Prognostic relevance of variant allele frequency for treatment outcomes in patients with acute myeloid leukemia: a study by the Spanish PETHEMA registry

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Supplemental material

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Table S1: Main characteristics of the diagnosis cohort. ECOG: Eastern Cooperative Oncology Group performance status. ELN2017: European LeukemiaNet. LDAC: low-dose cytarabine. Intensive treatments included '7+3' schemes. Low-dose cytarabine treatments contained cytarabine 70-200 mg/m2 daily as a bolus or infusion for 2-5 days, sometimes in combination with fludarabine in the FLUGA scheme. Patients treated in the hypomethylating group received it as monotherapy, without venetoclax.The type of induction treatment received was only available in 1,178 cases.

		TOTAL	INTENSIVE CHEMOTHERAPY	HYPOMETHYLATING AGENT	LDAC
		N=2,464	N=671	N=327	N=180
GENDER (MEN, (%)) N=2,464		1,399 (56.8%)	365 (54.4%)	101 (56.1%)	188 (57.5%)
AGE AT DIAGNOSIS (YEARS, MEDIAN (RANGE)) (N=2,464)		67 (18-98)	58 (18-78)	76 (64-96)	75 (45-98)
ECOG (N=1,299)	0	529 (40.7%)	271 (49.9%)	77 (26.6%)	67 (39.4%)
	1	527 (40.6%)	218 (40.1%)	132 (45.7%)	67 (39.4%)
	2	160 (12.3%)	36 (6.6%)	60 (20.8%)	25 (14.7%)
	3	63 (4.8%)	12 (2.2%)	20 (6.9%)	8 (4.7%)
	4	20 (1.5%)	6 (1.1%)	0 (0.0%)	3 (1.8%)
BLAST AT DIAGNOSIS IN BONE MARROW (%, MEDIAN (RANGE)) N=990		35 (3-100)	37 (3-100)	30 (5-96)	26,5 (4-98)
LEUKOCYTE COUNT AT DIAGNOSIS (10^9/L, MEDIAN (RANGE)) N=1,319		8.1 (0.3-374)	8.9 (0.3-374)	12.7 (0.4-284)	4.9 (0.6-219)
GENETIC RISK (ELN 2017) N=2,464	Favorable	369 (15.0%)	152 (22.7%)	34 (10.4%)	26 (14.4%)
	Intermediate	837 (34.0%)	223 (33.2%)	73 (22.3%)	58 (32.2%)
	Adverse	1,258 (51.1%)	296 (44.1%)	220 (67.3%)	96 (53.3%)
PRIMARY OR SECONDARY AML	Primary	905 (64.7%)	450 (74.9%)	174 (61.3%)	113 (63.5%)
	Secondary	494 (35.3%)	151 (25.1%)	110 (38.7%)	65 (36.5%)

Table S2. Mutational frequency according to diagnosis, age, gender, and ELN 2017 classification. These results correspond to all samples (diagnosis, refractoriness, and relapse). ELN: European leukemia-net. VAF: variant allele frequency.

