CCUS and Clonal Monocytosis of Clinical Significance

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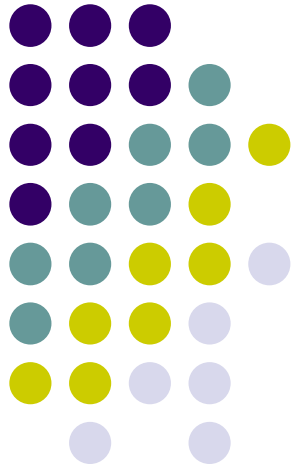
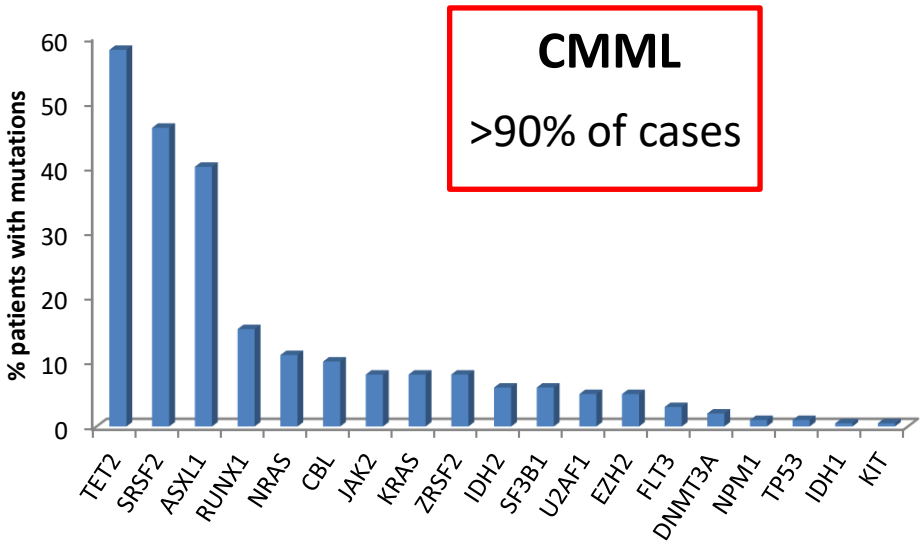


DISCLOSURE

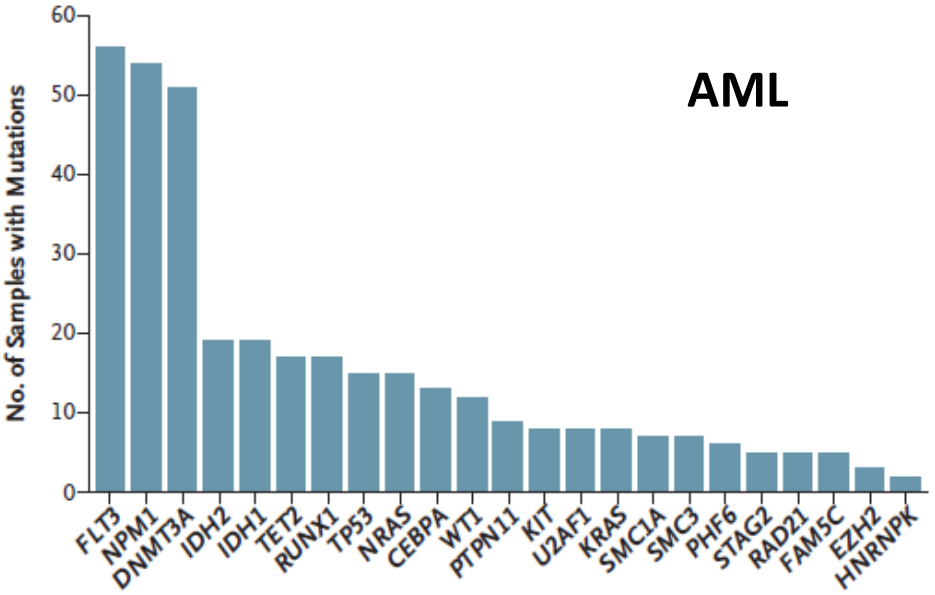
- I have the following financial relationships:
  Contracted Research: Celgene

Question

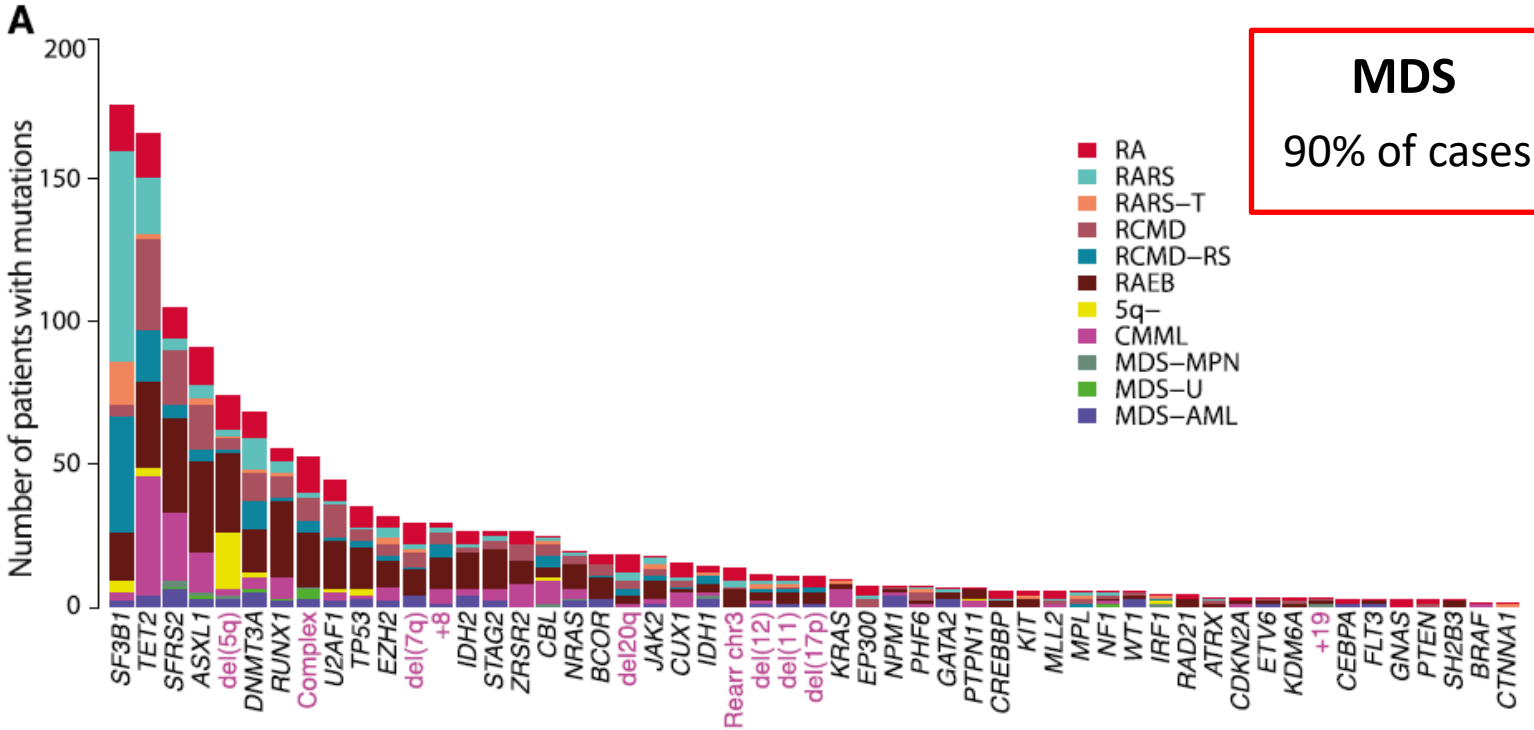
- In patients with cytopenia or monocytosis, who should undergo targeted DNA sequencing as part of their investigations?
  - All patients
  - All those referred to a haematologist
  - All those undergoing a bone marrow for investigation
  - Only those with borderline bone marrow features
  - None



