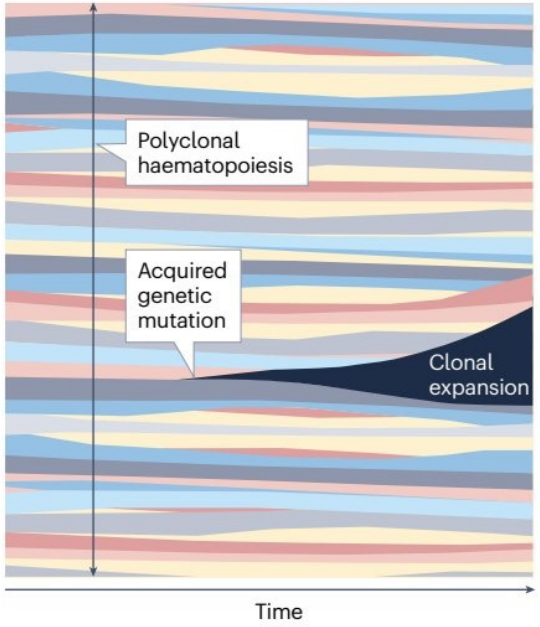
Clonal hematopoiesis in solid tumor patients: implications for secondary malignancies and diagnostics

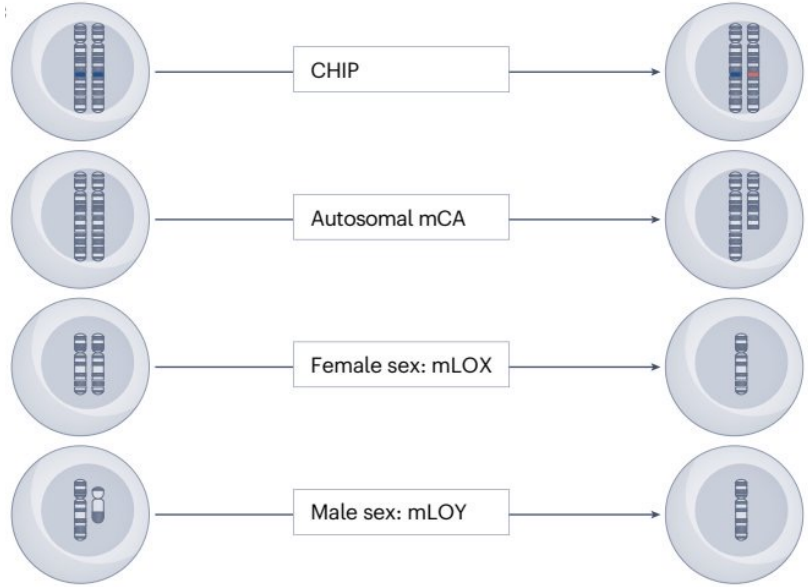
Ahmet Zehir, PhD
Executive Director, Precision Medicine
Precision Medicine & Biosamples
AstraZeneca

What is clonal hematopoiesis?

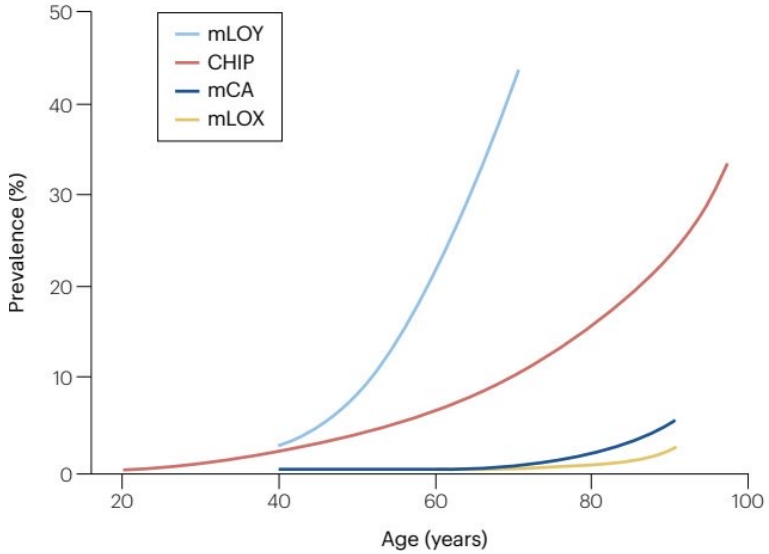
Clonal expansion of hematopoietic (stem) cells with genomic changes that increase cellular fitness



Acquired genetic modifications lead to clonal expansion over a background of polyclonal hematopoiesis



Main types of genetic changes are i) point mutations and small indels ii) mosaic chromosomal alterations, including mLOX and mLOY

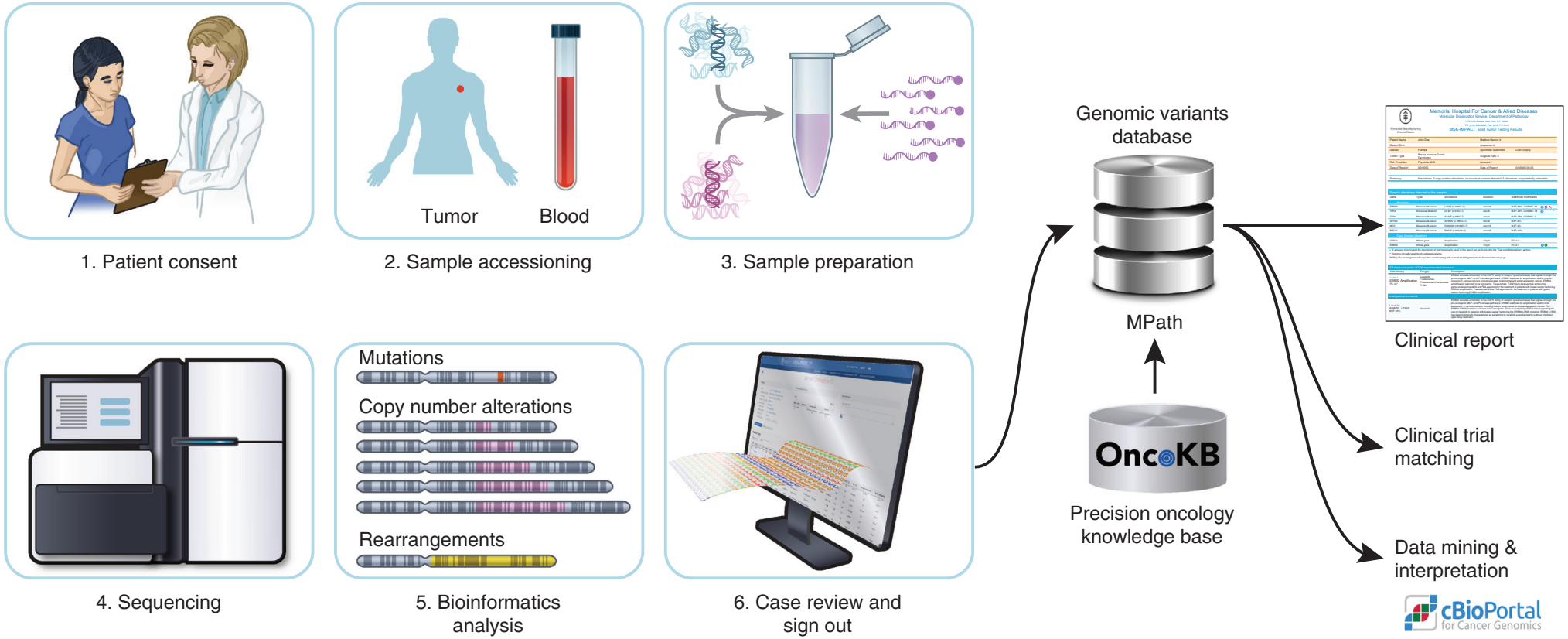


Prevalence estimates of clonal expansions through different genetic alterations across an individual's lifespan (highly dependent on limit of detection of the assays)

Vlasschaert C., *et al.,* Nat Reviews Neph 2023

Identification of CH mutations in solid tumor patients using MSK-IMPACT

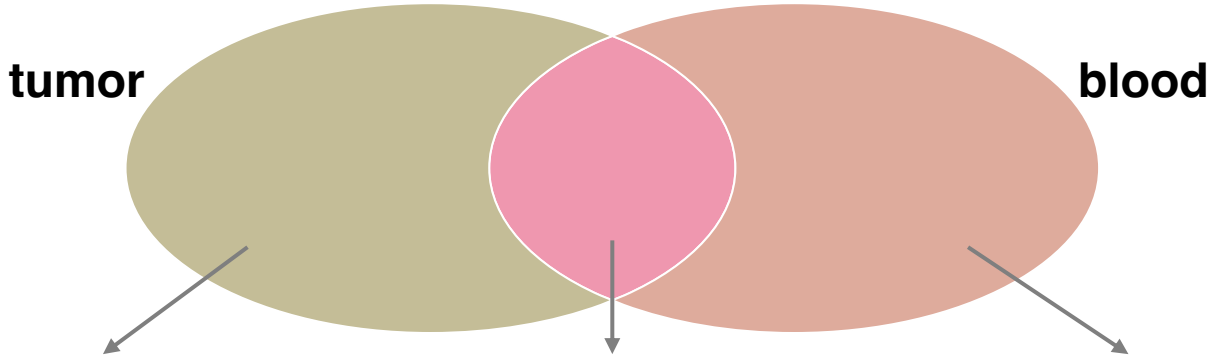
Identifies mutations, copy number alterations, structural variants in **505 genes** as well as biomarkers that could predict treatment response such as **microsatellite instability (MSI) status** or **tumor mutation burden (TMB)**



Zehir A, Benayed R *et al.*, Nature Med 2017

Paired tumor & matched blood sequencing

Subtracting tumor mutations from the blood allows for identifications somatic mutations in the blood



Tumor somatic alterations

- mutations (snvs/indels)
- copy number alterations
- structural variants
- microsatellite instability
- mutation burden

