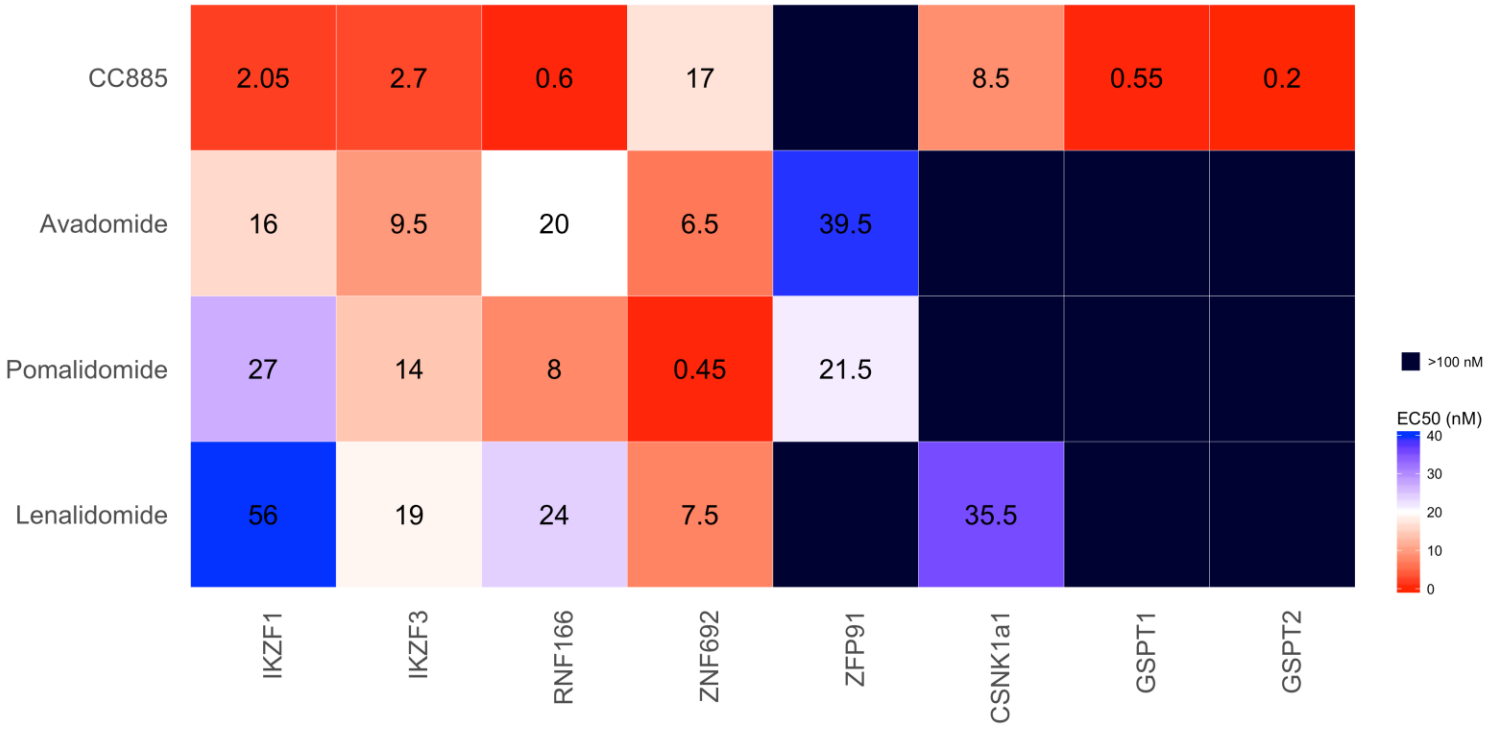


Proteome-wide C2H2 zinc finger screen



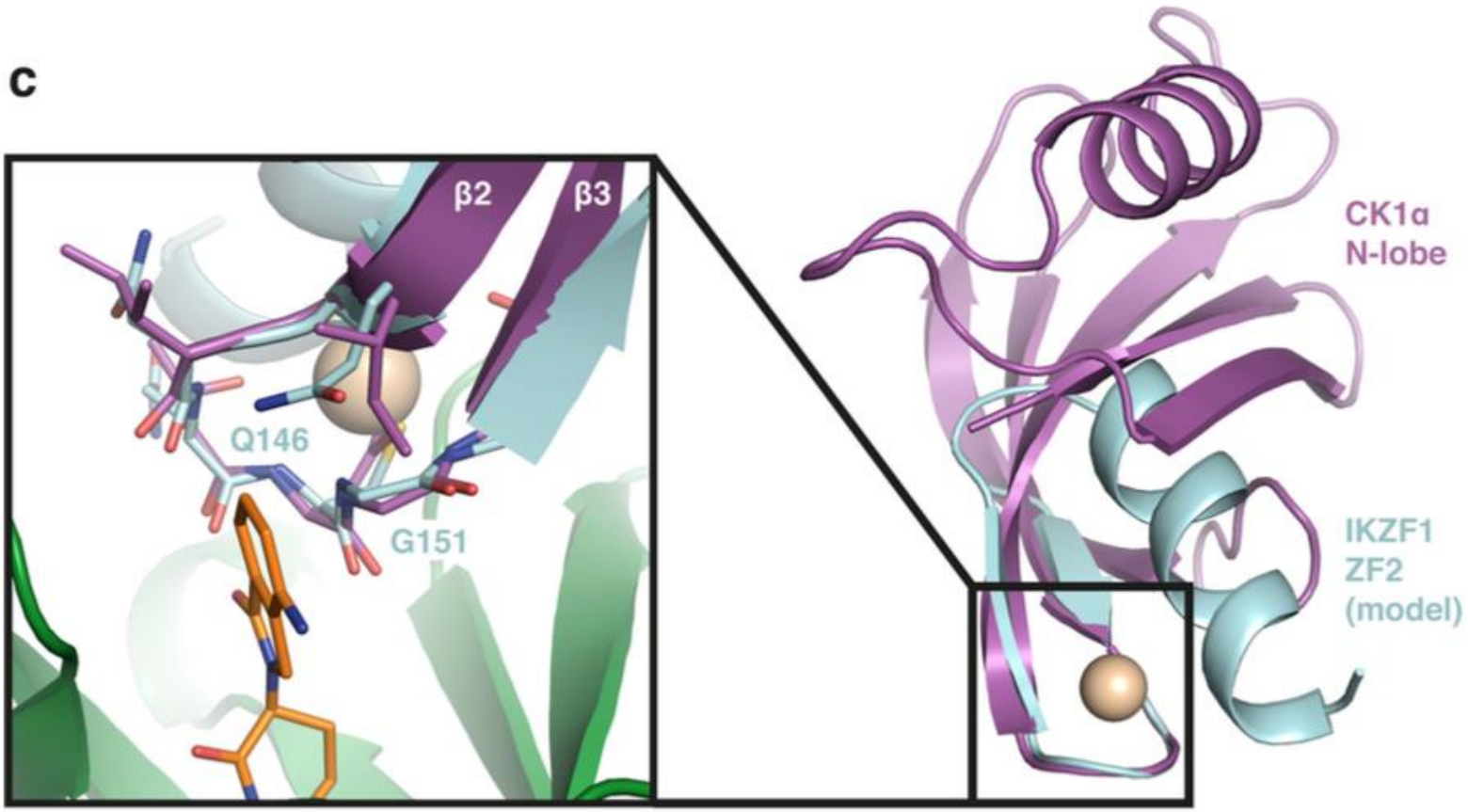
Sievers et al., *Science* 2018

Substrate-specific effects of different drugs



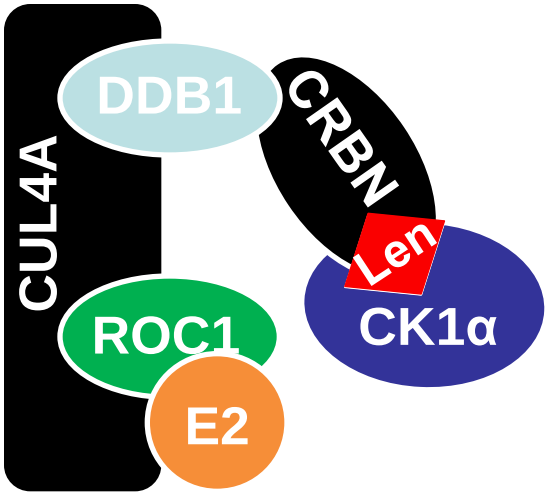
Sperling et al., *Blood* 2019

CK1α and GSPT1 have beta-beta fold degrons as well



Petzold et al., *Nature* 2016

Summary



$CRL4^{CRBN}$

- Thalidomide derivatives act as a molecular glue, inducing ubiquitination and degradation of substrates by the $CRL4^{CRBN}$ ubiquitin ligase
- Thalidomide derivatives target a zinc finger degron
- Different thalidomide derivatives target distinct substrates for degradation
- Multiple myeloma: lenalidomide induces degradation of IKZF1 and IKZF3
- Del(5q) MDS: lenalidomide induces degradation of CSNK1A1
- One non-conserved amino acid in mouse and human CRBN determines lenalidomide sensitivity

Acknowledgements

BWH/DFCI
Sidd Jaiswal
Coleman Lindsley
Zuzana Tothova
Quinn Sievers
Mikolaj Slabicki
Emma Fink
Jessica Gasser
Aline Renneville
Sidd Jaiswal
Ofir Wolaj
Chris Gibson
Rob Sellar
Coleman Lindsley
Zuzana Tothova
Roger Belizaire
Andrew Guirguis
Adam Sperling
James Kennedy
Steffen Boettcher
Mridul Agrawal
Josephine Kahn
Ellen Beauchamp
Sebastian Koochaki
Marie McConkey

Brigham Cardiology
Peter Libby

Broad Institute
Steve Carr
Namrata Udeshi

University of Ulm
Jan Krönke

Broad Institute
Sek Kathiresan
Pradeep Natarajan

FMI
Nico Thomä
Georg Petzold
Richard Bunker