CAPA Online Education Program Presentation
July 7th, 2024

Chinese American Pathologists Association: Excellence through Education, Professionalism and Connection

Molecular Hematopathology

Recent Updates and Practice Pearls

EMORY UNIVERSITY SCHOOL OF MEDICINE

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Disclosure

- **I have no commercial conflict of interest to disclose.**
- Most molecular genetic tests discussed in this presentation are not FDA-approved or cleared. You are responsible for investigating the test performances and related policies before considering them as clinical diagnostic tests.
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Objectives

1. Introduce the emerging molecular genetic methods applicable to hematopathology in clinical laboratories.
2. Analyze the application of mutation profiling in the diagnosis of MDS and the detection of MRD in AML.
3. Evaluate the clinical relevance and potential limitations of clonality testing for lymphoid neoplasms.
4. Discuss the recent advancements in mutation profiling of lymphoid neoplasms.

New Molecular Genetic Methods Coming to the Clinical Labs

ICC Classification of AML requires blast percentage

Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)/*PML*::*RARA* ≥ 10%

APL with other *RARA* rearrangements* ≥ 10%

AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1* ≥ 10%

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11* ≥ 10%

AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A* ≥ 10%

AML with other *KMT2A* rearrangements† ≥ 10%

AML with t(6;9)(p22.3;q34.1)/*DEK*::*NUP214* ≥ 10%

AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*; *MECOM*(*EVI1*) ≥ 10%

AML with other *MECOM* rearrangements‡ ≥ 10%

AML with other rare recurring translocations (see supplemental Table 5) ≥ 10%

AML with t(9;22)(q34.1;q11.2)/*BCR*::*ABL1*§ ≥ 20%

AML with mutated *NPM1* ≥ 10%

AML with in-frame bZIP *CEBPA* mutations ≥ 10%

AML and MDS/AML with mutated *TP53*† 10-19% (MDS/AML) and ≥ 20% (AML)

AML and MDS/AML with myelodysplasia-related gene mutations 10-19% (MDS/AML) and ≥ 20% (AML)

Defined by mutations in *ASXL1*, *BCOR*, *EZH2, RUNX1*, *SF3B1, SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*

AML with myelodysplasia-related cytogenetic abnormalities 10-19% (MDS/AML) and ≥ 20% (AML)

Defined by detecting a complex karyotype (≥ 3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurring genetic abnormalities), del(5q)/t(5q)/add(5q), −7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), −17/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities

AML not otherwise specified (NOS) 10-19% (MDS/AML) and ≥ 20% (AML)

WHO Classification, 5th edition

Table 7. Acute myeloid leukaemia.

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with *PML*::*RARA* fusion
Acute myeloid leukaemia with *RUNX1*::*RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB*::*MYH11* fusion
Acute myeloid leukaemia with *DEK*::*NUP214* fusion
Acute myeloid leukaemia with *RBM15*::*MRTFA* fusion
Acute myeloid leukaemia with *BCR*::*ABL1* fusion
Acute myeloid leukaemia with *KMT2A* rearrangement
Acute myeloid leukaemia with *MECOM* rearrangement
Acute myeloid leukaemia with *NUP98* rearrangement
Acute myeloid leukaemia with *NPM1* mutation
Acute myeloid leukaemia with *CEBPA* mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with other defined genetic alterations

How can we detect all the genetic changes at diagnosis?

Optical Genome Mapping

Fingerprinting chromosome segments

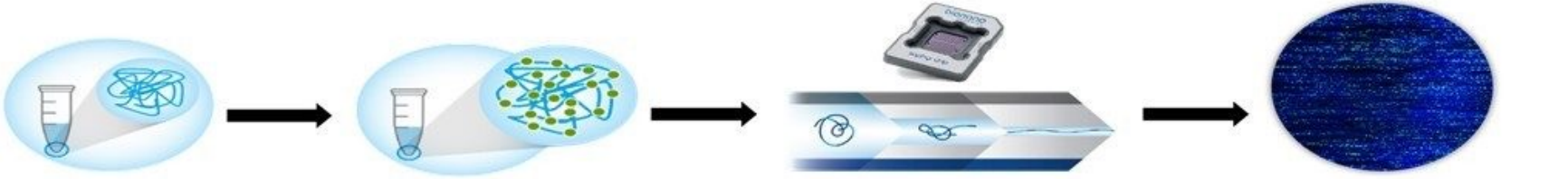
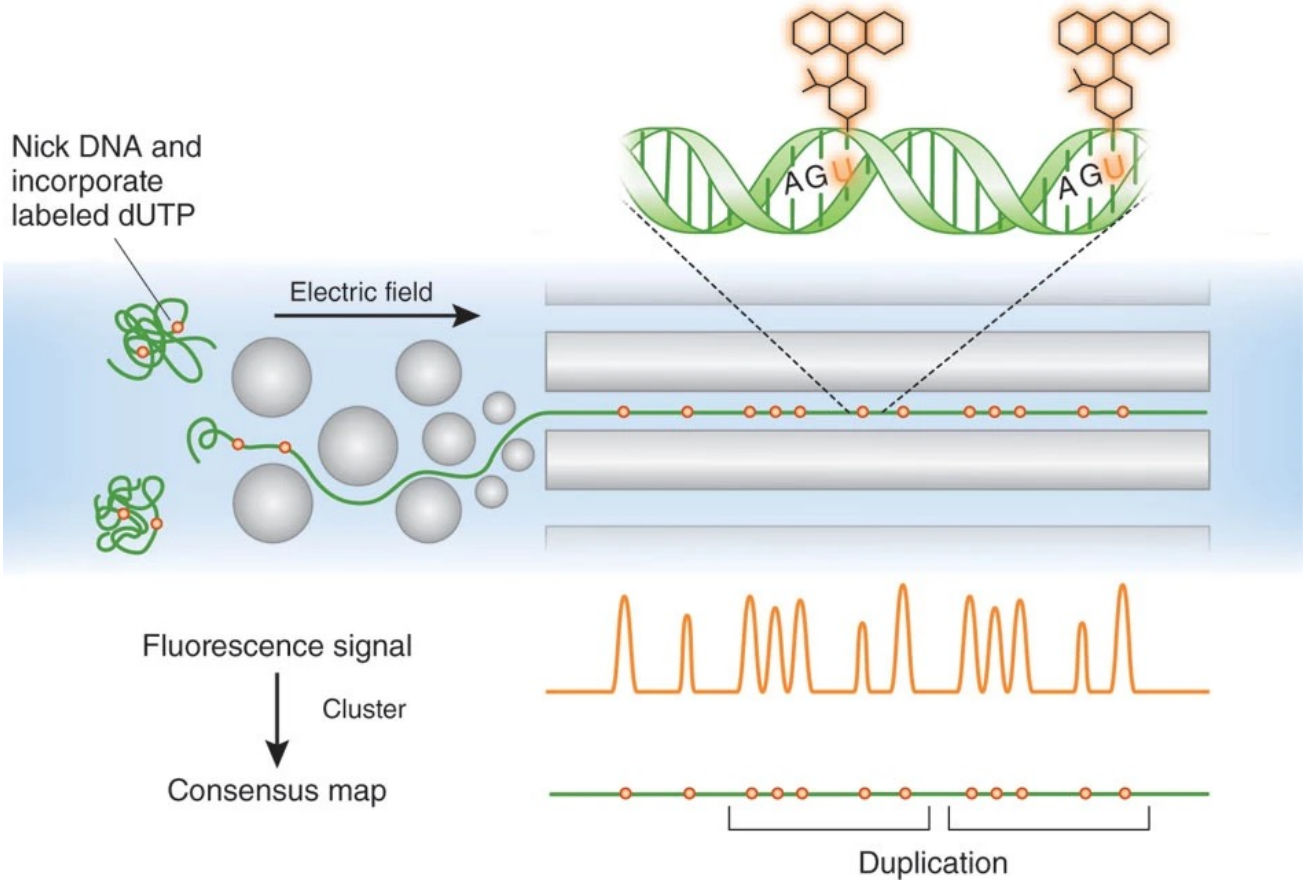


Image from MDACC website: https://www.mdanderson.org/research/research-resources/core-facilities/advanced-technology-genomics-core/services-and-fees/bionano-optical-genome-mapping.html

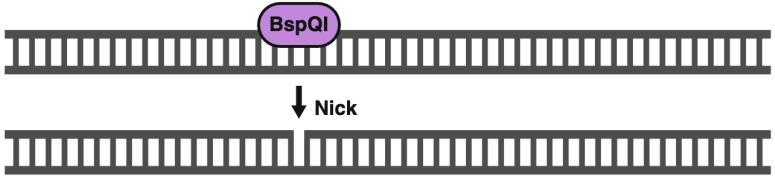
Optical Genome Mapping



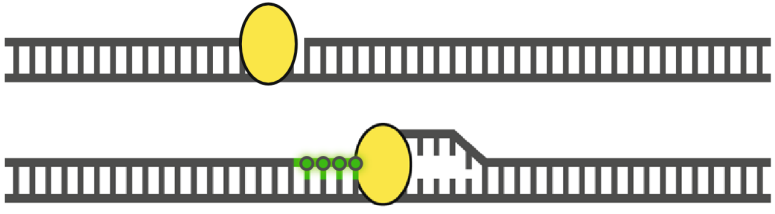
Yael Michaeli, Yuval Ebenstein. Channeling DNA for optical mapping. Nat Biotechnol. 2012 Aug;30(8):762-3. doi: 10.1038/nbt.2324.

OGM: Technical Workflow

1. Induce single-stranded breaks with nicking endonuclease (BspQI, BssSI)

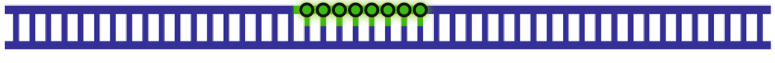


2. Taq Polymerase integrates fluorescent nucleotides at nicking site

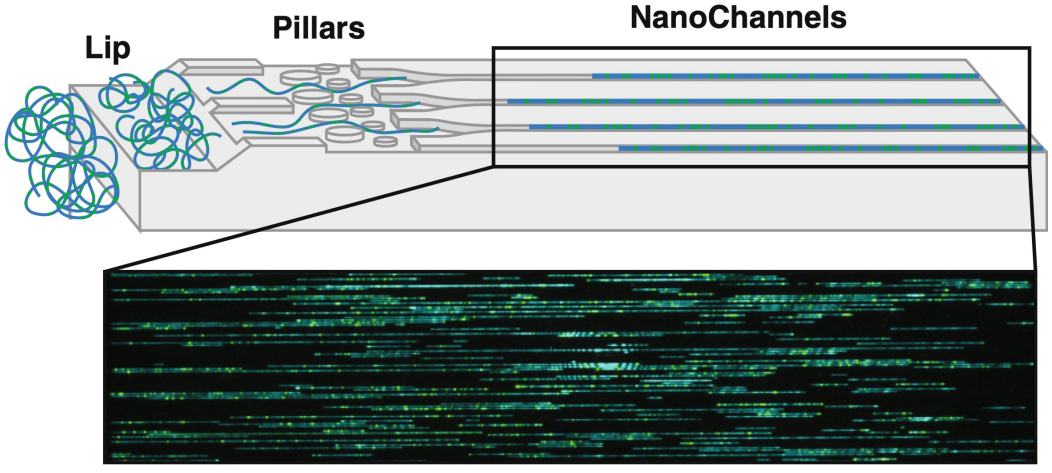


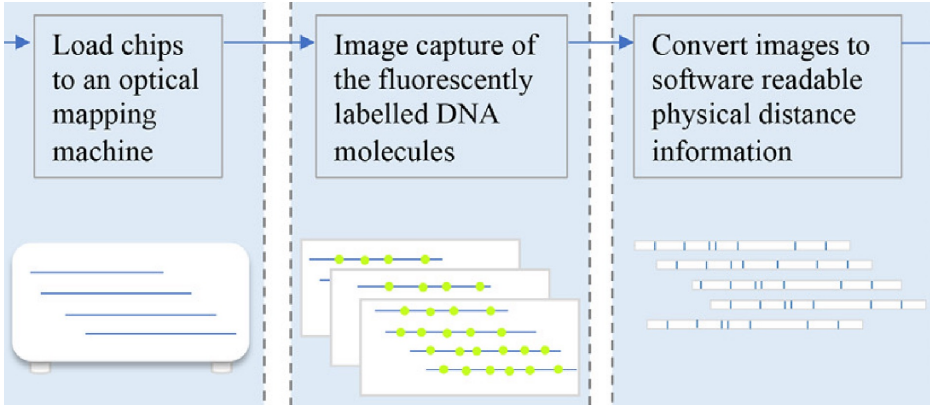
3. Ligation

4. DNA staining



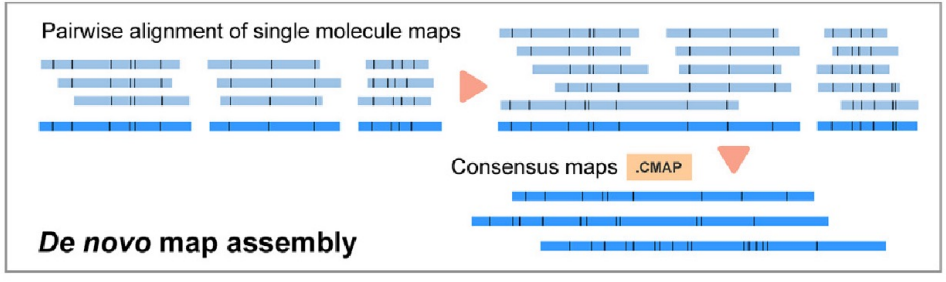
Genome Med. 2017;9(1):90. PMID: 29070057

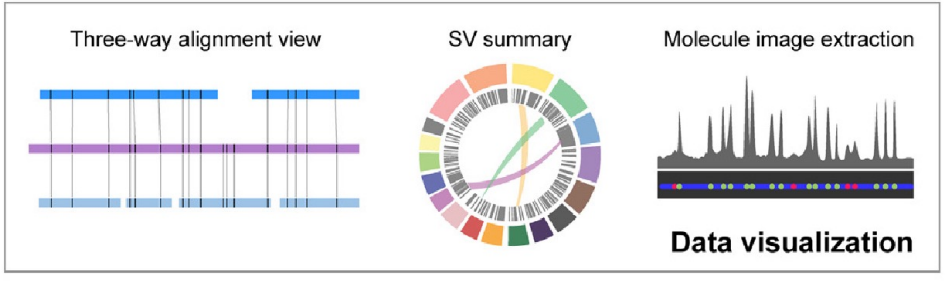


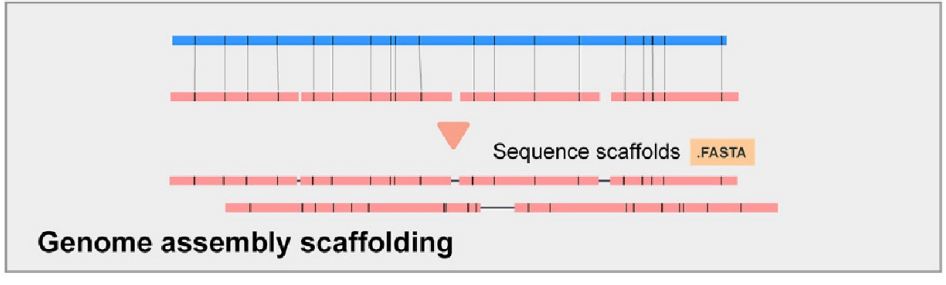


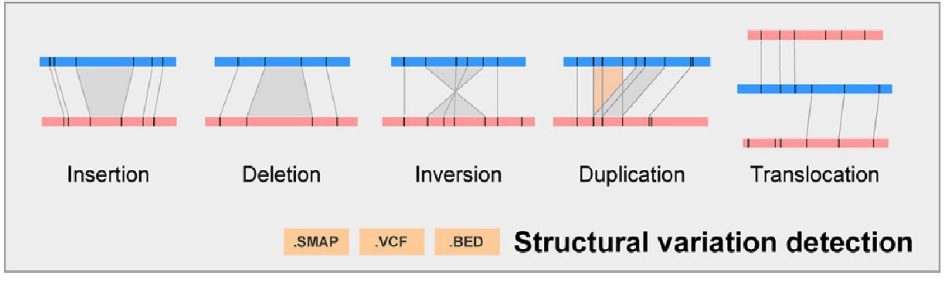
Comput Struct Biotechnol J. 2020;18:2051. PMID: 32802277

Mapping Structural Variations









Comput Struct Biotechnol J. 2020;18:2051. PMID: 32802277

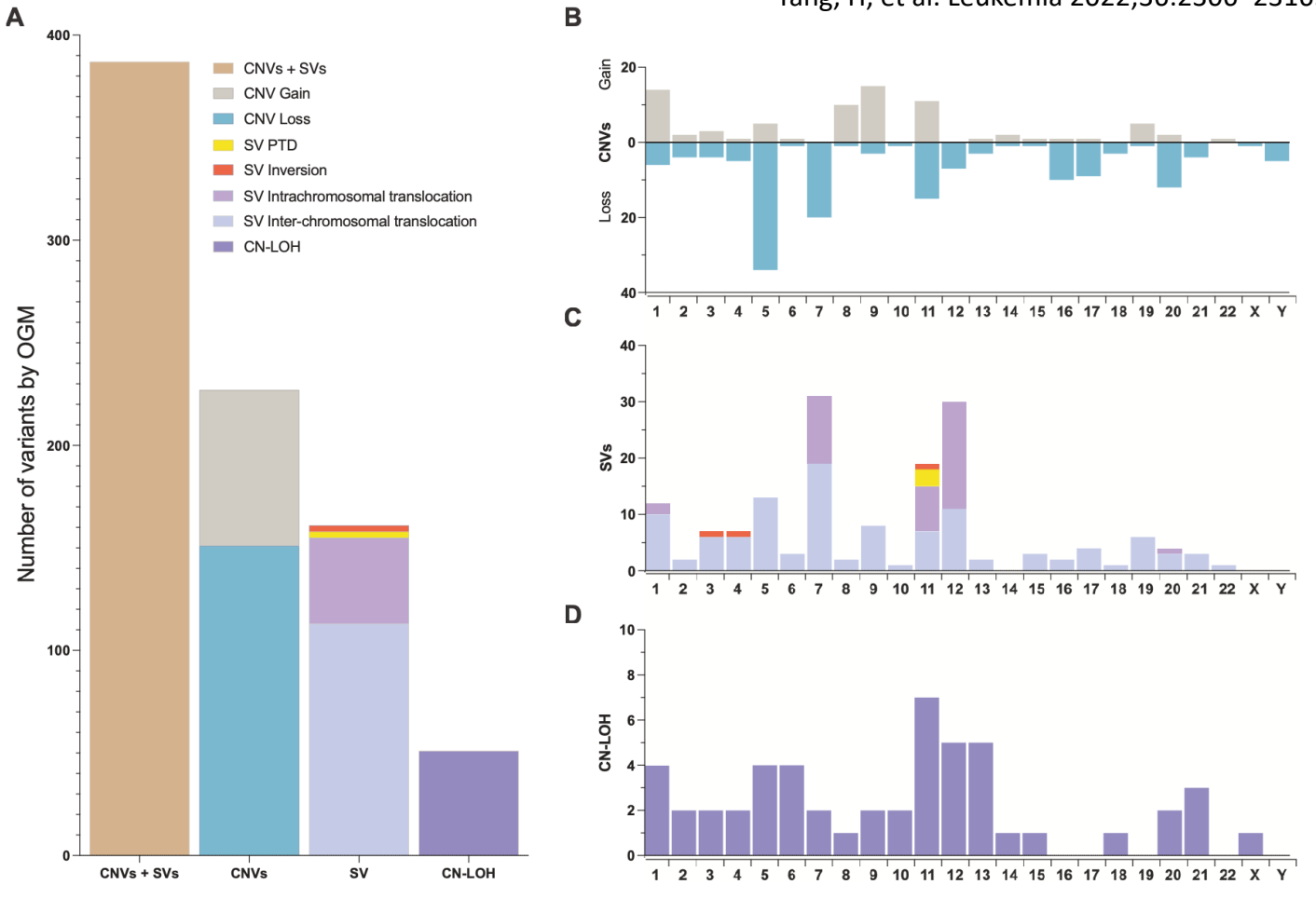
Comprehensive Genomic Methods

Technique	CG	FISH	CMA	OGM	Targeted	Exome	WGS	RNA-seq
Viable cells	Yes	No	No	No	No	No	No	No
Resolution	~5 Mb	100-200 kb	20-100 kb	5-50 kb	1 bp	1 bp	1 bp	1 bp
Coverage	Genome	Targeted	Genome	Genome	Targeted	Exome	Genome	Genome, Targeted
Alterations	CNV, SV	CNV, SV	CNV, LOH	CNV, SV	← SNV, Indel, CNV, SV, LOH →			Gene expression, SV
Sensitivity (VAF)	5%-10%	1%-5%	30%	5%	2%	5%-10%	10%	5%
TAT (days)*	2-21	1-3	3-14	4-7	5-14	5-14	3-14	5-14

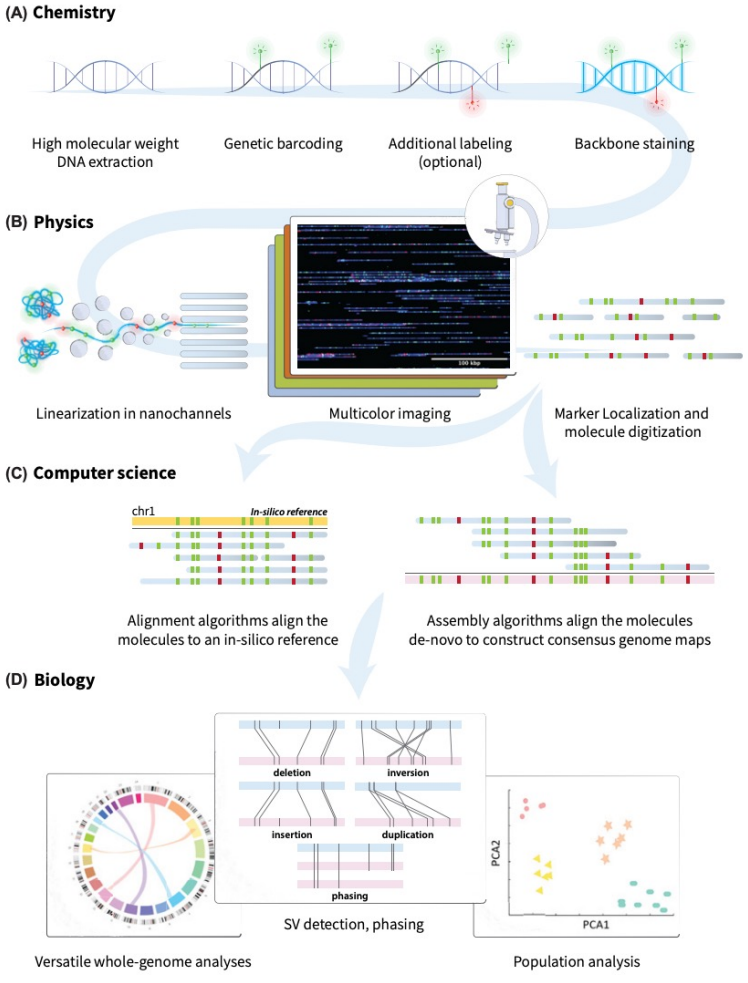
Blood (2022) 140 (21): 2228–2247

Clinically significant chromosomal aberrations in MDS detected by OGM

Yang, H, et al. Leukemia 2022;36:2306–2316. PMID: 35915143



OGM Summary



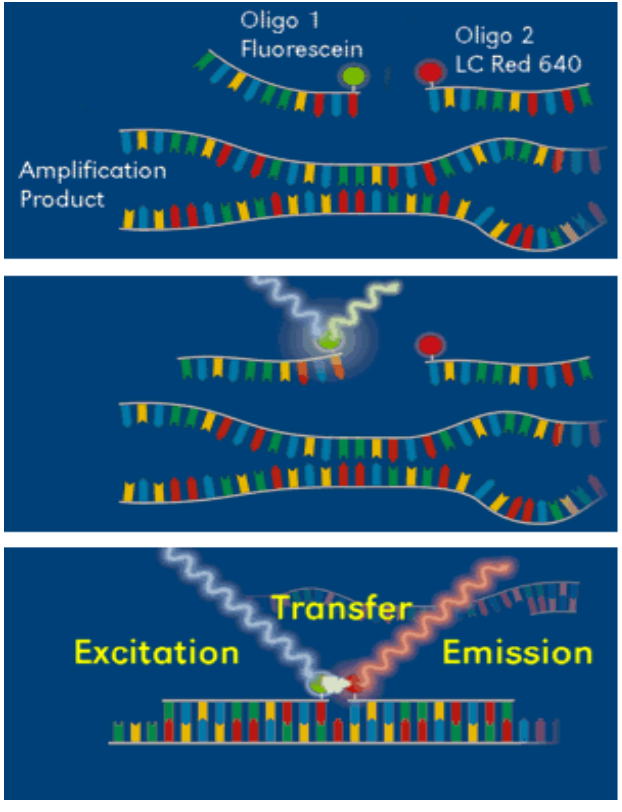
- Requires ultra-long genomic fragments (limited use for FFPE tissue)
- Single platform for high-throughput at a high resolution
- Single-molecule, no processing bias
- Genome-wide detection of all the types of SVs (CNVs, balanced and unbalanced structural variants)
- Genomic information that is otherwise inaccessible using sequencing, as low as 1% VAF
- Better resolution and better turnaround time than traditional karyotyping

Essays Biochem. 2021; 65(1): 51–66. PMID: 33739394

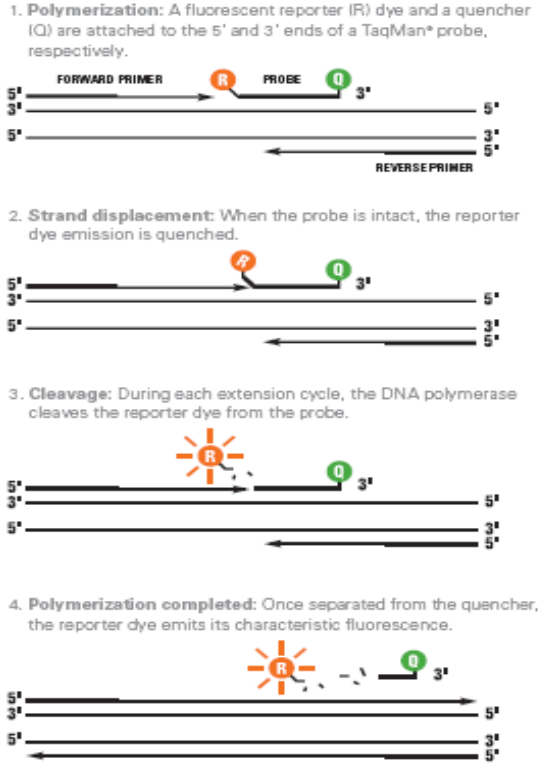
Polymerase Chain Reaction (PCR)

- End-point PCR
  - Detecting PCR products at the end of reaction
  - Useful to reveal fragment sizes
  - Can be multiplexed if product sizes are different
  - ➢ Usually needs to open the PCR tube and run the product with electrophoresis
  - ➢ Risk of contaminating the lab space
  - ➢ Not a quantitative method
- Real-time PCR
  - Detecting amount of amplicon real-time by reporter signal
  - Closed tube process, no risk of amplicon contamination
  - Easily quantitative
  - Multiplex by different fluorescence colors on the products
  - ➢ Cannot see the fragment sizes of products

Technologies qPCR (Examples)

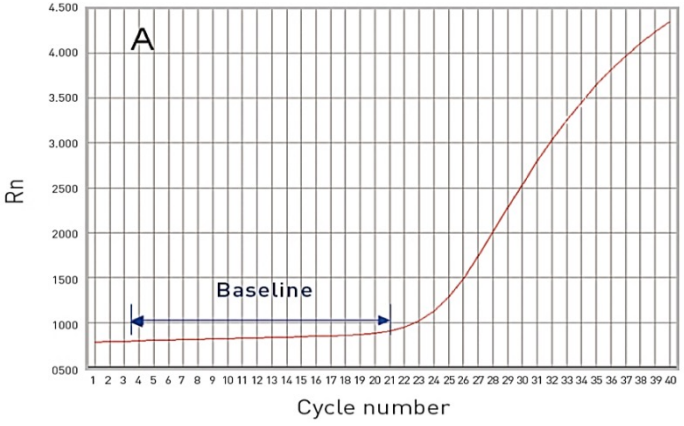


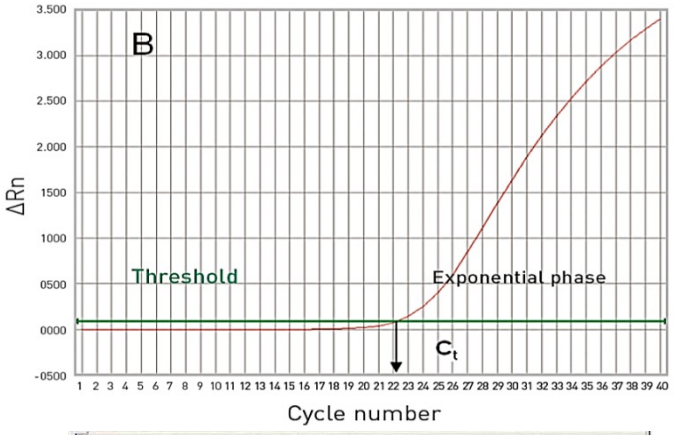
www.roche-mb.com/lightcycler.htm

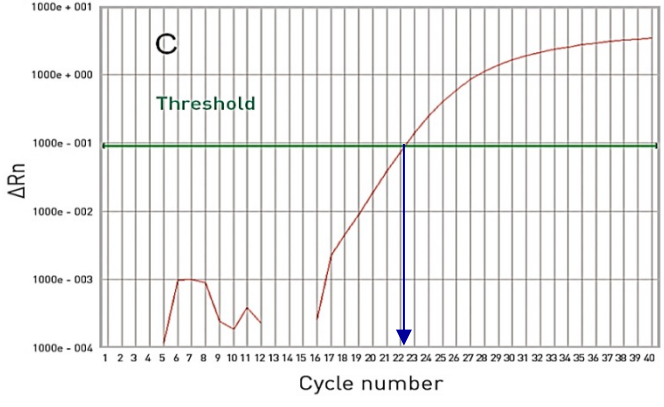


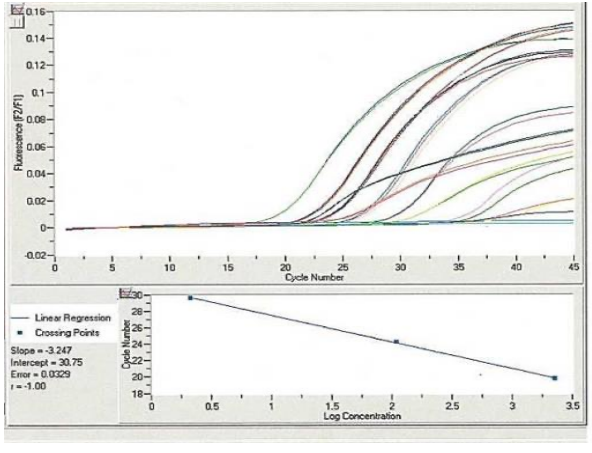
http://www3.appliedbiosystems.com/AB_Home/applicationstechnologies/Real-timePCR/TaqManvsSYBRGreenChemistries/index.htm?newGlobalNav=true

qPCR: Cycle Threshold and Quantitation





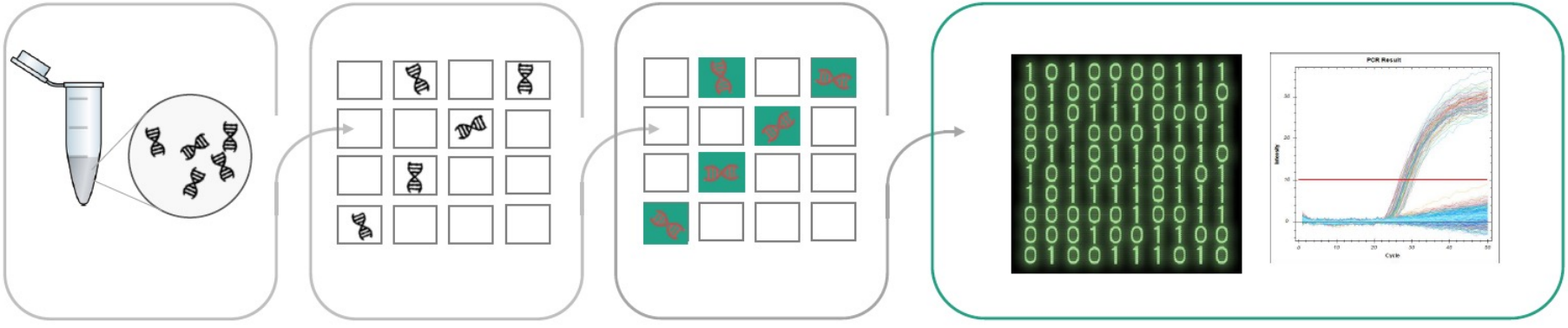




The cycle threshold converts to template concentration using a standard cure.

