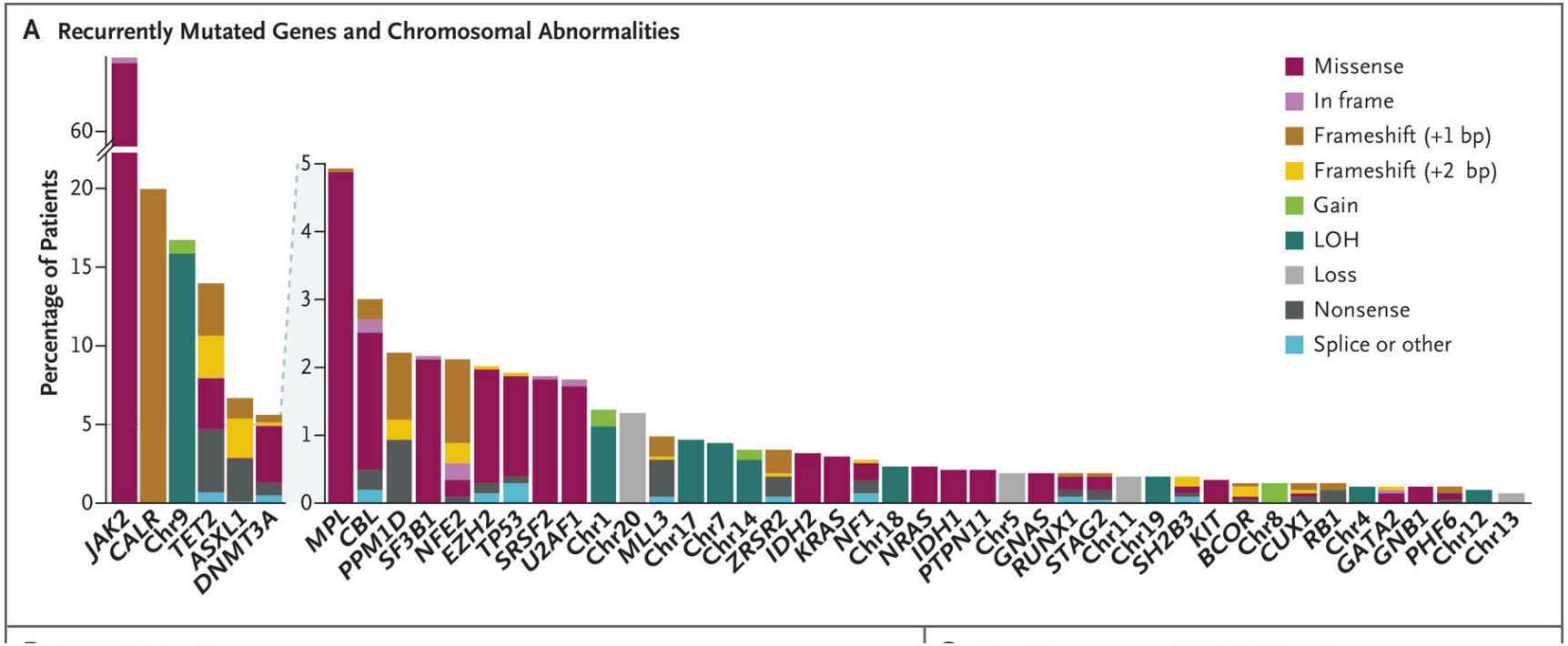
Drivers molecolari nelle MPNs Ph1 negative

Giovanni Barosi
Fondazione IRCCS Policlinico S. Matteo
Pavia

Nuovi target terapeutici in ematologia
San Giovanni Rotondo, 8 Novembre 2018

Recurrently mutated genes and chromosomal abnormalities in myeloprolifertive neoplasms (Grinfeld et al, NEJM, 2018)



