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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,*]; Mrinal M. Patnaik[1,*]

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

*EP, GMB, and MMP contributed equally to the manuscript.

Correspondence
Mrinal M. Patnaik, M.B.B.S.
Consultant in Hematology, Division of Hematology, Department of Medicine
Mayo Clinic, 200 First Street SW, Rochester, MN 55905
patnaik.mrinal@mayo.edu

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CMC collected data, performed the primary analyses, and drafted the manuscript. MG, RKS, KC, DH, TLL, CMF, CD, AN, AAM, AA, NG, AT, HA, GCM, RSK, NAA, EP, and GMB assisted with data collection and study design. EP, GMB, and MMP conceived the study. MMP provided principal oversight. All authors critically reviewed and approved the manuscript.

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TO THE EDITOR:

Truncating *ASXL1* mutations are a high-risk feature in chronic myelomonocytic leukemia (CMML)[1] and are associated with inferior overall survival (OS) and acute myeloid leukemia free survival (LFS).[2] Conversely, we previously showed that loss-of-function/hypomorphic mutations in *TET2* are associated with better outcomes, with the *ASXL1* wild type *TET2* mutant (*ASXL1*^{wt}/*TET2*^{mut}) genotype conferring a survival advantage independent of treatment.[3] However, contemporary prognostic scoring systems – including the Groupe Francophone des Myelodysplasies (GFM)[4], Mayo Molecular (Mayo-Mol)[5], and CMML-specific prognostic scoring system molecular (CPSS-Mol)[6] models – do not consider mutational variant allele fractions (VAF) or *TET2* mutational status. Here, we expand upon our prior work by assessing mutation VAF, reconsidering the use of binary mutation status, and integrating *TET2* into the prognostic models.

After Institutional Review Board approval, we cataloged CMML patients seen at two centers, Mayo Clinic (n=466, 52%) and MD Anderson Cancer Center (n=422, 48%). Next generation sequencing (NGS) was carried out as described at CMML diagnosis.[3,7] Variants were annotated against international normal allele and pathologic mutation databases, and variants of uncertain significance (VUS) were excluded from analysis. As *TET2* mutations occur in multiple clonal states[3,8], we considered the mutation with highest VAF when assessing the impact on outcomes. Copy number alterations and loss of heterozygosity data were only available for a small number of patients, as reported elsewhere[9], and thus were not considered for this analysis. Statistical analyses considered the parameters at the time of presentation to the respective institution. Categorical variables were compared by Fisher exact or Pearson χ^2 tests and continuous variables by Mann-Whitney U test or two-way ANOVA with Tukey *p*-value correction for pairwise comparisons. Univariate and multivariate analyses were performed using Cox proportional hazards regression models. Models were compared using concordance indices (C-statistic), where higher values indicate a better fit, and receiver operator curve (ROC) analyses.[10] Survival was assessed via the Kaplan-Meier method. *P*-values <0.05 were considered significant. Calculations were performed using BlueSky Statistics (v10.3.1) or MedCalc (v22.016).

The median age of the cohort (n=888) was 71 years (range 20 – 94), 33% were female, 46% had proliferative CMML (pCMML), and 19% had CMML-2 by current criteria[1,11] (**Table 1**). The most frequently mutated genes were *ASXL1* (45%), *TET2* (44%), *SRSF2* (41%), *NRAS* (15%), and *RUNX1* (15%). The median number of mutations in *ASXL1* was 1 (range 1 – 3) and in *TET2* was 1 (range 1 – 5); however, multiple *ASXL1* mutations were rare (3%) in comparison to multiple *TET2* mutations (47%, **Figure 1A**). Most patients had ≥1 mutation in an epigenetic regulator (79%) or spliceosome gene (57%). RAS pathway mutations were observed in 37%. Transformation to AML occurred in 168 patients (19%) and there were 586 deaths (66%). The median OS (mOS) and mLFS of the cohort were 31.8 and 28.4 months, respectively, with a median follow up of 63.1 months. Risk stratification according to the GFM, Mayo-Mol, and CPSS-Mol models is shown in **Figure S1A-C**.

To evaluate the impact of *ASXL1* and *TET2* mutations on OS and LFS, the cohort was divided into four genotype-based subgroups: *ASXL1*^{wt}/*TET2*^{wt} (n=244, 28%), *ASXL1*^{mut}/*TET2*^{wt} (n=254, 29%), *ASXL1*^{wt}/*TET2*^{mut} (n=241, 26%), and *ASXL1*^{mut}/*TET2*^{mut} (n=149, 17%) (**Table 1**). Patients with *ASXL1* mutations were more likely to be male (*p*=0.0135), have a higher white blood cell (WBC) count (*p*=0.0129), and harbor mutations in transcriptional and RAS pathways (*p*<0.0001). Patients with *TET2* mutations were more likely to have a higher hemoglobin (*p*<0.0001) and a normal karyotype (*p*=0.0005). As previously documented[3,12,13], those with

isolated *TET2* mutations had the longest mOS of 58 months whereas those with isolated *ASXL1* mutations had the shortest mOS of 21 months (**Figure 1B**). Patients with the *ASXL1*^{wt}/*TET2*^{wt} and *ASXL1*^{mut}/*TET2*^{mut} genotypes fared similarly with mOS of 30 and 27 months, respectively (**Figure S1F**). The same pattern was observed for LFS (**Figure 1C**).