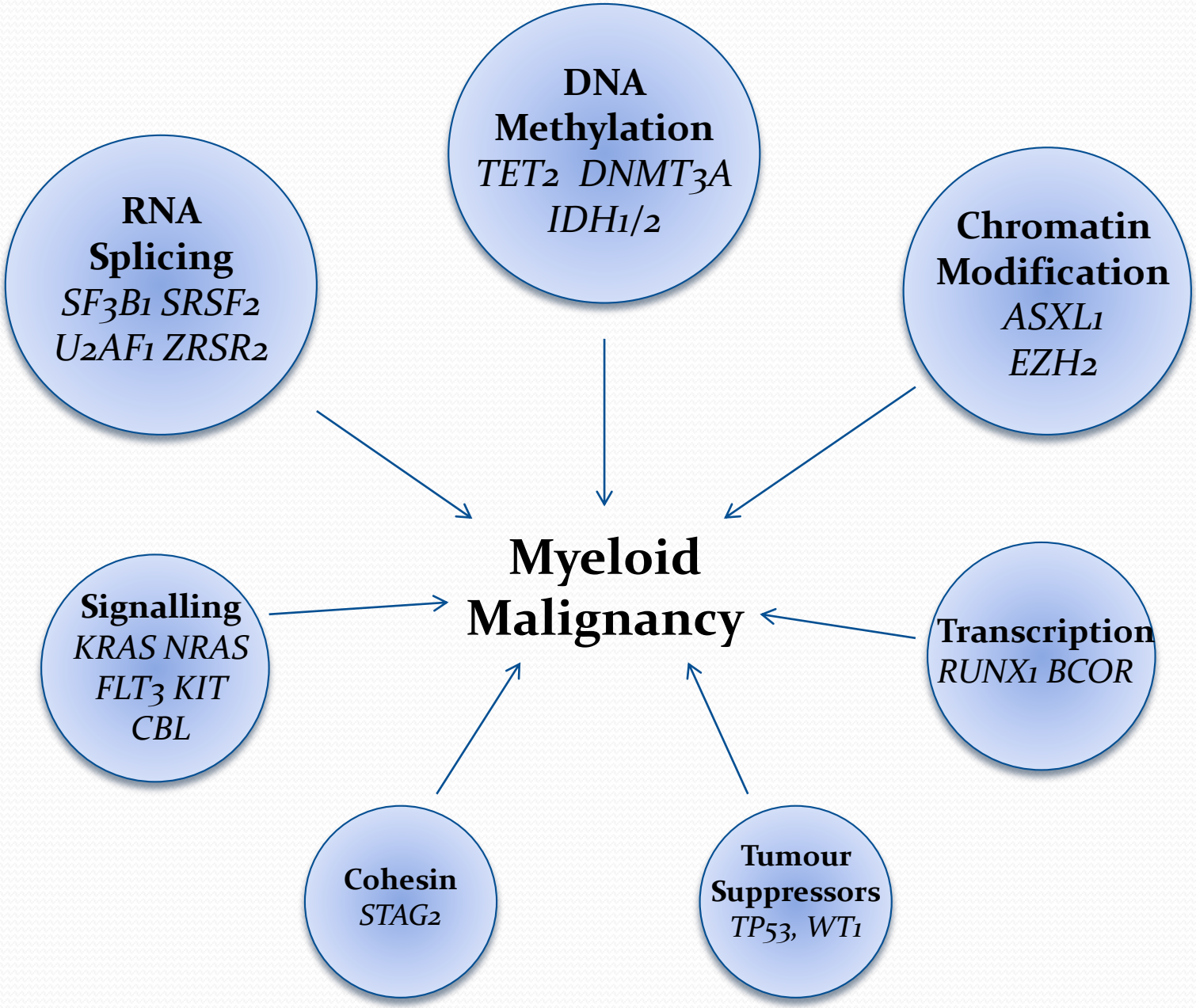
Adapted from Itzykson et al, 2013



Ley et al, 2013



Papaemmanuil et al, 2013



Adapted from Cazzola et al, 2013

Potential application in diagnosis of chronic myeloid malignancies…..

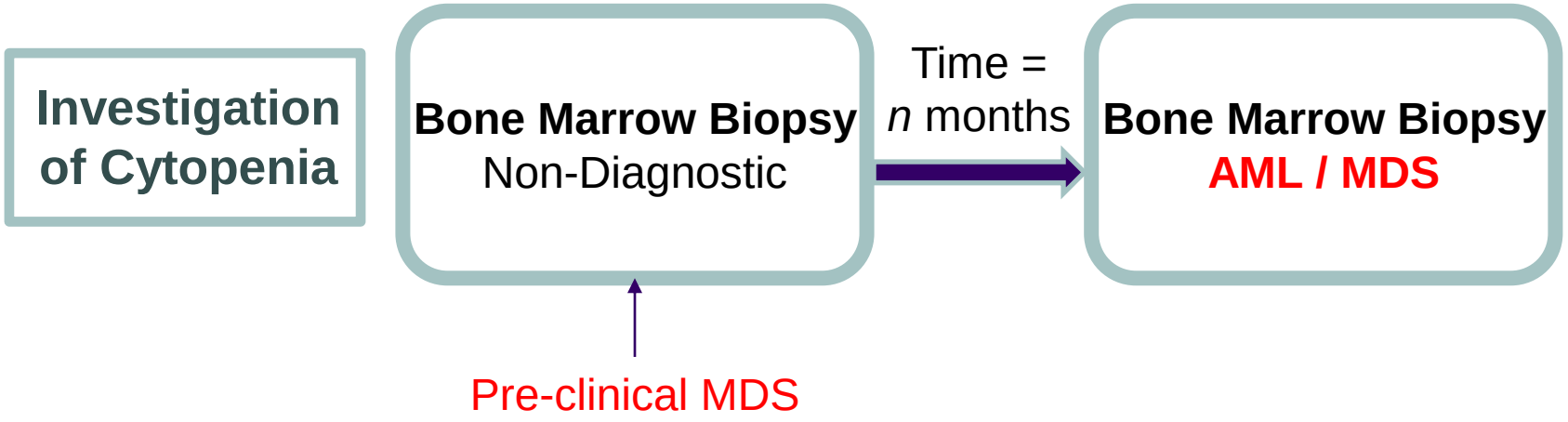
- Remains heavily reliant on morphology
- Challenging in those with <5% blasts
- Cytogenetic abnormality detected in only 30-50% of cases

- Idiopathic cytopenia with undetermined significance (ICUS)
  - Proportion progress to MDS or AML

Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression

Catherine A. Cargo,[1] Nicola Rowbotham,[1] Paul A. Evans,[1] Sharon L. Barrans,[1] David T. Bowen,[2] Simon Crouch,[3] and Andrew S. Jack[1]

- ***Aimed to molecularly characterise those cytopenic patients with the most clinically significant disease***
  - ***fail to meet current diagnostic criteria***



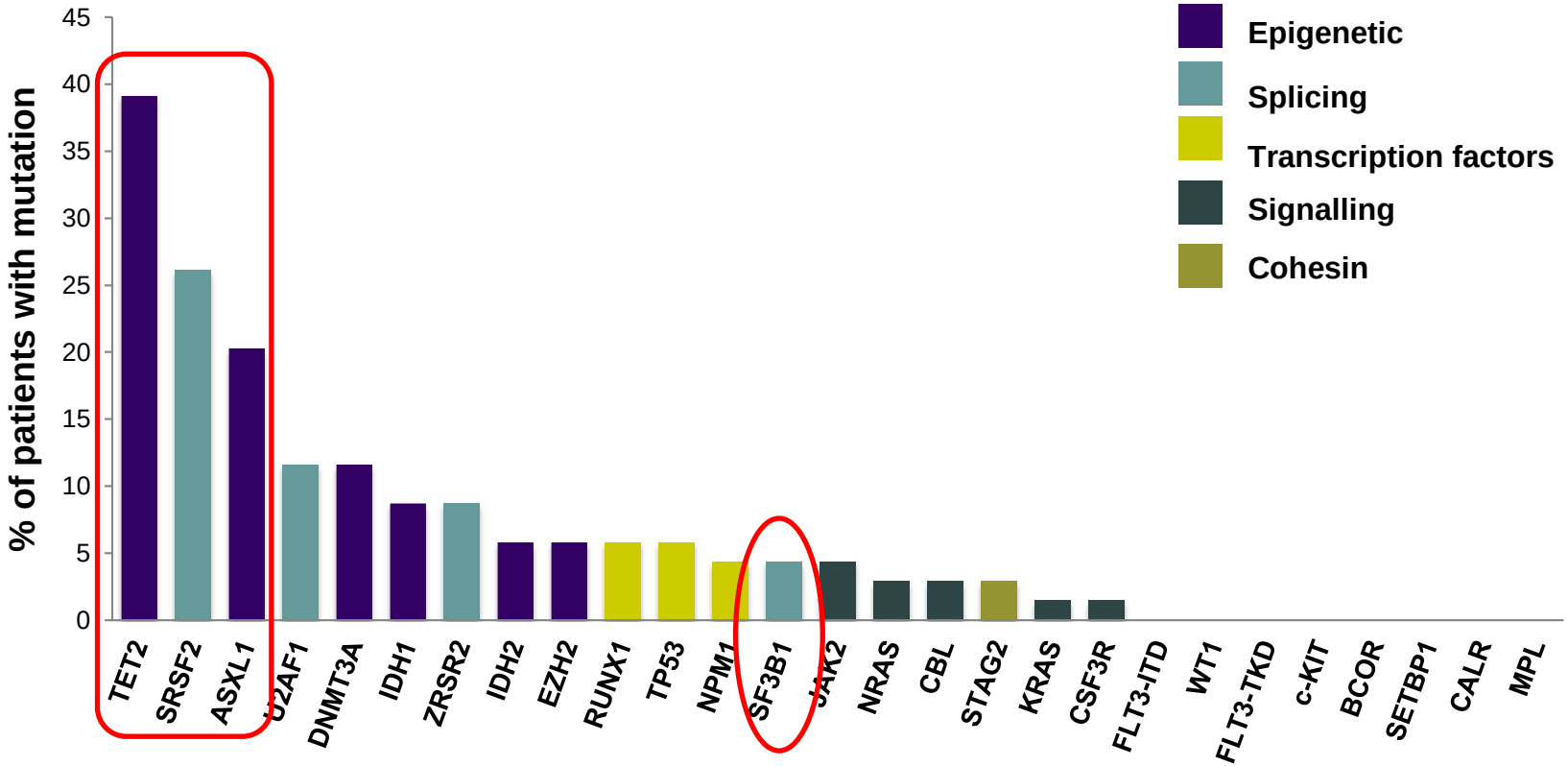
Cargo et al, Blood.2015; 126(21):2362-5

Targeted Sequencing Panel

Functional Pathway	Gene
DNA Methylation	TET2, DNMT3A, IDH1, IDH2
Chromatin Modification	ASXL1, EZH2
Splicing	SF3B1, SRSF2, U2AF1, ZRSR2
Transcription Factors	NPM1, RUNX1, BCOR, WTI, TP53
Signalling	FLT3, NRAS, KRAS, CBL, cKIT, JAK2, MPL, CSF3R
Cohesin complex	STAG2
Other	SETBP1, CALR

