Wissenschaftliches Symposium Myeloproliferative Neoplasien

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Hochmolekulare Risikomarker bei MPN – Implementierung in den klinischen Alltag ?

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Offenlegung Interessenskonflikte

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JAHRESTAGUNG 2017 STUTTGART 29.9.–3.10.

1. Anstellungsverhältnis oder Führungsposition: nein

2. Beratungs- bzw. Gutachtertätigkeit: Novartis, Janssen, Celgene, Baxalta

3. Besitz von Geschäftsanteilen, Aktien oder Fonds: nein

4. Patent, Urheberrecht, Verkaufslizenz: nein

5. Honorare: Novartis, Janssen, Celgene, Baxalta

6. Finanzierung wissenschaftlicher Untersuchungen: nein

7. Andere finanzielle Beziehungen: nein

8. Immaterielle Interessenkonflikte: nein

The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms

Daniel A. Arber, et al. Blood 2016 127:2391-2405

- Chronic myelogenous leukemia, *BCR-ABL1* positive (CML)
- Chronic neutrophilic leukemia (CNL)
- **Polycythaemia vera (PV)**
- **Essential thrombocythaemia (ET)**
- **Preprimary Myelofibrosis (prePMF)**
- **Overt primary Myelofibrosis**
- Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS)
- Myeloproliferative neoplasm, unclassifiable (MPN-U)

Driver Mutations in MPN

Driver mutations:

- implicated in the pathogenesis of MPN
- hyperproliferation of hemtapoetic cells > growth advantage
- functional relevant (e.g. cell lines, mouse models)
- required for maintenance of the disease

JAK2* V617F, *CALR*, *MPL

- mainly restricted to MPN
- essential for the myeloproliferative phenotype

➢ All three driver mutations abnormally activate the cytokine receptor/JAK2 pathway and their downstream effectors (STATs)

Non-Driver Mutations in MPN

Non-driver mutations:

- somatic mutations that do not primarly act on proliferation
- can modify and enhance effects of the driver mutation
- belong to different functional categories:
  - epigenetic regulators (TET2, DNMT3A, IDH1/2, EZH2, ASXL1)
  - signaling molecules (NF1, NRAS, KRAS, LNK, CBL, FLT3)
  - splicing complex (SF3B1, SRSF2, U2AF1,)
  - transcription factors (TP53, NFE2, CUX1, IKZF1, ETV6, RUNX1)
- not restricted to MPN, even more frequent in MDS
- modify differentiation and contribute to myelodysplastic features
- contribute to disease progression and leukemic transformation
- allowing the identification of high-risk patients

Non-Driver-Mutations in MPN: Prognostic Impact

- ***TET2*:** mutated in 12% of MPN (21/181 *JAK2*pos; 3/17 *JAK2*neg.); present in hematopoietic stem cells, can precede *JAK2* V617F
  Delhommeau FN et al., N Engl J Med. 2009;360(22):2289-301
- ***DNMT3A*:** mutated in 10% of MPN (12/115), most frequent in sAML and MF; co-occurrence with mutations in *JAK2*, *IDH1/2*, and *ASXL1*
  Stegel
- ***ASXL1***: 3/10, preP… rence with …
  Carbu…
- ***EZH2***: …4/30]; mostly homozygous mutations; association with worse outcome
  Ernst T et al., Nat. Genet. 2010;42(8):722-6
- ***IDH1/2*:** mutated in 4.2% of PMF and 21.6% of blast-phase MPN; predicts worse survival in blast-phase MPN
  Tefferi A et al., Leukemia. 2010;24(7):1302-9

Mutations in epigenetic modifiers

Non-Driver-Mutations in MPN

Gene	Protein function	Frequency	Consequences
ASXL1	Chromatin binding protein; associated with PRC1 and 2	25% PMF 1-3% ET/PV	Initiation, **rapid progression**
TET2	demathylation; oxidation of 5mC into 5hmC	10-20% MPN (ET, PV and PMF)	Inititation
DNMT3A	DNA methylation	5-10% MPN (ET, PV and PMF)	Initiation
SRSF2	Serine/arginin-rich pre- RNA splicing factor	<2% ET, 10-15% PMF	Initiation? **progression**
U2AF1	RNA splicing factor	10-15% PMF	Phenotypic change (anemia)
IDH1/IDH2	Neomorphic enzyme, generation of 2HG > blocking a-ketoglutarate-dependent enzymes	1-3% PMF, each	Initiation, **progression**
EZH2	H3K27 methyltransferase	5-10% PMF	Initiation, **progression**
TP53/RUNX1	Transcription factor	<5%/<3% each; 20%/10% sAML each	**Progression to leukemia**