PRINCIPLE OF DIGITAL PCR

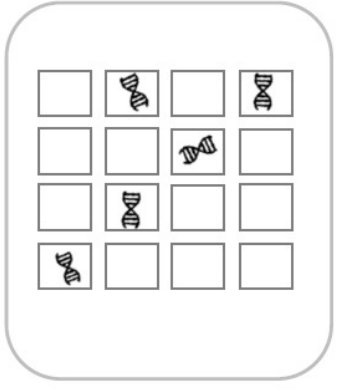
PCR REACTIONS THAT ARE DIGITALIZED



https://www.xboxlab.se/files/Produkter/whatisdigitalpcr.pdf

PRINCIPLE OF DIGITAL PCR

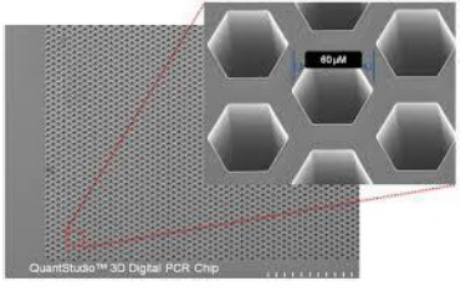
SAMPLE DISTRIBUTION



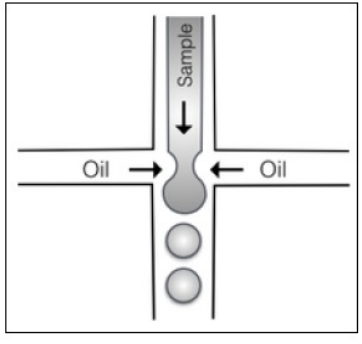
Distribution

a. Separation type

b. Droplet type



a. Separation type



b. Droplet type

https://www.xboxlab.se/files/Produkter/whatisdigitalpcr.pdf

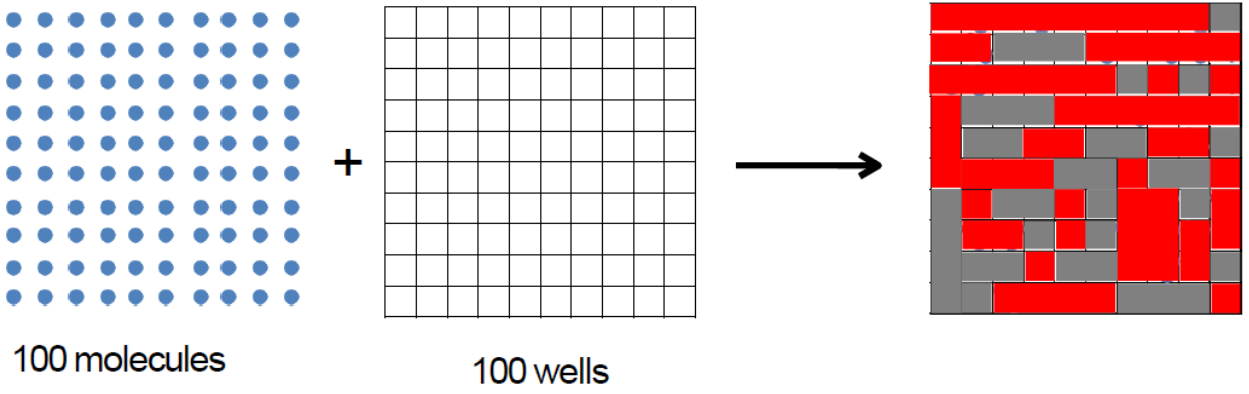
MEASURING TARGET CONCENTRATION

EVEN VS. RANDOM DISTRIBUTION

Positive well

Negative well

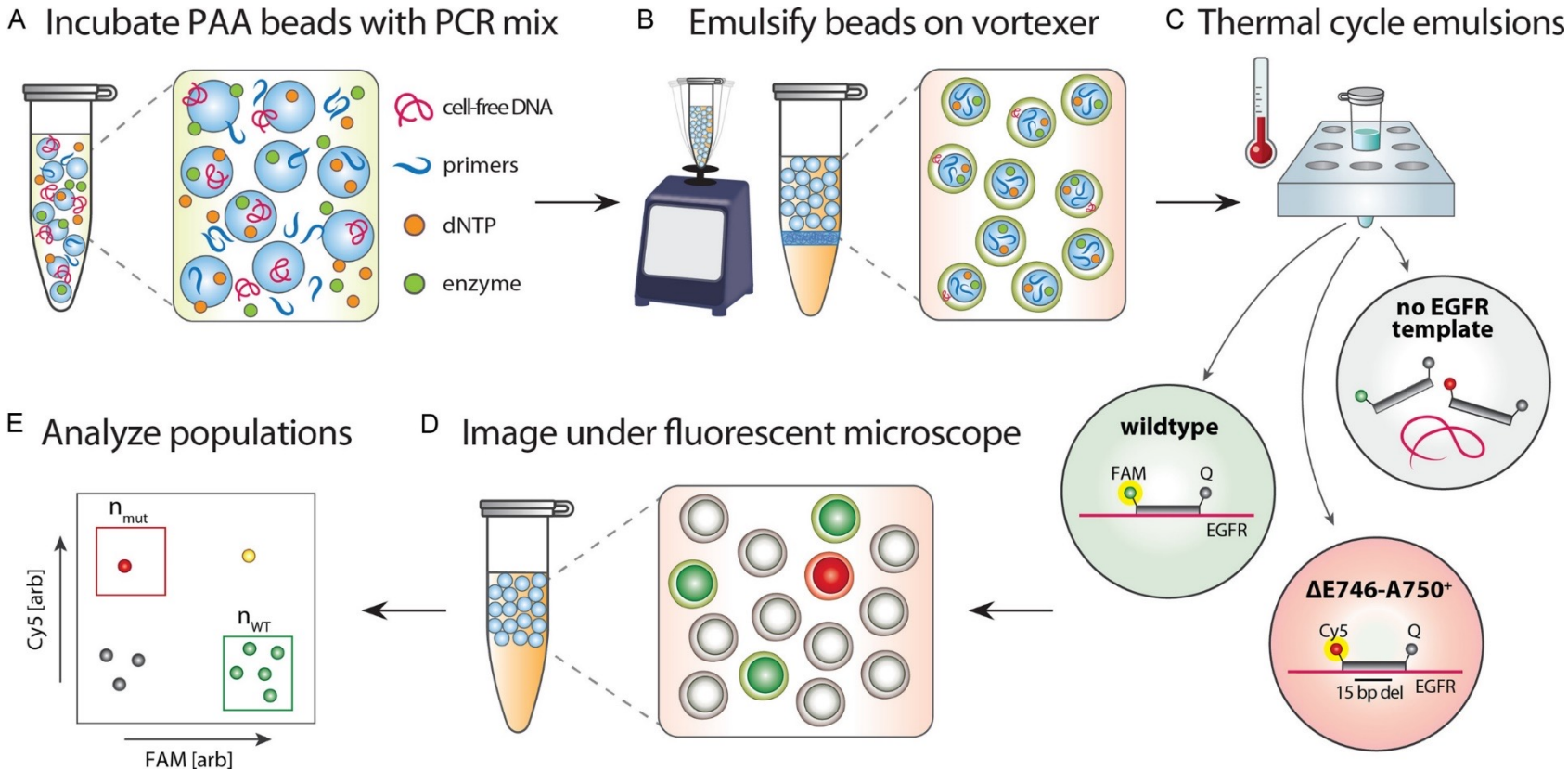
- Random distribution



37 wells with 0 molecule
63 wells with ≥1 molecules
Poisson calculated 99 molecules
95%CI 77 to 129 molecules

https://www.xboxlab.se/files/Produkter/whatisdigitalpcr.pdf

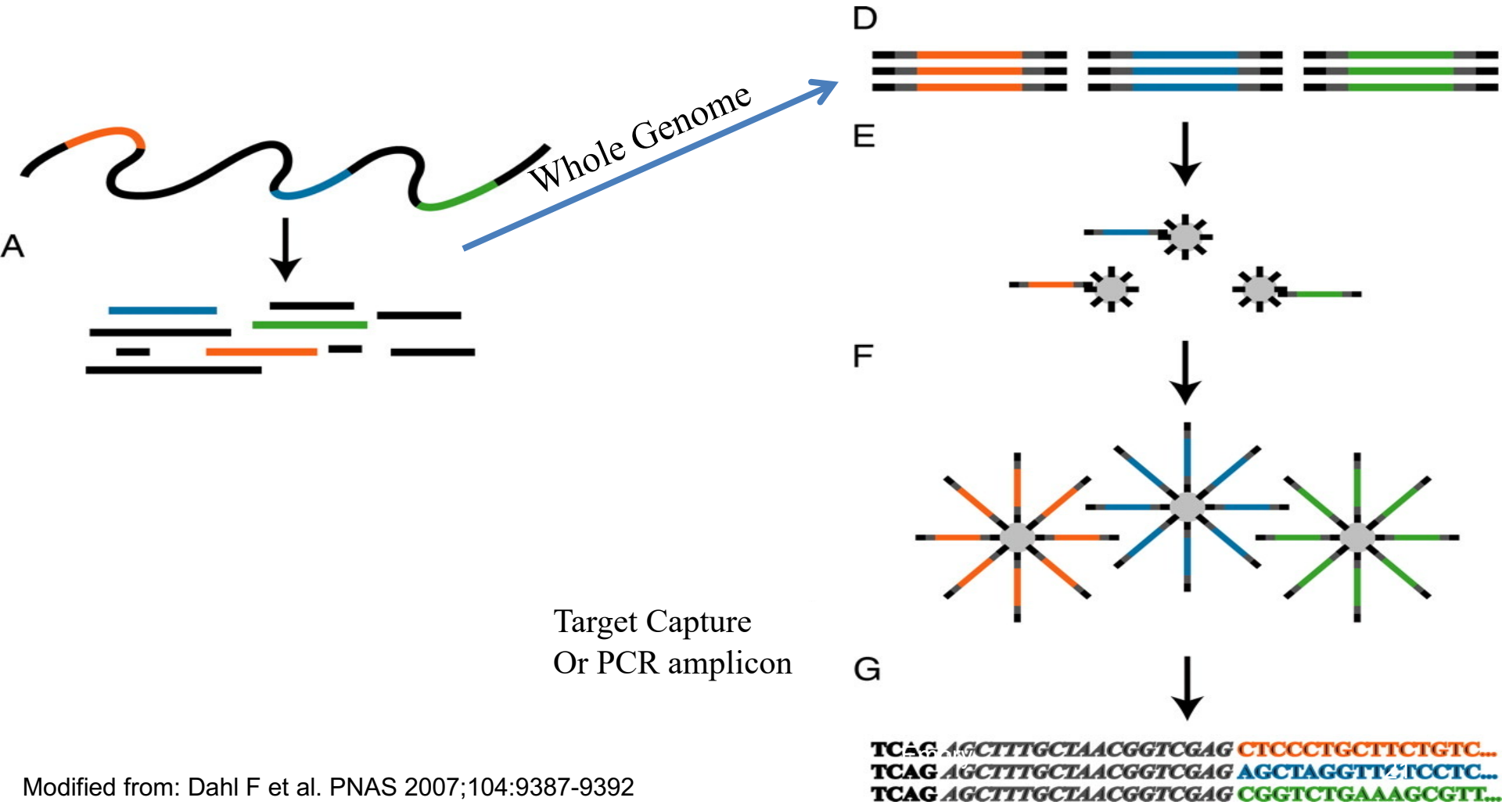
ddPCR based cfDNA mutation detection



Demaree et al. Methods in Cell Biology 2018; 148:119-131

Error Corrected NGS

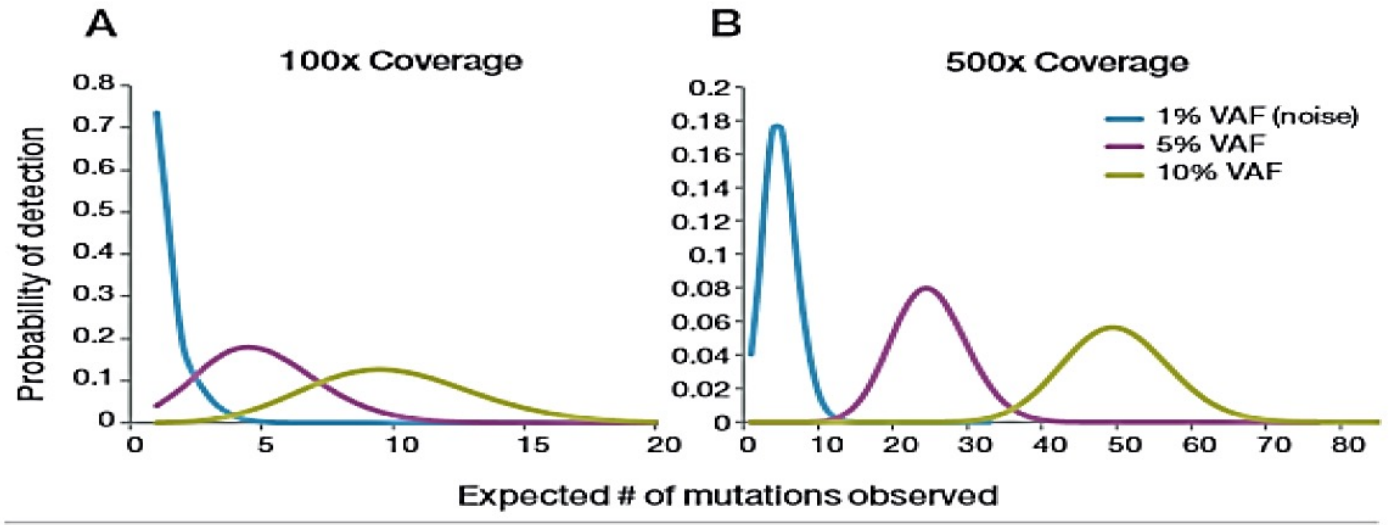
Next Generation/2nd Generation/Paralell Sequencing



Modified from: Dahl F et al. PNAS 2007;104:9387-9392

Sequencing Depth and Variant Frequency Estimation

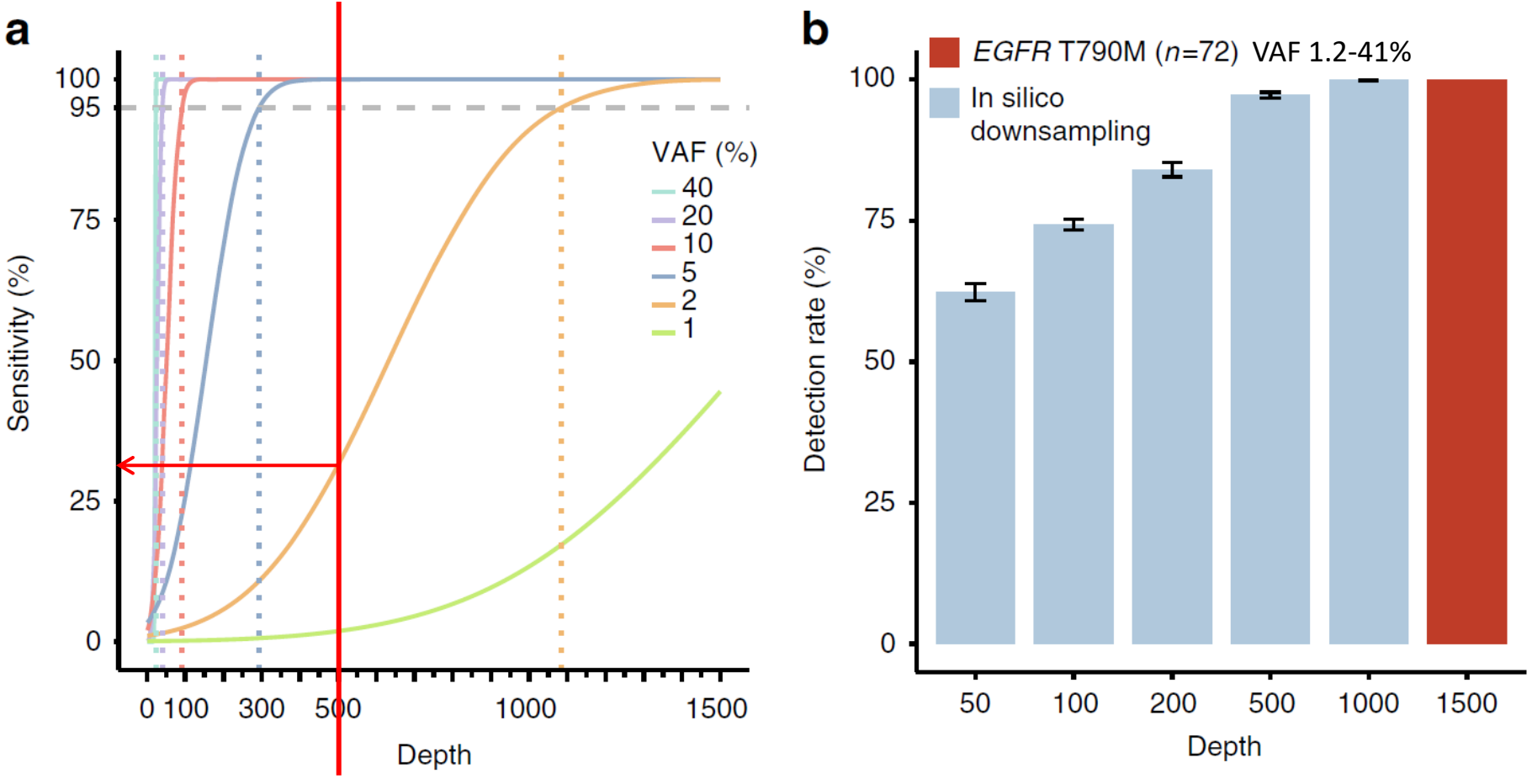
Figure 1: Impact of Coverage Depth on VAF Overlap



With only 100× coverage (panel A), there is considerable overlap between 5% and 1% VAF, inhibiting the ability to confidently call low-frequency variants below 5%. In contrast, variants below 5% frequency can be reliably called when coverage depth is increased to > 500× coverage (panel B).

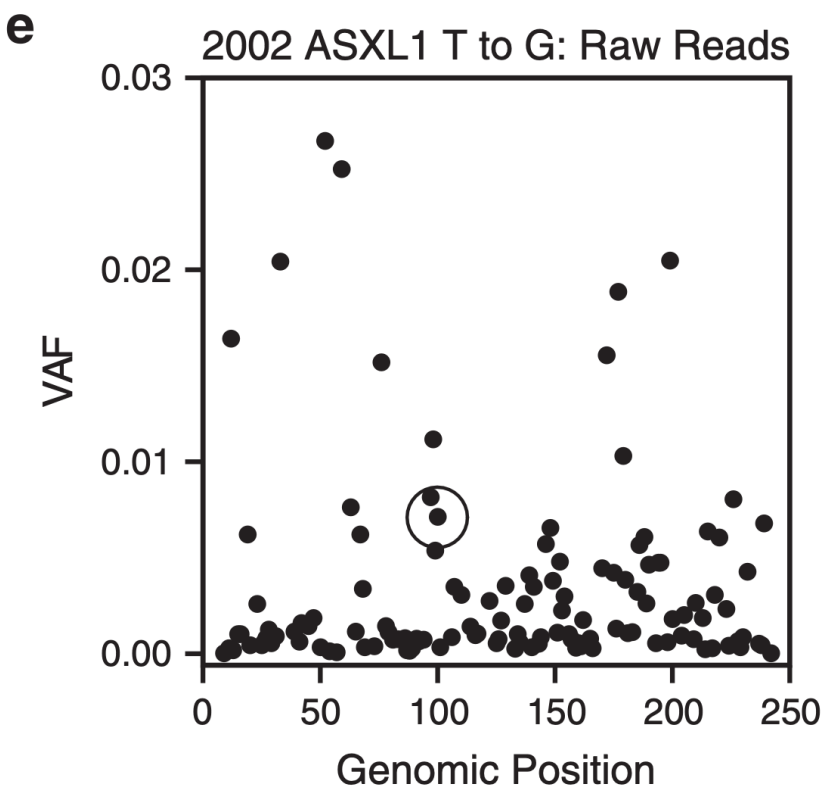
Frank Kuo, 2017 Society of Hematopathology Workshop

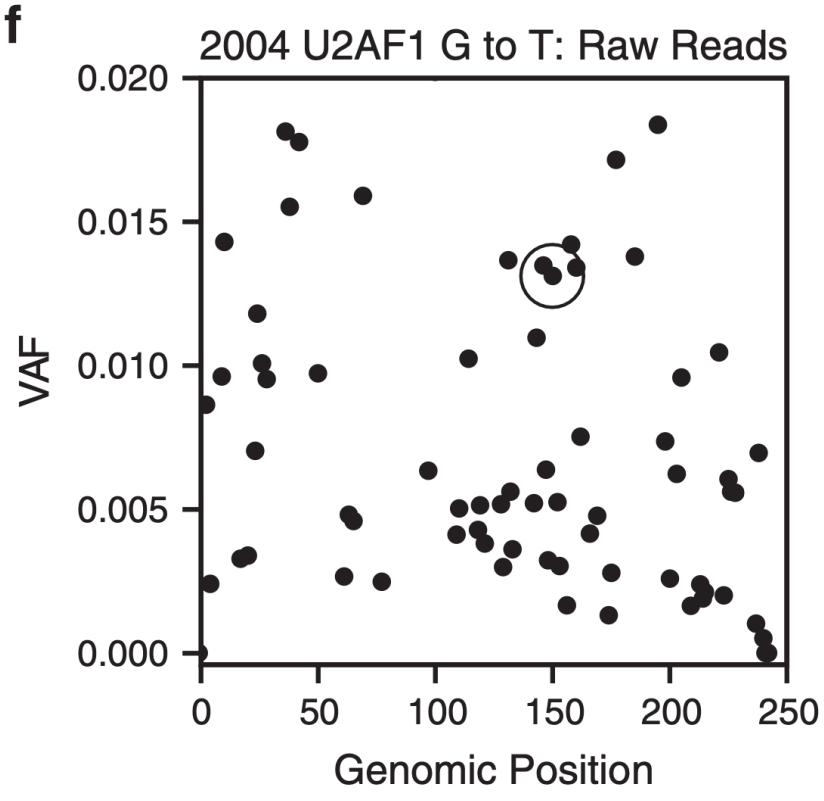
Sequencing depth and detection sensitivity



Shin H. et al. Nat Commun. 2017; 8: 1377

Noise of conventional NGS (Specificity)

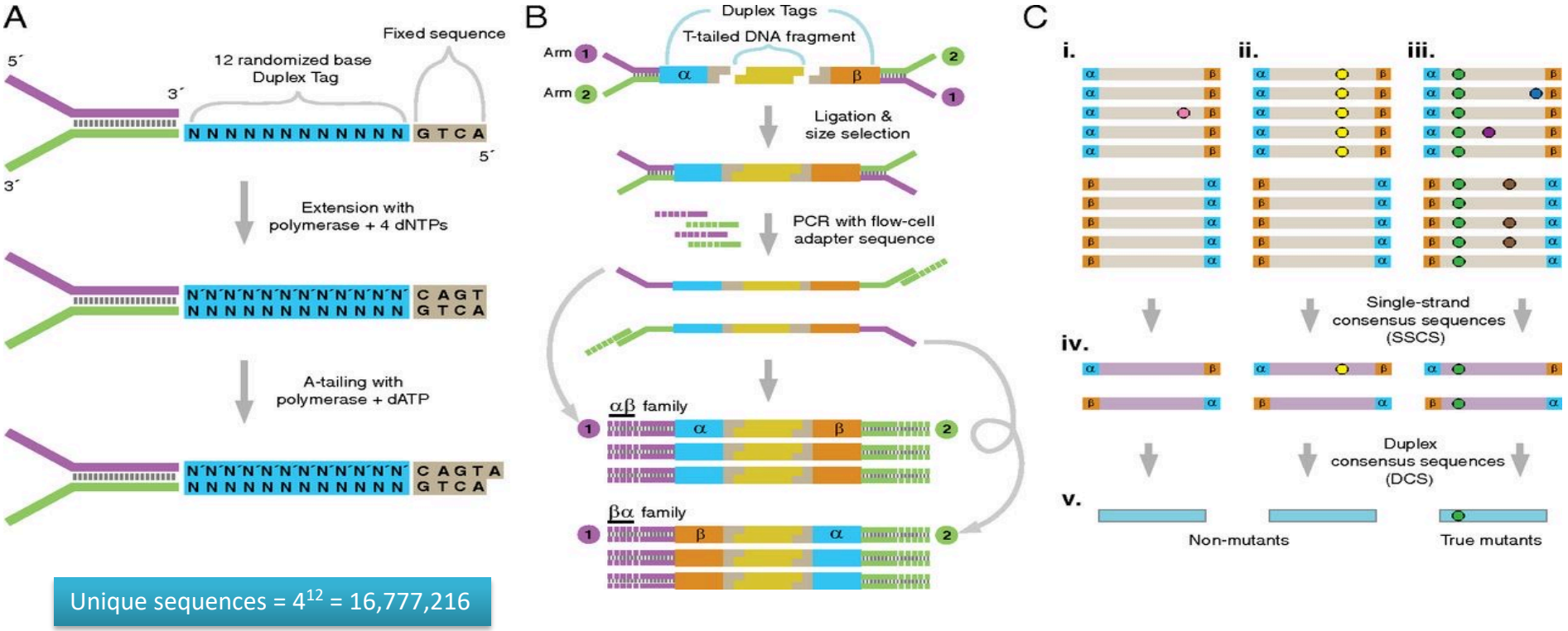




Leukemia 2015; 29:1608-11. PMID: 25644247

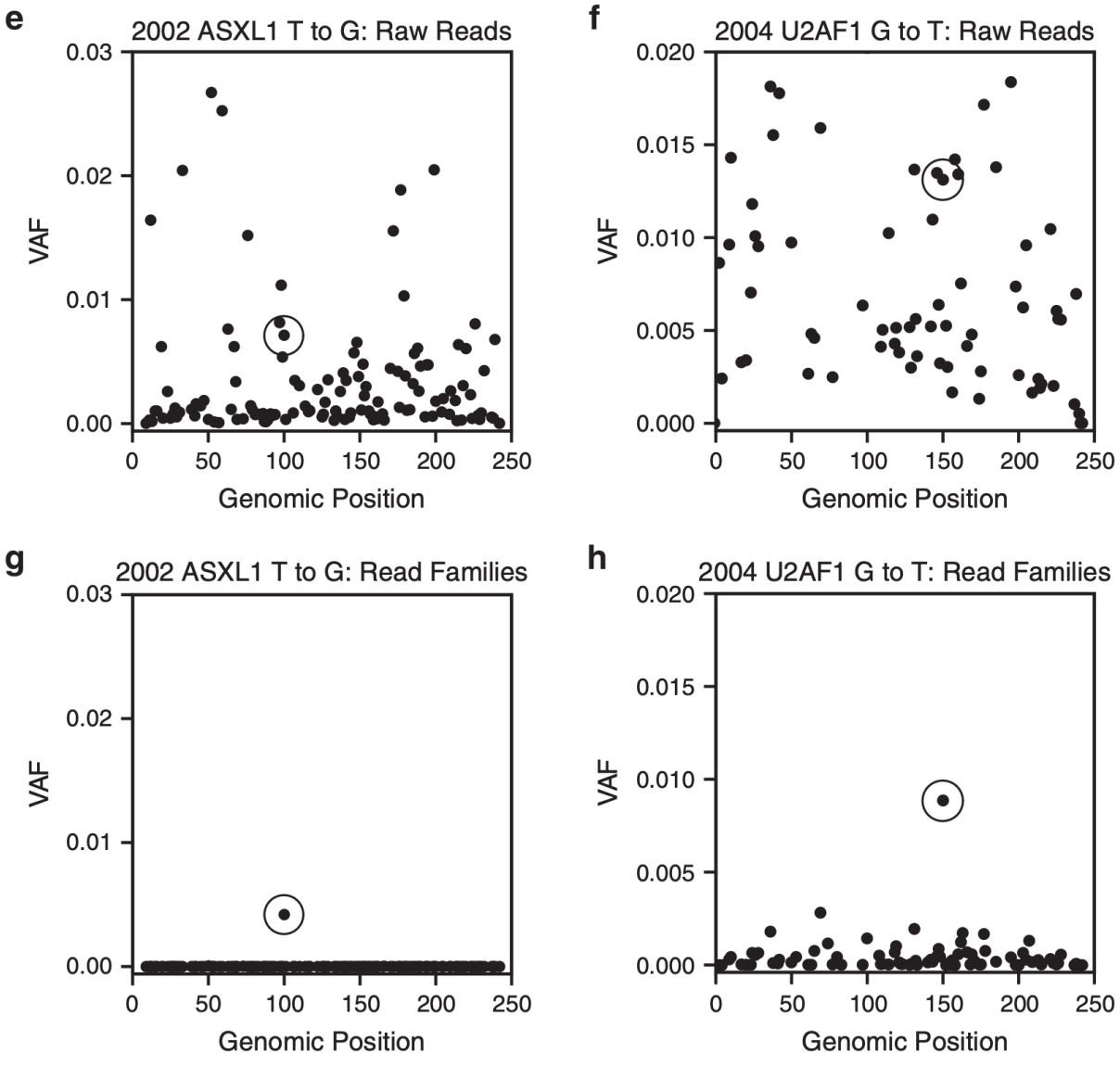
Routine NGS has an error rate of 0.5 ~ 2.0%

UMI Duplex Error Corrected NGS



Unique sequences = 4^{12} = 16,777,216

Michael W. Schmitt et al. PNAS 2012;109:14508-14513



Error corrected NGS cleared the noise

Sensitivity may reach <0.01%

Leukemia 2015; 29:1608-11

Frequently asked questions in practice

- How do you choose between different molecular and genetic tests?
- What is the utility of mutation results in diagnosing myeloid neoplasms (especially MDS, MPN and MDS/MPN)?
- How do I decide when to order clonality tests?
- How is a positive clonality result helping my diagnosis of lymphoma?
- Do you have an NGS mutation profiling test for lymphomas, and how helpful is it in your practice?

