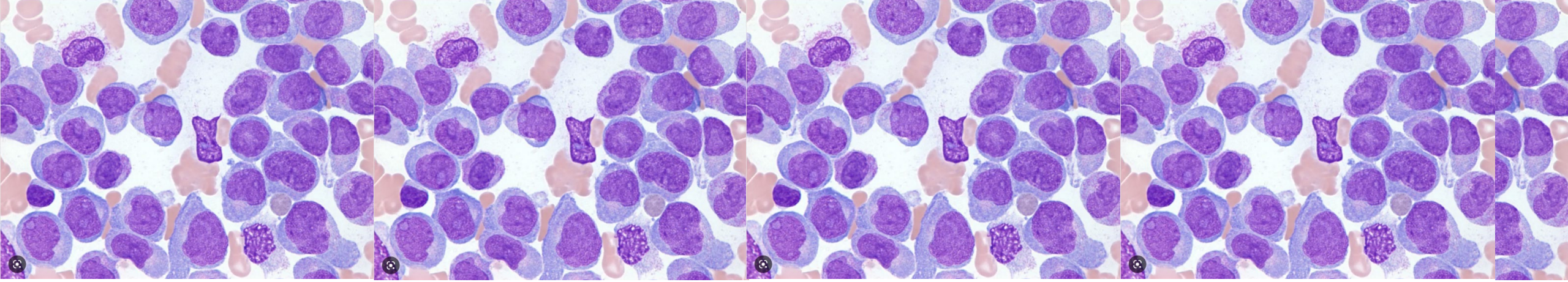


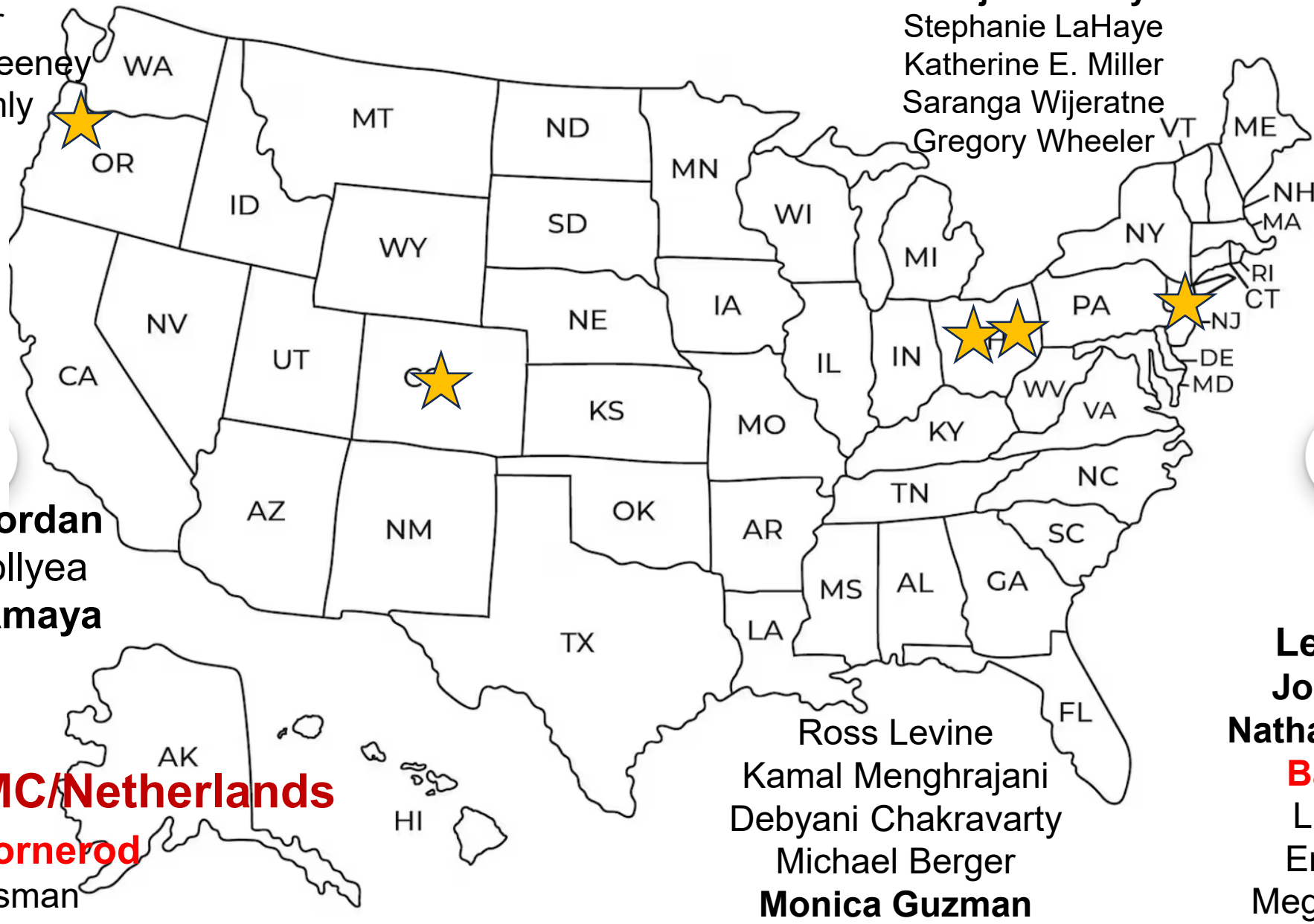
Genomic approaches to enable equitable care and precision medicine for patients with acute myeloid leukemia

Ann-Kathrin Eisfeld, MD

Associate Professor

Director, Clara D. Bloomfield Center for Leukemia Outcomes Research

The Ohio State University



Jeff Tyner
Shannon McWeeney
Dan Bottomly

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Maarten Fornerod
Lynn Aasman

Elaine Mardis
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Stephanie LaHaye
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Gregory Wheeler

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Bailee Kain
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Megan Johnston
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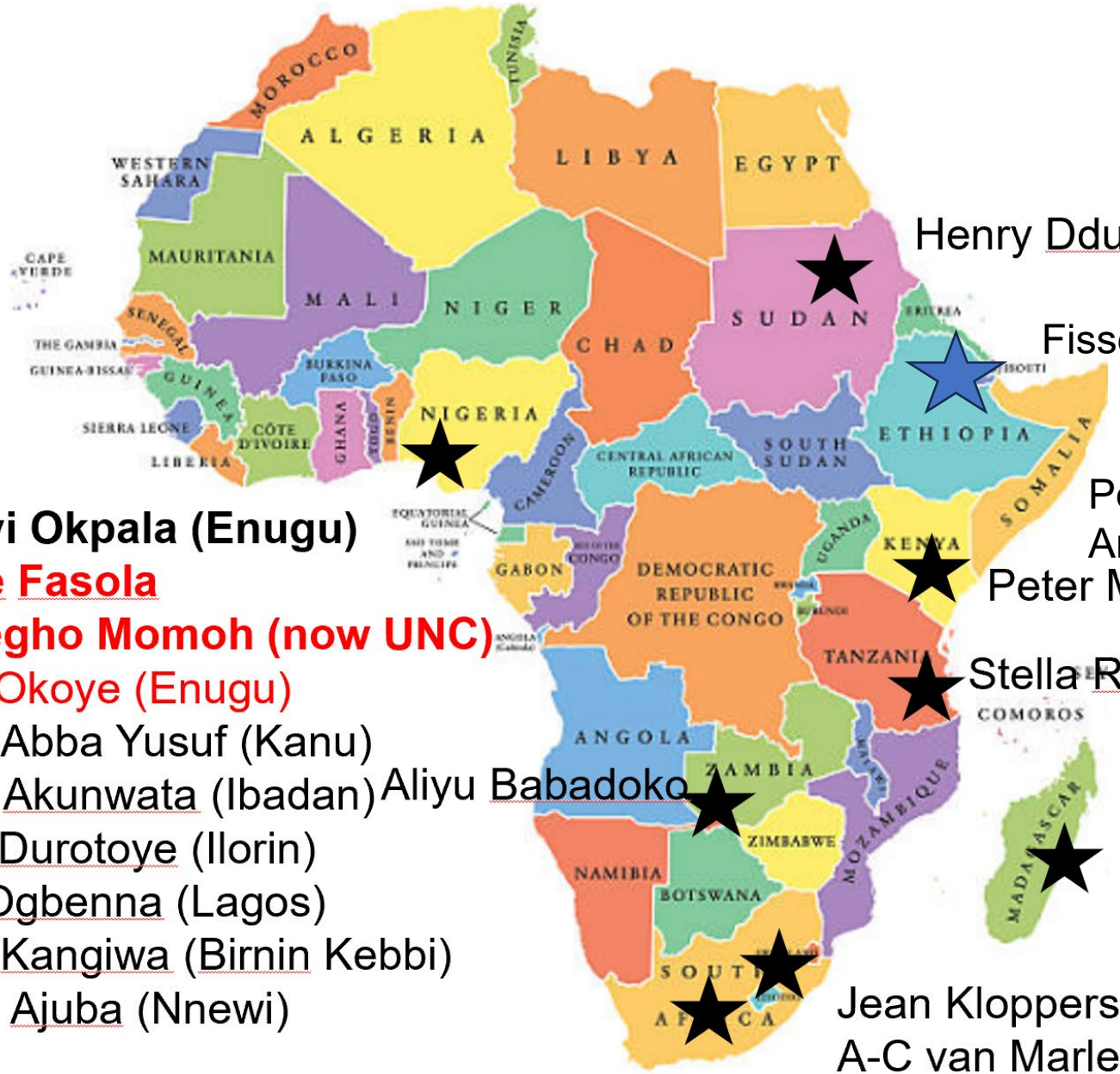

Henry Ddungu

Fisseha Tadesse*

Peter Omondi*
Anne Mwirigi

Peter Mwamba

Stella Rwezaura

Olivat Rakoto Alson

Jean Kloppers
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Chima Akunwata (Ibadan)
Idayat Durotoye (Ilorin)
Anna Ogbenna (Lagos)
Garba Kangiwa (Birnin Kebbi)
Ifeoma Ajuba (Nnewi)

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*via Rob Baiocchi/
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Rosana Pelayo
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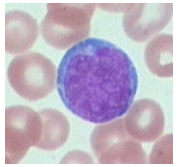
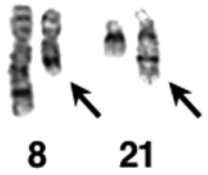
Acute myeloid leukemia (AML) disease classification

based on:

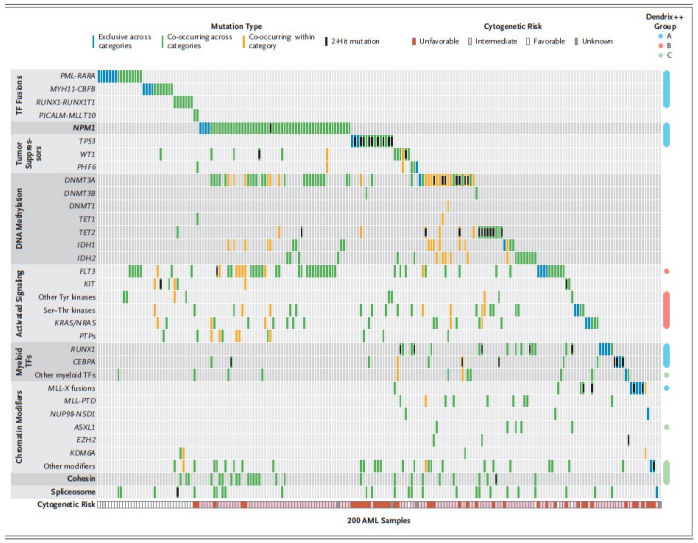
- the morphology of the leukemic blasts and associated dysplasia,



- specific chromosomal abnormalities



- recurrent gene mutations



European LeukemiaNet 2022 AML Genetic Risk Groups

FAVORABLE	INTERMEDIATE	ADVERSE
➢ t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* ➢ inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* ➢ Mutated *NPM1* without *FLT3*-ITD ➢ bZIP in-frame mutated *CEBPA*	➢ Mutated *NPM1* with *FLT3*-ITD ➢ Wild-type *NPM1* without *FLT3*-ITD (without adverse-risk genetic lesions) ➢ t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡ ➢ Cytogenetic abnormalities not classified as favorable or adverse	➢ t(6;9)(p23;q34.1); *DEK-NUP214* ➢ t(v;11q23.3); *KMT2A* rearranged ➢ t(9;22)(q34.1;q11.2); *BCR-ABL1* ➢ t(8;16); *KAT6A/CREBBP* ➢ inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1) ➢ -5 or del(5q); -7; -17/abn(17p) ➢ Complex karyotype,§ monosomal karyotype‖ ➢ Mutated *RUNX1*¶, *ASXL1, EZH2, BCOR, SF3B1, SRSF2, U2AF1, ZRSR2*¶ ➢ Mutated *TP53*#

Döhner H, et al. Blood. 2022;129:424-447.

* ELN: European LeukemiaNet

Acute myeloid leukemia (AML)
disease classification- how did we come up with this?

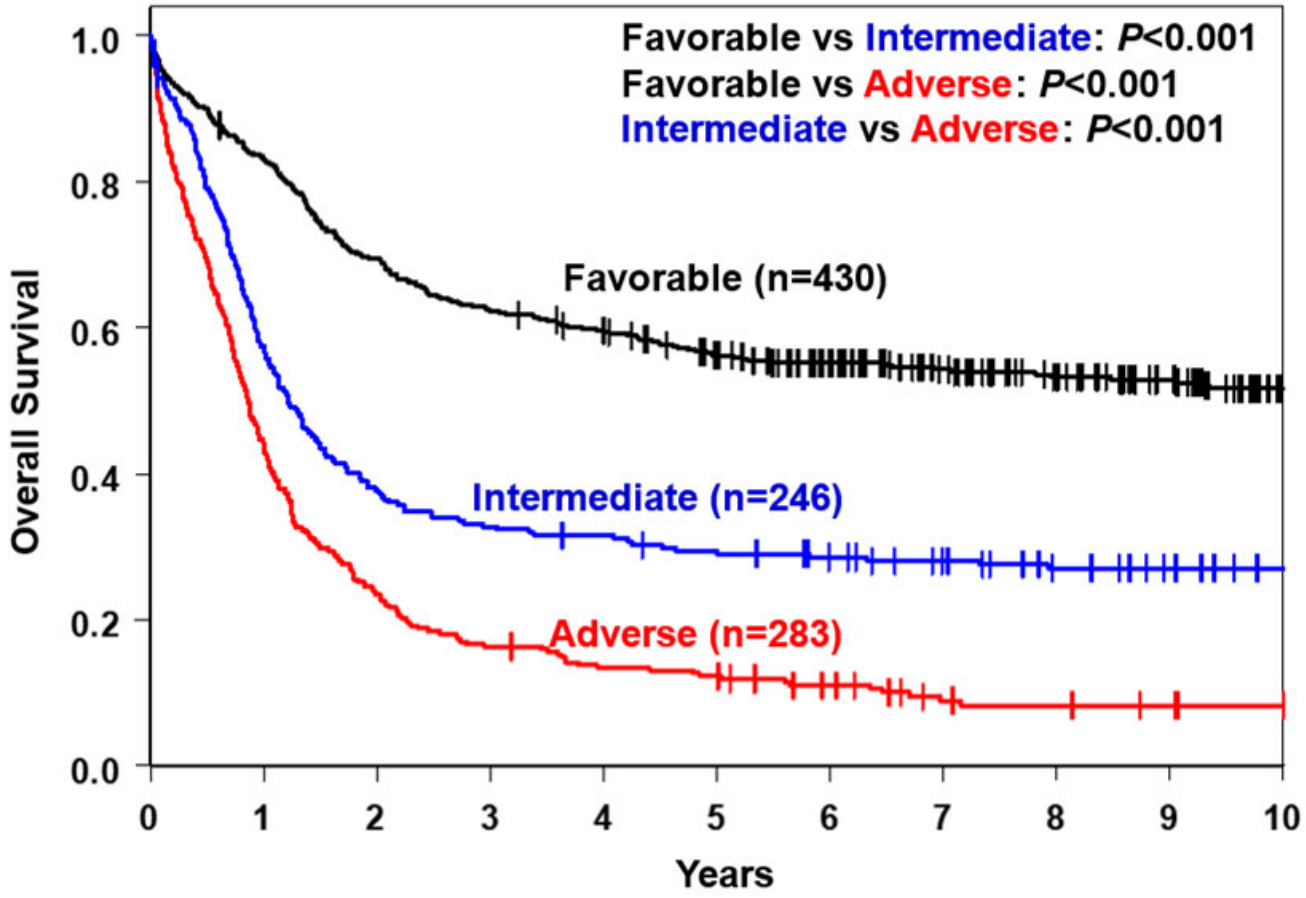
Patient presenting to CCC

Patient enrolling into biobanking (and clinical trial?)

Treatment (and biobanking) site large enough to contribute to pivotal studies

Resulting manuscripts informing ELN/NCCN/(..)