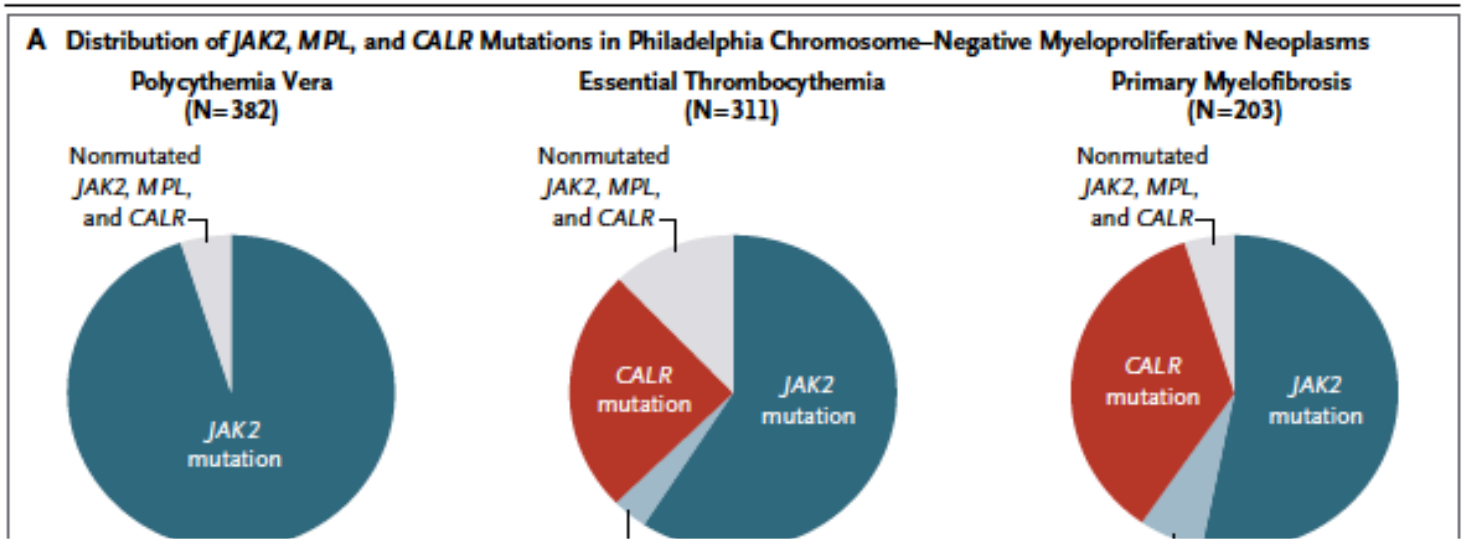
- Targeted sequencing for the full coding sequence of 69 genes and genomewide copy-number information in 2035 patients with MPNs
- A total of 33 genes had driver mutations in at least 5 patients
- Mutations in JAK2, MPN, and CALR were the sole abnormalities in 45% of the patients.

Le mutazioni che si associano alle MPNs sono complesse

- Interessano geni che hanno funzioni diverse
- Alcune mutazioni sono specifiche delle MPNs Ph1-negative classiche (PV,ET,MF), altre sono comuni a tutte le neoplasie mieloidi
- Alcune mutazioni sono presenti all'insorgenza della malattia, altre compaiono durate il decorso e la progressione della malattia.

Le mutazioni «driver» delle MPNs

- ❑ Comuni
- ❑ Discreta associazione con il fenotipo della malattia
- ❑ Il metodo di rilevazione è quello della PCR
- ❑ Metodi calibrati e standardizzati
- ❑ Possono essere ricercate in modo sequenziale perché sono quasi perfettamente mutuamente esclusive
- ❑ Diffuse nella pratica diagnostica
- ❑ Rimborsabilità illimitata



Le mutazioni «accessorie» delle MPN

- Elevata frequenza nella mielofibrosi (fino a 20%)
- Attualmente il loro utilizzo è al limite fra la ricerca e la pratica
- Non possono essere cercate in modo individuale e sequenziale perché non sono mutuamente esclusive
- La tecnica di rilevazione è il next generation sequencing (NGS) con piattaforme non ancora definite
- Rimborsabilità limitata

Genes assessed by targeted sequencing.

ASXL1	*	EP300		KSR2		PTEN	
ASXL3		ETV6		MBD1		PTPN11	*
ATRX		EZH2	*	MLL		RAD21	
BCOR	*	FAM47C		MLL2		RAD51	
BRAF		FARS2		MLL3	*	RB1	*
C2ORF39		FLT3		MLL5		RUNX1	*
CACNA2D3		GATA2	*	MPL	*	SARDH	
CALR	*	GNAS	*	NCL		SETBP1	
CBL	*	GNB1	*	NF1	*	SF3B1	*
CDKN2A		GRIN2B		NFE2	*	SH2B3	*
CEBPA		IDH1	*	NOTCH2		SRSF2	*
CHEK2		IDH2	*	NPM1		STAG2	*
CREBBP		IRF1		NRAS	*	TET2	*
CUX1	*	JAK2	*	PCDH15		TP53	*
DNMT3A	*	KDM6A		PHF6	*	U2AF1	*
DNMT3B		KIT	*	PPM1D	*	WT1	
ELF1		KRAS	*	PRKACB		WWOX	
						ZRSR2	*

Epigenetic regulation	*RAS pathway member/ regulator*	*mRNA splicing machinery*	
Cell cycle/ apoptosis	*Surface receptor signalling*	*Transcription regulation*	* denotes >4 high confidence mutations in cohort