CG vs FISH

Blood (2022) 140 (21): 2228–2247

Technique	CG	FISH	CMA	OGM	Targeted	Exome	WGS	RNA-seq
Viable cells	Yes	No	No	No	No	No	No	No
Resolution	~5 Mb	100-200 kb	20-100 kb	5-50 kb	1 bp	1 bp	1 bp	1 bp
Coverage	Genome	Targeted	Genome	Genome	Targeted	Exome	Genome	Genome, Targeted
Alterations	CNV, SV	CNV, SV	CNV, LOH	CNV, SV	← SNV, Indel, CNV, SV, LOH →			Gene expression, SV
Sensitivity (VAF)	5%-10%	1%-5%	30%	5%	2%	5%-10%	10%	5%
TAT (days)*	2-21	1-3	3-14	4-7	5-14	5-14	3-14	5-14

In adult AML, FISH rarely provides additional information when karyotyping is adequate (20 or more metaphases were analyzed).

R. He, et al. Am J Clin Pathol 2015;143(6):873-8. PMID: 25972330

FISH vs. PCR

Blood (2022) 140 (21): 2228–2247

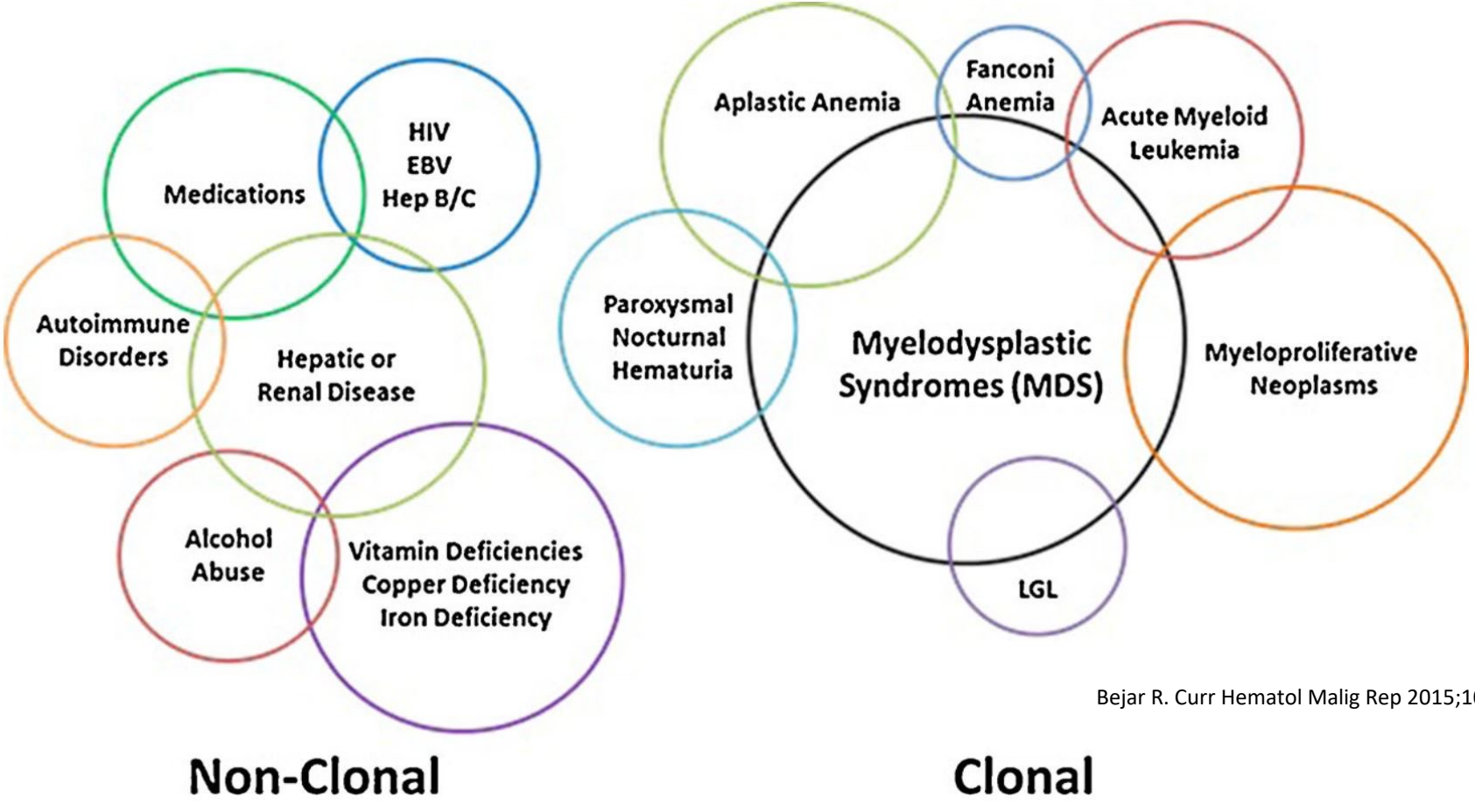
Technique	CG	FISH	CMA	OGM	Targeted	Exome	WGS	RNA-seq
Viable cells	Yes	No	No	No	No	No	No	No
Resolution	~5 Mb	100-200 kb	20-100 kb	5-50 kb	1 bp	1 bp	1 bp	1 bp
Coverage	Genome	Targeted	Genome	Genome	Targeted	Exome	Genome	Genome, Targeted
Alterations	CNV, SV	CNV, SV	CNV, LOH	CNV, SV	← SNV, Indel, CNV, SV, LOH →			Gene expression, SV
Sensitivity (VAF)	5%-10%	1%-5%	30%	5%	2%	5%-10%	10%	5%
TAT (days)*	2-21	1-3	3-14	4-7	5-14	5-14	3-14	5-14

When there is no fusion RNA product, FISH is clinically more sensitive than PCR for the diagnosis of hematolymphoid neoplasms.

- *PML::RARA*: both FISH and PCR are good for quick TAT test;
- *BCR::ABL1*: Always start with FISH test!
- Lymphoma fusions: FISH almost always better than PCR.

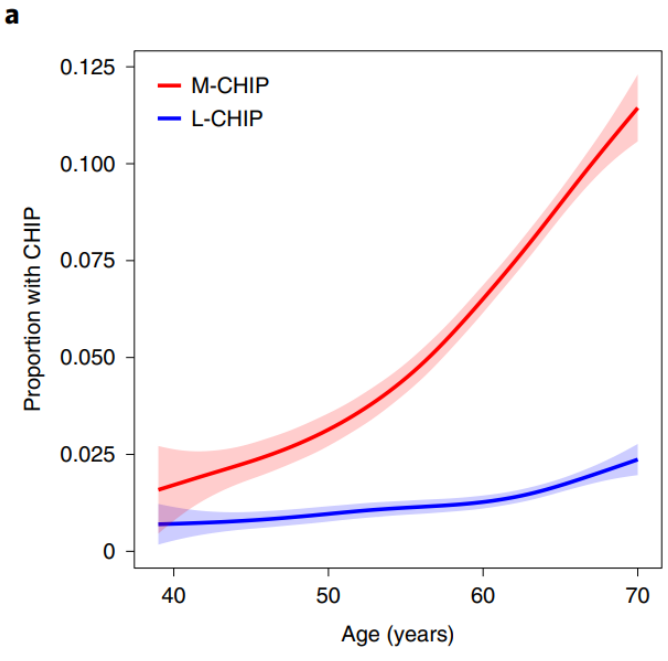
Cytopenia and MDS

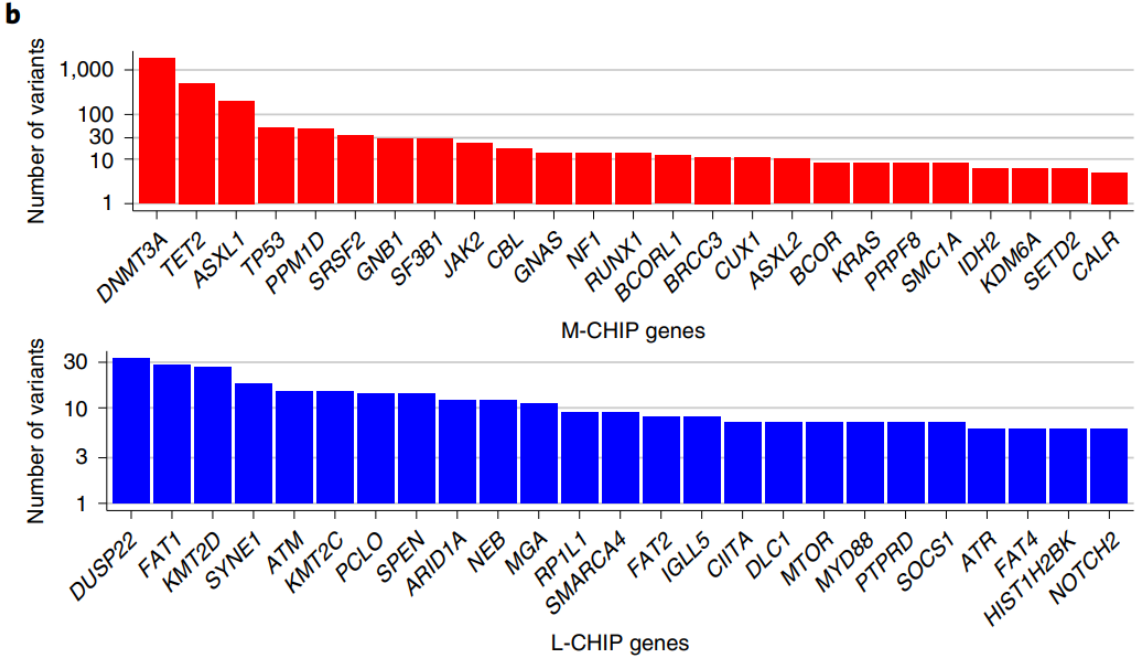
Differential Diagnosis of Cytopenia(s)



Bejar R. Curr Hematol Malig Rep 2015;10:282

Mutation accumulates with increased age

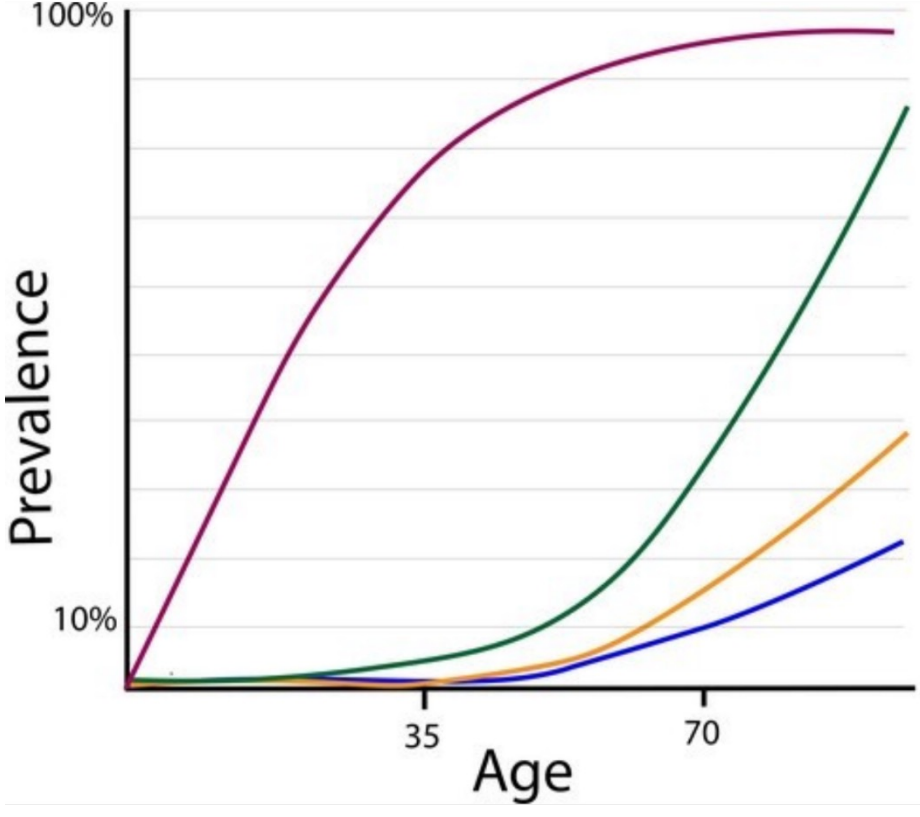




Total cases tested: 46,706 aged 40-70

Niroula A. et al. Nat Med. 2021 Nov;27(11):1921-1927. PMID: 34663986

Mutation accumulates with increased age



ECS (VAF > 0.01%)
Young et al., *Nat Comm* 2017

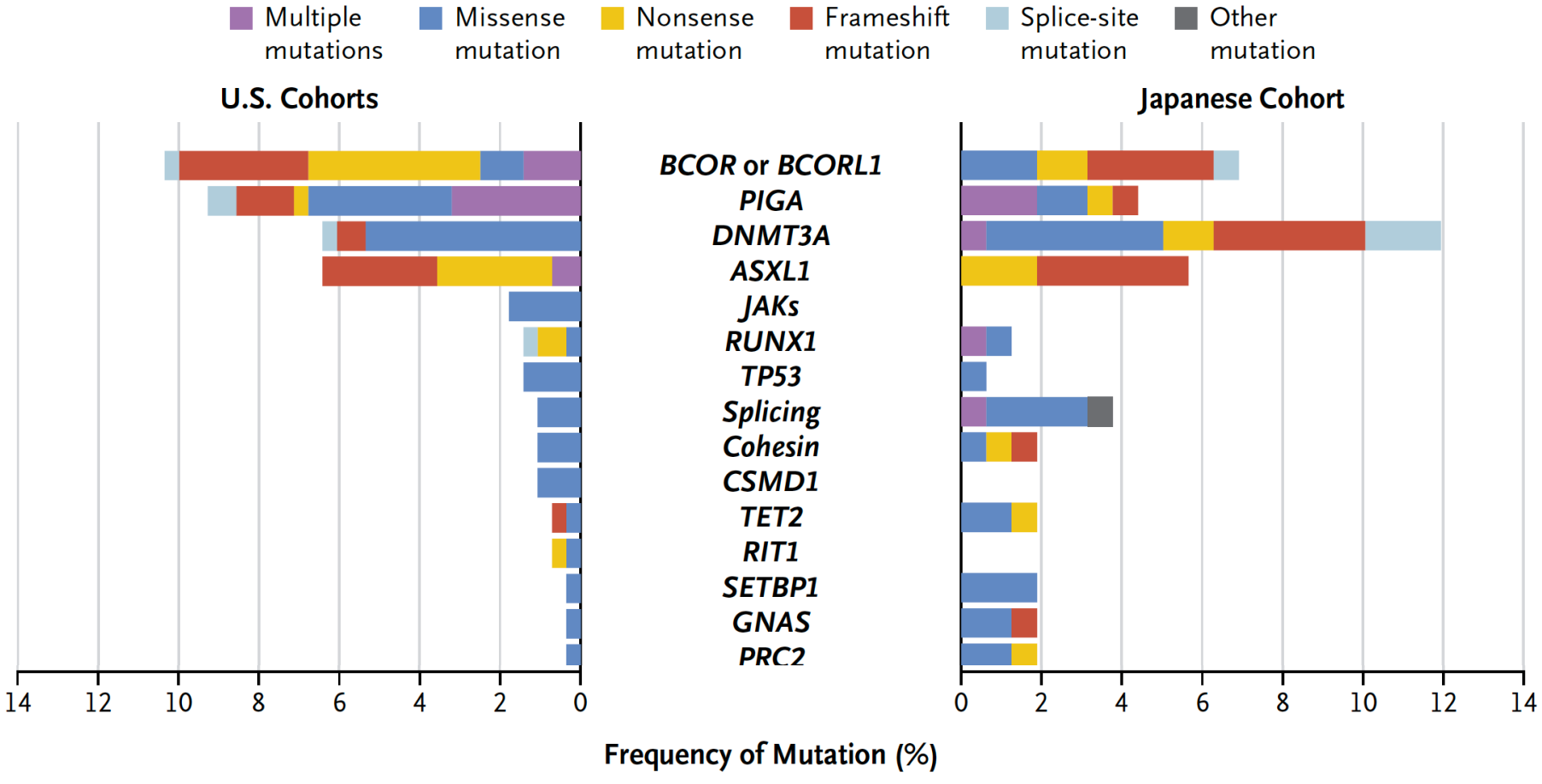
Targeted (VAF > 0.5%)
Abelson et al., *Nature* 2018
Desai et al., *Nat Med* 2018

WES (VAF > 3%)
Jaiswal et al., *NEJM* 2014
Genovese et al., *NEJM* 2014

WGS (VAF > 7%)
Zink et al., *Blood* 2017

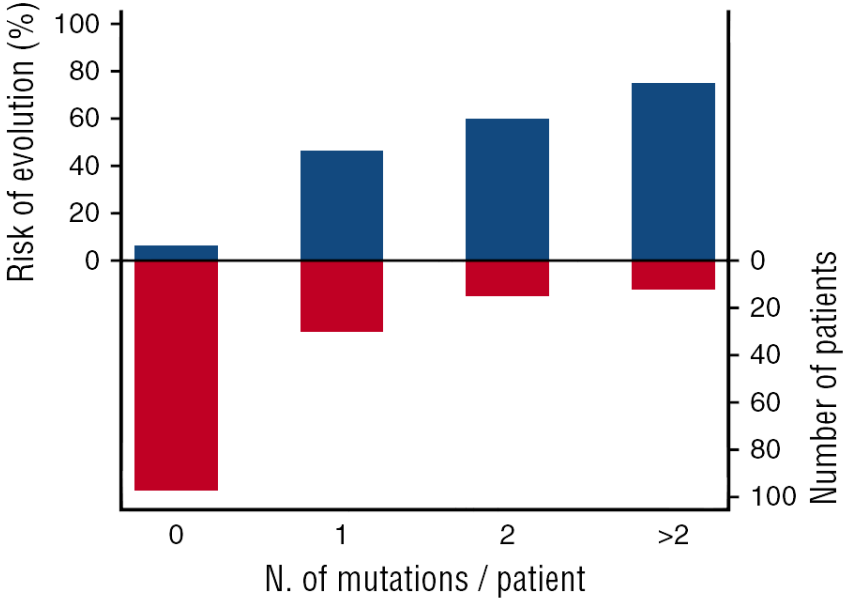
WHO-HAEM5 Blue Book Online

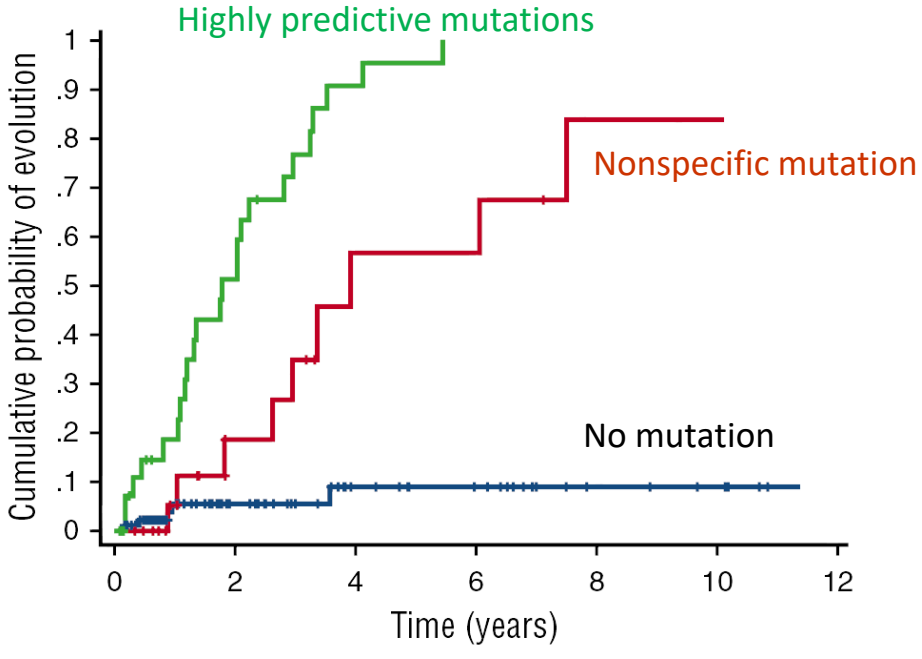
Somatic Mutations in Aplastic Anemia



Yoshizato T. et al. N Engl J Med 2015;373:35-47. PMID: 26132940; only top 15 are shown

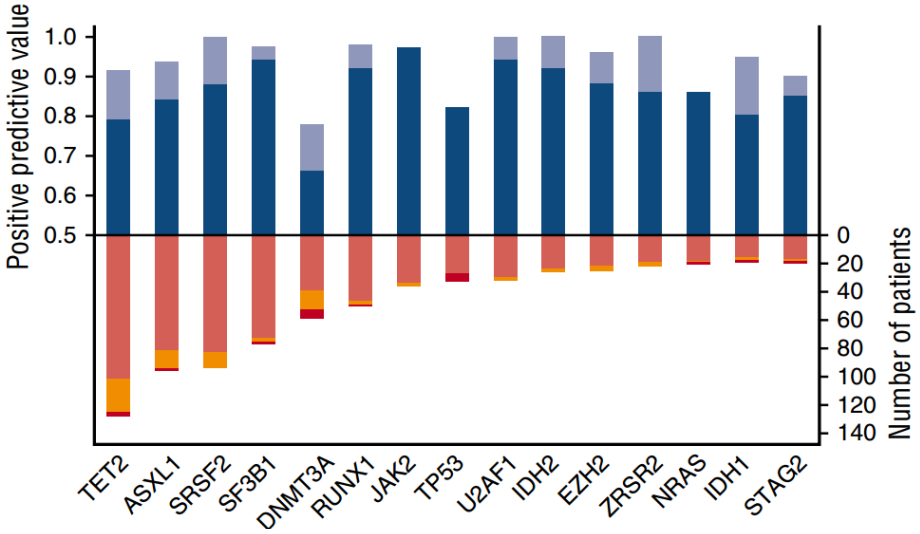
Mutation pattern is predictive of evolving to MDS

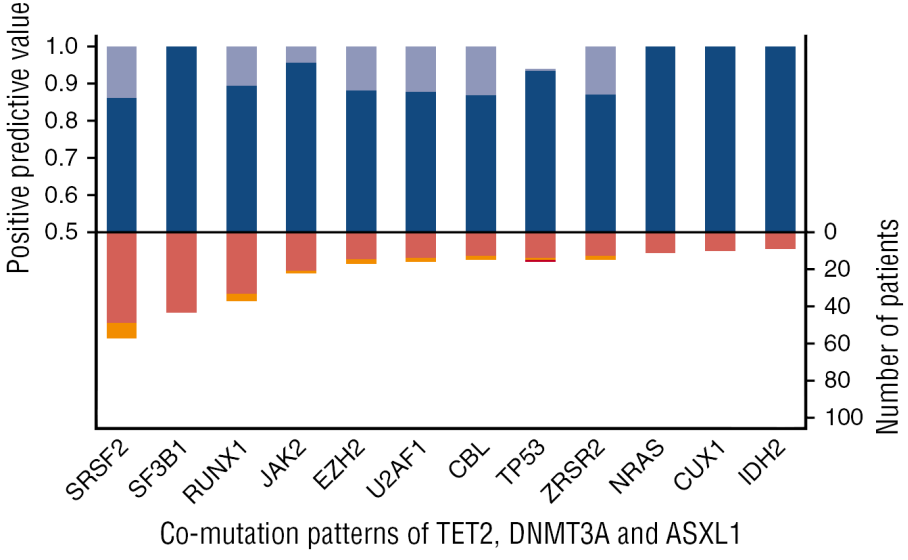




Malcovati, L. et al. Blood. 2017;129:3371-3378

CCUS: All mutations are not Equal





Mutations have high specificity for myeloid neoplasm with myelodysplasia:

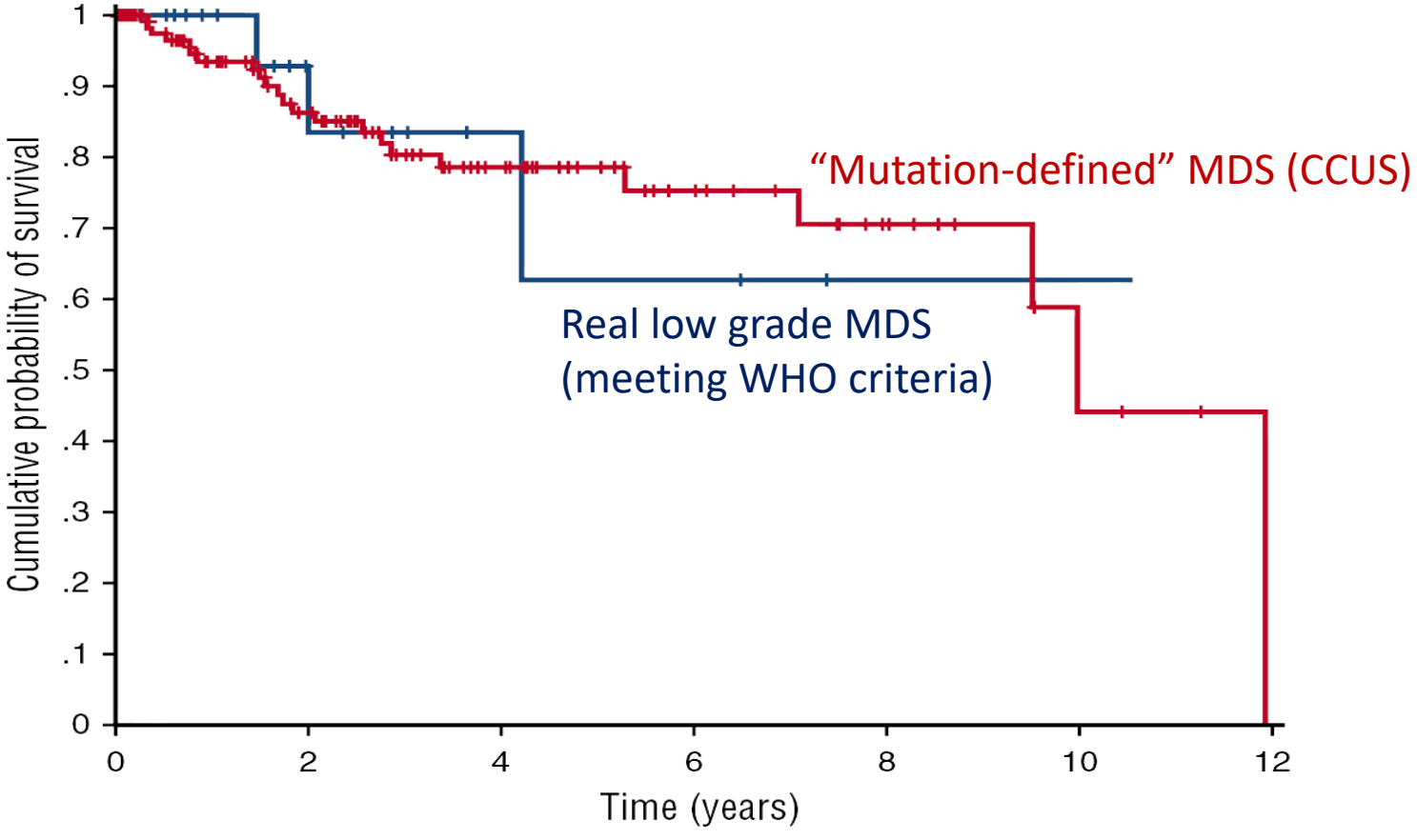
- Spliceosome genes*: SF3B1, ZRSR2, SRSF2*, *U2AF1* and *JAK2* (excluding *PMF*)
- Co-mutation patterns involving *TET2, ASXL1*, or *DNMT3A*:

  RUNX1, EZH2, CBL, BCOR, CUX1, TP53, or *IDH1/IDH2*

Malcovati, L. et al. Blood. 2017;129:3371-3378

OS of patients with CCUS and highly specific mutation pattern and of patients with myeloid neoplasm with myelodysplasia

Malcovati, L. et al. Blood. 2017;129:3371



Diagnostic performance of targeted NGS for cytopenias

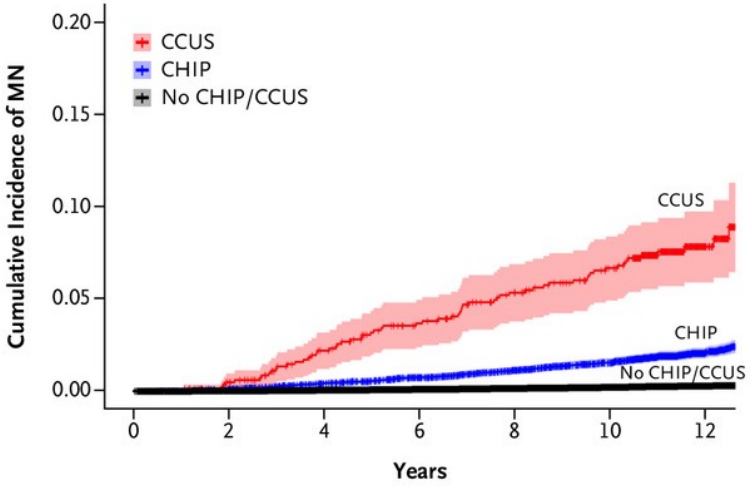
TABLE 2 Diagnostic performance of mutations for MDS with different cutoffs. The sensitivity, specificity, positive predictive value and negative predictive value with different VAF cutoffs and number of mutations for MDS in different NGS panels

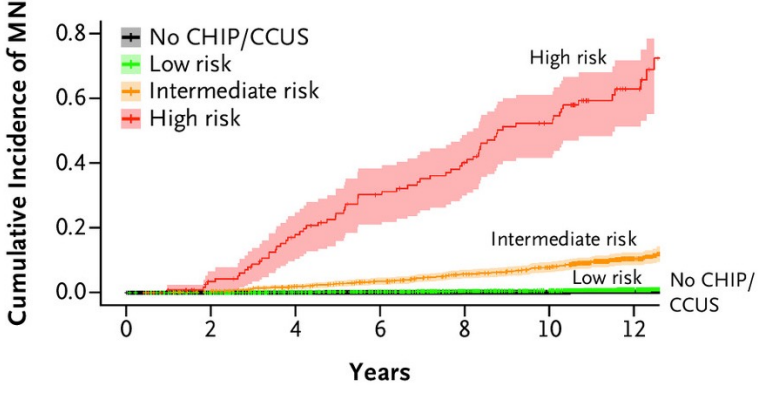
	Any mutations (VAF ≥ 1%)	VAF ≥ 20%	≥2 mutations	VAF ≥ 10% and ≥2 mutations
640 gene panel				
Sensitivity	98.3%	81.7%	84.3%	68.7%
Specificity	47.6%	95.3%	65.1%	95.3%
NPV	95.3%	79.6%	75.7%	69.5%
PPV	71.5%	95.9%	76.3%	95.2%
41 gene panel				
Sensitivity	95.7%	75.7%	69.6%	53.0%
Specificity	72.1%	100%	91.9%	100%
NPV	92.4%	75.4%	69.3%	61.4%
PPV	81.5%	100%	91.9%	100%

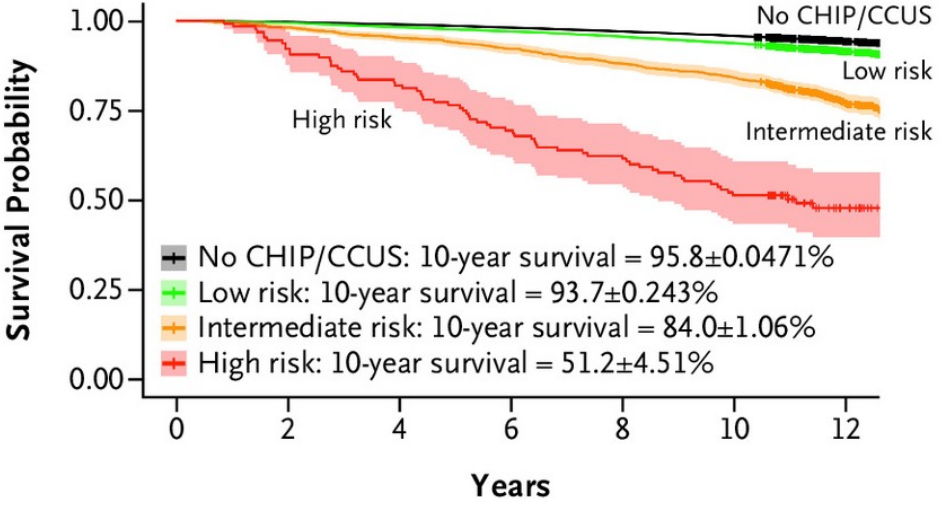
Abbreviations: NPV: negative predictive value; PPV: positive predictive value.