Vainchenker W and Kralovics R, Blood; 2017

High Molecular Risk Markers in PMF

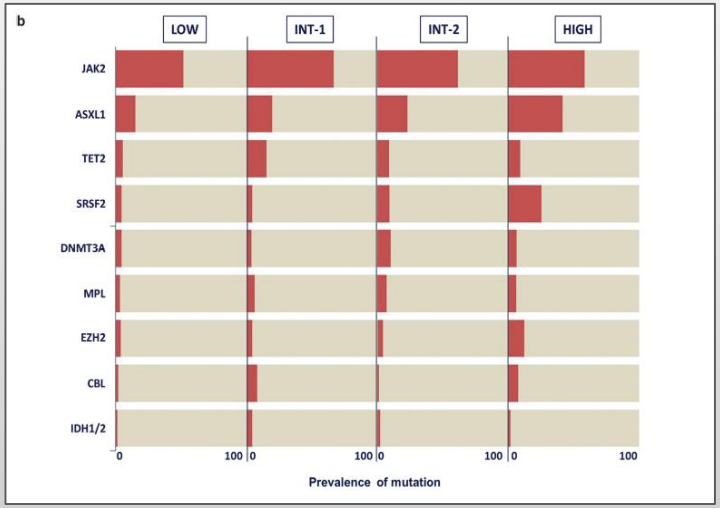
Vannucchi AM, et al. Leukemia. 2013;27(9):1861-9

- In total 879 PMF patients from two cohorts (European n=483, Mayo Clinic n=396) were studied
- Evaluation of the individual and combinatorial prognostic relevance of somatic mutations in *ASXL1, SRSF2, EZH2, TET2, DNMT3A, CBL, IDH1/2, MPL* and *JAK2*
- Validation of the findings from European cohort in Mayo Clinic cohort

Mutation frequency n=483 (%)

JAK2	59.2
ASXL1	21.7
TET2	9.7
SRSF2	8.5
DNMT3A	5.7
MPL	5.2
EZH2	5.1
CBL	4.4
IDH1/2	2.6

IPSS: LOW INT-1 INT-2 HIGH



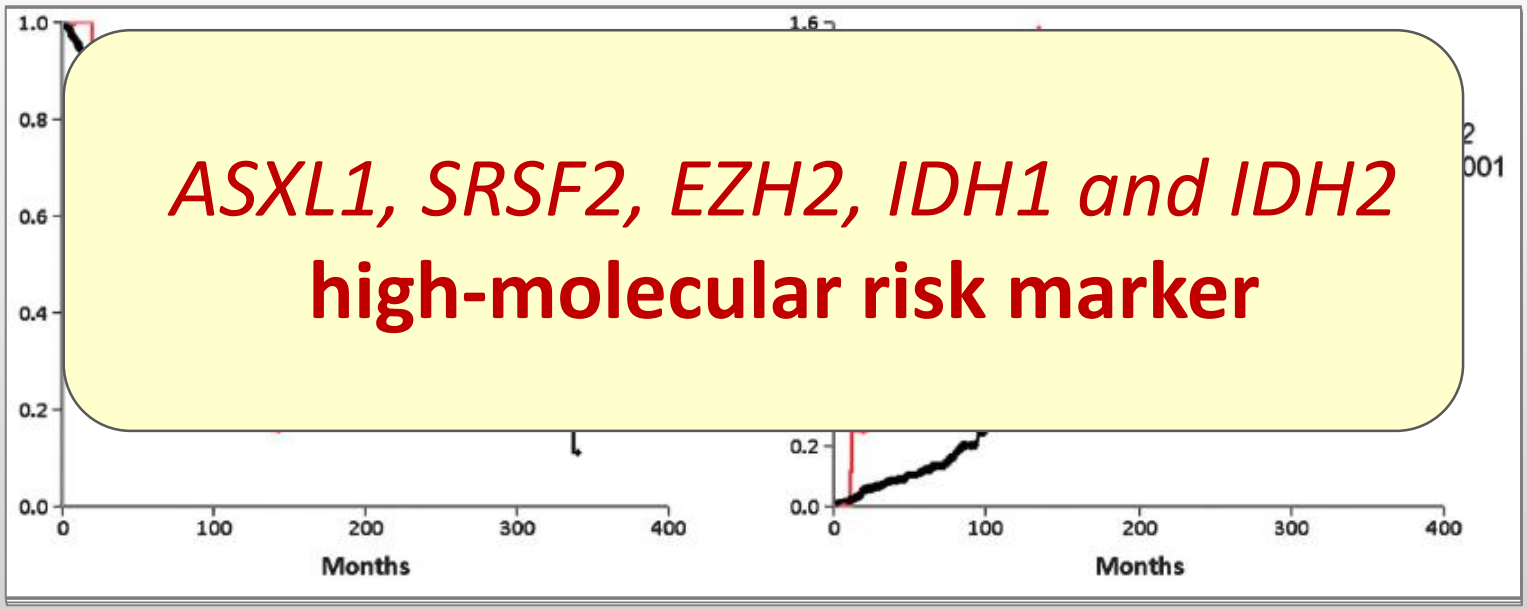
High Molecular Risk Markers in PMF

Vannucchi AM, et al. Leukemia. 2013;27(9):1861-9

- *ASXL1, SRSF2, EZH2:* inter-independently associated with significant shortened survial in univariate and multivariable analysis
- *ASXL1, IDH1/2, EZH2, SRSF2:* associated with adverse leukemia-free survival in univariate analysis; in multivariable analysis *ASXL1, IDH1/2, and SRSF2,* but not *EZH2* remained significant