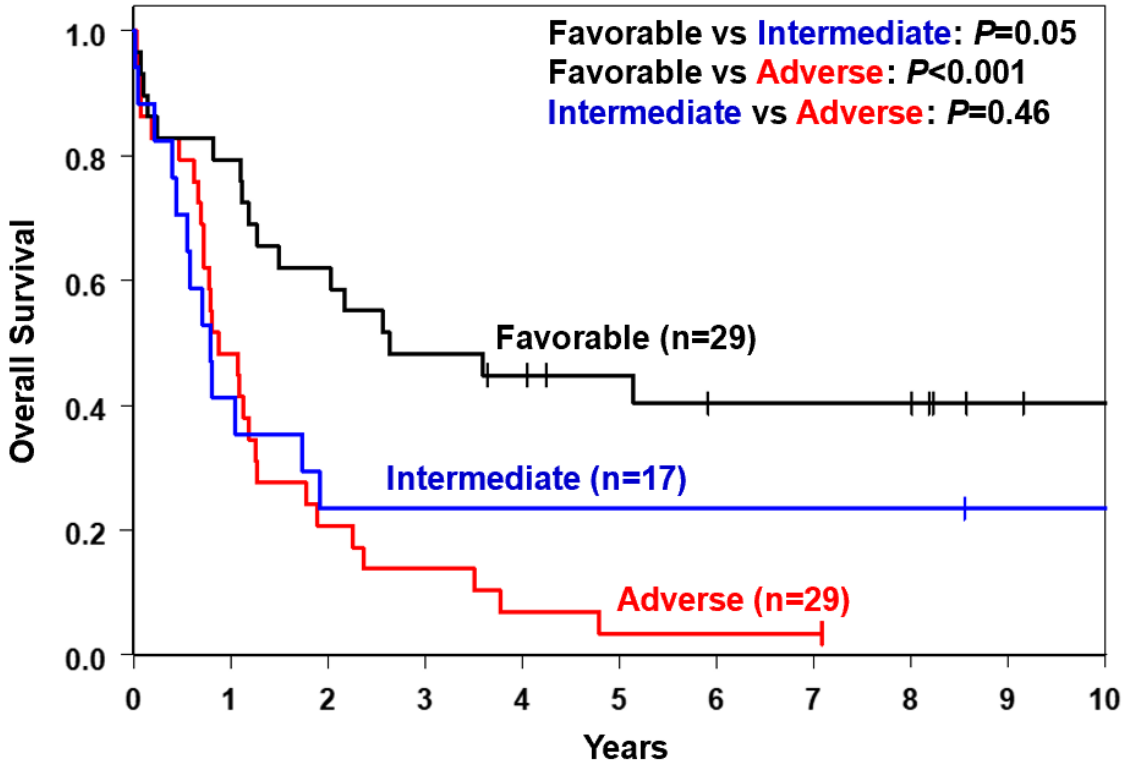
Overall survival of adult AML patients <60 years based on European LeukemiaNet 2022 AML genetic risk classification



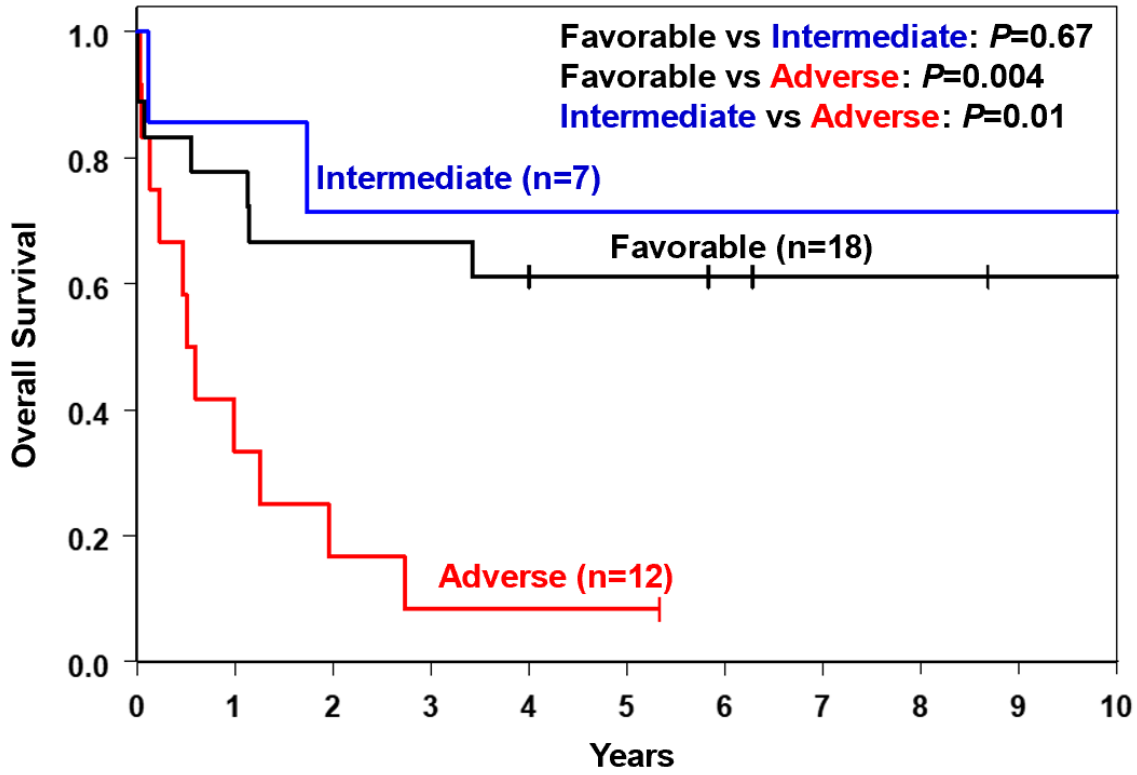
Mrozek K, *et al*.. Leukemia 2023, 32

Overall survival of adult AML patients <60 years based on European LeukemiaNet 2022 AML genetic risk classification

African-American patients

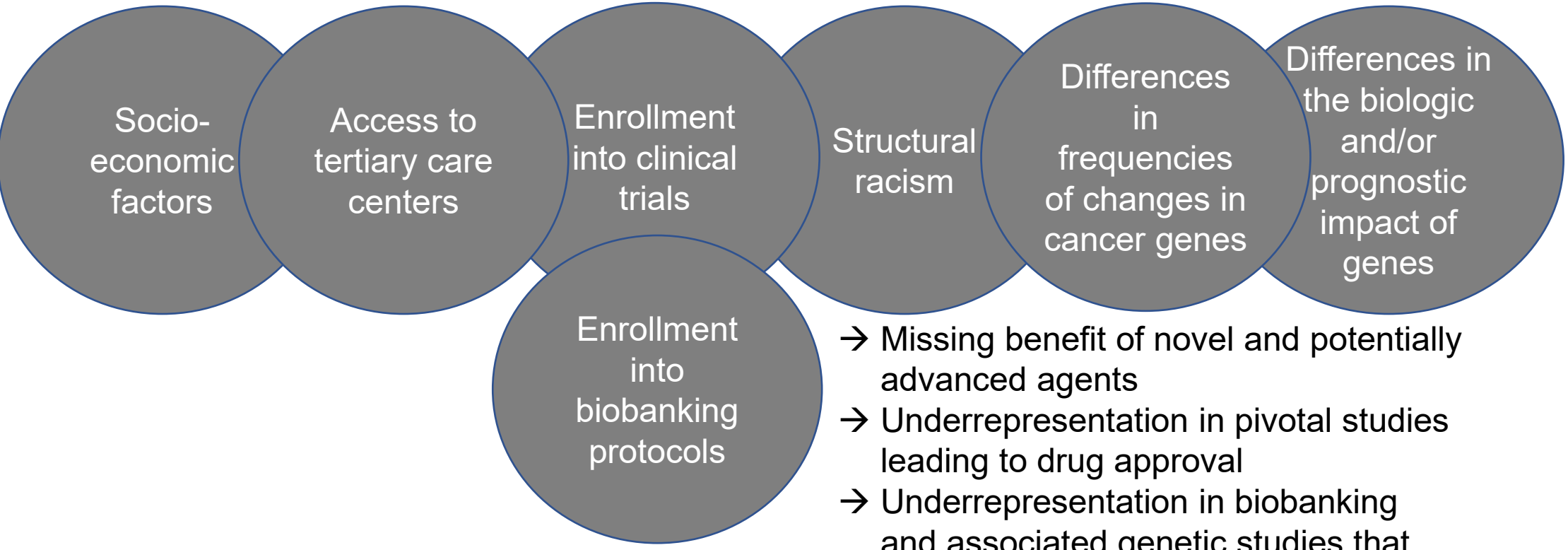


Hispanic patients



Mrozek K, *et al*.. Leukemia 2023, 32(6):in press

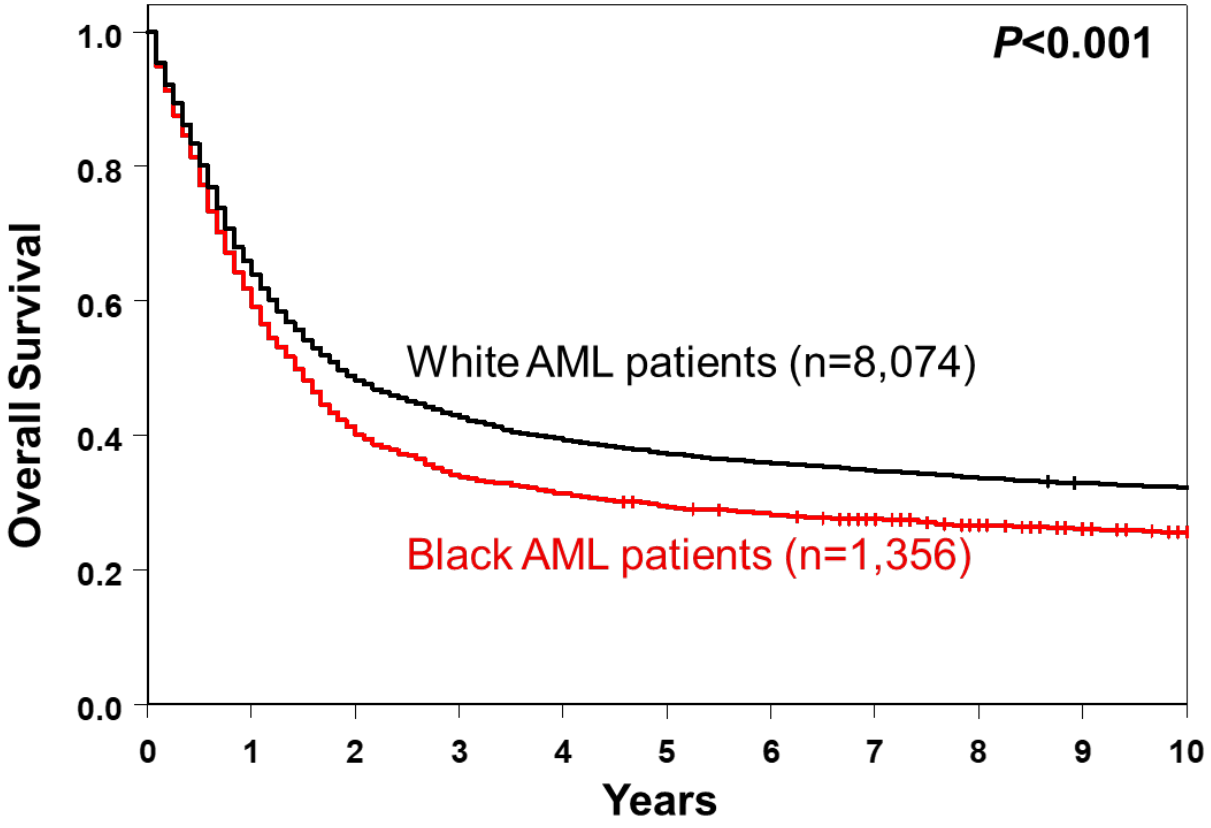
CONTRIBUTING FACTORS FOR RACIAL DISPARITIES IN TREATMENT OUTCOMES ARE MULTIFACTORIAL AND INTERCONNECTED



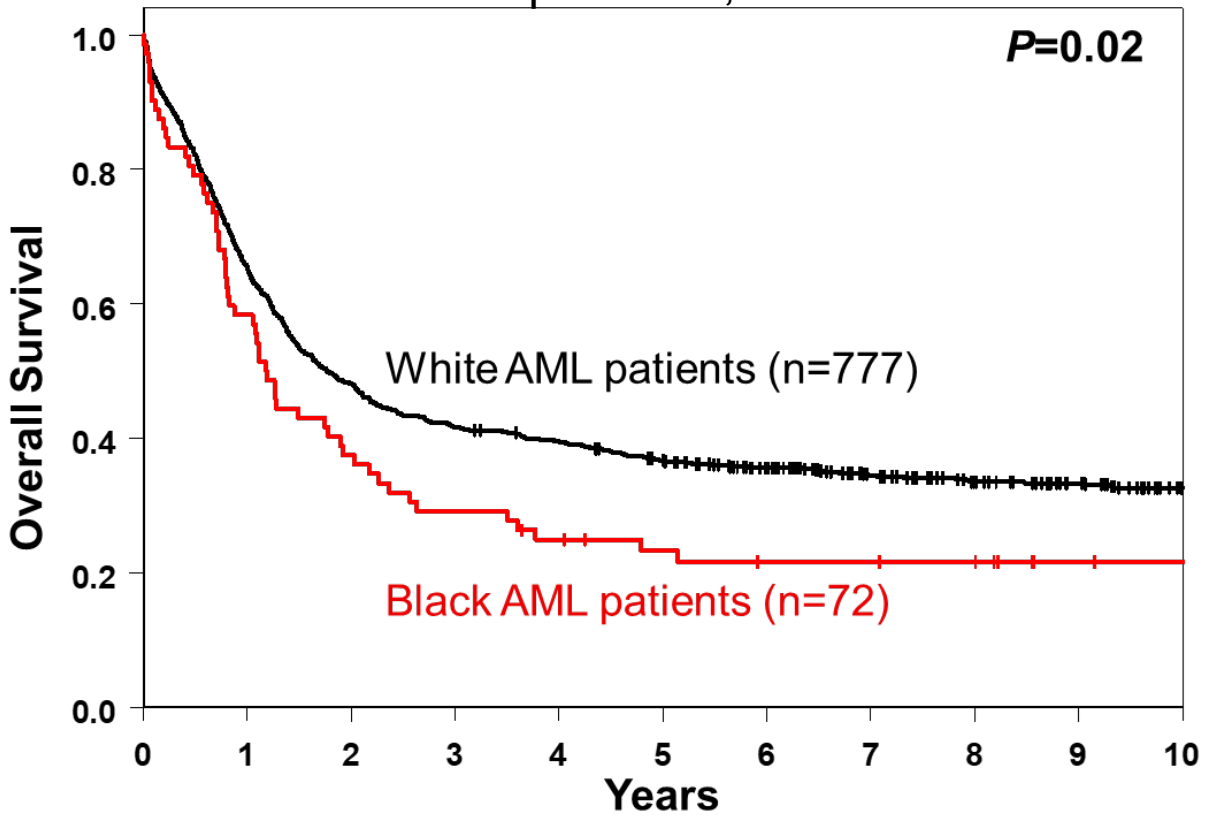
→ Missing benefit of novel and potentially advanced agents
→ Underrepresentation in pivotal studies leading to drug approval
→ Underrepresentation in biobanking and associated genetic studies that inform disease knowledge

KEY FINDING 1: Black AML patients have poor survival outcomes compared to White patients

Overall survival of AML patients in SEER, 1986-2015



Overall survival of AML patients treated on Alliance frontline protocols, 1986-2016

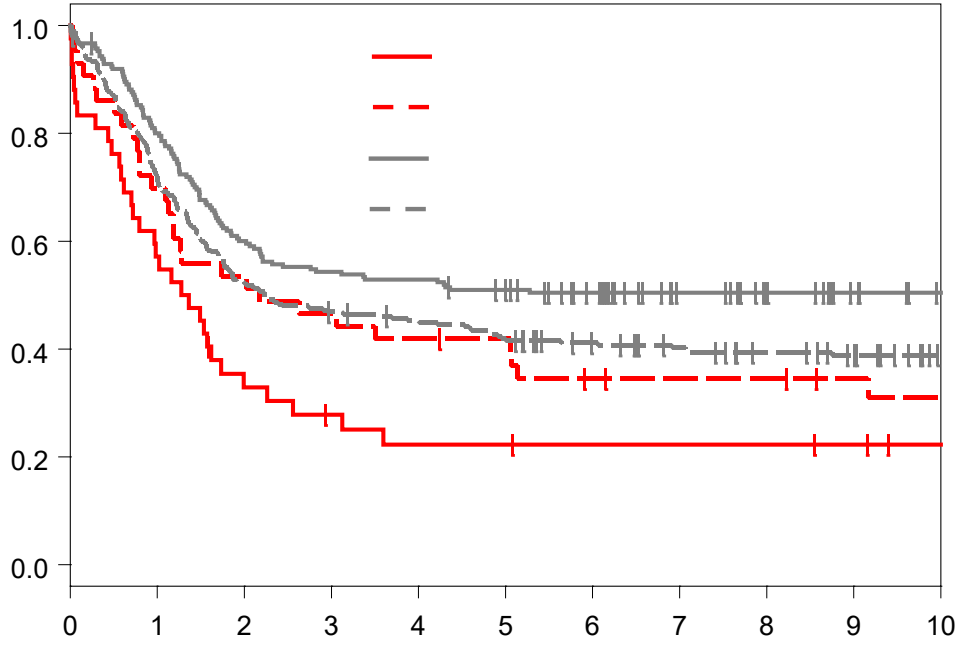


Bhatnagar B et al, *Cancer Discovery* 2021; 11:540–1

Key finding 2: The survival disparity also exists in very young patients treated on clinical trials

18-29 years			
Outcome end point	**Black patients n=44**	**White patients n=252**	***P***
Early death, %	16	3	**0.002**
Complete remission, %	66	83	**0.01**

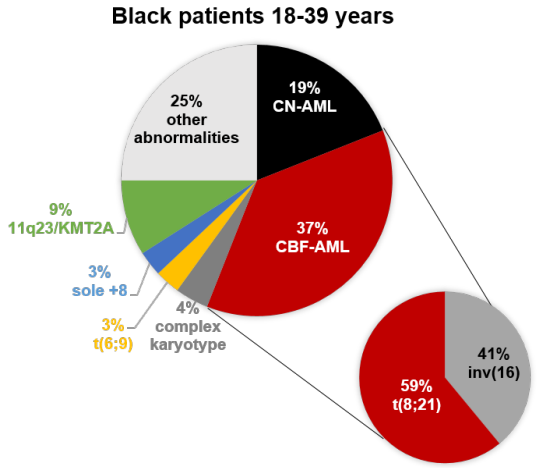
	18-29 years		30-39 years	
	Black patients	**White patients**	**Black patients**	**White patients**
OS Median (years)	**1.3**	**10.2**	2.2	2.2

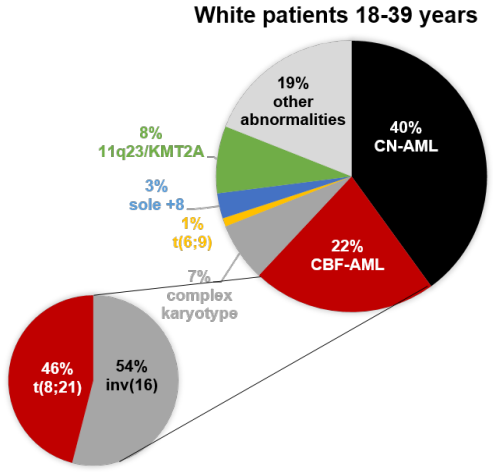


Larkin K et al, *Blood Advances 2022*

KEY FINDING 3: Differences in frequencies of established molecular features exist between Black and White AML patients

