SUPPLEMENTARY APPENDIX

Monitoring of clonal evolution of acute myeloid leukemia identifies the leukemia subtype, clinical outcome and potential new drug targets for post-remission strategies or relapse

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SUPPLEMENTAL DATA.

Detailed description of clinical treatments and follow-up.

Patient 1.

Female, diagnosed with *de* novo AML at the age of 31 years. Patient received cytabine (Cyt) plus idarubicine (Ida) (3+7 scheme) as first-line induction therapy, without response. She received a second regimen based on a high dose of cytabine (Cyt-HD) and amsacrine (Ams), resulting in the persitance of blasts (Rf1 sample). The patient then received a third induction regimen with mitoxantrone (Mtx), etoposide (Eto) and gemtuzumab ozogamicin (GO), reaching the first complete remission (CR). She subsequently underwent an allogeneic transplant (allo-HSCT) and currently continues in CR.

Patient 2.

Female, diagnosed with secondary AML from essential thrombocythemia at the age of 49 years with chromosome 7 monosomy. The patient received induction therapy with Cyt plus Ida (3+7 scheme), resulting in persistence of blasts (Rf1 sample). Resistance persisted after the second treatment regimen of Cyt-HD combined with Eto and GO, and after the third treatment regimen with clofarabine (Clo).

Patient 3.

Male, diagnosed with secondary AML from a myeloproliferative neoplasm with complex karyotype at the age of 69 years. The patient received azacytidine (Aza) without response (Rf1 sample). Unfortunately, the disease progressed and he died.

Patient 4.

Male, diagnosed with *de novo* AML at the age of 78 years with the translocation t(8;21)(q11;q22). Patient was treated with Cyt plus fludarabine (Flu), and showed refractoriness after the first cycle (Rf1 sample 1) and the second cycle (Rf1 sample 2). The patient died without reaching CR.

Patient 5.

Male, diagnosed with *de novo* AML with 5q and 17p deletions at the age of 63 years. He received induction treatment according to the 3+7 scheme but showed refractoriness (Rf1 sample 1). He started on a combined induction cycle with Flu, Cyt, Ida and plerixafor (Pxf) but also remained refractory (Rf1 sample 2). The patient received Aza as a therapeutic alternative but maintained refractoriness and died without reaching CR.

Patient 6.

Male, diagnosed with *de novo* AML with chromosome 13 trisomy and chromosome 21 monosomy at the age of 66 years. He received the first induction cycle with Cyt and Ida (3+7

scheme), showing partial remission (PR1 sample 1 and PR1 sample 2). The patient received a second identical cycle that allowed him to reach CR (CR1 sample 1). Subsequently, he received consolidation with Cyt-HD maintaining CR (CR1 samples 2 and 3). He underwent an autologous HSCT (auto-HSCT). He subsequently relapsed, and started rescue treatment based on decitabine (Dec) plus Cyt, to which he showed refractoriness. Unfortunately, because of the lack of response to the treatment, the patient died.

Patient 7.

Female, diagnosed with *de novo* AML, with a profile of myeloid sarcoma, at the age of 36 years with chromosome 16 monosomy and 1q duplication. She received the first induction cycle with Cyt and Ida (scheme 3+7), without obtaining response (PR1 sample). She then received the FLAG-Ida scheme treatment (Flu+Cyt+Ida). But unfortunately, the patient died because of the progression of the disease. No variants were detected at the time of diagnosis or during the refractoriness response.

Patient 8.

Male. Diagnosed with *de novo* AML at the age of 24 years with complex karyotype. He received induction treatment with Cyt and Ida (3+7 scheme), but showed refractoriness (PR1 sample 1). He received a second scheme based on Flu+Cyt+Ida+Pxf without obtaining response (PR1 sample 2) and finally died because of the disease. No variants were detected at the time of the diagnosis or during the refractoriness response.

Patient 9.

Male, diagnosed with *de novo* AML at the age of 42 years with inv(2). Patient received Cyt + daunorrubicine (Dau) + midostaurin (Mid) as first line induction treatment, showing initial refractoriness (Rf1 sample). He then received an identical scheme and reached CR (CR1 sample). Treatment was followed by 4 cycles of consolidation with Cyt-HD and Mid. The patient relapsed early (R1 sample) and received rescue treatment based on FLAG-Ida scheme (Flu+Cyt+Ida), which allowed him to reach CR (CR2 sample 1). The patient underwent an allo-HSCT, maintaining CR for 3 years (CR2 sample 2 and 3), but he subsequently experienced extramedullary relapses several times and finally died of the disease.