Overall Survival

Transformation to Leukemia



ASXL1, SRSF2, EZH2, IDH1 and IDH2

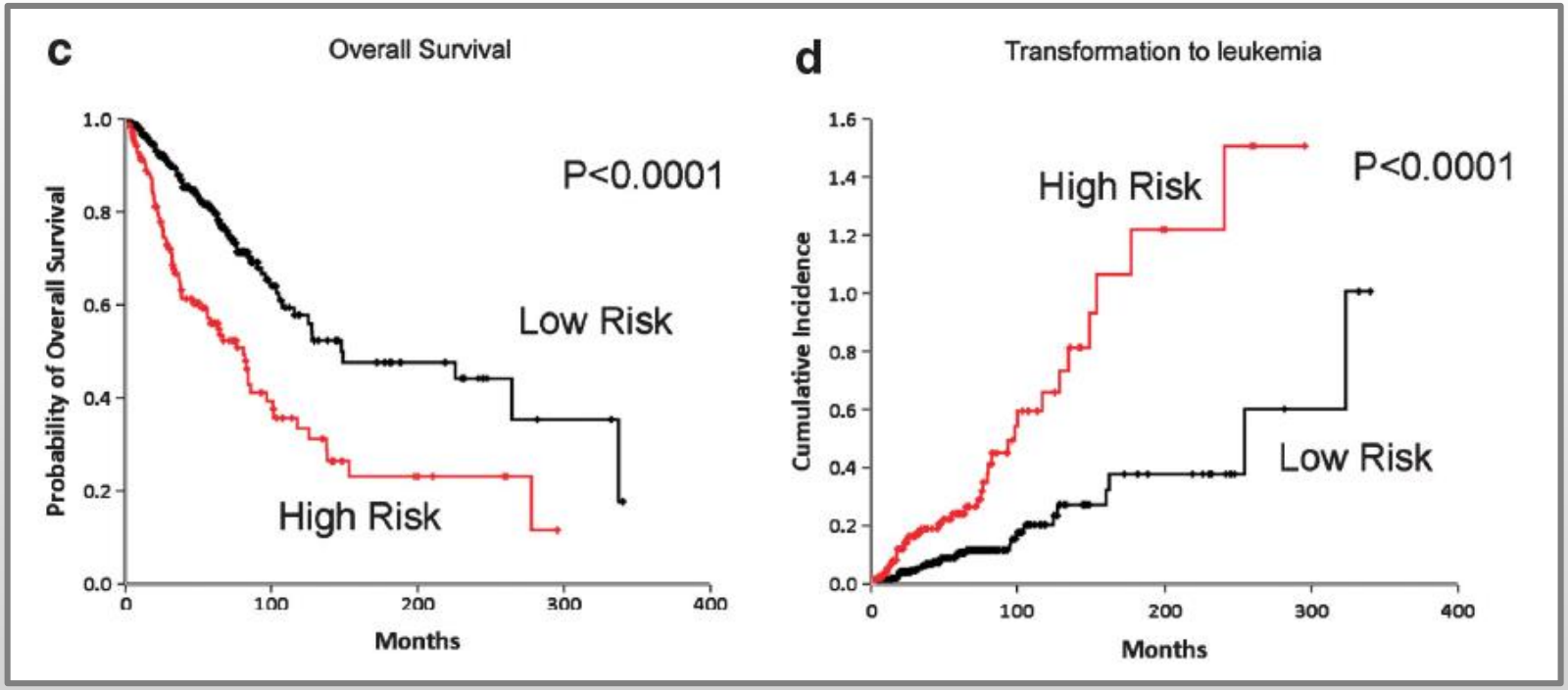
high-molecular risk marker

High Molecular Risk Markers in PMF

Vannucchi AM, et al. Leukemia. 2013;27(9):1861-9

ASXL1, SRSF2, EZH2, IDH1 and *IDH2*

- High risk: mutated in at least one of the five HMR genes
- Low risk: no HMR mutation
- Multivariable analysis for survival: independent prognostic value of the mutationally risk groups