We hypothesized that the *ASXL1* or *TET2* mutation VAF would be more predictive of outcomes than a binary metric. The respective median VAFs were 37% and 45% (**Figure 1A**). When treated as a continuous variable, there was no correlation between VAF and OS or LFS by either Pearson linear or Cox regression (p>0.39 for all correlations in both models; **Figure 1D**). Similarly, amongst patients with multiple *ASXL1* or *TET2* mutations, there was no association between the number of mutations and OS or LFS (p≥0.06). There was also no survival difference between those with 1 versus ≥2 mutations in either gene (p>0.05 for each). Although prior studies have inconsistently shown associations between the number of *TET2* mutations and survival[3,8], these results support the practice of considering *ASXL1* and *TET2* mutation status as binary metrics in prognostic models.

Unlike in the overall cohort, the *ASXL1*/*TET2* genotypes did not accurately stratify patients with pCMML, CMML-2, or those considered high-risk by the prognostic models (**Figure S1J-K**). In contrast, patients considered intermediate and low-risk by the Mayo-Mol and CPSS-Mol models were further stratified by the *ASXL1*/*TET2* genotypes. Therefore, we sought to improve the existing molecular models by incorporating *TET2* mutation status as a favorable prognosticator. Given that *TET2* mutations balanced detrimental *ASXL1* mutations in the Kaplan-Meier analyses, *TET2* mutation status was given equal weight as *ASXL1* in the GFM (-2 points), Mayo-Mol (-1.5 points), and the genetic risk scoring of the CPSS-Mol models (-1 point) (**Table S1A**). Sex-specific hemoglobin thresholds were used as a surrogate for transfusion dependency in the CPSS-Mol model[6,14,15].

After adjusting the risk category cutoffs to accommodate *TET2* scoring (**Table S1A**), the number of patients down-staged was 122 (18.4%), 215 (25.3%), and 97 (14.6%) in the Mayo-Mol, CPSS-Mol, and GFM models, respectively (**Figure 2**). Although 2% of patients in the Mayo-Mol and 6% in the CPSS-Mol were upstaged, no patients with *TET2* mutations were upstaged. With the addition of *TET2* status, the intermediate-1 and intermediate-2 risk groups were not significantly different in the Mayo-Mol model (p=0.49), whereas the low and intermediate-1 risk groups were not significantly different in the CPSS-Mol model (p=0.084); thus, these were each combined into a single group, yielding a three-tiered stratification in both models. In the GFM with *TET2* mutational status, this resulted in a mOS of 42, 21, and 14 months for the low, intermediate, and high-risk groups, respectively. In the Mayo-Mol with *TET2*, the mOS was 58, 31, and 15 months, respectively. In the CPSS-Mol with *TET2*, the mOS was 63, 30, and 16 months, respectively. (**Figure 1E-G**). Similar results were obtained when patients in the Mayo Clinic subgroup (where hematopoietic cell transplantation data were available, n=18, 4%) were censored at the time of transplant. In all three models, the addition of *TET2* mutation status improved prognostication compared to the parental model, as indicated by higher concordance indices for each model (**Table S1B**). Likewise, the models with *TET2* status performed similar to or better than the parental models in ROC analyses.

These findings were then validated in an external database from Moffitt Cancer Center (n=265, 31% female) with median age 71 years (range 17 – 88 years) and 55% pCMML and 15% CMML-2 cases (**Table S2**). The mOS and mLFS of the external cohort were 41 (95% CI, 33 – 51) and 37 (28 - 46) months, respectively, with 55 (21%) blast transformation events and 136 (51%) deaths. The external cohort was grouped by *ASXL1*/*TET2* genotype, providing: *ASXL1*^{wt}/*TET2*^{wt} (n=50, 19%), *ASXL1*^{mut}/*TET2*^{wt} (n=44, 17%), *ASXL1*^{wt}/*TET2*^{mut} (n=105, 40%),

and *ASXL1*^{mut}/*TET2*^{mut} (n=66, 25%). As in the primary cohort, the *ASXL1*^{wt}/*TET2*^{mut} genotype conferred the longest mOS (61 months) and the *ASXL1*^{mut}/*TET2*^{wt} genotype the shortest mOS (22 months; **Figure S1L**). The same trend was observed for LFS. Again, the pCMML (p = 0.056) and CMML-2 (p = 0.12) subgroups were not stratified by the genotypes, and there was no correlation between *ASXL1* or *TET2* VAF with either OS or LFS (p >0.25 for all comparisons). While patients were stratified by existing molecular models (as expected), the addition of *TET2* mutation status to the Mayo-Mol and CPSS-Mol models again defined three risk groups (low, intermediate, and high) with respective mOS values of 77, 39, and 20 months for the Mayo-Mol (p<0.0001) and 77, 39, and 22 months for the CPSS-Mol model (p<0.0001; **Figure 1I-J**). The mOS with the GFM model incorporating *TET2* status was 61, 31, and 15 months, respectively (p<0.0001; **Figure 1H**). Again, models incorporating *TET2* mutation provided higher concordance indices and similar AUC values compared to parental models (**Table S1B**).

In summary, our data validates the positive prognostic impact of *TET2* mutations in CMML, highlighting the importance of considering the *ASXL1/TET2* co-mutational status for prognostication.[3,12,13] Expanding upon prior work, we further show that *ASXL1* and *TET2* mutational VAF does not impact prognostic outcomes, supporting the ongoing practice of binary assessments for molecularly-based CMML prognostication. Furthermore, in a large database and an external validation cohort, the addition of binary *TET2* mutation status to existing molecularly-integrated CMML prognostic models simplified and refined risk stratification. Regardless of whether they are statistically superior, by downstaging some patients and harmonizing the models into three-tiered systems, these refined models may simplify risk stratification and clinical decision making. In this regard, the low-risk tiers of these models represent the lowest-risk patients whereas the intermediate- and high-risk tiers identify “higher-risk” patients. Finally, the favorable impact of *TET2* mutations in hematological neoplasms is largely associated with CMML[3] and biological studies understanding the underlying mechanism are needed.