Zheng, G. et al. Am J Hematol. 2019; 94(10):1141. PMID: 31350794

Risk of developing myeloid neoplasms: Groups

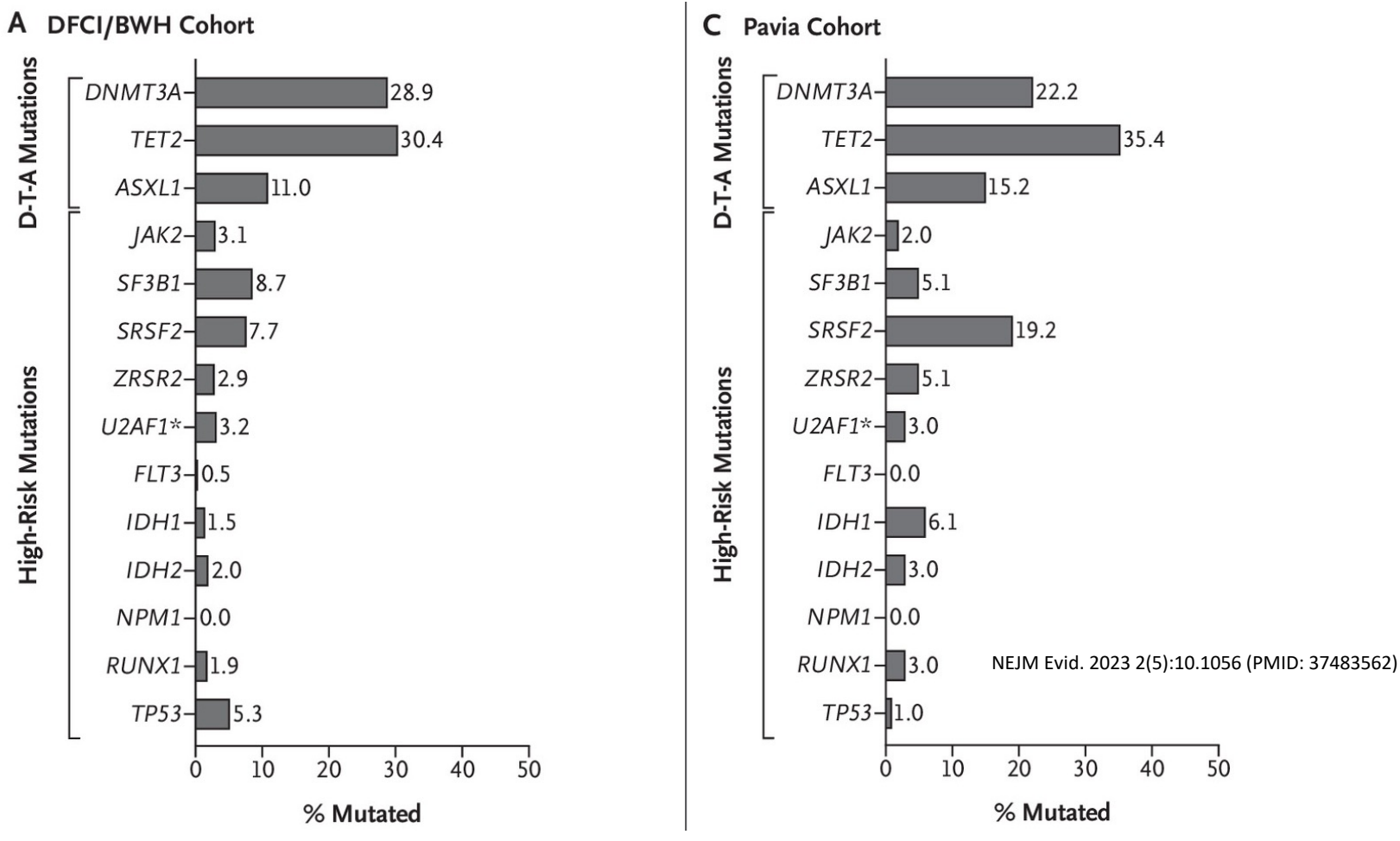






NEJM Evid. 2023 2(5):10.1056 (PMID: 37483562)

Risk of developing myeloid neoplasms: Genes

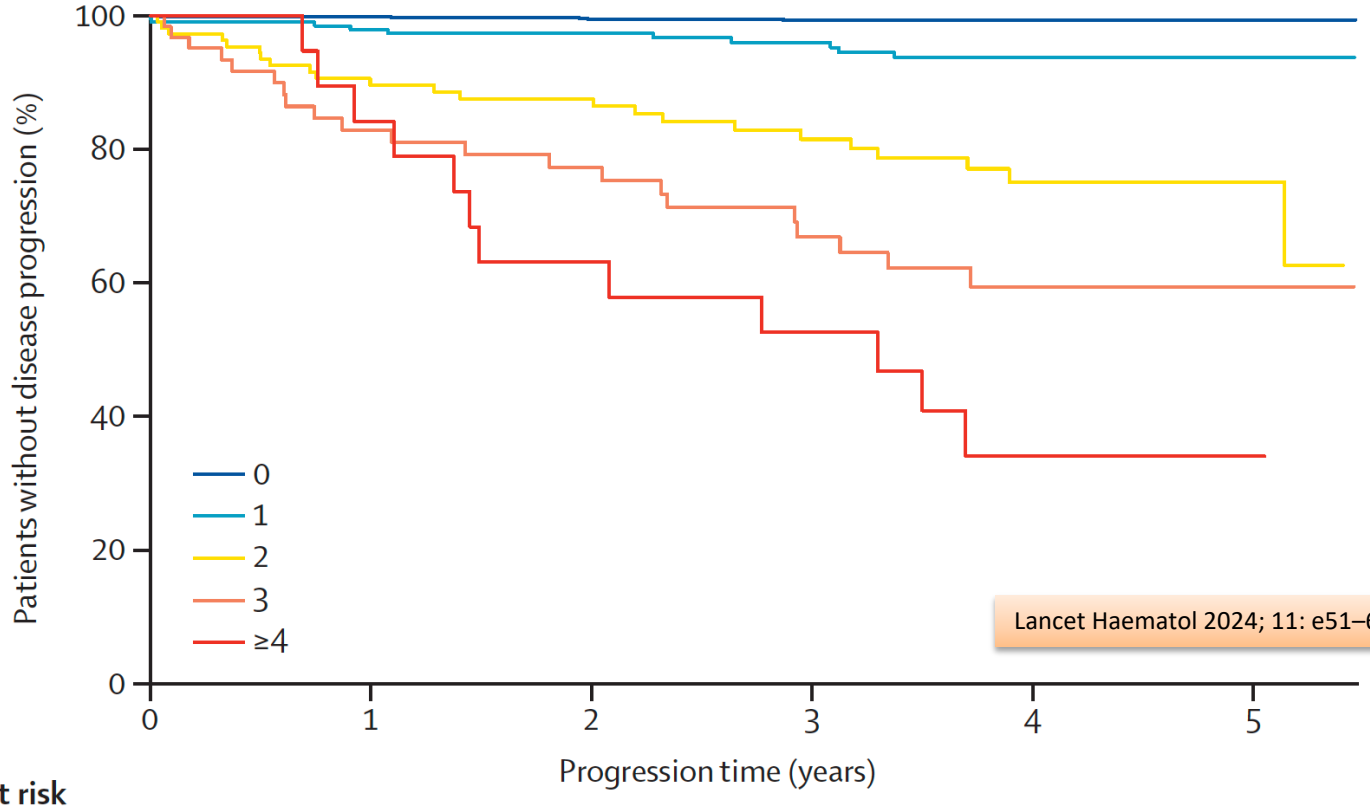


NEJM Evid. 2023 2(5):10.1056 (PMID: 37483562)

Time to progression univariate regression analysis for samples non-diagnostic of MDS

Variable	HR (95% CI)	p-value
ASXL1	**11.22 (6.34-19.87)**	**p<0.0001**
BCOR	**20.11 (8.04-50.3)**	**p<0.0001**
DNMT3A	1.3 (0.52-3.23)	p=0.5706
IDH1	**12.91 (5.19-32.11)**	**p<0.0001**
RUNX1	**19 (9.4-38.39)**	**p<0.0001**
SRSF2	**11.89 (7.38-19.16)**	**p<0.0001**
TET2	**8.43 (5.31-13.4)**	**p<0.0001**
U2AF1	**7.75 (3.85-15.6)**	**p<0.0001**
ZRSR2	**6.57 (2.84-15.16)**	**p<0.0001**

Lancet Haematol 2024; 11: e51–61 (PMID: 38135373)



Lancet Haematol 2024; 11: e51–61 (PMID: 38135373)

Number at risk (number censored)

0	1085 (0)	946 (139)	874 (211)	817 (268)	577 (508)	202 (883)
1	205 (0)	176 (29)	153 (52)	131 (74)	86 (119)	33 (172)
2	110 (0)	99 (11)	87 (23)	67 (43)	43 (67)	8 (102)
3	64 (0)	51 (13)	43 (21)	38 (26)	23 (41)	6 (58)
≥4	21 (0)	17 (4)	14 (7)	13 (8)	9 (12)	1 (20)

Figure 4: **Time-to-progression analysis in non-diagnostic patients according to number of mutations**

Proposed minimal diagnostic criteria of MDS

Oncotarget. 2017;8(43):73483. PMID: 29088721

A. Prerequisite Criteria (both must be fulfilled)

1. Persistent (4 months) peripheral blood cytopenia in one or more of the following lineages: Eythroid cells, neutrophils, platelets

Exception: In the presence of a blast cell excess and MDS-related cytogenetic abnormalities the diagnosis of MDS can be established without delay

2. Exclusion of all other hematopoietic or non-hematopoietic disorders as primary reason for cytopenia/dysplasia

B. MDS-Related (Major) Criteria (at least one must be fulfilled)

1. Dysplasia in at least 10% of all cells in one of the following lineages in the bone marrow smear: erythroid; neutrophilic; megakaryocytic
2. ≥15% ring sideroblasts (iron stain)
   or ≥5% ring sideroblasts (iron stain) in the presence of *SF3B1* mutation
3. 5-19% myeloblasts on bone marrow smears (or 2-19% myeloblasts on blood smears)
4. Typical chromosome abnormality(ies) by conventional karyotyping or FISH

C. Co-Criteria

For patients fulfilling A but not B, and otherwise show typical clinical features, e.g. macrocytic transfusion-dependent anemia; two or more of these co-criteria must be fulfilled for considering a provisional diagnosis of MDS)

1. Abnormal findings in histologic and/or immunohistochemical studies of bone marrow biopsy sections supporting the diagnosis of MDS (ALIP, CD34+ clusters, micromegakaryocytes, etc.)
2. Abnormal immunophenotype of bone marrow cells by flow cytometry, with multiple MDS-associated phenotypic aberrancies indicating the presence of a monoclonal population of erythroid and/or myeloid cells
3. Evidence of a clonal population of myeloid cells determined by molecular (sequencing) studies revealing MDS-related mutations

Mutations in cytopenic patients without definitive evidence of MDS

- Single mutation
- Low variant allele frequency (<10%)
- Mutation in common ARCH genes
- Mild cytopenias

- Multiple mutations
- Higher variant allele frequency (>20%)
- Mutation in genes more commonly associated with MDS
- Cytopenia, especially progressive

Where is the threshold?

Malcovati, L. et al. Blood. 2017;129:3371-3378

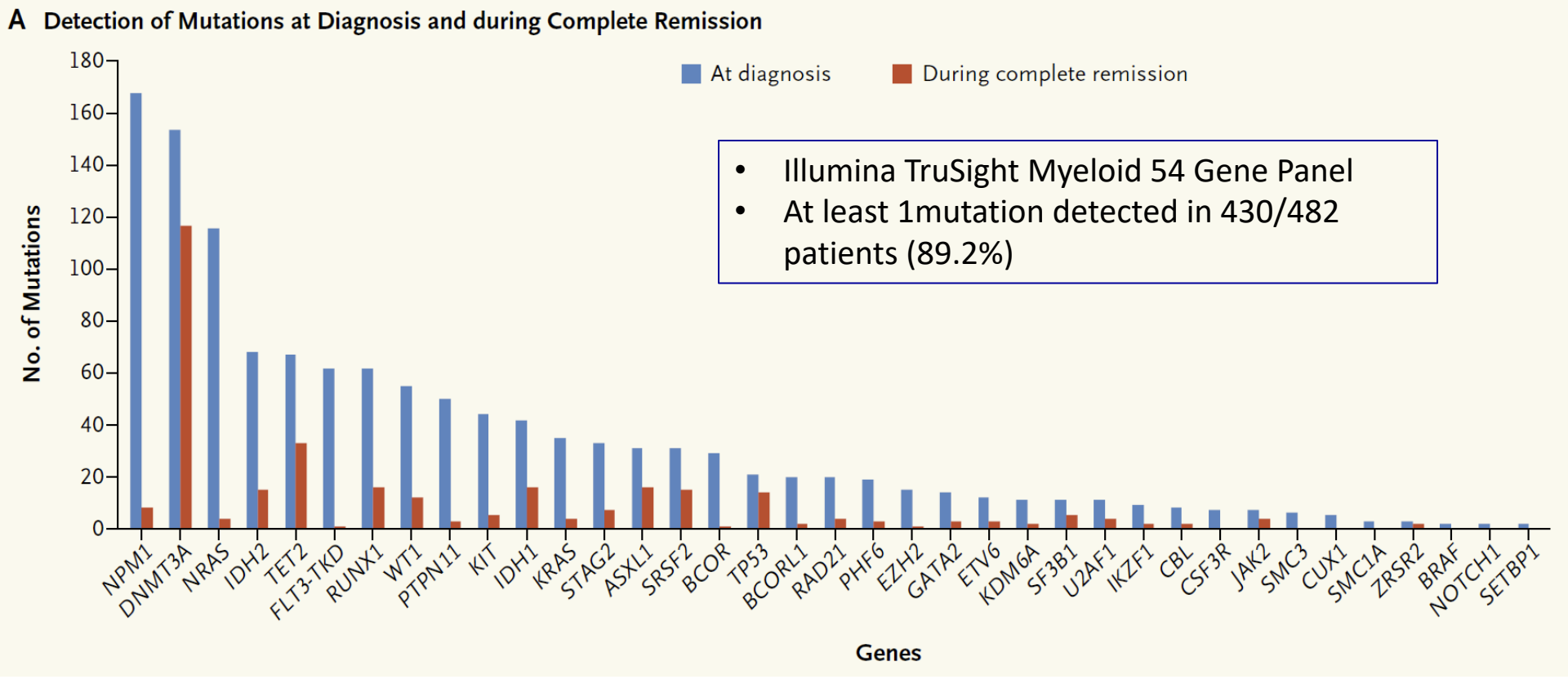
Modified from Steensma D. Hematology: ASH Education Program 2016
AMP 2016 presentation

AML Novel Molecular Targeting Therapies

Protein kinase inhibitors	FLT3 inhibitors (midostaurin, quizartinib, gilteritinib, crenolanib)
	KIT inhibitors
	PI3K/AKT/mTOR inhibitors
	Aurora and polo-like kinase inhibitors, CDK4/6 inhibitors, CHK1, WEE1, and MPS1 inhibitors
	SRC and HCK inhibitors
Epigenetic modulators	HDAC inhibitors; New DNA methyltransferase inhibitors (SGI-110)
	IDH1 and IDH2 inhibitors
	DOT1L inhibitors
	BET-bromodomain inhibitors
Mitochondrial inhibitors	Bcl-2, Bcl-xL, and Mcl-1 inhibitors; Caseinolytic protease inhibitors
Therapies targeting oncogenic proteins	Fusion transcripts targeting
	EVI1; NPM1; Hedgehog (Glasdegib)
Targeting environment	CXCR4 and CXCL12 antagonists; Antiangiogenic therapies

Modified from Blood. 2017; 129:424. PMID: PMID: 27895058

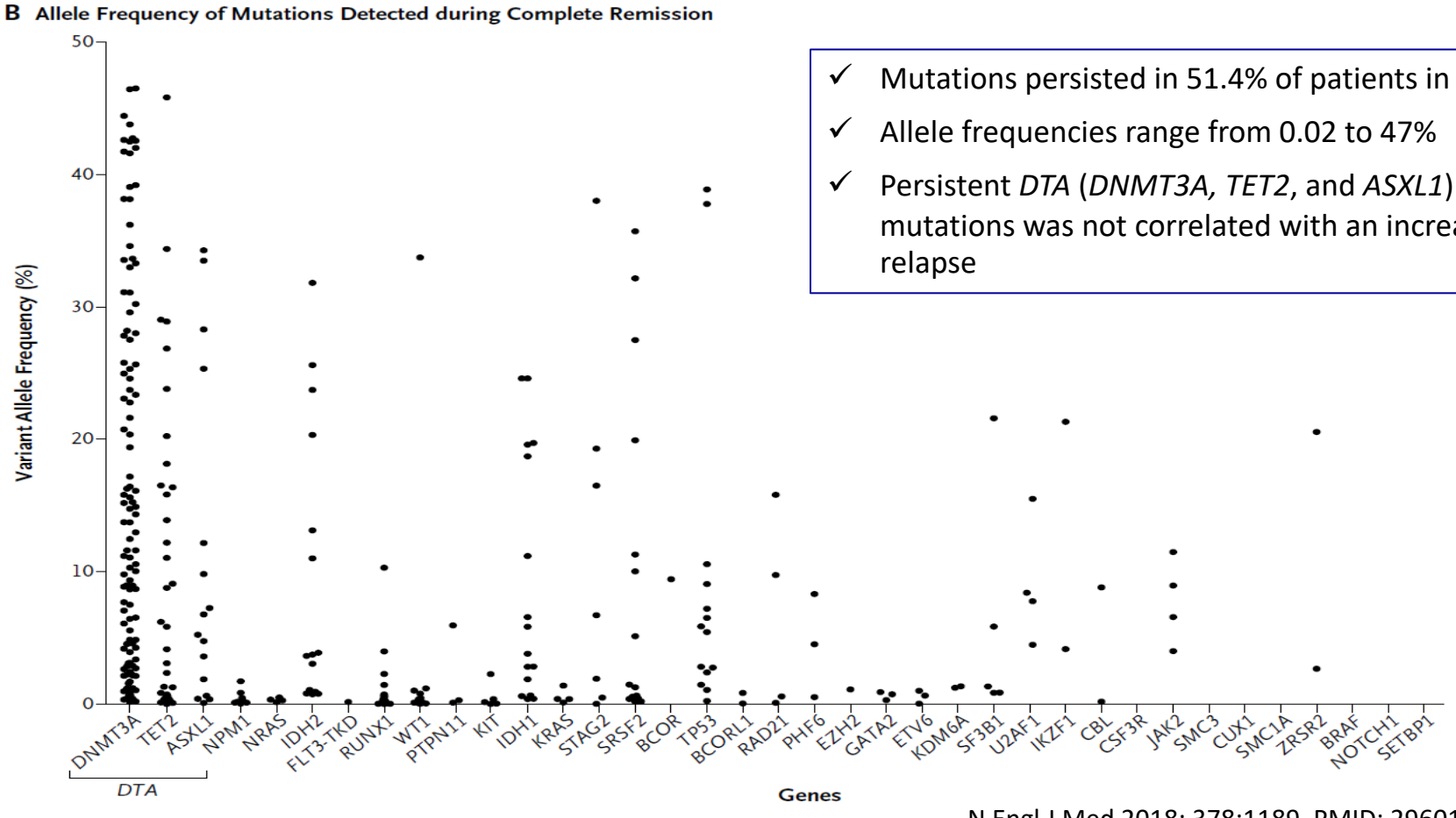
NGS-based Mutation Profile of AML



- Illumina TruSight Myeloid 54 Gene Panel
- At least 1mutation detected in 430/482 patients (89.2%)

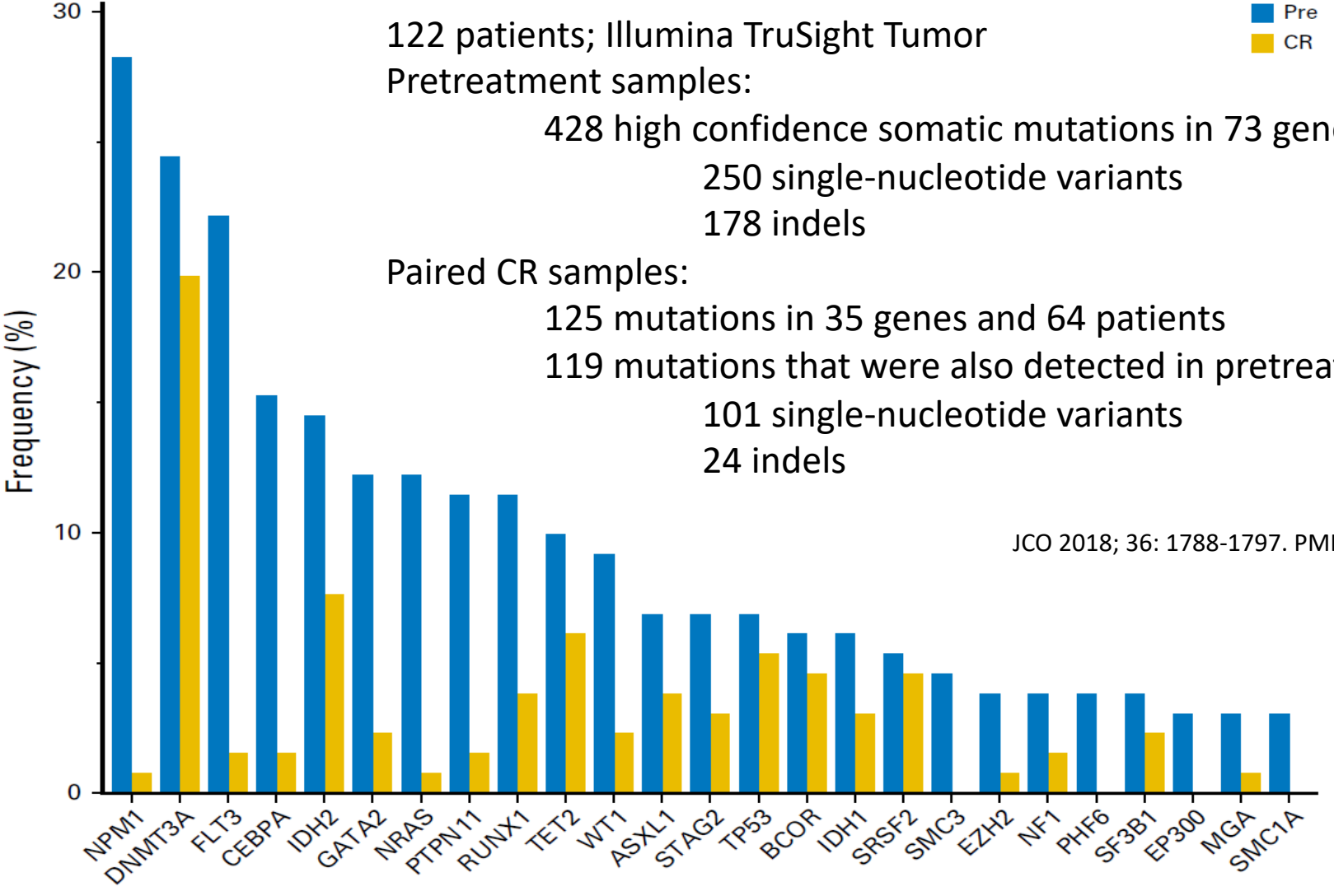
N Engl J Med 2018; 378(13):1189-1199. PMID: 29601269

Molecular Assessment of Residual Disease in AML



- ✓ Mutations persisted in 51.4% of patients in CR
- ✓ Allele frequencies range from 0.02 to 47%
- ✓ Persistent *DTA* (*DNMT3A, TET2*, and *ASXL1*) mutations was not correlated with an increased relapse

N Engl J Med 2018; 378:1189. PMID: 29601269



122 patients; Illumina TruSight Tumor

Pretreatment samples:

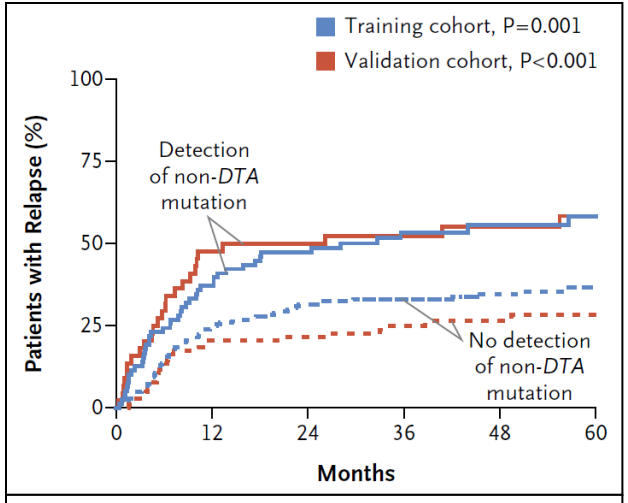
- 428 high confidence somatic mutations in 73 genes
  - 250 single-nucleotide variants
  - 178 indels

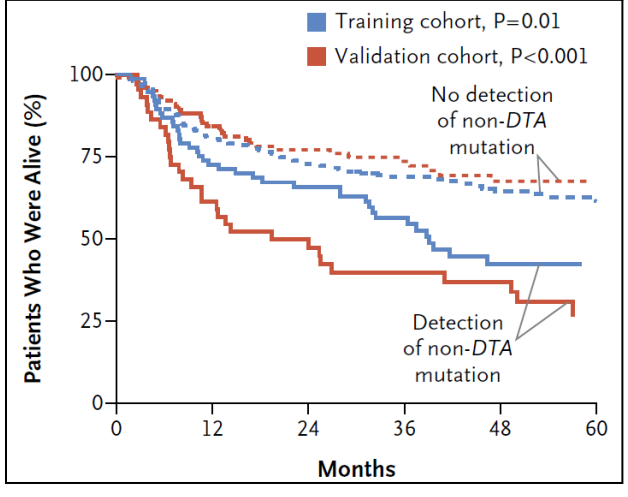
Paired CR samples:

- 125 mutations in 35 genes and 64 patients
- 119 mutations that were also detected in pretreatment
  - 101 single-nucleotide variants
  - 24 indels

JCO 2018; 36: 1788-1797. PMID: 29702001

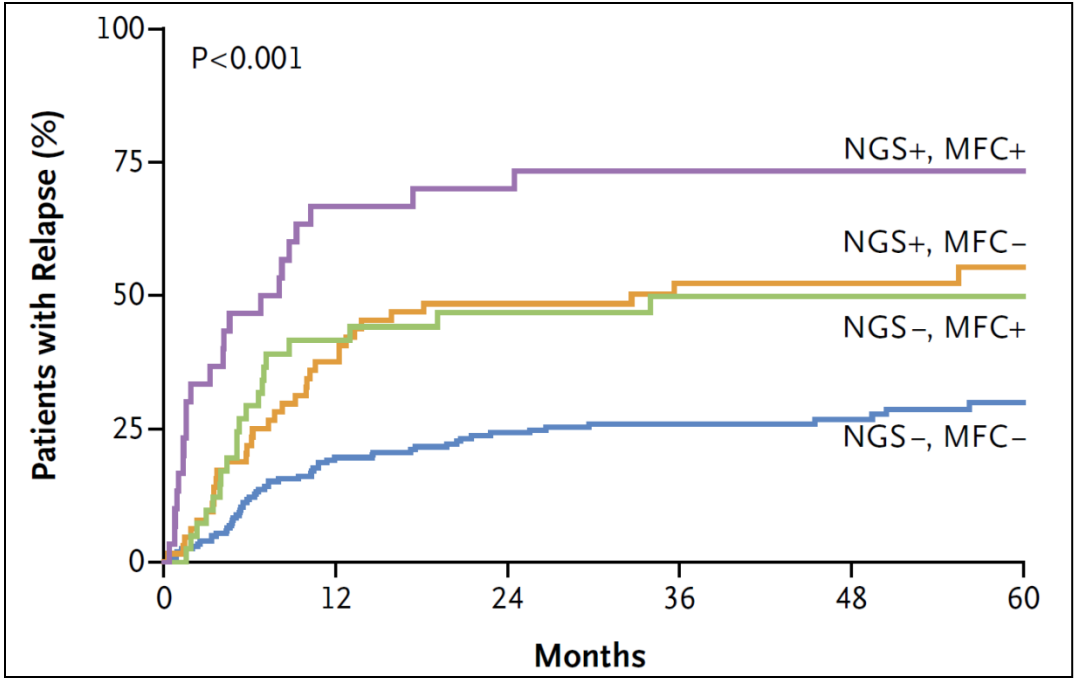
Molecular Residual Disease and Clinical Outcome





Non-DTA mutations!