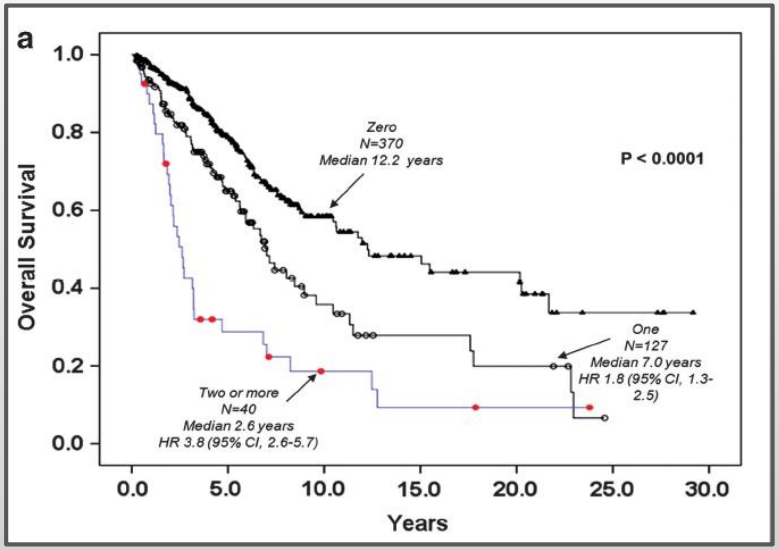


High Molecular Risk Markers in PMF
- The „number“ is prognostically relevant -

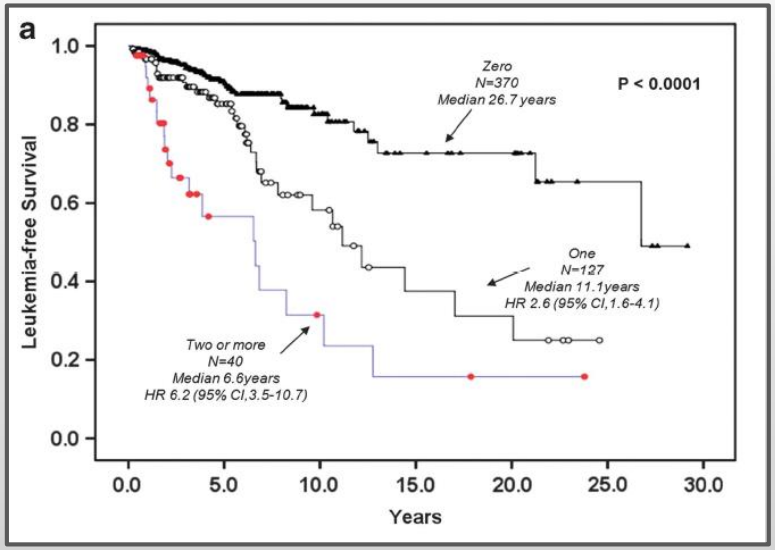
Guglielmelli P et al. Leukemia. 2014; 28:1804–1810

- A total of 797 PMF patients were recruited from Europe (n=537) and the Mayo Clinic (n=260)
- 167 (31%) patients of the Europe cohort had HMR; 127 (23.6%) had one and 40 (7.4%) had two or more HMR mutations
- The presence of ≥ 2 HMR mutations predicted the worst survival (median 2.6 years) and shortened leukemia-free survival