						VAF BY AGE			GENDER			PRIMARY OR SECONDARY			VAF BY ELN2017 CLASSIFICATION			
	Mutated patients		Median	Mean	Range VAF	≤ 65 years	> 65 years	P-value	Male	Female	P-value	Primary	Secondary	P-value	Favorable	Intermediate	Adverse	P-value
ABL1	12	0.40%	0.49	0.48	0.35-0.53	0.5	0.47	0.164	0.5	0.47	0.183	0.5	0.49	0.308	0.48	0.48	0.51	0.236
ASXL1	440	14.60%	0.43	0.38	0.01-0.90	0.35	0.39	**0.011**	0.38	0.37	0.575	0.38	0.39	0.426	0.39	0.38	0.36	0.635
BRAF	18	0.60%	0.46	0.37	0.03-0.55	0.38	0.37	0.884	0.41	0.34	0.493	0.43	0.24	0.797	0.03	0.46	0.49	**0.029**
CALR	55	1.80%	0.49	0.49	0.04-0.96	0.48	0.49	0.713	0.49	0.48	0.776	0.5	0.52	**0.014**		0.48	0.49	0.921
CBL	109	3.60%	0.34	0.35	0.01-0.97	0.37	0.33	0.331	0.33	0.38	0.398	0.34	0.34	0.101	0.18	0.4	0.32	0.199
CEBPA	184	6.10%	0.4	0.34	0.01-0.97	0.38	0.31	**0.018**	0.35	0.34	0.812	0.38	0.27	0.871	0.22	0.34	0.39	0.381
CSF3R	87	2.90%	0.46	0.38	0.02-0.89	0.39	0.37	0.578	0.42	0.34	**0.045**	0.36	0.44	0.341	0.49	0.42	0.4	0.886
DNMT3A	734	24.30%	0.44	0.41	0.01-0.98	0.41	0.41	0.836	0.42	0.41	0.174	0.4	0.42	0.465	0.42	0.4	0.42	0.484
ETV6	96	3.20%	0.44	0.38	0.03-0.97	0.38	0.38	0.943	0.39	0.35	0.348	0.4	0.36	**0.003**	0.39	0.35	-	0.375
EZH2	161	5.30%	0.46	0.45	0.01-0.99	0.34	0.49	**0.004**	0.46	0.44	0.786	0.41	0.51	0.835	0.07	0.43	0.46	0.453
FLT3	51	1.70%	0.31	0.32	0.02-0.76	-	0.32	-	-	0.32	0.713	0.31	0.35	0.818	0.32	0.3	-	0.199
FLT3-TKD	166	5.50%	0.16	0.2	0.01-0.62	0.21	0.2	0.765	0.2	0.21	0.934	0.21	0.18	0.927	0.06	0.26	0.14	**0.002**
FLT3-ITD	452	15.00%	0.26	0.28	0.01-0.99	0.3	0.26	0.069	0.27	0.29	0.228	0.3	0.26	0.194	0.26	0.31	0.29	0.814
GATA2	92	3.00%	0.34	0.31	0.01-0.98	0.3	0.31	0.86	0.3	0.31	0.759	0.3	0.33	0.976	-	0.32	0.35	0.616
IDH1	313	10.40%	0.41	0.34	0.01-0.76	0.35	0.33	0.424	0.34	0.34	0.715	0.35	0.32	**0.037**	0.46	0.33	0.34	0.251
IDH2	449	14.90%	0.43	0.4	0.01-0.96	0.37	0.42	**0.001**	0.39	0.4	0.539	0.39	0.42	0.412	0.42	0.39	0.41	0.701
JAK2	158	5.20%	0.46	0.38	0.01-0.99	0.41	0.37	0.324	0.39	0.37	0.632	0.35	0.43	0.306	0.51	0.39	0.33	0.623
KIT	102	3.40%	0.26	0.26	0.01-0.60	0.26	0.25	0.844	0.28	0.23	0.138	0.26	0.29	0.971	0.21	0.26	0.25	0.688
KRAS	222	7.40%	0.16	0.19	0.01-0.70	0.19	0.19	0.948	0.19	0.19	0.969	0.19	0.17	0.335	0.23	0.15	0.26	**0.003**
MPL	52	1.70%	0.46	0.37	0.01-0.91	0.38	0.35	0.529	0.32	0.4	0.194	0.37	0.44	0.554	0.48	0.33	0.47	0.169
NPM1	678	22.50%	0.37	0.35	0.01-0.69	0.34	0.36	**0.009**	0.35	0.35	0.653	0.35	0.34	0.122	0.34	0.34	0.36	0.06
NRAS	464	15.40%	0.21	0.23	0.01-0.94	0.23	0.25	0.145	0.24	0.22	0.16	0.23	0.26	0.498	0.24	0.25	0.26	0.9
PTPN11	170	5.60%	0.18	0.21	0.01-0.51	0.23	0.19	0.109	0.21	0.21	0.995	0.23	0.19	0.687	0.05	0.23	0.19	0.356
RUNX1	566	18.80%	0.41	0.39	0.02-0.99	0.38	0.4	0.2	0.4	0.37	0.173	0.41	0.39	0.826	0.29	0.4	0.4	0.674
SETBP1	98	3.20%	0.41	0.34	0.01-0.72	0.31	0.35	0.21	0.34	0.33	0.823	0.35	0.29	0.644	-	0.31	0.34	0.608
SF3B1	168	5.60%	0.41	0.35	0.02-0.51	0.33	0.36	0.214	0.35	0.34	0.43	0.34	0.36	0.811	0.48	0.33	0.38	0.241
SRSF2	486	16.10%	0.46	0.42	0.01-0.96	0.39	0.42	**0.008**	0.41	0.42	0.894	0.41	0.43	0.618	0.42	0.41	0.44	0.37
TET2	640	21.20%	0.46	0.43	0.01-0.99	0.4	0.44	**0.014**	0.42	0.44	0.098	0.43	0.44	0.217	0.27	0.43	0.42	0.262
TP53	509	16.90%	0.5	0.52	0.01-0.99	0.48	0.54	**0.029**	0.52	0.53	0.555	0.52	0.52	**0.036**	0.02	0.5	0.53	0.169
U2AF1	191	6.30%	0.43	0.37	0.02-0.53	0.36	0.38	0.28	0.38	0.36	0.438	0.38	0.36	0.614	0.45	0.36	0.38	0.747
WT1	177	5.90%	0.31	0.3	0.01-0.98	0.3	0.3	0.93	0.29	0.31	0.614	0.30	0.32	**0.039**	0.2	0.28	0.34	0.255
SH2B3	4	0.10%	0.09	0.1	0.01-0.22	0.1	0.09	0.919	0.11	0.09	0.812	-	-	-	0.05	0.22	0.11	0.247
ZRSR2	2	0.10%	0.1	0.1	0.02-0.17	0.17	0.02	-	1	-	-	-	-	-	-	0.02	0.17	-

