ASXL1/TET2 genotype-based risk stratification outperforms ASXL1 mutational impact and is independent of mutant variant allele fractions in chronic myelomonocytic leukemia

Clifford M. Csizmar,[1] Mark Gurney,[1] Rashmi Kanagal-Shamanna,[2] Kelly Chien,[3] Danielle Hammond,[3] Terra L. Lasho,[1] Christy M. Finke,[1] Christopher Dean,[1] Anuya Natu,[1] Abhishek A. Mangaonkar,[1] Aref Al-Kali,[1] Naseema Gangat,[1] Ayalew Tefferi,[1] Hassan Alkhateeb,[1] Guillermo Garcia-Manero,[3] Rami S. Komrokji,[4] Najla A. Ali,[4] Eric Padron,[4#] Guillermo Montalban-Bravo[3#] and Mrinal M. Patnaik[1#]

[1]Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN; [2]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX; [3]Division of Cancer Medicine, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX and [4]Department of Malignant Hematology, H. Lee Moffitt Cancer Center, Tampa, FL, USA

[#]EP, GM-B, and MMP contributed equally as senior authors.

Correspondence:
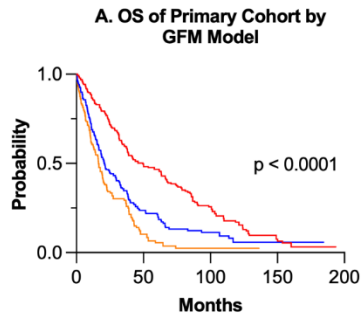M.M. PATANIK – patnaik.mrinal@mayo.edu

Supplemental Information for:

ASXL1/TET2 genotype-based risk stratification outperforms *ASXL1* mutational impact and is independent of mutant variant allele fractions in chronic myelomonocytic leukemia
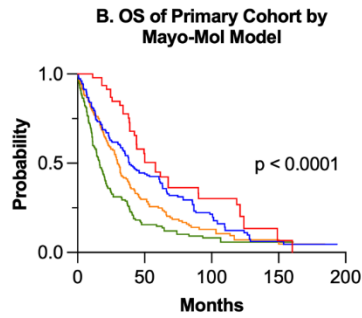
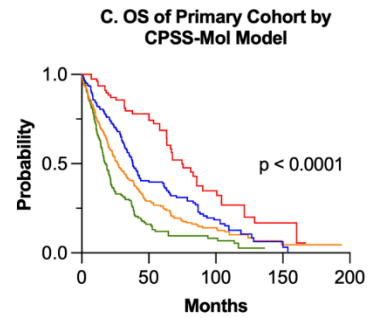Csizmar CM, *et al.*

Table of Contents

A. OS of Primary Cohort by GFM Model

p < 0.0001

- Low, n = 275, mOS 50 (39 - 63) mo.
- Inter, n = 243, mOS 21 (18 - 29) mo.
- High, n = 148, mOS 16 (14 - 20) mo.

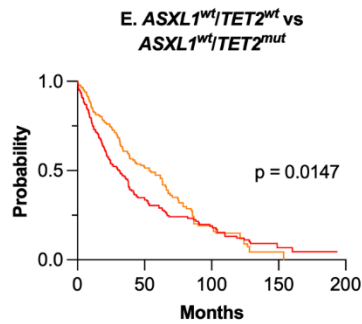**B. OS of Primary Cohort by Mayo-Mol Model**

p < 0.0001

- Low, n = 55, mOS 58 (44 - 90) mo.
- Int-1, n = 192, mOS 39 (34 - 61) mo.
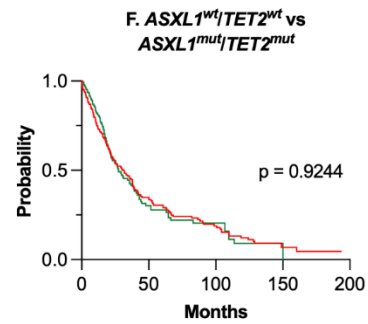- Int-2, n = 192, mOS 30 (27 - 34) mo.
- High, n = 227, mOS 16 (14 - 20) mo.