Overall Survival



Transformation to Leukemia

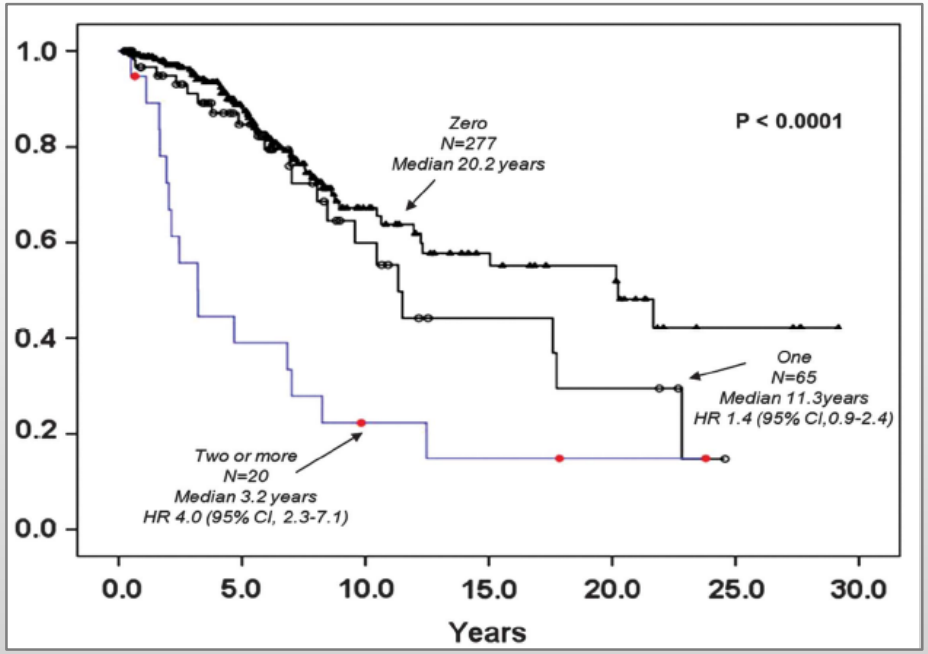


High Molecular Risk Markers in PMF
- The „number“ is prognostically relevant -

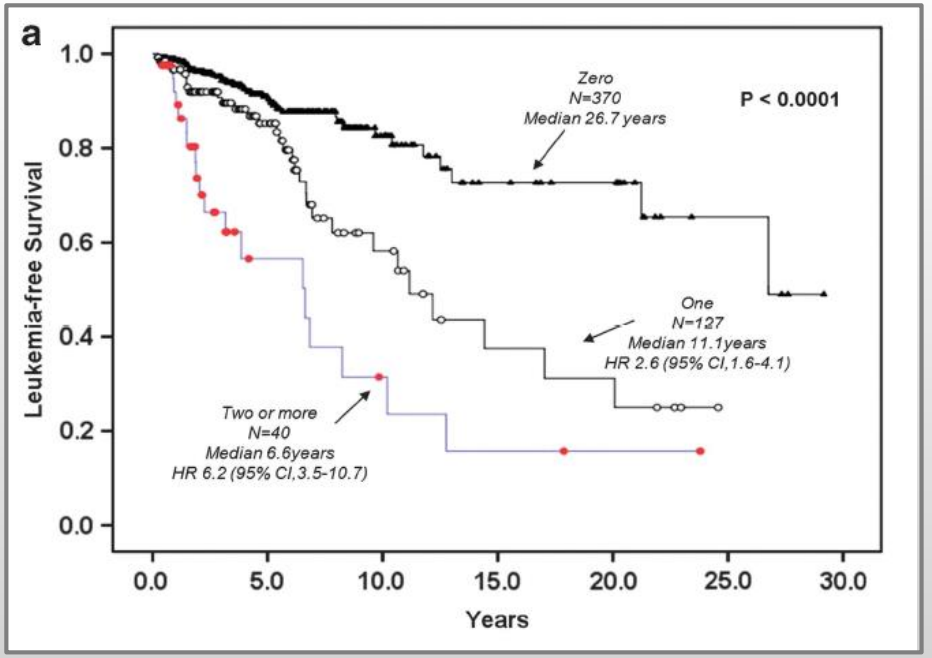
Guglielmelli P et al. Leukemia. 2014; 28:1804–1810

Low plus intermediate-1 risk IPSS categories

Overall Survival



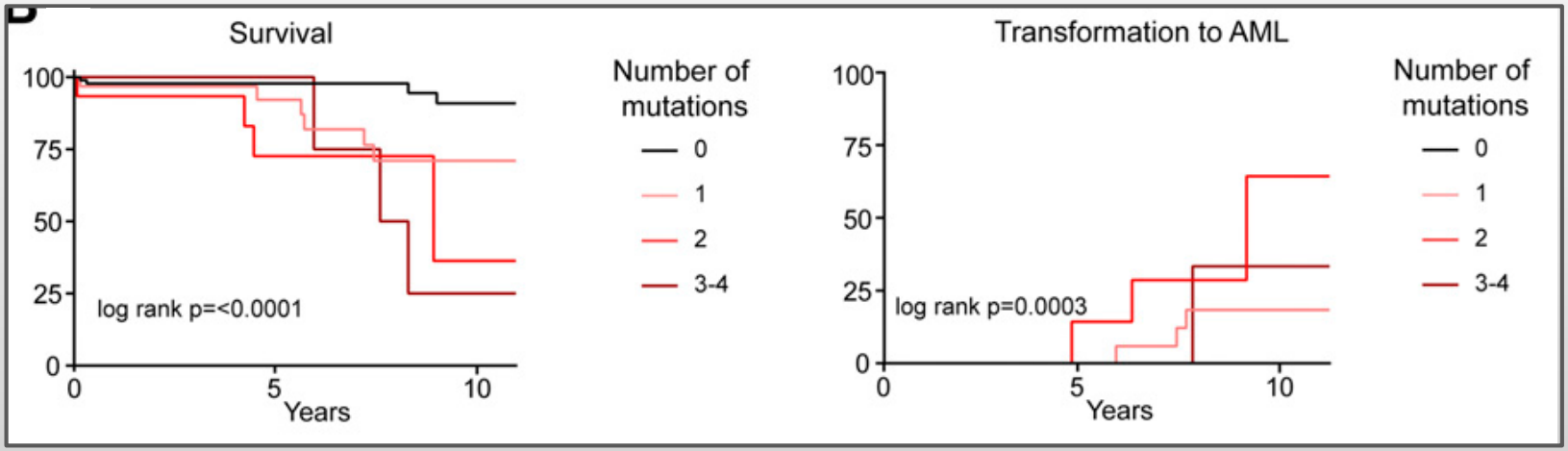
Leukemia-free survival



Prognostic Impact of Somatic Mutations in MPN

Lundberg P et al. Blood. 2014; 123(14):2220-8

- Targeted NGS approach of 104 genes in 197 MPN [PV n=94; ET n=69; PMF n=34] pts to evaluate clinical outcome and clonal evolution
- Somatic mutations in 90% of the pts; 37% other than *JAK2* V617F and *CALR*
- NGS in serial samples: no significant change in number of mutations during a long follow-up > low mutation rate
- Presence of ≥2 mutations: significantly reduced OS and increased risk of transformation to leukemia