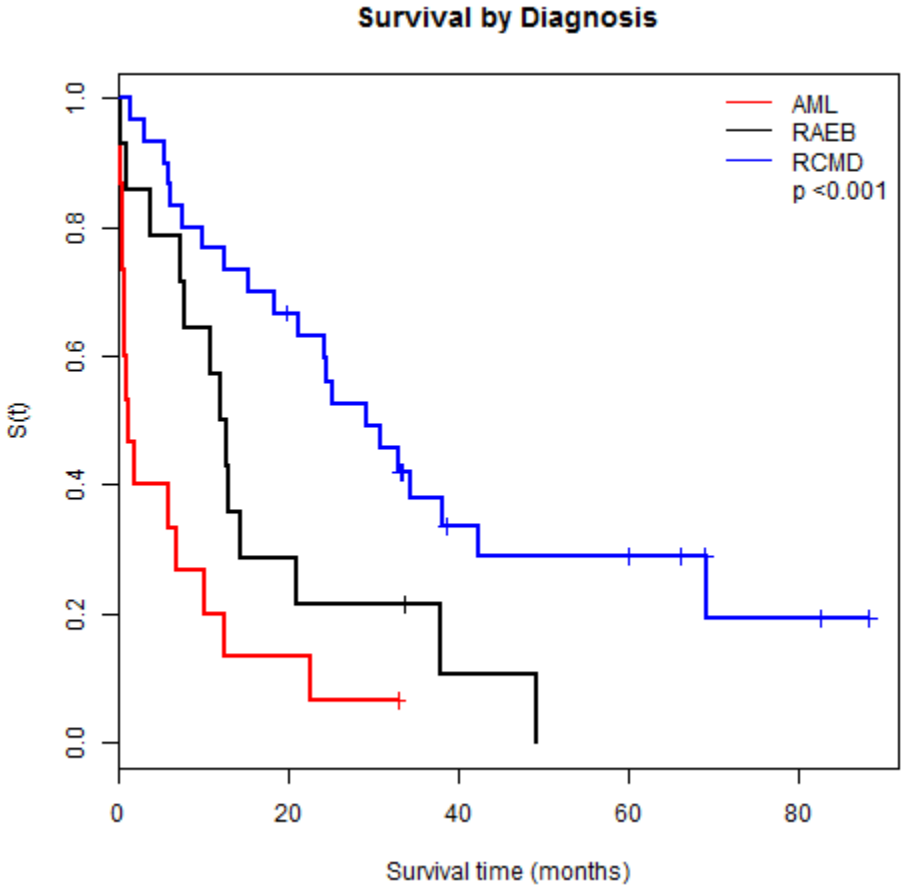
Results – pre-diagnostic sample

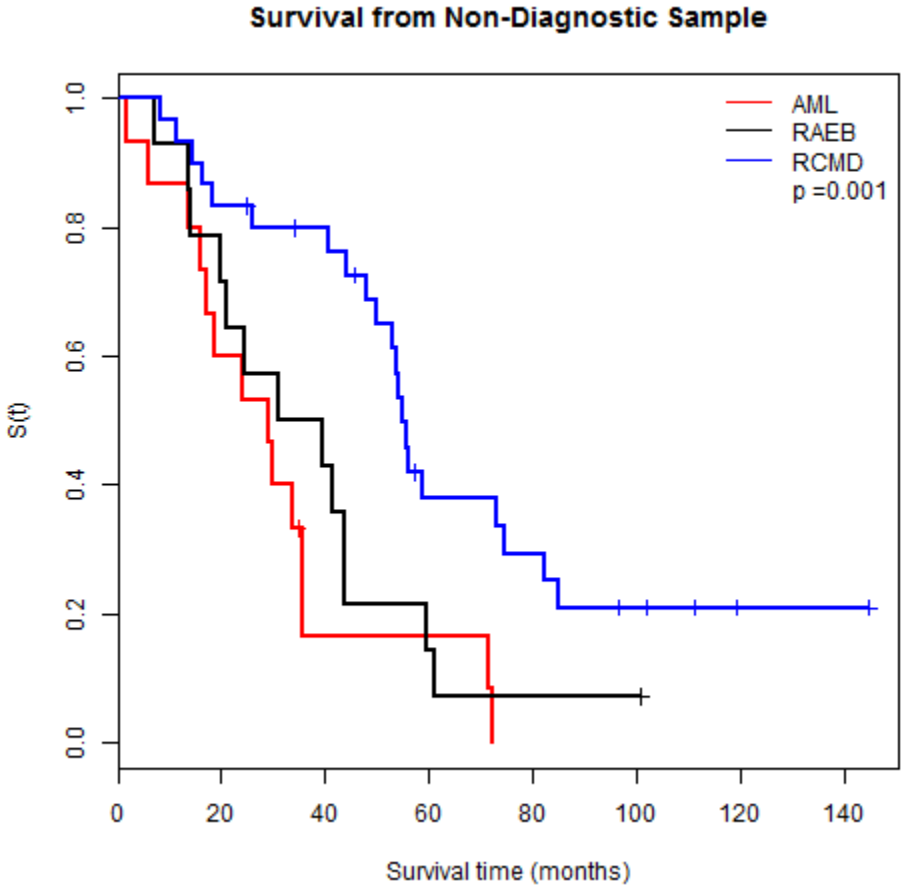
- Driver mutation and/or structural variant identified in **91%** of non-diagnostic samples (63/69)



Cargo et al, Blood.2015; 126(21):2362-5

Is there a clinical benefit to detecting mutations early?





Cargo et al, Blood.2015; 126(21):2362-5

Mutation Patterns in PB can predict a myeloid malignancy in BM

Gene	N. of Mutated Patients			Positive predictive value		
	Total Number	Isolated N (%)	Co-Mutated N (%)	Overall (95% CI)	Isolated (95% CI)	Co-Mutated (95% CI)
TET2	128	25 (19.5)	103 (80.5)	0.79 (0.71-0.86)	0.56 (0.35-0.76)	0.85 (0.76-0.91)
ASXL1	96	14 (14.6)	82 (85.4)	0.84 (0.76-0.91)	0.57 (0.29-0.82)	0.89 (0.80-0.95)
SRSF2	94	6 (6.4)	88 (93.6)	0.88 (0.80-0.94)	1.0 (0.54-1.0)	0.88 (0.79-0.94)
SF3B1	77	31 (40.3)	46 (59.7)	0.94 (0.86-0.98)	0.87 (0.70-0.96)	0.98 (0.89-1.0)
DNMT3A	59	17 (28.8)	42 (71.2)	0.66 (0.53-0.78)	0.29 (0.10-0.56)	0.81 (0.66-0.91)
RUNX1	50	2 (4)	48 (96)	0.92 (0.81-0.98)	0.50 (0.01-0.99)	0.94 (0.83-0.99)
JAK2	35	9 (25.7)	26 (74.3)	0.97 (0.85-1.0)	1.0 (0.66-1.0)	0.96 (0.80-1.0)
TP53	33	14 (42.4)	19 (57.6)	0.82 (0.65-0.93)	0.71 (0.42-0.92)	0.90 (0.67-0.99)
U2AF1	32	8 (25)	24 (75)	0.94 (0.79-0.99)	0.88 (0.47-1.0)	0.96 (0.79-1.0)
IDH2	25	1 (4)	24 (96)	0.92 (0.74-0.99)	1.0 (0.03-1.0)	0.92 (0.73-0.99)
EZH2	25	3 (12)	22 (88)	0.88 (0.69-0.98)	0.67 (0.09-0.99)	0.91 (0.71-0.99)
ZRSR2	22	4 (18.2)	18 (81.8)	0.86 (0.65-0.97)	0.75 (0.19-0.99)	0.89 (0.65-0.99)
NRAS	21	3 (14.3)	18 (85.7)	0.86 (0.64-0.97)	0.33 (0.01-0.91)	0.94 (0.73-1.0)
IDH1	20	4 (20)	16 (80)	0.80 (0.56-0.94)	0.75 (0.19-0.99)	0.81 (0.54-0.96)
STAG2	20	4 (20)	16 (80)	0.85 (0.62-0.97)	0.50 (0.07-0.93)	0.94 (0.70-1.0)

RUNX1, EZH2, CBL, TP53, NRAS, CUX1 or *IDH2 – PPV 0.86-1.0*

Malcovati et al, Blood. 2017; 129(25): 3371-8

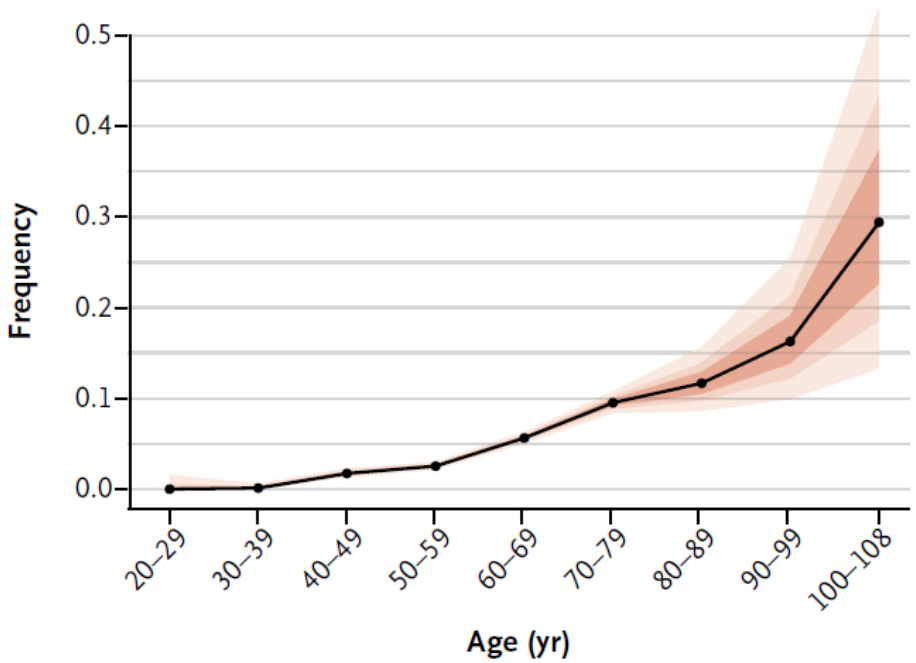
Is the presence of a mutation diagnostic of MDS?

is not yet fully understood; thus, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia, where these mutations may be commonly found.[62] Further study is required to determine the optimal management and monitoring of such patients and

Arber et al, Blood 2016; 127(20): 2391-2405

Somatic mutations in healthy individuals

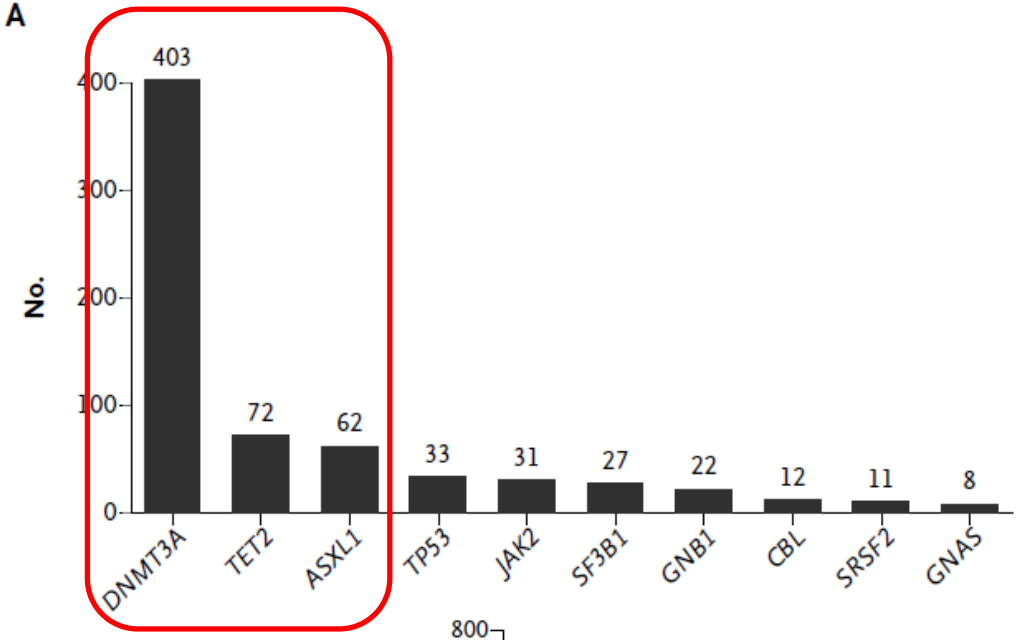
- Somatic mutations identified in small proportion of healthy individuals
- Increase in frequency with age