C. OS of Primary Cohort by CPSS-Mol Model

p < 0.0001

- Low, n = 83, mOS 75 (63 - 101) mo.
- Int-1, n = 206, mOS 38 (34 - 44) mo.
- Int-2, n = 337, mOS 27 (23 - 33) mo.
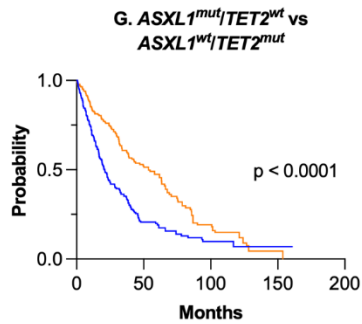- High, n = 224, mOS 18 (15 - 21) mo.
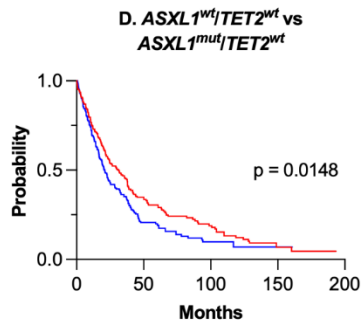
D. $ASXL1^{wt}/TET2^{wt}$ vs $ASXL1^{mut}/TET2^{wt}$

p = 0.0148

- ASXL1 wt TET2 wt
- ASXL1 mut TET2 wt

E. $ASXL1^{wt}/TET2^{wt}$ vs $ASXL1^{wt}/TET2^{mut}$

p = 0.0147

- ASXL1 wt TET2 wt
- ASXL1 wt TET2 mut

F. $ASXL1^{wt}/TET2^{wt}$ vs $ASXL1^{mut}/TET2^{mut}$

p = 0.9244

- ASXL1 wt TET2 wt
- ASXL1 mut TET2 mut

G. $ASXL1^{mut}/TET2^{wt}$ vs $ASXL1^{wt}/TET2^{mut}$

p < 0.0001

- ASXL1 mut TET2 wt
- ASXL1 wt TET2 mut

H. $ASXL1^{mut}/TET2^{wt}$ vs $ASXL1^{mut}/TET2^{mut}$

p = 0.0371

- ASXL1 mut TET2 wt
- ASXL1 mut TET2 mut

I. $ASXL1^{wt}/TET2^{mut}$ vs $ASXL1^{mut}/TET2^{mut}$

p = 0.0084

- ASXL1 wt TET2 mut
- ASXL1 mut TET2 mut

J. OS by $ASXL1/TET2$ Genotype in the pCMML Subgroup

p = 0.34

- ASXL1 wt TET2 wt
- ASXL1 mut TET2 wt
- ASXL1 wt TET2 mut
- ASXL1 mut TET2 mut

K. OS by $ASXL1/TET2$ Genotype in the CMML-2 Subgroup

p = 0.24

- ASXL1 wt TET2 wt
- ASXL1 mut TET2 wt
- ASXL1 wt TET2 mut
- ASXL1 mut TET2 mut

L. OS by $ASXL1/TET2$ Genotype in the External Cohort

p < 0.0001
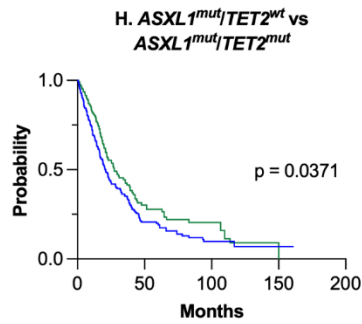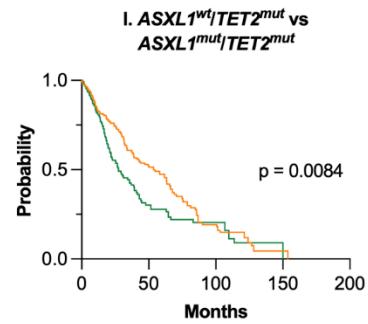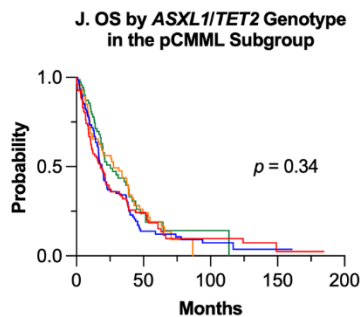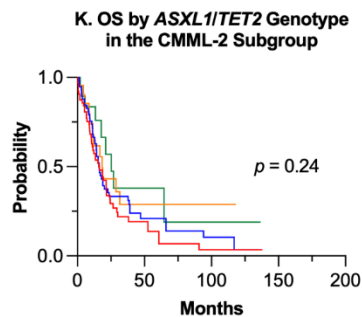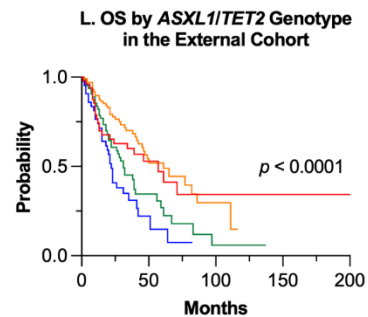
- ASXL1 wt TET2 wt, n = 50, mOS 57 (34 - NR) mo.
- ASXL1 mut TET2 wt, n = 44, mOS 22 (18 - 41) mo.
- ASXL1 wt TET2 mut, n = 105, mOS 61 (46 - NR) mo.
- ASXL1 mut TET2 mut, n = 66, mOS 31 (22 - 56) mo.

