Society for Hematopathology
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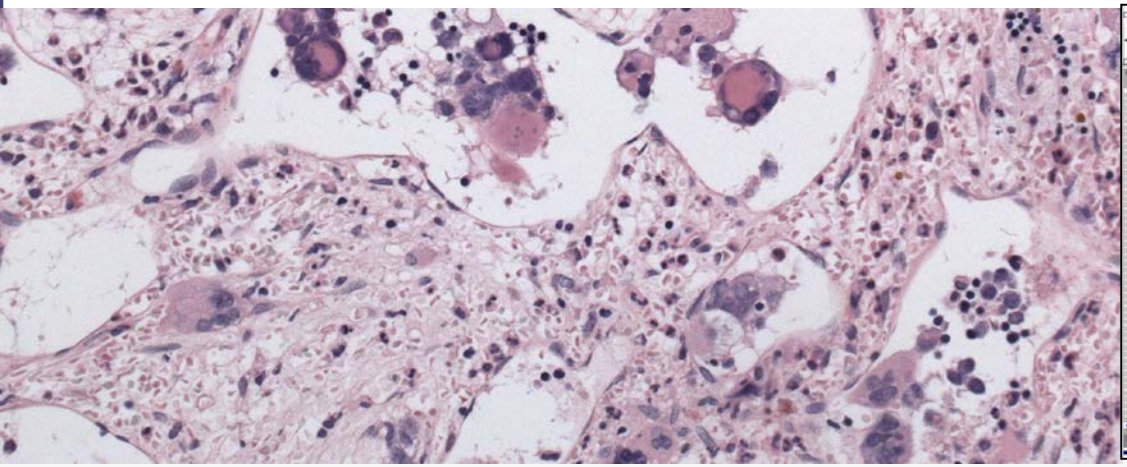

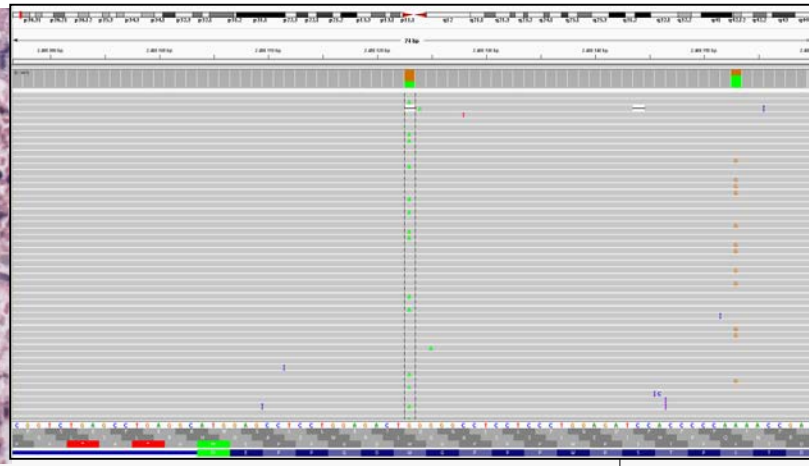

Summary Session 6

Genetics Revealing the Biology of Myeloid Neoplasms (excluding acute leukemias)

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UKT

Grouping of summarized cases

CML

- Accelerated phase (AP) and blast crisis (BC) with unusual genetics
- CML with Ph- MPN or other hematological neoplasms

Ph- MPN with unusual genetic and molecular findings

- Suggesting overlap with MDS or MDS/MPN
- In progression and transformation

MDS/MPN with unusual genetic and molecular findings

MDS with unusual genetic and molecular findings

- Suggesting overlap with MPN or MDS/MPN

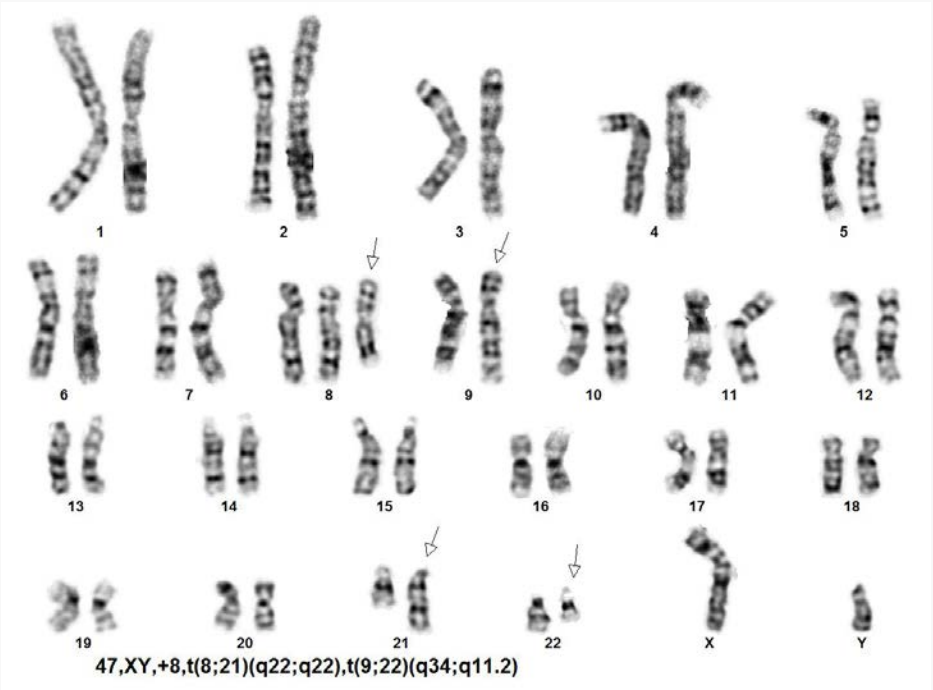
Chronic myelogenous leukemia
unusual phenotypic or genetic findings in progression

Case	Submitter	Panel Dx
59	Nejati, R	CML, BCR-ABL1+, AP (with MYC rearrangement)
68	Al-Ghamdi, Y	CML, BCR-ABL1+, BC with PML-RARA
182	Yin, C	CML,BCR-ABL1+, in T lymphoid blast crisis
185	Li, S	CML, BCR-ABL1+ in myeloid blast crisis [with inv(16)]
215	Wasserman, A	CML, BCR-ABL1+ in myeloid BC[with t(8;21)]
257	Thakral, B	CML, BCR-ABL1+ in B-lymphoid BC (CRLF2 rearrangement)

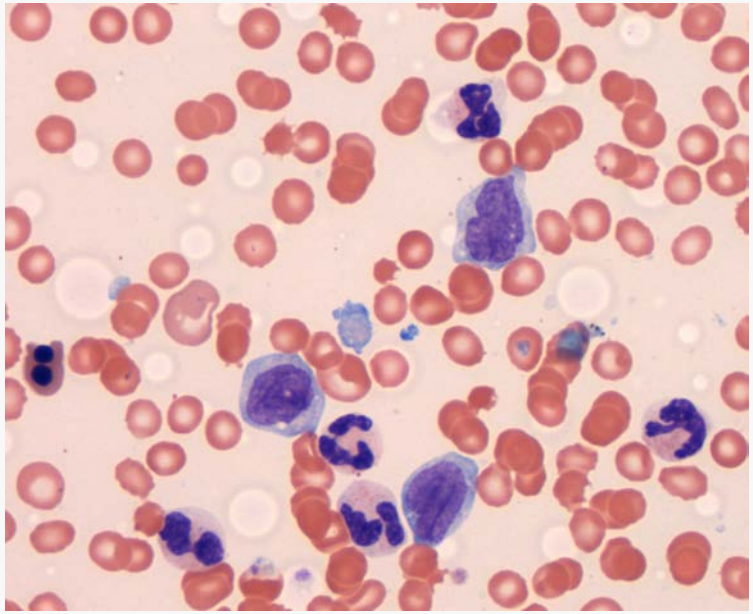
CML blast crisis with unusual genetic findings

CML BC with AML-type translocations are frequently associated with blast morphology/phenotype typical for genetic alteration

Mostly only few case reports



#0215 Wasserman, CML BC with t(8;21)



#0185, Li, CML BC with inv(16)

Chronic myelogenous leukemia
associated with other hematologic neoplasms

Case	Submitter	Panel Dx	Comment
205	Tang, G	1. CML, BCR-ABL1+ in CP 2. CLL	
298	Viswanathan, K	1. CML, BCR-ABL1+ in CP 2. CLL	
149	Xia, D	1. CML, BCR-ABL1+ in CP 2. ET	*CALR* 52 bp del.
231	Soderquist, C	1. CML, BCR-ABL1+ in CP 2. ET	*JAK2* V617F
344	Idrees, A	1. CML, BCR-ABL1+ in AP 2. ET	*JAK2* V617F (at onset)

Ph+ CML and MPN with JAK2 or CALR mutation

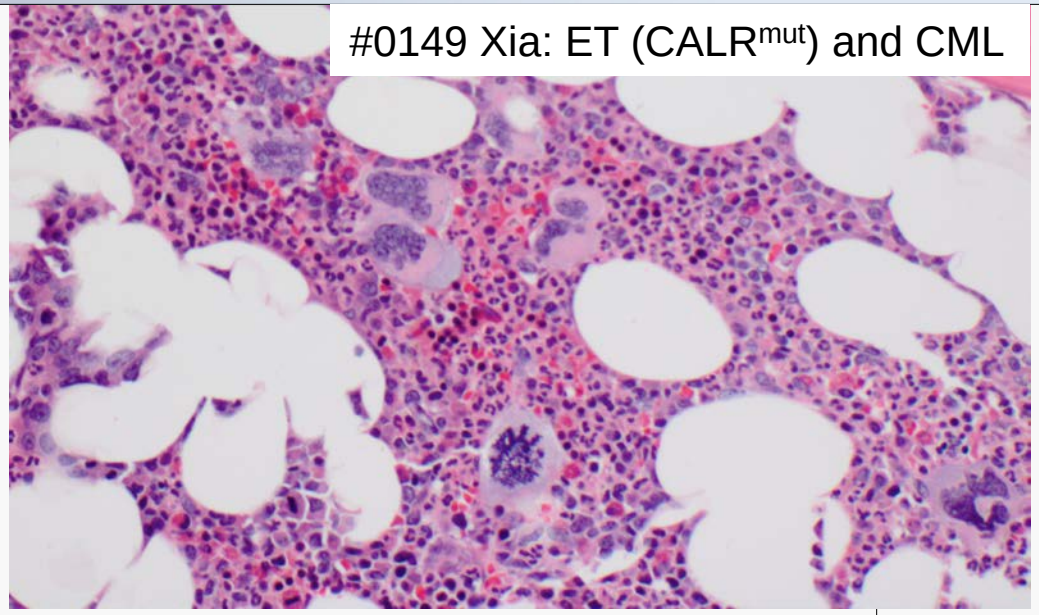
Probably both present in same population as well as separate clones

Temporal sequence of mutations variable:

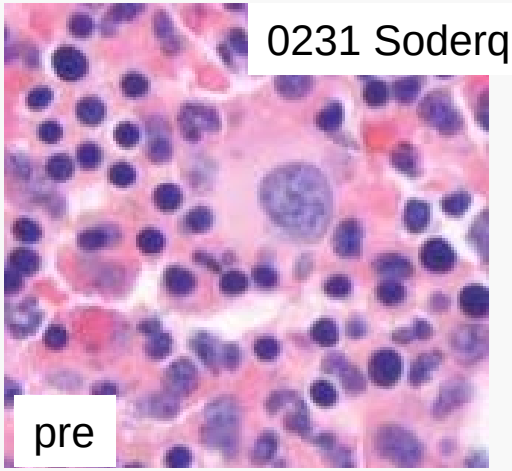
- CML may appear at later time
- Treatment with TKI unmasks second clone

Clinical and morphological changes suggestive of second MPN

BM biopsy and molecular studies necessary to discern from TKI resistance or CML/CNL-like progression



#0149 Xia: ET ($CALR^{mut}$) and CML



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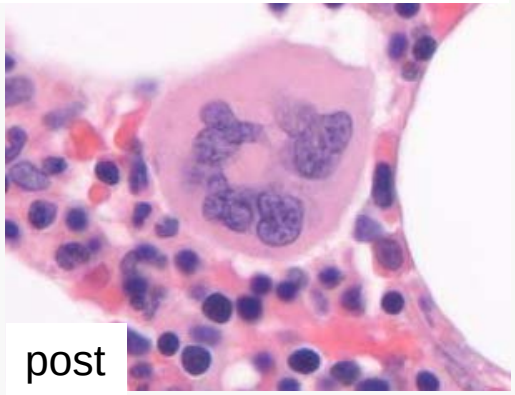


post

0231 Soderquist: CML and ET $JAK2^{mut}$

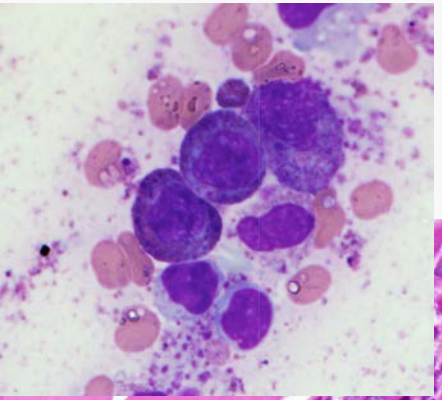
Ph- Myeloproliferative Neoplasms
Unusual genetic findings

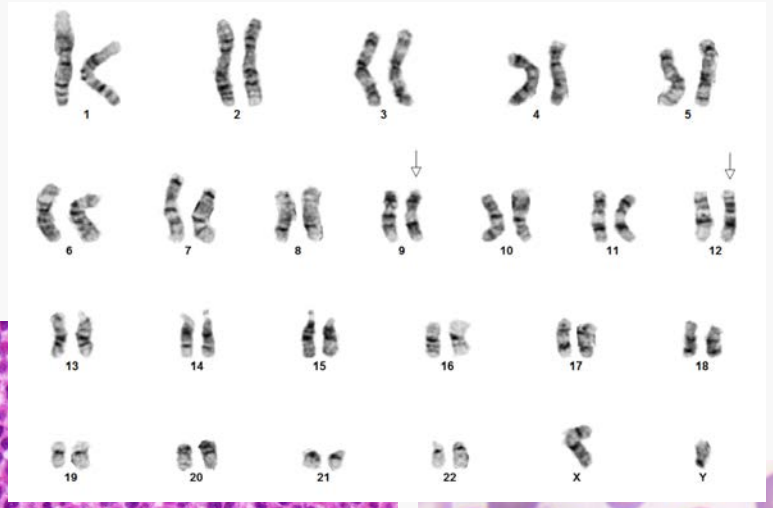
Case	Submitter	Panel Dx	Comment
84	Qualtieri, J	MPN-U (with MYC rearrangement)	
229	Omman, R	MPN-U (with likely germline JAK2 SNP)	JAK2 G571S described as germline SNP
250	Thakral, B	Pre-fibrotic PMF (with non-canonical MPL mutation)	*MPL* S204P prev. identified as somatic in MPN
303	Chan, A	MPN-U (with ETV6-ABL1 rearrangement) and progression to blast crisis	ETV6-ABL1 in ALL and aCML-like MDS/MPN
340	Bradley, K	MPN-U (with ETV6-ABL1 rearrangement)	"
375	Mayordomo-Aranda, E	CNL (with PTPN11 mutation)	

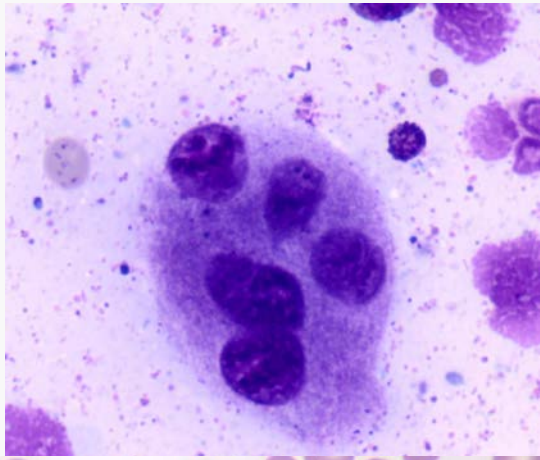
MPN-U with t(9;12)/ETV6-ABL1

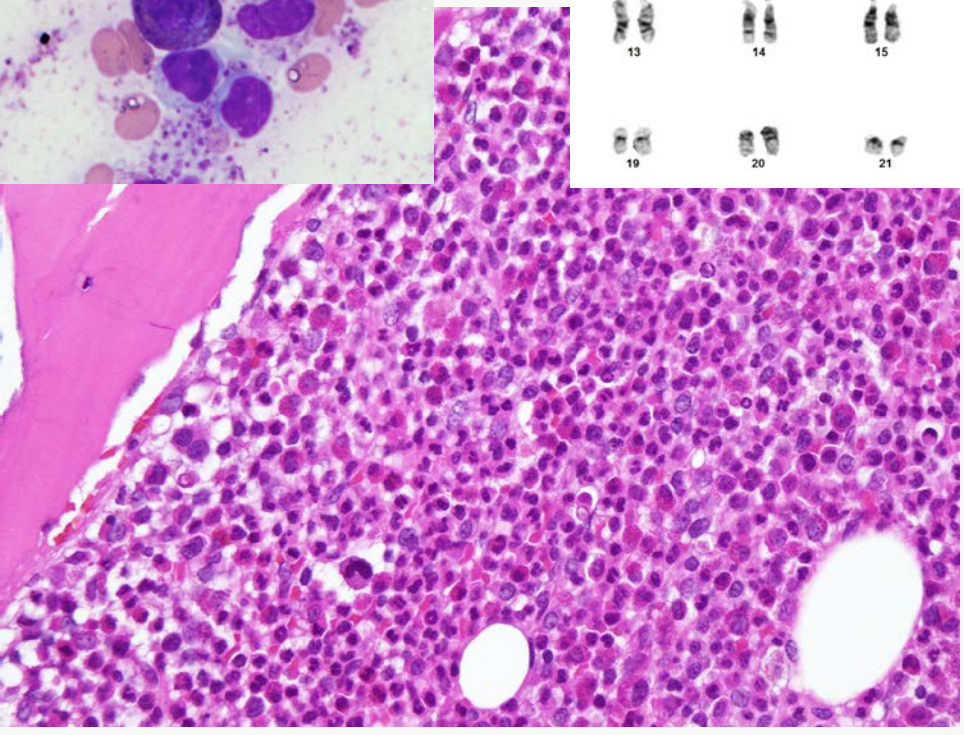
#303 Chan MPN-U
with massive thrombocytosis, increase in atypical eo/baso
Progression to AML with baso-philic differentiation

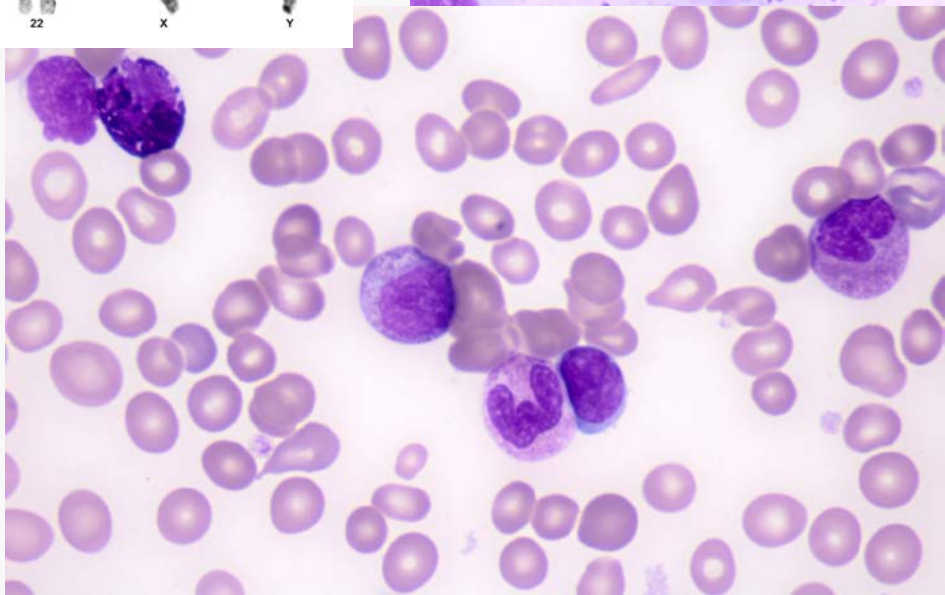
#0340 Bradley
MPN-U with leukocytosis with CML-like PB, abnormal basophils











Neoplasms with ETV6-ABL1

Results from t(9;12) or complex rearrangements, may be missed by cytogenetics, requires at least 3 chromosomal breaks

50% are B-ALL (<1% of all cases), predominantly in children

50% myeloid neoplasms (mostly MPN, some AML) strongly reminiscent of BCR-ABL1+ CML

Eosinophilia virtually constant feature in ETV6-ABL1+ MPN

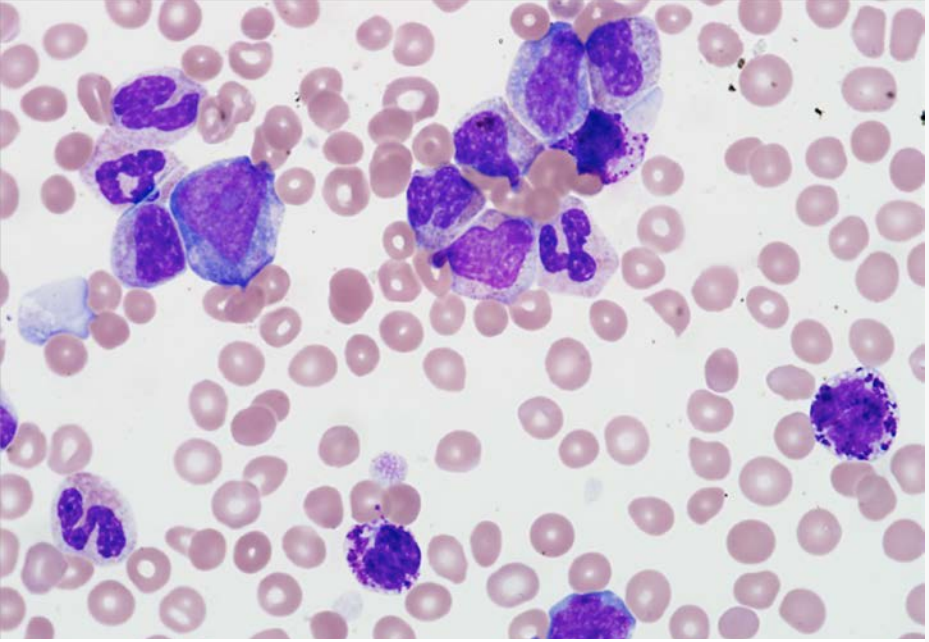
Poor prognosis, MPN responsive to imatinib/nilotinib