Rilevanza delle mutazioni somatiche nella diagnosi delle MPNs

Revision of WHO diagnostic criteria for myeloproliferative neoplasms

	Polycythemia vera (PV)	Essential thrombocythemia (ET)	Primary myelofibrosis (PMF)
Major criteria			
1	Hemoglobin >16.5 g/dl (men) >16 g/dl (women) or hematocrit >49% (men) >48% (women)	Platelet count ≥450 x 10^9/l	Megakaryocyte proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis, or
2	BM trilineage myeloproliferation with pleomorphic megakaryocytes	Megakaryocyte proliferation with large and mature morphology	Not meeting WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm
3	**Presence of *JAK2* mutation**	Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm	**Presence of *JAK2*, *CALR* or *MPL* mutation**
4		**Presence of *JAK2*, *CALR* or *MPL* mutation**	
Minor criteria			
1	Subnormal serum erythropoietin level	Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis	**Presence of a clonal marker (e.g. ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2, SF3B1)** or absence of evidence for reactive bone marrow fibrosis
2			Presence of anemia or palpable splenomegaly
3			Presence of leukoerythroblastosis, or increased lactate dehydrogenase

Tefferi, Thiele, Vannucchi & Barbui. *Leukemia.* 2014; 28:1407-13

ELN 2018 revised guidelines (Barbui et al, Leukemia 2018)

- ✓ *Peripheral blood or BM screening for driver mutations, i.e. JAK2V617F, CALR and MPL, is recommended in any patient who may have a Ph-neg MPN.*
- ✓ *Search for complementary clonal markers, such as ASXL1, EZH2, IDH1/IDH2, and SRSF2 is recommended in patients who tested negative for the three driver mutations and have BM features and a clinical phenotype consistent with MF.*
- ✓ *There was no consensus concerning the search of additional clonal markers such as TP53, TET2, DNMT3A and CBL in MF, and no consensus concerning the need for searching for complementary clonal markers in ET. Thus, this decision should follow individual institutional preference.*

Criticità nella classificazione diagnostica WHO delle MPNs

- Il margine di discriminazione fa ET e PV è incerto;
- La distinzione fra mielofibrosi prefibrotica e trombocitemia essenziale viene attualmente fatta con un errore elevato
- Vi sono forme transizionali fra PV e mielofibrosi che sono classificate in modo diverso in diverse istituzioni.
- Alcune serie hanno un esageratamente alto numero di casi non classificati
- La categorizzazione di malattie mielodisplastiche/mieloproliferative è in alcuni casi ingiustificata

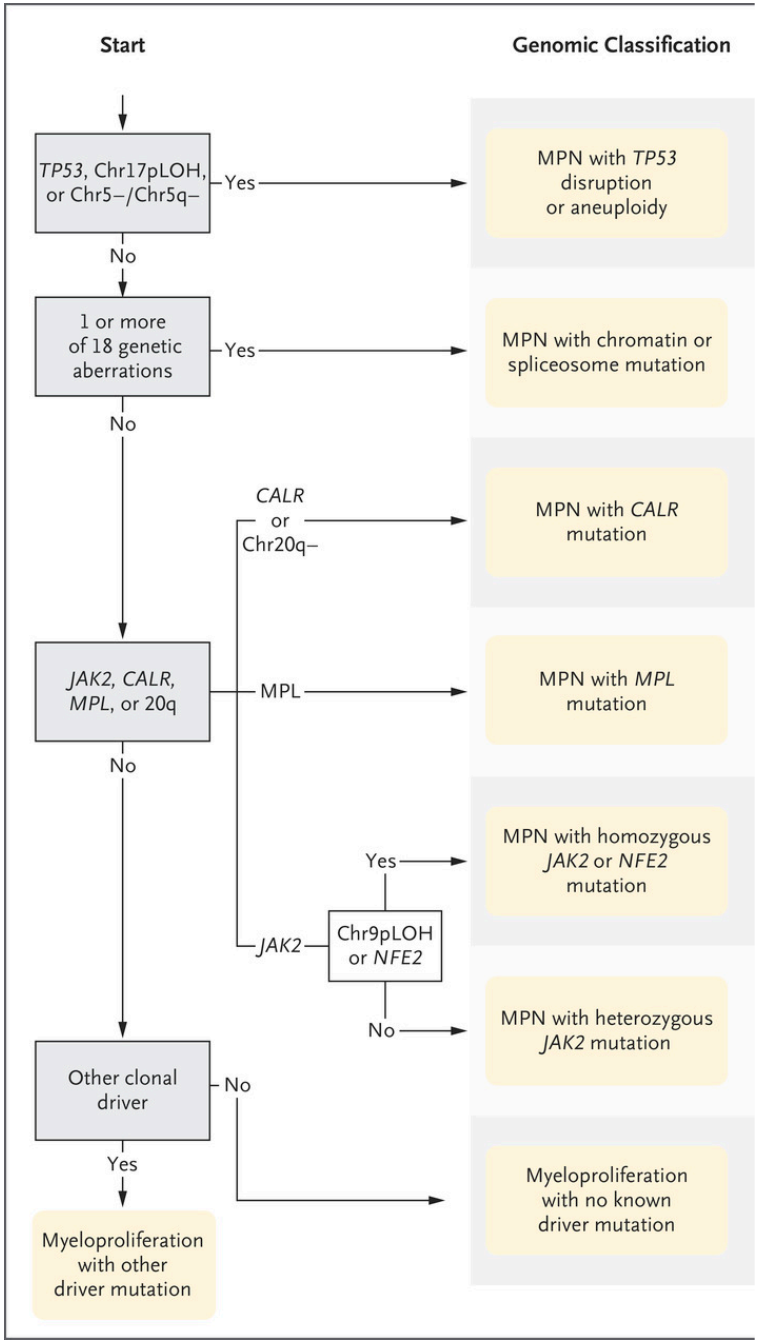
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Classification and Personalized Prognosis in Myeloproliferative Neoplasms