Germline alterations

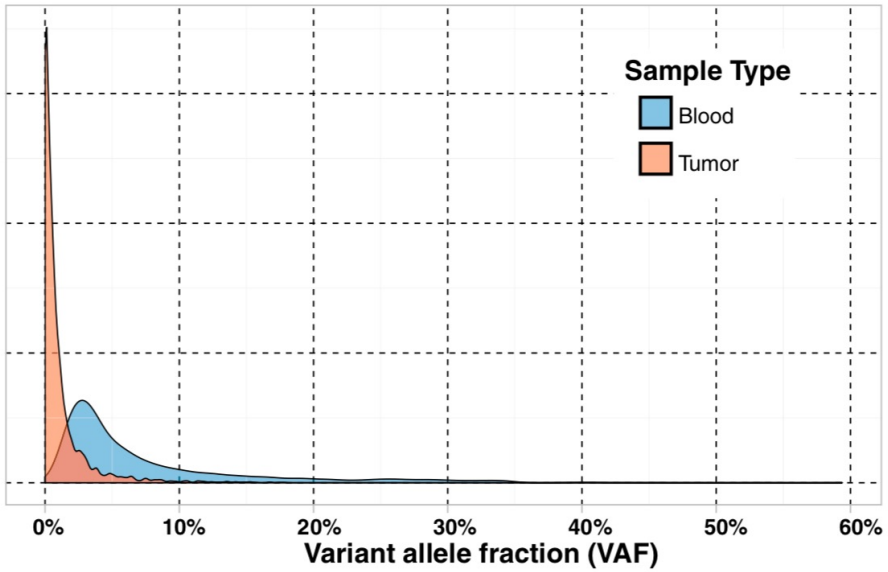
- In 90 genes known to be involved in hereditary cancers
- mutations (snvs/indels)
- copy number alterations
- expert curation by molecular geneticists
- patient consultations via clinical genetics service

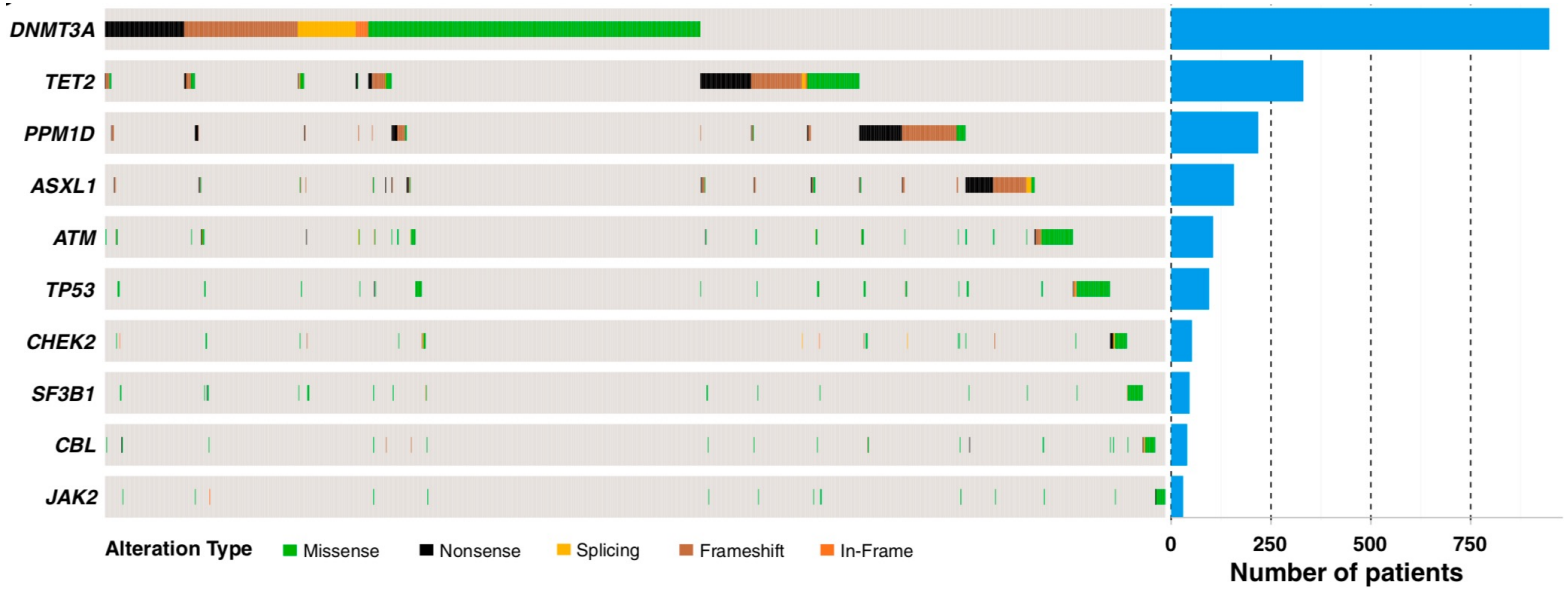
Clonal hematopoiesis (CH/CHIP)

- mutations (snvs/indels)
- copy number alterations

Clonal hematopoiesis is common in cancer patients

CH is observed in ¼ patients, occurring in genes commonly mutated in heme malignancies



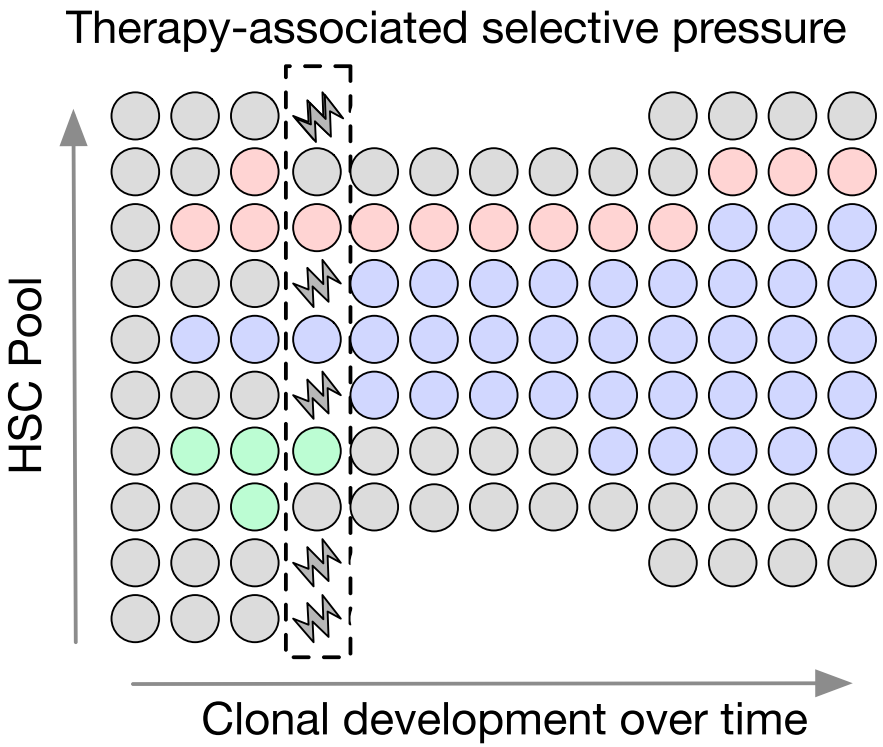


identified CH in ~ 24% of advanced cancer patients (n = 8,810)

Coombs CC, Zehir A., *et al*, *Cell Stem Cell* 2017

Clonal hematopoiesis

Oncologic therapy can shape further establishment and development of certain clones



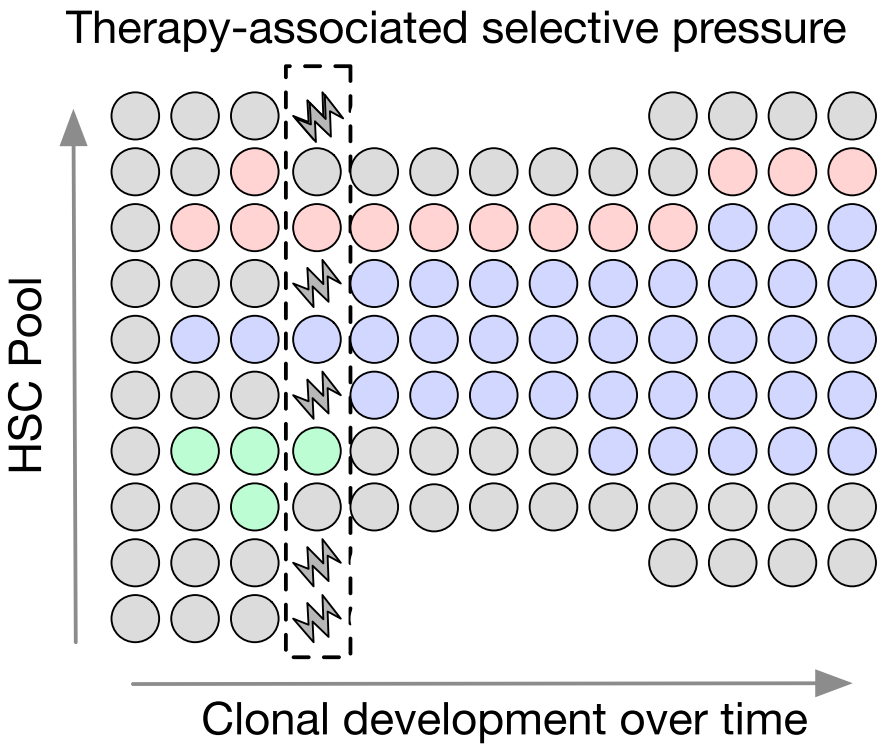
Bowman LB., *et al*, 2018 Cell Stem Cell Review

	Overall	CH	No CH	p value
Number of patients	5,649	1,353	4,296	–
Age (y)	58.3	66.1	55.8	<0.001
Gender (% male)	49	50	49	0.40
Associations with prior toxic exposures				
Current or former smoker (%)	46	53	44	<0.001
Prior chemotherapy (%)	64	64	64	0.89
Prior RT (%)	37	41	35	<0.001