Targeted Next-Generation Sequencing in PMF:
Number of non-driver mutations is prognostically relevant

- Targeted NGS approach of 27 genes in 180 PMF pts to identify additional mutations and to evaluate the prognostic value of number of mutations
- *TET2, DNMT3A, IDH1, IDH2, ASXL1, EZH2, SUZ12, SRSF2, SF3B1, ZRSR2, U2AF1, PTPN11, Tp53, SH2B3, RUNX1, CBL, NRAS, JAK2, CSF3R, FLT3, KIT, CALR, MPL, NPM1, CEBPA, IKZF,* and *SETBP1*
- Mutations other than *JAK2, CALR* or *MPL* in 150 (83%) of the pts
- DIPSS-plus high/intermediate-2 risk: higher number of mutations (*P*=.0004), higher mutational frequencies for *ASXL1* (*P*=0.02), *SRSF2* (*P*=.004), and *CBL* (P=0.02)

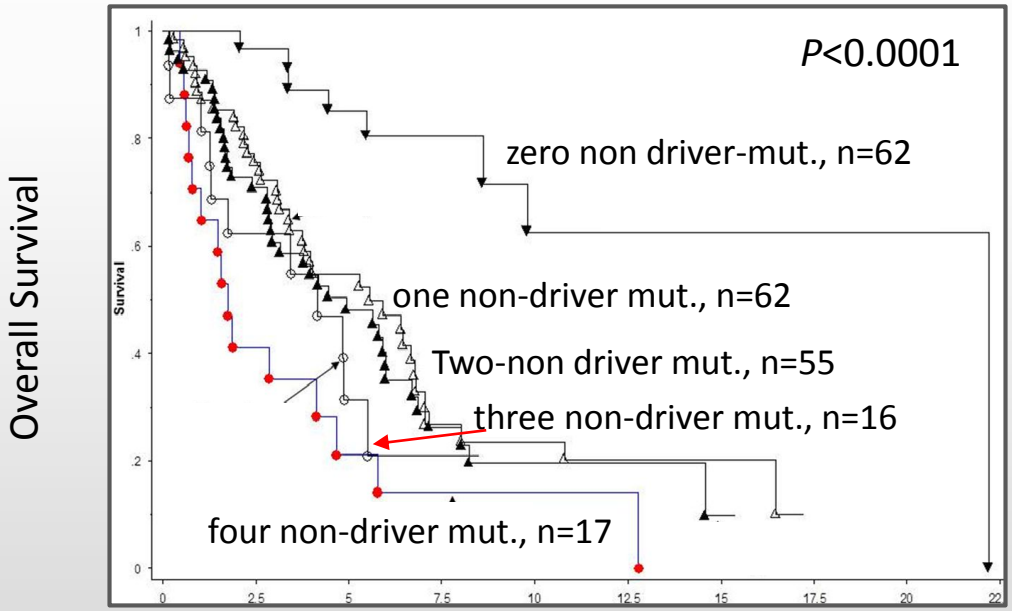
Mutation frequencies

ASXL1	36%
TET2	18%
SRSF2	17%
U2AF1	17%
ZRSR2	11%
SF3B1	10%
DNMT3A	9%
TP53	7%
CBL	5%
RUNX1	3%

Tefferi A, et al. Blood 2015 126:#350, ASH

Targeted Next-Generation Sequencing in PMF:
Number of non-driver mutations is prognostically relevant

- **Multivariate analysis:** 1-3 mutations, ≥4 mutations, *RUNX1*, *CBL*, *ASXL1* and *SRSF2* mutations were independently associated with shortened survival



Tefferi A, et al. Blood 2015 126:#350, ASH

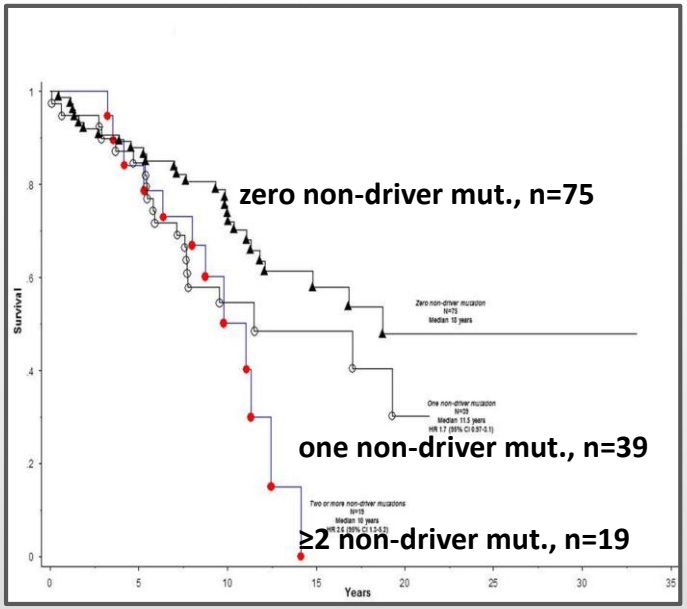
Targeted Next-Generation Sequencing in PV and ET:
Number of non-driver mutations is prognostically relevant