Figure S1. Overall Survival of Key CMML Subgroups. Panels **A-C** depict the overall survival (OS) of the primary CMML cohort stratified by the Groupe Francophone des Myelodysplasies (GFM), Mayo Molecular (Mayo-Mol), and CMML-specific prognostic scoring system molecular (CPSS-Mol) models, respectively. Panels **D-I** show individual OS comparisons between the four *ASXL1*/*TET2* genotypes; all comparisons are significant ($p < 0.05$ as shown) except for the *ASXL1*^{wt}/*TET2*^{wt} and *ASXL1*^{mut}/*TET2*^{mut} genotypes, which performed similarly ($p = 0.9244$). Panels **J-K** demonstrate that the *ASXL1*/*TET2* genotypes do not accurately stratify patients with proliferative CMML (pCMML) or CMML-2. Panel **L** depicts the OS of the external cohort stratified by the *ASXL1*/*TET2* genotypes; the comparison of *ASXL1*^{wt}/*TET2*^{wt} vs *ASXL1*^{mut}/*TET2*^{mut} genotypes is not statistically significant ($p = 0.83$). Survival data are presented as median OS (mOS) (95% confidence interval) with log-rank (Mantel-Cox) p values.

Table S1. Incorporation of *TET2* Mutation Status into Contemporary Prognostic Models.

Section A. Calculation of Risk Scores and Categories Incorporating *TET2* Mutation Status	
GFM Model with *TET2*	
Risk Calculation	**Points**
Age > 65 years	+2
WBC > 15 x10^9/L	+3
Hemoglobin < 11 g/dL (males) or < 10 g/dL (females)	+2
Platelet count < 100 x10^9/dL	+2
ASXL1 mutation	+2
TET2 mutation	-2
GFM Risk Categories	**Score**
Low	≤ 4
Intermediate	5 – 7
High	≥ 8

Mayo Molecular Model with *TET2*	
Risk Calculation	**Points**
Hemoglobin < 10 g/dL	+2
AMC > 10 x10^9/L	+2
IMC Present	+2
Platelet count < 100 x10^9/L	+1.5
ASXL1 mutation	+1.5
TET2 mutation	-1.5
Mayo Molecular Risk Categories	**Score**
Low	≤ 1
Intermediate	1.5 – 3.5
High	≥ 4

CPSS-Molecular Model with *TET2*	
Genetic Risk Group Calculation	**Points**
Spanish Cytogenetic Risk Category	
Low	+0
Intermediate	+1
High	+2
ASXL1 mutation	+1
NRAS mutation	+1
RUNX1 mutation	+2
SETBP1 mutation	+1
TET2 mutation	-1
Genetic Risk Group	**Score**
Low	-1
Intermediate-1	0
Intermediate-2	1
High	≥ 2
CPSS Molecular Score Calculation	**Points**
CPSS Genetic Risk Group	
Low	+0
Intermediate-1	+1
Intermediate-2	+2
High	+3
Bone marrow blasts ≥ 5%	+1
WBC ≥ 13 x10^9/L	+1
Hemoglobin < 9 g/dL (males) or < 8 g/dL (females)	+1
CPSS-Molecular Risk Categories	**Score**
Low	0 – 1
Intermediate	2 – 3
High	≥ 4

Section B. Concordance Indices and AUC Values of Original Prognostic Models and New Models Incorporating *TET2* Mutation Status.				
	Primary Cohort		External Cohort	
	Original Model	*TET2* Model	Original Model	*TET2* Model
Overall Survival (OS)				
Concordance Indices				
GFM	0.6239	0.6391	0.6706	0.6794
Mayo-Mol	0.6222	0.6363	0.6939	0.7020
CPSS-Mol	0.6151	0.6391	0.6626	0.6748
Area Under the Curve (AUC) Values				
GFM	0.5900	0.5900	0.6220	0.6510
Mayo-Mol	0.5460	0.5540	0.6550	0.6450
CPSS-Mol	0.5590	0.5880	0.6620	0.6630
Acute Myeloid Leukemia Free Survival (LFS)				
Concordance Indices				
GFM	0.6255	0.6407	0.6605	0.6724
Mayo-Mol	0.6296	0.6463	0.6850	0.6944
CPSS-Mol	0.6172	0.6429	0.6517	0.6711

In section A, items in blue indicate parameters that are new (TET2) or changed (score cutoff values for risk categories) compared to the parental model. In section B, concordance indices were determined via Cox regression modeling for overall survival (OS) and acute myeloid leukemia free survival (LFS); receiver operator curve (ROC) analyses were used to determine the area under the curve (AUC) values for each OS model.