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Table 1. Characteristics of the Four *ASXL1*/*TET2* Genotypes within the CMML Cohort

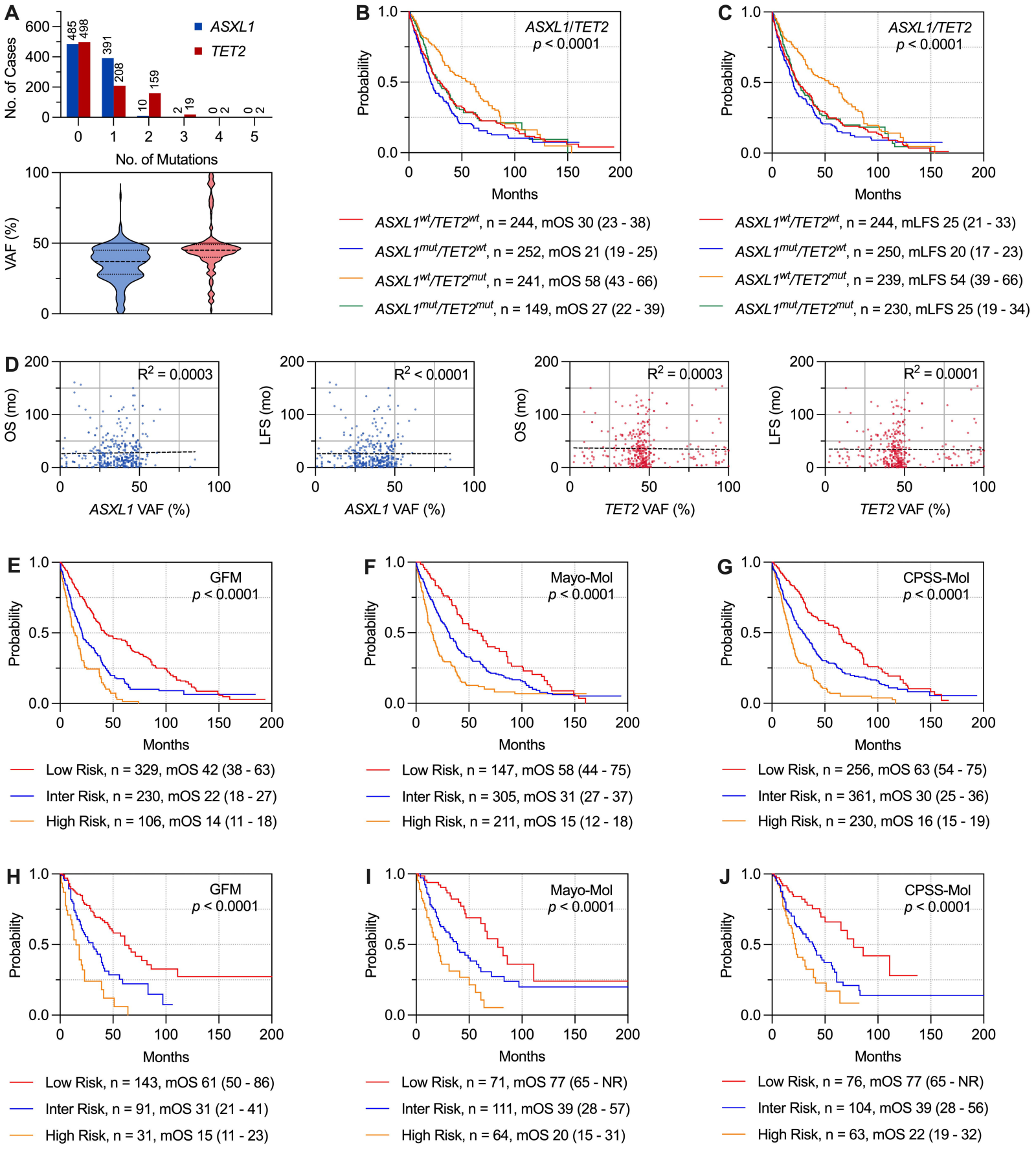
Variable	Cohort	*ASXL1*^{wt}/*TET2*^{wt}	*ASXL1*^{mut}/*TET2*^{wt}	*ASXL1*^{wt}/*TET2*^{mut}	*ASXL1*^{mut}/*TET2*^{mut}	*P* value[a]
n	888	244	254	241	149	
Demographics						
Age	71 (20 – 94)	71 (20 - 94)	70 (27 - 90)	70 (36 - 90)	71 (39 - 91)	0.1027
Male	593 (66.8%)	151 (61.9%)	183 (72%)	150 (62.2%)	109 (73.2%)	0.0135
Female	295 (33.2%)	93 (38.1%)	71 (28%)	91 (37.8%)	40 (26.8%)	
Laboratory Parameters						
Hemoglobin (g/dL)	10.8 (4.2 – 17.3)	10.4 (4.2 - 17.3)	10.2 (5.3 - 16.9)	11.6 (6.6 - 16.0)	11.6 (6.3 - 15.8)	< 0.0001
MCV (fL)	92.4 (59.0 – 121.0)	92.8 (59.0 - 119.4)	91.7 (59.0 - 121)	93.3 (60.6 - 114.5)	91.1 (69.0 - 120.1)	0.2723
Platelets (x10^9/L)	97 (8 – 1264)	95 (8 - 820)	105 (10 - 1264)	103 (12 - 840)	81 (10 - 308)	0.0033
WBC (x10^9/L)	12 (1.2 – 264.8)	11.1 (1.2 - 235.0)	14.8 (2.0 - 264.8)	9.2 (1.8 - 185.7)	13 (1.8 - 264.8)	0.0129
ANC (x10^9/L)	5.9 (0.0 – 151.0)	5.2 (0.0 - 67.5)	7.7 (0.0 - 151.0)	4.3 (0.0 - 142.9)	7.2 (0.2 - 142.9)	0.0283
AMC (x10^9/L)	2.3 (0.0 – 47.5)	2 (0.0 - 37.9)	2.8 (0.3 - 37.8)	2 (0.0 - 39.5)	2.7 (0.6 - 47.5)	0.0027
ALC (x10^9/L)	1.8 (0.0 – 22.0)	2 (0.3 - 11.0)	1.9 (0.4 - 22.0)	1.7 (0.0 - 11.0)	1.9 (0.0 - 7.9)	0.1914
IMC present	437 (49.5%)	115 (47.3%)	146 (57.9%)	97 (40.4%)	79 (53.7%)	0.0015
Peripheral blasts (%)	0 (0 – 19)	0 (0 - 16)	0 (0 - 19)	0 (0 - 12)	0 (0 - 14)	< 0.0001
Marrow blasts (%)	4 (0 – 31)	4 (0 - 31)	4 (0 - 20)	3 (0 - 17)	3 (0 - 18)	< 0.0001
Ringed sideroblasts	68 (15.8%)	17 (14.2%)	19 (14.6%)	22 (19.8%)	10 (14.5%)	0.6162
LDH (units/L)	246 (84 – 6075)	268 (98 - 6075)	256 (109 - 3615)	225 (85 - 1808)	247 (84 - 4464)	0.2315
Subtype						
Dysplastic	474 (53.6%)	133 (54.7%)	108 (42.7%)	160 (66.9%)	73 (49%)	0.0005
Proliferative	410 (46.4%)	110 (45.3%)	145 (57.3%)	79 (33.1%)	76 (51%)	
WHO Category						
CMML-1	708 (80.7%)	117 (48.5%)	189 (75%)	216 (90.4%)	126 (86.9%)	0.0005
CMML-2	169 (19.3%)	64 (26.6%)	63 (25%)	23 (9.6%)	19 (13.1%)	
Karyotype						
Normal	569 (66.9%)	124 (54.4%)	155 (62.2%)	178 (76.7%)	112 (78.9%)	0.0005
Abnormal	282 (33.1%)	104 (45.6%)	94 (37.8%)	54 (23.3%)	30 (21.1%)	
Spanish Cytogenetic Risk Category						
Low	591 (69.4%)	128 (56.1%)	158 (63.5%)	196 (84.5%)	115 (81%)	0.0005
Intermediate	124 (14.6%)	43 (18.9%)	42 (16.9%)	24 (10.3%)	15 (10.6%)	