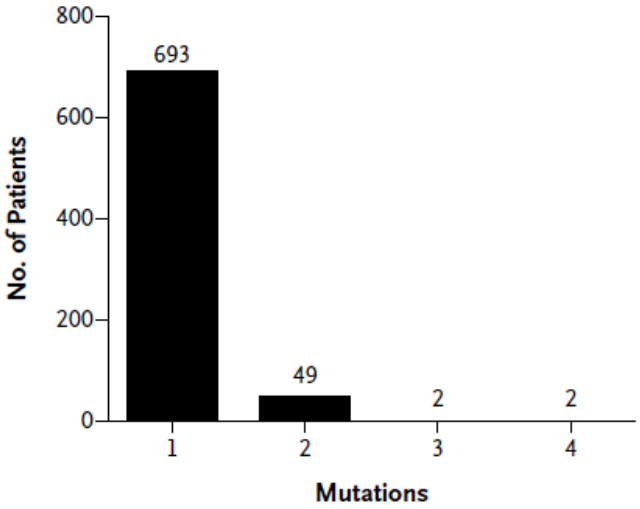


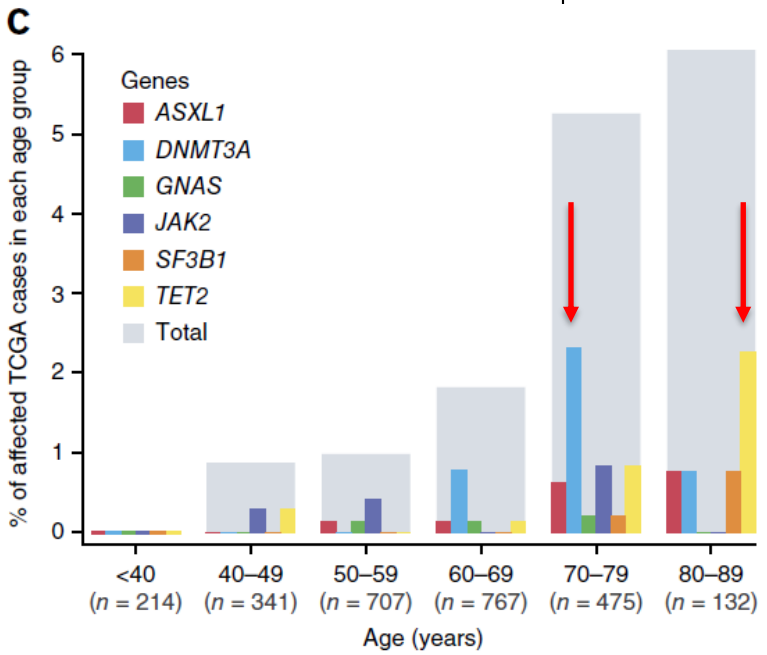
No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

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

Somatic mutations in healthy individuals



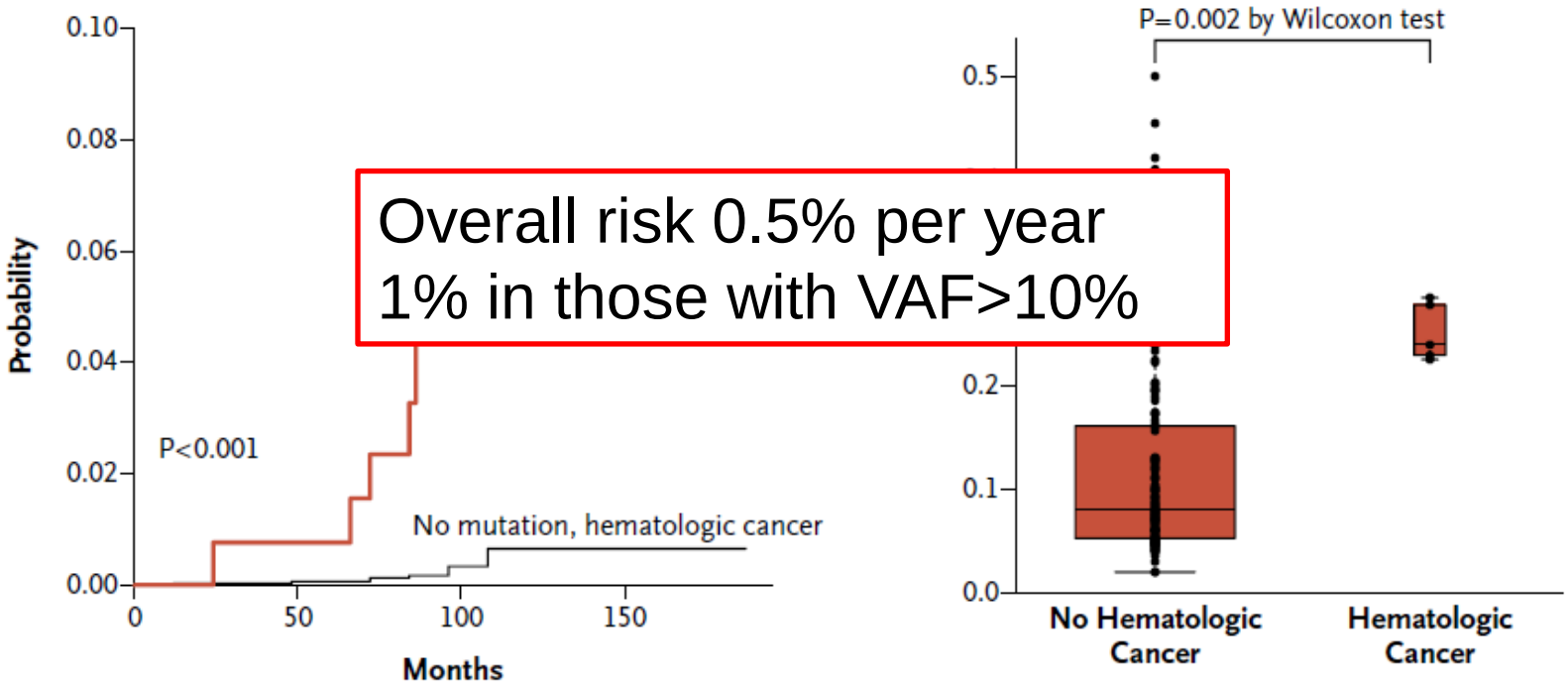




Jaiswal et al, NEJM, 2014;
Xie et al, Nat Med, 2014;

Risk of progression?

- Presence of a mutation
  - Haematological malignancy more common by factor of 11
  - VAF >10% - increased by factor of 50



Jaiswal et al, NEJM, 2014

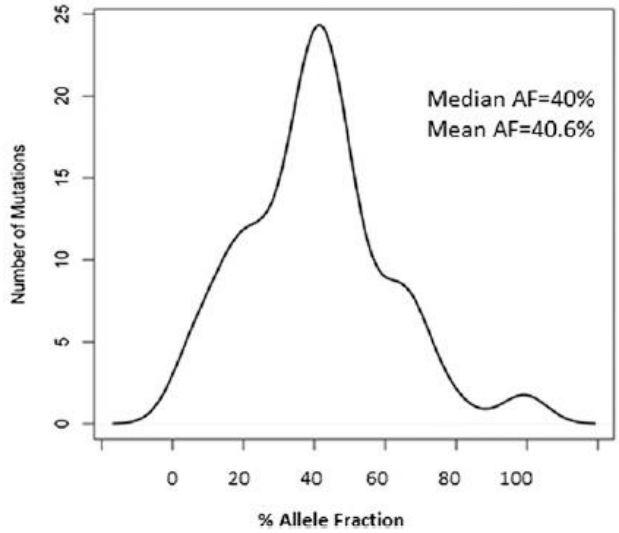
“CHIP”
(Clonal Haematopoiesis of Indeterminate Potential)

- Absence of definitive morphological evidence of a haematological neoplasm
- Does not meet diagnostic criteria for PNH, MGUS or MBL
- Presence of a somatic mutation associated with haematological neoplasia at a variant allele fraction of at least 2%

Steensma et al, Blood, 2015

Does the genomic profile differ between healthy individuals and suspected MDS?