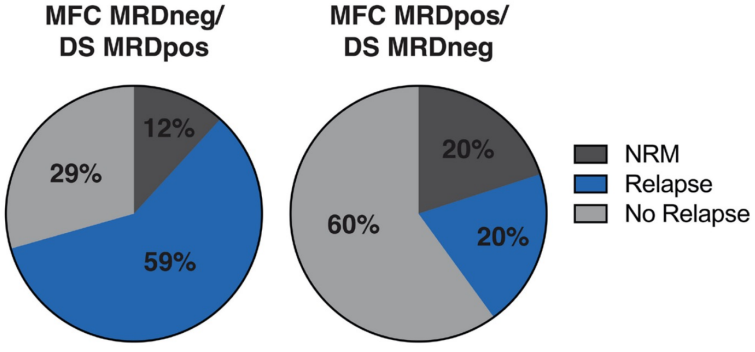
N Engl J Med 2018; 378:1189

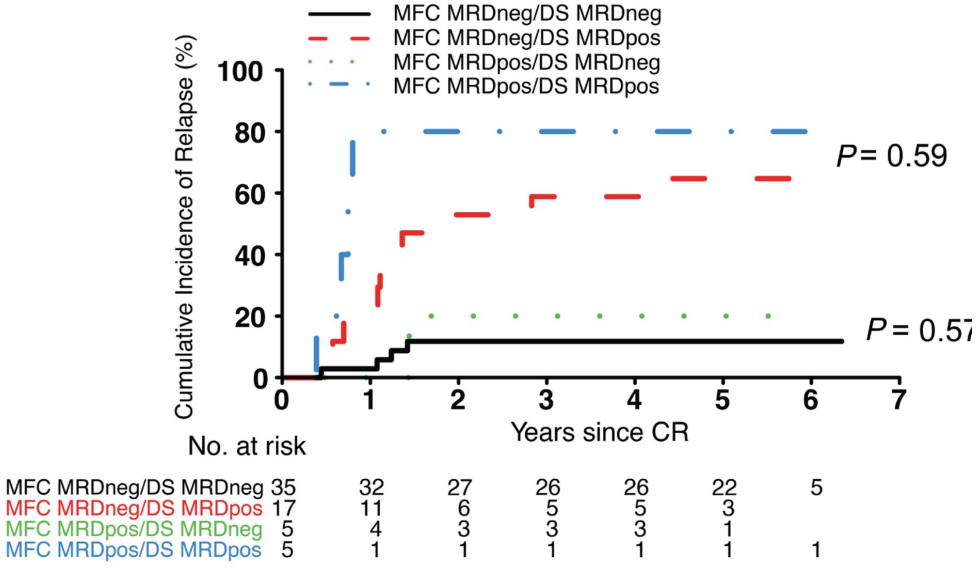


MRD detection with NGS vs. MFC

Sensitive NGS MRD is better than MFC

	MFC MRDneg (N=52)	MFC MRDpos (N=10)	Total
DS MRDneg	35 (67%)	5 (50%)	40 (64.5%)
DS MRDpos	17 (33%)	5 (50%)	22 (35.5%)





Dillon Haematologica, 2024 Feb 1;109(2):401-410. PMID: 37534515

Methods for detection of MRD in AML

Status	Method	Target	Sensitivity	Applicable % AML	TRT(d)	Limitations/problems
Established	MFC	Leukemia-associated immunophenotype (LAIP) or different from normal (DfN)	10^{-3} ~ 10^{-4}	85-90	2	Less sensitive, more subjective analysis
Established	RT-qPCR	**Robust data:** *NPM1, CBFB::MYH11, RUNX1::RUNX1T1* **Less validated:** *KMT2A::MLLT3, DEK::NUP214, BCR::ABL1, WT1*	10^{-4} ~ 10^{-5}	40-50	3-5	Limited applicability
Exploratory	NGS	Potentially any somatic mutation	10^{-2} ~ 10^{-4}	~100	5-10	Less sensitive, costly, technically challenging
Exploratory	dPCR	Specific targeted mutations	10^{-3} ~ 10^{-4}	~70	3-5	Specific assay necessary for every mutation, limited sensitivity

Modified from: Blood. 2022;140(12):1345-1377. PMID: 35797463

NGS MRD Testing for AML: Targets

- Specific mutations identified at diagnosis *vs* agnostic panel approaches both can be considered.
- Mutations in signaling pathway genes (*FLT3-ITD, FLT3-TKD, KIT*, and *RAS*, among others)
  - Likely represent residual AML when detected.
  - They are often subclonal and have a low negative predictive value.
  - These mutations are best used in combination with additional MRD markers.
- Molecular marker that is targeted (FLT3 inhibitors and IDH1/IDH2 inhibitors) should be included.
- Emerging variants not found at diagnosis should be reported only if confidently detected above background noise.
- Considering all detected mutations as potential MRD markers, exclude:
  - Germline mutations (*ANKRD26, CEBPA, DDX41, ETV6, GATA2, RUNX1*, and *TP53*)
  - Mutations in *DNMT3A, TET2*, and *ASXL1* (DTA).

Blood. 2021;138(26):2753-2767. PMID: 34724563

Molecular MRD Testing for AML: Methods

- LOD of 10^{-3} or lower: qPCR, dPCR, or error-corrected NGS using UMIs is recommended.
- 5 mL of BM aspirate from the first pull.
- Leukemia-specific PCR assays (eg, for *NPM1*, *PML::RARA*, or *CBF* AML).
- There is no uniform bioinformatics pipeline/platform for NGS-MRD variant calling.
- Potential cross-sample sequence contamination
  - Never run a high positive sample with MRD sample
  - Contamination by pooling samples should be bioinformatically evaluated.

Blood. 2021;138(26):2753-2767. PMID: 34724563

Molecular MRD Testing for AML: Technical Recommendations

- PCR recommendations:
  - Sufficient template input (100ng DNA, 1μg total RNA, or RT volume corresponding to 100 ng of RNA)
  - For ddPCR:
    - number of total copies > 32,000
    - total droplet count > 15,000
    - empty droplets > 100
  - Duplicate or triplicate
  - >40 cycles; Ct ≤40 cycles
  - Determine LLOD and LOB
- NGS and bioinformatics recommendations
  - Error correction NGS: ≥10,000 read families and >10 mutant reads
  - Non-error-corrected NGS: ≥60,000 reads and >60 mutant reads

Blood. 2021;138(26):2753-2767. PMID: 34724563

Chinese American Pathologists Association (CAPA)
10th Annual Diagnostic Course

Saturday, October 19th, and Sunday, October 20th, 2024. Las Vegas

Meeting site: Plaza hotel and Casino, 1 Main Street, Las Vegas, Nevada, 89101

This Diagnostic Course does **NOT** Provide Virtual Option.

Hotel reservation open on 5/15/2024: Visit CAPA Official Website to get information.

Reserved hotel rooms are very limited and may not be the best price. We recommend you search around the meeting site to find the hotel best fit your need.

Grand Canyon West Tour with Hoover Dam Stop and Optional Skywalk (Monday, 10/21/2024, from 6 AM to 6 PM):
Luggage is **NOT ALLOWED** for Hoover Dam Stop. Therefore, if you need to leave Las Vegas Monday night after the tour (*returning flights **after 9:30 PM** can be considered*), check out hotel, ask the hotel Bell Desk to hold your luggage until you come back from the tour.
More information about the tour pending.

- **Concurrent session #2: Hematopathology / Pediatric pathology / Dermatopathology. Moderator: Dr. Di Jing**
- 1:00 - 1:25 PM Eosinophilia: What are the Causes? By ***Dr. Yan Liu***. Loma Linda University Health
- 1:25 - 1:50 PM Somatic UBA1 Mutation Predispose Male Patient to Low Grade MDS. By ***Dr. Peng Li***. University of Utah
- 1:50 - 2:00 PM Break
- 2:00 - 2:25 PM Nodular Lymphocyte Predominant Hodgkin Lymphoma in Children. ***Dr. Dehua Wang***. Rady Children's Hospital UCSD
- 2:25 - 2:50 PM Diagnosis of CTCL: Challenge and Tips. By ***Dr. Jing Zhang***. Carolinas Dermatology and Plastic Surgery

3:00 PM Group photos

Poster Session (Chair: Dr. Xiaoying Liu):
Recent and valuable posters, regardless of whether they were previously presented at other conferences or not, will be considered for the poster session (limited space available).

Dinner Party at Rio KJ Dim Sum & Seafood

10/20/2024 Sunday (Las Vegas Local Time)

- **Concurrent session #3: Anatomic Pathology. Moderator: Dr. Haodong Xu**
- 8:00 -8:25 Hepatobiliary pathology:
    1. Defining Atypical Hepatocellular Adenoma: an Existing Or Arbitrary Entity At the Transition of Benign Versus Malignant Hepatocellular Neoplasm. By ***Dr. Lee-Ching Zhu***. University of North Carolina at Chapel Hill
    2. What's New in Cholangiocarcinoma: Unveiling a Versatile Liver Lump. By ***Dr. Xiaotang Du***. UCLA department of pathology and lab medicine
- 8:25 - 8:50 WHO Reporting System for Pancreaticobiliary Cytopathology: Review, Application, and Ancillary Testing. ***By Dr. Qun Wang***. Emory University School of Medicine
- 8:50 - 9:00 Break
- 9:00 - 9:25 Undifferentiated Small Round Cell Sarcomas. By ***Dr. Shaoxiong Chen***. Indiana University
- 9:25 - 9:50 Cervical Lesions: Is It a Carcinoma? By ***Dr. Yanjun Hou***. Wake Forest University

- **Concurrent session #4: Clinical Pathology, Molecular Genetic Pathology and Digital Pathology. Moderator: Dr. Linsheng Zhang**
- 8:00 -8:25 Molecular Pathology Updates on Solid Tumors. By ***Dr. Wei Zhang***. University of Kansas Medical Center.
- 8:25 - 8:50 Enhancing Cytology Practice: Integrating AI and Recent ASC Guidelines for Precision Diagnosis. By ***Dr. Xiaoying Liu***. Dartmouth Hitchcock Medical Center.
- 8:50 - 9:00 Break
- 9:00 - 9:25 Achieving Success in the MolDx: The Molecular Hematopathology Perspective. By ***Dr. Yi Ding***. Geisinger Medical Center
- 9:25 - 9:50 Clinical Applications of Liquid Biopsy for Cancer: Challenges and Opportunities. By ***Dr. Gang Zheng***. Mayo Clinic

- **General session #2 Moderator: Dr. Y. Helen Zhang**
- 10:00 - 10:30 Methylation Profile in CNS Tumor Diagnostics. By ***Dr. Liam Chen***. University of Minnesota
- 10:30 - 11:00 Ancillary Studies and Artificial Intelligence: Are They Useful in Urine Cytology? By ***Dr. Juan Xing***. Cleveland Clinic
- 11:15 AM End of meeting

Visit https://tinyurl.com/capa10d for more information.

Molecular Diagnostics of Lymphoid Neoplasms

- Proof of clonality
- Supporting lineage determination
- Relatedness of lymphomas
  - Different sites: are they from the same clone?
  - Progression/relapse
- Diagnosis, Prognosis, and Subclassification
  - Disease specific/characteristic translocation/mutation
  - Subclassification by mutation profiling
  - Patient specific clonal marker and MRD detection
- Targeting therapy and resistance prediction

Evidence of Clonality

- Flow cytometry or IHC
  - Ig light chain for B cells/plasma cells
  - Vβ for T cells
- Fusion genes or other specific mutations
  - FISH
  - PCR
- *TCR/IGH* clonal rearrangement
  - Southern blot
  - PCR
  - NGS

Clonality Test by T/B Cell Receptor Gene Rearrangement

- Lineage infidelity
  - Clonality can support but not prove lineage
  - Clonal rearrangements of TCR genes can be found in B-cell neoplasms and vice versa
- PCR based tests have analytic sensitivity (~5%), not optimal for MRD

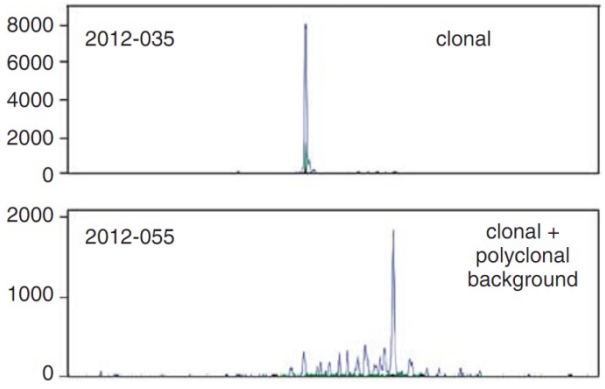
Clonality assessment

- Arbitrary definition of a positive peak (amplicon)
- Clonality judgment is subjective
- Clonality ≠ Malignancy
  - Pseudoclonality
  - Infection/inflammation (predominant clones)
- Not all clones will be detected by any technique
  - Somatic hypermutation
  - Poor DNA quality (perform amplification control)
  - Sampling artifact

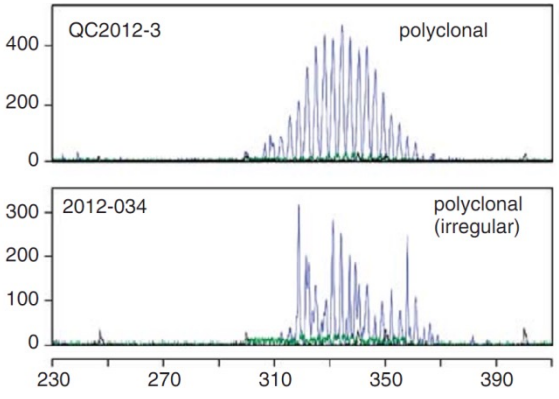
BIOMED-2 PCR Testing for B/T Cell Lymphomas

- 107 different primers in 18 multiplex PCR tubes
- B cell
  - VH–JH 3
  - DH–JH 2
  - Ig kappa (*IGK*) 2
  - Ig lambda (*IGL*) 1
- T cell
  - TCR beta (*TCRB*) 3
  - TCR gamma (*TCRG*) 2
  - TCR delta (*TCRD*) 1
- Fusion genes
  - *BCL1*-Ig heavy chain (*IGH*) 3
  - *BCL2-IGH* 1
- Clonality assessment by heteroduplex analysis or GeneScanning.

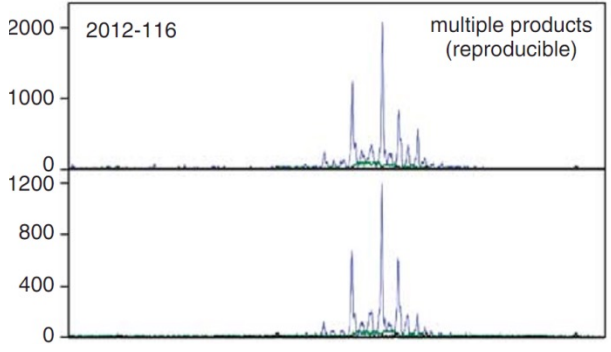
Common Patterns of *IGH/TCR*



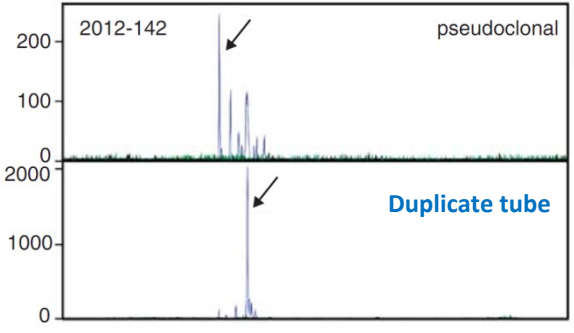
Monoclonal



Polyclonal



Oligoclonal



Pseudoclonal

IGH FR1

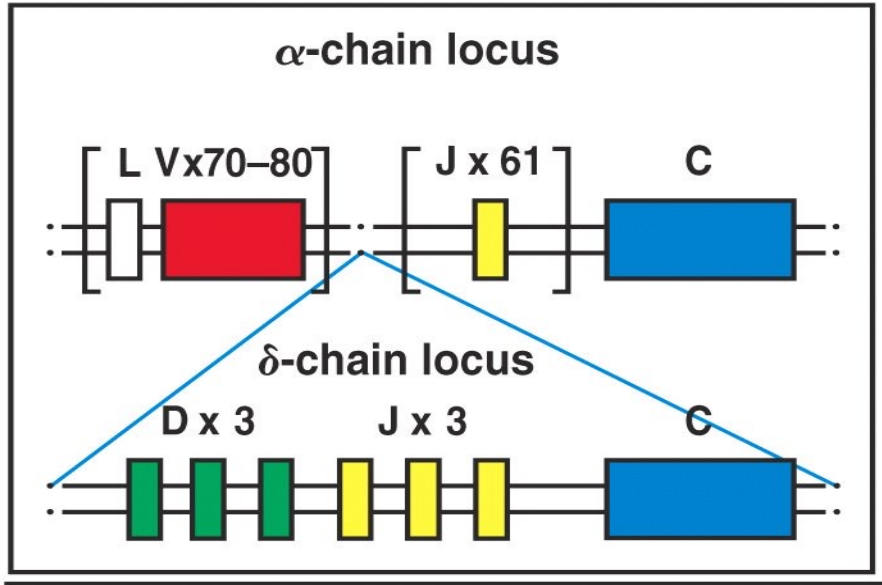
TCRB, tube A

Complementarity of Ig targets for clonality detection

Table from: Leukemia 2007; 21: 201

	IGH			*IGK*	*IGH+IGK*
	VH–JH	*DH–JH*	*VH–JH+DH–JH*	*Vκ–Jκ+Kde*	
MCL(%)	100	11	100	100	100
B-CLL(%)	100	43	100	100	100
FL(%)	84	19	86	84	100
MZL(%)	88	51	95	83	100
DLBCL(%)	79	30	85	80	98
TOTAL(%)	88	28	91	88	99

IGH/IGK clonality studies reported sensitivity 42-79% (FF) and 9-94% (FFPE) in HL (CHL & NLPHL)
Specificity studies on IGH/IGK are limited. False positive Ig/TCR clonality estimated approximately 10%



TCRD is deleted after *TCRA* rearrangement

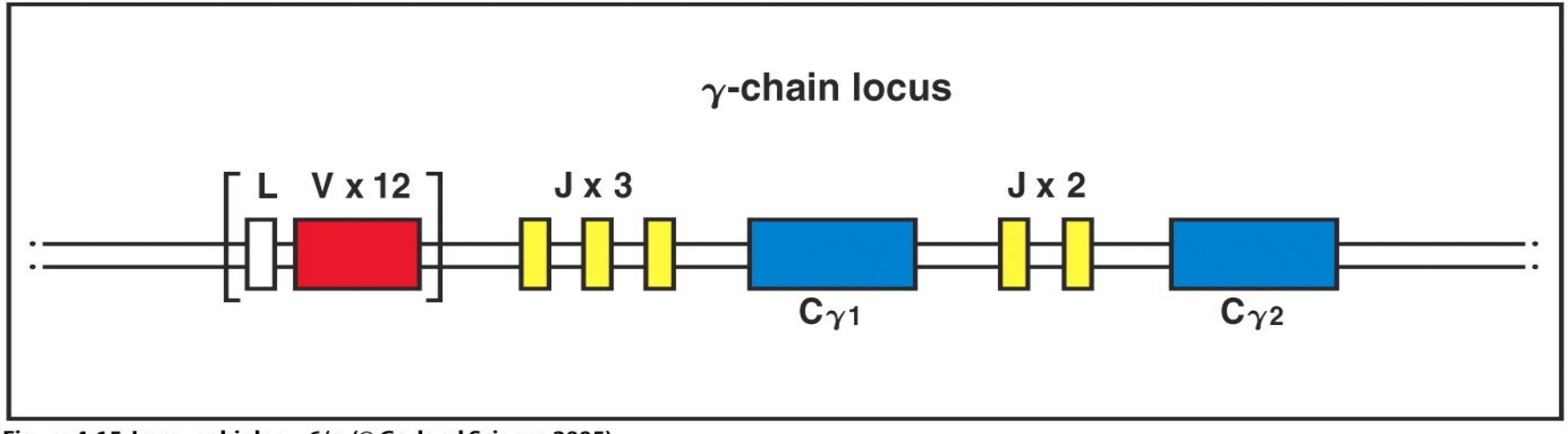
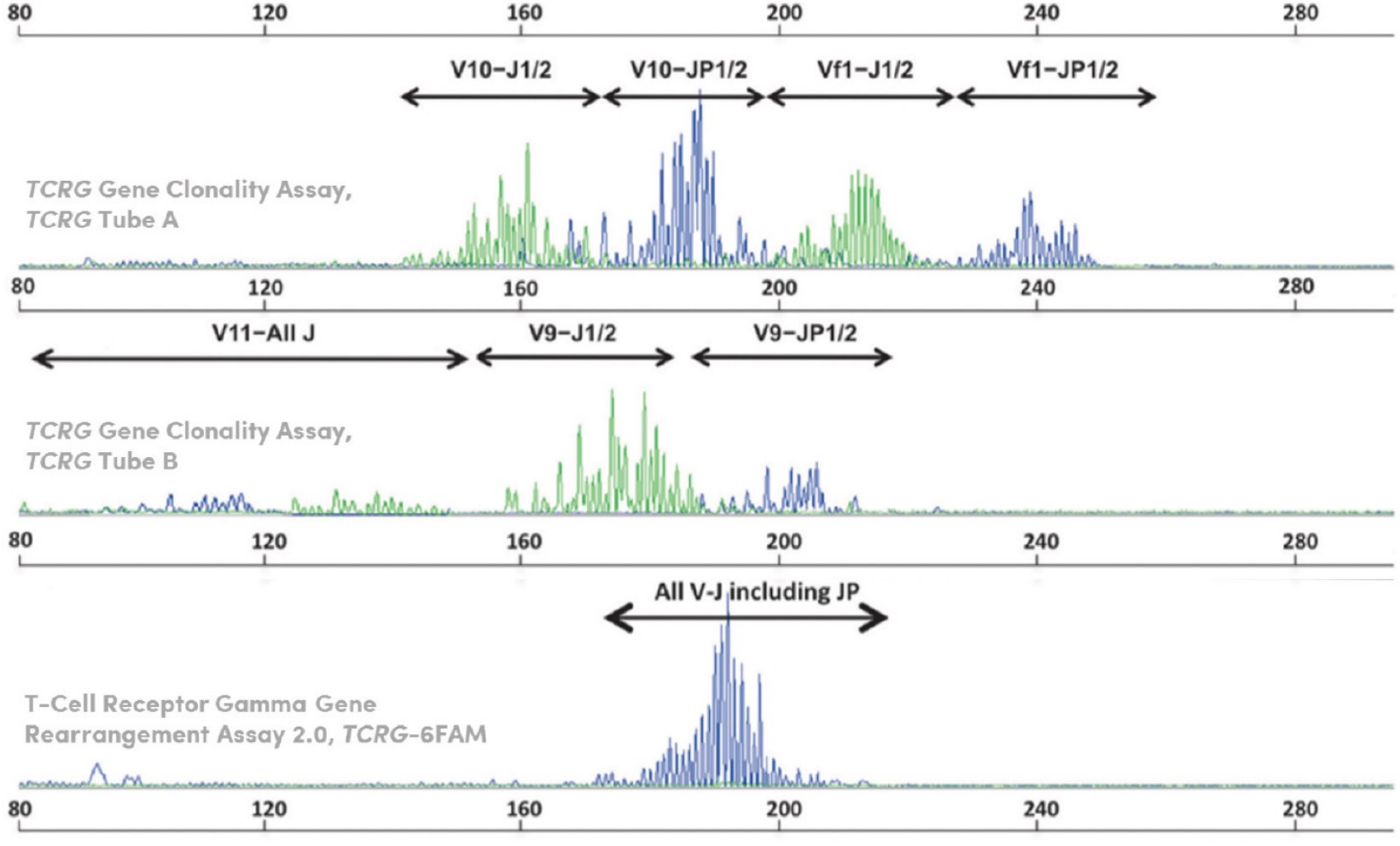
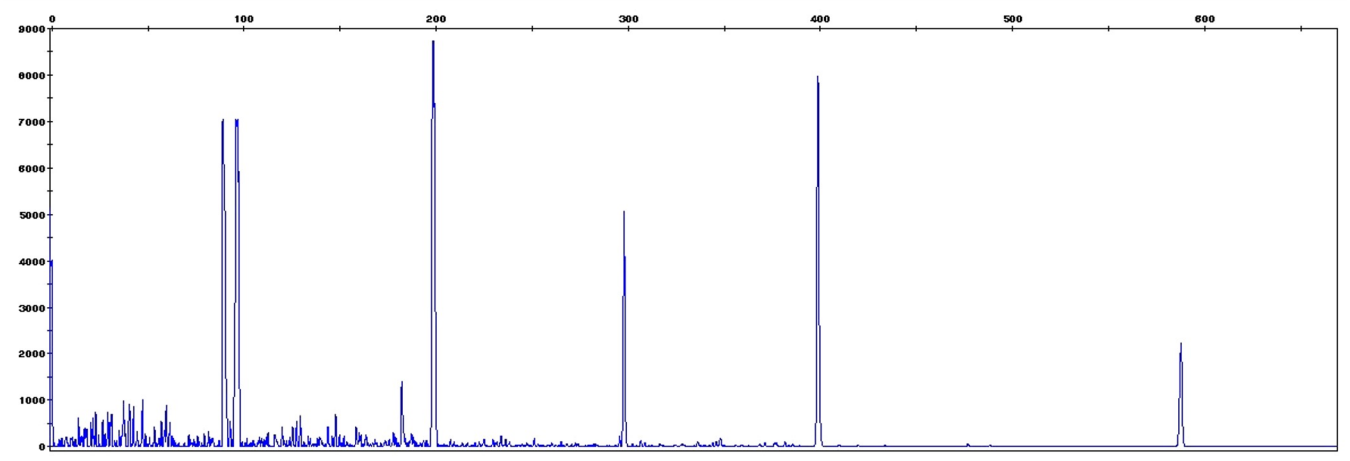


Figure 4-15 Immunobiology, 6/e. (© Garland Science 2005)