Table S2. Characteristics of the Four *ASXL1*/*TET2* Genotypes within the External CMML Cohort

Variable	Cohort	*ASXL1*wt/*TET2*wt	*ASXL1*mut/*TET2*wt	*ASXL1*wt/*TET2*mut	*ASXL1*mut/*TET2*mut	P value[a]
n	265	50	44	105	66	
Demographics						
Age	71 (17 - 88)	69 (17 – 88)	71 (38 – 85)	72 (53 - 87)	72 (42 - 85)	0.0071
Male	183 (69.1%)	34 (68.0%)	30 (68.2%)	70 (66.7%)	49 (74.2%)	0.7816
Female	82 (30.9%)	16 (32.0%)	14 (31.8%)	35 (33.3%)	17 (25.8%)	
Laboratory Parameters						
Hemoglobin	11.0 (3.4 - 15.6)	10.9 (5.7 – 15.6)	9.9 (3.4 – 15.2)	11.3 (5.7 – 15.4)	10.4 (7.0 – 14.7	0.0085
Platelet Count	102 (2 - 1945)	154 (5 – 712)	115 (9 – 1945)	96 (5 - 443)	101 (2 - 730)	0.0045
WBC Count	14.2 (2.4 - 288.6)	13.4 (4.1 – 114.1)	18.5 (5.3 – 288.6)	9.1 (2.4 – 100.0)	19.9 (2.7 – 141.4)	0.0006
ANC	6.7 (0.1 - 155.6)	5.7 (1.3 – 69.6)	10.6 (1.0 – 155.6)	4.1 (0.1 – 64.7)	11.2 (0.2 – 94.1)	0.0002
AMC	2.71 (0.40 - 35.6)	2.7 (0.8 – 20.0)	2.9 (0.8 – 27.7)	1.9 (0.4 – 21.7)	3.2 (0.9 – 35.6)	0.0019
IMC	150 (60%)	29 (58.0%)	29 (65.9%)	47 (44.8%)	45 (68.2%)	0.0065
PB Blasts (%)	0 (0 - 15)	0 (0 – 15)	0 (0 – 11)	0 (0 – 15)	0 (0 - 12)	0.0406
BM Blasts (%)	3 (0 - 19)	4 (0 – 19)	3 (1 – 15)	3 (0 – 16)	2 (0 - 14)	0.0117
Ringed Sideroblasts	93 (35.1%)	14 (28.0%)	5 (11.3%)	16 (15.4%)	7 (10.6%)	0.5143
LDH (elevated)	144 (55.8%)	31 (62.0%)	25 (56.8%)	49 (46.7%)	39 (59.1%)	0.2134
FAB Subtype						
Dysplastic	118 (44.5%)	23 (46.0%)	12 (27.2%)	63 (60.0%)	20 (30.3%)	0.0005
Proliferative	147 (55.5%)	27 (54.0%)	32 (72.7%)	42 (40.0%)	46 (69.7%)	
WHO Category						
CMML-1	207 (84.8%)	36 (72.0%)	35 (79.5%)	82 (78.1%)	54 (81.8%)	0.1469
CMML-2	37 (15.2%)	12 (24.0%)	7 (15.9%)	12 (11.4%)	6 (9.1%)	
Karyotype						
Normal	198 (75.6%)	32 (64.0%)	30 (68.2%)	88 (83.8%)	48 (72.7%)	0.0480
Abnormal	64 (24.4%)	18 (36.0%)	13 (29.5%)	17 (16.2%)	16 (24.2%)	
Spanish Cytogenetic Risk Category						
Low	198 (75.6%)	32 (64.0%)	30 (68.2%)	88 (83.8%)	48 (72.7%)	0.1529
Intermediate	30 (11.5%)	10 (20.0%)	5 (11.4%)	7 (6.7%)	8 (12.1%)	
High	34 (12.8%)	8 (16.0%)	8 (18.2%)	10 (9.5%)	8 (12.1%)	
GFM Risk Category						
Low	100 (37.7%)	28 (56.0%)	4 (9.1%)	61 (58.1%)	7 (10.6%)	0.0005
Intermediate	105 (39.6%)	18 (36.0%)	19 (43.2%)	40 (38.1%)	28 (42.4%)	