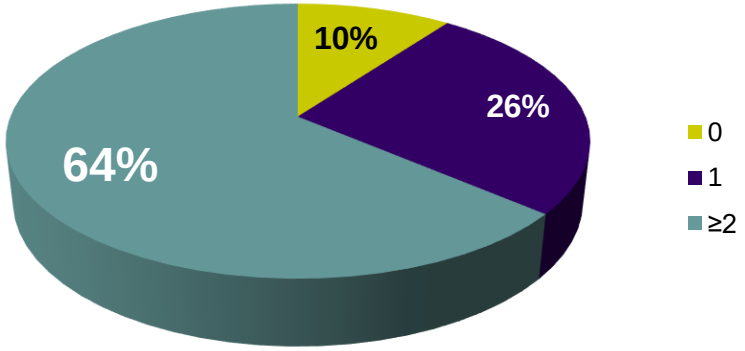
- Allele fraction



- Median AF=39.9% (vs 9-10%[1])
- Only 1 patient harboured an isolated mutation <20%

- Healthy individuals who subsequently developed a haematological malignancy – mean VAF 25.2%[1]

- No. of mutations per patient

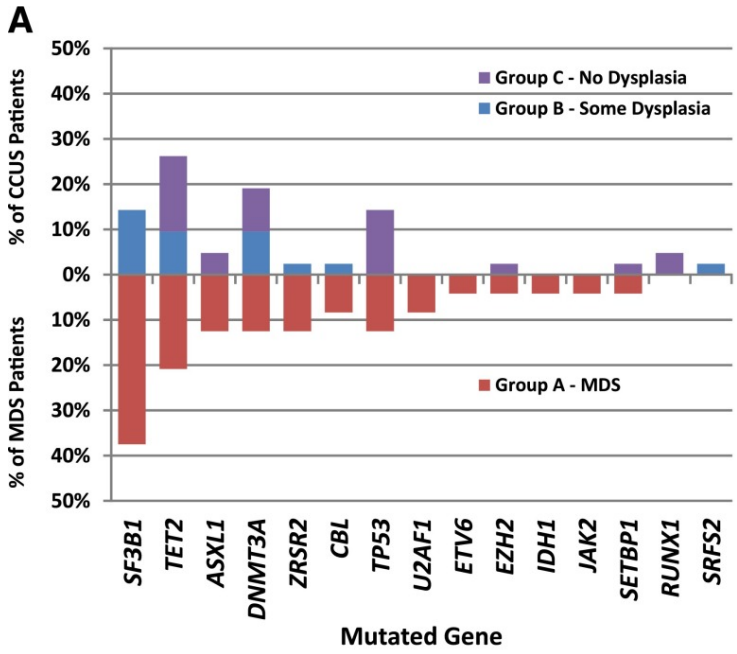


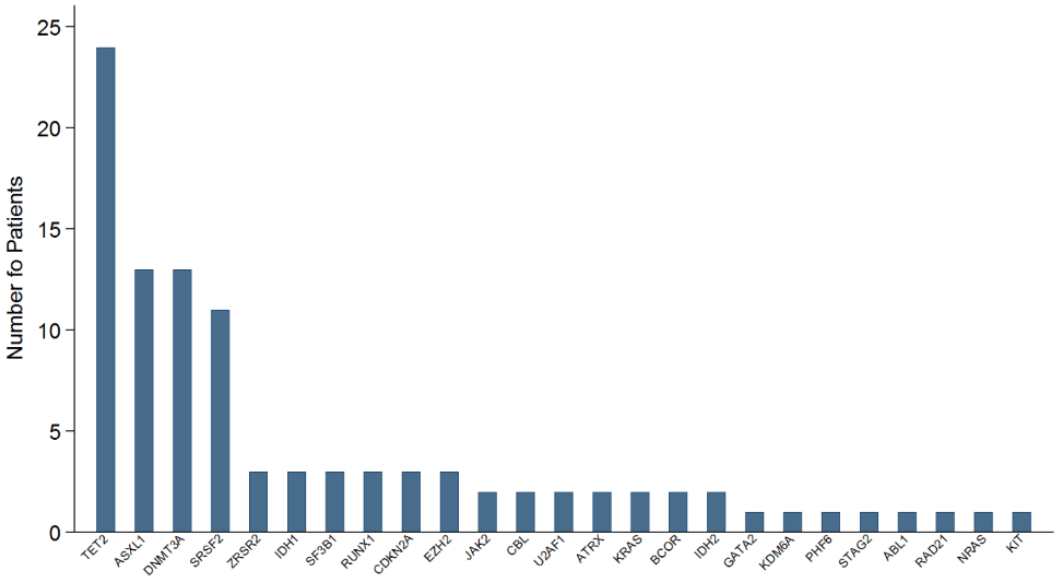
- Majority of patients had ≥2 mutations
- Only 8% of those with age related clonal haematopoiesis had ≥2 mutations[1]

[1]Jaiswal et al, NEJM, 2014; DOI: 10.1056/NEJMoa1408617

How frequent are somatic mutations in cytopenic patients?

- 35-38% of ICUS patients have ≥1 mutation
  - Cohort size 144-249 patients





Kwok et al, Blood. 2015
Malcovati et al, Blood. 2017

Clonal Cytopenia of Undetermined Significance

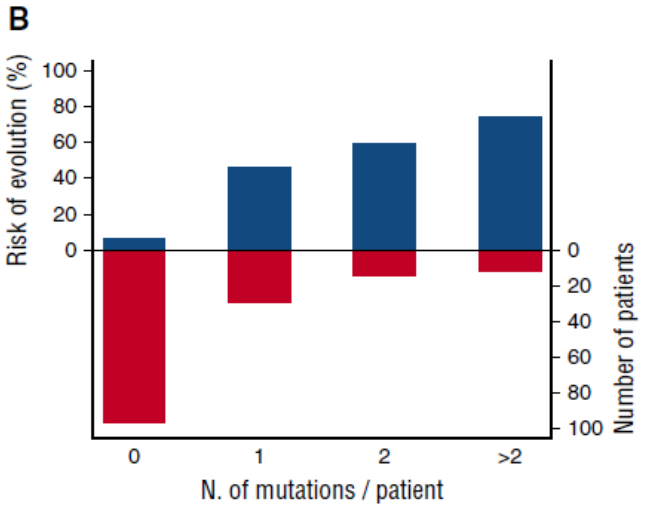
	CHIP	ICUS	CCUS	Lower risk MDS
Cytopenia	-	+	+	+
Dysplasia	-	-	-	+
Clonality	+	-	+	+
% Blasts	<5%	<5%	<5%	<5%
Overall risk	Very low	Very low	????	Low

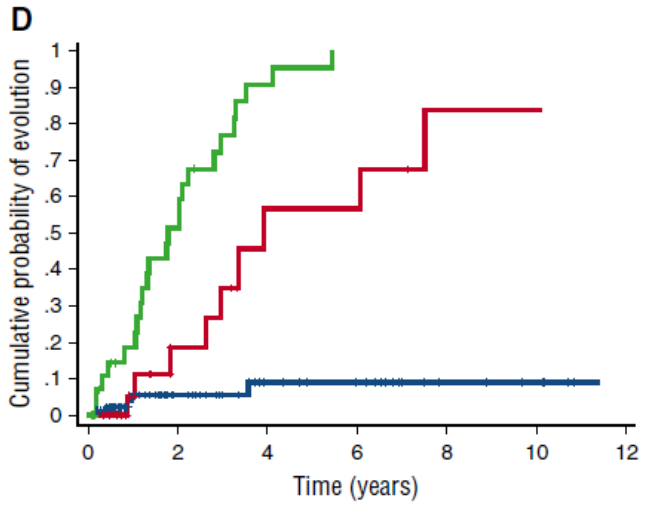
Adapted from - Steensma et al, Blood. 2015; 126(1): 9-16

Are mutations clinically relevant in cytopenic patients?

- 56/154 (36%) had ≥1 mutation
  - Mutated vs Unmutated - HR 13.93 (P<0.001)
  - 10yr probability of progression 96% vs 15% (p<0.001)

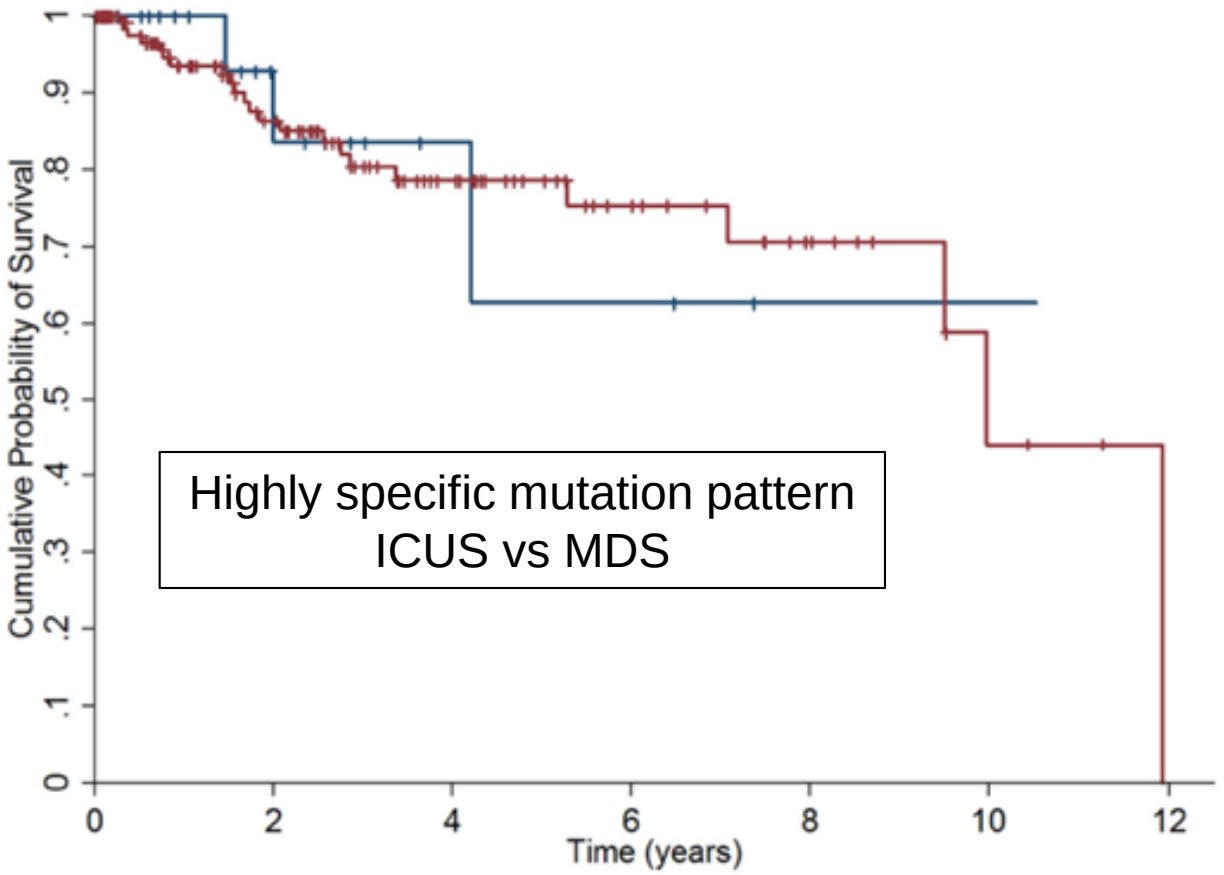
- Highly specific mutation pattern
  - Spliceosome mutations
  - Co-mutation patterns involving TET2, ASXL1 or DNMT3A
    - *RUNX1, EZH2, CBL, BCOR, CUX1, TP53* or *IDH1/IDH2*