J. Grinfeld, J. Nangalia, E.J. Baxter, D.C. Wedge, N. Angelopoulos, R. Cantrill, A.L. Godfrey, E. Papaemmanuil, G. Gundem, C. MacLean, J. Cook, L. O'Neil, S. O'Meara, J.W. Teague, A.P. Butler, C.E. Massie, N. Williams, F.L. Nice, C.L. Andersen, H.C. Hasselbalch, P. Guglielmelli, M.F. McMullin, A.M. Vannucchi, C.N. Harrison, M. Gerstung, A.R. Green, and P.J. Campbell

NEJM 2018,379:1416



Grinfeld et al, NEJM, 2018

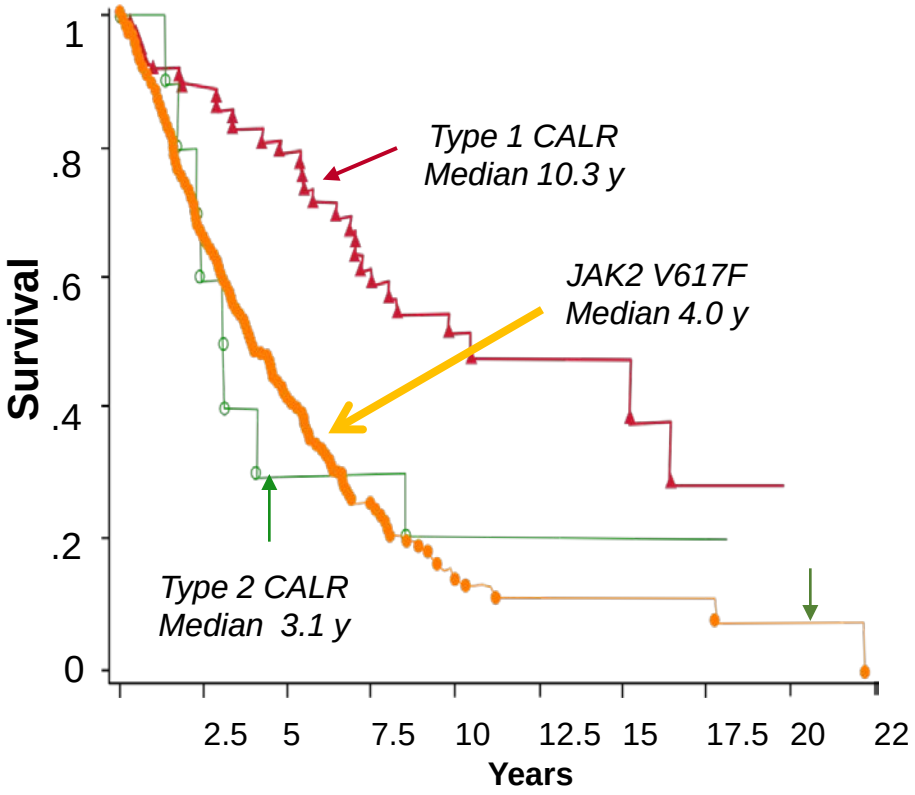
Rilevanza delle mutazioni somatiche nella prognosi delle MPNs