Zalilova et al, Haematologica 2016; Reiter & Gottlib, Blood 2017

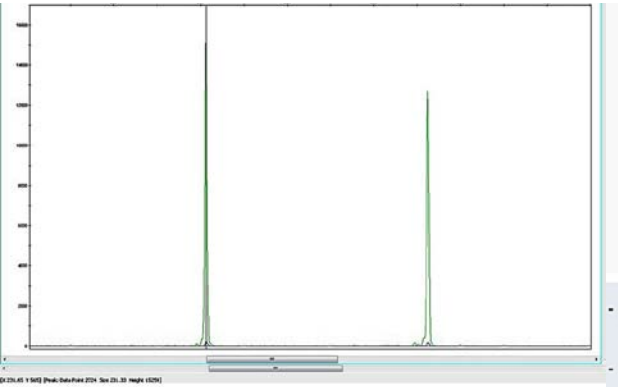
Myeloproliferative Neoplasms

Genetic findings suggesting overlap with MDS/MPN or MDS Progression of Ph- MPN

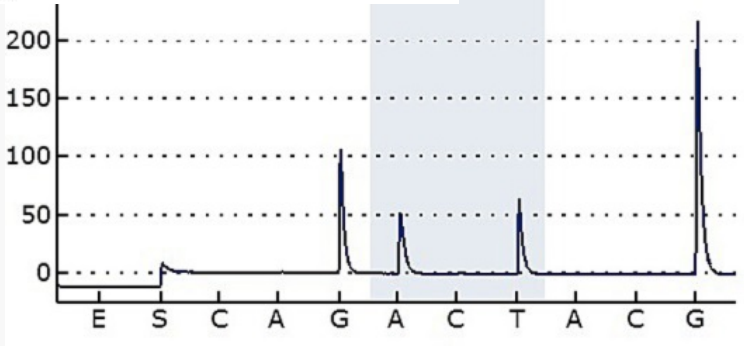
Case	Submitter	Panel Dx	Comment
238	Boiocchi, L	Pre-fibrotic PMF (with RS and SF3B1 mutation)	*JAK2* V617F, *SF3B1* K666N
255	Nakashima, M	Essential thrombocythemia with del(5q)	*JAK2* V617F, 5q-
29	Vasef, M	Post-ET MF in blast phase	*MPL* W515R *CALR* 52pb del
166	Teruya-Feldstein, J	PMF, overt fibrotic stage, in accelerated phase	*JAK2* V617F Del 20q
207	Nam, A	Post-ET MF in accelerated phase with CMML-like monocytosis	*JAK2* V617F (at onset only)