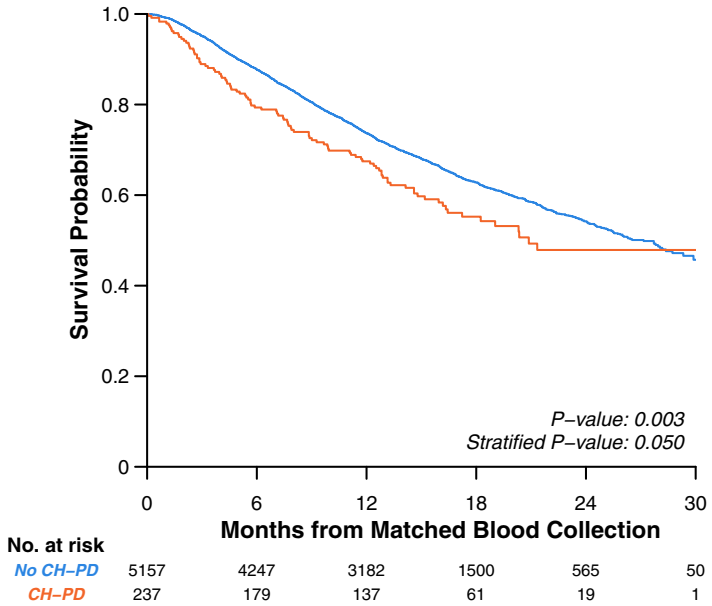
Coombs CC, Zehir A., *et al*, 2017 Cell Stem Cell

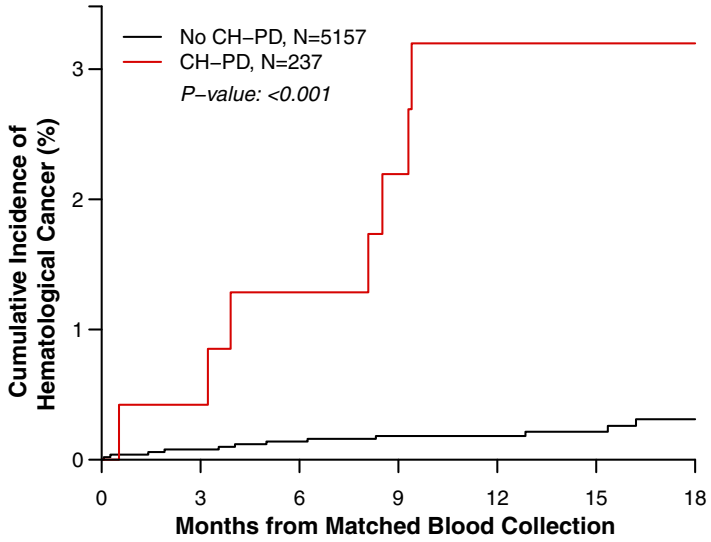
Clonal hematopoiesis

Oncologic therapy can shape further establishment and development of certain clones



Bowman LB., *et al*, 2018 Cell Stem Cell Review





Coombs CC, Zehir A., *et al*, 2017 Cell Stem Cell

First CH clinic + real time CH reporting

These findings have led to the formation of the first CH clinic in the world, and we started reporting CH clinically

Somatic mutations detected in the blood (for investigational use - see assessment section):

Gene	Type	Alteration	Location	Additional Information
TET2	Missense	C1378Y *(c.4133G>A)*	exon 9	MAF: 13.7%
TET2	Frameshift Deletion	P333Hfs*14 *(c.998del)*	exon 3	MAF: 19.7%
ASXL1	Frameshift Deletion	L731Yfs*13 *(c.2191del)*	exon 12	MAF: 18.3%
PMS1	Frameshift Deletion	R760Sfs*19 *(c.2280del)*	exon 10	MAF: 15.2%

MSK-IMPACT report

MSK Opens New Clinic to Monitor People with a Genetic Risk for Developing Blood Cancer

By Julie Grisham, **Tuesday, January 23, 2018**

Ross Levine

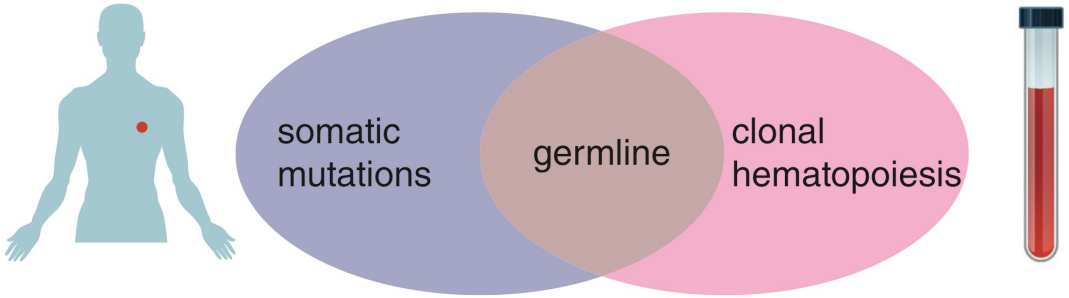
Kelly Bolton

CH clinic utilizes MSK-IMPACT data to identify patients who harbor CH mutations in real time.
Patients are consulted and followed up if necessary

Bolton KL. et al., Hematol Oncol Clin North Am, 2020

Refining how oncologic therapy shapes CH in cancer patients

Identified CH in 24,354 patients and combined with deep phenotypic data on treatment



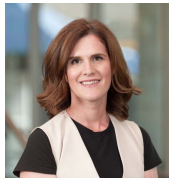
raw variant call set

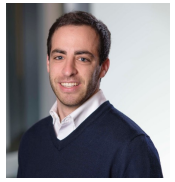
filter sequencing artifacts
- remove if a variant is present more than 3 times with >= 1.5% VAF in PON*
- remove if a variant is present at all with >= 2% VAF in PON
- 1 bp deletions within a homopolymer stretch (>= 3bp)
- SNVs completing a >= 5bp homoplymer
- ins/dels in highly repetitive regions
- variants with strand bias (fisher test)

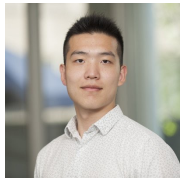
filter low quality samples
require variants called by both `VarDict` and `MuTect`
require VAF_normal > VAF_tumor * 1.5 (if bipsy is from lymph node)
VAF_normal > VAF_tumor * 2 (otherwise)
VAF ≥ 2% | AD ≥ 10 | DP ≥50
remove any variant if present in gnomad > 0.005 MAF

final variant call set

* PON: Pool of normals. 300 blood samples from persons < 20 yrs old


Kelly Bolton


Ryan Ptashkin

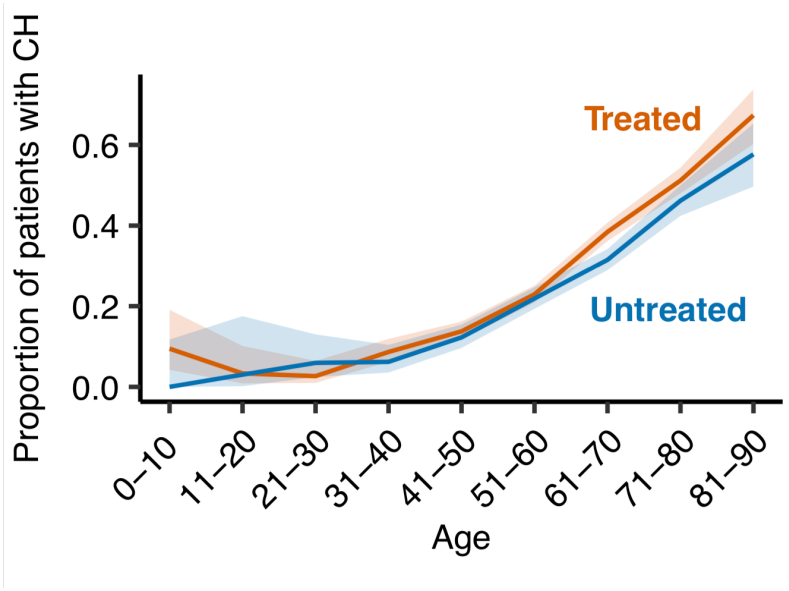

Teng Gao

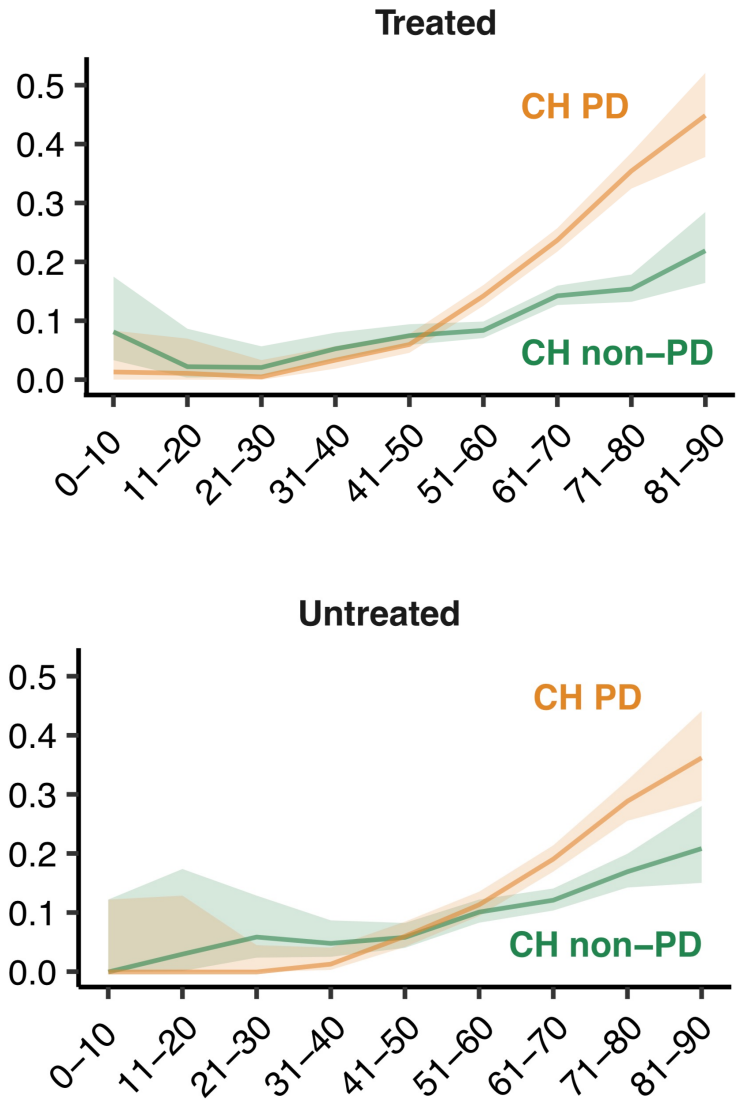

Elli Papaemmanuil

Bolton KL. et al., *Nat Genetics* 2020

Oncologic treatment affect CH rates across ages

CH in cancer driver genes (PD) shows the strongest selection relative to non-presumptive driver mutations