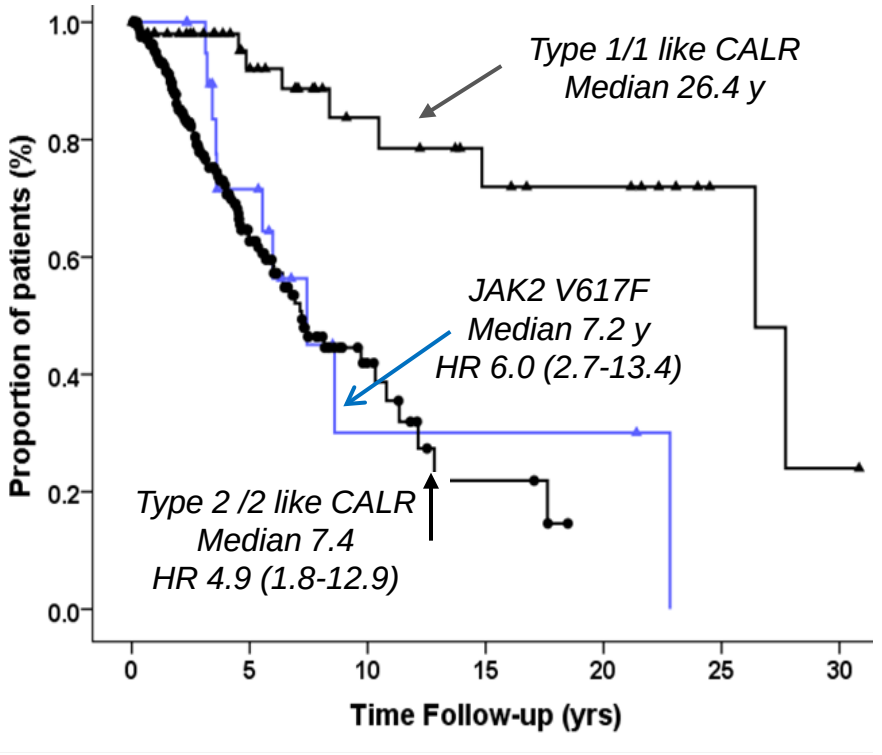
Clinical Scores for Risk Stratification in PMF

Variable	IPSS	DIPSS	DIPSS-plus
Age >65 y	√	√	**If DIPSS:**
Constitutional symptoms	√	√	
Hemoglobin <10 g/dL	√	√	**Low= 0**
Leukocyte count >25x10^9/L	√	√	**Int-1= 1**
Circulating blasts ≥ 1%	√	√	**Int-2=2**
			High= 3
Platelet count <100x10^9/L			√
RBC transfusion need			√
Unfavorable karyotype +8,-7/7q-,i(17q),inv(3), -5/5q-,12p-, 11q23 rearr.			√

Cervantes F, et al. *Blood.* 2009;113:2895-901
Passamonti F, et al. *Blood.* 2010; 115:1703-8
Gangat N, et al. *J Clin Oncol.* 2011; 29:392-7

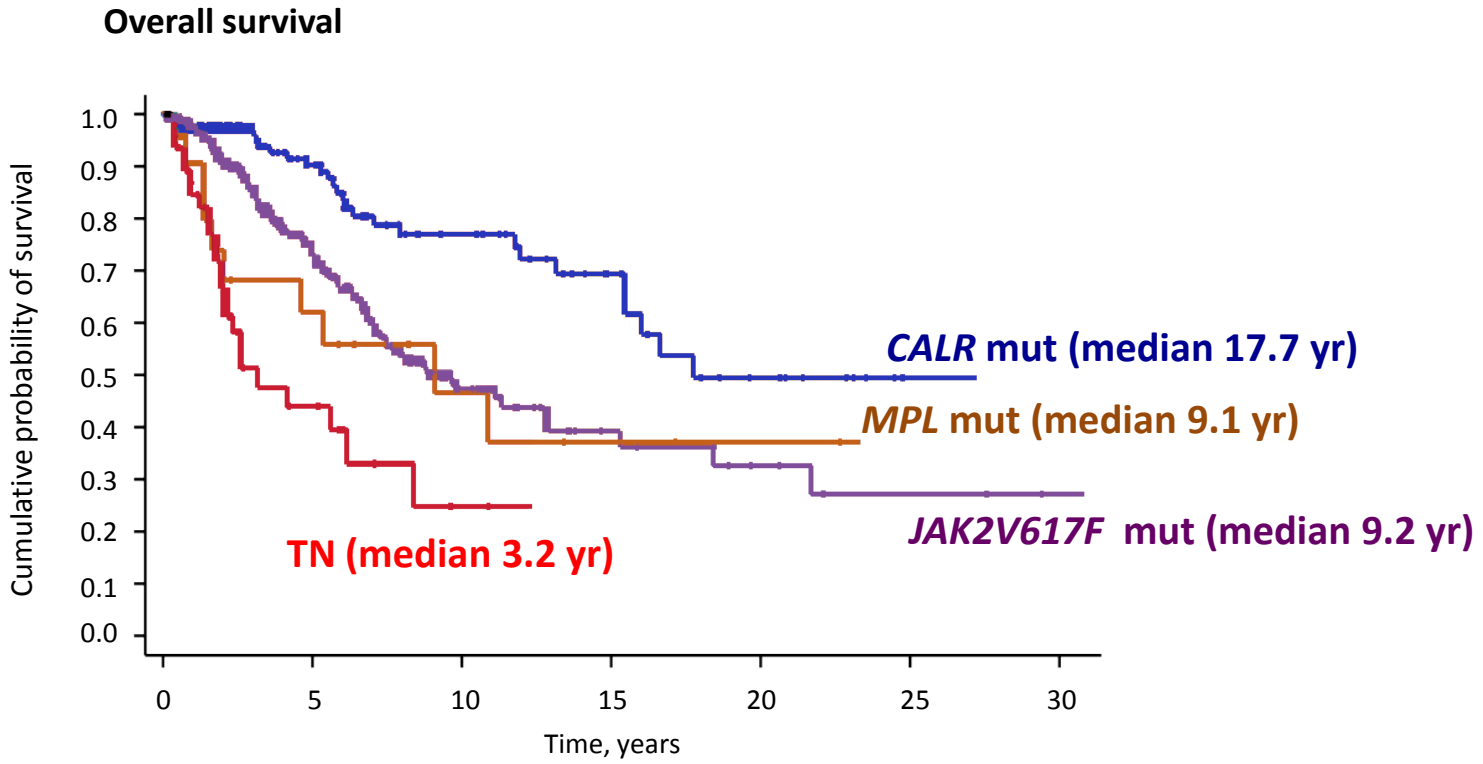
CALR Type 1/1-like *vs* Type 2/2-like Mutations in PMF Make a Difference