Table S3. Mixed-effects ML regression for OS in global AML cohort. To avoid the impact of negative cases on the analyses of the VAF effect, we carried out a mixed-effects ML regression. OR: odds ratio. CI: confidence interval. Effects regression was used to analyze data where there are fixed effects and random effects. This approach is commonly used in studies with hierarchical or nested data, where replications or variability between different levels can be observed. In retrospective studies, where data have already been collected and are analyzed with the aim of finding associations, mixed-effects regression allows modelling both fixed effects (GEN, relapse and death) and random effects (unobserved variability between groups, such as individual differences not explained by the measured variables). Our aim was to control for variability not explained by the observed variables, as there were differences between analysis groups, heterogeneity, which we tried to correct for with random effects. We used the bootstrap cross-validation technique to verify that the model is not over-fitted and that the results are robust and generalizable. This technique allowed us to generate multiple subsets of the data by sampling with replacement. The model is then trained and evaluated on each subset generated. In addition, an assessment of the variance explained by the fixed and random effects was carried out at each iteration of cross-validation, to ensure that the model generalizes well and does not overfit the specific characteristics of the training data. Mixed-effects regression is a useful and common tool in retrospective studies, especially when there is repeated or nested data. However, as with any statistical model, it is important to employ techniques to avoid overestimating effects or artificially increasing statistical significance.

GENE	OR	P-VALUE	95% CI LOW	95% CI HIGH
ASXL1	1.317	**0.036**	0.084	2.550
BRAF	0.582	0.355	-0.651	1.816
CALR	0.132	0.834	-1.101	1.365
CBL	0.201	0.749	-1.032	1.434
CEBPA	-0.363	0.564	-1.597	0.870
CSF3R	0.191	0.762	-1.042	1.424
DNMT3A	-1.156	0.066	-2.389	0.077
ETV6	0.521	0.408	-0.713	1.754
EZH2	0.943	0.134	-0.290	2.176
FLT3	1.382	**0.028**	0.148	2.615
***FLT3*-TKD**	-0.373	0.554	-1.606	0.860
***FLT3*-OTHER**	-0.306	0.626	-1.539	0.927
***FLT3*-ITD**	-0.902	0.152	-2.135	0.331
GATA2	0.107	0.865	-1.126	1.340
HRAS	0.077	0.902	-1.156	1.311
IDH1	-0.279	0.657	-1.513	0.954
IDH2	-0.113	0.858	-1.346	1.120
JAK2	1.400	**0.026**	0.167	2.633
KIT	0.173	0.783	-1.060	1.407
KRAS	-0.096	0.879	-1.329	1.137
MPL	-0.288	0.647	-1.521	0.945
NPM1	-2.417	**<0.001**	-3.651	-1.184
NRAS	0.587	0.351	-0.646	1.821