High	136 (16%)	57 (25%)	49 (19.7%)	15 (6.5%)	15 (10.6%)	
GFM Risk Category						
Low	275 (41.3%)	103 (55.4%)	28 (14.4%)	120 (68.6%)	24 (21.6%)	0.0005
Intermediate	243 (36.5%)	67 (36%)	84 (43.3%)	46 (26.3%)	46 (41.4%)	
High	148 (22.2%)	16 (8.6%)	82 (42.3%)	9 (5.1%)	41 (36.9%)	
Mayo Molecular Risk Category						
Low	55 (8.3%)	24 (12.9%)	0 (0%)	31 (17.7%)	0 (0%)	0.0005
Intermediate-1	192 (28.8%)	73 (39.2%)	26 (13.4%)	77 (44%)	16 (14.4%)	
Intermediate-2	192 (28.8%)	51 (27.4%)	55 (28.4%)	47 (26.9%)	39 (35.1%)	
High	227 (34.1%)	38 (20.4%)	113 (58.2%)	20 (11.4%)	56 (50.5%)	
CPSS-Molecular Risk Category						
Low	83 (9.8%)	32 (14%)	0 (0%)	51 (22.1%)	0 (0%)	0.0005
Intermediate-1	206 (24.2%)	53 (23.2%)	29 (11.6%)	91 (39.4%)	33 (23.2%)	
Intermediate-2	337 (39.6%)	98 (43%)	101 (40.6%)	77 (33.3%)	61 (43%)	
High	224 (26.4%)	45 (19.7%)	119 (47.8%)	12 (5.2%)	48 (33.8%)	
Mutation Profile						
Number of mutations	3 (0 – 8)	1 (0 - 5)	3 (1 - 7)	2 (1 - 6)	4 (2 - 8)	< 0.0001
ASXL1	403 (45.4%)	0 (0%)	254 (100%)	0 (0%)	149 (100%)	< 0.0001
BCOR	18 (2%)	4 (1.6%)	8 (3.1%)	4 (1.7%)	2 (1.3%)	0.5899
BRAF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	N/A
CALR	7 (0.8%)	3 (1.2%)	0 (0%)	4 (1.7%)	0 (0%)	0.0800
CBL	118 (13.3%)	15 (6.1%)	34 (13.4%)	36 (14.9%)	33 (22.1%)	< 0.0001
CEBPA	31 (3.5%)	8 (3.3%)	11 (4.3%)	7 (2.9%)	5 (3.4%)	0.8588
CSF3R	7 (1.5%)	1 (0.8%)	2 (1.4%)	3 (2.4%)	1 (1.3%)	0.84.9
CUX1	1 (9.1%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0.5455
DNMT3A	51 (5.7%)	21 (8.6%)	10 (3.9%)	17 (7.1%)	3 (2%)	0.0172
ETNK1	10 (1.1%)	1 (0.4%)	4 (1.6%)	4 (1.7%)	1 (0.7%)	0.5128
ETV6	2 (0.9%)	0 (0%)	2 (4.1%)	0 (0%)	0 (0%)	0.1392
EZH2	34 (3.8%)	2 (0.8%)	15 (5.9%)	3 (1.2%)	14 (9.4%)	< 0.0001
FLT3	15 (1.7%)	1 (0.4%)	7 (2.8%)	1 (0.4%)	6 (4%)	0.0062
GATA2	1 (0.5%)	1 (2.3%)	0 (0%)	0 (0%)	0 (0%)	0.2028
IDH1	11 (1.2%)	4 (1.6%)	5 (2%)	2 (0.8%)	0 (0%)	0.3127
IDH2	49 (5.5%)	21 (8.6%)	27 (10.6%)	0 (0%)	1 (0.7%)	< 0.0001
JAK2	52 (5.9%)	15 (6.1%)	14 (5.5%)	13 (5.4%)	10 (6.7%)	0.9383
KIT	23 (2.6%)	7 (2.9%)	7 (2.8%)	2 (0.8%)	7 (4.7%)	0.1038
KRAS	73 (8.2%)	19 (7.8%)	22 (8.7%)	19 (7.9%)	13 (8.7%)	0.9749
MPL	14 (1.6%)	3 (1.2%)	1 (0.4%)	7 (2.9%)	3 (2%)	0.1191
NPM1	18 (2.0%)	14 (5.7%)	0 (0%)	4 (1.7%)	0 (0%)	< 0.0001
NRAS	137 (15.4%)	27 (11.1%)	50 (19.7%)	33 (13.7%)	27 (18.1%)	0.0361
PHF6	20 (2.3%)	2 (0.8%)	6 (2.4%)	7 (2.9%)	5 (3.4%)	0.2460
PTPN11	37 (4.2%)	11 (4.5%)	17 (6.7%)	7 (2.9%)	2 (1.3%)	0.0463
RAD21	2 (0.9%)	1 (2.3%)	1 (2%)	0 (0%)	0 (0%)	0.3228