Malcovati et al, Blood. 2017; 129(25): 3371-8

Impact on survival



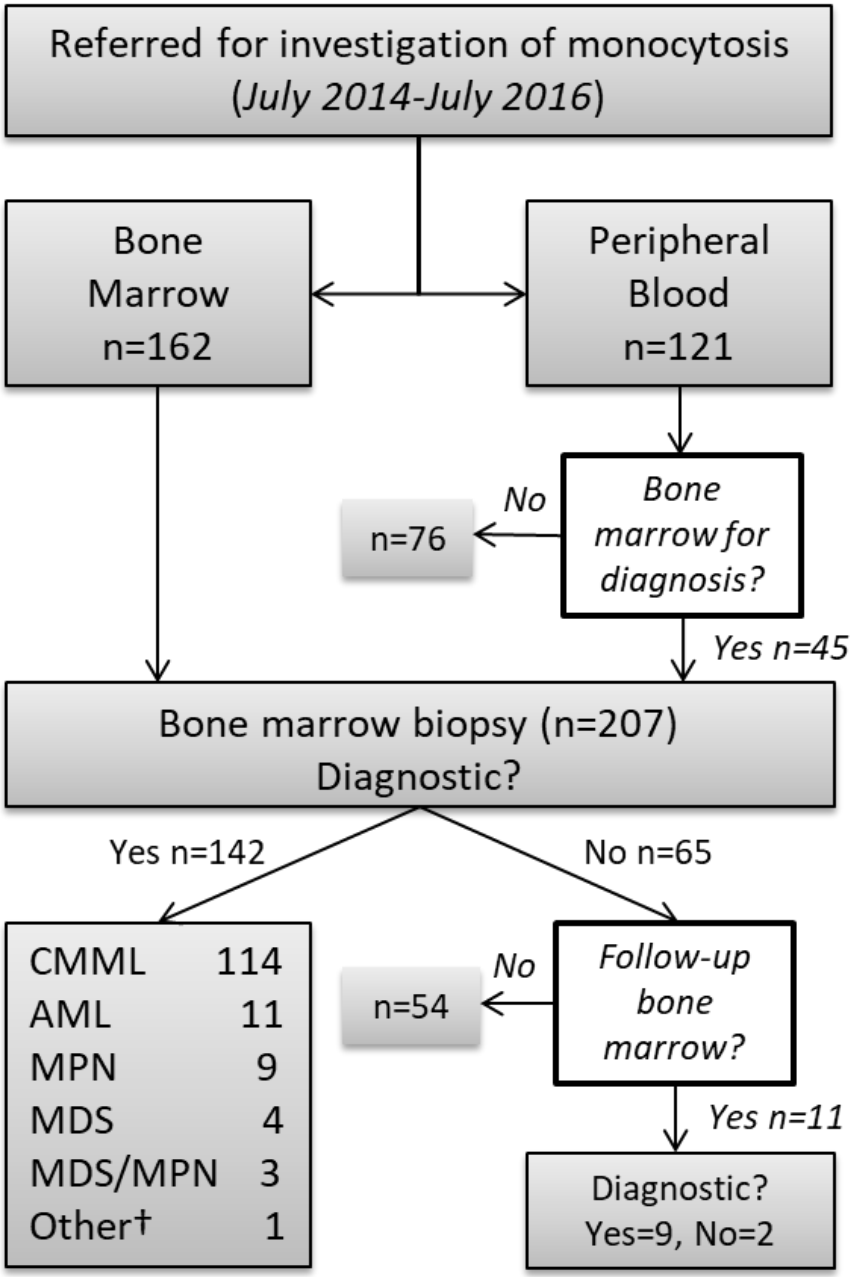
Highly specific mutation pattern
ICUS vs MDS

Malcovati et al, Blood. 2017; 129(25): 3371-8

Diagnostic criteria for CMML

Table 11. Diagnostic criteria for CMML

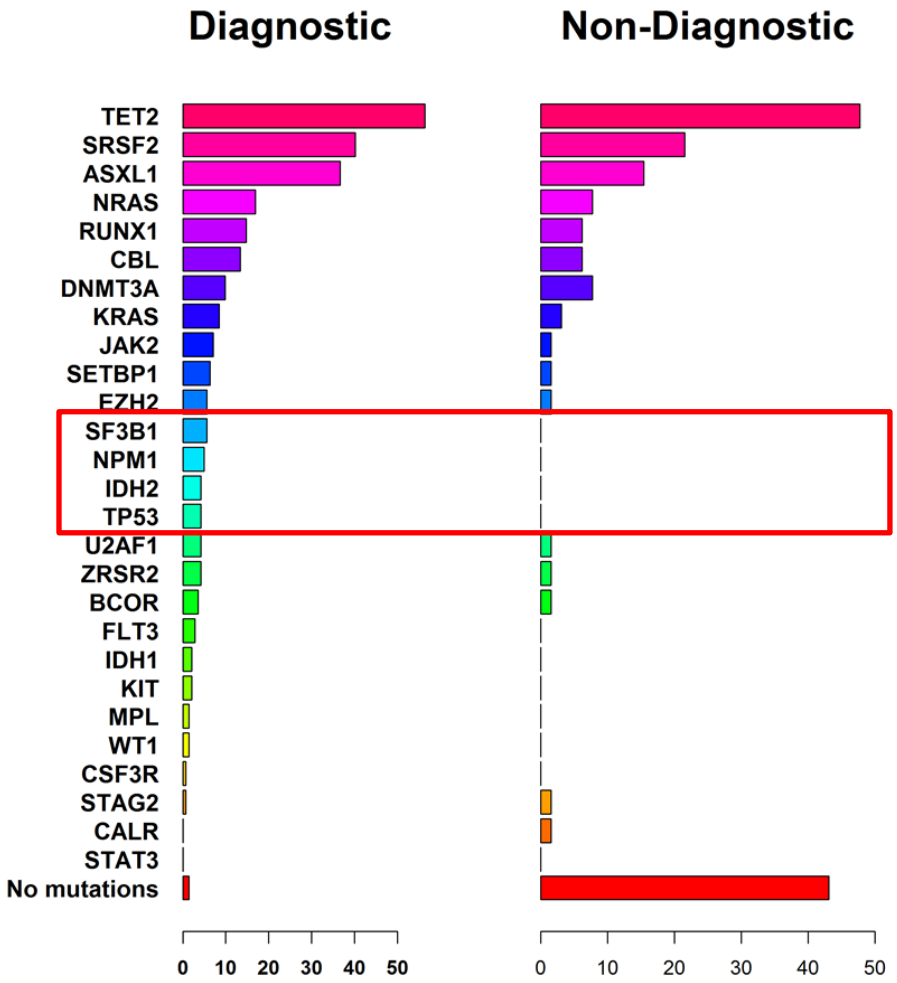
CMML diagnostic criteria
• Persistent PB monocytosis ≥1 × 10^9/L, with monocytes accounting for ≥10% of the WBC count
• Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PMF, PV, or ET*
‡The presence of mutations in genes often associated with CMML (eg, *TET2*, *SRSF2*, *ASXL1*, *SETBP1*) in the proper clinical contest can be used to support a diagnosis. It should be noted however, that many of these mutations can be age-related or be present in subclones. Therefore, caution would have to be used in the interpretation of these genetic results.
• An acquired clonal cytogenetic or molecular genetic abnormality is present in hemopoietic cells‡
or
• The monocytosis (as previously defined) has persisted for at least 3 mo and
• All other causes of monocytosis have been excluded



Cargo et al, Blood, 2019; 133(12):1325-1334

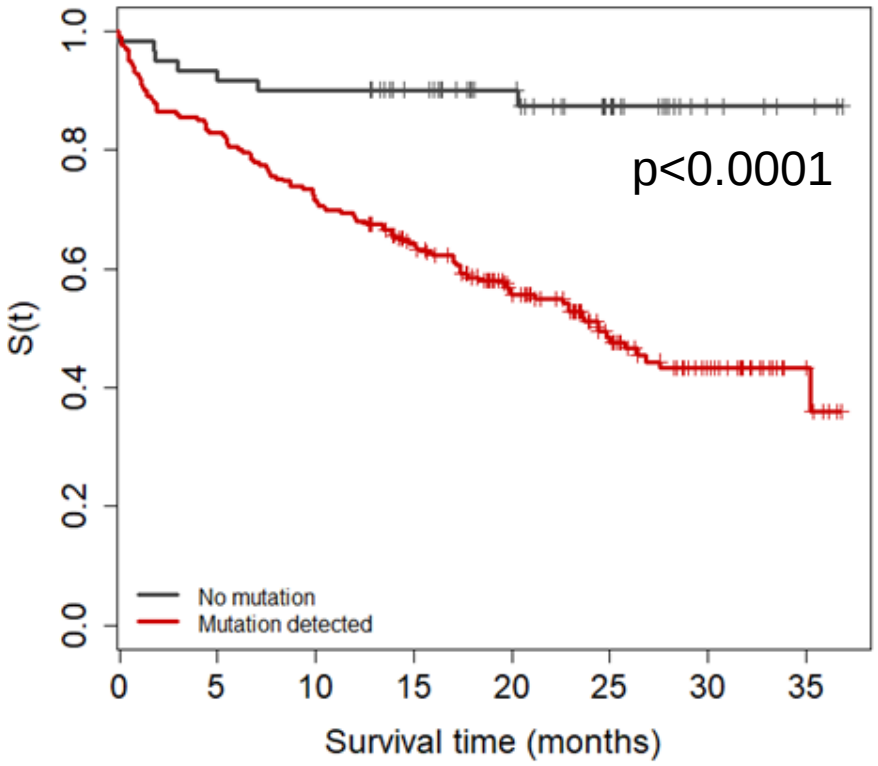
Molecular profile in bone marrow samples