- Same targeted NGS approach of 27 genes in PV (n=133) and ET (n=181) pts to identify additional mutations and to evaluate the prognostic value of number of mutations

Polycythemia Vera:

- Mutations other than *JAK2, CALR* or *MPL* in 58 (44%) of the pts (18% *TET2*, 11% *ASXL1*, 5% *SH2B3*, 3% *SF3B1*)
- Number of mutations was significantly associated with OS and MF-free survival

Survial of 133 PV pts. stratified by number of non-driver mutations

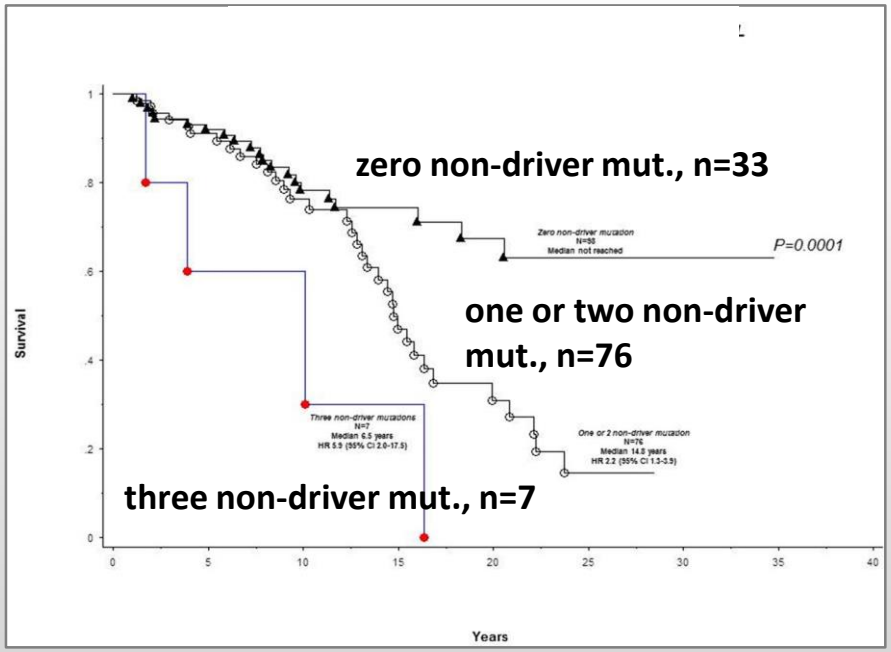


Tefferi A, et al. Blood 2015 126:#354, ASH

Targeted Next-Generation Sequencing in PV and ET:
Number of Non-driver Mutations is prognostically relevant

Essential Thrombocythemia:

- Mutations other than *JAK2, CALR* or *MPL* in 83 (46%) of the pts (13% *TET2*, 11% *ASXL1*, 6% *DNMT3A,* 5% *SF3B1*)
- Number of mutations was significantly associated with OS but not with MFS or LFS



Survial of 181 ET pts stratified by number of non-driver mutations

Tefferi A, et al. Blood 2015 126:#354, ASH

Molecular Markers for Disease Progression

- Mutations in genes involved in transcription or DNA damage are associated with leukemic transformation:
  - *RUNX1* (acquired at the time of transformation) > not predictive
  - *TP53* > hemizygous / homozygous >> rapid progression