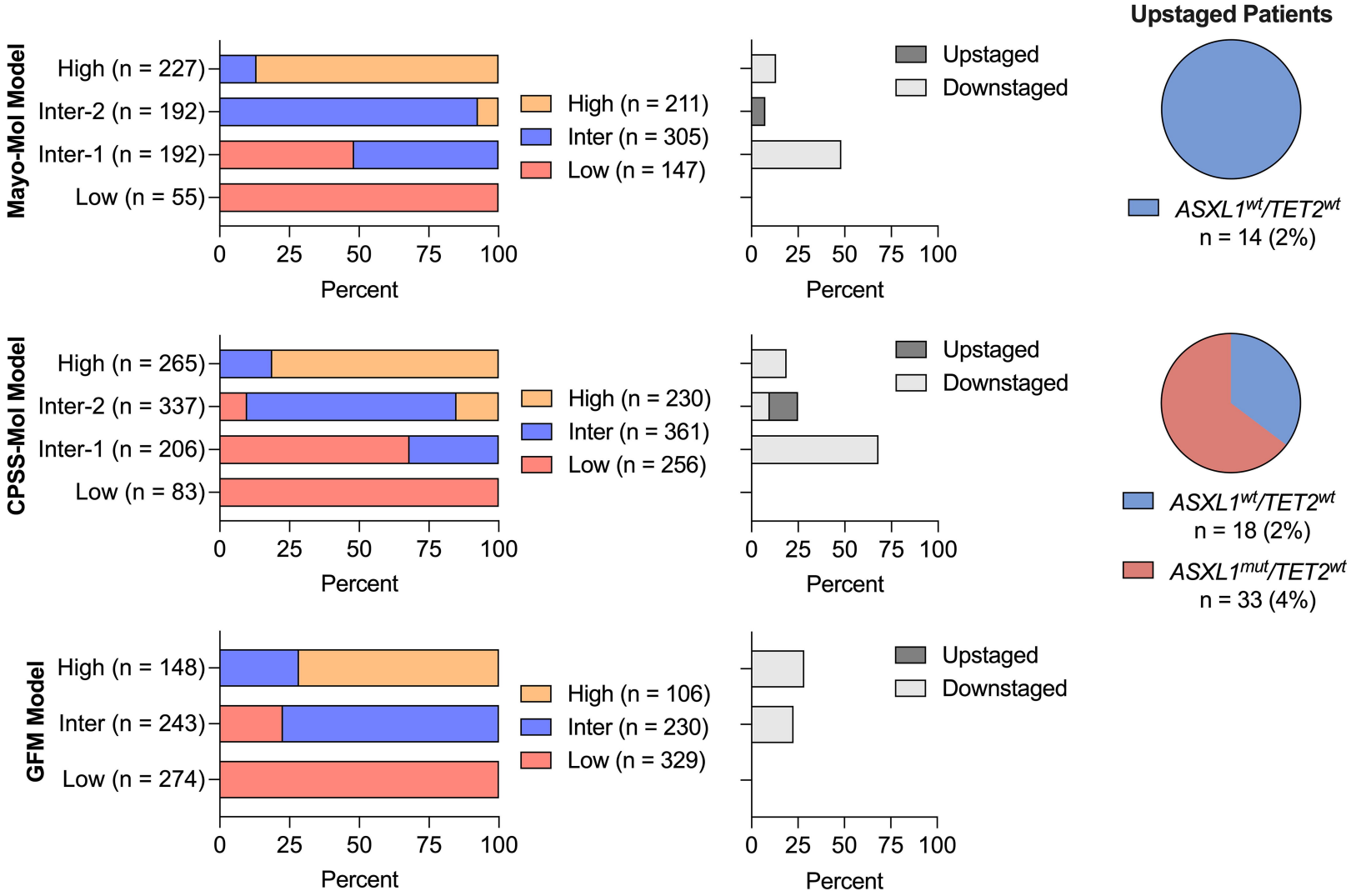
RUNX1	129 (14.5%)	27 (11.1%)	47 (18.5%)	24 (10%)	31 (20.8%)	0.0025
SETBP1	82 (9.2%)	12 (4.9%)	52 (20.5%)	7 (2.9%)	11 (7.4%)	< 0.0001
SF3B1	45 (5.1%)	18 (7.4%)	3 (1.2%)	22 (9.1%)	2 (1.3%)	< 0.0001
SH2B3	4 (0.9%)	0 (0%)	2 (1.4%)	2 (1.6%)	0 (0%)	0.4739
SRSF2	363 (40.9%)	70 (28.7%)	108 (42.5%)	106 (44%)	79 (53%)	< 0.0001
STAG2	12 (1.4%)	2 (0.8%)	6 (2.4%)	0 (0%)	4 (2.7%)	0.0226
SUZ12	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	N/A
TET2	390 (43.9%)	0 (0%)	0 (0%)	241 (100%)	149 (100%)	< 0.0001
TP53	37 (4.2%)	24 (9.8%)	1 (0.4%)	7 (2.9%)	5 (3.4%)	< 0.0001
U2AF1	69 (7.8%)	19 (7.8%)	33 (13%)	7 (2.9%)	10 (6.7%)	0.0004
WT1	4 (0.5%)	2 (0.8%)	2 (0.8%)	0 (0%)	0 (0%)	0.4413
ZRSR2	44 (5.0%)	6 (2.5%)	8 (3.1%)	17 (7.1%)	13 (8.7%)	0.0083
Mutation Groups						
RAS Oncogenes	326 (36.7%)	60 (24.6%)	113 (44.5%)	85 (35.3%)	68 (45.6%)	< 0.0001
Epigenetic Regulators	698 (78.6%)	54 (22.1%)	254 (100%)	241 (100%)	149 (100%)	< 0.0001
Spliceosome Components	505 (56.9%)	108 (44.3%)	151 (59.4%)	145 (60.2%)	101 (67.8%)	< 0.0001
Signaling Pathways	26 (2.9%)	2 (0.8%)	11 (4.3%)	6 (2.5%)	7 (4.7%)	0.0392
Transcription Factors	227 (25.6%)	43 (17.6%)	102 (40.2%)	36 (14.9%)	46 (30.9%)	< 0.0001
Tumor suppressors	41 (4.6%)	26 (10.7%)	3 (1.2%)	7 (2.9%)	5 (3.4%)	< 0.0001
Outcomes						
Transformation	168 (18.9%)	45 (18.4%)	50 (19.7%)	36 (14.9%)	37 (24.8%)	0.1108
Death	586 (66.0%)	177 (72.5%)	181 (71.3%)	133 (55.2%)	95 (63.8%)	0.0001