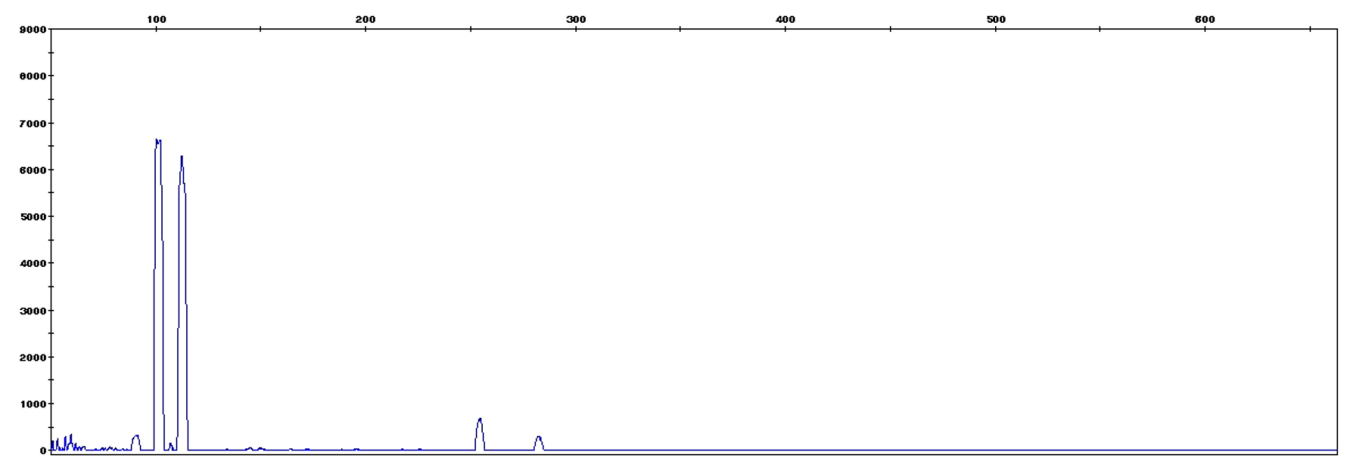
TRG V1 (BIOMED2) vs. V2



Quality Control for FFPE Specimen

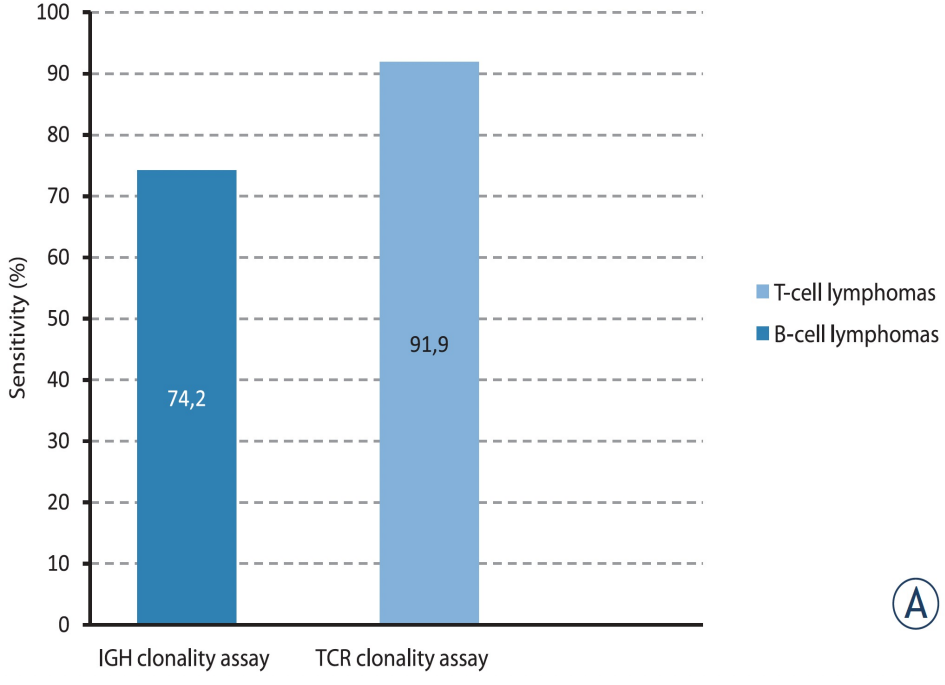


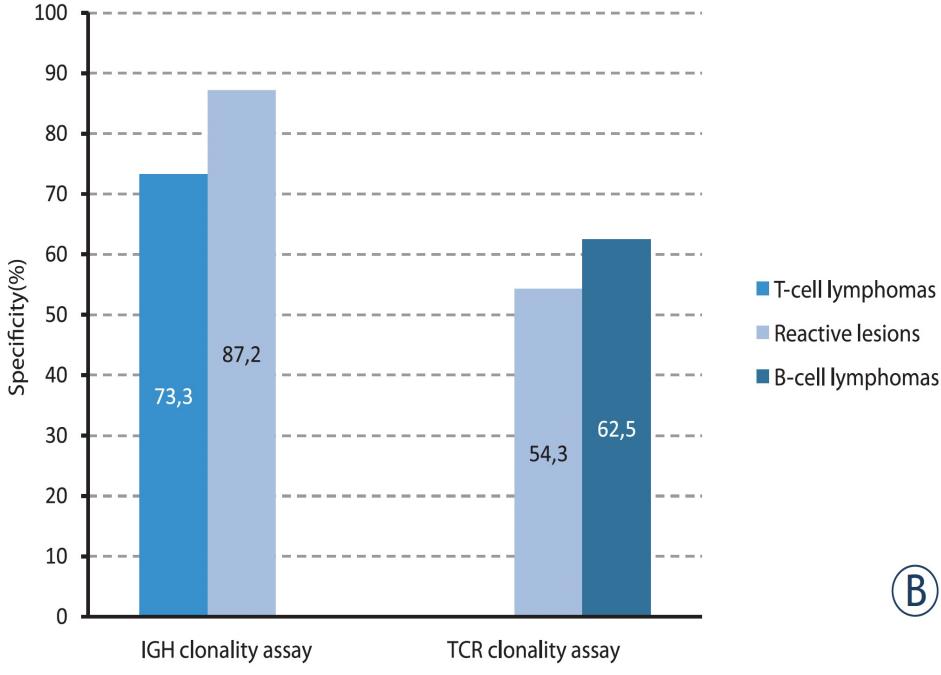
Good DNA quality



Poor DNA quality

Sensitivity and Specificity Issues

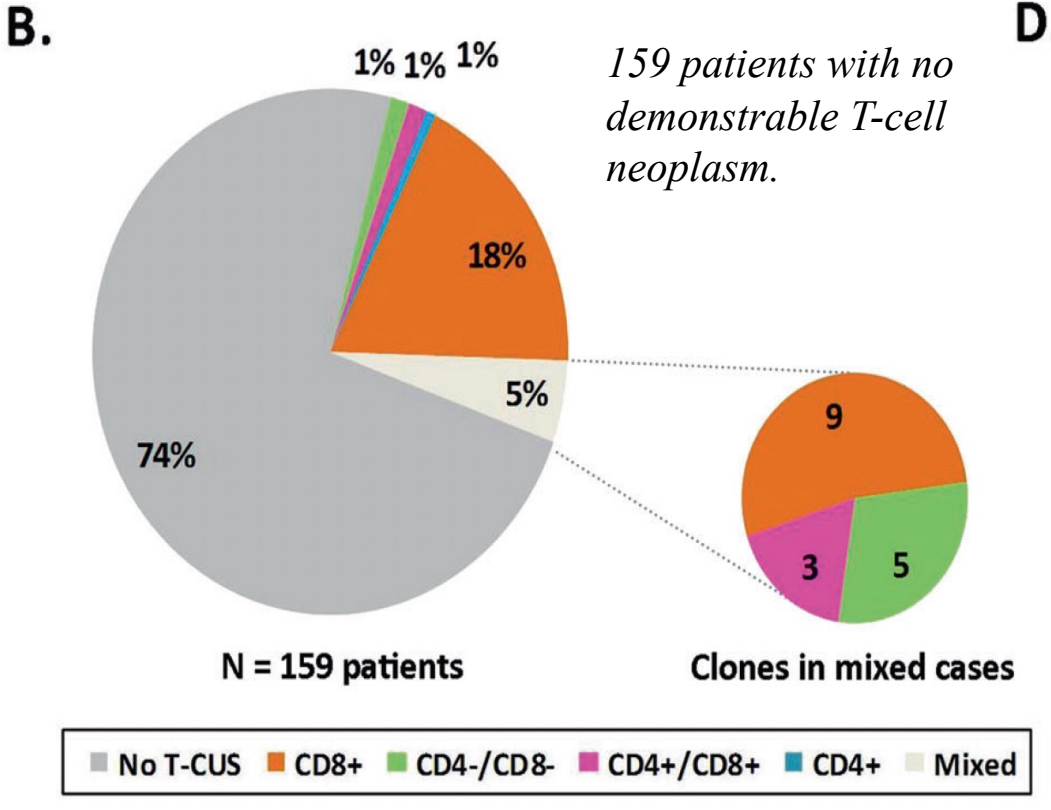




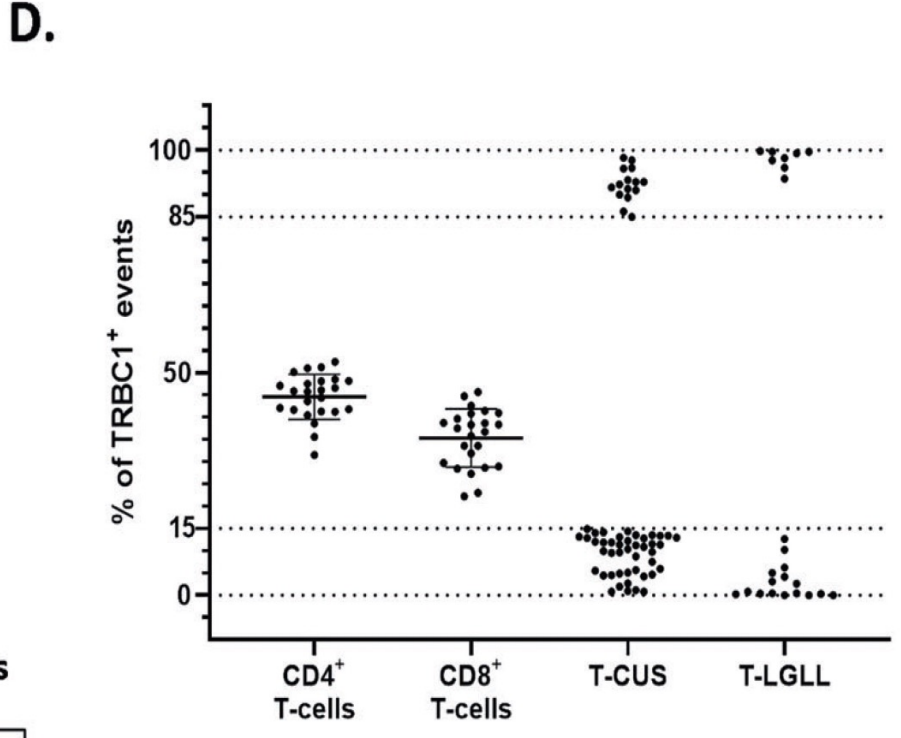
Case #: 96 *IGH* (pos 32, neg 64); 119 *TCRG/B* (pos 38, neg 71)

Kokovic, I et al. *Radiol Oncol* 2014; 48(2): 155-162.

T-cell clones of uncertain significance (Flow cytometry study)



159 patients with no demonstrable T-cell neoplasm.



Shi, M. et al. *Modern Pathology* (2020) 33:2046–2057

T-cell clones of uncertain significance

Table 2 Monoclonal Results for *TCRG/TCRB* Genes in Different Groups of Patients

Patient Group	*TCRG*, n (%)	TCRB Dβ-Jβ, n (%)	*TCRB Vβ-Jβ*, n (%)	*TCRB Dβ-Jβ/ Vβ-Jβ*, n (%)	*TCRG* and/or *TCRB*, n (%)
T-LGLαβ (n = 30)	25 (83.3)	17 (56.7)	29 (96.7)	30 (100)	30 (100)
T-LGLγδ (n = 12)	11 (91.7)	5 (41.7)	4 (33.3)	7 (58.3)	11 (91.7)
Healthy (n = 62)	7 (11.3)	3 (4.8)	0 (0)	3 (4.8)	9 (14.5)
RA/SLE (n = 14)	3 (21.4)	2 (14.3)	0 (0)	2 (14.3)	5 (35.7)
Reactive CD8+ lymphocytosis (n = 17)	3 (17.6)	2 (11.8)	0 (0)	2 (11.8)	5 (29.4)
Total control group (n = 93)	13 (14)	7 (7.5)	0 (0)	7 (7.5)	19 (20.4)

Abbreviations: RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; T-LGL leukemia = T-cell large granular lymphocytic leukemia.

Sidorova YV et al. *Clinical Lymphoma, Myeloma & Leukemia.* 2020; 20:203-8

T-cell clonality test: S/S and PPV

Table 3 Sensitivity, Specificity, and Positive Predictive Value of T-cell Clonality Testing for Differential Diagnosis in αβ-T-LGL Leukemia

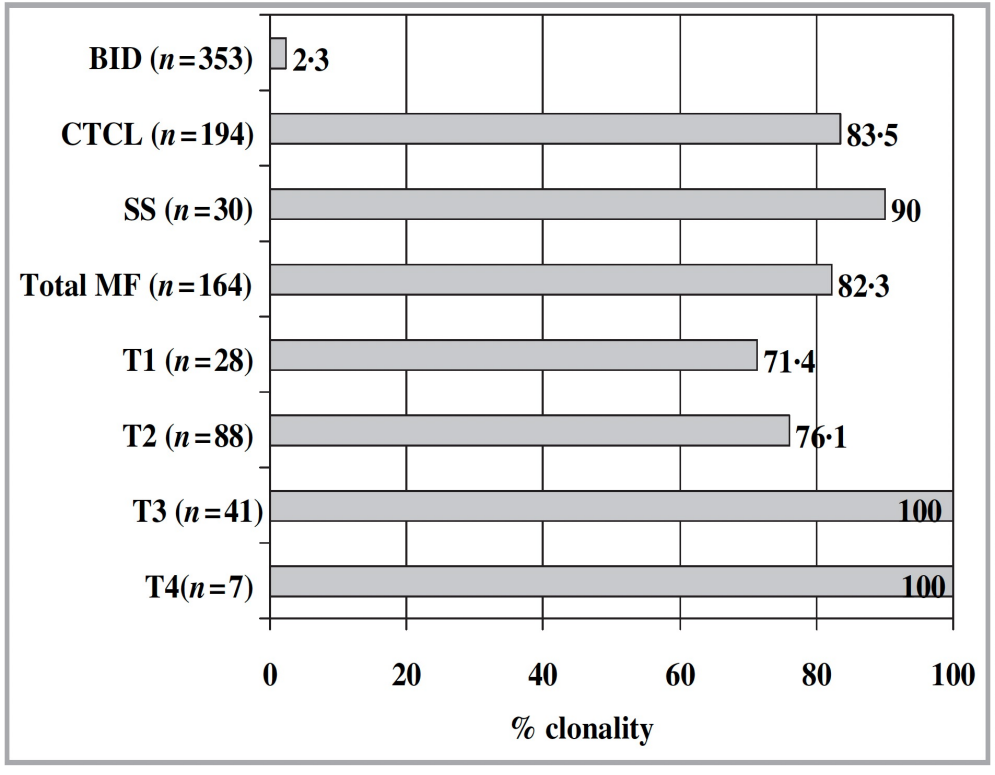
Method	Sensitivity, %	Specificity, %	Positive Predictive Value, %
TCRG	83.3	86	85.4
TCRB (Dβ-Jβ)	56.7	92.5	83.7
TCRB (Vβ-Jβ)	96.7	100	99.2
TCRB (*Dβ-Jβ* and/or *Vβ-Jβ*)	100	92.5	94.3
Both *TCRG* + *TCRB*	83.3	98.9	95.1

Abbreviation: T-LGL leukemia = T-cell large granular lymphocytic leukemia.

- Clonal peaks present only in CD8+CD57+ cells
- Studied blood samples only
- Vast majority of T-LGLL cases are α/β T-cells

Sidorova YV et al. *Clinical Lymphoma, Myeloma & Leukemia.* 2020; 20:203-8

TRG clonality test: Studies on CTCL



- Specificity: 97.7% (95% CI 96.2–99.3%)
- Sensitivity 83.5% (78.3–88.7%)
- PPV:95.% (92.1–98.5%)
- NPV: 91.5% (88.7–94.3%)
- diagnostic accuracy: 92.7% (95% CI 90.5–94.8%).

Only *TRG* rearrangement test was performed.

Ponti et al. *Br J Derm* 2005;153:565–573

T-cell clonality test: *TCRG* vs *TCRB*

Table 2. Correlation between *TCRG* and *TCRB* Test Results

	TCRG			
	Monoclonal	Polyclonal	Oligoclonal	Total
TCRB				
Monoclonal	43	22	1	66
Polyclonal	21	107	0	128
Oligoclonal	2	0	6	8
Total	66	129	7	202

Concordance rate of *TCRG* and *TCRB* assays = (43 + 107 + 6)/202 = 77.2%.

Zhang, B. et al. *JMD*. 2010;12:320-327. PMID: 20203005

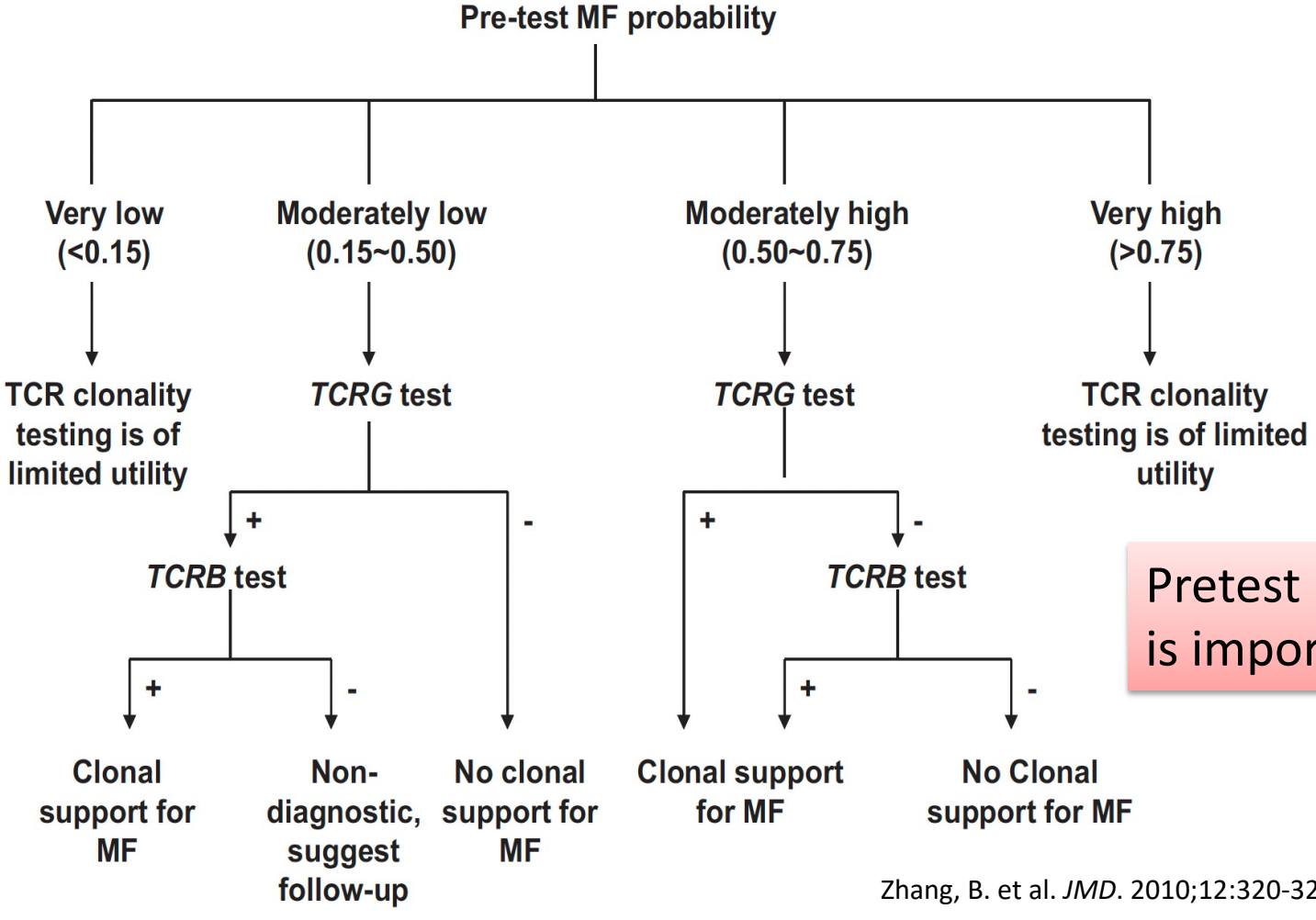
T-cell clonality test: S/S on CTCL

Table 3. Test Results of *TCRG*, *TCRB*, and Combined Use of *TCRG* and *TCRB* When Interpreting Oligoclonality as Either Negative or Positive

Zhang, B. et al. *JMD*. 2010;12:320-327. PMID: 20203005

Classification of oligoclonal pattern	Clonality test(s) used	Test interpretation	Definitions of "positive" and "negative"	# of MF	# of ID	Sensitivity	Specificity
As negative	*TCRG* alone	Positive	Monoclonal	44	22	64%	84%
		Negative	Oligoclonal or polyclonal	25	111		
	TCRB alone	Positive	Monoclonal	44	22	64%	84%
		Negative	Oligoclonal or polyclonal	25	111		
	TCRG and *TCRB*	Positive	Both tests are monoclonal	34	9	49%	93%
		Negative	At least one test is not monoclonal	35	124		
	TCRG and *TCRB*	Positive	At least one test is monoclonal	54	35	78%	74%
		Negative	Neither test is monoclonal	15	98		
As positive	*TCRG* alone	Positive	Monoclonal or oligoclonal	51	22	74%	84%
		Negative	Polyclonal	18	111		
	TCRB alone	Positive	Monoclonal or oligoclonal	51	23	74%	83%
		Negative	Polyclonal	18	110		
	TCRG and *TCRB*	Positive	Both tests are monoclonal or oligoclonal	40	9	58%	93%
		Negative	At least one test is polyclonal	29	124		
	TCRG and *TCRB*	Positive	At least one test is monoclonal or oligoclonal	62	36	90%	73%
		Negative	Both tests are polyclonal	7	97		
			Total #	69	133		

T-cell clonality test: Algorithm from CTCL Study



Pretest probability is important

Zhang, B. et al. *JMD*. 2010;12:320-327. PMID: 20203005

Clonality Test by Next Generation Sequencing

- Amplify and sequence the v-j regions
- Detect clonal sequence by 1) % of total (>2.5%) and above background (e.g. >3x ~ 10x of the 3rd or 4th most abundant clonotype)
- Better analytic sensitivity and specificity
- Provide detail sequence information of the rearrangements
- Accurate information for clonal relation of different lesions
- Easily designed for MRD detection
  - Require diagnostic index sequence to compare the clonal rearrangement
  - Analytic precision dependent on sequencing read-depth, but limited by Poisson sampling and sequencing error
  - Clonal sequences may not be stable after therapy
- Bias introduced by PCR could be a major issue

Technical limitations and non-malignant causes of false-positive IG/TCR gene rearrangement results

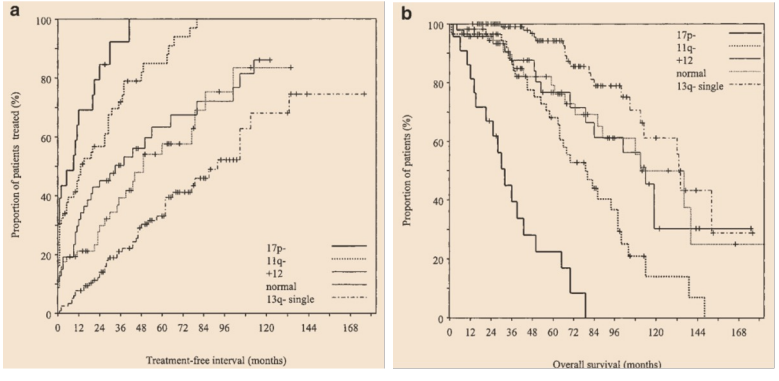
Mendoza H. et al. *Pathology.* 2021; 53(2):157–165

Non-neoplastic causes of positive IG gene rearrangement studies	T-cell neoplasms
	Immunosuppression
	Autoimmunity
Non-neoplastic causes of positive TCR gene rearrangement studies	B-cell neoplasms
	Viral infections
	Benign reactive lesions
	Benign skin disorders
	Oligoclonal T-cell populations in elderly
	Recovery from chemotherapy or stem cell transplantation
Technical limitations affecting both IG and TCR gene rearrangement studies	Use of non-preferred fixatives
	Small tissue samples
	Rare rearrangements not covered by available primer sets
	Small monoclonal populations outside of sensitivity of current tests
	Undersized and oversized PCR products

Mutation Profiling for Lymphoma

CLL/SLL: Established Markers and Targets

- FISH or array test:
  - del 17p (*TP53*)
  - del 11q (*ATM*)
  - del 13q (miR-15a, miR-16-1)
  - trisomy 12

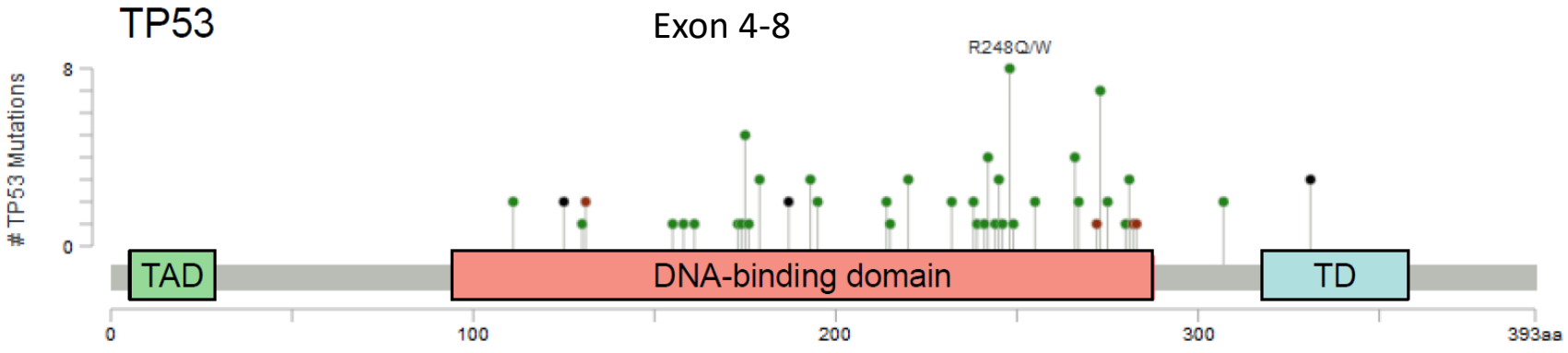


Leukemia 2002; 16: 993

- Molecular test:
  - *IGHV* Somatic hypermutation
  - *TP53* mutation
  - NGS mutation profiling for targeting therapy and drug resistance:
    - BTK inhibitors: Ibrutinib, Acalabrutinib
    - PI3K inhibitors: Idelalisib, Duvelisib
    - BCL2 inhibitor: Venetoclax

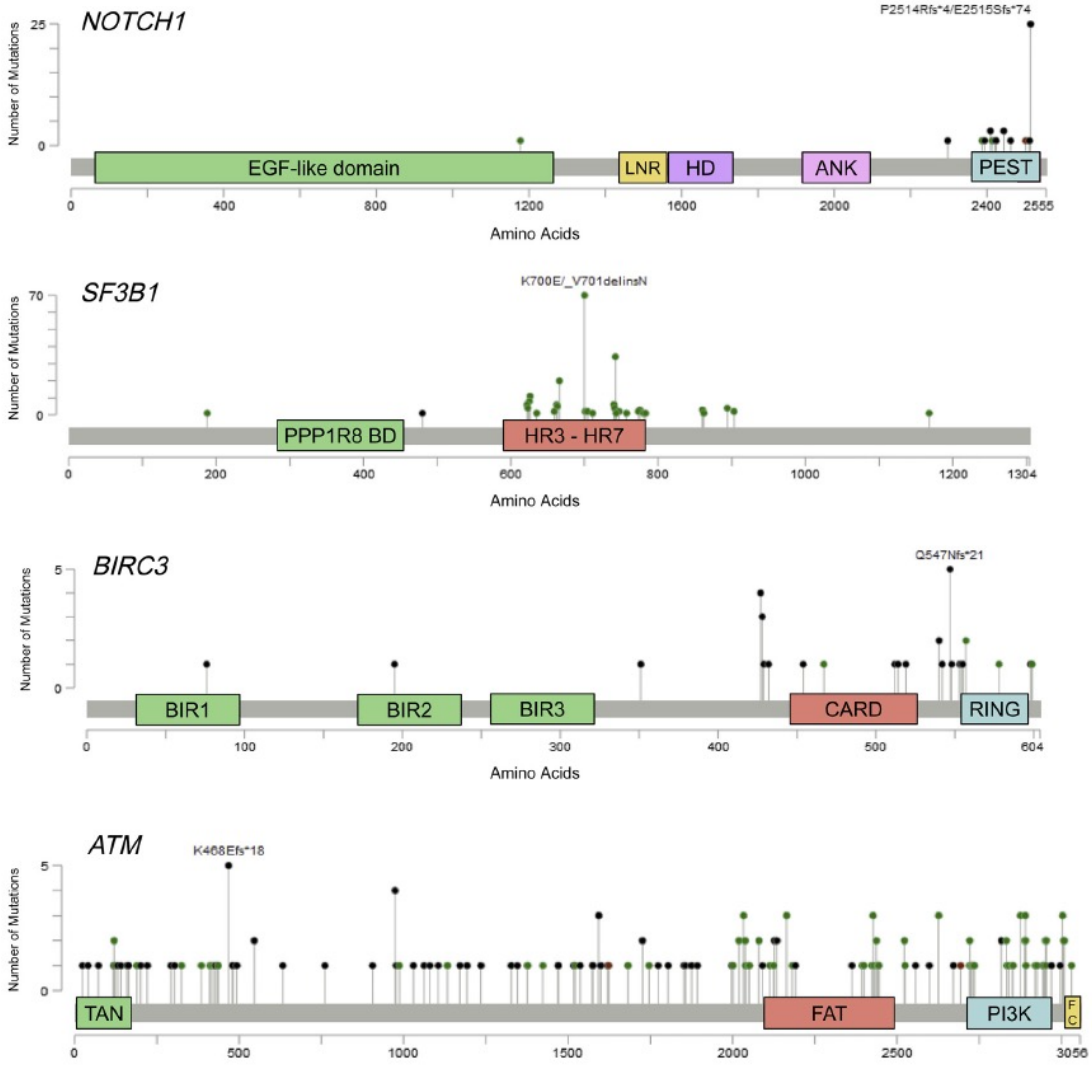
CLL/SLL: *TP53* Mutations

Lee J & Wang YL. JMD. 2020;22(9):1114-1125



- Green circles: missense mutations; Black: truncating mutations; Brown, inframe mutations
- Frequency:
  - Del(17p): untreated ~6%, treated ~16%
  - Mutation: untreated: ~8%, treated 21%
- Functional consequences: inactivation, dominant negative effect
- Clinical significance: poor survival, poor response to chemotherapy

CLL/SLL: Emerging Markers



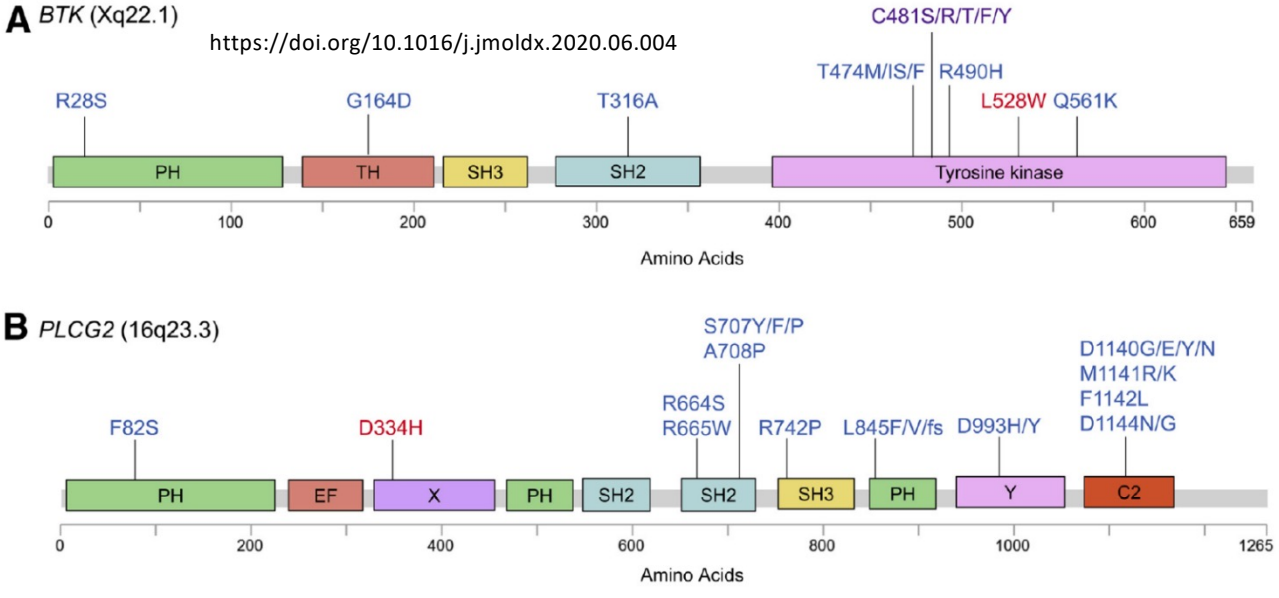
Lee J & Wang YL. JMD. 2020;22(9):1114-1125

CLL/SLL: Emerging Markers

		Time to Treatment	Overall Survival
TP53	Clonal	No impact	Shorter OS
	Subclonal	No impact	Shorter OS
SF3B1	Clonal	Shorter TTT	Trend for a shorter OS
	Subclonal	No impact	No impact
BIRC3	Clonal	No impact	Trend for a shorter OS
	Subclonal	No impact	No impact
NOTCH1	Clonal	Shorter TTT	Shorter OS
	Subclonal	Shorter TTT	No impact
ATM	Clonal	Shorter TTT	No impact
	Subclonal	*	*

Lee J & Wang YL. JMD. 2020;22(9):1114-1125

CLL/SLL: Mutations Associated with Resistance



- **<u>BTKI resistance</u>**: *BTK* mutation (70%) and *PLCG2* mutation (10%) is considered major mechanism.
- Preexisting subclonal mutation is predictive of evolving into resistance and relapse.
- **<u>Venetoclax resistance:</u>** *BCL2* G101V and D103Y, *CDKN2A* and *BTG1* mutations, *MCL1*, *PRKAB2* amplification.