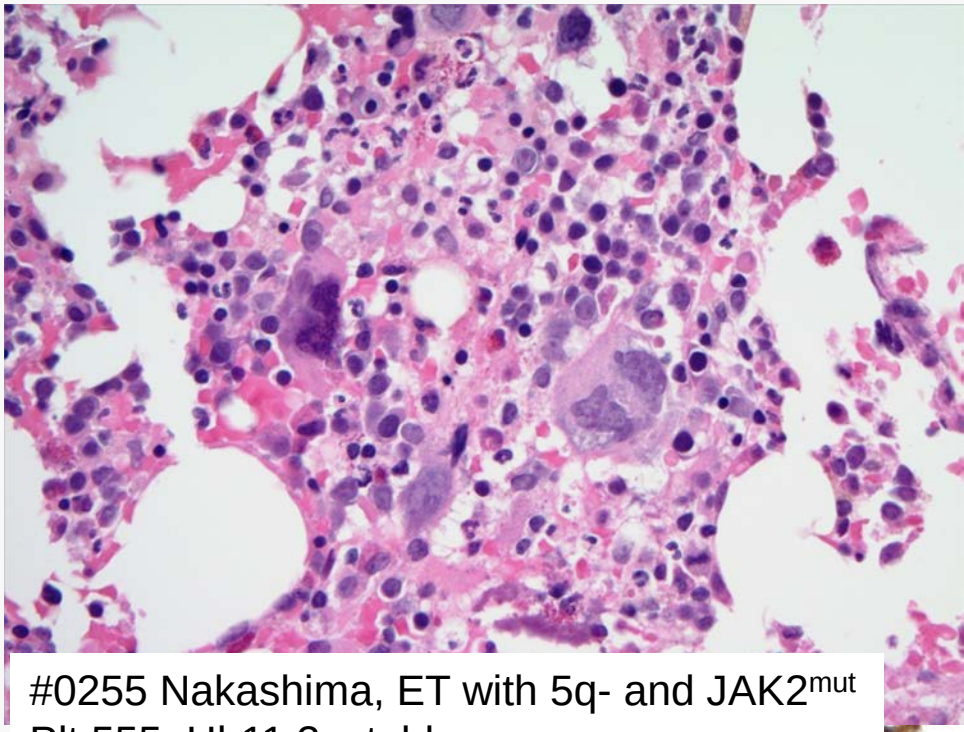


#0029 Vasef: post-ET MF with progression BC

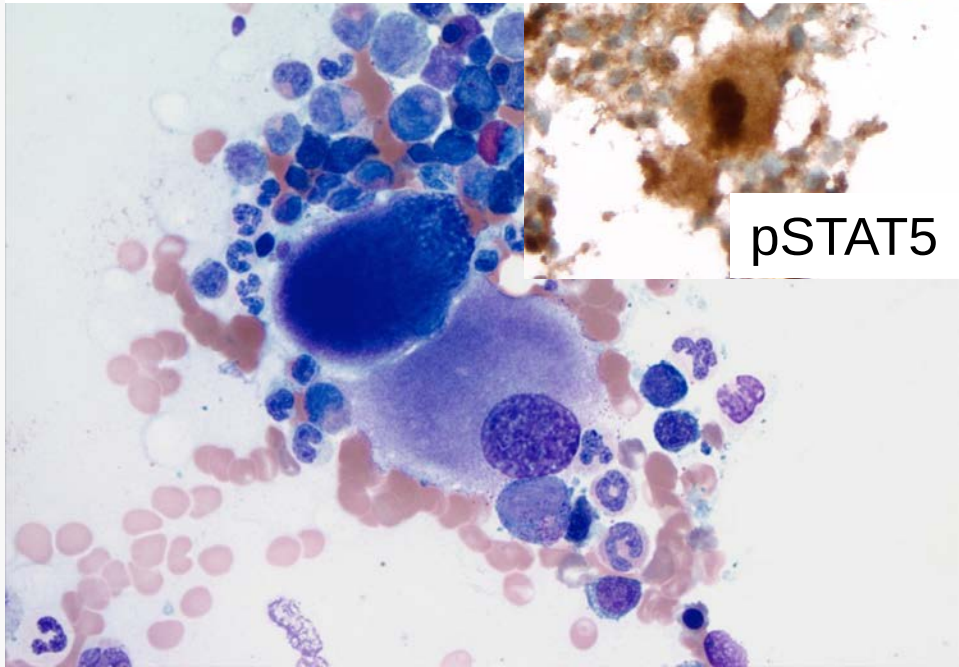


CALR 52 bp del



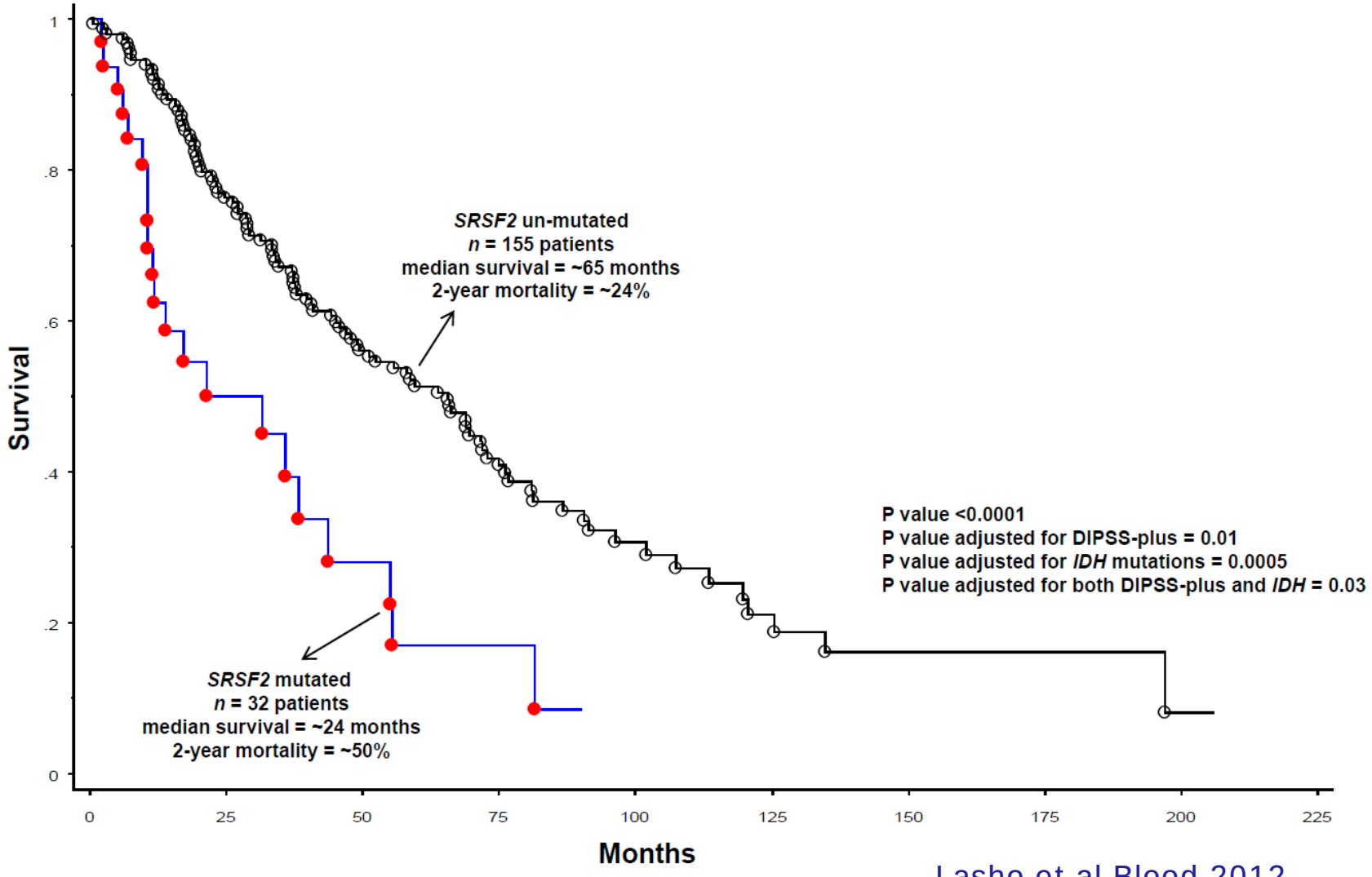


#0255 Nakashima, ET with 5q- and JAK2^{mut}
Plt 555; Hb11,3, stable course



Secondary mutations and prognosis in PMF

SRSF2 and association with IDH1/2 mutations



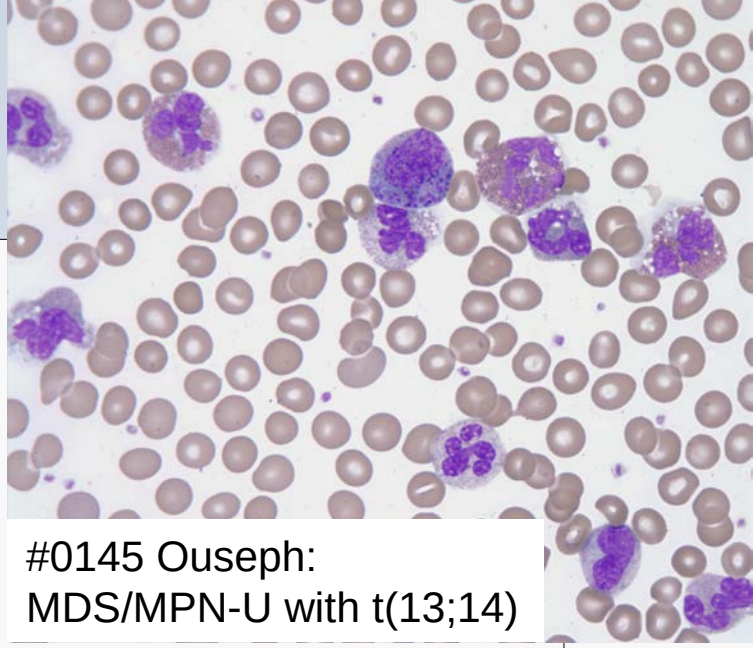
Lasho et al Blood 2012

Myelodysplastic/myeloproliferative Neoplasms and other MN

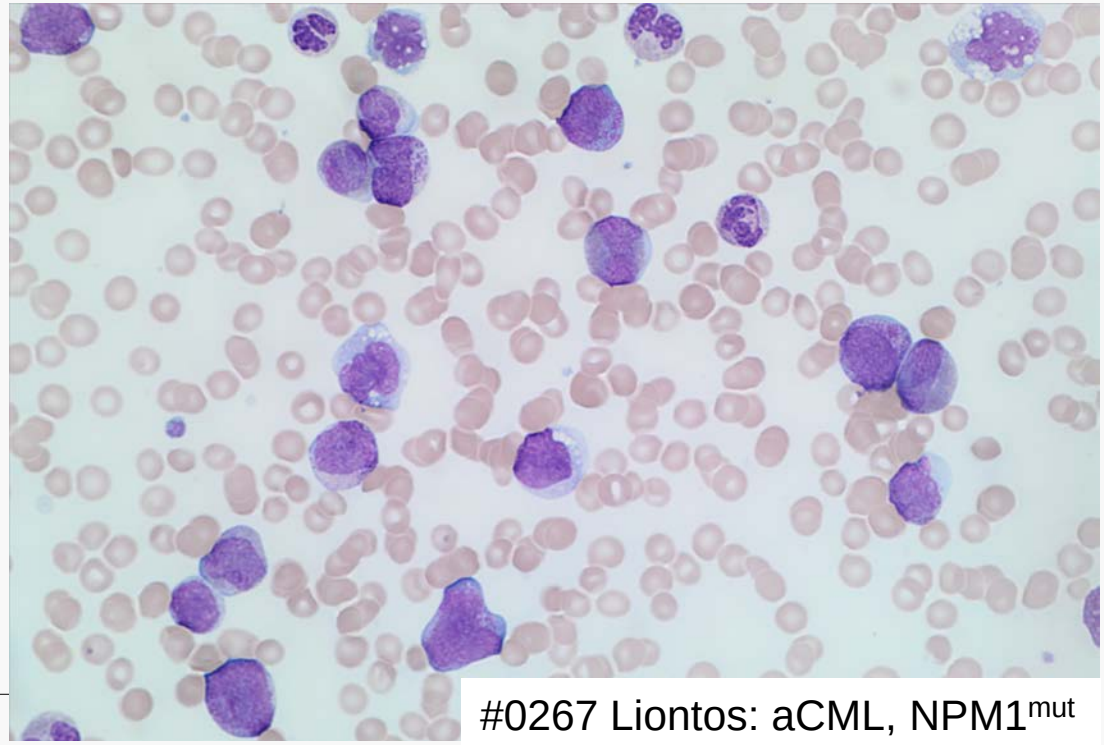
Case	Submitter	Panel Dx
145	Ouseph, M	MDS/MPN-U progressed to myeloid sarcoma
192	Climent, F	CMML-0 (with *NPM1* mutation)
267	Liontos, L	aCML, BCR-ABL1 negative
355	Chen, W	MDS/MPN-U (with ETV-ACSL6 rearrangement)
372	Alvares, C	MDS/MPN-U (with RUNX1 mutation)
293	Zhou, J	BM: SM-AHN (CMML-0) Skin: BPDCN
353	Oberley, M	Myeloid/lymphoid neoplasm with PDGFRA rearrangement (presenting as T-ALL/LBL)

MDS/MPN with unusual molecular findings

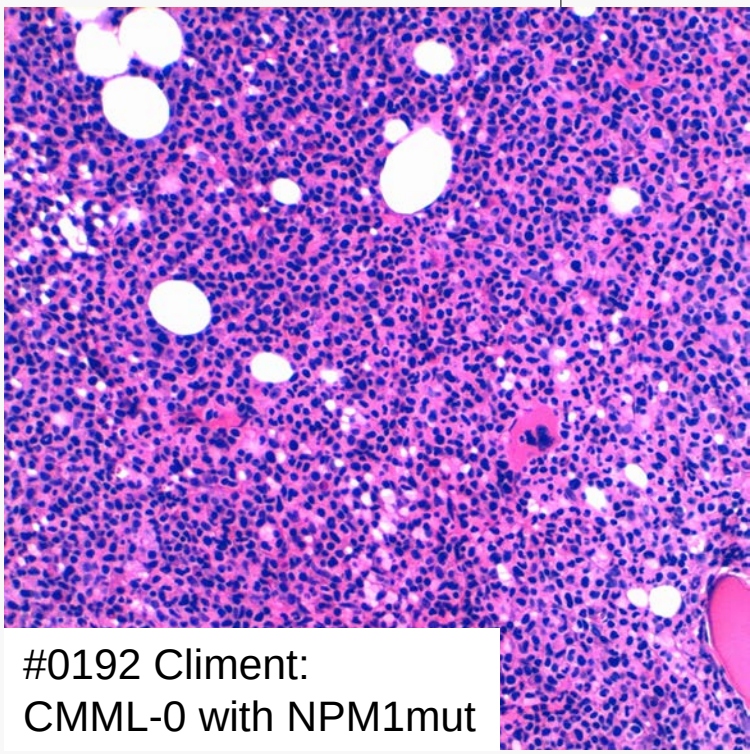
- NPM1 mutations rare in chronic myeloid disorders
  - <5% of cases of CMML, more frequent evolution to AML
  - Occasionally observed in aCML



#0145 Ouseph: MDS/MPN-U with t(13;14)