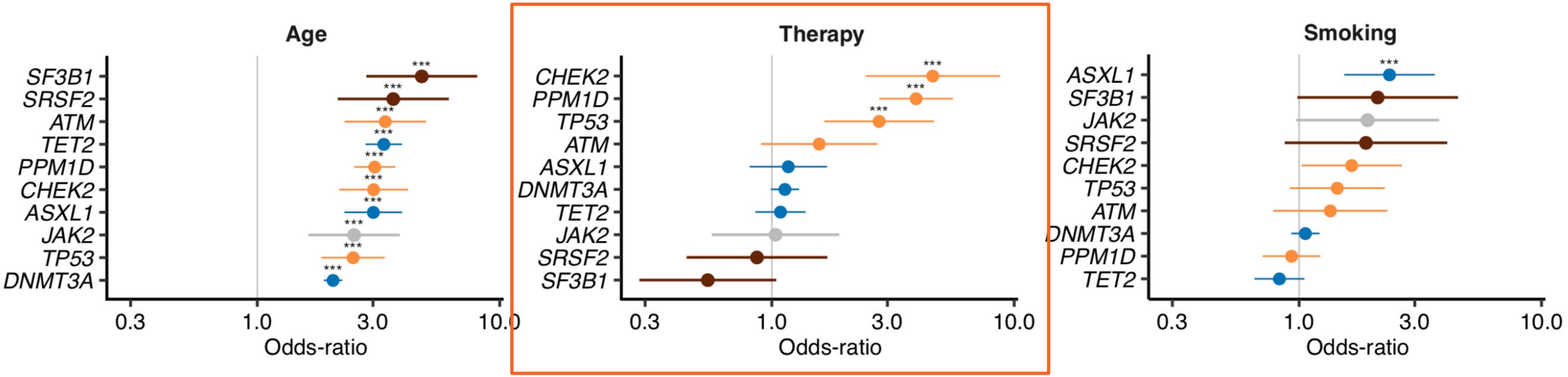




Bolton KL. et al., *Nat Genetics* 2020

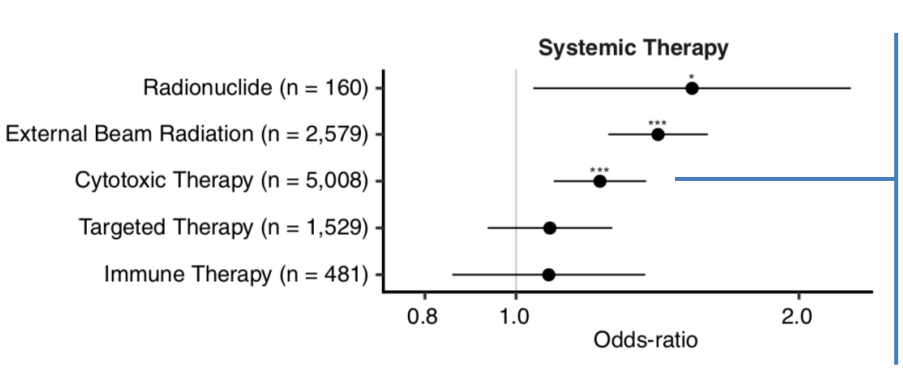
Individual genes associate differently with selective pressures

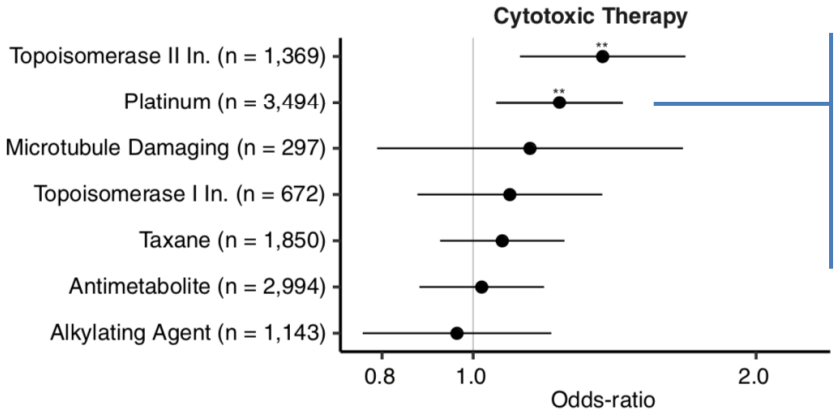
While aging is associated with CH across all genes, therapy is specifically associated with CHEK2, PPM1D and TP53 and smoking is associated with ASXL1

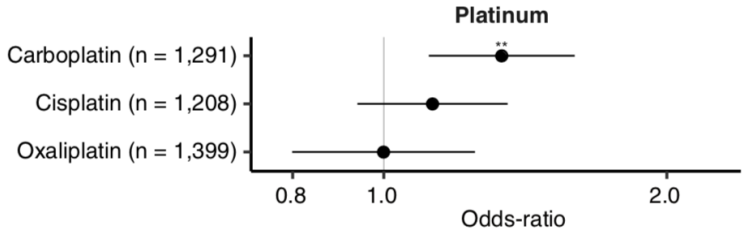


Bolton KL. et al., *Nat Genetics* 2020

CH-PD is associated with specific therapies



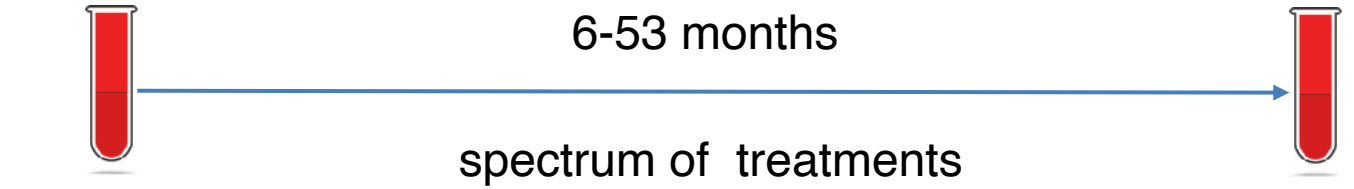




Multivariable logistic regression adjusted for age, gender, time from diagnosis to blood sequencing, race, all therapy subclasses

Bolton KL. et al., *Nat Genetics* 2020

Does ongoing treatment promote pre-existing CH or induce new mutations?