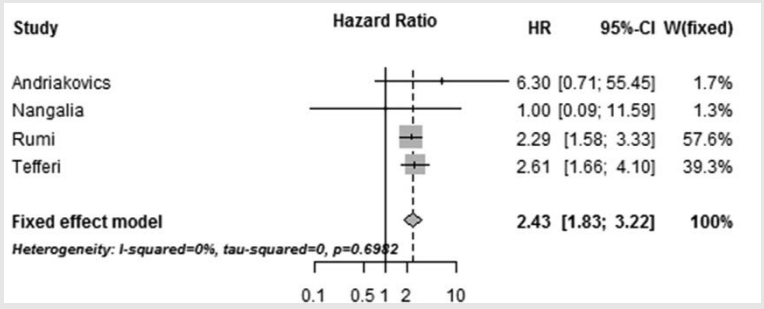




Tefferi A, et al. Leukemia. 2014; 28:1568-70;
Guglielmelli P et al, BCJ, 2015, Online

Phenotype Driver Mutations Have a Strong Prognostic Impact in PMF





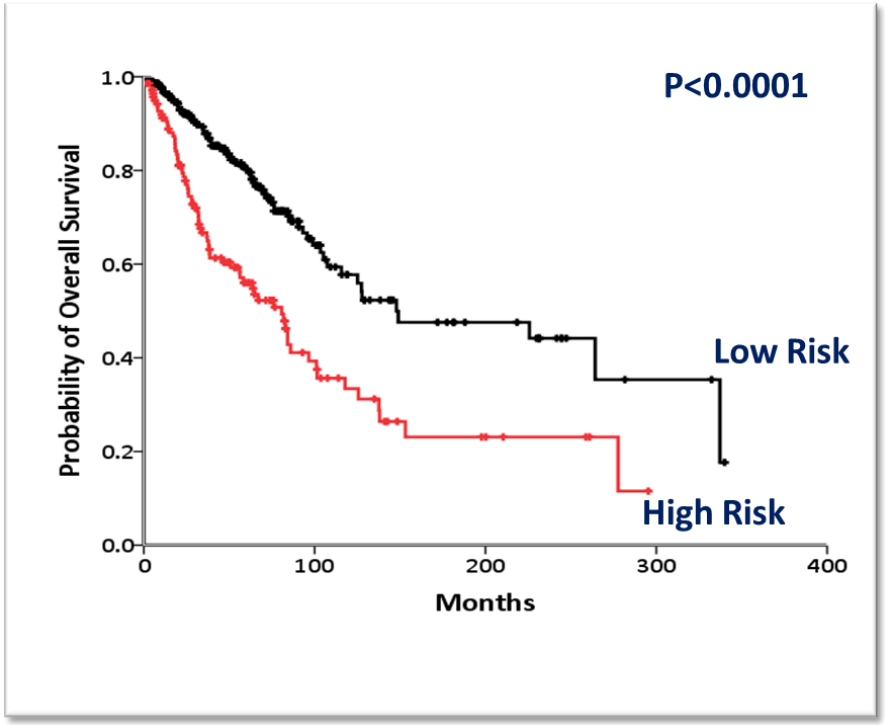
JAK2 mutated patients had shorter overall survival compared with those CALR^{+} (Meta-analysis combined hazard ratio, 2.43; 95% CI, 1.83-3.22; P= < .001).