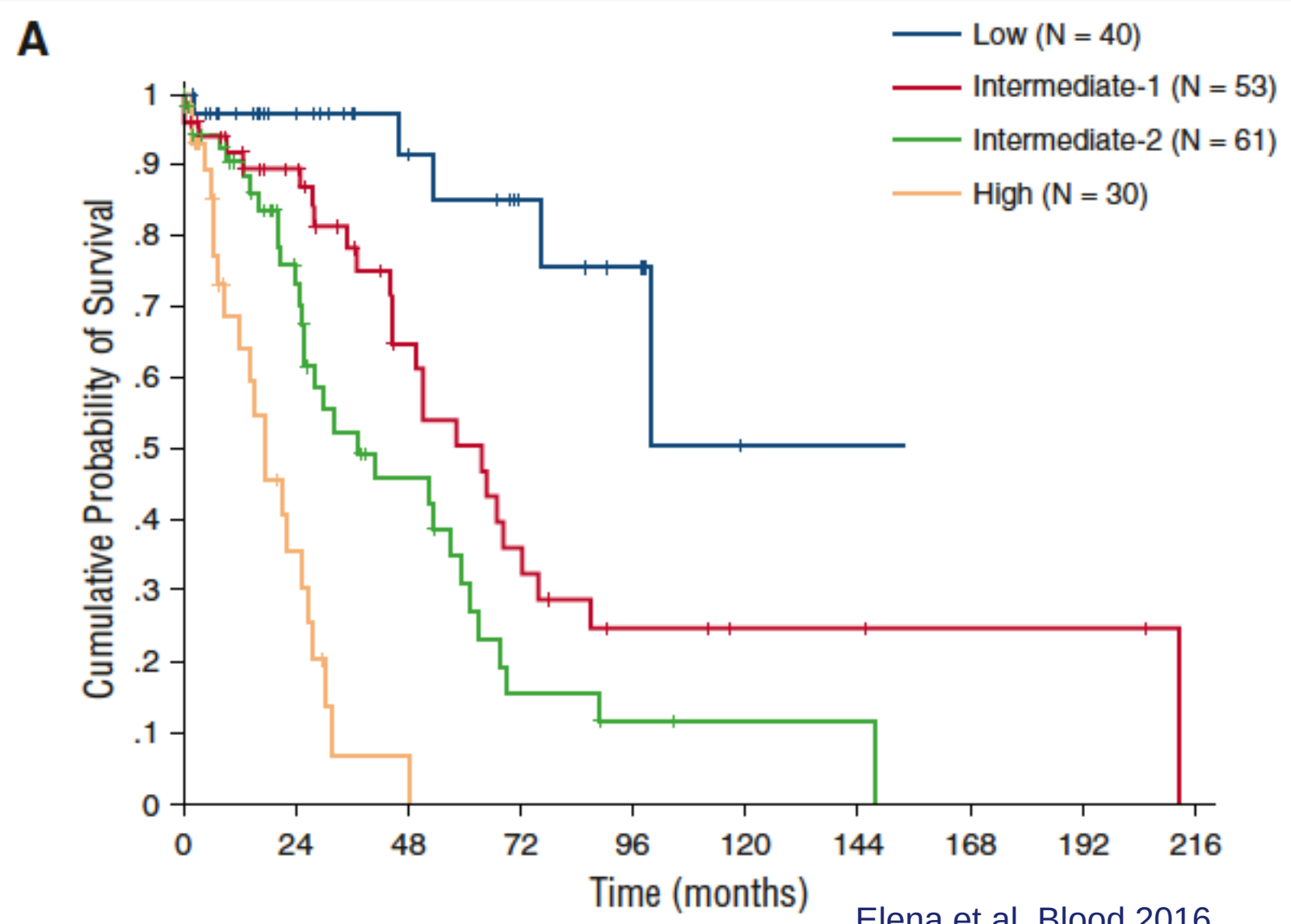


#0267 Liontos: aCML, $NPM1^{mut}$



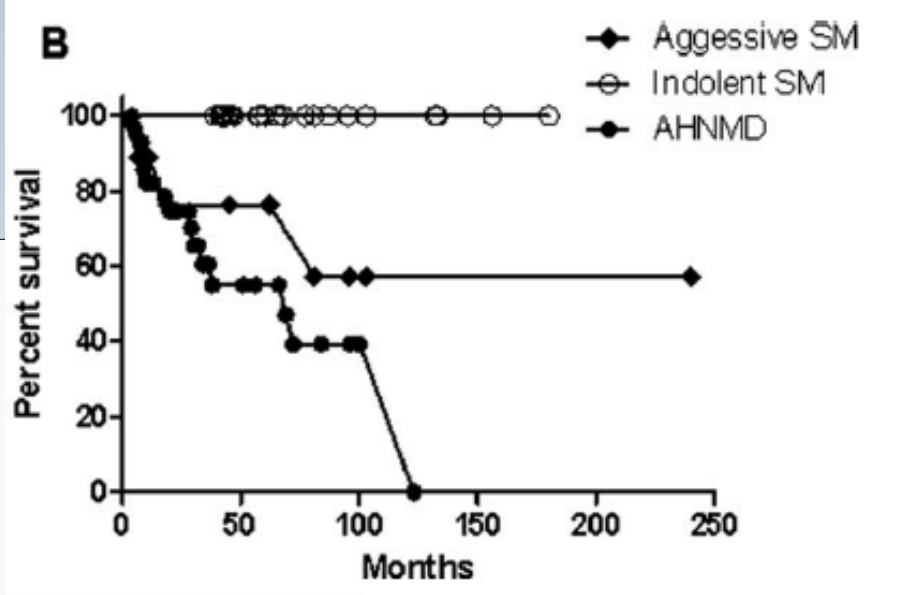
#0192 Climent: CMML-0 with NPM1mut

Risk model for CMML integrating mutations for *RUNX1, ASXL1, SETBP1* and *NRAS*



Elena et al, Blood 2016

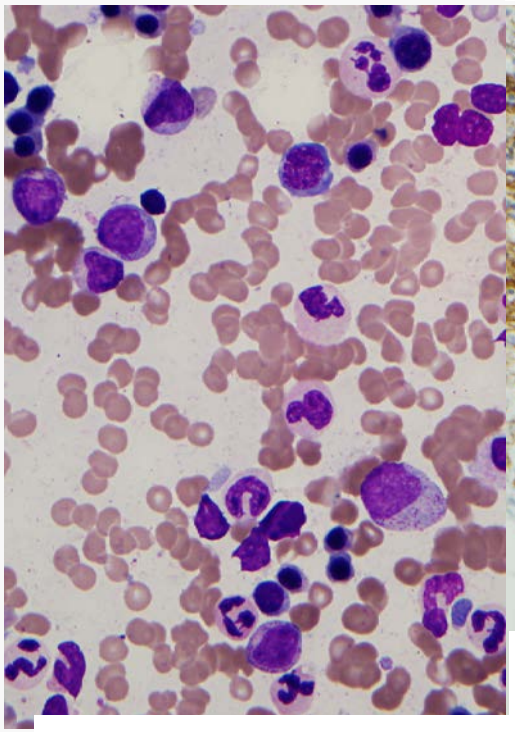
SM-AHM

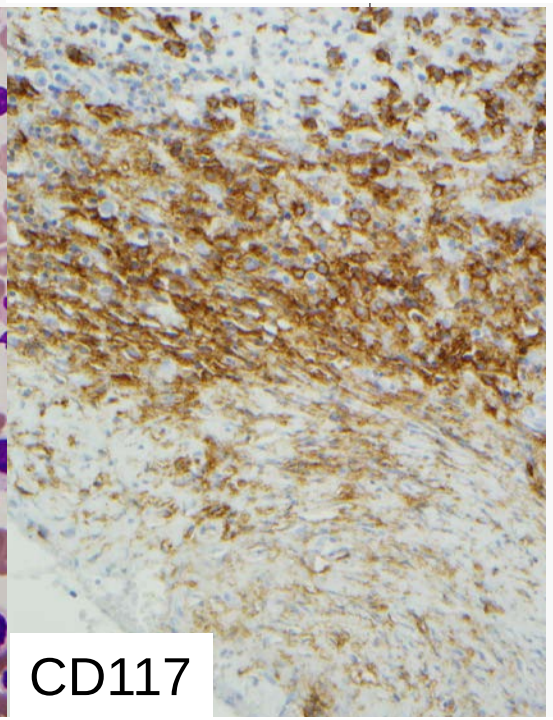


Wang SA, Am J Hematol 2012

- Approximately 40% of SM
- Associated with older age, constitutional symptoms and hematological abnormalities, less frequent skin involvement
- CMML is the most common associated neoplasm (30-40%) and shares genetic abnormalities, whereas lymphoid neoplasms likely represent coincidence
- Prognosis worse than for conventional (indolent) SM, due to AHN

Schwaab et al, Blood 2013, Hanssens et al, Haematologica 2014, Federmann et al, Hum Pathol 2013, Wang et al, AJH 2012

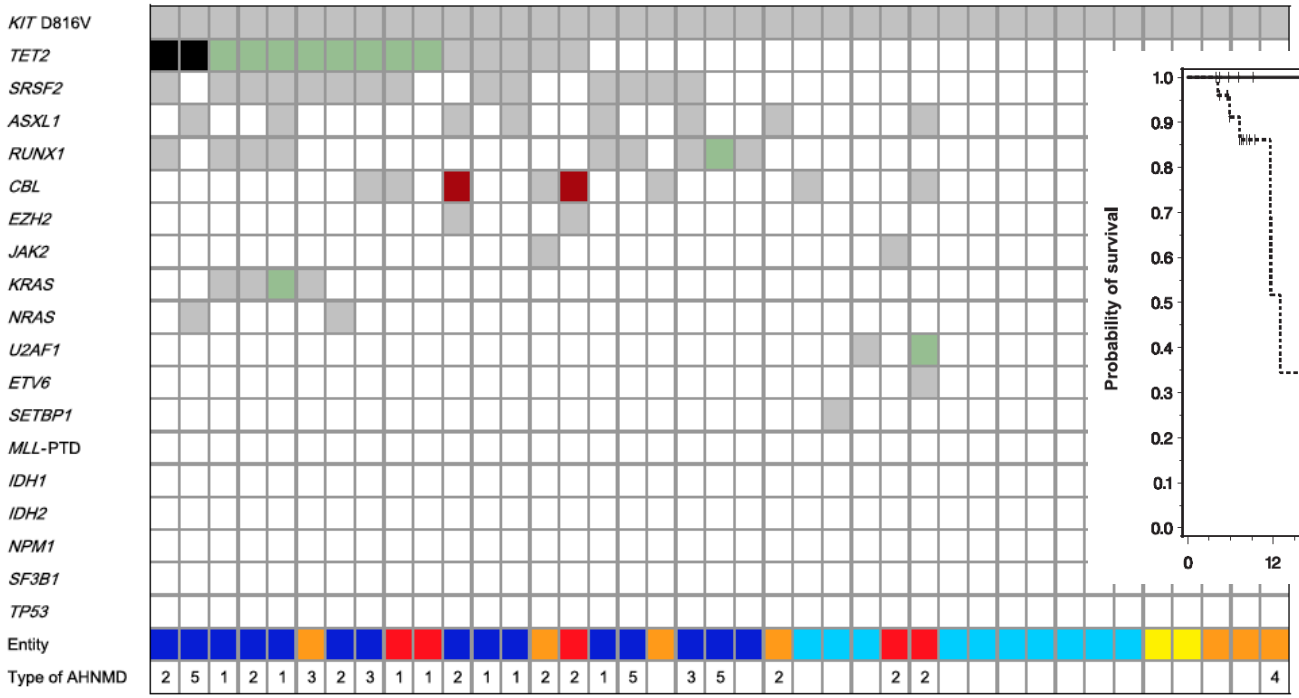




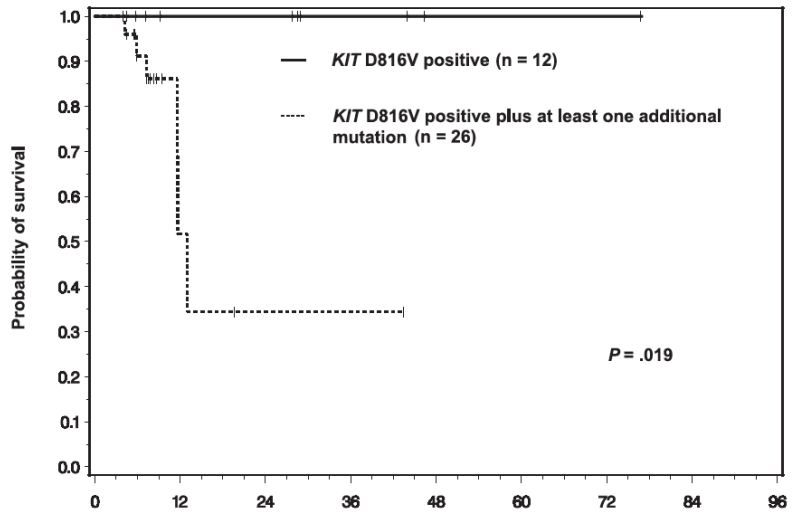
CD117

#0293 Zhou; SM-AHN (CMML-0) and BPDCN

Additional mutations have significant impact on survival in SM
SM-AHN usually shows additional mutations