Patient 10.

Female, diagnosed with *de novo* AML at the age of 62 years with complex karyotype. She received the first cycle of induction with Cyt and Ida (3+7 scheme) without response (Rf1 sample). She then received a second identical scheme reaching CR (CR1 sample 1). The patient received consolidation with Cyt-HD maintaining CR (CR1 sample 2) and underwent auto-HSCT, maintaining CR (CR1 sample 3). However, the patient relapsed (R1 sample) and was started on

a rescue treatment based on Flu+Cyt+Ida without obtaining a response and finally died of the disease.

Patient 11.

Female, diagnosed with *de novo* AML at the age of 61 years with normal karyotype. The patient received an induction cycle with Cyt and Ida (scheme 3+7), reaching CR. She then received consolidation treatment consisting of two cycles of Cyt-HD, maintaining CR during 96 months. She subsequently relapsed (R1 sample).

Patient 12.

Male, diagnosed with *de novo* AML at the age of 28 years with inv(16). He received an induction cycle according to a standard 3+7 scheme achieving CR. He then received Cyt-HD plus Ida and Cyt-HD plus Ams, maintaining CR in all cases. The patient received an auto-HSCT but experienced a relapse (R1 sample). He then received a rescue treatment based on Flu+Cyt+Ida+GO, reaching CR again, and underwent an allo-HSCT, maintaining CR.

Patient 13.

Female, diagnosed with *de novo* AML at the age of 54 years with chromosome 13 trisomy. The patient received induction treatment according to 3+7 scheme achieving partial response (PR), and then received a second induction cycle based on Cyt+Ida+GO, reaching CR. The consolidation treatment consisted of Cyt-HD and an auto-HSCT, which maintained CR. The patient relapsed (R1 sample), and received a rescue treatment based on Flu+Cyt+Ida, reaching CR again (CR1 sample). She then started Aza treatment.

Patient 14.

Female, diagnosed with *de novo* AML with normal karyotype at the age of 60 years. She received the first induction cycle according to the 3+7 scheme with Cyt and Ida, reaching CR, followed by a second induction cycle with the same scheme plus GO, and a consolidation cycle based on Cyt-HD and an auto-HSCT, always maintaining CR (CR1 sample). After a few months the patient relapsed (R1 sample).

Patient 15.

Male, diagnosed with secondary AML from a myelodysplastic syndrome at the age of 63 years. The patient was treated with two cycles of induction 3+7, reaching CR (CR1 sample). But the disease progressed early (R1 sample). He received an allo-HSCT and achieved and maintained CR (CR2 sample) until he died.

Patient 16.

Female, diagnosed with *de novo* AML with *MLL* deletion at the age of 34 years. She received standard induction treatment according to 3+7 scheme (Cyt+Ida) with PR. The patient then received re-induction (scheme 3+7) and Cyt-HD, reaching CR (CR1 sample). The patient then received an auto-HSCT, but relapsed (R1 sample). Subsequently she received a rescue treatment according to the FLAG-Ida scheme (Flu+Cyt+Ida). However, the patient died without reaching CR again.

Patient 17.

Male, diagnosed with *de novo* AML at the age of 71 years with normal karyotype. He received the first cycle of induction (Cyt+Ida, scheme 3+7) showing PR, and so he received a second identical cycle, reaching CR. He then received the consolidation regimen based on Cyt-HD; however, the patient relapsed early (R1 sample) that was sustained (R2 sample). After that, he received Aza, but died soon after.

Patient 18.

Male, diagnosed with *de novo* AML at the age of 51 years with inv(16). The patient received a first cycle of chemotherapy according to the 3+7 scheme, reaching CR (CR1 sample 1). The patient received a new cycle 3+7 maintaining CR (CR1 sample 2), and then consolidation treatment was established with Cyt-HD, followed by an auto-HSCT, and maintaining CR (CR1 sample 3) for some years. However, the patient relapsed (R1 sample) and received FLAG-Ida (Flu+Cyt+Ida) as a rescue treatment, being refractory initially but ultimately reaching CR.

Patient 19.

Male diagnosed with *de novo* AML at the age of 75 years with chromosome 6 trisomy and t(20;6)(p12;q13). The patient received two cycles of chemotherapy 3+7 reaching CR. Consolidation treatment with Cyt-HD was given, maintaining CR. However, the patient relapsed (R1 sample), and started a rescue treatment based on Flu+Cyt+Ida without reaching CR, and he died soon after.

Patient 20.