Rumi E, et al. Blood. 2014 Aug 14;124(7):1062-9; Kourie HR et al, Clin Lymphoma Myeloma Leuk. 2016 Feb 6

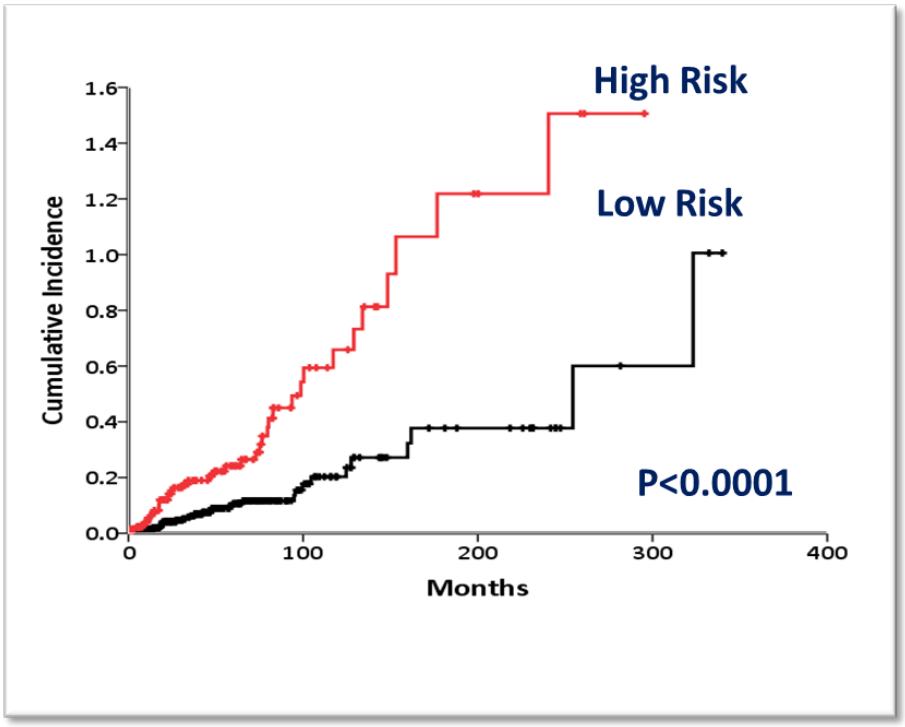
High Molecular Risk Prognostic Category

harboring ≥1 mutation in any one of ***ASXL1, EZH2, SRSF2, IDH1/2***

Overall Survival



Blast Transformation



- A HMR status is associated with reduced OS and increased risk of blast transformation in PMF patients independent of IPSS/DIPPS-plus

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

I modelli prognostici più recenti della mielofibrosi primaria

MIPSS70 (mutation enhanced international prognostic scoring system for transplant-age patients): include le variabili cliniche di rischio in aggiunta alle mutazioni.

MIPSS70+ include la informazione citogenetica.

MIPSS70+ versione 2.0: aggiunge l'uso di U2AF1Q157 come mutazione ad alto rischio e ha messo soglie di emoglobina diverse.

GIPSS (genetically inspired prognostic system for primary myelofibrosis): è basato solo sulla genetica e dipende solo dal cariotipo e dalle mutazioni

MIPSS70 (mutation-enhanced international prognostic system for transplant-age patients (age <=70 years)

Genetic variables:

One HMR mutation	(1 point)
>= 2 HMR mutations	(2 points)
Type 1/like CALR absent	(1 point)

Clinical Variables

Hemoglobin < 10 g/dl	(1 point)
Leukocytes > 25 x 10^9/L	(2 points)
Platelet < 100 x10^9/L	(2 points)
Circulating blasts >=2	(1 point)
Constitutional symptoms	(1 point)
Bone marrow fibrosis grade >=2	(1 point)

HMR mutations: ASXL1, SRSF2, EZH2, IDH1, IDH2

Guglielmelli et al. J Clin Oncol 2018;36:310

ELN 2018 revised guidelines (Barbui et al, Leukemia 2018)

- ✓ *In MF, the IPSS, based on hematological and clinical variables, is the recommended prognostic system and should be scored in all patients at diagnosis.*
- ✓ *There is increasing evidence that integration of IPSS with additional genetic information, i.e. cytogenetics and molecular parameters, allows a more detailed individualized prognostic classification.*
- ✓ *For this reason, cytogenetic studies, classification of CALR mutations into type 1/like and type 2/like, and screening for non-driver additional mutations including at least ASXL1 and SRSF2, has become current practice in research centers.*
- ✓ *The Panel agreed that a complete genetic assessment should be encouraged in all patients for the prognostic assessment at diagnosis. However, the Panel also claimed that failure to perform a full genetic characterization at the time of diagnosis is acceptable in clinical practice.*