Schwaab et al, Blood 2013



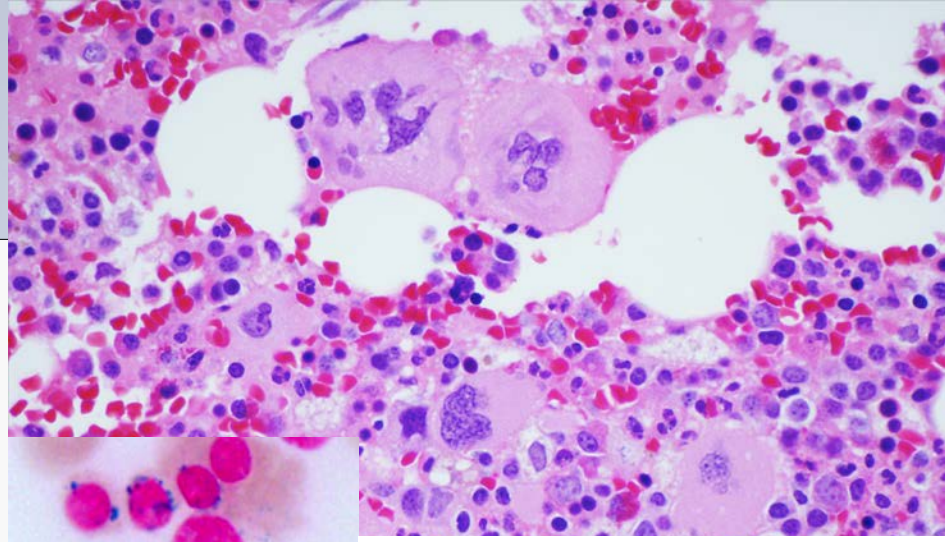
Myelodysplastic syndromes

with molecular findings suggesting overlap with MPN or MDS/MPN

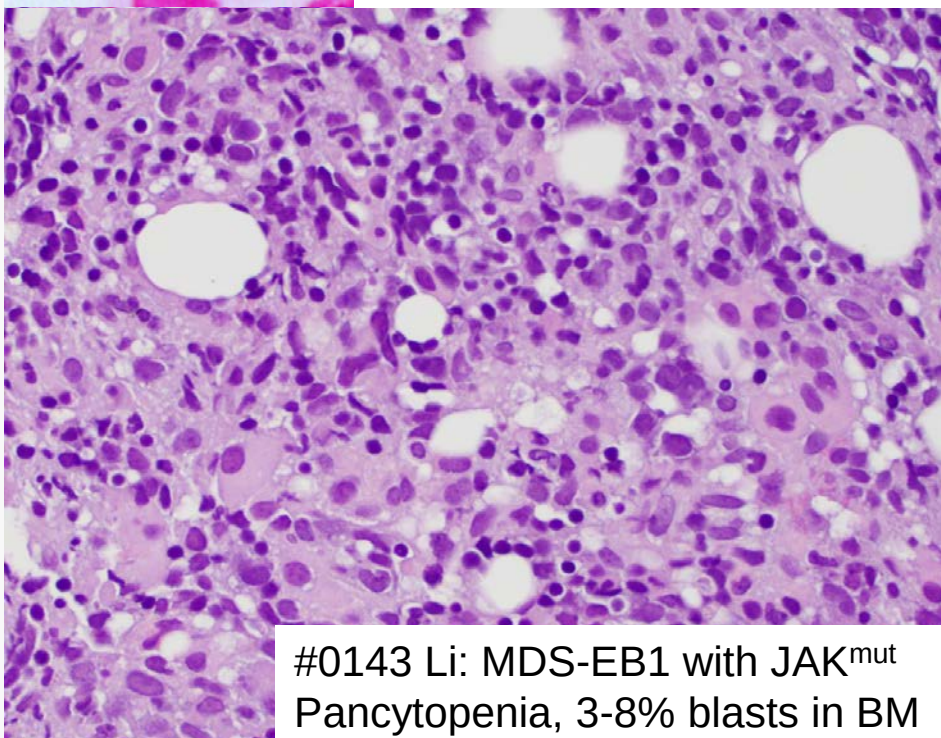
Case	Submitter	Panel Dx	Comment
61	Zheng, G	MDS with isolated del(5q)(with *JAK2* mutation)	*JAK2* V617F 5q-
140	Baste, N	MDS with isolated del(5q) (with non-canonical *MPL* mutation)	*MPL* Y591D, *TET2* (2x) and *ASXL1* mut 5q-
143	Li, S	MDS-EB1 (with *JAK2* mutation)	*JAK2* V617F
159	Lewis, N	Myeloid neoplasm with features of both MDS with isolated del(5q) and MDS/MPN-RS-T	*MPL* W515S *SF3B1* K666Q
239	Boiocchi, L	1. MDS-RS-SLD (with JAK2 mutation) 2. MGUS	*JAK2* V617F *SF3B1* T663I

JAK2 in MDS and MDS/MPN other than MDS/MPN-RS-T

- JAK2 V617F mutation occurs in 3-5% of MDS – no prognostic impact
- May reside in subclone/separate clone
- Diagnosis of MDS/MPN-RS-T requires thrombocytosis ≥450 K and ≥15% RS
- MDS/MPN-RS-T frequently associated with JAK2 V617F, less common with CALR or MPL mutation
- CALR and MPL mutations usually associated with thrombocytosis



#0239 Boiocchi: MDS-RS-SLD (with JAK2^{mut}, SF3B1^{mut})
Plt 110-125; mild splenomegaly



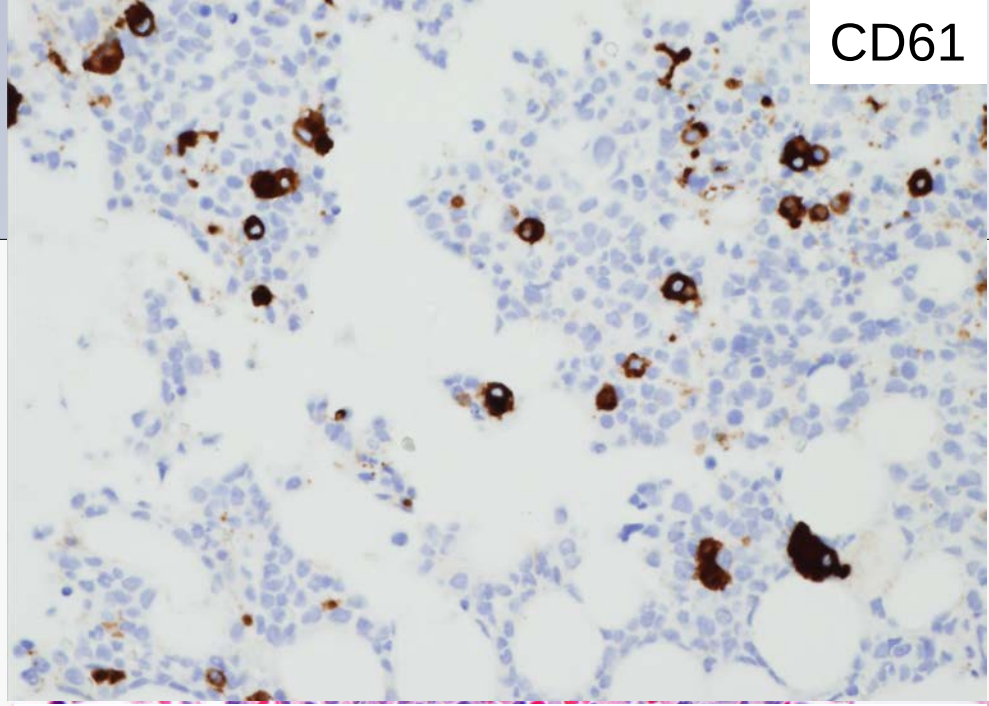
#0143 Li: MDS-EB1 with JAK^{mut}
Pancytopenia, 3-8% blasts in BM

Myelodysplastic syndromes
with unusual clinical and/or molecular findings

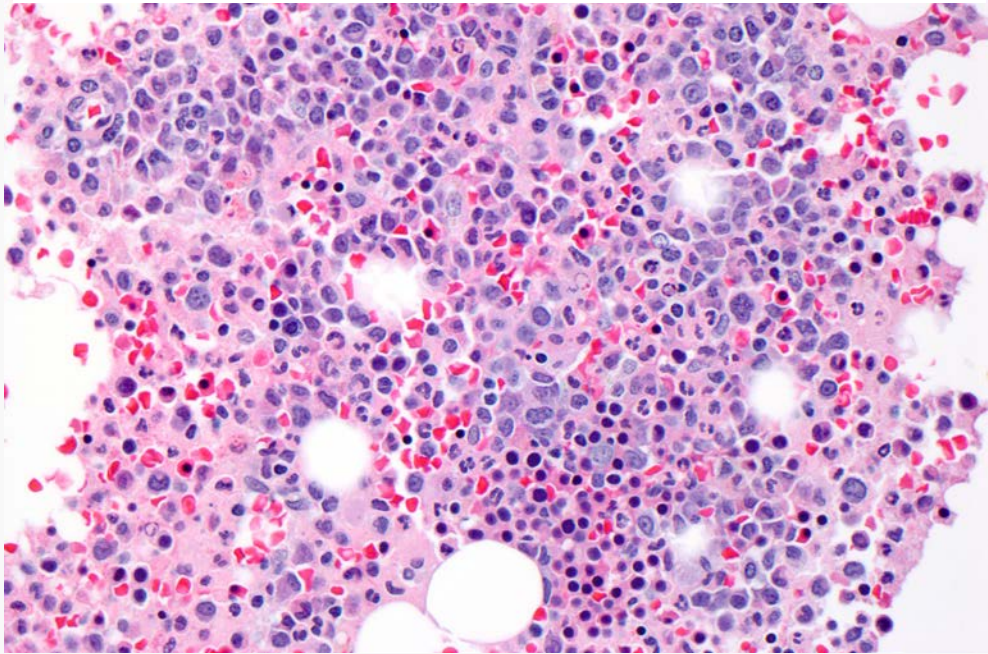
Case	Submitter	Panel Dx	Comment
226	Dulau-Florea, A	MDS-RS (with PRCA and clonal T-cell population of undetermined significance)	*SF3B1* K666N
244	Mroz, P	MD-EB2 and clonally-related mediastinal malignant germ cell tumor	i(12)(p10)
249	El Hussein, S	MDS-MLD	Inv(12)
321	Hidalgo-Lopez, J	SM-AHN (CMML-0) followed by MDS-MLD (donor derived)	
330	Stuart, L	MDS-MLD (donor derived)	*DNMT3A R882C*

Myeloid neoplasms of donor origin

- Cytopenias are common after SCT
  - Reactive, e.g. infections or toxicities
  - Poor engraftment
  - Relapse
- Donor-derived MDS/AL rare
  - 0.5% of SCT recipients
  - May be due to unrecognized germline mutations
- Donor-engrafted CHIP is common among SCT recipients with unexplained cytopenias (Gibson et al, Blood 2017)
- Cytogenetics and mutational analysis may aid in diagnosing donor related MN



#0330 Stuart: MDS-MLD donor derived



What is the role of extended genetic testing in myeloid neoplasms with chronic evolution?