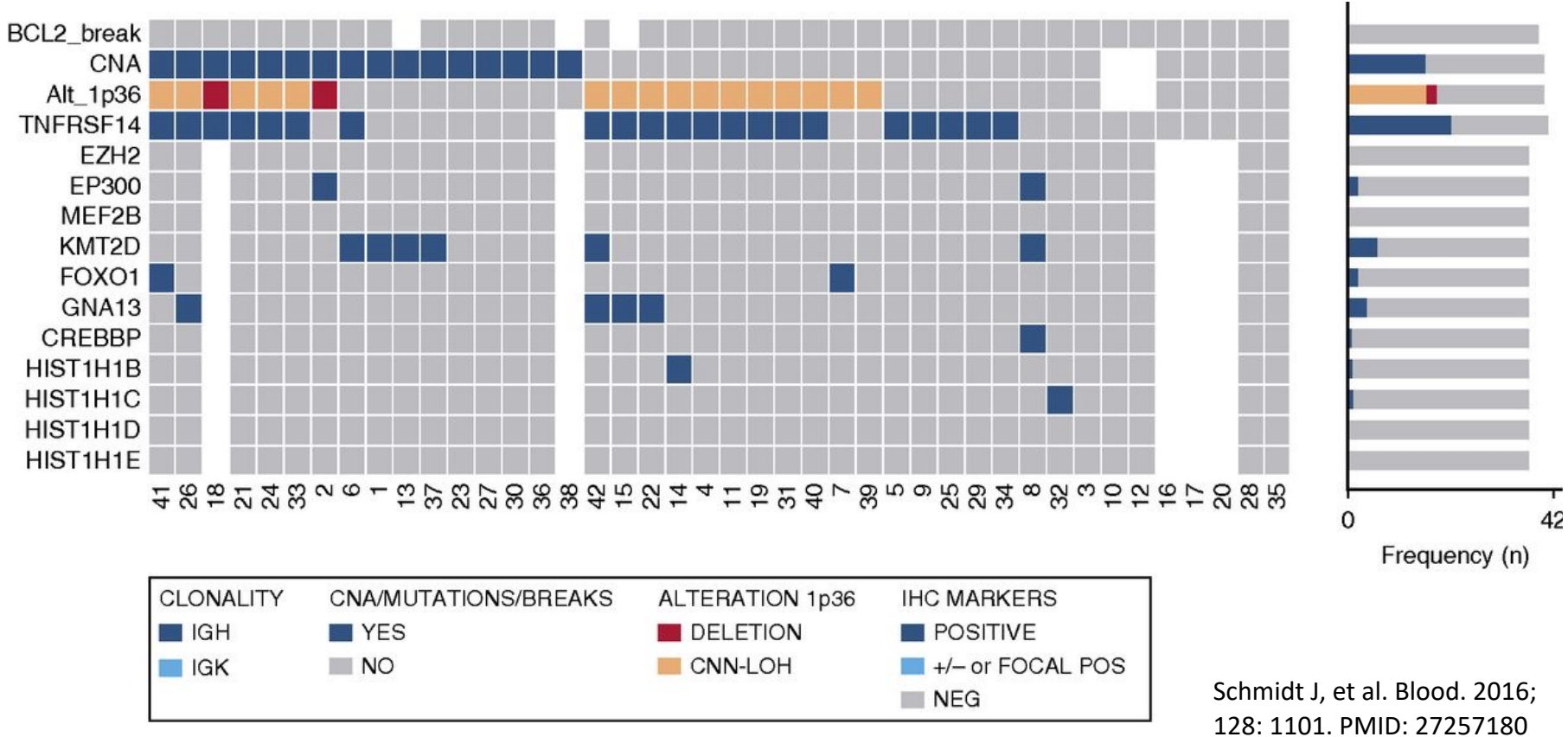
  Red color: associated with Richter transformation

Lee J & Wang YL. JMD. 2020;22(9):1114-1125

Follicular Lymphoma: Mutation Profile

- ***IGH::BCL2* rearrangement** with overexpression of BCL2 is a typical molecular feature of follicular lymphoma.
- ***IGH::BCL2* negative FL**:
  - Recurrent alterations of gene and/or 1p36, *CREBBP,* and *EZH2*
  - **Diffuse type, CD23+:** *STAT6* mutation and del or CNLOH 1p36.
  - **Pediatric type**; low genetic complexity with mutation in *TNFRSF14*
  - **PCFCL:** mutations more frequent in *TNFAIP3;* similar occurrences in *TNFRSF14* or del1p36 deletions, less frequent in *CREBBP, EP300, EZH2 KMT2D.*
- ***EZH2* Activating mutation, CNV**: resulting in aberrant methylation of histone H3 lysine 27 (H3K27m3) in 20–25% of FL.
  - *H3K27* methylation: expression is a useful surrogate for *EZH2* alteration
  - Overexpressed H3K27m3 in 89% of FL and 100% of PCFCL (independently of BCL2).
  - 95% of FH and 100% of PTFL cases lacked H3K27m3 overexpression.
  - H3K27m3 overexpression not specific for FL.

Molecular Genetic Findings in Pediatric FL

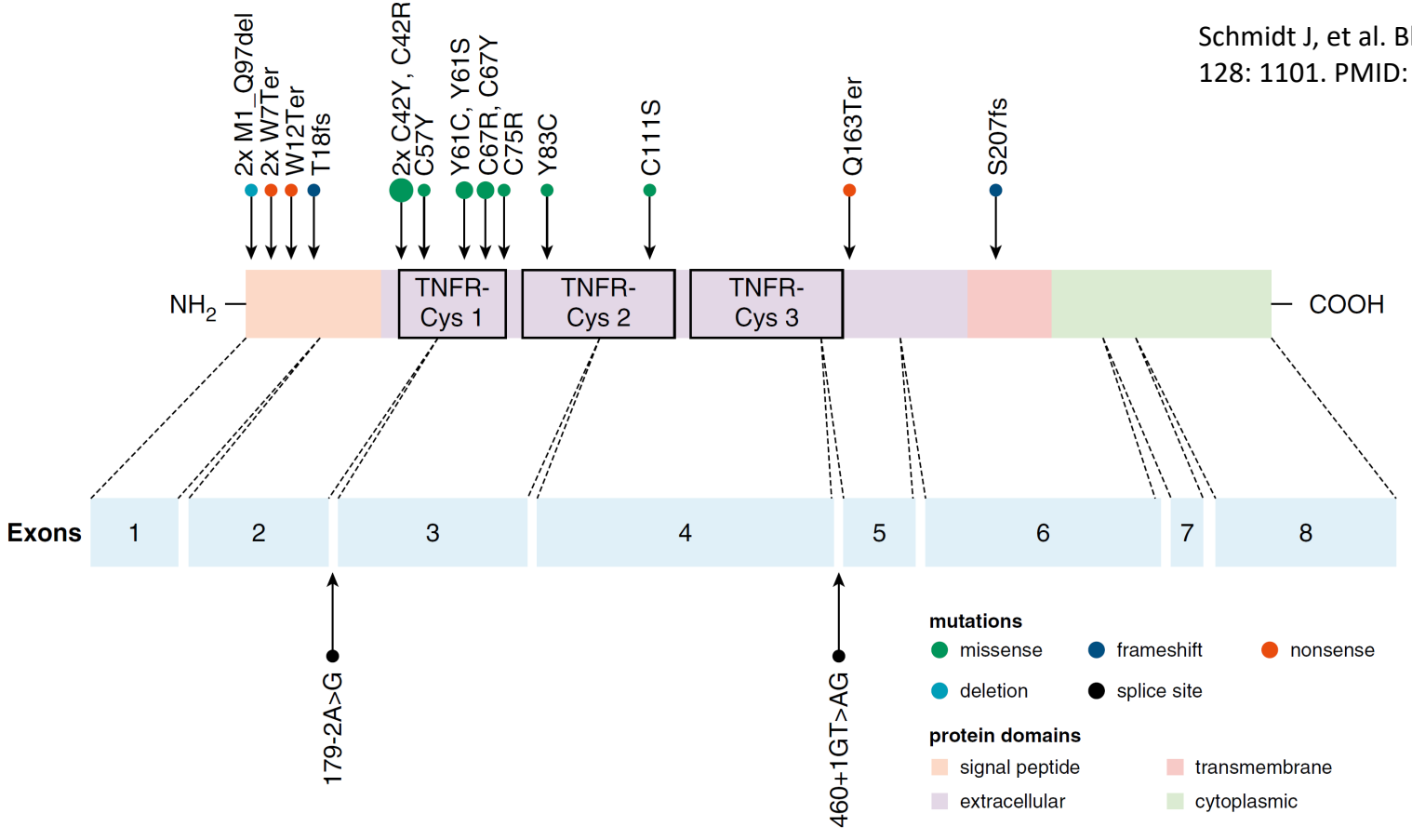


Schmidt J, et al. Blood. 2016; 128: 1101. PMID: 27257180

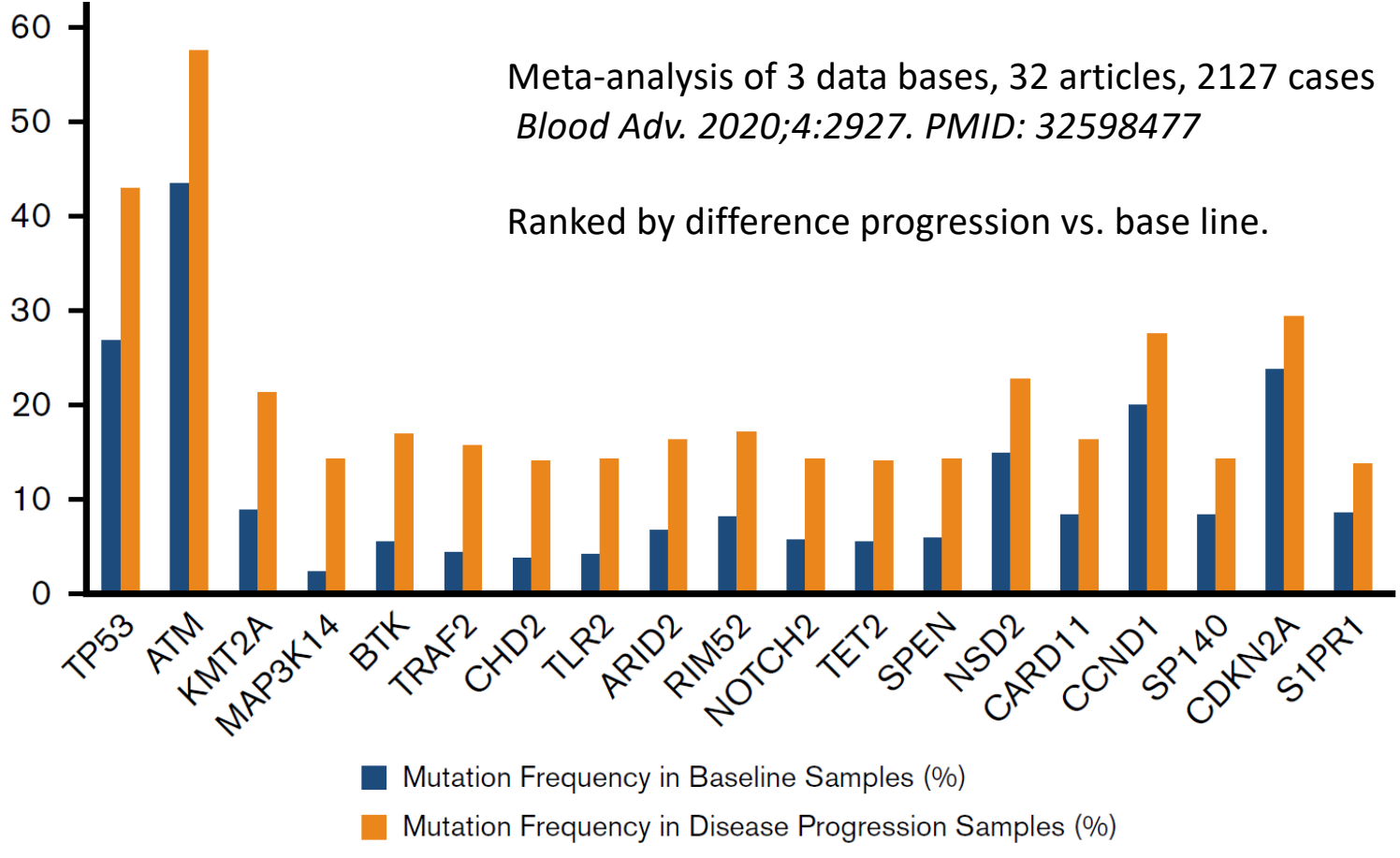
- 18 of 40 cases analyzed (45%) carried deletions (2 cases) or CNN-LOH (16 cases) of 1p36.32 including the *TNFRSF14*.
- 10 cases (24%) carried 1p36 alterations (9 cases with CNN-LOH and 1 case deletion) as the only genetic abnormality.

TNFRSF14 mutation in Pediatric FL

Schmidt J, et al. Blood. 2016; 128: 1101. PMID: 27257180



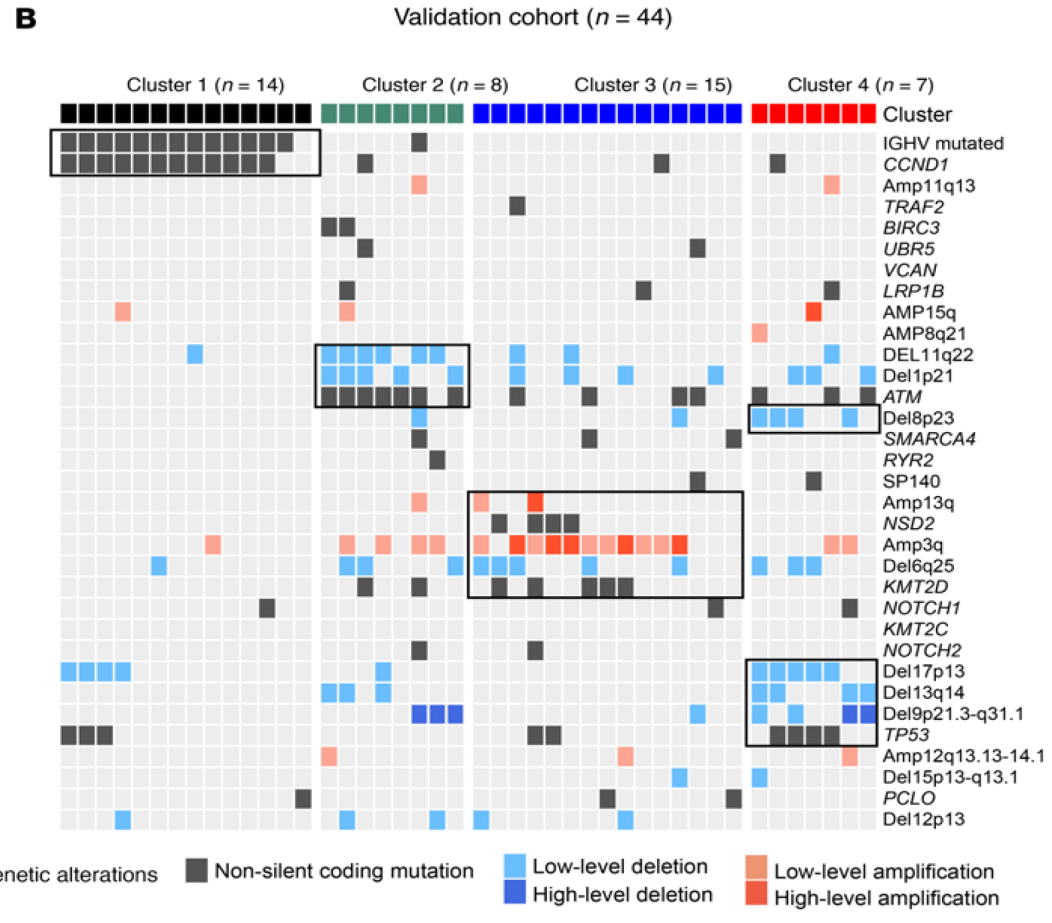
Mantle Cell Lymphoma: Mutation Prevalence

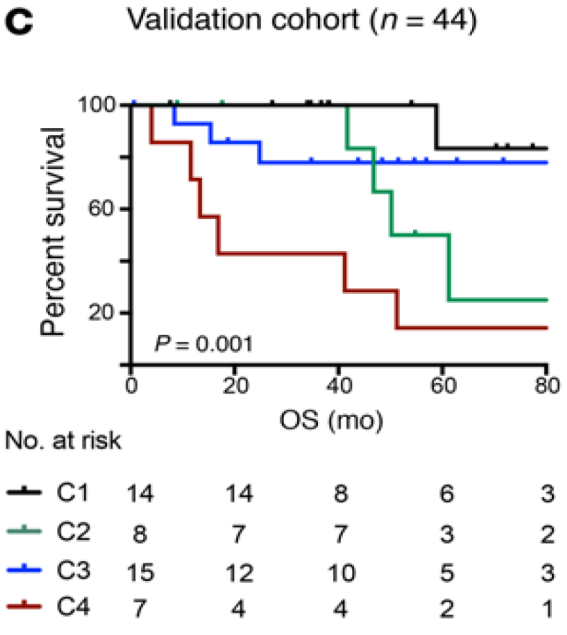


Meta-analysis of 3 data bases, 32 articles, 2127 cases
Blood Adv. 2020;4:2927. PMID: 32598477

Ranked by difference progression vs. base line.

Mantle Cell Lymphoma: Genetic Clusters





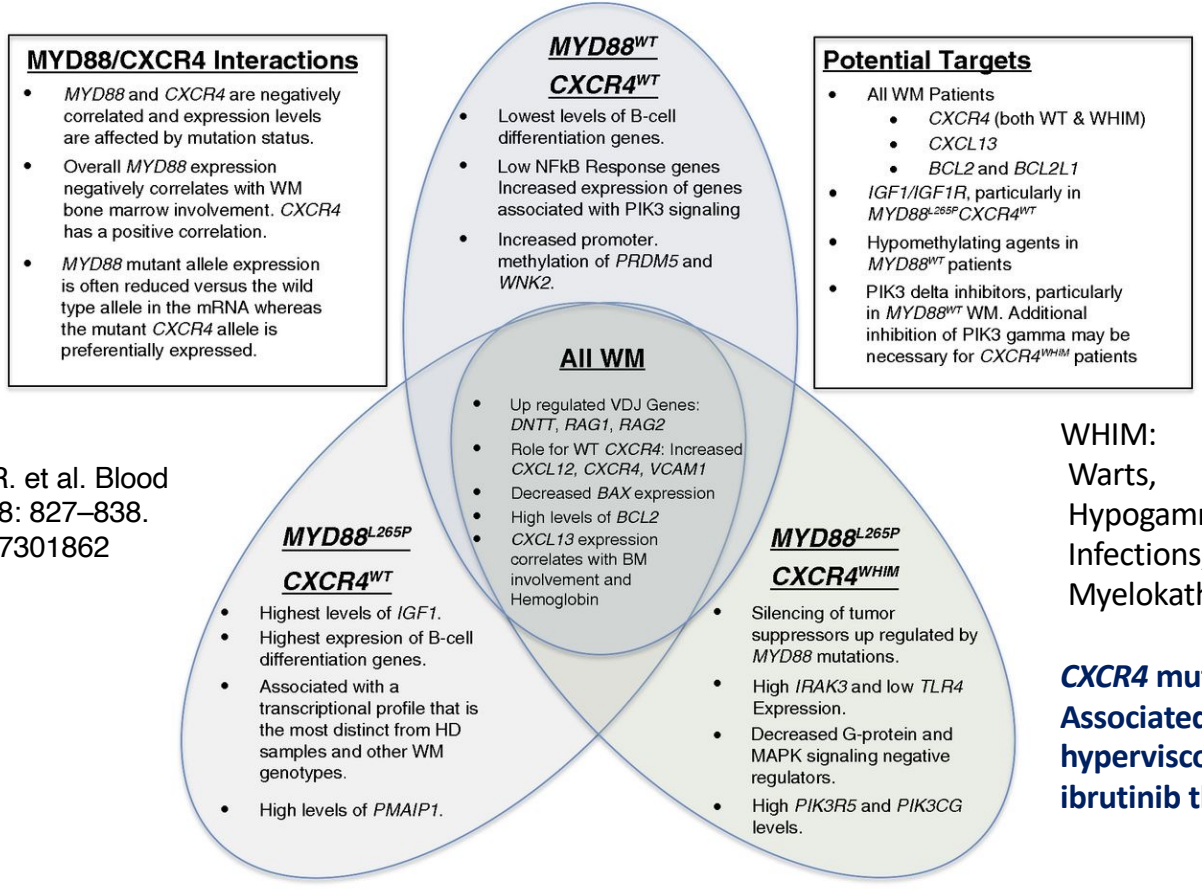
J Clin Invest. 2022;132(3):e153283. PMID: 34882582

MCL: Mutations and Prognosis/Treatment Response

- **Unfavorable outcome:** Unmutated *IGHV*, complex karyotype, mutation in ***TP53****, ANK2, NOTCH1/2, BIRC3, CDKN2A* (deletion)*, NSD2 (WHSC1), CCND1*, and *MYC* overexpression
- **Poor response to ibrutinib:** *BIRC3* aberrations (mutation, deletion), *SWI/SNF* (*SMARCA4*)
- **Acquired ibrutinib resistance:** chromosomal complexity, *NSD2, NOTCH2, UBR5, BIRC3, TRAF2, MAP2K14, KMT2D, CARD11, SMARCA4*, and ***BTK***. Activation of *PI3K/AKT* and the integrin-β1 signaling pathway.

Ref: PMID: 30963600, 32770099

Lymphoplasmacytic Lymphoma/WM

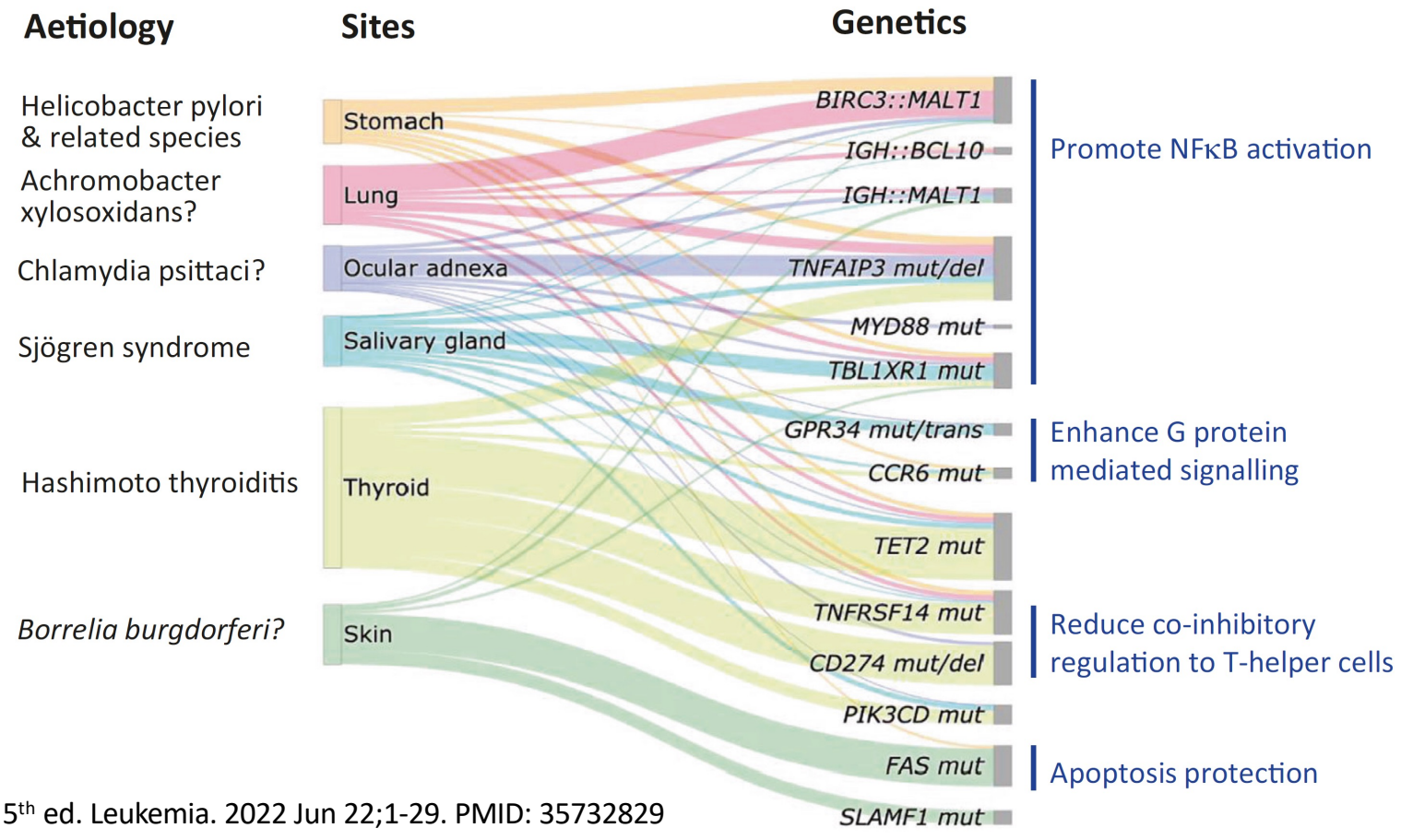


Hunter R. et al. Blood 2016;128: 827–838. PMID: 27301862

WHIM:
Warts,
Hypogammaglobulinemia,
Infections,
Myelokathexis

***CXCR4* mutation (40% of LPL): Associated with symptomatic hyperviscosity and resistance to ibrutinib therapy.**

Etiology and recurrent genetic abnormalities: Extranodal MZL



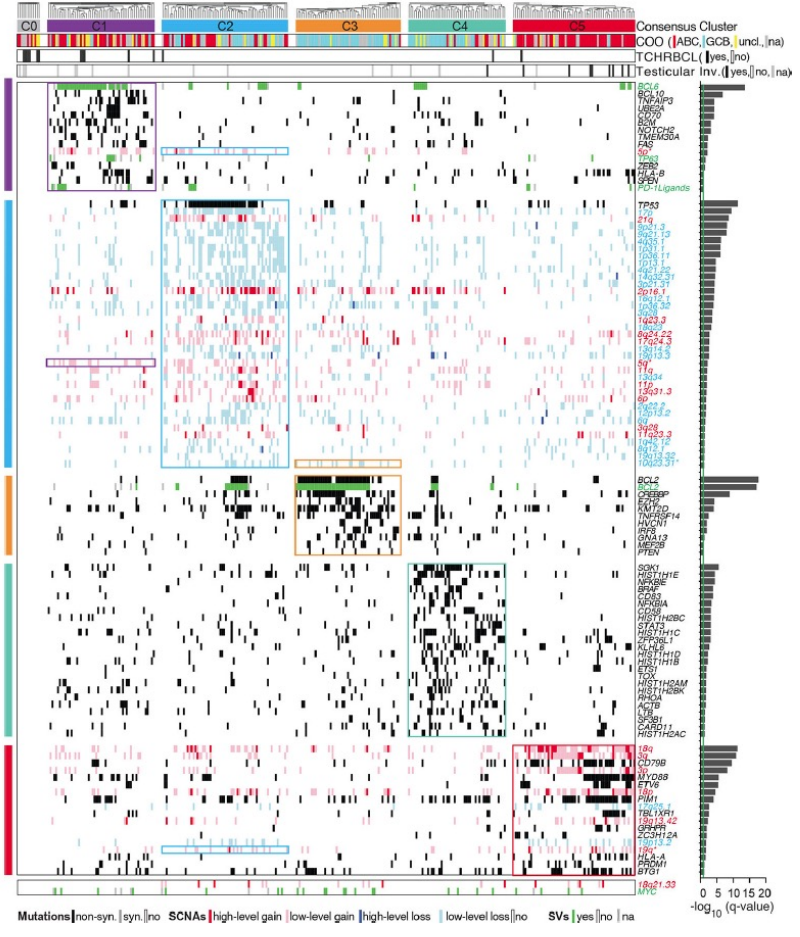
WHO 5th ed. Leukemia. 2022 Jun 22;1-29. PMID: 35732829

DDx of Small B-cell Lymphomas CD5 & CD10-negative,*BCL2*-R–negative

- Favor FL:
  - ✓ del1p36, *BCL6* rearrangement
  - ✓ Mutation in *CREBBP*, *EZH2, TNFRSF14, STAT6*
  - ✓ (PCFCL) *TNFAIP3*
- Favor MZL:
  - ✓ Characteristic translocations involving *MALT1*, *FOXP1*, and *BCL10*;
  - ✓ Mutations in *KLF2, NOTCH2, PTPRD, CARD11, IRF8,* or *MAP2K1*
- Favor LPL: *MYD88* and *CXCR4*
- HCL: *BRAF* mutation (V600E)

Blood 2022;140:2193
PMID: 36001803

Complexity of Genetic Abnormalities in DLBCL



- **Cluster 0:** No defining genetic drivers. THRLBCL, probably low tumor percentage
- **Cluster 1**: low-risk ABC, possibly MZL origin. *BCL6* SVs with mutations of NOTCH2 pathway; NF-kB pathway, *BCL10, TNFAIP3* and *FAS*. *MYD88* non-L265P
- **Cluster 2**: an ABC/GCB independent group, bi-allelic inactivation of *TP53*, and loss of *CDKN2A*, *RB1*, associated genomic instability.
- **Cluster 3**: high-risk GCB DLBCLs with *BCL2::IGH*, inactivating mutations and/or copy loss of PTEN and mutations in chromatin modifiers, *KMT2D*, *CREBBP* and *EZH2*, B-cell TFs and BCR signaling. Also includes DH/TH LBCL
- **Cluster 4**: low-risk GCB DLBCLs with alterations in multiple histone genes, *JAK/STAT, BRAF, STAT3*, and *RHOA*
- **Cluster 5**: high-risk ABC DLBCLs, *BCL2* copy gain, *MYD88* L265P, *CD79B* mutations, and extra-nodal tropism

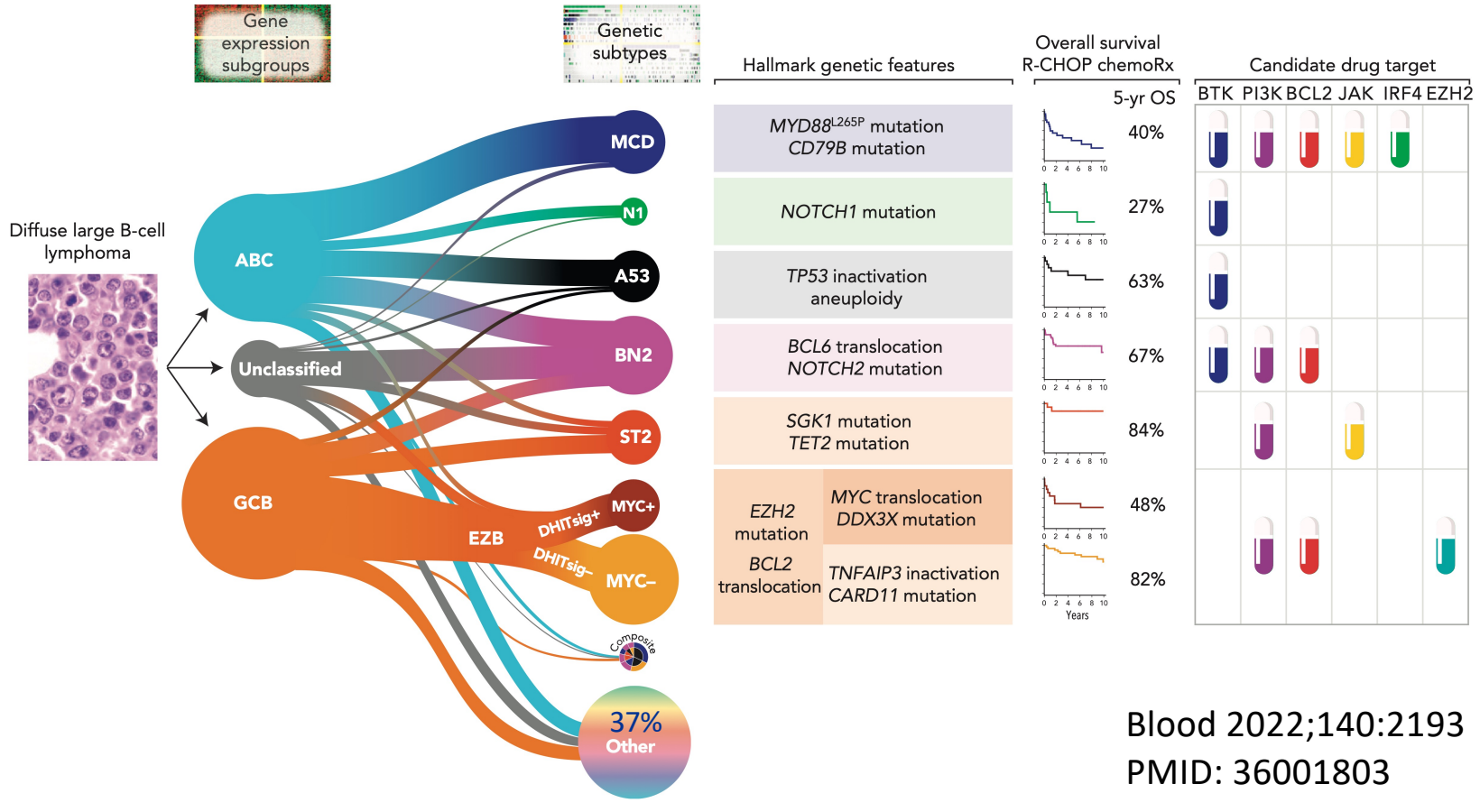
Nat Med. 2018;24:679. PMID: 29713087

DLBCL genetic subtypes: comparison of equivalent subtypes

LymphGen	Modified HMRN	Harvard	Main Genetic Alterations	COO	Clinical Outcome	Related Lymphoma
MCD	**MYD88**	**C5**	*MYD88*^{L265P}, *CD79B*, *PIM1*, *ETV6*, *CDKN2A*	ABC	Poor	Primary CNS lymphoma, Primary testis lymphoma
EZB	**BCL2**	**C3**	*BCL2*-R, *EZH2*, *CREBBP*, *KMT2D*, *TNFRSF14*	GCB	Good	Follicular lymphoma
EZB-MYC+	**BCL2-MYC**				Poor	Double hit lymphoma
BN2	**NOTCH2**	**C1**	*BCL6*-R, *NOTCH2*, *BCL10*, *SPEN*, *CD70*, *TNFAIP3*	ABC, GCB, UC	Intermediate	Marginal zone lymphoma
ST2	**TET2/SGK1**	**C4**	*TET2*, *SGK1*, *KLHL6*, *BRAF*	GCB	Good	Nodular lymphocyte predominant Hodgkin lymphoma
	SOCS1/SGK1		*SOCS1*, *SGK1*, *CD83*, *NFKBIA*, *NFKBIE*, *STAT3*	GCB	Very good	Primary mediastinal B cell lymphoma
Other	NEC	**C0**				
N1	**NOTCH1**	NA	*NOTCH1*, *ID3*	ABC	Poor	Chronic lymphocytic leukaemia
A53	NA	**C2**	*TP53*, aneuploidy	Mixed	Intermediate	