Gene mutation	Black, %	White, %	*P*
NPM1	25	38	0.04
WT1	3	10	0.05
IDH2	17	8	0.03





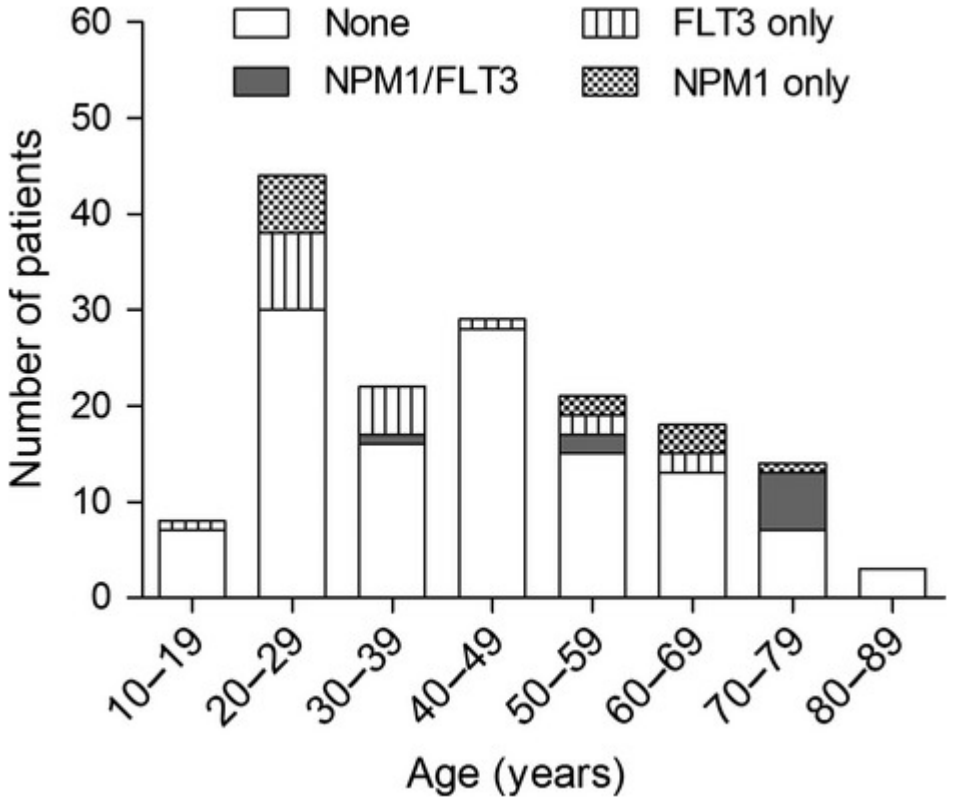
Bhatnagar B et al, *Cancer Discovery* 2021; 11:540–1

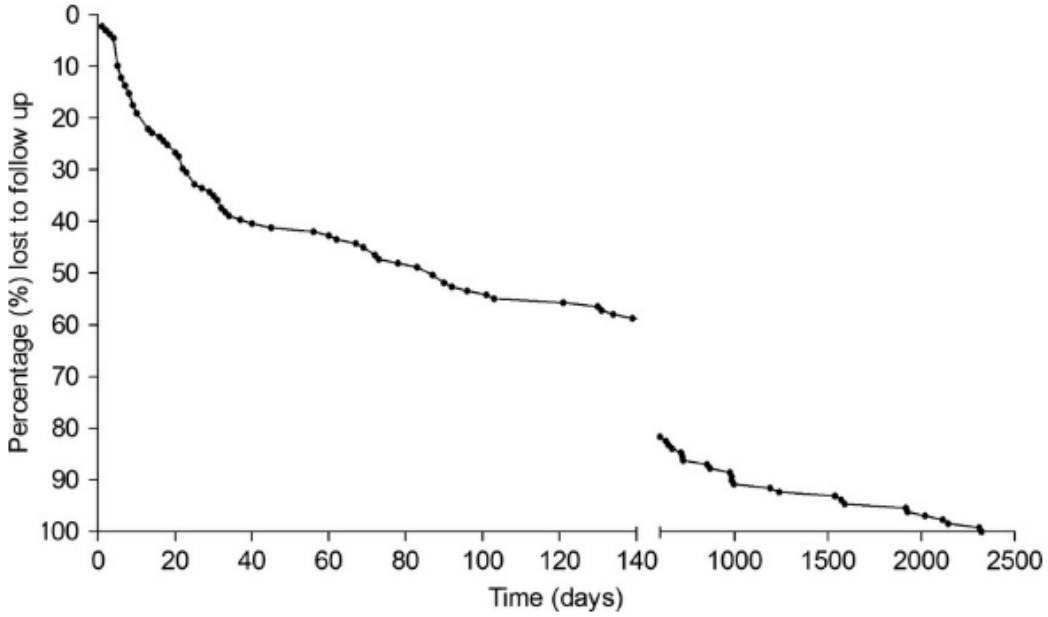
Larkin K et al, *Blood Advances 2022*

KEY FINDING 3: Differences in frequencies of established molecular features exist between Black and White AML patients

→ Profiling of n=160 *de novo* AML patients seen in Central South Africa for FLT3-ITD and *NPM1* mutations

→ *NPM1* mutations only seen in 7.5% of patients

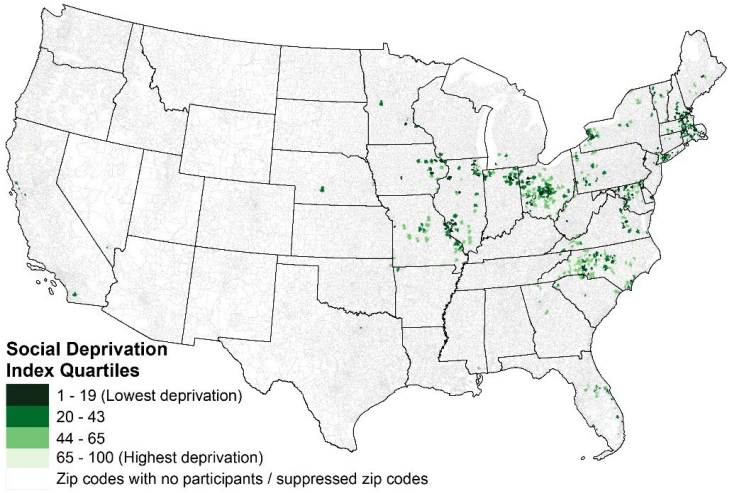




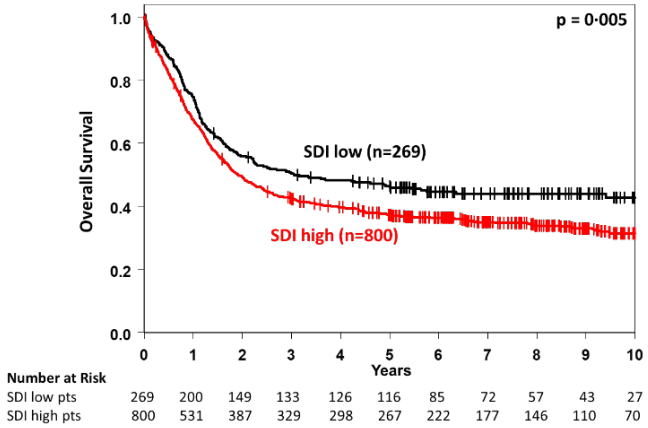
Marshall RC et al, *Int J Lab Hematology* 2014

Key finding 4: Socioeconomic features impact on survival but race remains an independently impactful

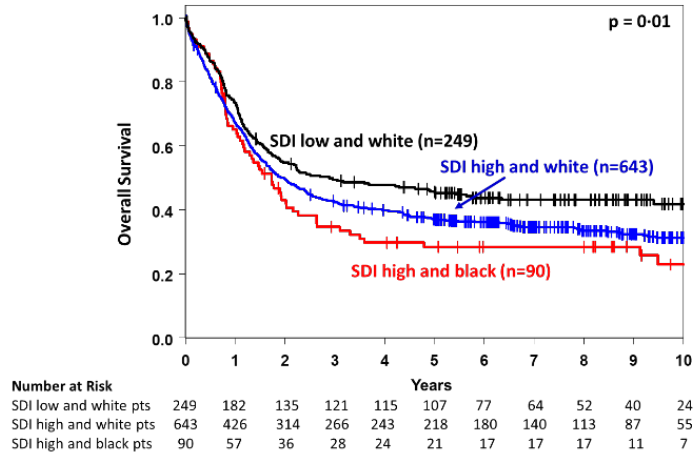
SDI assignment



Overall survival based on SDI



Overall survival based on SDI ~~and race~~

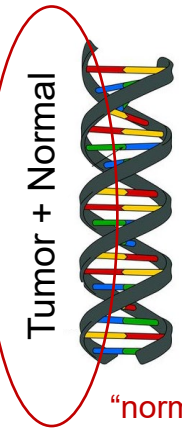


Variable	Categories	p-value	Hazard ratio (95% CI)
SDI score	More than 50 *v* less than 50	.005	1.33 (1.09, 1.64)
ELN			
	Intermediate *v* Favorable	<.001	2.48 (1.90, 3.24)
	Adverse *v* Favorable	<.001	3.56 (2.79, 4.54)

Rebechi et al, *Blood Advances* 2023; in press

THE "AFRICAN-AMERICAN AML PROJECT"- with the IGM/Elaine Mardis

- → 100 African-American AML* patients, with genotype-confirmed ancestry
- → (*hereafter referred to as Black patients)
- → similarly treated with intensive induction chemotherapy on CALGB/Alliance frontline protocols

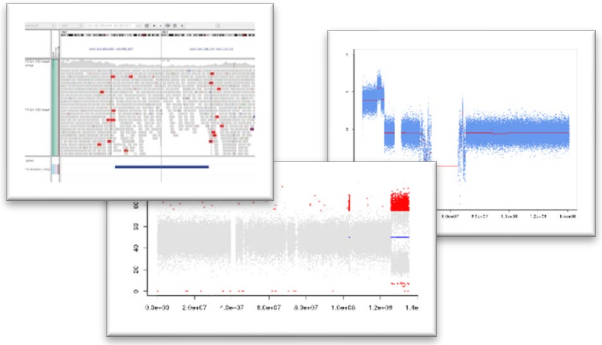


eWES

250x

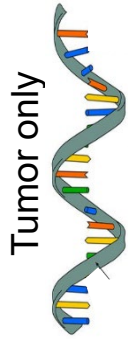
Churchill alignment*

"normal"= flow-sorted B/T cells



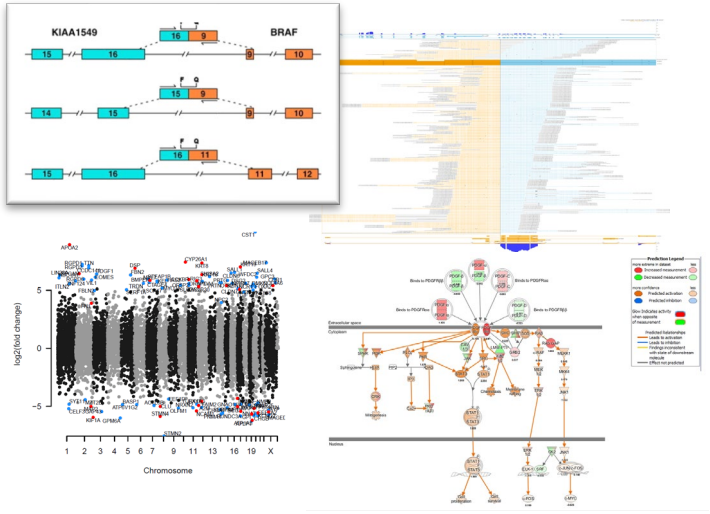
Germline + Somatic

SNP, INDEL, SV, CNV, LOH



RNA-Seq

DESeq
Salmon
PCA



Fusion Detection With Ensemble Approach**

Variant Calling

Outlier Expression
TME/deconvolution
ssGSEA
PCA-based Diagnosis

* Kelly B.J. et al., *Genome Biol.* 2015

** LaHaye et al., *BMC Genomics* 2021

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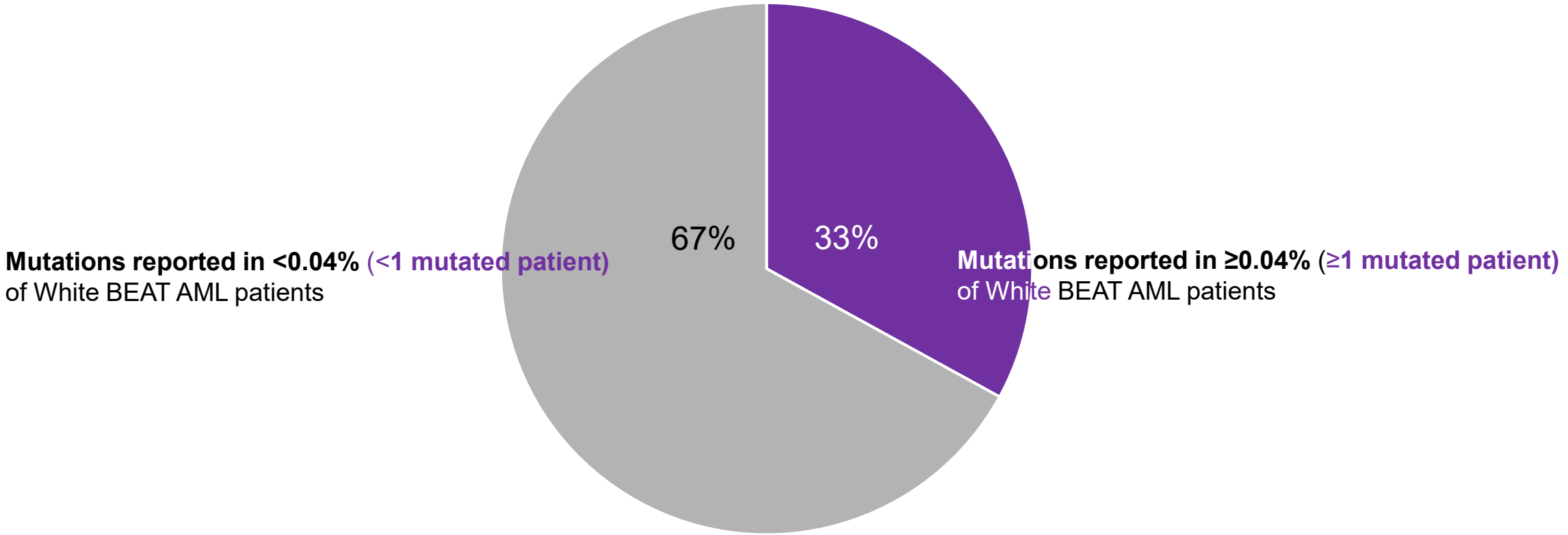
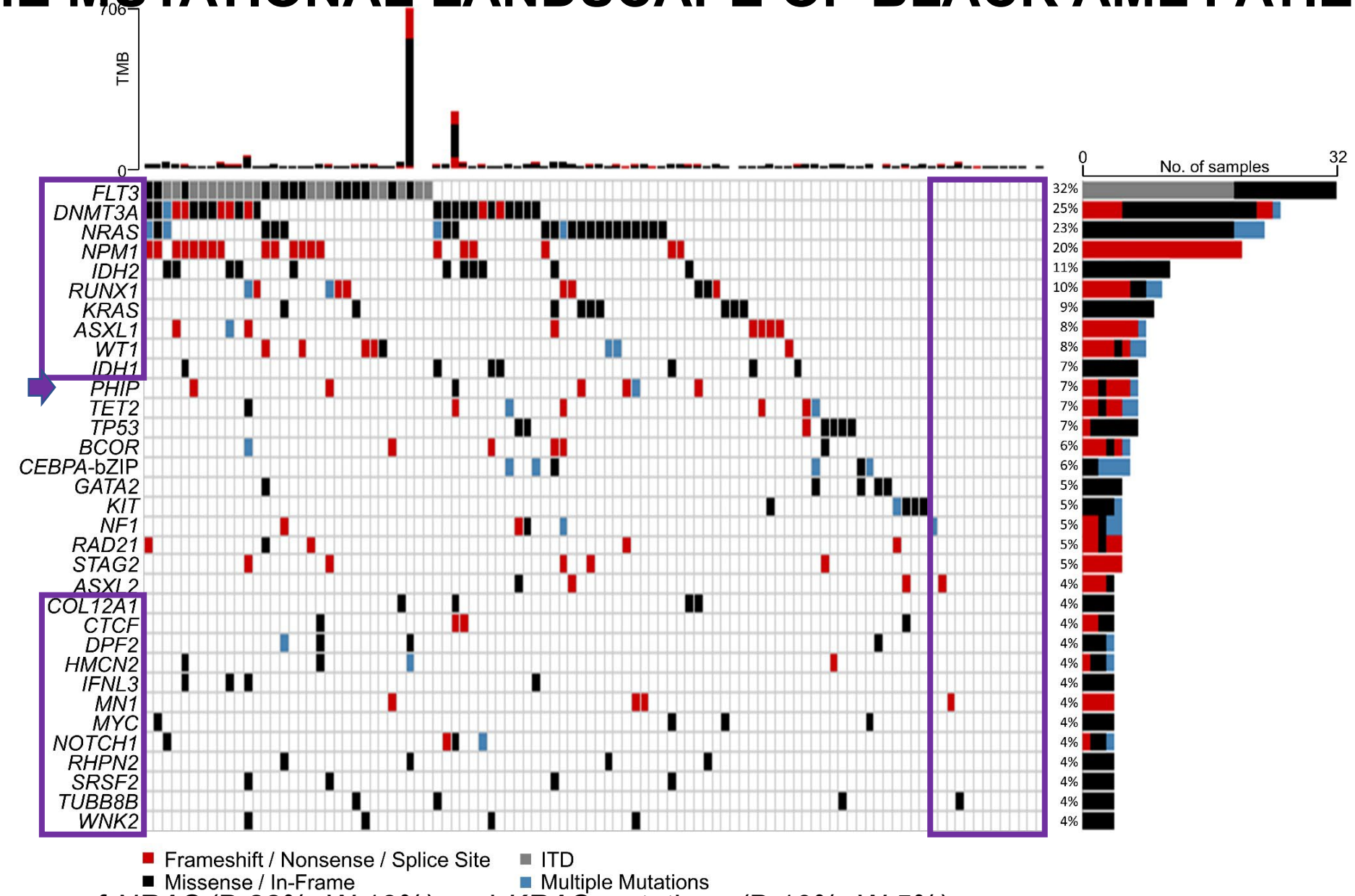
FREQUENCIES OF SHARED VS. ANCESTRY-ASSOCIATED VARIANTS

n=168 recurrently mutated genes in Black AML patients



n=55/168 genes mutated in ≥0.04% (**≥1 mutated patient)** of White BEAT AML patients
n=113/168 genes found mutated in <0.04% **(<1 mutated patient)**