Data are presented as either median (range) or n (%), as appropriate. [a]P values represent two-way ANOVA comparisons with Tukey corrections between the four genotypes. Oncogenic RAS mutations include *NRAS, KRAS, BRAF, CBL,* and *PTPN11*. Epigenetic mutations include *ASXL1, TET2, BCOR, DNMT3A, EZH2, IDH1/2, PHF6,* and *STAG2*. Spliceosome mutations include *SF3B1, SRSF2, U2AF1,* and *ZRSR2*. Signaling mutations include *CSF3R, FLT3, JAK2,* and *SH2B3*. Transcription mutations include *CEBPA, ETV6, GATA2, RUNX1,* and *SETBP1*. Tumor suppressor mutations include *TP53* and *WT1*. Abbreviations: MCV, mean corpuscular volume; WBC, white blood cell; ANC, absolute neutrophil count; AMC, absolute monocyte count; ALC, absolute lymphocyte count; IMC, immature myeloid cells; LDH, lactate dehydrogenase.

Figure 1. Prognostic implications of *ASXL1*/*TET2* co-mutation status in chronic myelomonocytic leukemia. (**A**) The upper panel (histogram) shows the distribution of cases with the indicated number of mutations in *ASXL1* or *TET2*. The lower panel (violin plot) shows the distribution of mutational variant allele fractions (VAF) for *ASXL1* in blue (median 37%, quartiles 28% – 45%) and *TET2* in red (median 45%, quartiles 40% – 49%). (**B**) Median overall survival (mOS) and (**C**) acute myeloid leukemia free survival (LFS) of the cohort stratified by *ASXL1*/*TET2* genotype. (**D**) No correlation was observed between mutation VAF and either OS or LFS. $P > 0.39$ for all correlations by both Pearson linear regression and Cox proportional hazard modeling. The mOS of the primary cohort stratified into low, intermediate (inter), and high-risk subgroups by the (**E**) Groupe Francophone des Myelodysplasies (GFM), (**F**) Mayo-Molecular (Mayo-Mol), and (**G**) CPSS-Molecular (CPSS-Mol) models each with the addition of *TET2* mutation status The mOS of the external cohort stratified by the (**H**) GFM, (**I**) Mayo-Mol, and (**J**) CPSS-Mol models each with the addition of *TET2* mutation status. In all Kaplan-Meier analyses, data are presented as median survival (95% confidence interval) in months with log-rank *p* values.