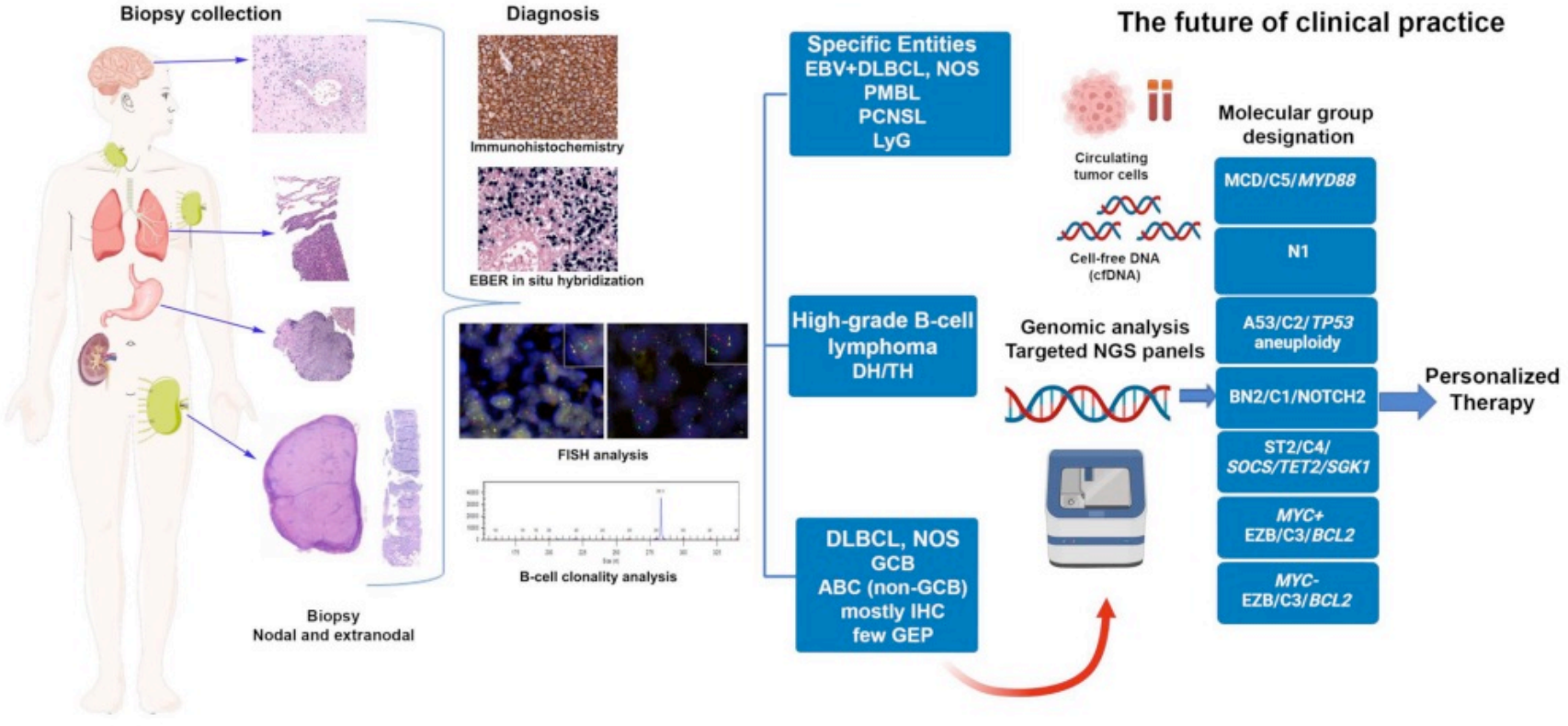
Mod Pathol. Jan 2023;36(1):100007. PMID: 36788062

Genetic subgroups of DLBCL: LymphGen Algorithm



Blood 2022;140:2193
PMID: 36001803

Algorithm for the diagnostic work-up of aggressive B-cell lymphomas

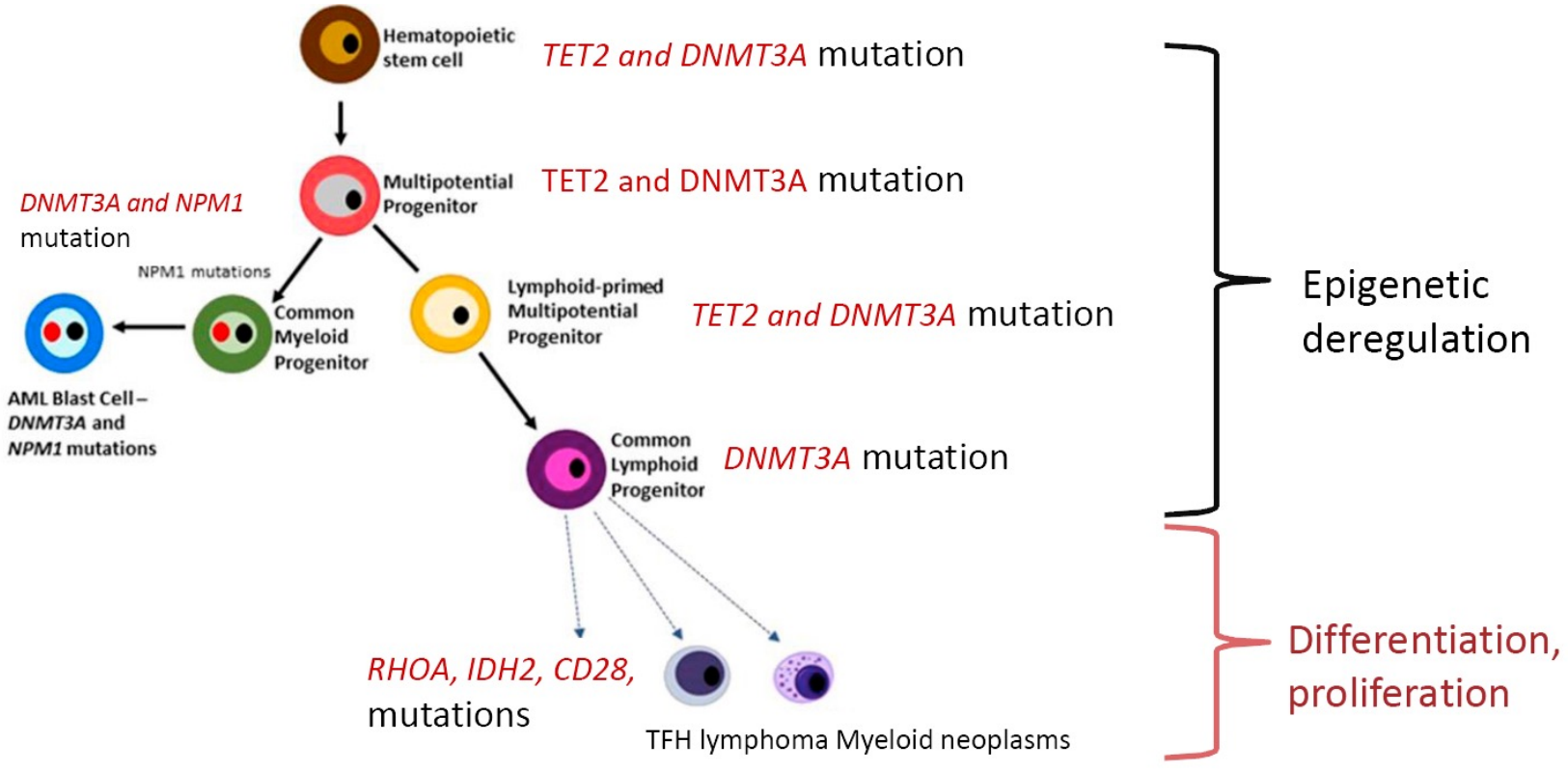


WHO 5th ed. Leukemia. 2022 Jun 22;1-29. PMID: 35732829

TABLE 2 Mutations associated with nodal follicular helper T cell lymphomas.

Genes	Frequency			Front Oncol. 2023;13:1105651. PMID: 36793612
	AITL	nTFH-NOS	nTFH-FL	
GTPase				
RHOA^{G17V}	50-70	25-50	60	• G17V specific to AITL/PTCL-TFH
				• Not associated with prognosis
Epigenetic regulators				
TET2	40-80	50-75	75	• Found in other neoplasms (myeloid)
DNMT3A	20-30	7-18	25	• *TET2* co-occur w/ *DNMT3* and *IDH2* mutations is specific to TFH lymphomas
IDH2^{R172}	20-45	0	0	• *IDH2* mutations mostly restricted to AITL • Presence of clear cells • More pronounced TFH signature • Strong CD10 and CXCL13 expression • Chr 5 and 21 gains • More aberrant genome than IDH2 negative cases • Clinical trial: enasidenib
TCR signaling pathway				
PLCγ	8-14	6.25	N/A	• Not specific (PTCL-NOS)
CD28	10-12	0	N/A	• Worse prognosis in AILT

Multistep model of pathogenesis TFH-derived TCL



Megan Lim. AMP 2018 Presentation. Modified from Koeffler HP and Leong G. Leukemia 2017; 31: 534

Implications of Epigenetic Gene Mutations

- Helpful for the DDx of TFH lymphoma vs. other PTCL
- Background CHIP interferes with interpretation
- Clonal relationship with concurrent or secondary myeloid neoplasms
- Potential therapeutic targets

HDACi: TFHL vs PTCL, Multicentr, Phase 2

Table 3. Response to oral azacytidine and romidepsin across study populations

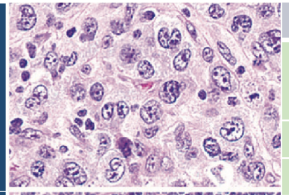
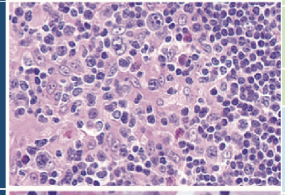
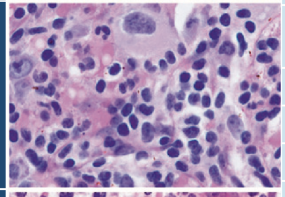
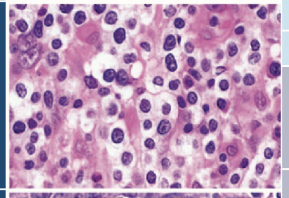
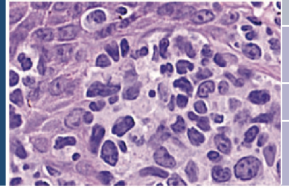
Response	All patients (n = 23)	Treatment-naïve patients (n = 10)	R/R disease (n = 13)	tTFH phenotype (n = 15)	Other subtypes (n = 8)
Overall response	14 (61)	7 (70)	7 (54)	12 (80)	2 (25)
Complete response	10 (43)	5 (50)	5 (38)	9 (60)	1 (12.5)
Partial response	4 (17)	2 (20)	2 (15)	3 (20)	1 (12.5)
Stable disease	5 (22)	2 (20)	3 (23)	2 (13)	3 (37.5)
Progressive disease	4 (17)	1 (10)	3 (23)	1 (7)	3 (37.5)
Not evaluable	2	2	0	2	0

Falchi L. et al. Blood. 2021;137(16):2161-2170. PMID: 33171487

JAK-STAT Pathway Mutations in Mature TCL

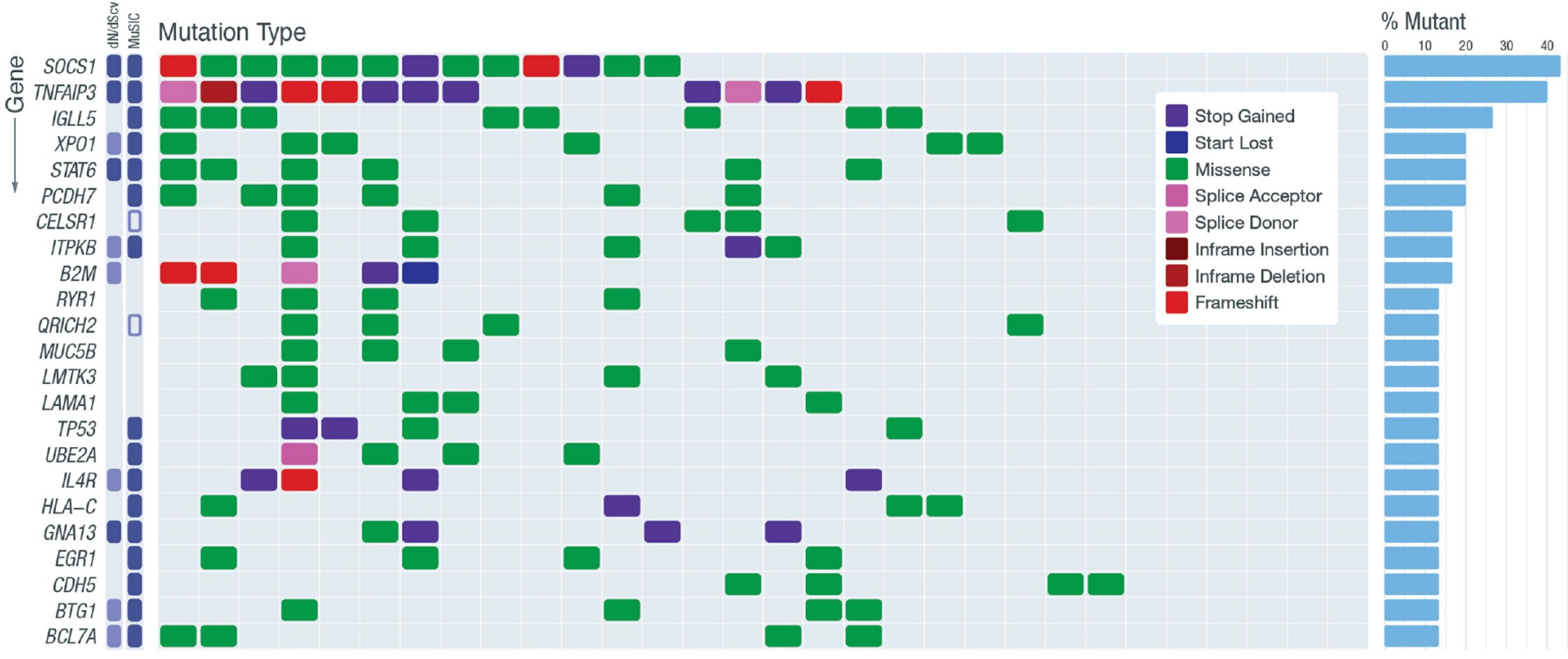
- **ALCL, ALK-negative:** activation of STAT3
- **TPLL**: *TCL1A*-R or *MTCP1*-R
  - Mutually exclusive mutations affecting *IL2RG, JAK1, JAK3*, or *STAT5B*
- **GI Lymphomas:**
  - EATL: *JAK1* and *STAT3* mutation more common
  - MEITL: ***SETD2****, GNAI2, JAK3,* and *STAT5B* mutations more common
  - *STAT3::JAK2* fusions in **indolent** CD4+ **T-cell lymphoproliferative disorder** of the gastrointestinal tract: 4/5 cases
- **Hepatosplenic T-cell lymphoma**: i7q, +8
  - Mutations in *SETD2, INO80, PIK3CD, TET3, SMARCA2* and *STAT5B* or *STAT3.*
- **T-LGLL:**
  - Gain of function mutations in *STAT3* and *STAT5B*
  - *STAT3* mutation in CD8+ T-LGLL: associated with neutropenia and poorer overall survival.
  - *STAT5B* has no prognostic impact in CD4+ T-LGLL and gamma/delta T-LGLL.

Recurrent genetic lesions in mature NK- and T-cell neoplasms with potential therapeutic intervention

		NK and T-cell neoplasms	Genetic lesions	Mechanism	Potential therapeutic intervention	
ALCL		TFHL, PTCL NOS, CTCL, ATLL	***CD28 FYN CARD11 PLCG1 RHOA* mutations**	TCR signaling activation	PI3K inhibitors (duvelisib, copanlisib), mTOR inhibitors (everolimus, temsirolimus), TKI (dasatinib), ITK inhibitor (CPI-818) (a,c)	TCR
		TFHL, CTCL, ATLL	***CD28* fusions**	Increased CD28 signaling	CTLA4 blockade (ipilimumab) (*CTLA4::CD28*) (b, c)	
		TFHL, PTCL NOS	***FYN::TRAF3IP2***	NF-kappaB activation	IkB kinase inhibitors (c)	
PTCL-NOS		TFHL	***ITK::SYK***	SYK and JAK3/STAT5 activation	JAK3 inhibitor (tofacitinib), dual SYK and JAK inhibitor (cerdulatinib) (c)	
		ALK- ALCL, PTCL NOS, ATLL	***VAV1* fusions**	VAV1 and RAC1 activation	RAC1 inhibitor (azathioprine) (c)	
T-LGLL		T-LGLL, NK-LGLL, T-PLL, MEITL, EATL, HSTL, ENKTCL, ALK- ALCL, BIA-ALCL, PTCL NOS	***JAK1 JAK2 JAK3 STAT3 STAT5B SOCS1* mutations**	STAT3 phosphorylation	JAK inhibitors (ruxolitinib, tofacitinib, gandotinib, momelotinib), dual SYK and JAK inhibitor (cerdulatinib) (a, c)	JAK/STAT
		ALK- ALCL, BIA-ALCL, CD30+ PTCL NOS, ITLPD-GI	***JAK2* fusions**	STAT5 phosphorylation		
		ALK+ ALCL	***ALK* fusions**	STAT3 phosphorylation	ALK inhibitors (crizotinib, alectinib) (a)	
		ALK- ALCL	***FRK* fusions**	STAT3 phosphorylation	Kinase inhibitor (dasatinib) (c)	
		PTCL NOS, TFHL-F	***ITK::FER***	STAT3 phosphorylation	JAK3 inhibitor (tofacitinib), kinase inhibitors (c)	
AITL		ALK- ALCL	***ROS1* fusions**	STAT3 phosphorylation	ROS1 inhibitor (JNJ-ROS1i-A) (c)	
		ALK- ALCL	***TYK2* fusions**	STAT1 phosphorylation	JAK inhibitors, TYK2 inhibitor (deucravacitinib) (c)	
		TFHL, PTCL NOS, CTCL, ATLL	***TET2 DNMT3A IDH2* mutations**	DNA hypermethylation Oncometabolite production ($IDH2^{R172}$)	Hypomethylating agents (5-azacytidine, decitabine), histone deacetylase inhibitors (romidepsin, belinostat, chidamide, vorinostat); IDH2 inhibitors (enasidenib) (a, b)	Epigenetics
		MEITL, HSTL	***SETD2* mutations deletions**	Loss of H3K36me3	Wee1 inhibitor (adavosertib) (c)	
ATLL		ENKTCL, ATLL	***CD274* CNA or SV**	PD-L1 overexpression	Anti-PD1 antibodies (pembrolizumab, nivolumab) (b, c)	Others
		ATLL	***CCR4* mutations**	Increased CCR4 expression	Anti-CCR4 antibody (mogamulizumab) (b)	
		ALK- ALCL	***ERBB4* fusions or truncated transcripts**	ERBB4 overexpression	Inhibitors of ERBB-family kinases (lapatinib) (c)	

Blood 2022;140:2193. PMID: 36001803

Recurrently mutated genes in CHL



Total number of cases: 31; mutations seen in >3 cases are shown. Ultra-Deep Sequencing, not R-S cell sequencing

Cancer Res Commun. 2023;3(11):2312-2330. PMID: 37910143

Table 1 Genes Included in the NGS for Lymphoid Malignancies Panel

ARAF	*CARD11*	*CSF1R*	*FAS*	*JAK1*	*MYD88*	*PTEN*	*TNFAIP3*
ARID1A	*CCND1*	*CUL4A*	*FBXW7*	*JAK3*	*NOTCH1*	*RHOA*	*TNFRSF14*
ARID1B	*CCND3*	*CUL4B*	*FOXO1*	*JUNB*	*NOTCH2*	*SF3B1*	*TP53*
ATM	*CCR4*	*CXCR4*	*FYN*	*KLF2*	*NRAS*	*SPEN*	*TRAF3*
B2M	*CD58*	*DDX3X*	*GNA13*	*KMT2D*	*NSD2*	*STAT3*	*VAV1*
BCL2	*CD79A*	*DIS3*	*ID3*	*KRAS*	*PIK3CA*	*STAT5B*	*XBP1*
BIRC3	*CD79B*	*DNMT3A*	*IDH1*	*MAP2K1*	*PIM1*	*STAT6*	*XPO1*
BRAF	*CDKN2A*	*EGFR*	*IDH2*	*MEF2B*	*PLCG1*	*TCF3*	
BTG1	*CRBN*	*EP300*	*IKZF1*	*MSC*	*PLCG2*	*TENT5C*	
BTK	*CREBBP*	*EZH2*	*IKZF3*	*MYC*	*PRDM1*	*TET2*	

Highlighted genes not in TSO500
Missing *SOCS1* (CHL)

Mayo Clinic Lymphoma NGS panel

J Mol Diagn 2024, 26: 583e598;
https://doi.org/10.1016/j.jmoldx.2024.03.008

Liquid Biopsy (cfDNA test) for Lymphoma

- Advantages and encouraging findings
  - At initial diagnosis: helpful when
    - Lymphoma cells are few (CHL, THRLBC)
    - Location difficult to get biopsy (PCNSL – CSF test; IVLBCL)
  - Follow up and MRD
    - cfDNA mutation level may reflect tumor burden.
    - NGS-based clonality test may be used to identify MRD
    - EMR and CMR reported to be useful indicator of long term outcome in DLBCL, CHL
- Issues and controversies
  - At diagnosis:
    - No mutation profile is entirely specific;
    - CHIP associated mutations frequently seen in old age patients
    - Currently no standard guideline for clinical diagnosis/classification
  - Follow up and MRD
    - Need more study on the clinical correlation of MRD
    - Test techniques need to be standardized.

NGS for Lymphoma Mutation Profiling: Conclusions and Future Trends

- Mutational profiling enables a better understanding of the molecular pathobiology of lymphoma and refines the classification of lymphomas.
- Some genetic alterations are becoming classification markers or effective targets of novel treatment.
- Lymphoma profiling panels are evolving but will likely become popular soon.
- Liquid biopsy (cfDNA tests) may be a practical tool for challenging cases and post-treatment follow-up.

Most frequently mutated genes by entity

Myeloid neoplasms

MDS		MDS/MPN-U		MPN		CML		aCML		CMML		MLN-eo		AML		s-AML		t-AML	
SF3B1	31	*ASXL1*	60	*JAK2*	68	*ASXL1*	21	*ASXL1*	86	*TET2*	67	*RUNX1*	11	*NPM1*	23	*SRSF2*	44	*KRAS*	38
TET2	25	*TET2*	30	*ASXL1*	20			*SRSF2*	48	*ASXL1*	58	*FANCL*	6	*DNMT3A*	19	*ASXL1*	31	*NRAS*	13
ASXL1	18	*CSF3R*	30	*TET2*	18			*TET2*	34	*SRSF2*	46			*TET2*	19	*SF3B1*	31	*CSF3R*	13
SRSF2	13	*SF3B1*	20	*CALR*	11			*SETBP1*	34	*NRAS*	22			*FLT3-ITD*	16	*RUNX1*	31	*TP53*	6
DNMT3A	11	*DNMT3A*	20	*SRSF2*	10			*EZH2*	20	*PTPN11*	17			*NRAS*	14	*TET2*	25	*RUNX1*	6
TP53	11	*JAK2*	20	*DNMT3A*	6			*RUNX1*	18	*SETBP1*	17			*ASXL1*	14	*NRAS*	25	*PTPN11*	6
RUNX1	7	*NRAS*	20	*U2AF1*	5			*NRAS*	16	*KRAS*	13			*SRSF2*	12	*IDH2*	19	*WT1*	6
U2AF1	6	*RUNX1*	20					*CUX1*	10	*RUNX1*	13			*TP53*	11	*FLT3*	19	*BCOR*	6
ZRSR2	5	*CEBPA*	20					*KRAS*	8	*EZH2*	13			*CEBPA*	10	*STAG2*	13	*KIT*	6
STAG2	5	*STAG2*	20					*STAG2*	8	*CBL*	13			*IDH2*	9	*TP53*	13	*ASXL2*	6
		SRSF2	10					*CBL*	8	*CUX1*	13			*RUNX1*	9	*DNMT3A*	13	*DIS3*	6
		EZH2	10					*GATA2*	8	*NF1*	8			*KRAS*	8	*JAK2*	13	*LRP1B*	6
		BRAF	10					*CSF3R*	8					*FLT3*	8	*KRAS*	13	*FAT4*	6
		NF1	10					*SF3B1*	6					*WT1*	8	*FLT3-ITD*	13	*SMC1A*	6
		ATM	10					*NF1*	6					*PTPN11*	6	*EZH2*	13		
		SETBP1	10											*IDH1*	5	*PTPN11*	13		
		SH2B3	10											*SF3B1*	5	*BCOR*	13		
		GNAS	10											*STAG2*	5	*GATA2*	13		
		PRPF8	10													*CUX1*	13		
																NPM1	6		
																U2AF1	6		

Mixed myeloid/lymphoid

AUL		MPAL	
SRSF2	46	*TP53*	33
TET2	38	*DNMT3A*	27
RUNX1	38	*FLT3-ITD*	17
ASXL1	29	*NRAS*	10
BCOR	25	*RUNX1*	10
DNMT3A	13	*FLT3*	10
KRAS	13	*WT1*	10
EZH2	13	*NF1*	7
STAG2	8	*TET2*	7
ZRSR2	8	*KRAS*	7
GATA2	8	*STAG2*	7
		PHF6	7

Plasma cell neoplasms

MGUS		MM	
NRAS	17	*KRAS*	26
KRAS	8	*NRAS*	21
TRAF3	8	*DIS3*	6
SUZ12	8	*TP53*	6

122 genes in 3096 cases of
28 hematological malignancies
Blood Adv. 2021;5(21):4426-4434.
PMID: 34570179

0%, n.a.
1-5%
5-10%
10-15%
15-20%
20-25%
25-30%
30-35%
35-40%
40-45%
45-50%
>50%

Lymphoid neoplasms

B-ALL		T-ALL		T-NHL		NK		B-NHL		BL		CLL		FL		MCL		LPL		HCL		HCL-v	
TP53	17	*NOTCH1*	38	*STAT3*	52	*STAT3*	23	*TP53*	50	*TP53*	60	*TP53*	13	*KMT2D*	87	*TP53*	24	*MYD88*	98	*BRAF*	100	*BIRC3*	23
NRAS	10	*PHF6*	30	*TET2*	9	*TET2*	13	*MYD88*	17	*MYC*	30	*NOTCH1*	12	*CREBBP*	73	*ATM*	18	*CXCR4*	51	*ARID1A*	7	*TP53*	12
KRAS	8	*DNMT3A*	13	*DNMT3A*	6	*ASXL1*	5	*ARID1A*	13	*ID3*	30	*SF3B1*	11	*ARID1A*	27	*CCND1*	18	*ARID1A*	20			*TET2*	9
KMT2D	5	*WT1*	12	*PHF6*	6	*KRAS*	5	*CXCR4*	13	*KMT2D*	20	*MYD88*	7	*BCL2*	13	*WHSC1*	12	*DNMT3A*	11			*BCOR*	9
PTPN11	5	*RUNX1*	11	*LRP1B*	6	*PPM1D*	5	*BIRC3*	10	*CD79B*	15	*ATM*	7	*EZH2*	11	*DNMT3A*	6	*TET2*	9			*CXCR4*	9
		BCOR	10	*CTCF*	6	*FAS*	5	*DNMT3A*	7	*MYD88*	10	*BIRC3*	5	*TNFRSF14*	11	*KMT2D*	6	*TP53*	9			*ASXL1*	7
		ASXL1	7	*JAK1*	6			*KLF2*	7	*CREBBP*	10			*EP300*	8	*CREBBP*	6	*CD79B*	5			*DNMT3A*	7
		IDH2	5							*BCL2*	10			*TP53*	6	*NOTCH1*	6					*ARID1A*	7
										FOXO1	10			*CARD11*	5	*ETV6*	6					*ATM*	7
										TBL1XR1	10			*CD79A*	5	*BIRC3*	6					*MAP2K1*	7
										ASXL1	5					*DIS3*	6					*KRAS*	5
										NRAS	5					*RB1*	6					*BCORL1*	5
										U2AF1	5					*LRP1B*	6					*ZBTB7A*	5
										BCOR	5					*NFKBIE*	6					*NOTCH2*	5
										ARID1A	5					*NOTCH2*	6						
										XPO1	5					*FOXO1*	6						
										RB1	5												
										CARD11	5												
										CDKN2A	5												
										KLHL6	5												

Other

BPDCN		PPBL	
TET2	67	*DNMT3A*	10
ASXL1	27		
SRSF2	27		
U2AF1	13		
ZRSR2	13		
TP53	7		
DNMT3A	7		
NRAS	7		
RUNX1	7		
PTPN11	7		
ATM	7		
PHF6	7		
CUX1	7		
DIS3	7		
KDM6A	7		
XPO1	7		
RB1	7		
KDM5A	7		

Acknowledgments

- My fellow hematopathologists in the Hematopathology Focus Interest Group
- CAPA Online Education Subcommittee and Hematopathology Subcommittee

The presentation file in PDF format is available for download on my personal webpage:

https://tinyurl.com/lszhang

Emory University