THE MUTATIONAL LANDSCAPE OF BLACK AML PATIENTS

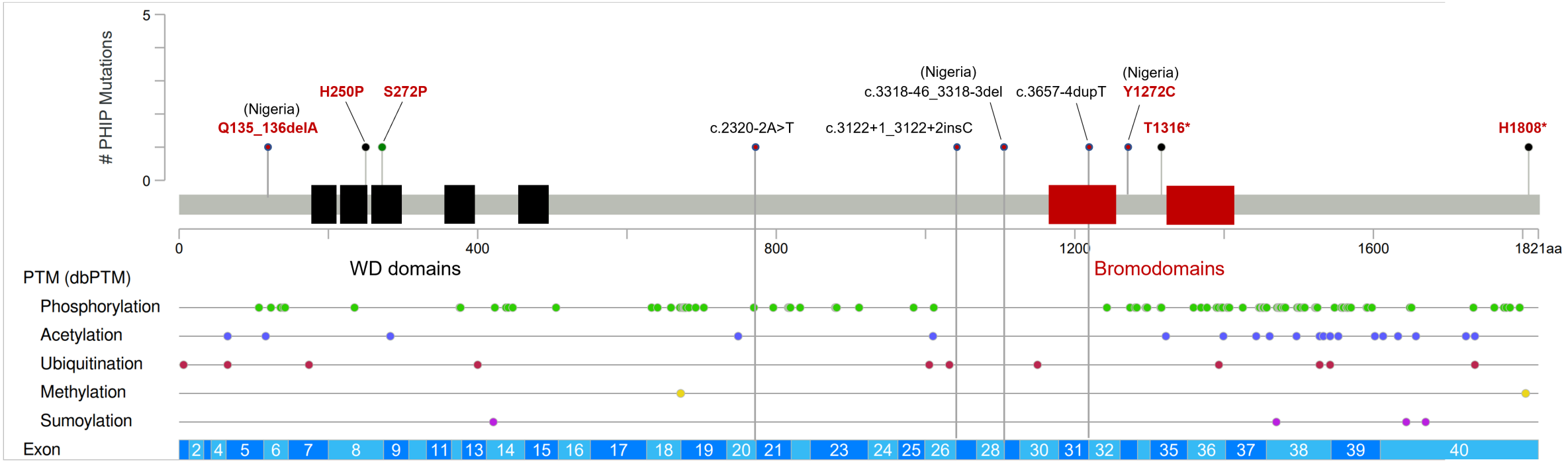


High frequency of *NRAS* (B:22%, W:13%) and *KRAS* mutations (B:10%, W:5%)

Paucity of *SRSF2* (B:4%, W:12%), *U2AF1*(B:2%, W:5%), *TET2* (B:7%, W:14%)

Stiff A et al, Nature Genetics, in press

PHIP mutations in 7% of Black AML patients (<1% in Whites)

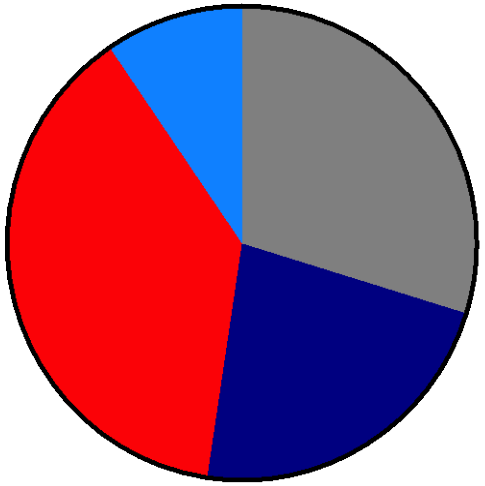


- previously reported mutations in myelodysplastic syndromes, blast crisis CML as well as clonal hematopoiesis (no functional data, just reported mutation).
- not reported as recurrently mutated in AML
- In BEAT AML cohort, only 1 White patient with a *PHIP* mutation was identified out of 741 patients (7% vs. 0.3%, P<.001).
- PHIP mutation frequency was validated in a cohort of 38 Nigerian AML patients (3 mutations detected), while 0/23 Black AML patients from South Africa carried mutations

Stiff A et al, Nature Genetics, in press

PHIP: AN UNDERAPPRECIATED GENE IN MYELOID DISEASES?

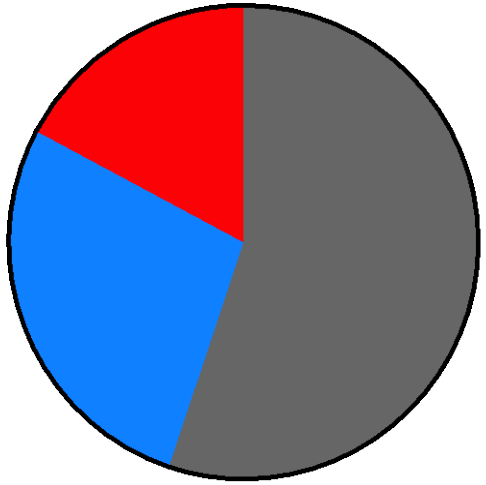
Chung-Jansen Syndrome



n=84

Jansen et al 2018
Kampmeier et al 2023
Sudnawa et al 2024

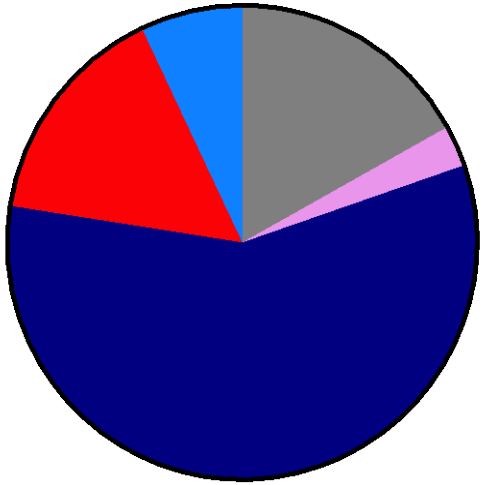
CHIP



n=58

UK Biobank
Bick et al Nature 2020

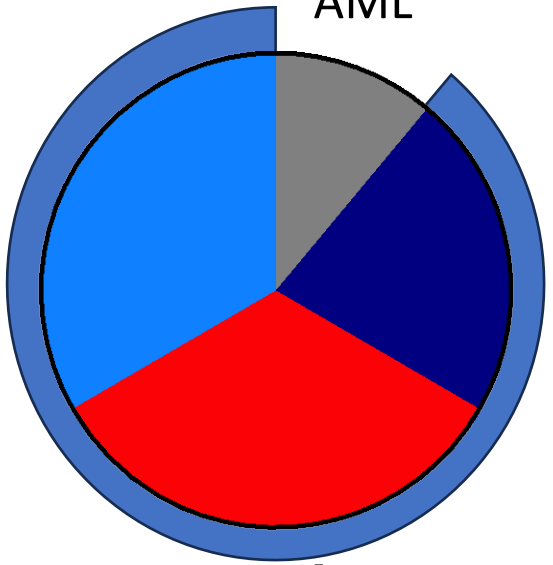
MDS



n=71

cBioPortal for
Cancer Genomics

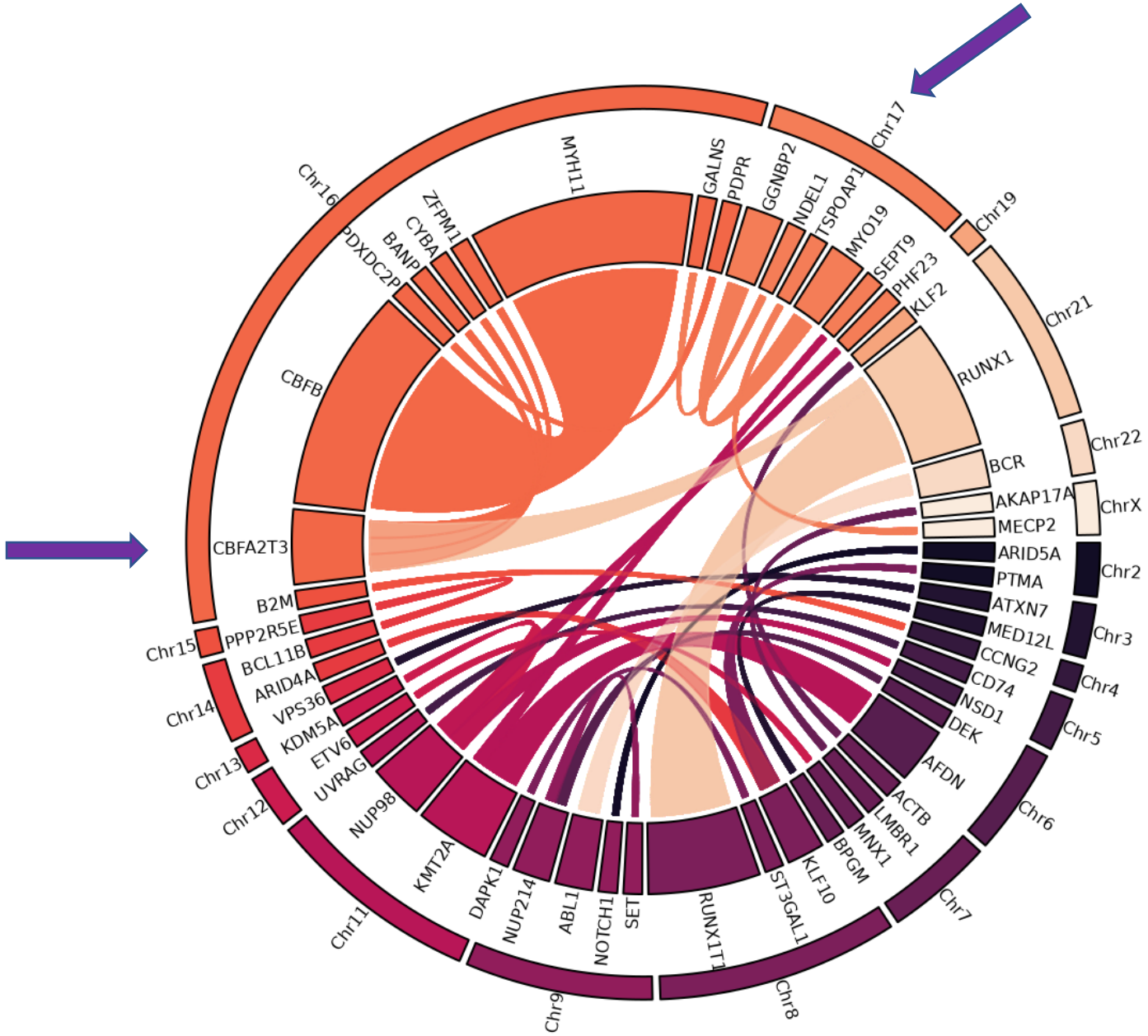
AML



n=9

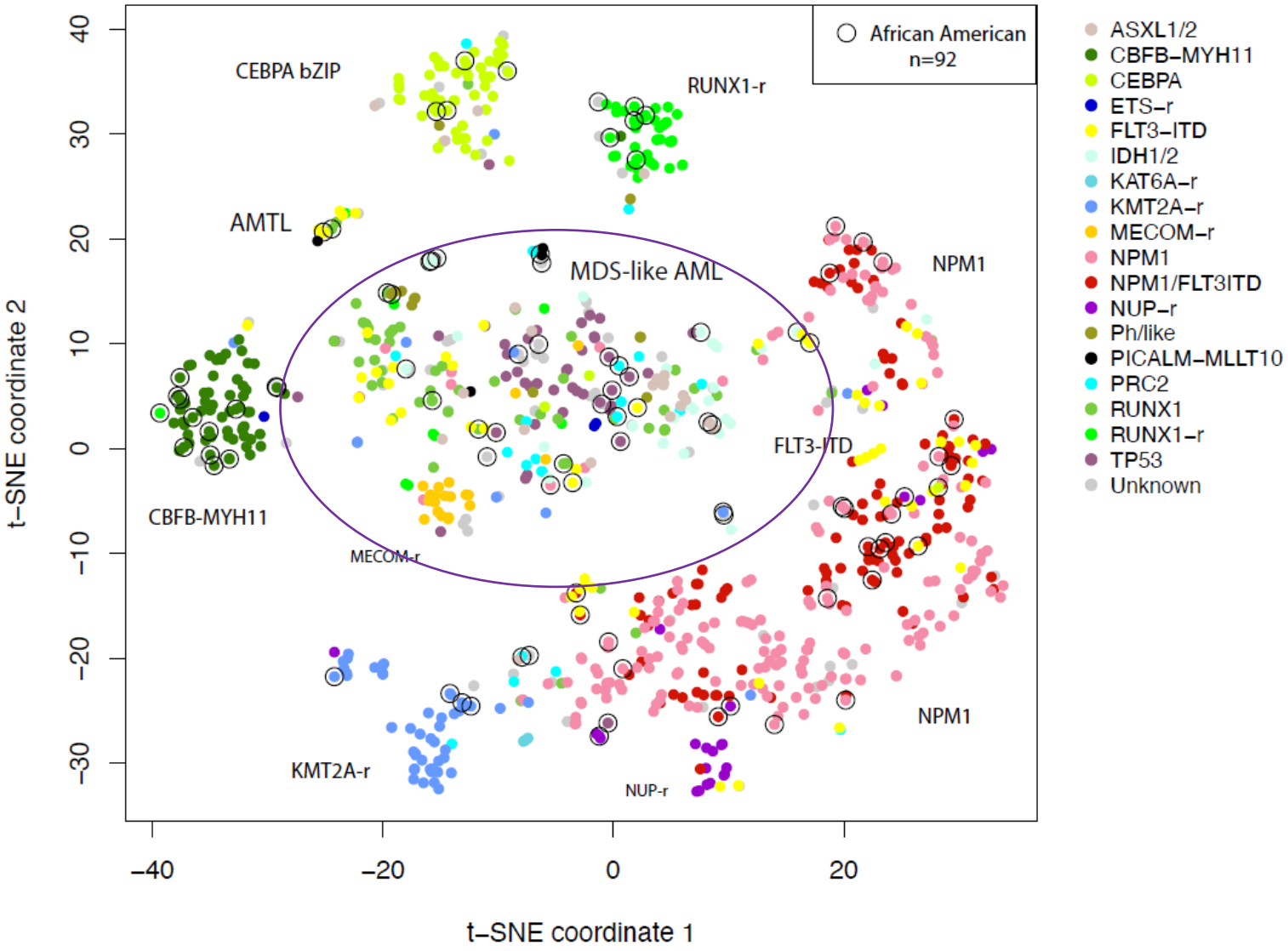
Frame shift
In frame (insertion or deletion)
Missense
Nonsense
Splice site