PTPN11	-0.125	0.842	-1.358	1.108
RUNX1	2.215	**<0.001**	0.982	3.448
SETBP1	0.501	0.426	-0.732	1.734
SF3B1	0.284	0.657	-0.969	1.537
SRSF2	3.263	**<0.001**	2.030	4.496
TET2	2.662	**<0.001**	1.429	3.896
TP53	4.712	**<0.001**	3.479	5.946
U2AF1	1.270	**0.044**	0.036	2.503
WT1	0.662	0.292	-0.571	1.896

Table S4. Empirical cut point estimation for VAF regarding OS, using Youden method. CI: confidence interval. NPV: negative predict. PPV: positive predictive value. VAF: variant allele frequency. Genes with the highest predictive ability have been highlighted, based on Youden index and area under the curve. FLT3 (which includes DIT and SNV) has not been highlighted, because the laboratories that perform amplicon enrichment underestimate the VAF of FLT3-ITD, compared to capture methods; therefore, the distribution of VAF of FLT3-ITD is not homogeneous.

GEN	EMPIRICAL OPTIMAL CUTPOINT (VAF)	YOUDEN INDEX	SENSITIVITY AT CUTPOINT	SPECIFICITY AT CUTPOINT	AREA UNDER ROC	PPV	PPV (95% CI)		NPV	NPV (95 % CI)	
ABL1	0.353	0	0.00	1.00	0.50	25.0%	5.5%	57.2%	66.8%	65.0%	68.5%
ASXL1	0.475	0.031	0.06	0.97	0.52	49.2%	40.3%	58.2%	67.5%	65.8%	69.2%
BRAF	0.114	0.011	0.01	1.00	0.51	85.7%	57.2%	98.2%	67.0%	65.3%	68.7%
CALR	0.533	0.003	0.00	1.00	0.50	66.7%	22.3%	95.7%	66.9%	65.1%	68.6%
CBL	0.118	0.004	0.03	0.97	0.50	36.7%	26.1%	48.3%	66.9%	65.1%	68.6%
CEBPA	0.628	0.002	0.00	1.00	0.50	44.4%	13.7%	78.8%	66.8%	65.1%	68.5%
CSF3R	0.080	0.006	0.03	0.98	0.50	38.6%	27.2%	51.0%	66.9%	65.2%	68.6%
DNMT3A	0.478	0.022	0.07	0.95	0.51	42.0%	34.3%	50.0%	67.3%	65.5%	69.0%
ETV6	0.460	0.013	0.02	0.99	0.51	53.7%	37.4%	69.3%	67.1%	65.3%	68.8%
EZH2	0.181	0.02	0.05	0.97	0.51	44.1%	34.9%	53.5%	67.2%	65.5%	69.0%
FLT3	0.025	0.042	0.04	1.00	0.52	88.0%	75.7%	95.5%	67.7%	66.0%	69.4%
FLT3-ITD	0.772	0.003	0.01	0.99	0.50	44.4%	21.5%	69.2%	66.9%	65.1%	68.6%
GATA2	0.472	0.006	0.01	1.00	0.50	35.4%	23.9%	48.2%	66.8%	65.1%	68.6%
IDH1	0.477	0.014	0.03	0.99	0.51	50.0%	36.3%	63.7%	67.1%	65.4%	68.8%
IDH2	0.463	0.013	0.05	0.96	0.51	40.7%	31.7%	50.1%	67.1%	65.3%	68.8%
JAK2	0.038	0.03	0.07	0.96	0.52	47.5%	39.0%	56.1%	67.5%	65.7%	69.2%
KIT	0.454	0.009	0.01	1.00	0.50	59.1%	36.4%	79.3%	67.0%	65.3%	68.7%
KRAS	0.029	0.005	0.06	0.94	0.50	35.2%	28.2%	42.7%	66.9%	65.1%	68.7%
MPL	0.503	0.001	0.00	1.00	0.50	36.4%	10.9%	69.2%	66.8%	65.1%	68.5%
NPM1	0.468	0.016	0.04	0.98	0.51	47.3%	35.6%	59.3%	67.2%	65.4%	68.9%
NRAS	0.408	0.019	0.04	0.98	0.51	47.3%	36.7%	58.0%	67.2%	65.5%	69.0%
PTPN11	0.480	0.004	0.01	1.00	0.50	71.4%	29.0%	96.3%	66.9%	65.1%	68.6%
RUNX1	0.043	0.042	0.21	0.83	0.52	38.4%	34.2%	42.6%	67.9%	66.0%	69.8%
SETBP1	0.005	0.015	0.04	0.97	0.51	43.3%	33.3%	53.7%	67.1%	65.4%	68.9%
SF3B1	0.468	0.011	0.02	0.99	0.51	52.9%	35.1%	70.2%	67.1%	65.3%	68.8%
SRSF2	0.028	0.073	0.21	0.87	0.54	43.4%	38.9%	48.0%	68.7%	66.9%	70.5%
TET2	0.030	0.054	0.24	0.81	0.53	39.0%	35.1%	42.9%	68.3%	66.4%	70.2%
TP53	0.024	0.079	0.22	0.86	0.54	43.9%	39.4%	48.4%	68.9%	67.0%	70.7%
U2AF1	0.011	0.028	0.08	0.95	0.51	43.2%	36.0%	50.7%	67.5%	65.7%	69.2%
WT1	0.035	0.019	0.07	0.95	0.51	40.9%	33.2%	48.9%	67.2%	65.5%	69.0%