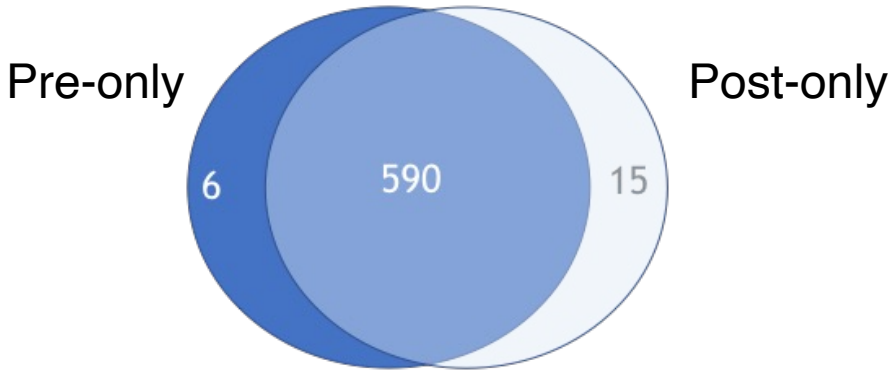


525 patients

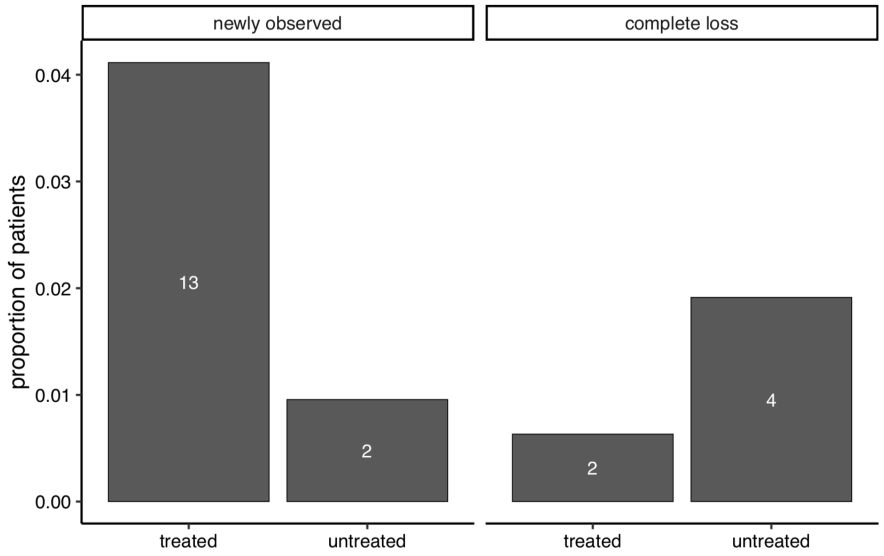
389 CH+

136 CH-

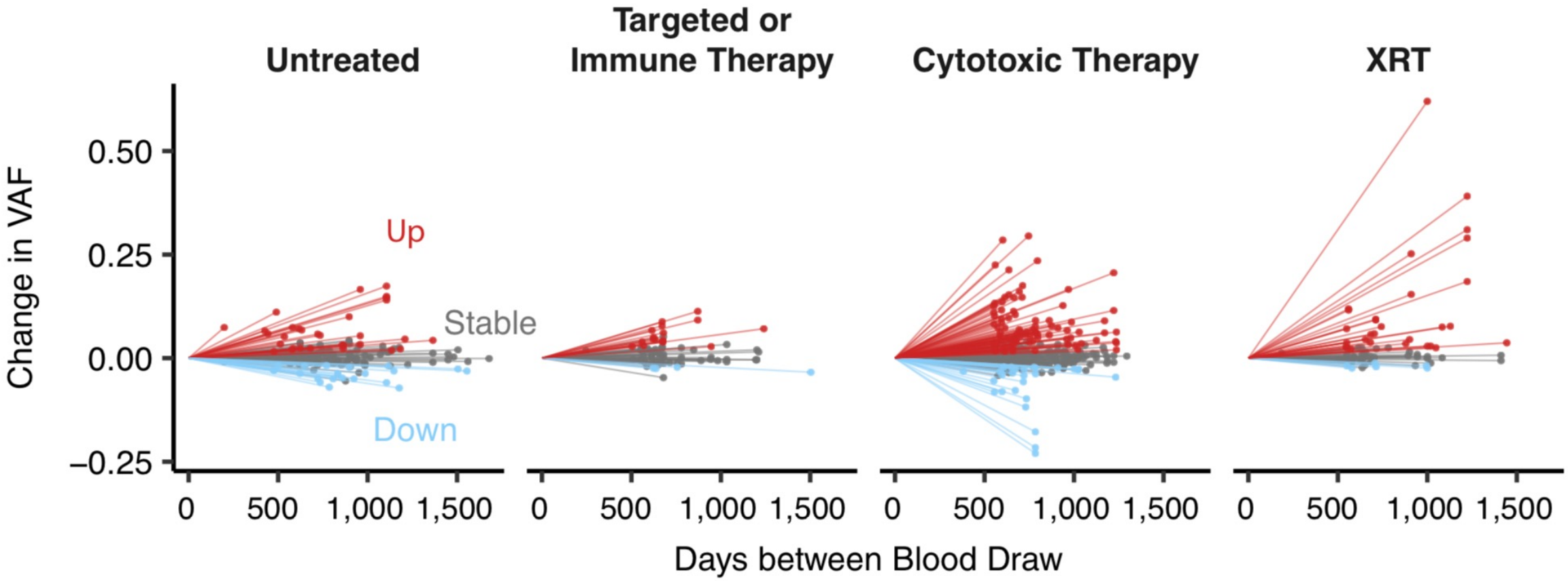
CH ?



95% of the CH variants are detected at both time points

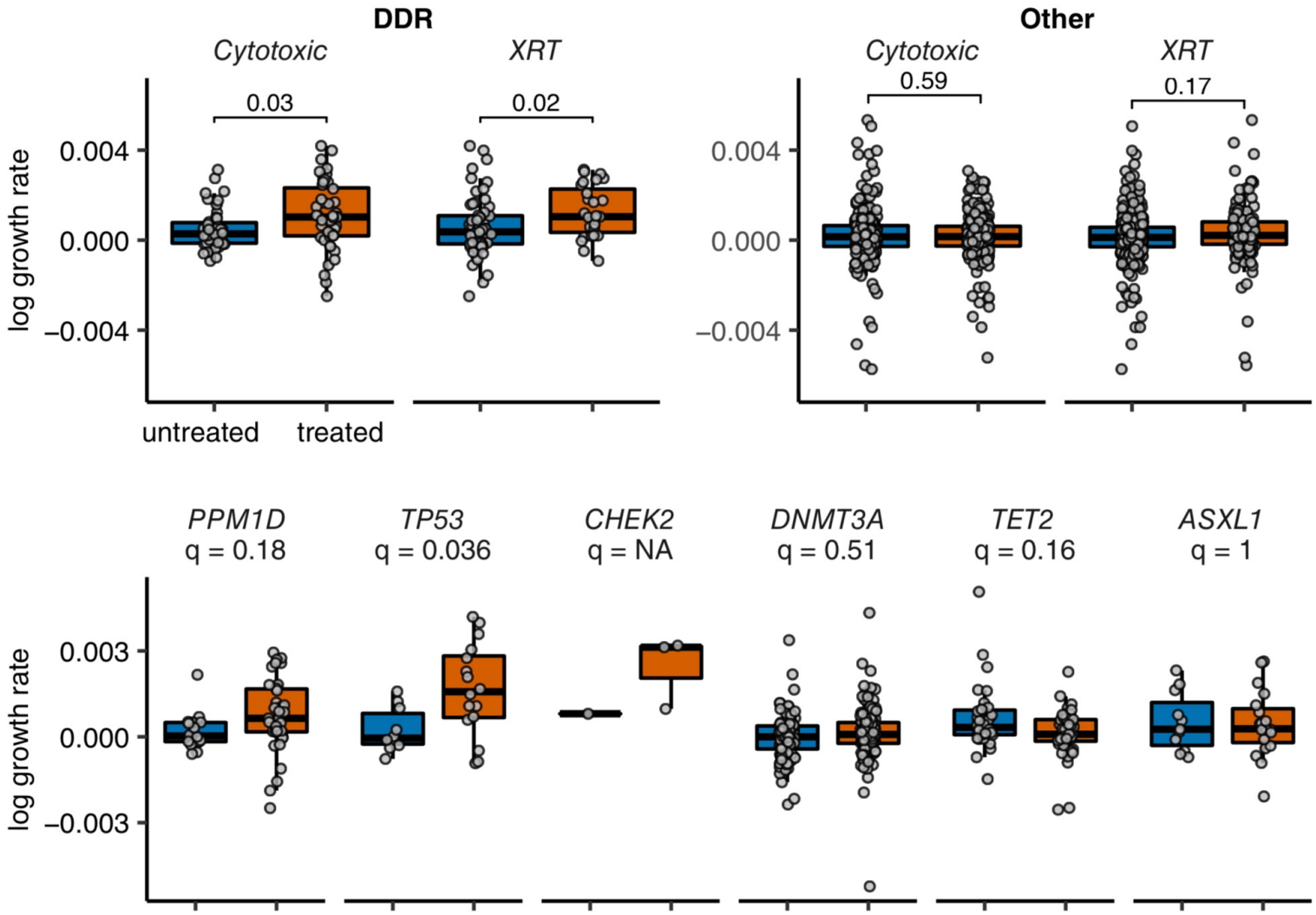


Cytotoxic therapy and radiation promote growth of existing CH clones



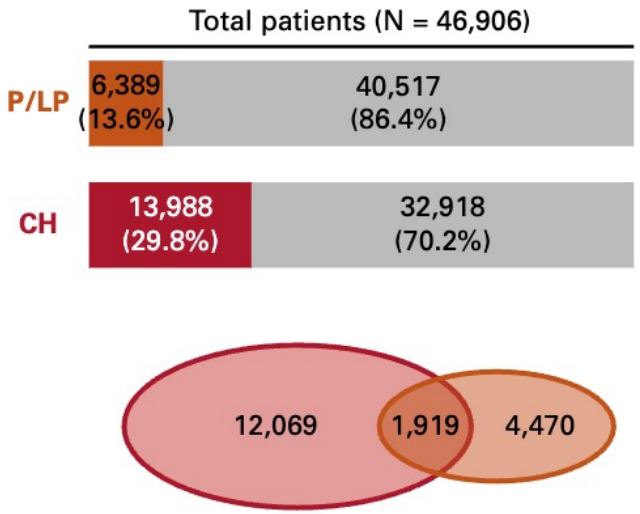
Cytotoxic therapy and radiation promote growth of existing CH clones

DDR pathway genes are specifically promoted

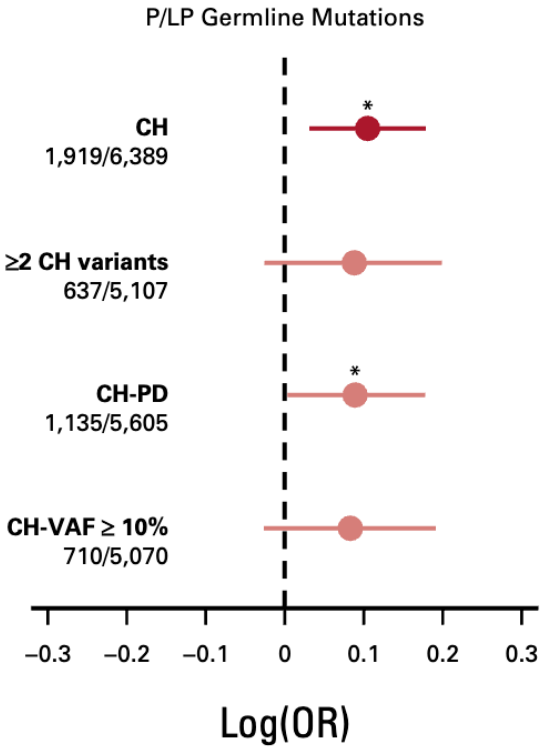


CH associations with inherited cancer predisposition mutations

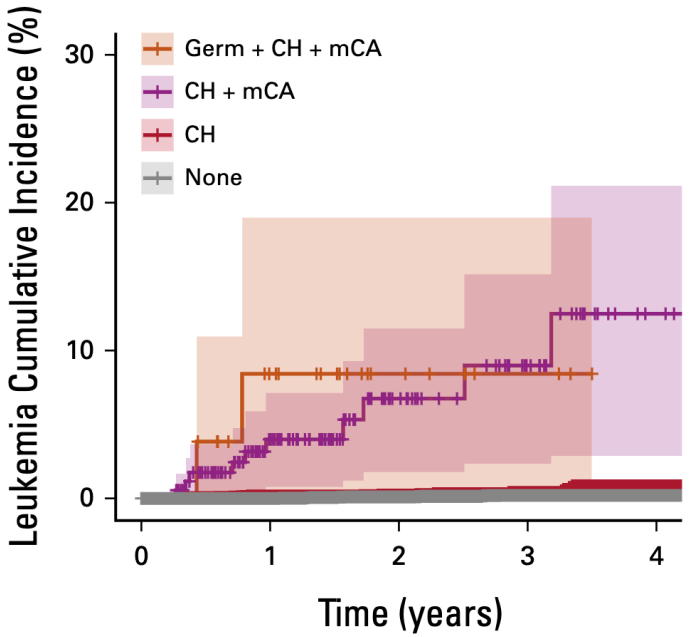
Cohort of 46,906 patients with CH and pathogenic/likely pathogenic germline mutations suggest additional population of patients who might need screening and monitoring



Amongst patients with P/LP and CH mutations, 10% ot patients have both



Inherited cancer predisposition mutations are associated with presence of CH and CH-PD