FUSION GENES: KNOWN AND NOVEL



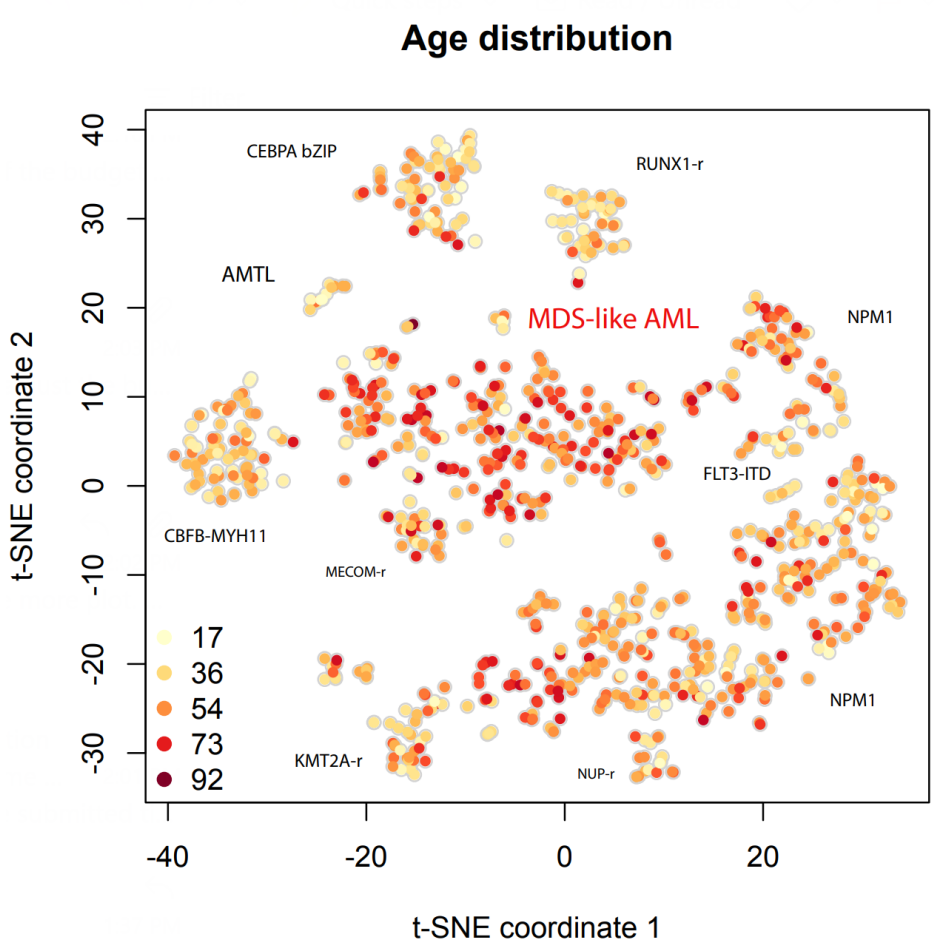
unpublished, please do not post

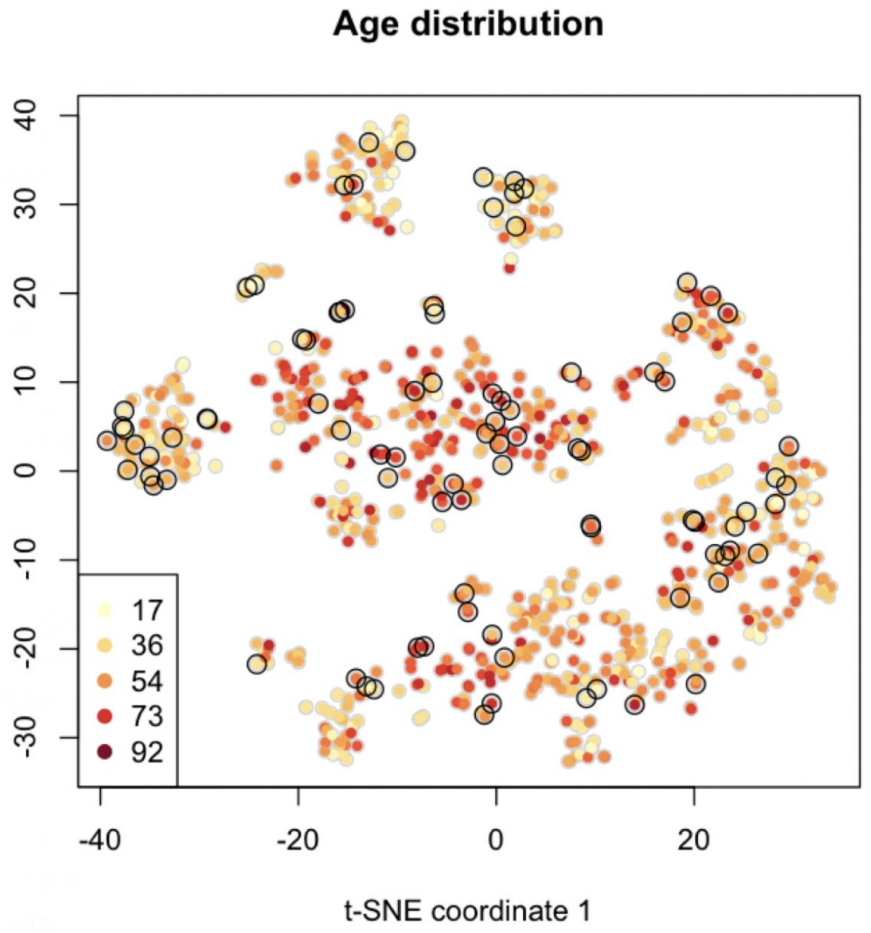
GROSSLY CONCORDANT CLUSTERING OF BLACK AND WHITE AML PATIENTS

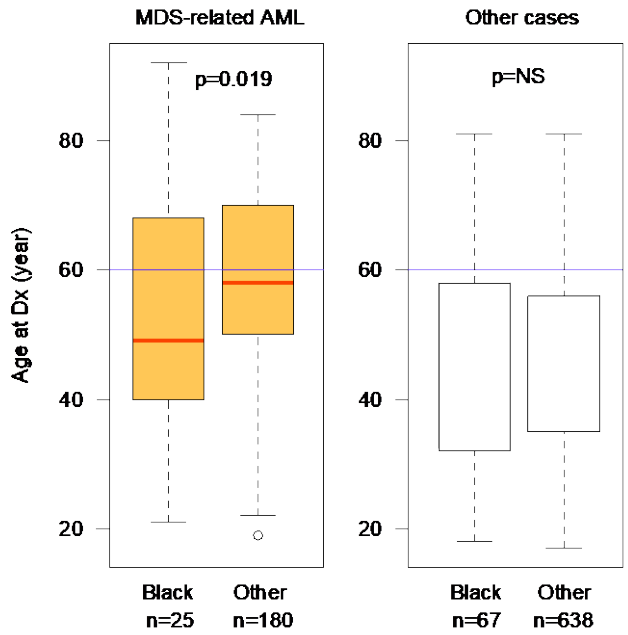


Stiff A et al, Nature Genetics, in press

EARLY ONSET OF MYELODYSPLASIA-RELATED AML

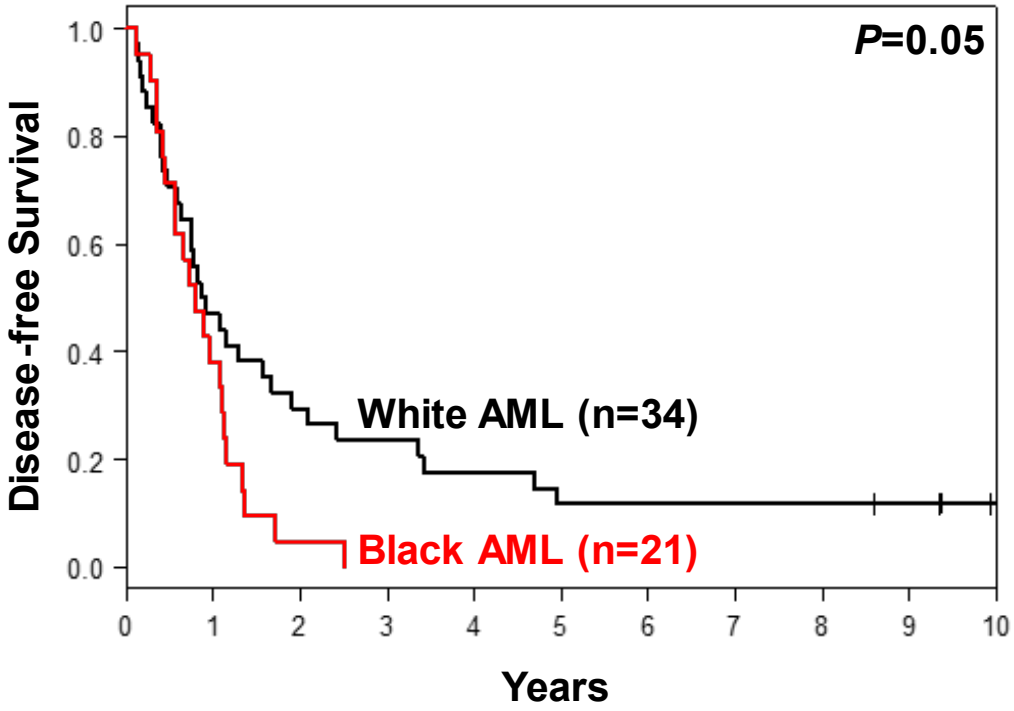






Stiff A et al, Nature Genetics, in press

POOR SURVIVAL OF MYELODYSPLASIA-RELATED AML



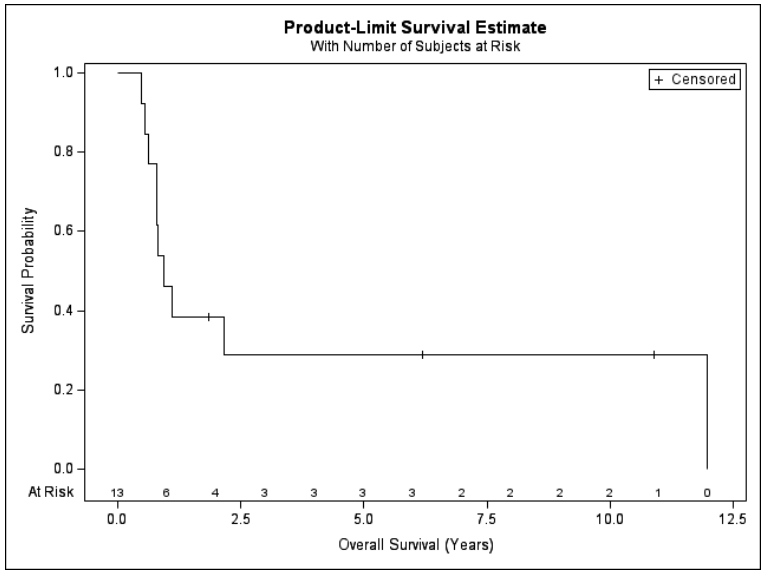
	Black patients (n=31)	White patients (n=62)	*P*
ED	2 (6%)	5 (8%)	1.00
CR	21 (67%)	34 (55%)	0.27
Relapse	20 (95%)	23 (68%)	0.02

Stiff A et al, Nature Genetics, in press

INCIDENCE AND RELAPSE RISK OF BLACK AML PATIENTS WITH CLONAL HEMATOPOIESIS

DNMT3A, n=4
TET2, n=3
TP53, n=2
ASXL1, n=2
MPL, n=2
CBL, n=1
SRSF2, n=1
SMC1A, n=1
SH2B3, n=1 pt with t(8;21) AML
→*not found mutated in BEAT AML cohort*
→ *lymphocyte-specific adapter protein*

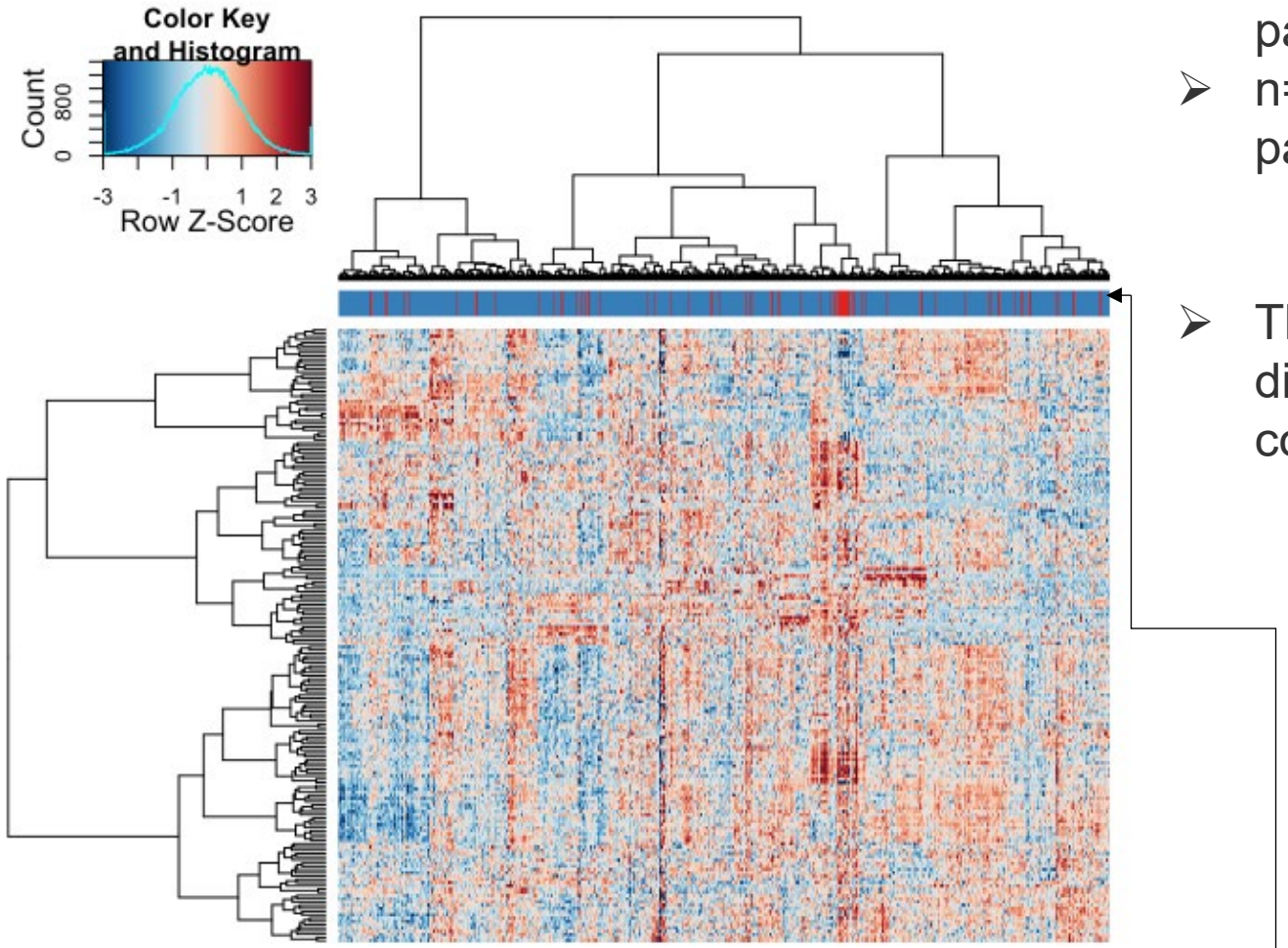
15% (23/156) of Black AML patients have evidence of underlying CH



~80% of Black AML patients with underlying CH experience disease relapse

Stiff A et al, Nature Genetics, in press

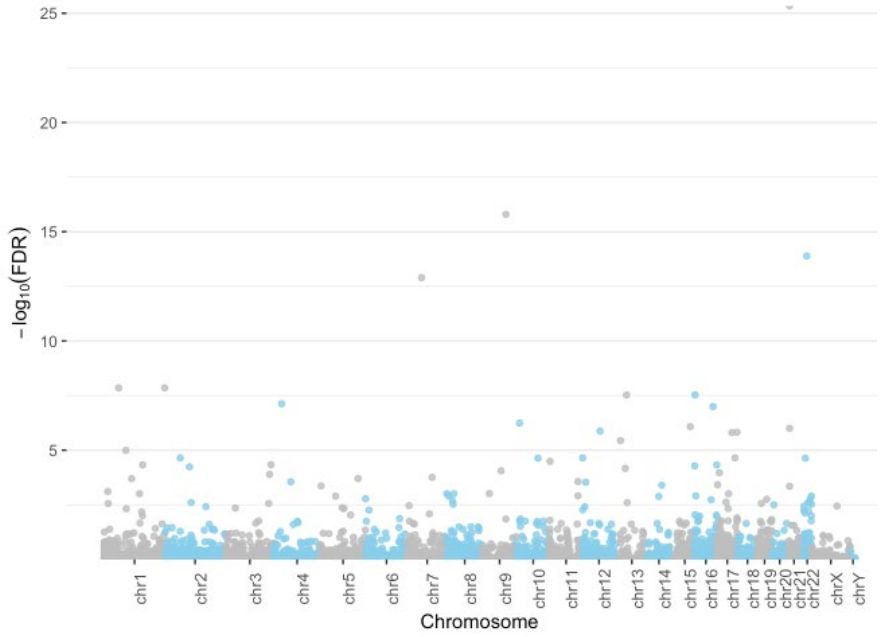
THE EXPRESSION PROFILE OF LONG NON-CODING RNAS DIFFERS WITH RESPECT TO ANCESTRY



Blue along the top dendrogram indicates White patients
Red along the top dendrogram indicates Black patients

- n=135 lncRNAs were **up-regulated** in Black AML patients and
- n=55 lncRNAs were **down-regulated** in Black AML patients compared to White AML patients

- The differentially expressed lncRNAs were equally distributed throughout the genome, indicating high conservation as shown in the Manhattan plot below:

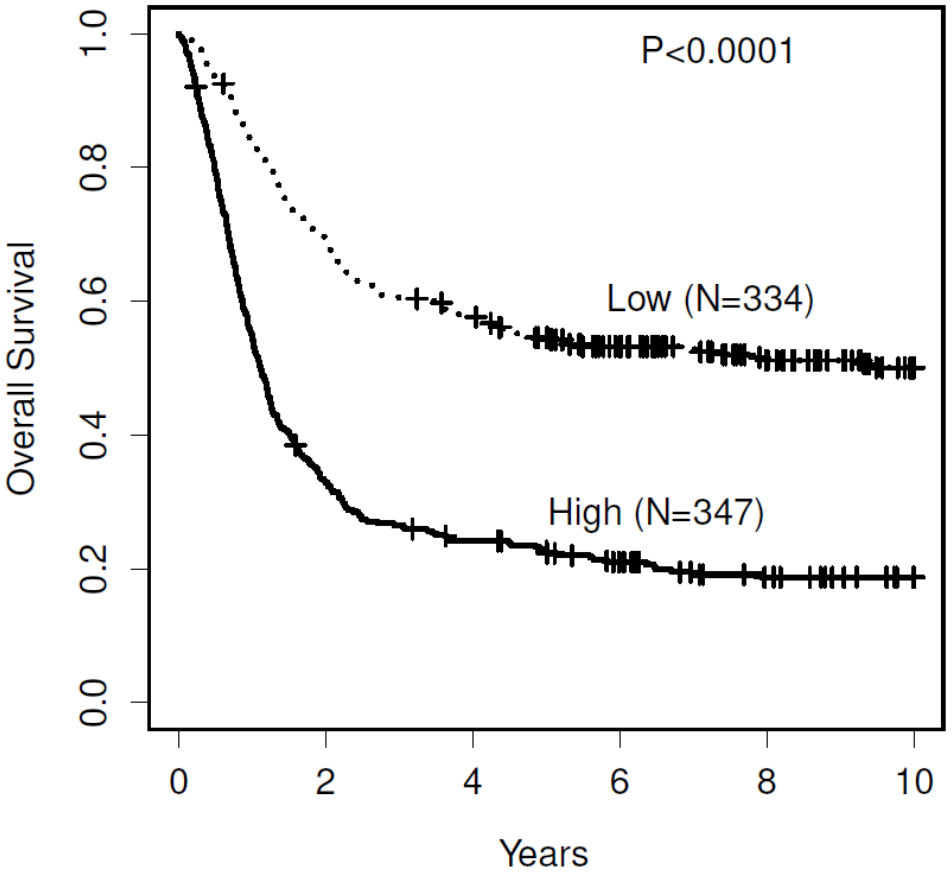


LNC RNAS ABERRANTLY EXPRESSED IN BLACK PATIENTS ASSOCIATE WITH POOR SURVIVAL

- Given the shorter survival of Black compared to White AML patients, even in the same genetic risk groups, we hypothesized that aberrantly expressed lncRNAs may carry prognostic significance, irrespective of race/ethnicity