Table S5. Univariate analysis, comparing VAF average between responder and no responder patients. VAF: variant allele frequency. SD: standard deviation.

	RESPONDER		**NO RESPONDER**		
	Mean	SD	Mean	SD	p-value
AGE	57.58	13.95	68.23	12.86	0.009
LEUKOCYTE COUNT	37.3	56.83	29.62	53.38	0.012
***ASXL1* VAF**	**3.31**	**0.1188**	**0.0729**	**0.1764**	**<0.001**
***BRAF* VAF**	**0.0001**	**0.0016**	**0.0051**	**0.049**	**<0.001**
***CSF3R* VAF**	0.0101	0.0656	0.0062	0.0577	0.047
***DNMT3A* VAF**	**0.1248**	**0.2028**	**0.0913**	**0.1873**	**<0.001**
***EZH2* VAF**	0.0185	0.0994	0.0317	0.1477	0.001
***FLT3* VAF**	**0.0057**	**0.0471**	**0.0201**	**0.09**	**<0.001**
***FLT3*-TKD VAF**	**0.0178**	**0.0727**	**0.0039**	**0.0296**	**<0.001**
***FLT3*-ITD VAF**	**0.0531**	**0.1447**	**0.0302**	**0.1122**	**<0.001**
***IDH2* VAF**	**0.0506**	**0.1439**	**0.0748**	**0.1828**	**<0.001**
***JAK2* VAF**	**0.0122**	**0.0745**	**0.0316**	**0.1295**	**<0.001**
MPL VAF	0.0039	0.0427	0.0096	0.0717	0.003
***NPM1* VAF**	**0.1369**	**0.183**	**0.0603**	**0.1491**	**<0.001**
***PTPN11* VAF**	**0.0212**	**0.0809**	**0.0115**	**0.065**	**<0.001**
***RUNX1* VAF**	**0.0648**	**0.1682**	**0.099**	**0.2182**	**<0.001**
***SETBP1* VAF**	**0.0082**	**0.0614**	**0.0169**	**0.0867**	**<0.001**
***SRSF2* VAF**	**0.0365**	**0.1248**	**0.1046**	**0.1984**	**<0.001**
***TET2* VAF**	**0.0768**	**0.1758**	**0.1166**	**0.2226**	**<0.001**
***TP53* VAF**	**0.0394**	**0.1527**	**0.126**	**0.2809**	**<0.001**
***U2AF1* VAF**	**0.0114**	**0.0662**	**0.0331**	**0.1166**	**<0.001**