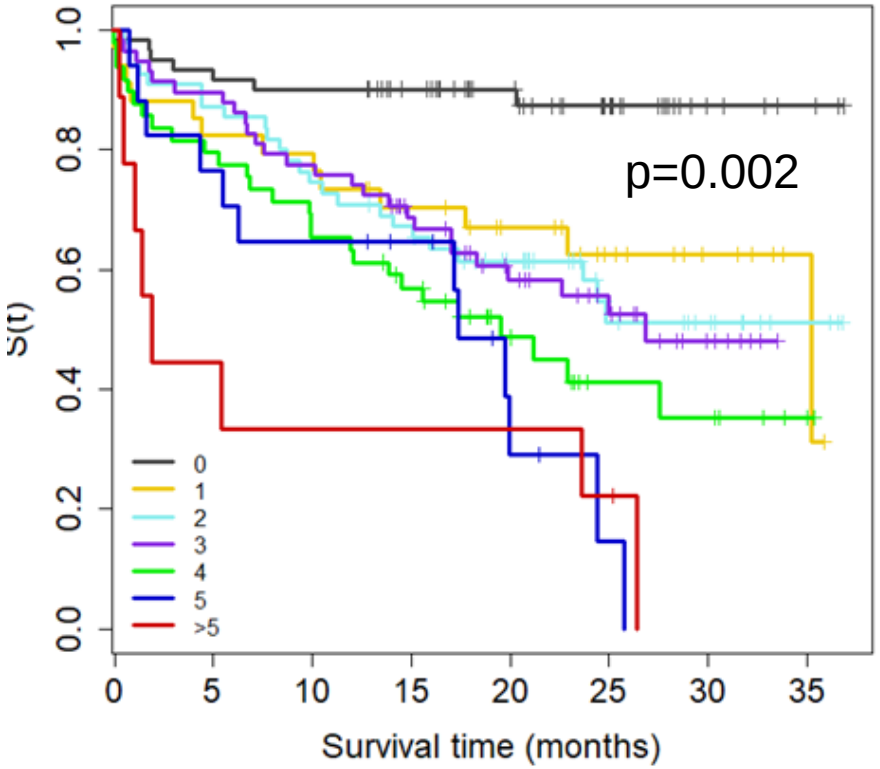
- A total of 207 patients had a BM performed
- Of those with a diagnosis
  - Mutation detected in 99%
- Of those without a diagnosis
  - Mutation detected in 57%
- In those with a mutation
  - No difference in no. of mutations or VAF between diagnostic and non-diagnostic groups



Cargo et al, Blood, 2019; 133(12):1325-1334

OS correlated strongly with no. of mutations



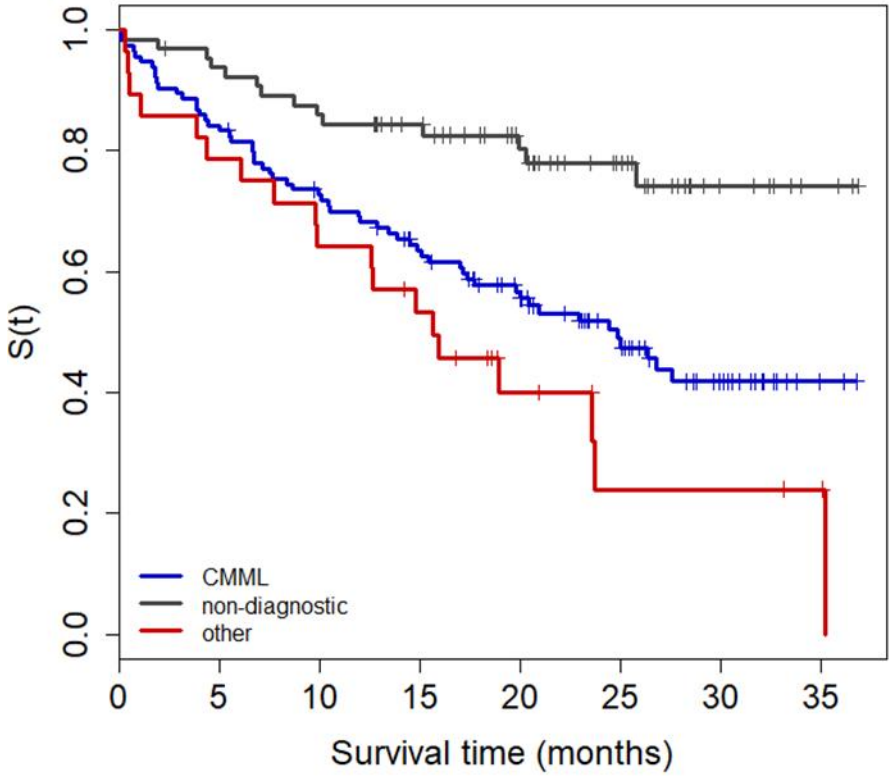


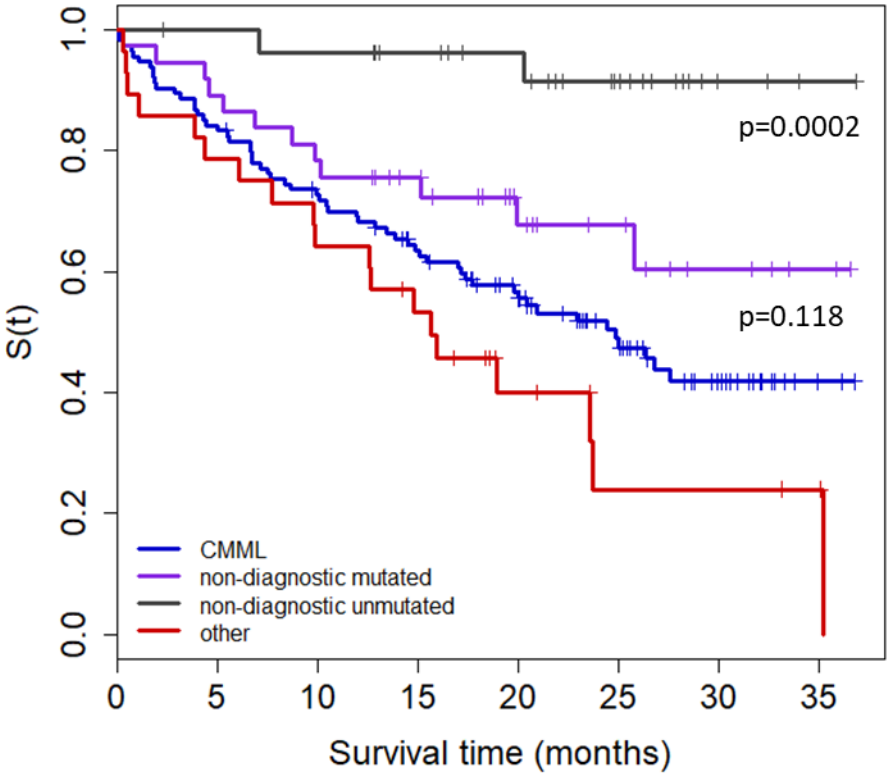
Cargo et al, Blood, 2019; 133(12):1325-1334

Variable	Univariate Analysis		Multivariate Analysis	
	p-value	OR, 95% CI	p-value	OR, 95% CI
Age	p<0.0001	1.04(1.02-1.06)	p<0.0001	1.04(1.02-1.06)
ASXL1	p<0.0001	2.10(1.45-3.05)	p=0.023	1.59(1.07-2.38)
CBL	p=0.003	2.12(1.30-3.47)	p=0.002	2.24(1.35-3.73)
DNMT3A	p<0.0001	2.87(1.73-4.76)	p<0.0001	2.82(1.68-4.73)
KRAS	p=0.13; NS†	1.69(0.85-3.33)	-	
NRAS	p=0.002	2.11(1.32-3.36)	p=0.014	1.85(1.13-3.01)
RUNX1	p<0.0001	2.75(1.74-4.37)	p=0.001	2.20(1.37-3.54)
SRSF2	p=0.41; NS†	1.18(0.80-1.72)	-	
TET2	p=0.30; NS†	0.82(0.57-1.19)	-	
EZH2	p<0.0001	4.88(2.52-9.44)	-	
STAG2	p<0.0001	22.39(7.48-67.01)	-	

Cargo et al, Blood, 2019; 133(12):1325-1334

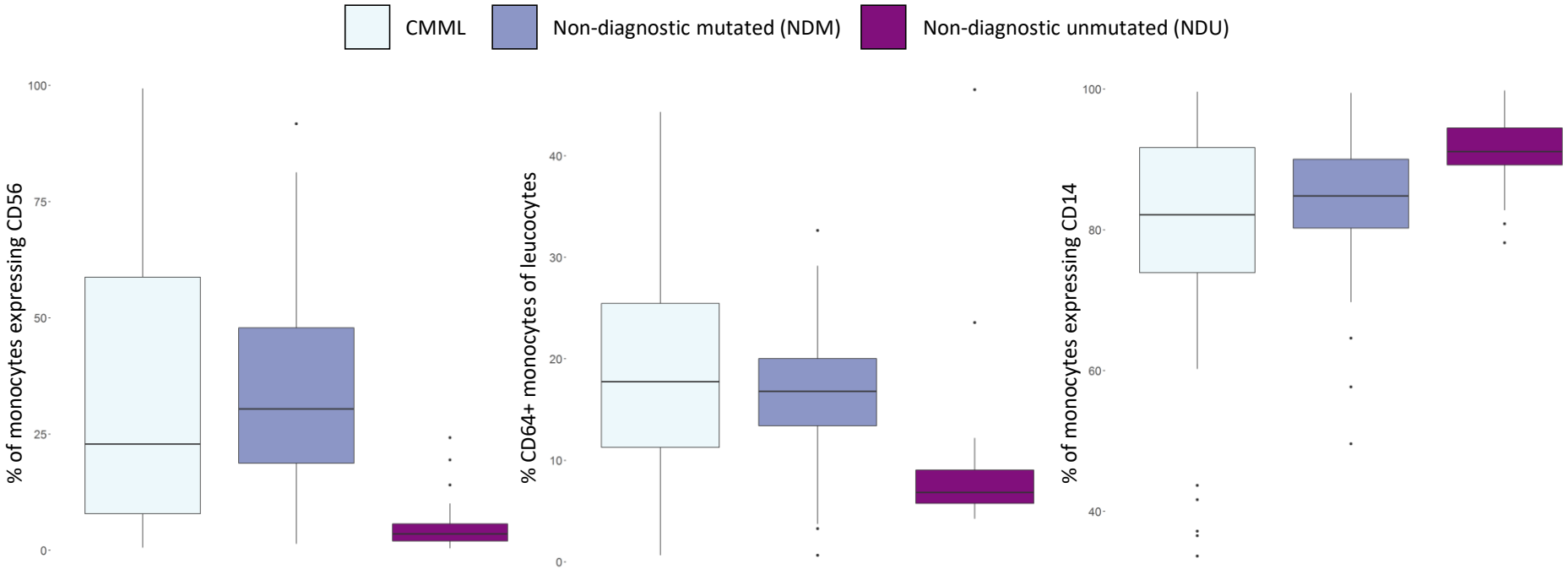
What is the clinical phenotype of non-diagnostic^{mut} patients?