Indications

- Diagnostic
- Prognostic
- Predictive

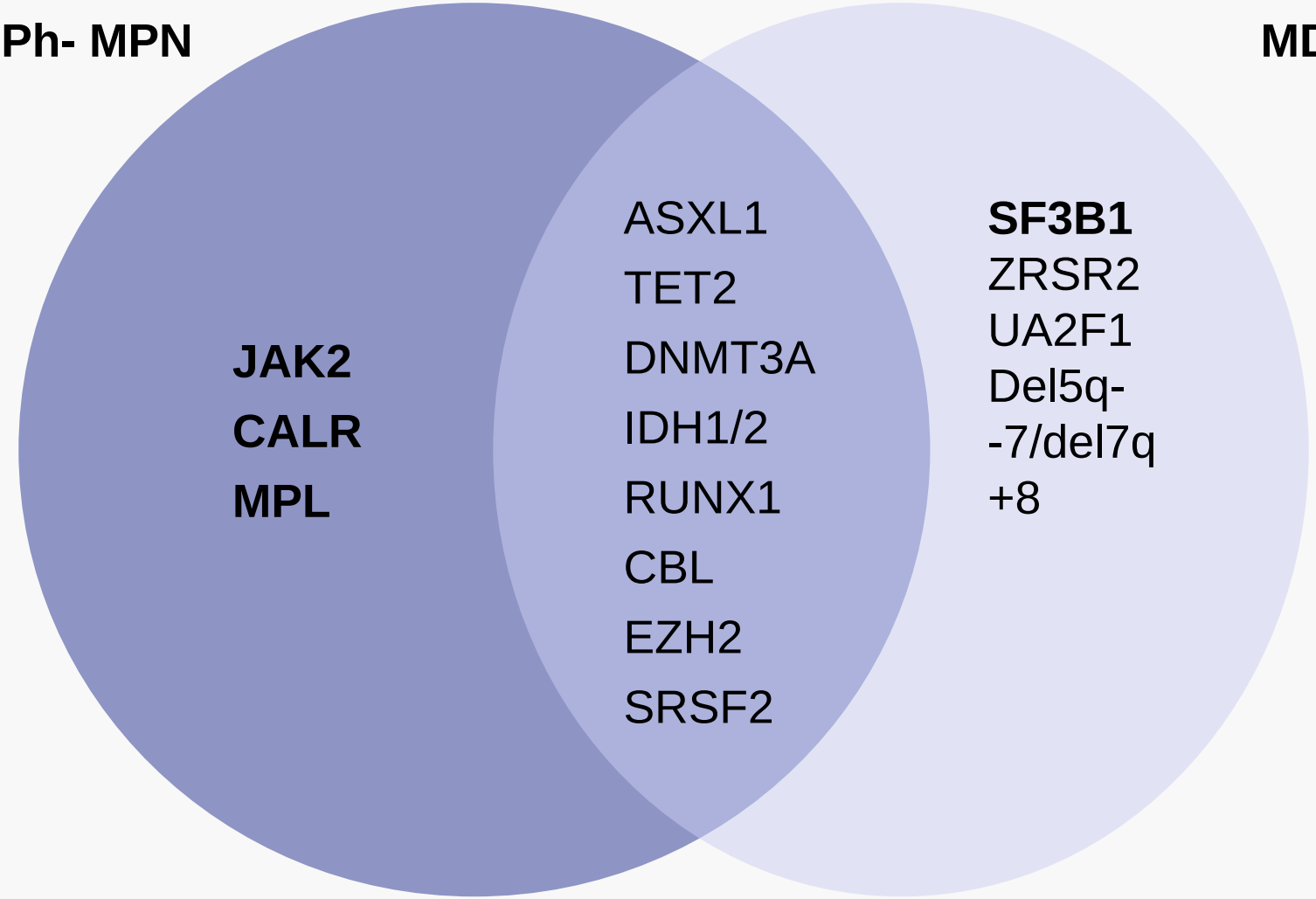
Time points for testing

- At diagnosis
- Before starting therapy
- At relapse/progression/transformation
- After allo-TX

What is the role of extended genetic testing in myeloid neoplasms with chronic evolution?

Diagnostic	Examples for indications	Limitations
Confirmation of MN	MDS with borderline dysplasia, CMML, early MPN	Separation from CCUS requires integration of other findings
Subtyping of MN	CNL, MDS-RS, MN with atypical features	Lack of specificity of common mutations
MN with clinical/morphological/phenotypical overlap	MN with ring sideroblasts, MDS with fibrosis, SM-AHN…	Overlap result of mutational pattern
MN presenting as suspected AP or BC	Identification of underlying/antecendent chronic myeloid neoplasm (CML, Ph-MPN)	Primary mutation (e.g. JAK2 V617F) may disappear, unusual variants of progression
At progression/transformation/relapse	Identification of resistance mutations (ABL1), clonal evolution	Primary mutation (e.g. JAK2 V617F) may disappear, unusual variants of progression
After allo-SCT	Relapse vs. second neoplasm	CHIP may also occur in transplant

The mutational profile is insufficient to define disease entities



Modified from Hussein et al

Extended genetic testing in CML

Chronic phase

- Test for JAK2/MPL/CALR in cases with unusual clinical or morphological features or change during TKI therapy

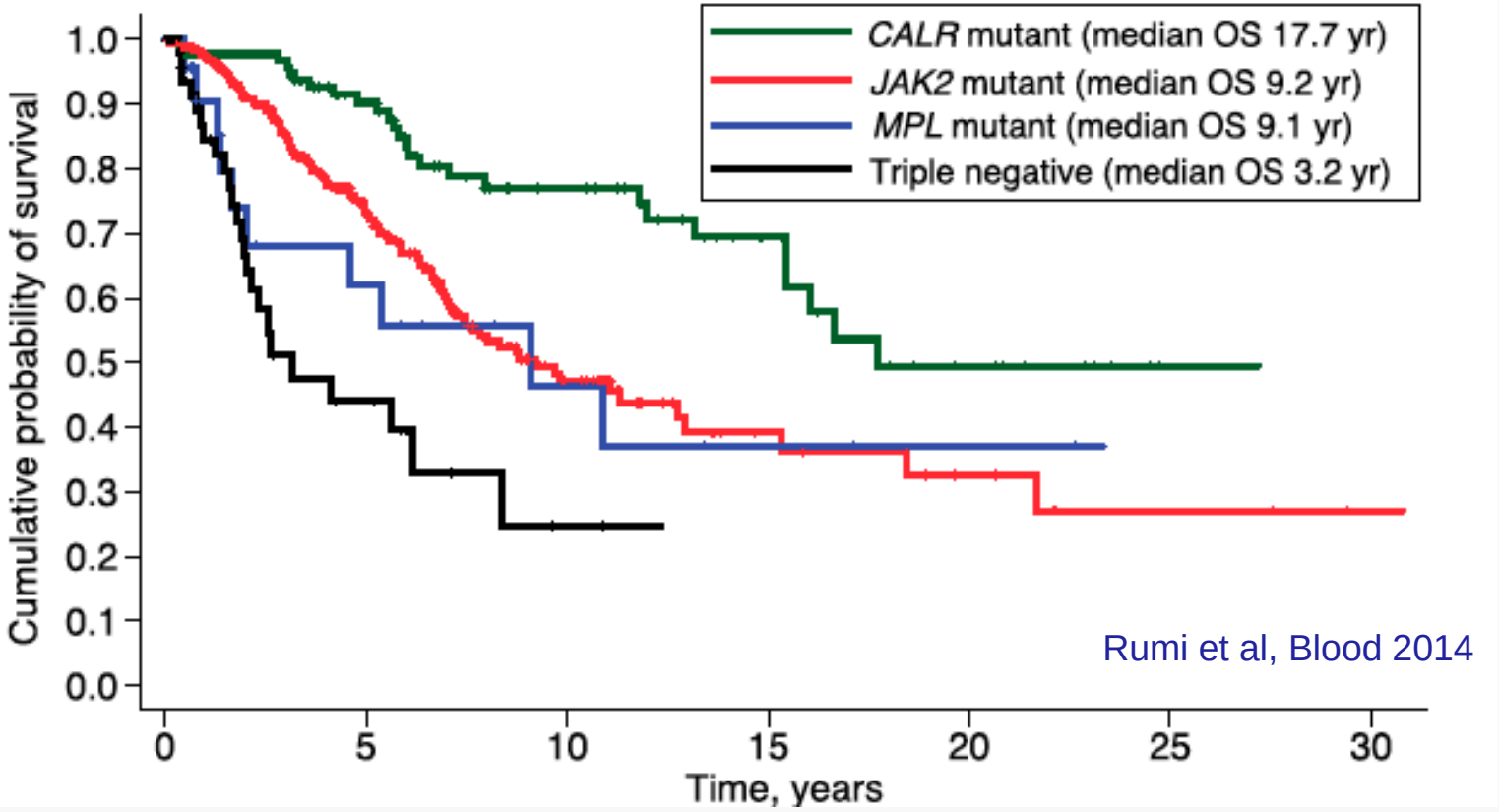
Blast crisis/accelerated phase

- Cytogenetics may reveal secondary alterations amenable for specific therapies
- Check for ABL1 mutations may not be enough in TKI resistance

MPN with CML-like features and negativity for BCR/ABL

- PDGFRA/B translocations
- ETV6-ABL1 translocations
- Mutations frequent in CNL and aCML (SETBP1, CSF3R..)

Genetic testing in Ph- MPN
Driver mutations and prognosis of Ph-



Rumi et al, Blood 2014

- CALR mutated PMF has superior diagnosis, “triple-negative” PMF the worst prognosis, even if corrected for age
- Different prognostic impact of type 1 and type 2 CALR mutations
- Determination of JAK2, CALR and MPL status essential for prognostication

What is the role of other mutations in Ph- MPN?

Mutation	**Prevalence**	**Prognostic Impact**
JAK2 Exon 12	5% PV	
MPL W515K/L	5-10% PMF, 1-6% ET; 0% PV	
CALR Ex. 9 frameshift	25-33% ET; 25-35% PMF; 0% PV	Good
„triple negative“	5-10% of ET and PMF	Poor
CSF3R T618I	Chronic neutrophilic leukemia (60-100%)	
SETBP1	Atypical CML (25-30%)	
Other mutations	**not resticted to MPN**	
ASXL1	7% PV, 4% ET, 22% PMF	Poor (PMF)
SRSF2	8-17% PMF	Poor (PMF)
DNMT3A	7% PV, 3% ET, 15% PMF	Frequent in CHIP
EZH2	8% PMF	Poor (PMF)
IDH1/2	2-4% in MPN	Poor
TET2	16% PV, 4% ET, 14% PMF	
CBL	8% PMF, others rare	

Extended genetic testing in Ph- MPN

Diagnostic markers

- JAK2, CALR, MPL
- In triple negative cases, include non-canonical mutations
- Check for BCR/ABL1 in case of CML/CNL-like progression
- Assess SF3B1 if ring sideroblasts are encountered
- Overlap cases: look for CNL/aCML-type mutations

Prognostic markers

- Important disease modifiers (*ASXL1, SRSF2, IDH2….*)
- Risk markers for transformation/progression (RUNX1, TP53(?))