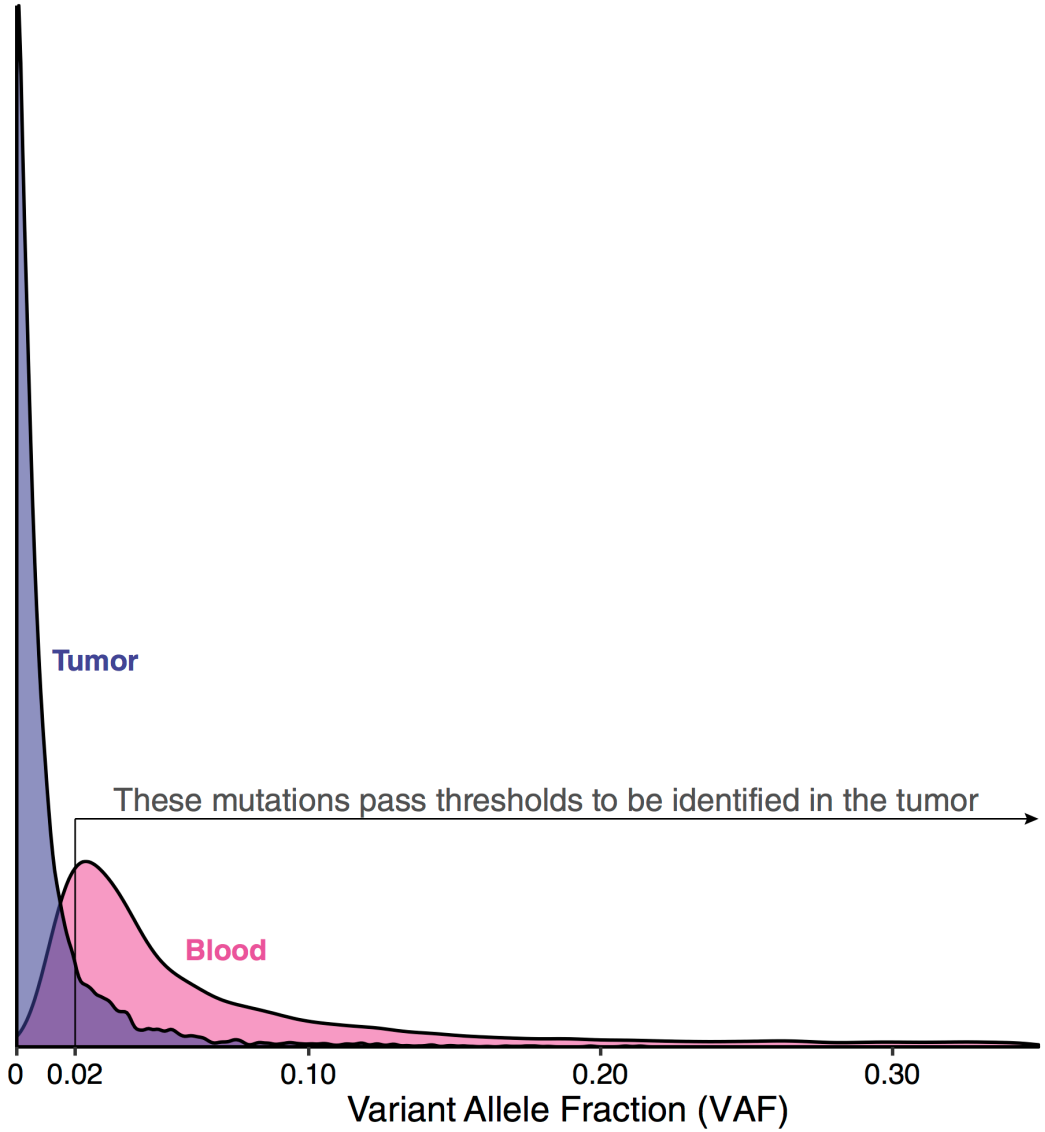


Slightly increased leukemia incidence for Germline + CH + mCA – unfortunately small numbers

What are the implications of CH mutations in solid tumor diagnostics?

Identification of CH related mutations in the solid tumors

26.5% of patients have CH in their blood



Total # of patients = 17,469

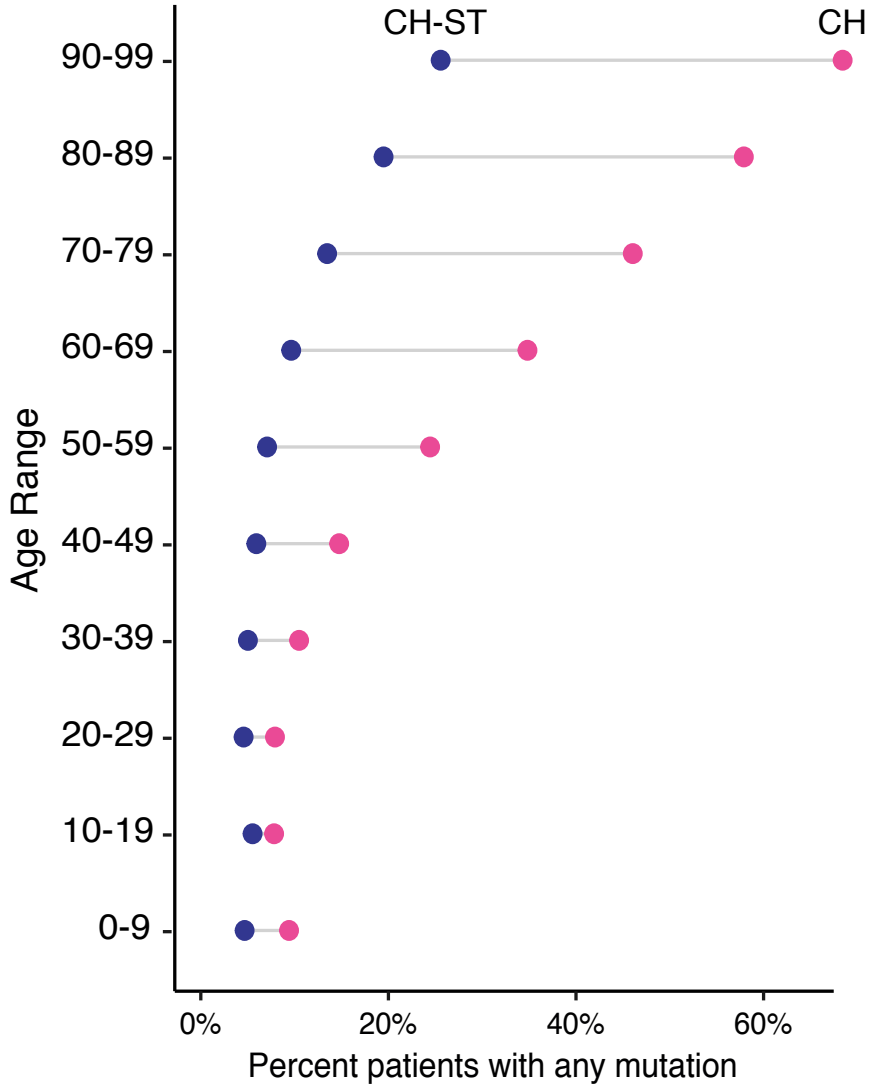
Identified 7,608 CH mutations in 4,628 patients (26.5%)

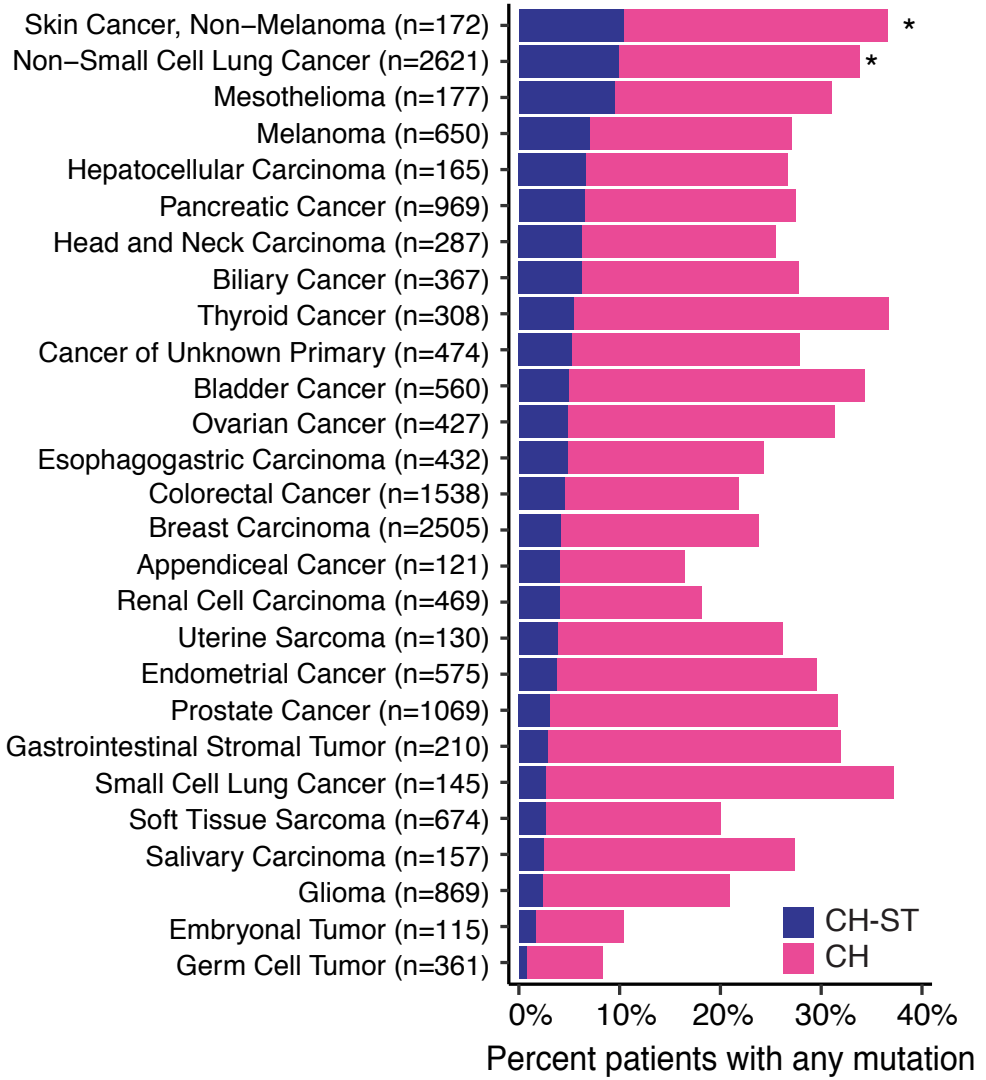
Identified 1,075 CH mutations in tumors of 912 patients (5.2%)