High	60 (22.6%)	4 (8.0%)	21 (47.7%)	4 (3.8%)	31 (46.7%)	
Mayo Molecular Risk Category						
Low	22 (8.9%)	7 (14.0%)	0 (0%)	15 (14.3%)	0 (0%)	0.0005
Intermediate-1	72 (29.3%)	18 (36.0%)	5 (11.4%)	44 (41.9%)	5 (7.6%)	
Intermediate-2	79 (32.1%)	16 (32.0%)	13 (29.5%)	31 (29.5%)	19 (28.8%)	
High	73 (29.7%)	7 (14.0%)	23 (52.3%)	6 (5.7%)	37 (56.1%)	
CPSS-Molecular Risk Category						
Low	35 (14.3%)	9 (18.0%)	0 (0%)	26 (24.8%)	0 (0%)	0.0005
Intermediate-1	60 (24.5%)	12 (24.0%)	5 (11.4%)	34 (32.4%)	9 (13.6%)	
Intermediate-2	103 (42%)	20 (40.0%)	19 (43.2%)	30 (28.6%)	34 (51.5%)	
High	47 (19.2%)	8 (16.0%)	18 (40.9%)	5 (4.8%)	16 (24.2%)	
Mutation Statistics						
Number of Mutations	3 (0 - 7)	2 (0 – 5)	3 (1 – 5)	2 (1 - 6)	4 (2 - 7)	< 0.0001
Mutation Profile						
ASXL1	110 (41.5%)	0 (0%)	44 (100%)	0 (0%)	66 (100%)	0.0005
CBL	39 (14.7%)	7 (14.0%)	5 (11.4%)	12 (11.4%)	15 (22.7%)	0.2094
DNMT3A	18 (6.8%)	11 (22.0%)	2 (4.5%)	4 (3.8%)	1 (1.5%)	0.0005
ETV6	11 (4.2%)	1 (2.0%)	2 (4.5%)	4 (3.8%)	4 (6.1%)	0.7796
EZH2	34 (12.8%)	3 (6.0%)	11 (25.0%)	7 (6.7%)	13 (19.7%)	0.0020
IDH1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	N/A
IDH2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	N/A
JAK2	20 (7.5%)	8 (16.0%)	3 (6.8%)	5 (4.8%)	4 (6.1%)	0.0830
KIT	13 (4.9%)	0 (0%)	3 (6.8%)	5 (4.8%)	5 (7.6%)	0.2689
KRAS	13 (4.9%)	3 (6.0%)	1 (2.3%)	6 (5.7%)	3 (4.5%)	0.8451
MPL	3 (1.1%)	0 (0%)	1 (2.3%)	2 (1.9%)	0 (0%)	0.4598
NPM1	4 (1.5%)	3 (6.0%)	0 (0%)	0 (0%)	1 (1.5%)	0.0350
NRAS	43 (16.2%)	10 (20.0%)	6 (13.6%)	18 (17.1%)	9 (13.6%)	0.7646
PHF6	9 (3.4%)	1 (2.0%)	3 (6.8%)	3 (2.9%)	2 (3.0%)	0.6237
RUNX1	51 (19.2%)	8 (16.0%)	13 (29.5%)	18 (17.1%)	12 (18.2%)	0.2909
SETBP1	24 (9.1%)	5 (10.0%)	9 (20.5%)	2 (1.9%)	8 (12.1%)	0.0015
SF3B1	21 (7.9%)	11 (22.0%)	2 (4.5%)	7 (6.7%)	1 (1.5%)	0.0005
SRSF2	101 (38.1%)	5 (10.0%)	13 (29.5%)	51 (48.9%)	32 (48.5%)	0.0005
TET2	171 (64.5%)	0 (0%)	0 (0%)	105 (100%)	66 (100%)	0.0005
TP53	7 (2.6%)	3 (6.0%)	1 (2.3%)	1 (1.0%)	2 (3.0%)	0.3733
U2AF1	19 (7.2%)	4 (8.0%)	8 (18.2%)	2 (2.9%)	4 (6.1%)	0.0125
ZRSR2	19 (7.2%)	1 (2.0%)	3 (6.8%)	11 (10.5%)	4 (6.1%)	0.2629
Outcomes						
Transformation	55 (20.8%)	11 (22.0%)	8 (18.2%)	17 (16.2%)	19 (28.8%)	0.2487
Death	136 (51.3%)	24 (48.0%)	31 (70.5%)	40 (38.1%)	41 (62.1%)	0.0006