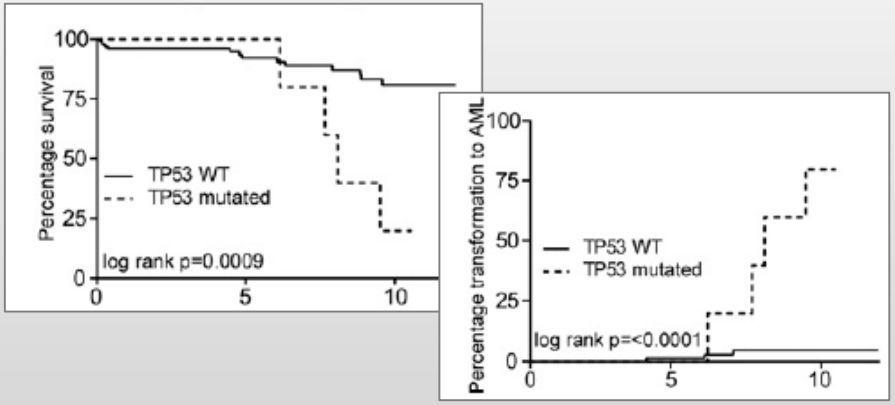


Table 4. Intrinsic risk factors for disease transformation in PV and ET

Transformation	*Clinical risk factors*	*Genetic risk factors*
Post-PV MF	Age Leukocytosis Disease duration Reticulin fibrosis Splenomegaly	*JAK2V617F* allele burden
Post-PV Leukemia	Age Leukocytosis Reticulin fibrosis Splenomegaly	Abnormal karyotype *TP53* *RUNX1*
Post-ET MF	Age Leukocytosis Anemia Reticulin fibrosis	Absent *JAK2V617F* mutation *ASXL1*
Post-ET leukemia	Age Leukocytosis Anemia Reticulin fibrosis Thrombosis Platelets $\geqslant 1000 \times 10^9$/l	*TP53* *RUNX1*

Abbreviations: ET, essential thrombocythemia; MF, myelofibrosis; PV, polycythemia vera.

Cerquozzi S and Tefferi. Blood Cancer Journal 2015; Lundberg P et al., Blood 2014

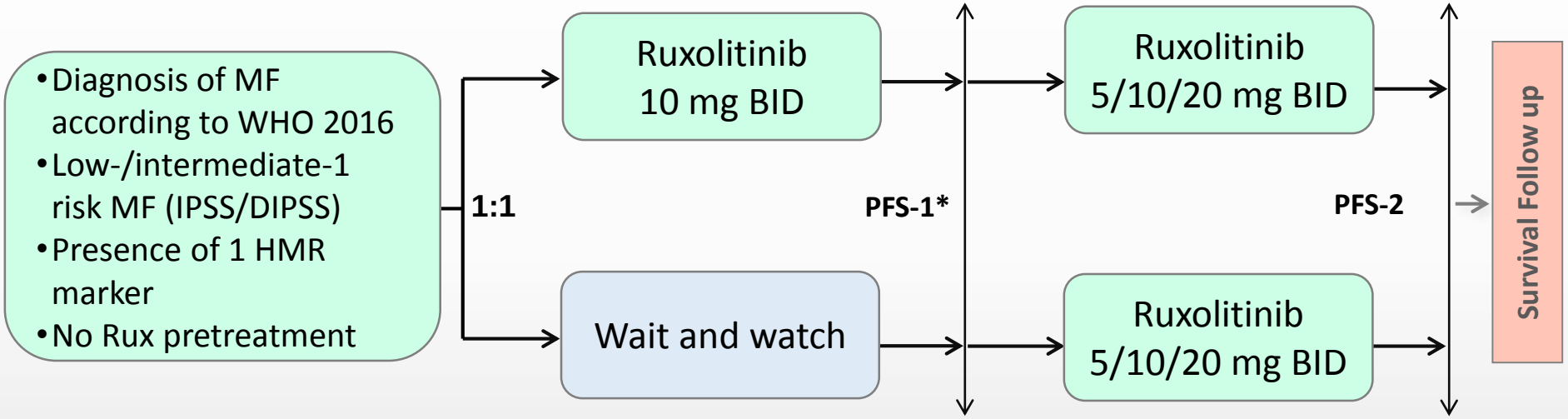
Summary and Perspective

- In PMF 5 „high molecular risk“ (HMR) markers have been identified that are significantly associated with survival and transformation risk to leukemia : *ASXL1, SRSF2, EZH2, IDH1* and *IDH2*
- In PMF HMR markers should be considered for implementation into the current risk-stratification sytems and they should be considered for treatment decisions, in particular within the context of alloSCT
- In MF low- and intermediate-1 risk (IPSS/DIPSS) patients with HMR mutations further studies are needed to evaluate if early treatment delays disease progression
- In ET and PV the number of non-driver mutations might be of prognostic relevance; however the prognostic and predictive value has to be determined in prospective clinical studies
- *TP53* and *RUNX1* mutations are clearly associated with disease progression and thus can be used for treatment decisions (alloSCT)

GSG-MPN03-17 Study Proposal

randomized, open-label, multicenter, Phase III study investigating the efficacy and safety of ruxolitinib versus wait and watch in low and intermediate-1 risk (IPSS/DIPSS) myelofibrosis patients harboring high molecular risk marker mutations

- Evaluation of safety and efficacy of Ruxolitinib in patients with low-and intermediate-risk 1 (IPSS/DIPSS) MF harbouring HMR mutations



Population

- Low-/intermediate-1 risk MF (IPSS/DIPSS)
- ANC ≥ 1.000/µl
- Platelet count > 75.000/µl
- MPN-SAF TSS ≤ 50
- Not considered for alloSCT

Primary endpoint

*Progression free survival (PFS-1) from date of randomization until disease progression (modified criteria)

Secondary endpoint

- PFS-2, safety and tolerance, symptoms, QOL, OS

* Disease progression >> patients will be treated in PFS-2 with RUX 5/15/20 mg BID

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https://www.cto-im3.de/gsgmpn/

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