Ryan Ptashkin

CH mutations in solid tumors (CH-ST) is common

CH-ST incidence increase with age and varies by cancer type

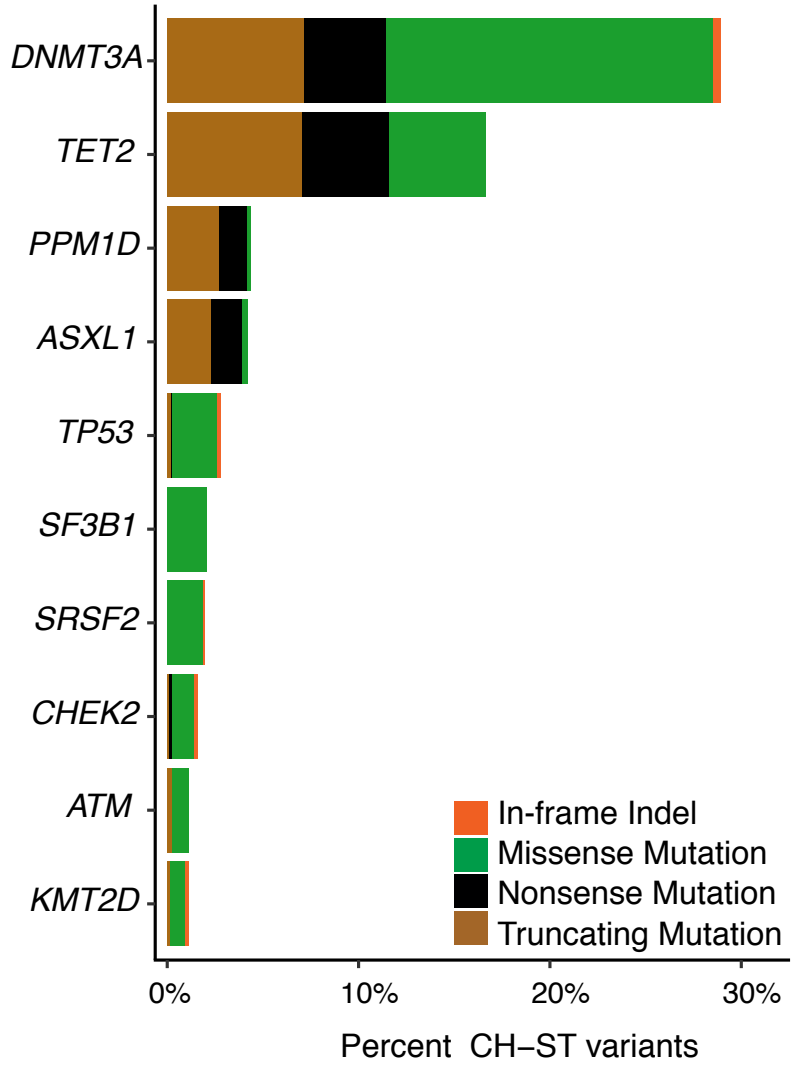


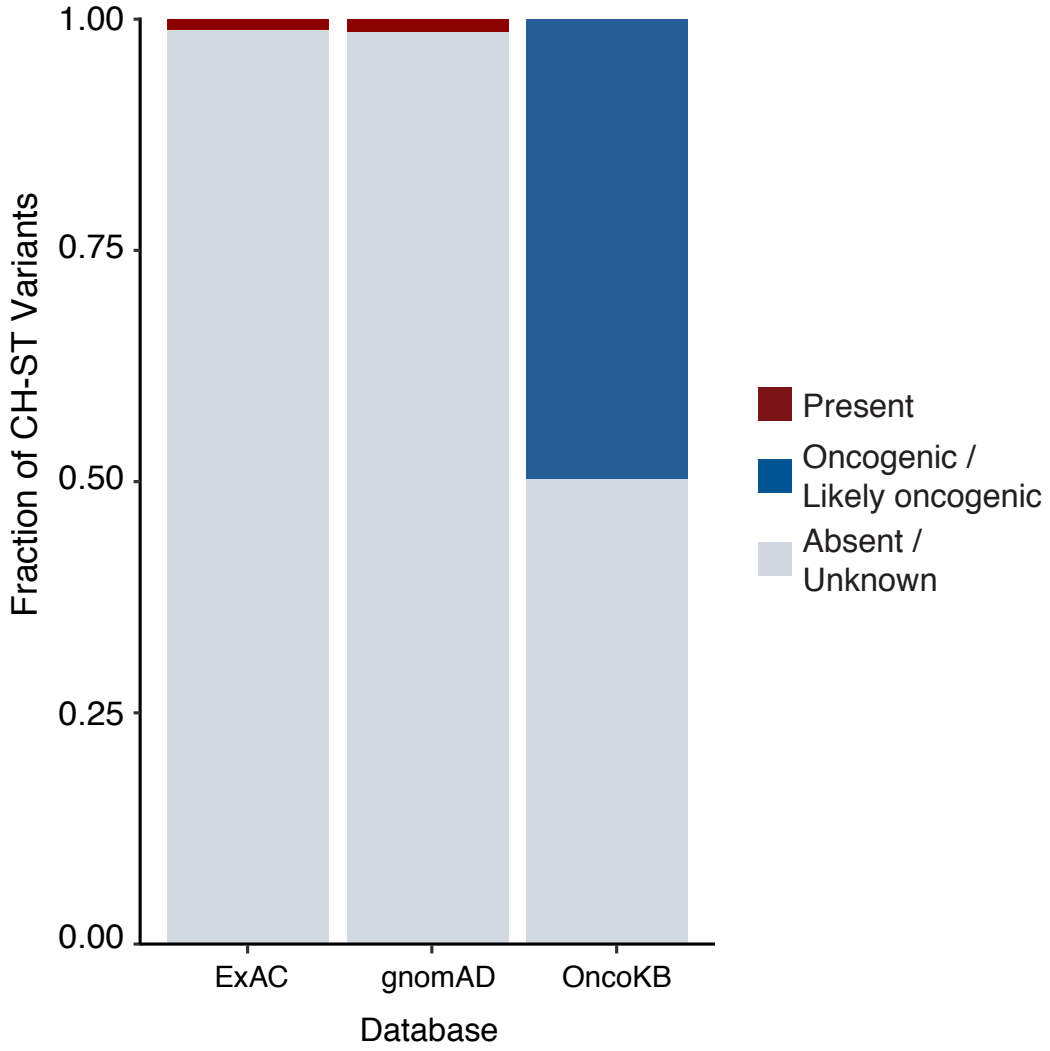


Ptashkin RN., *et al*, *Jama Oncology*, 2018

CH-ST mutations are absent in population databases

CH-ST are observed in 206 genes, mostly absent in population databases and half are oncogenic/likely oncogenic

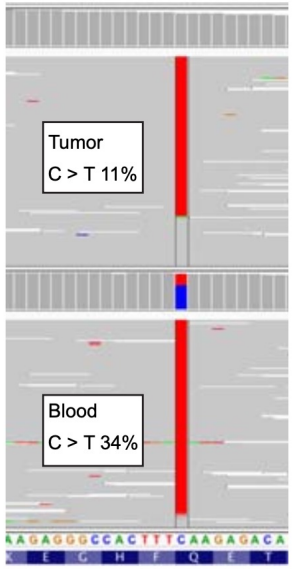




Ptashkin RN., *et al*, *Jama Oncology*, 2018

CHIP as a confounding factor for germline mutations

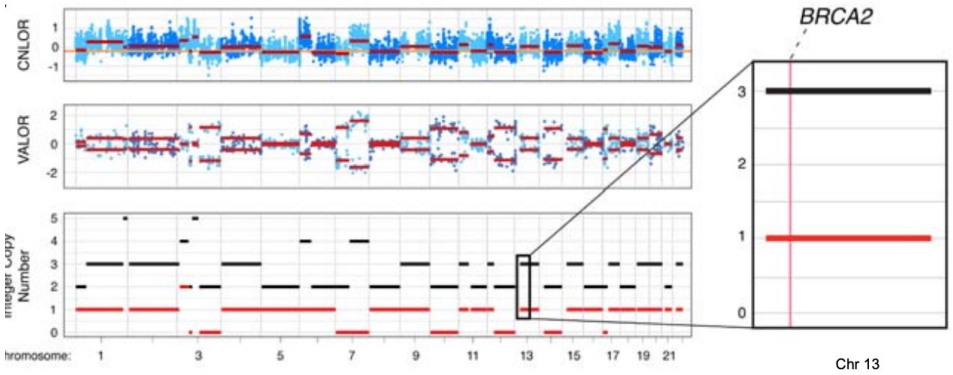
BRCA2 pathogenic variant with implications for PARPi therapy



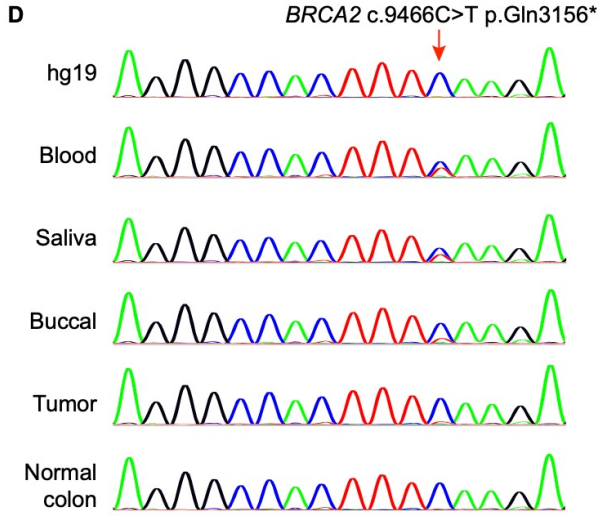
BRCA2
c.9466C>T p.Gln3156*

Looks somatic in tumor-only sequencing

Looks germline in blood-only sequencing



Tumor-blood data together suggests loss-of-heterozygosity in BRCA2 locus in tumor, however, both copies are present