Figure 2. Risk re-stratification from existing chronic myelomonocytic leukemia prognostic models to models incorporating *TET2* mutation status. Stacked colored bar plots show the re-stratification of existing prognostic models to the updated models incorporating *TET2* mutation status for patients whom both scores could be calculated. Each bar corresponds to one existing risk category while colors represent the new risk categories with *TET2* status. The gray bar plots represent the percentage of re-stratified patients within each contemporary model's stratum. The pie charts depict the *ASXL1*/*TET2* genotypes of the patients who were upstaged in each model. The addition of *TET2* status to the GFM model did not upstage any patients, and therefore no pie chart is depicted for this model. Abbreviations: inter, intermediate.


A
No. of Cases
ASXL1
TET2
485
498
391
208
10
159
2
19
0
2
0
2
No. of Mutations
VAF (%)
B
ASXL1/TET2
p < 0.0001
Probability
Months
ASXL1wt/TET2wt, n = 244, mOS 30 (23 - 38)
ASXL1mut/TET2wt, n = 252, mOS 21 (19 - 25)
ASXL1wt/TET2mut, n = 241, mOS 58 (43 - 66)
ASXL1mut/TET2mut, n = 149, mOS 27 (22 - 39)
C
ASXL1/TET2
p < 0.0001
Probability
Months
ASXL1wt/TET2wt, n = 244, mLFS 25 (21 - 33)
ASXL1mut/TET2wt, n = 250, mLFS 20 (17 - 23)
ASXL1wt/TET2mut, n = 239, mLFS 54 (39 - 66)
ASXL1mut/TET2mut, n = 230, mLFS 25 (19 - 34)
D
R2 = 0.0003
OS (mo)
ASXL1 VAF (%)
R2 < 0.0001
LFS (mo)
ASXL1 VAF (%)
R2 = 0.0003
OS (mo)
TET2 VAF (%)
R2 = 0.0001
LFS (mo)
TET2 VAF (%)
E
GFM
p < 0.0001
Probability
Months
Low Risk, n = 329, mOS 42 (38 - 63)
Inter Risk, n = 230, mOS 22 (18 - 27)
High Risk, n = 106, mOS 14 (11 - 18)
F
Mayo-Mol
p < 0.0001
Probability
Months
Low Risk, n = 147, mOS 58 (44 - 75)
Inter Risk, n = 305, mOS 31 (27 - 37)
High Risk, n = 211, mOS 15 (12 - 18)
G
CPSS-Mol
p < 0.0001
Probability
Months
Low Risk, n = 256, mOS 63 (54 - 75)
Inter Risk, n = 361, mOS 30 (25 - 36)
High Risk, n = 230, mOS 16 (15 - 19)
H
GFM
p < 0.0001
Probability
Months
Low Risk, n = 143, mOS 61 (50 - 86)
Inter Risk, n = 91, mOS 31 (21 - 41)
High Risk, n = 31, mOS 15 (11 - 23)
I
Mayo-Mol
p < 0.0001
Probability
Months
Low Risk, n = 71, mOS 77 (65 - NR)
Inter Risk, n = 111, mOS 39 (28 - 57)
High Risk, n = 64, mOS 20 (15 - 31)
J
CPSS-Mol
p < 0.0001
Probability
Months
Low Risk, n = 76, mOS 77 (65 - NR)
Inter Risk, n = 104, mOS 39 (28 - 56)
High Risk, n = 63, mOS 22 (19 - 32)


Mayo-Mol Model
High (n = 227)
Inter-2 (n = 192)
Inter-1 (n = 192)
Low (n = 55)
0 25 50 75 100
Percent
High (n = 211)
Inter (n = 305)
Low (n = 147)
Upstaged
Downstaged
0 25 50 75 100
Percent
Upstaged Patients
ASXL1wt/TET2wt
n = 14 (2%)
CPSS-Mol Model
High (n = 265)
Inter-2 (n = 337)
Inter-1 (n = 206)
Low (n = 83)
0 25 50 75 100
Percent
High (n = 230)
Inter (n = 361)
Low (n = 256)
Upstaged
Downstaged
0 25 50 75 100
Percent
ASXL1wt/TET2wt
n = 18 (2%)
ASXL1mut/TET2wt
n = 33 (4%)
GFM Model
High (n = 148)
Inter (n = 243)
Low (n = 274)
0 25 50 75 100
Percent
High (n = 106)
Inter (n = 230)
Low (n = 329)
Upstaged
Downstaged
0 25 50 75 100
Percent