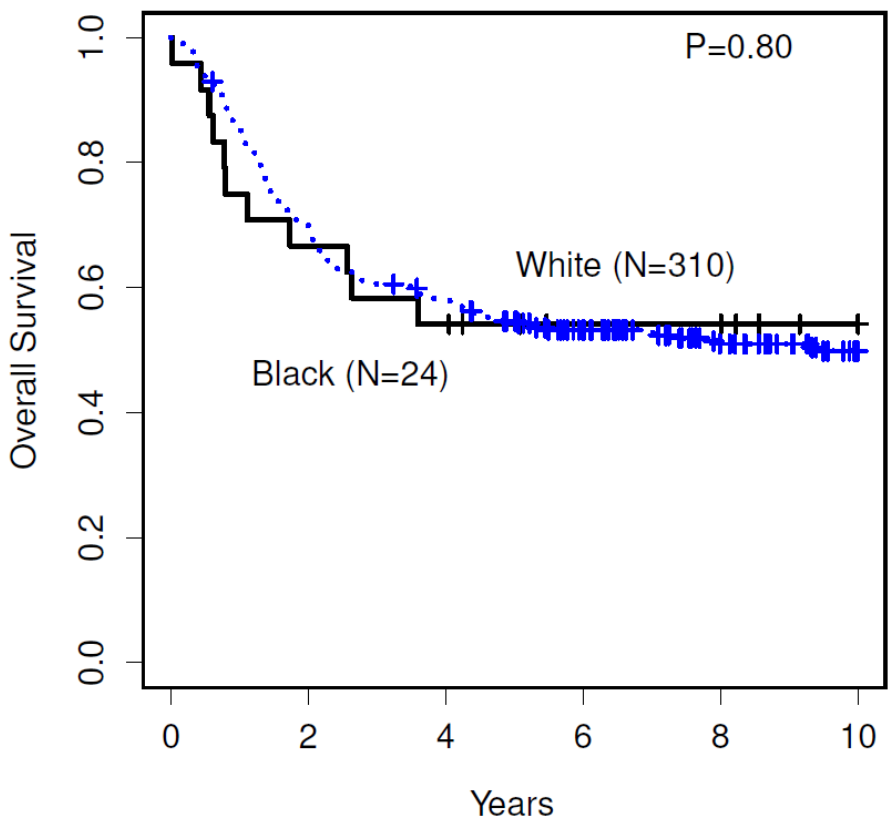
- Using the 190 aberrantly expressed lncRNAs in Black compared to White patients, we derived a prognostic score (lnC Score)

lncRNA signature (n=190 genes) → Sparse regression analyses → **lncRNA Score** (n=13 lncRNAs)

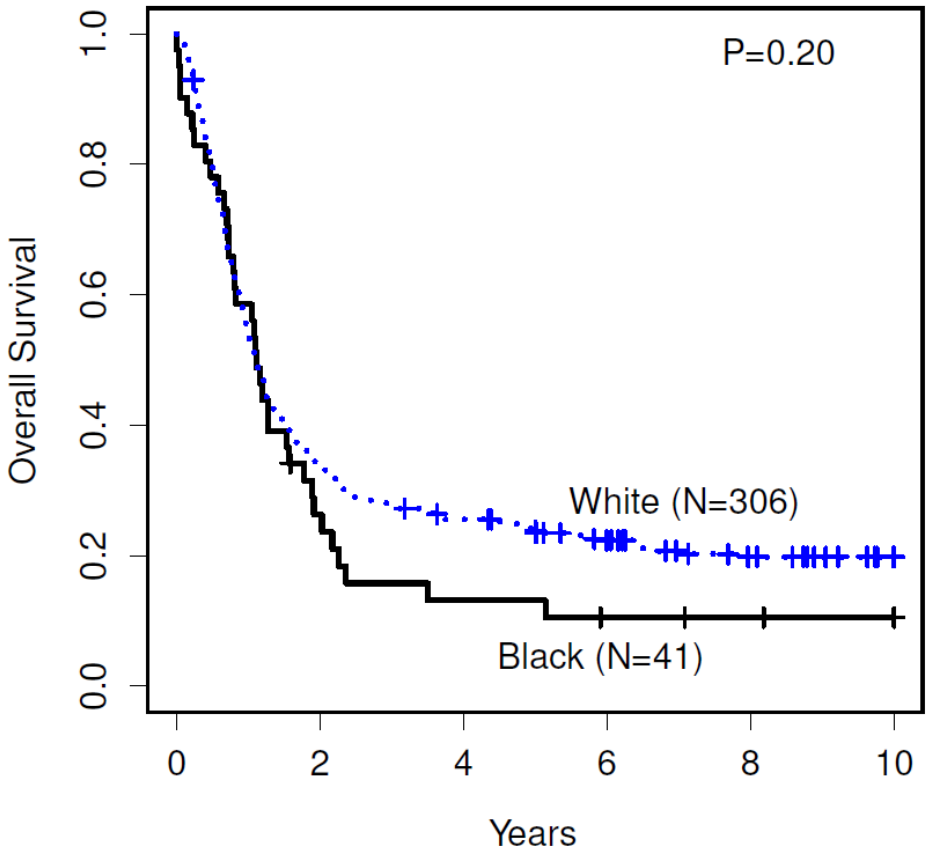


LNC RNA SCORE ASSOCIATES WITH SURVIVAL IRRESPECTIVE OF ANCESTRY

Low lncRNA score



High lncRNA score

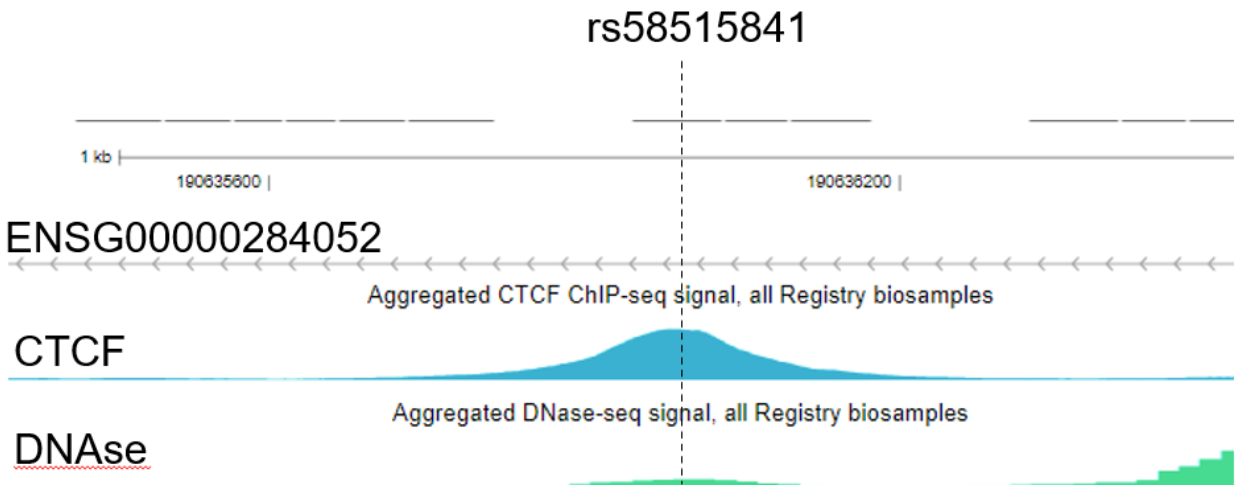


ANCESTRY-ASSOCIATED POLYMORPHISMS MAY MODULATE LNC RNA EXPRESSION

- Examination of the genomic context of regulatory regions within the 13 lncRNAs revealed multiple ancestry-associated SNPs that putatively altered transcription factor (TF) / chromatin regulator binding sites in cis-regulatory regions.

1) rs58515841 in a CTCF binding site within the lncRNA (ENSG00000284052) with minor allele frequency 24% vs 9% in White vs Black patients

2) rs1400262 in a REST binding site upstream of the lncRNA (NCK1-DT) with minor allele frequency 62% vs 10% in White vs Black patients



ANCESTRY-ASSOCIATED SURVIVAL PROGNOSTICATORS MAY REFINE GENETIC RISK ASSIGNMENT FOR BLACK AML PATIENTS

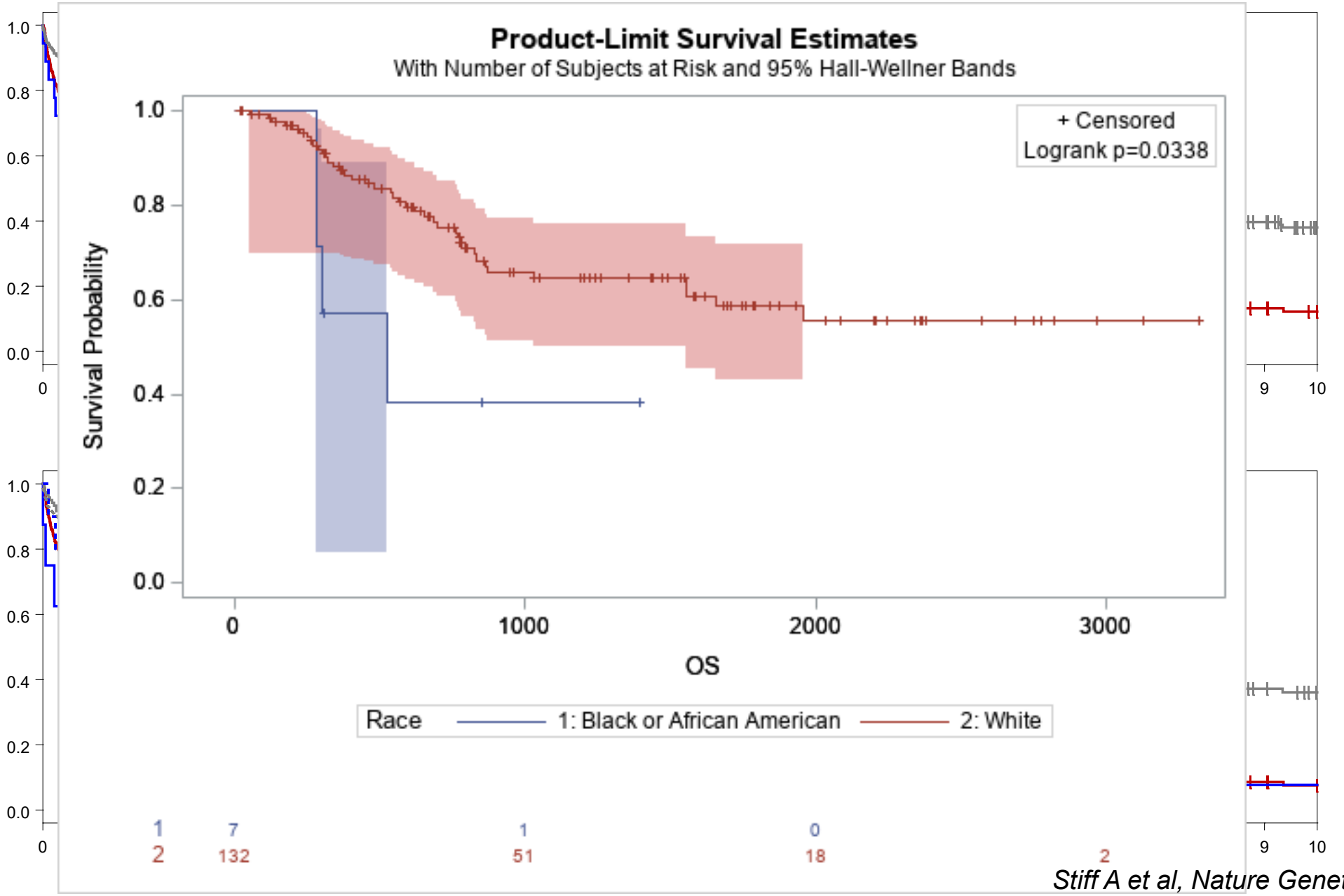
Multivariable models, final analyses

	Variable	*P*-value	HR(95% CI)
Disease-free survival	*NPM1*	0.003	2.67 (1.41, 5.06)
	NRAS	0.02	2.26 (1.17, 4.37)
Overall survival	*IDH1/2*	0.05	1.73 (1.01, 2.97)

Additional markers in final DFS MVA: WBC, complex karyotype, MR-AML genes
Additional markers in final OS MVA: WBC, CBF AML

Stiff A et al, Nature Genetics, in press

NPM1, *NRAS* AND *IDH1/2* MUTATIONS CONFER ADVERSE RISK



Stiff A et al, Nature Genetics, in press

Acute myeloid leukemia (AML)
PROGNOSIS ASSESSMENT

FAVORABLE

- t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
- inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- Mutated *NPM1* without *FLT3*-ITD
- bZIP in-frame mutated *CEBPA*

INTERMEDIATE

- Mutated *NPM1* with *FLT3*-ITD
- Wild-type *NPM1* without *FLT3*-ITD (without adverse-risk genetic lesions)
- t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
- Cytogenetic abnormalities not classified as favorable or adverse

ADVERSE

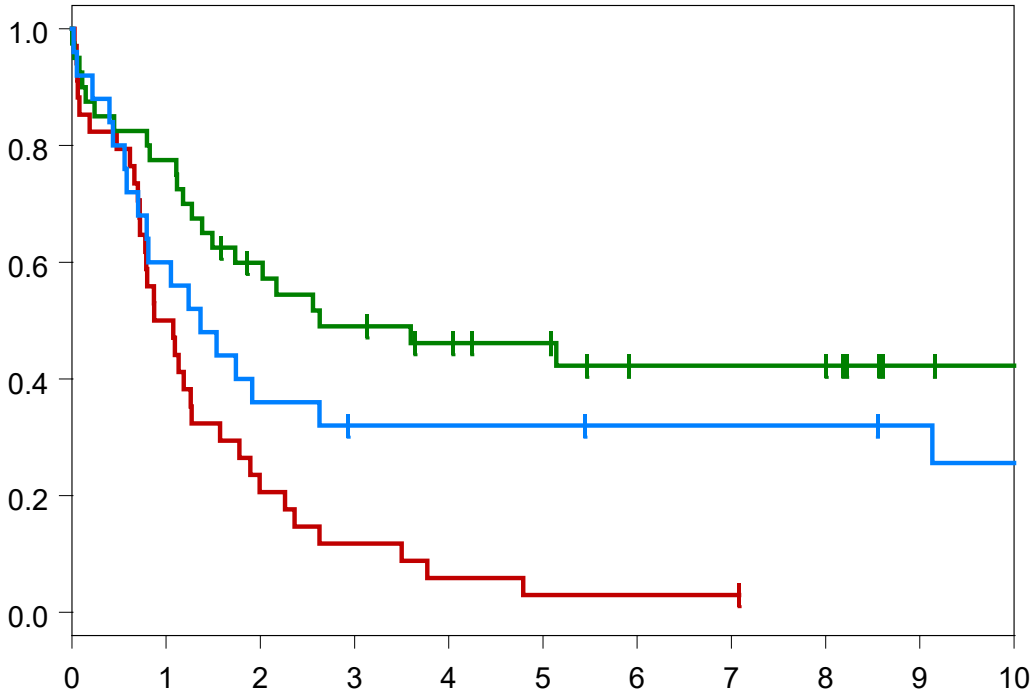
- t(6;9)(p23;q34.1); *DEK-NUP214*
- t(v;11q23.3); *KMT2A* rearranged
- t(9;22)(q34.1;q11.2); *BCR-ABL1*
- t(8;16); *KAT6A/CREBBP*
- inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1)
- -5 or del(5q); -7; -17/abn(17p)
- Complex karyotype,§ monosomal karyotype‖
- Mutated *RUNX1*¶, *ASXL1, EZH2, BCOR, SF3B1, SRSF2, U2AF1, ZRSR2*¶
- Mutated *TP53*#

Mutated IDH1/2
Mutated NRAS

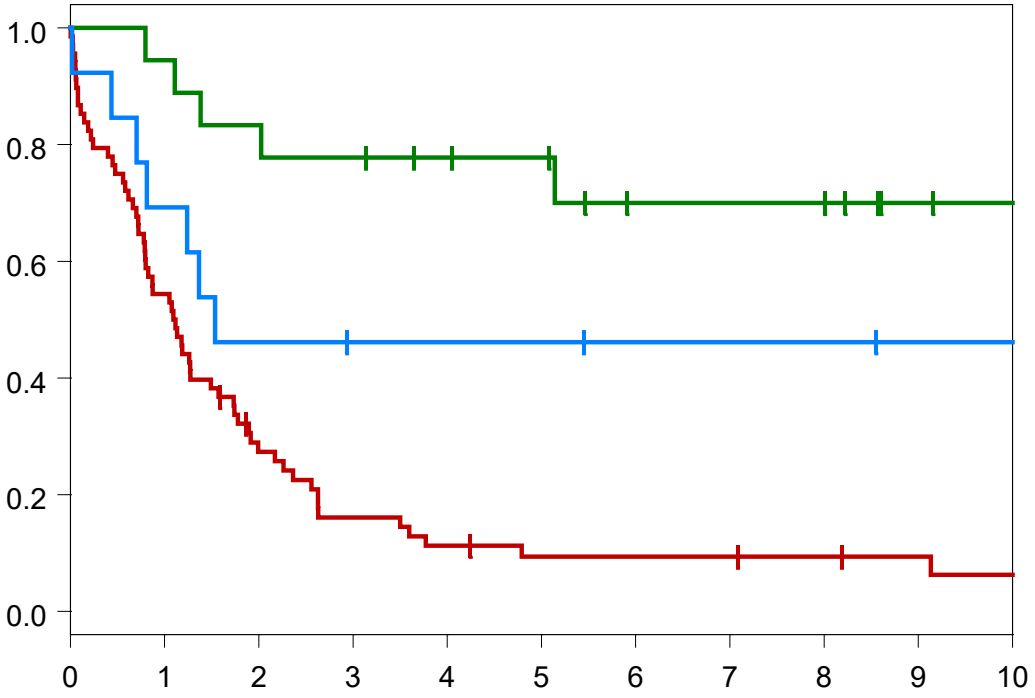
Döhner H, et al. Blood. 2022;129:424-447.
* ELN: European LeukemiaNet