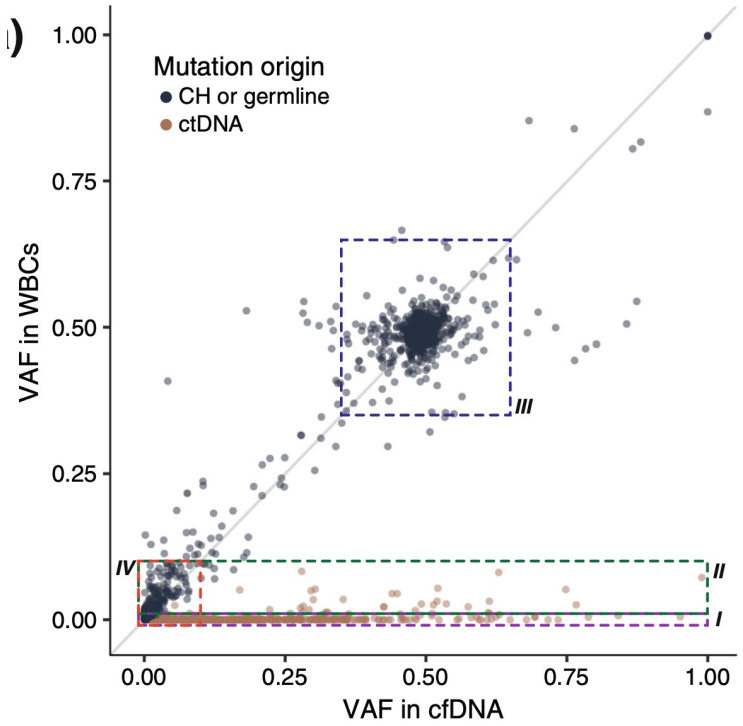


Sanger sequencing of different tissues show the mutation is a CH mutation

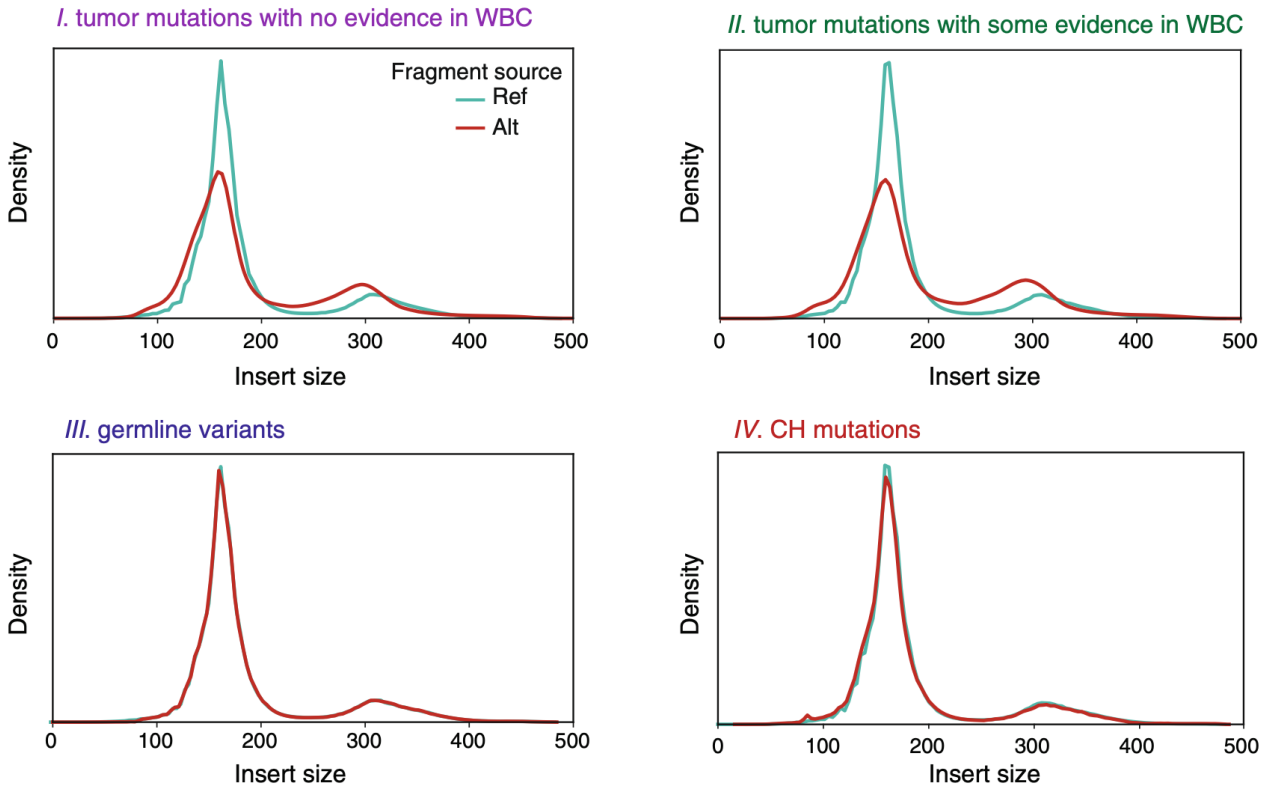
Ptashkin RN., *et al*, *Jama Oncology*, 2018

Presence of CH mutations in liquid biopsies

Identification of genomic alterations in cell-free tumor-derived DNA (ctDNA) by sequencing white blood cells



Analysis of 617 plasma & WBC pairs, using MSK-ACCESS, shows up to 77% of variants removed through use of WBC data (< 10% VAF)



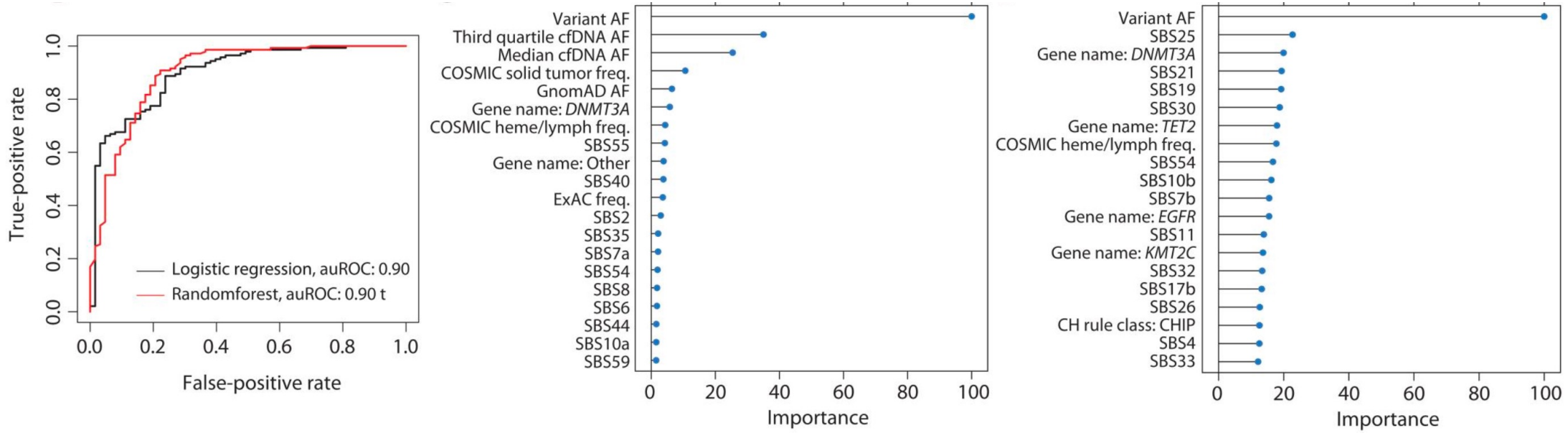
Fragments supporting tumor-derived mutations in plasma have shorter size distribution compared to the size of fragments supporting wild-type mutations.

Size distribution of fragments supporting germline and CH mutations do not differ from the background

Brannon AR, Jayakumaran G. *et al. Nature Comms*, 2021

Presence of CH mutations in liquid biopsies

Machine learning approach to differentiate CH derived mutations from tumor mutations

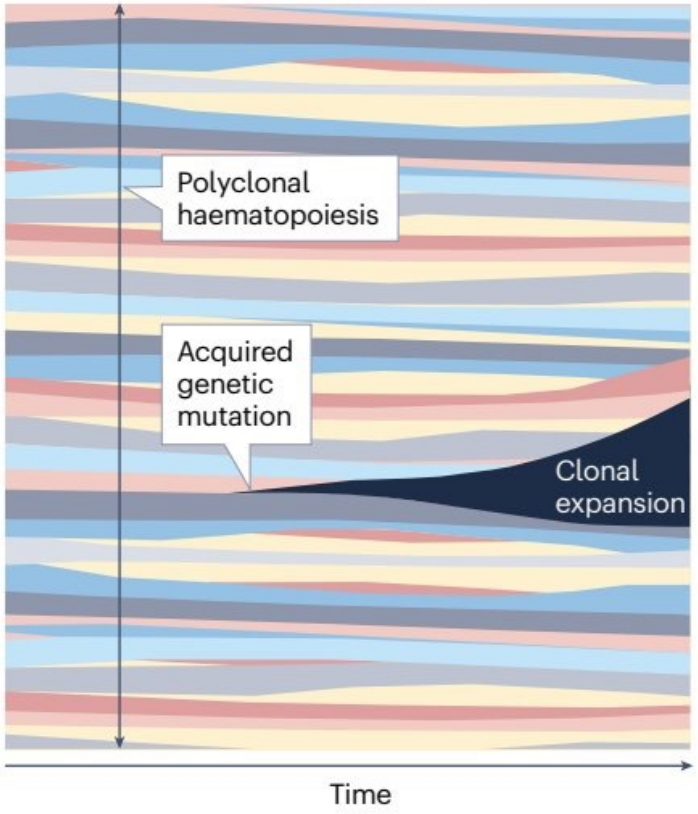


Variables for 1,400 SNVs with known CHIP/somatic status are used to train a logistic regression or random forest classifier. Feature importance suggest mutation VAF, gene name and signature are critical. Data lacks use of fragment size information

Fairchild L. *et al. STM,* 2023

Conclusions

CH mutations can be confounders in diagnostic assays without appropriate controls and have implications for solid tumor patients



i. noise for tumor-only sequencing (both ffpe & ctDNA)
ii. noise for blood-only germline sequencing

iii. signal for selecting patients at high-risk for hematological malignancies

Remaining questions for the community

1) Can we use CH as a biomarker to identify high-risk cancer patients who might develop secondary malignancies? How would this look in practice? Can we treat these patients prophylactically?

2) What is the most optimal way to identify and remove CHIP derived noise in liquid biopsies in the absence of white blood cell sequencing?

3) Does presence of CH mutations lead to epigenetic changes thus confound methylation based liquid biopsy assays?

4) What is the extend of CHIP confounding in the setting of MRD?