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**

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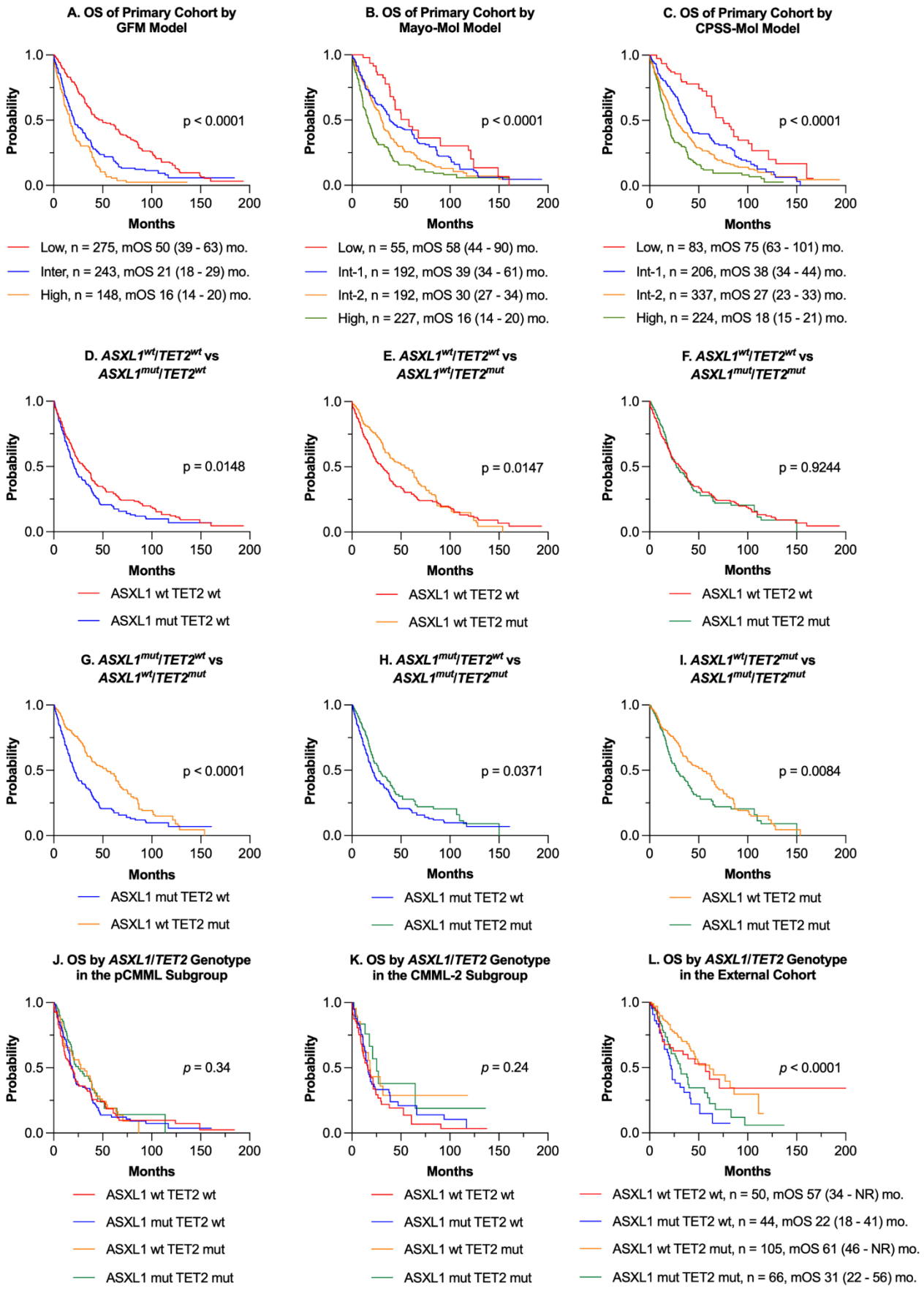
A. OS of Primary Cohort by GFM Model
Probability
1.0
0.5
0.0
p < 0.0001
0
50
100
150
200
Months
Low, n = 275, mOS 50 (39 - 63) mo.
Inter, n = 243, mOS 21 (18 - 29) mo.
High, n = 148, mOS 16 (14 - 20) mo.
B. OS of Primary Cohort by Mayo-Mol Model
Probability
1.0
0.5
0.0
p < 0.0001
0
50
100
150
200
Months
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
Probability
1.0
0.5
0.0
p < 0.0001
0
50
100
150
200
Months
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. ASXL1wt/TET2wt vs ASXL1mut/TET2wt
Probability
1.0
0.5
0.0
p = 0.0148
0
50
100
150
200
Months
ASXL1 wt TET2 wt
ASXL1 mut TET2 wt
E. ASXL1wt/TET2wt vs ASXL1wt/TET2mut
Probability
1.0
0.5
0.0
p = 0.0147
0
50
100
150
200
Months
ASXL1 wt TET2 wt
ASXL1 wt TET2 mut
F. ASXL1wt/TET2wt vs ASXL1mut/TET2mut
Probability
1.0
0.5
0.0
p = 0.9244
0
50
100
150
200
Months
ASXL1 wt TET2 wt
ASXL1 mut TET2 mut
G. ASXL1mut/TET2wt vs ASXL1wt/TET2mut
Probability
1.0
0.5
0.0
p < 0.0001
0
50
100
150
200
Months
ASXL1 mut TET2 wt
ASXL1 wt TET2 mut
H. ASXL1mut/TET2wt vs ASXL1mut/TET2mut
Probability
1.0
0.5
0.0
p = 0.0371
0
50
100
150
200
Months
ASXL1 mut TET2 wt
ASXL1 mut TET2 mut
I. ASXL1wt/TET2mut vs ASXL1mut/TET2mut
Probability
1.0
0.5
0.0
p = 0.0084
0
50
100
150
200
Months
ASXL1 wt TET2 mut
ASXL1 mut TET2 mut
J. OS by ASXL1/TET2 Genotype in the pCMML Subgroup
Probability
1.0
0.5
0.0
p = 0.34
0
50
100
150
200
Months
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
Probability
1.0
0.5
0.0
p = 0.24
0
50
100
150
200
Months
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
Probability
1.0
0.5
0.0
p < 0.0001
0
50
100
150
200
Months
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