Rilevanza delle mutazioni somatiche nella terapia delle MPNs

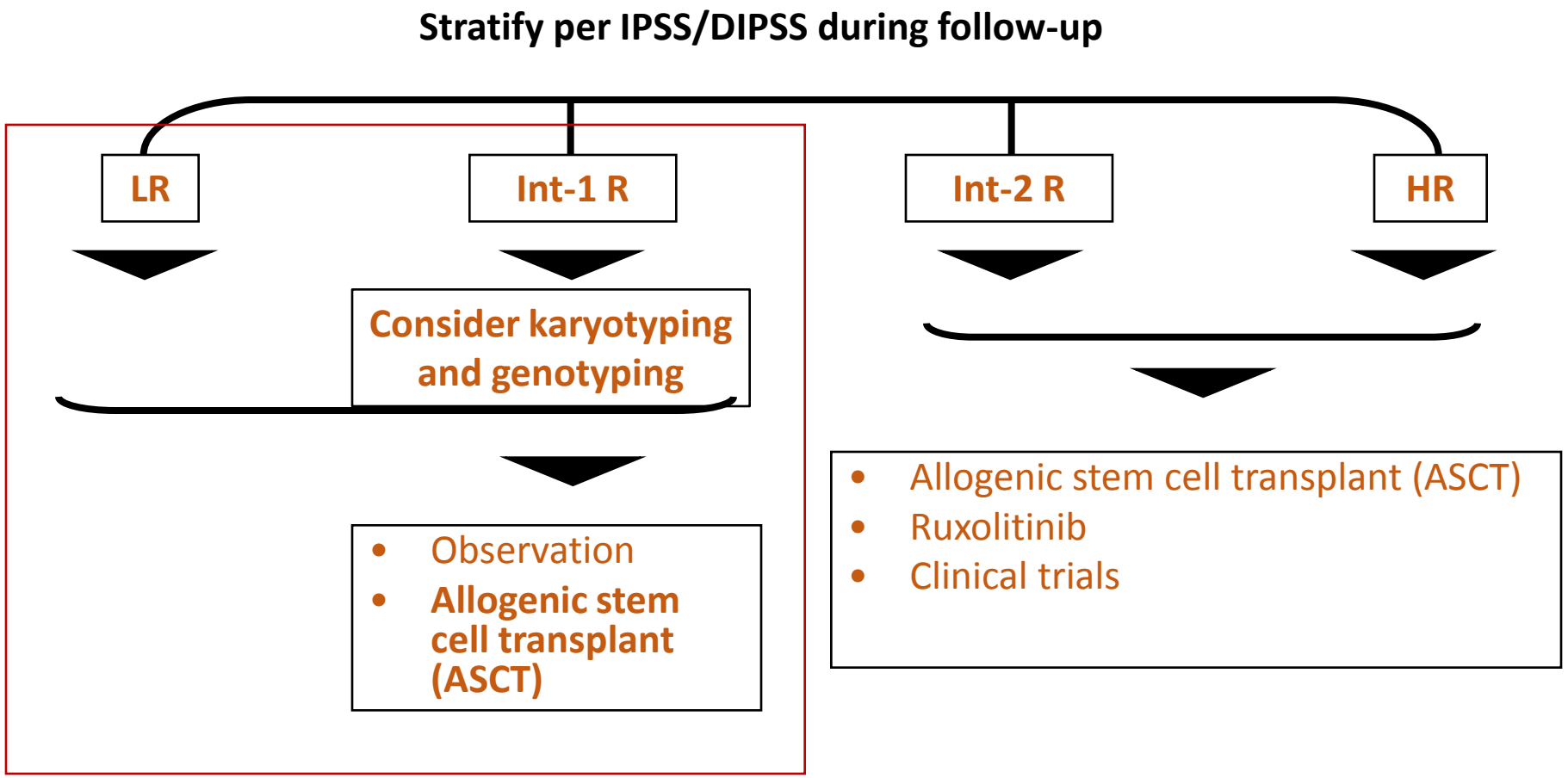
Precision medicine – a new paradigm

Prevention and treatment strategies that take individual variabilty into account

Technological facilities

- Development of large scale biological data (human genome sequence)
- Powerful methods for characterizing patients (proteomics, metabolomics, genomics..)
- Computational tools for analyzing large sets of data

Personalized approach to MF: HSCT for DIPSS INT-1 disease



ELN 2018 revised guidelines (Barbui et al, Leukemia 2018)

- ✓ *We recommend considering allogeneic stem cell transplant for all transplant-eligible patients with IPSS/DIPSS/DIPSS-plus high or intermediate-2 risk.*
- ✓ *The Panel also recommended consideration of allogeneic stem cell transplantation for transplant eligible patients with IPSS/DIPSS/DIPSS Plus intermediate-1 risk score who present with either refractory, transfusion-dependent anemia, ~~or~~ a percentage of blasts in peripheral blood >2% in at least two repeated manual measurements, adverse cytogenetics, or high-risk mutations,*
- ✓ *In this situation, the transplant procedure should be performed in a controlled setting (registries, clinical trial).*

Prognostic markers validation

Analytical validity: test accuracy (reproducibility)

Clinical validity: test result correlates with a clinical endpoint (response to therapy, survival) – Usually established in a retrospective study.

Clinical utility: the use of the prognostic marker results in improved outcome for patients - Usually requires conduction of a prospective clinical trial.