NPM1, *NRAS* AND *IDH1/2* MUTATIONS CAN REFINE ELN 2022 GENETIC RISK GROUP ASSIGNMENTS FOR BLACK AML PATIENTS

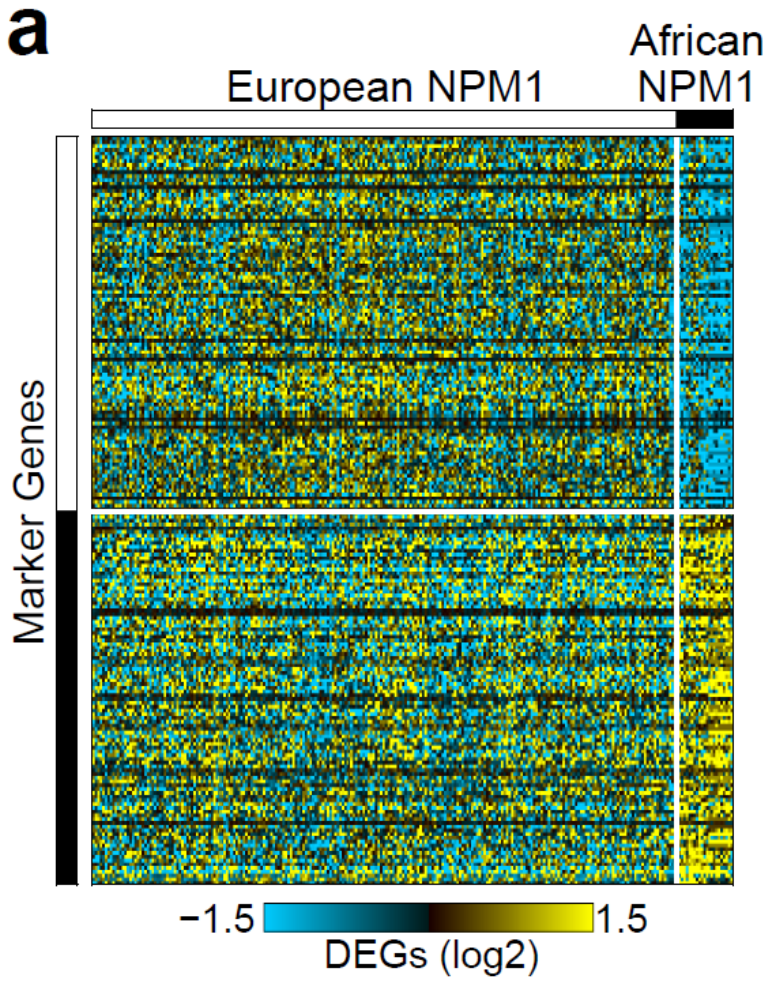
ELN 2022, Black AML patients <60y

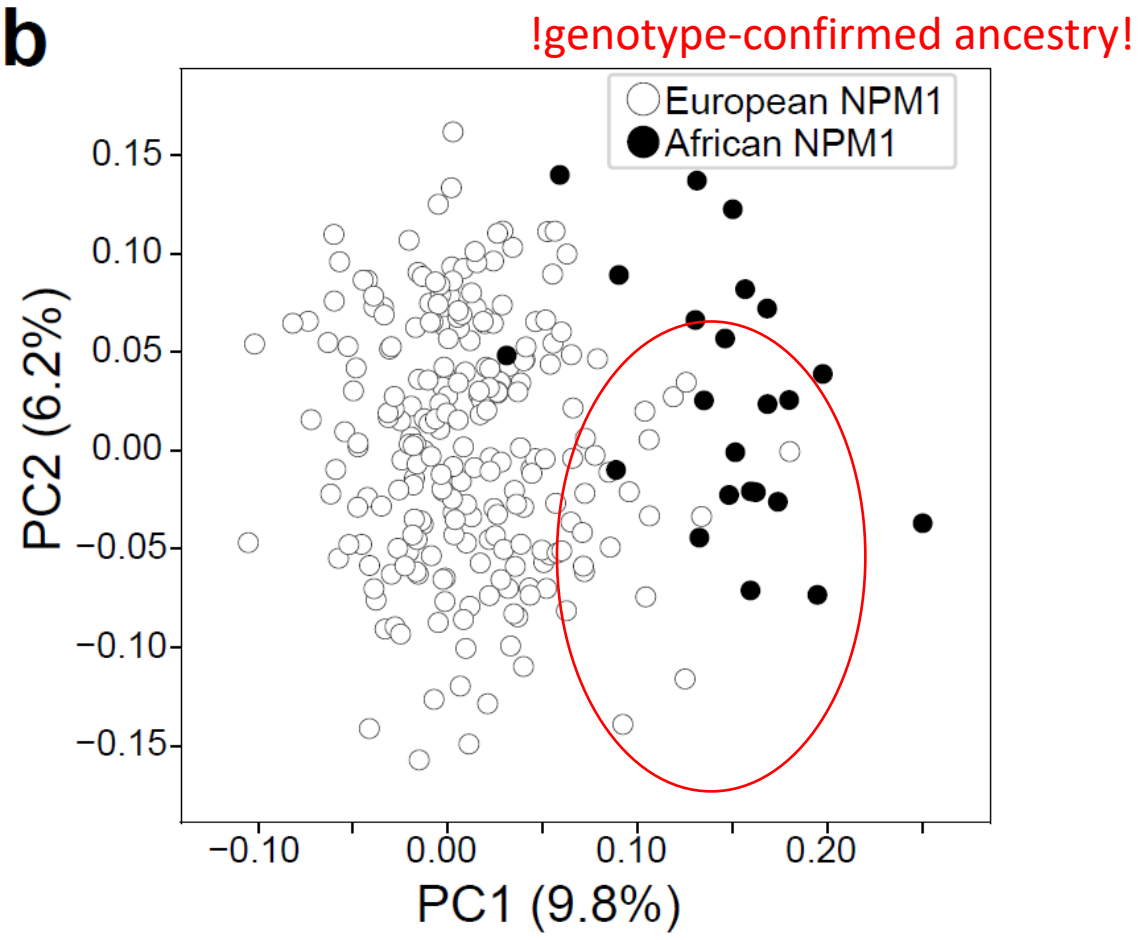


ELN 2022, Black AML patients <60y
-refined with NPM1, NRAS, IDH1/2 as adverse risk-



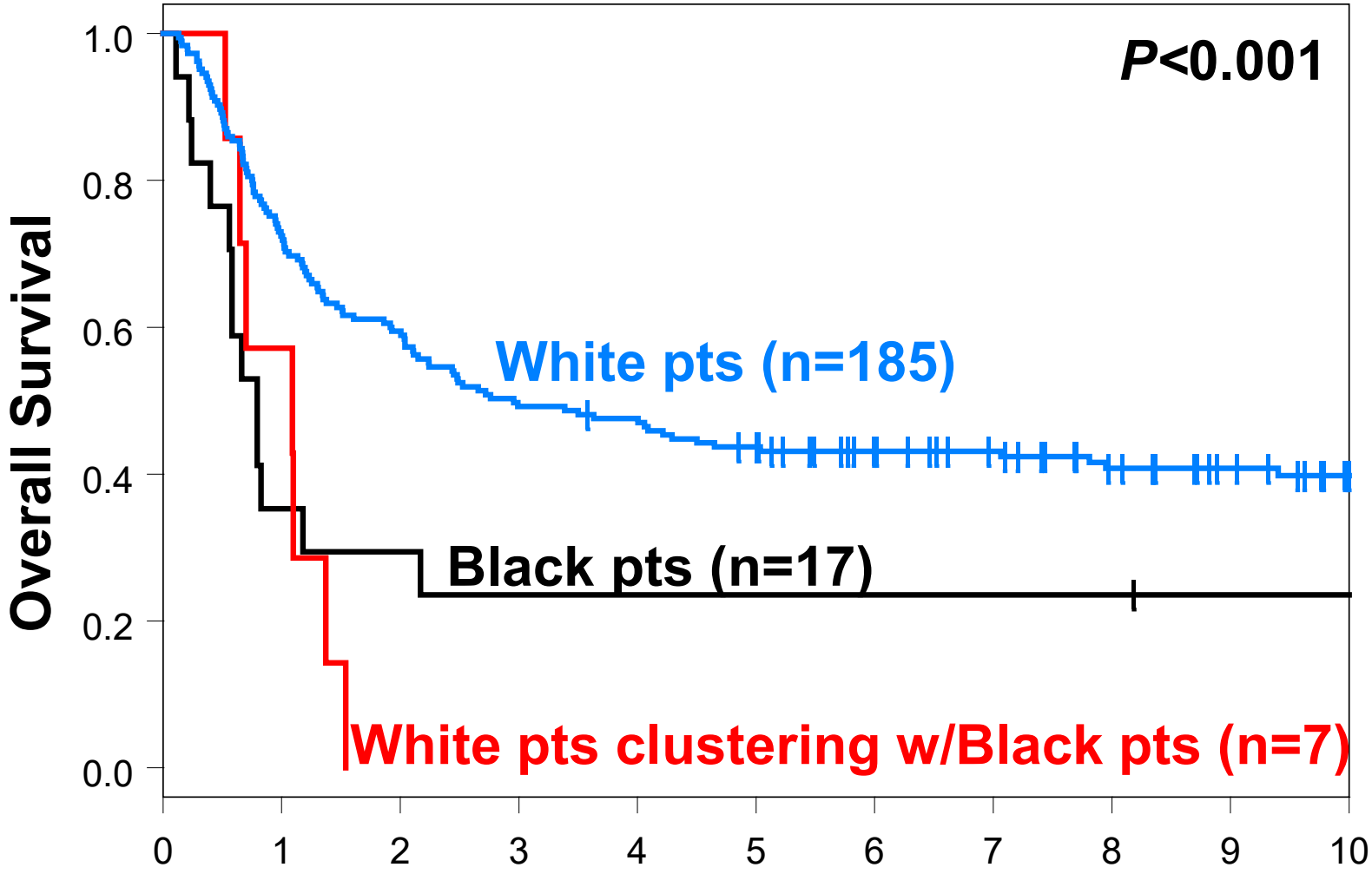
UNDERSTANDING THE DIFFERENCES IN NPM1-ASSOCIATED SURVIVAL





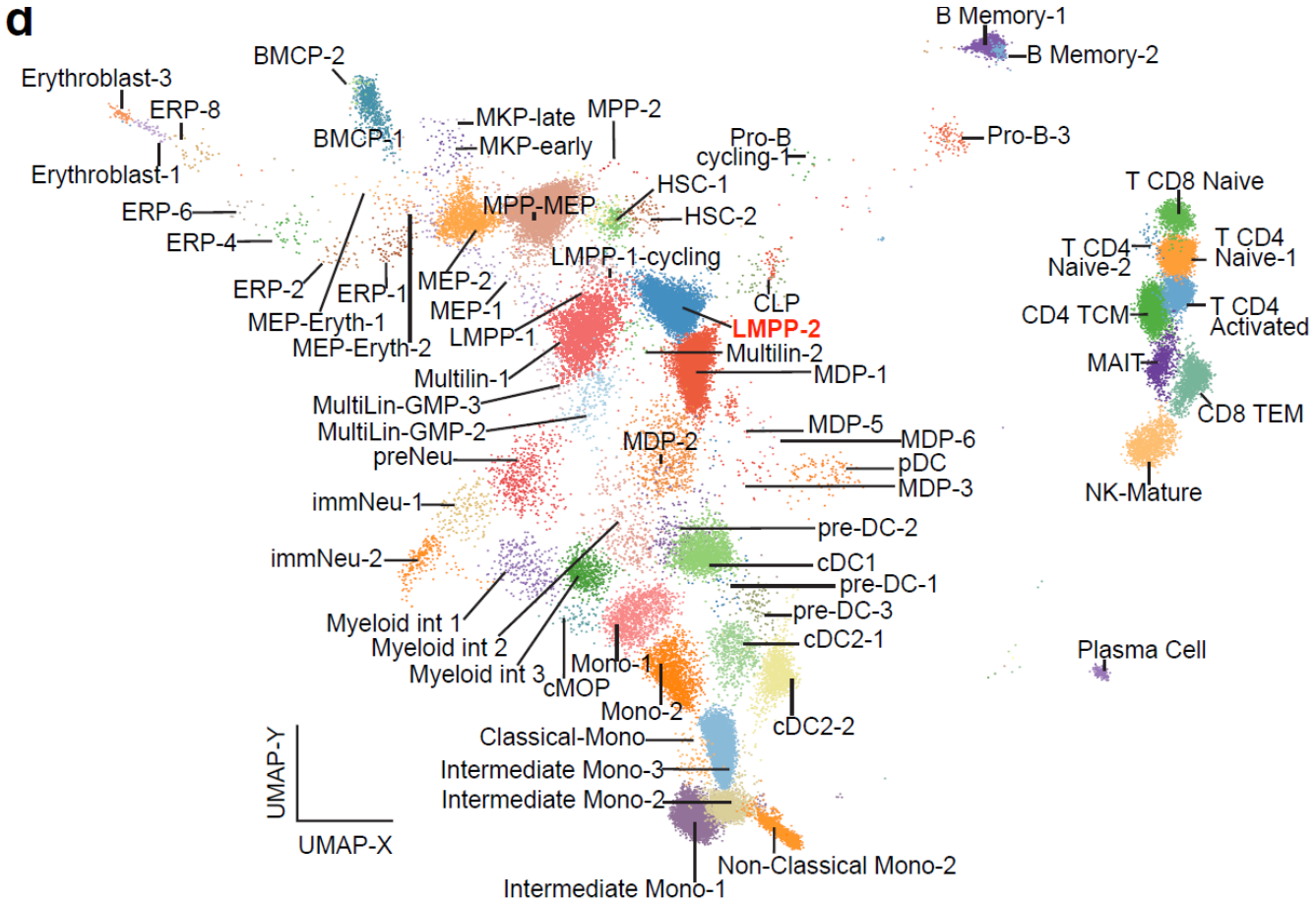
Stiff A et al, Nature Genetics, in press

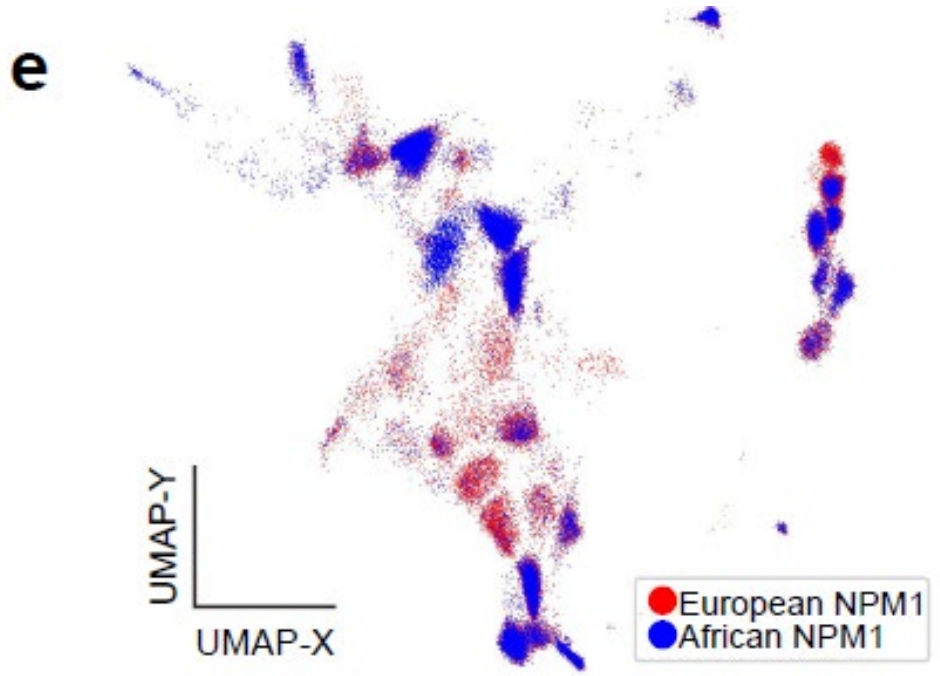
WHITE NPM1 PATIENTS THAT ARE "GENOMIC PHENOCOPIES" ALSO SHARE THE INFERIOR SURVIVAL