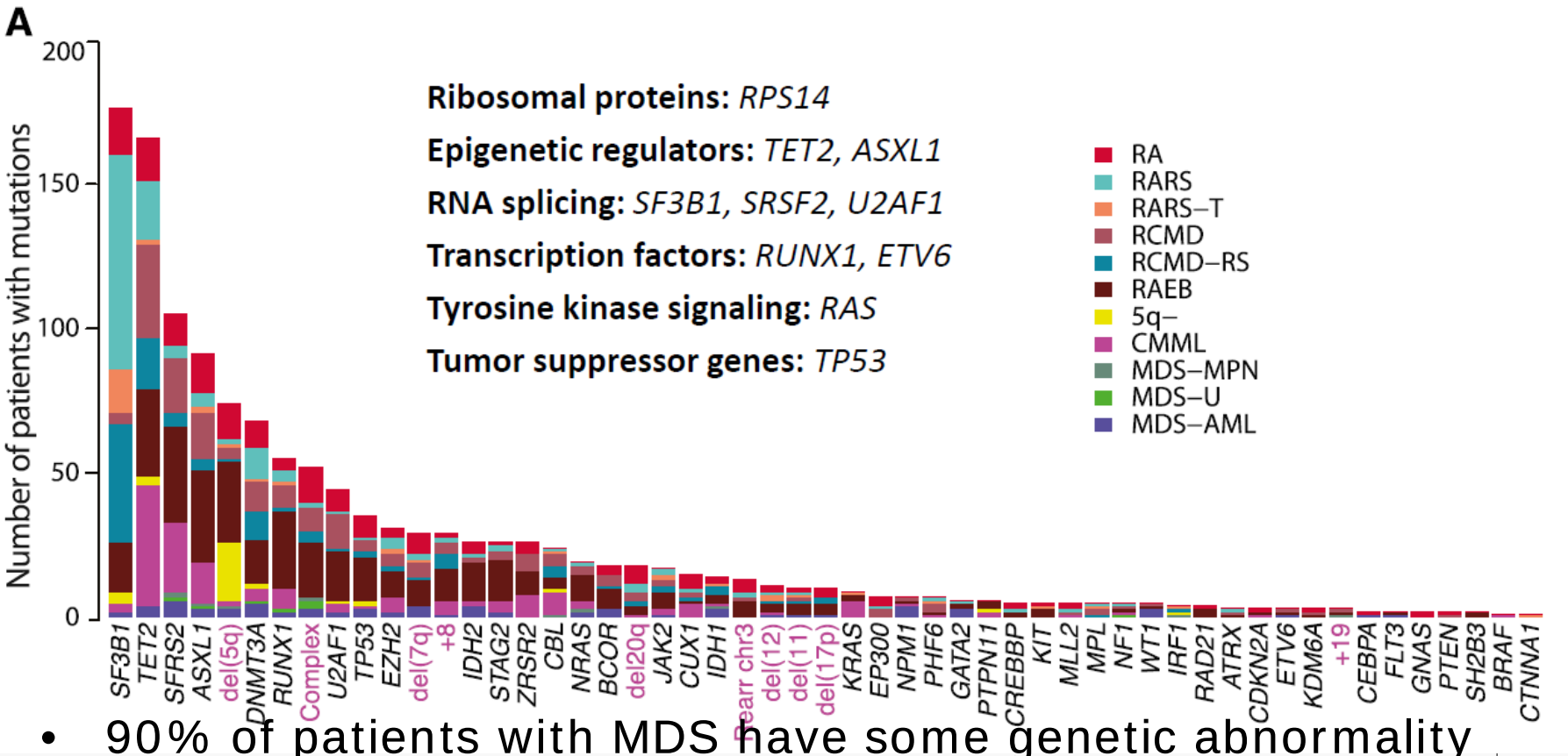
Predictive markers?

Somatic mutations in MDS

HIGH-THROUGHPUT TARGETED GENE RESEQUENCING IN MDS

PAPAEMMANUIL et al

BLOOD, 21 NOVEMBER 2013 • VOLUME 122, NUMBER 22



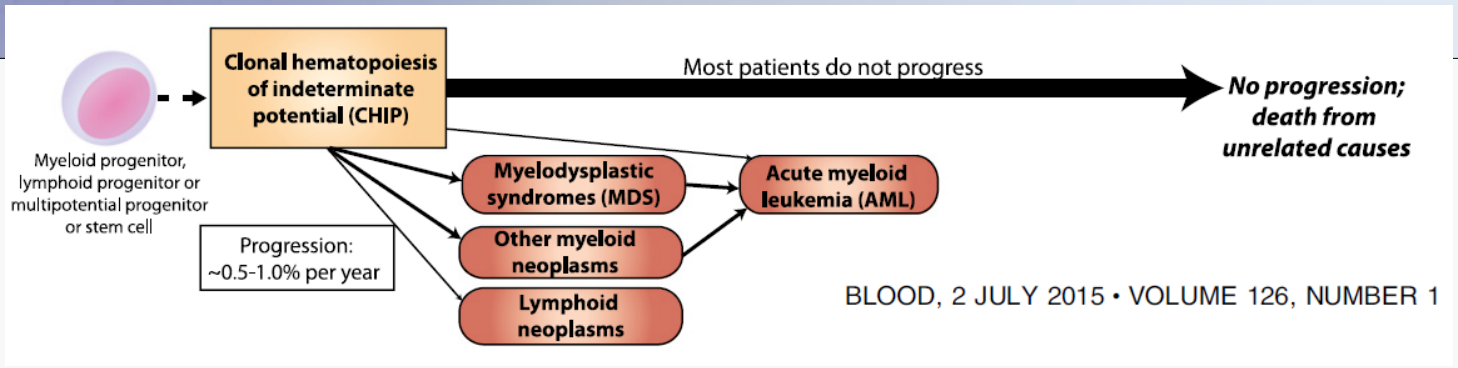
- 90% of patients with MDS have some genetic abnormality
- Broad spectrum of mutations

Prognostic impact of common mutations in MDS

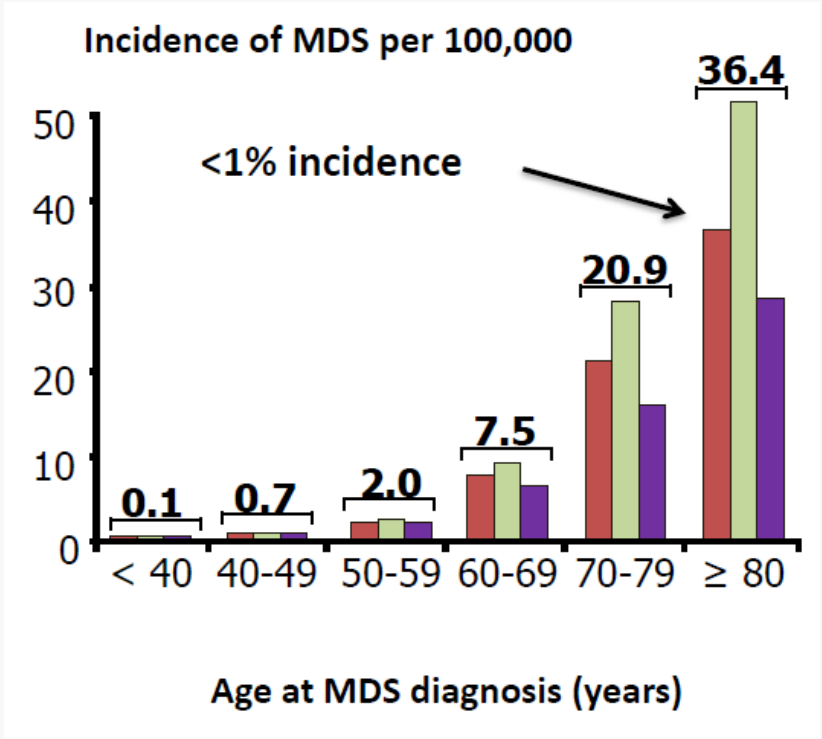
Gene	Frequency	Prognostic impact
ASXL1	14-29%	poor
EZH2	6-8%	poor
TET2	12-23%	None (?)
DNMT3A	13-18%	None (?)
IDH2/IDH1	4-13%	None/poor (?)
SF3B1	9-75%	good
SRSF2	12-15%	None/poor (?)
U2AF1	12-16%	None/poor (?)
ZRSR2	3-11%	None/poor (?)
TP53	2-21%	Poor
RUNX1	9-16%	Poor
NRAS	3-4%	Poor
CBL	2%	Poor
STAG2	6-8%	Poor

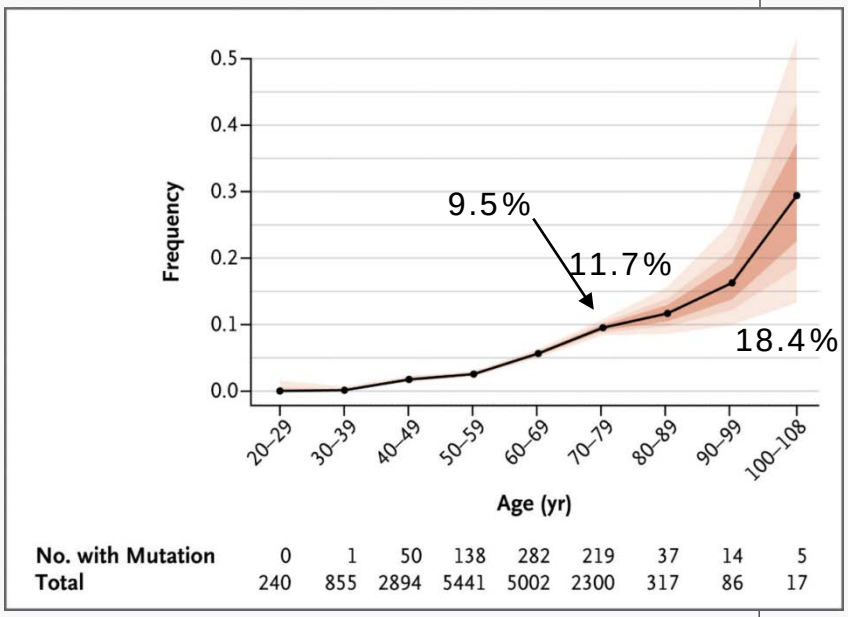
- Number of mutations has prognostic impact
- Influence of combination of mutations and cytogenetics

CHIP is far more frequent than MDS



10-15% CHIP





N Engl J Med. 2014 December 25; 371(26): 2488–2498.

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Extended molecular testing in MDS and MDS/MPN

In addtion to conventional markers (blast count, IPSS-R, cytogenetics), mutational analysis can provide diagnostic help and better prognostication

- Normal/unavailable cytogenetics
- Low grade MDS with borderline dysplasia vs. ICUS

Prognostic markers

- SF3B1
- ASXL1, RUNX1, IDH1/2, TP53…..
- Number of mutations

Keep clinical consequences in mind

Overlap with CHIP/CCUS

- DNMT3A, TET2, ASXL1, others

What is sufficient to diagnose MDS according to the 2016 WHO update?

Feature	Sufficient to diagnose MDS alone?
Dysplastic morphology (>10%)	**Yes**, provided possible secondary causes of cytopenia and dysplasia are excluded clinically
Excess marrow blasts (>5%)	**Yes**, provided marrow recovery or growth factor effects are excluded
Cytogenetic abnormality	**Yes** (excluding +8, -7, del 20q)
Flow cytometry abnormality	**No**, but can support a MDS diagnsis
MDS-type mutation	**No** (because of CHIP) but might support the diagnosis together with other features

Mutation testing in MDS is a complementary tool, but currently does not replace other diagnostic procedures

Take home messages

Clinical, laboratory and morphological features govern classification of myeloid neoplasms with chronic evolution for now

Except for BCR-ABL1 (and PDGFR alterations), mutations are not disease-specific

Mutational profiling aids in subclassification and prognostication of MPN, MDS/MPN and MDS

The high frequency of CHIP and CCUS in elderly populations precludes the use of mutational analysis as stand-alone test

Remaining controversies and future questions

Is there a “standard” mutational panel for MN with chronic evolution – and when should we use it?

Will we adopt a molecular classification of MPN and MDS/MPN?

- JAK2+, MPL+, CALR+, triple neg. MPN

Can mutations change conventionally obtained diagnoses, and at what threshold?

- ICUS to MDS
- MDS with SF3B1 and JAK2 to MDS/MPN-RS-T
- Ologomonocytic CMML

How to deal with germline findings?

Will we look for CHIP as a risk factor for cardiovascular disease?

Thank you!