Cargo et al, Blood, 2019; 133(12):1325-1334

Non-diagnostic^{mut} patients are indistinguishable from CMML



A

	% CD56 expression on monocytes
CMML vs NDM	p=0.45; NS
CMML vs NDU	p<0.0001
NDM vs NDU	p<0.0001

B

	% CD64+ monocytes of leucocytes
CMML vs NDM	p=0.36; NS
CMML vs NDU	p<0.0001
NDM vs NDU	p<0.0001

C

	% CD14 expressing monocytes
CMML vs NDM	p=0.22; NS
CMML vs NDU	p=0.0004
NDM vs NDU	p=0.006

Cargo et al, Blood, 2019; 133(12):1325-1334

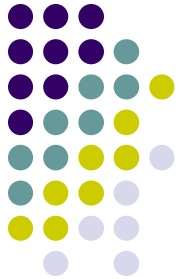
Peripheral Blood is predictive of a bone marrow diagnosis

- Peripheral blood mutation analysis highly predictive of diagnosing a myeloid malignancy in bone marrow
  - PPV 0.97, NPV 1.0
- High concordance between mutations detected in PB and BM (96%)

BM \ PB		Mutation detected in PB?	
		Y	N
Confirmed diagnosis in subsequent BM?	Y	33	0
	N	1	11

Cargo et al, Blood, 2019; 133(12):1325-1334

Clonal Monocytosis of Clinical Significance

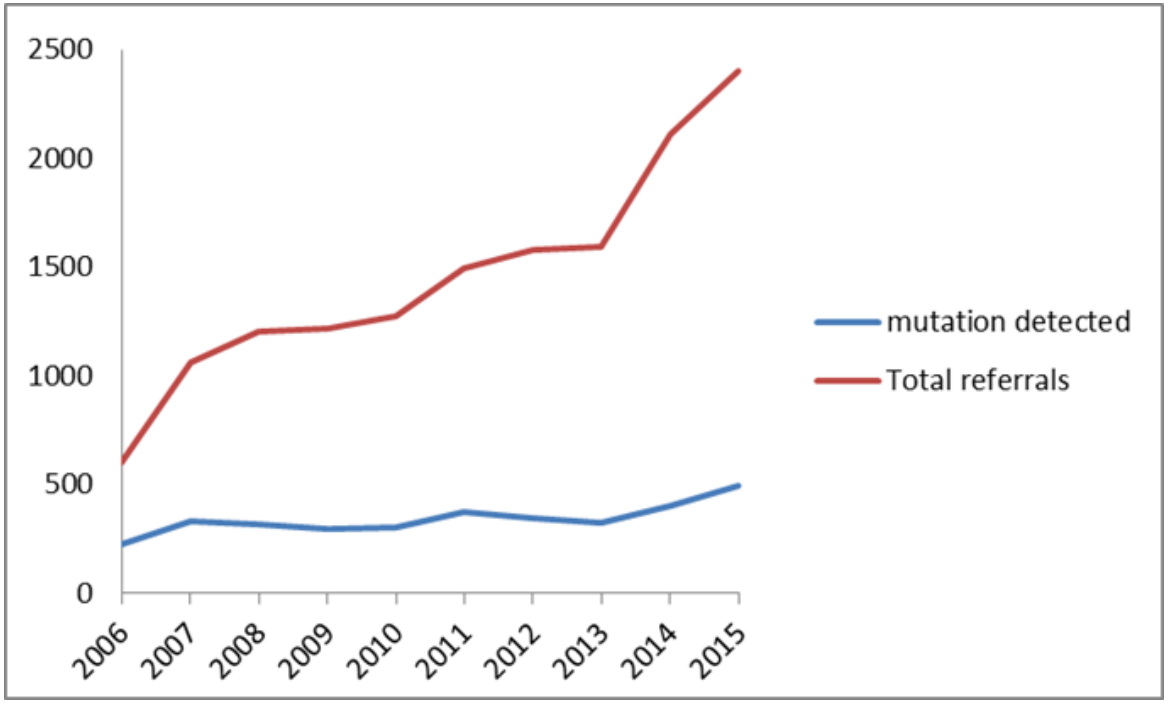


- Patients with a persistent monocytosis failing to meet morphological criteria of CMML (or other myeloid malignancy)
  - Presence of a somatic mutation associated with CMML (most commonly TET2, SRSF2, ASXL1)

- Mutation spectrum, immunophenotypic features and overall survival indistinguishable from CMML
  - ?presumptive evidence of disease

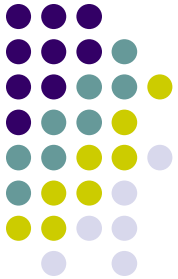
Who should have sequencing performed?

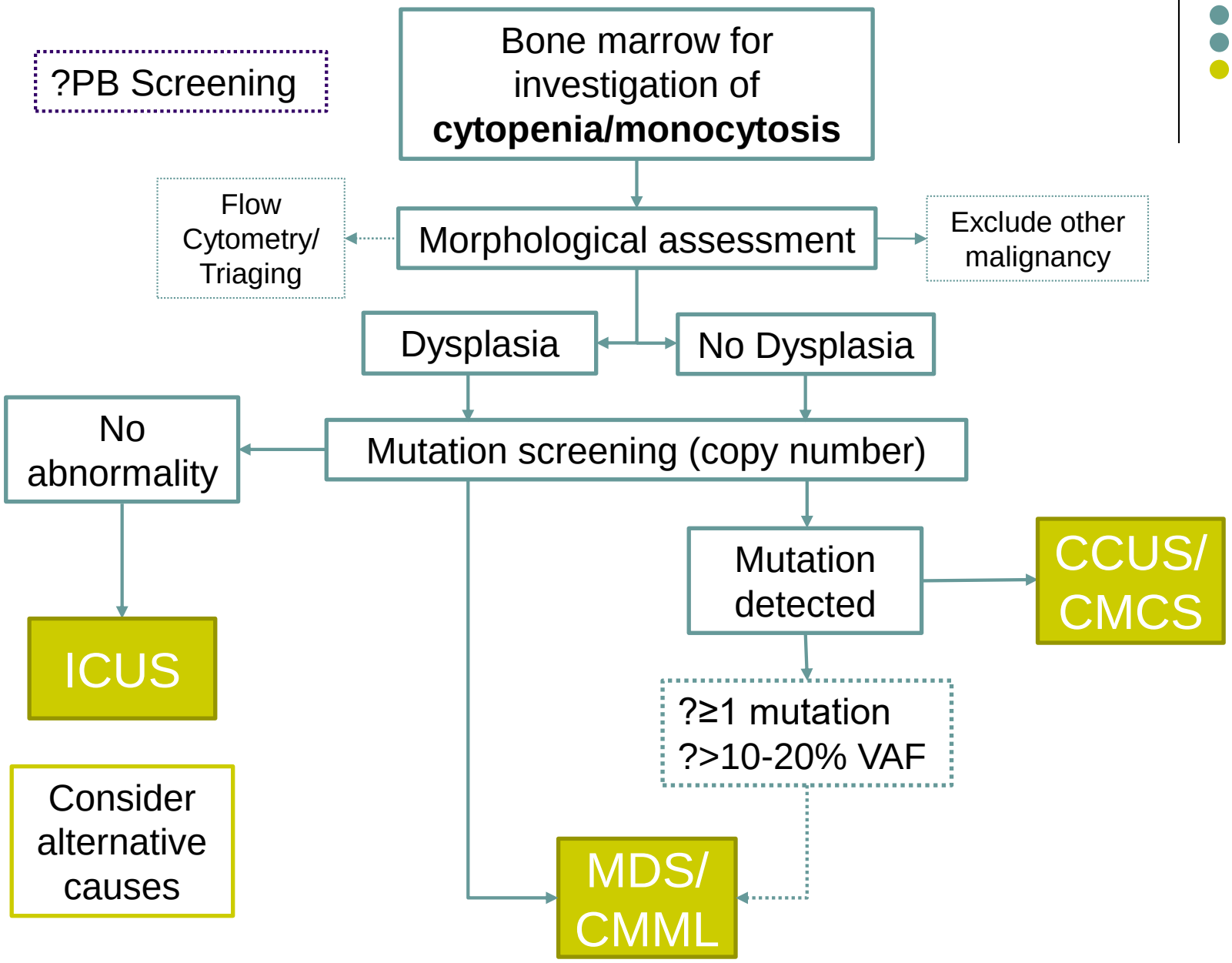
- Caution is required when introducing new test



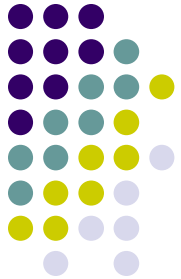
Referrals for suspected MPN 2006-2015 (HMDS)

Diagnostic algorithm





Impact on laboratory and clinical practice



- Would require a standardised approach
  - Panel design and minimum genes to sequence
  - Analysis and interpretation
    - grading of mutations
- Appropriate infrastructure within laboratories
  - Data processing and storage
  - Bioinformatic support

- Clinical impact
  - High frequency of mutations would significantly increase the number of patients attending haematology clinic

Summary

- *Mutation analysis provides key information even in those failing to meet current diagnostic criteria*

- CCUS
  - Patients have worse overall survival and progressive cytopenias even in the absence of morphological disease
  - Mutations can predict the development of overt disease
  - Absence of a mutation has a high negative predictive value
  - Provides potential window of opportunity for intervention
    - Therapeutic intervention

- CMCS
  - Mutation profile, immunophenotypic features and outcome indistinguishable from CMML
  - Peripheral blood is clinically informative

Acknowledgements

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HMDS
haematological malignancy diagnostic service
education & training

mds foundation
the myelodysplastic syndromes foundation, inc.

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Poster

- Abstract #1712
  - Targeted sequencing predicts the development of myeloid malignancies and clinical outcome in patients with unexplained cytopenia
  - Saturday, December 7
  - 5.30-7.30pm

Question

- In patients with cytopenia or monocytosis, who should undergo targeted DNA sequencing as part of their investigations?
  - All patients
  - All those referred to a haematologist
  - All those undergoing a bone marrow for investigation
  - Only those with borderline bone marrow features
  - None