Stiff A et al, Nature Genetics, in press

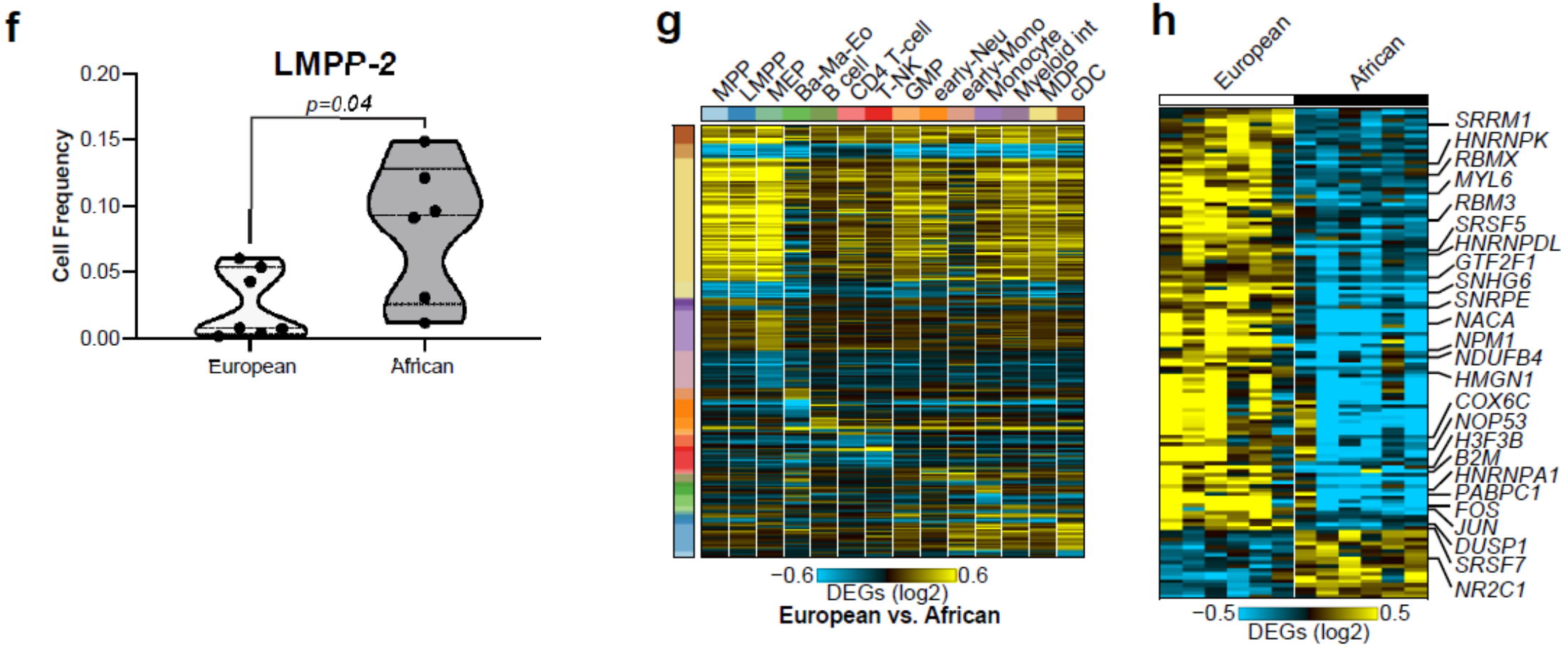
UNDERSTANDING THE DIFFERENCES IN NPM1-ASSOCIATED SURVIVAL





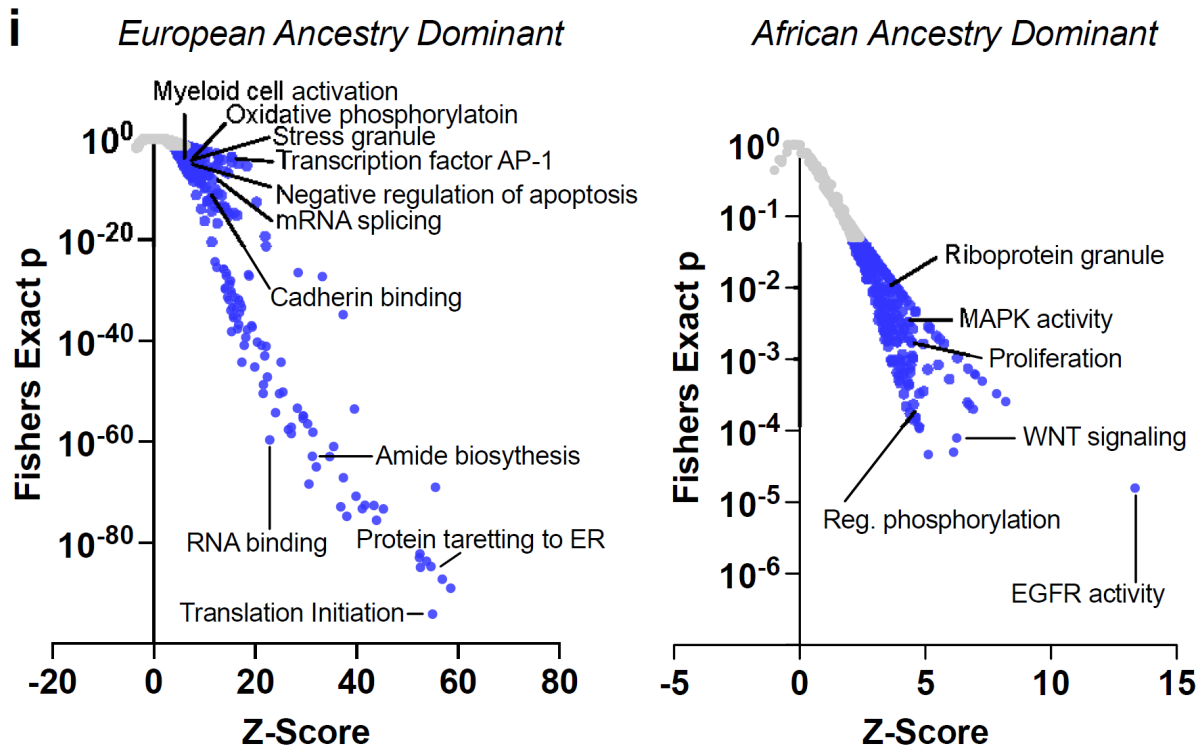
Stiff A et al, Nature Genetics, in press

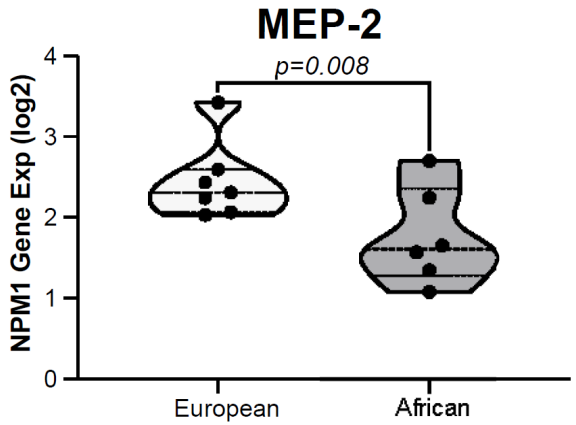
UNDERSTANDING THE DIFFERENCES IN NPM1-ASSOCIATED SURVIVAL



Stiff A et al, Nature Genetics, in press

UNDERSTANDING THE DIFFERENCES IN NPM1-ASSOCIATED SURVIVAL





Stiff A et al, Nature Genetics, in press

CONCLUSIONS

- *NPM1*-mutated AML has contrasting prognostic impact in patients of African compared to European ancestry
- There are distinct differences in cell proportions (→ increase of LMPPs), and cell state/lineage specific programs (→ *NPM1* downregulation in MEPs)
- White patients who share this “phenotype” also share the poor survival

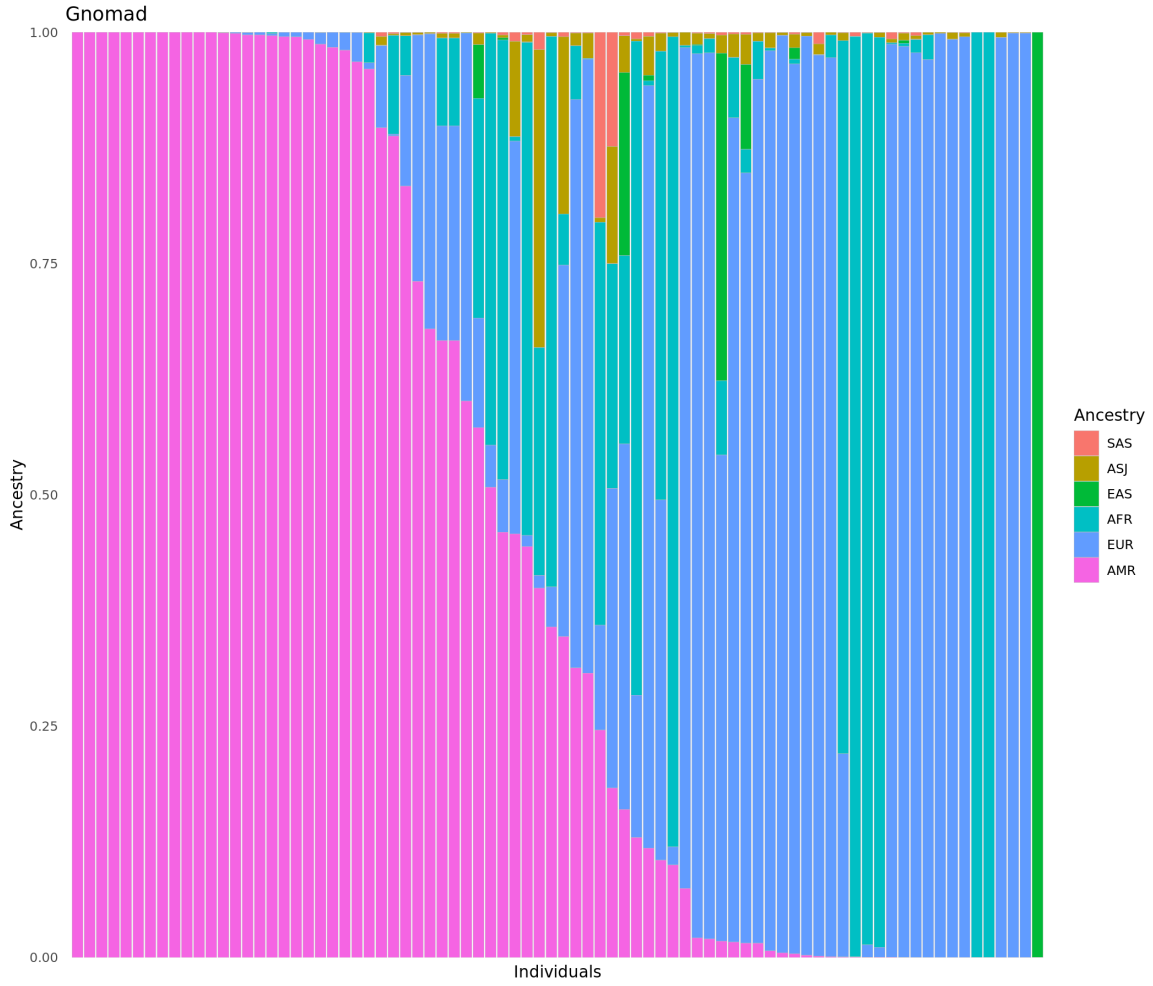
Together, this suggests a biologic rationale for the poor survival of Black patients with NPM1 mutations, that is mirrored in the rare White patients presenting with the same features.

TOWARDS ANCESTRY-INCLUSIVITY: "HISPANIC" AML

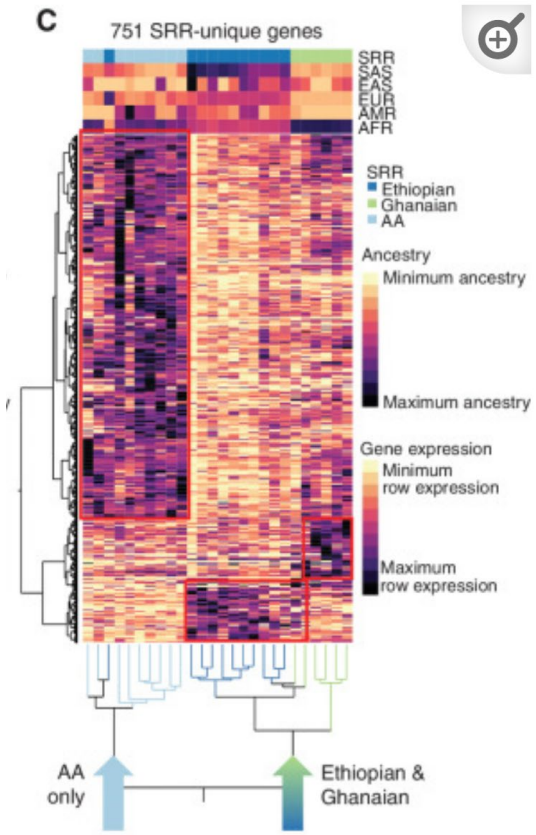
- "Hispanic or Latino": a person of Spanish culture or origin-regardless of race.

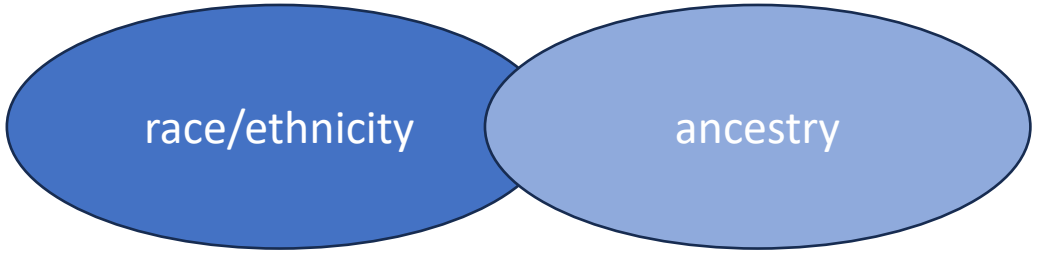
- The commonality among Hispanic patients (and associated knowledge and care disparities) is heavily influenced by social, economic and/or environmental disadvantages that are shared among them

- However, this ancestral diversity is likely further increasing the number of thus far unrecognized genetic and genomic features in Hispanic patient, as each geographically and/or ancestral defined group likely presents with distinct genetic features.



RACE AND ETHNICITY PROVIDE ADDITIVE INFORMATIION





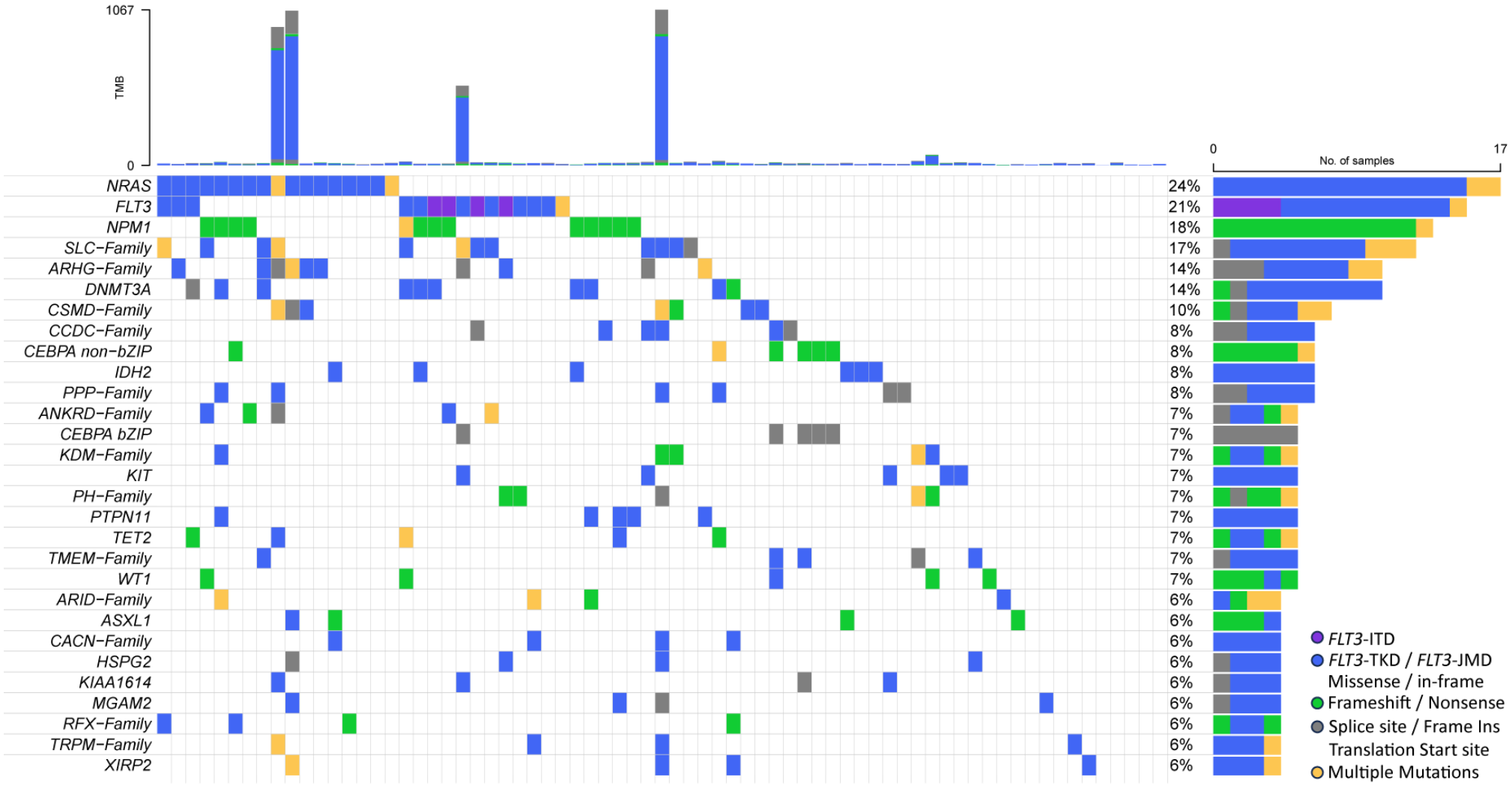
exogenous
"lived experience"

endogenous
constitutional genetic background

! enrichment of genes in known canonical pathways associated with environmental exposures and/or comorbidities.

Martini et al, Cancer Discov. 2022 Nov 2; 12(11): 2530–2551.

SOMATIC GENETIC LANDSCAPE OF HISPANIC AML



CONTRIBUTING FACTORS FOR RACIAL DISPARITIES IN TREATMENT OUTCOMES ARE MULTIFACTORIAL AND INTERCONNECTED

→we are decades behind in our knowledge regarding AML biology in basically all patients that are not of European ancestry
→we need to be **mindful about our knowledge gap** when taking care of patients with myeloid malignancies from different racial/ethnic backgrounds
> more patients that are “marker negative”
> potential differences in survival associations of established markers
→we need to be **mindful about our knowledge gap and possible limitations** when we interpret our experimental data

From a cancer genetics perspective
→we need to study more patients, especially patients that do NOT reside in the US or Europe.
→we need to partner with other countries
→we need to know which of these variants are recurrent, which ones are functionally important and which ones might be even targetable
→We need more and better model systems

Acknowledgments

Patients and Families

Clara D. Bloomfield Center for Leukemia Outcomes Research

Deedra Nicolet
Andrew Stiff
Krzysztof Mrózek
Lisa Sterling
Victoria Klein
Jill Buss
Karilyn Larkin
Alice S. Mims

OSU

Electra Paskett
Jesse Plascak

OHSU

Jeff Tyner
Shannon McWeeney
Dan Bottomly

Institute for Genomic Medicine

Elaine Mardis
Benjamin Kelly
Stephanie LaHaye
Katherine E. Miller
Saranga Wijeratne
Gregory Wheeler

UC and Cincinnati Children's

Lee Grimes
John C. Byrd
Nathan Salomonis
Bailee Kain

University of Colorado

Craig Jordan
Dan Pollyea
Maria Amaya

Alliance for Clinical Trials in Oncology

Richard M. Stone
Andrew J. Carroll
William Blum
Bayard L. Powell
Richard A. Larson
Jonathan E. Kolitz
Geoff Uy
Wendy Stock

The Leukemia Tissue Bank

NIH National Cancer Institute
Leukemia Research Foundation
Pelotonia
American Cancer Society
Leukemia & Lymphoma Society
American Society of Hematology