Male, diagnosed with *de novo* AML at the age of 59 years with normal karyotype. The patient received two cycles of the 3+7 scheme, reaching CR (CR1 sample 1). The patient received a consolidation cycle based on Cyt-HD maintaining CR (CR1 sample 2), and finally an auto-HSCT. He subsequently relapsed (R1 sample) and received 3+7 and also Flu+Cyt+Ida, reaching again CR (CR2 sample), although he died some months later.

Patient 21.

Female, diagnosed with *de novo* AML at the age of 65 years with normal karyotype. The patient received two cycles of the 3+7 scheme as induction treatment, achieveing CR (CR1 sample 1). The patient then received a consolidation scheme based on Cyt-HD, maintaining CR (CR1 sample 2 and 3). However, the patient relapsed (R1 sample) and started second-line treatment composed of a combined induction cycle of Flu+Cyt+Ida+Pxf, reaching CR (CR2 samples 1 and 2). She then received a first consolidation cycle composed of Cyt+Pxf, maintaining CR (CR2 sample 2). Despite that, the patient experienced a second relapse, (R2 sample), but did not receive any other alternative treatment due to the adversity of clinical features, and she died two months later.

Patient 22.

Male, diagnosed with *de novo* AML at the age of 42 years with normal karyotype. Patient received induction treatment according to the 3+7 scheme, entering CR (CR1 sample 1), and continued on the standard consolidation treatment based on an identical cycle (3+7) and Cyt-HD, maintaining CR (CR1 sample 2), culminating in an auto-HSCT, and after that showed PR (PR1 sample). The patient relapsed (R1 sample) and started a rescue treatment based on Cyt+Ida+Flu+Pxf achieving PR (PR2 sample). The patient then received a consolidation regimen consisting of Cyt+Pxf, acheiving PR (Rf2 sample). After that, he underwent allo-HSCT but died a few months later.

Patient 23

Female, diagnosed with *de novo* AML at the age of 35 years with t(9;11)(p22;q23). She received two cycles of the 3+7 scheme and Cyt-HD before reaching CR (CR1 sample 1). She then underwent an auto-HSCT, maintaining CR (CR1 samples 1, 2 and 3) Finally, the patient relapsed (R1 sample), and received a rescue treatment based on Cyt+Ida+Flu+Pxf, showing refractoriness (Rf1 and Rf2 samples). She then started Aza treatment without obtaining response (Rf3 sample). She then received a new rescue treatment based on Cyt+Eto+Mtx without success, and finally died of the disease. No allelic variants were detected in any of the 7 samples studied.

SUPPLEMENTAL TABLES & FIGURES

Supplemental Table S1. Genes included in the sequencing panel

List of the genes included in the custom NGS panel. The table indicates the pathway to which the gene belongs (Pathways), the gene name (Gene), the number of the chromosome (Chr), start genomic coordinates (Start), end genomic coordinates (End), numbers of amplicons that are included (Amplicons), the percentage of the gene that the sequencing covered (Coverage %) and the number of exons (Exons).

Pathways	Gene	Chr	Start	End	Amplicons	Coverage (%)	Exons
CALR	***CALR***	19	13049314	13055076	23	86	9
Transcriptional regulation	***ASXL1***	20	30954090	31025087	52	91	13
	EZH2	7	148504653	148544423	44	99	21
	PHF6	X	133511597	133559416	22	98	11
Epigenetic regulation	***DNMT3A***	2	25457019	25523119	51	91	25
	TET2	4	106155047	106197701	64	99	10
	IDH1	2	209101751	209116313	22	98	8
	IDH2	15	90627407	90634952	21	87	11
	KDM6A	X	44732713	44970702	64	93	29
	KMT2A	11	118339409	118392930	145	96	37
Splicing	***SF1***	11	64532722	64545911	30	80	19
	SF3A1	22	30730553	30752852	37	94	18
	SF3B1	2	198256947	198299851	66	97	26
	SRSF2	17	74732208	74733231	5	70	2
	U2AF1	21	44513107	44524598	15	87	10
	ZRSR2	X	15808511	15841407	26	97	11
	PRPF40B	12	50024310	50037977	54	95	26
Cytokine signaling & JAK/STAT way	***EPOR***	19	11488599	11495009	21	93	8
	FLT3	13	28578144	28644774	53	97	24
	JAK2	9	5021946	5126885	57	97	23
	KIT	4	55524151	55604786	51	99	22
	SH2B3	12	111855922	111886159	15	64	7
	MPL	1	43803438	43818424	30	92	12
	CBL	11	119077153	119170540	41	93	16
RAS pathway	***HRAS***	11	532519	534348	10	83	5
	NRAS	1	115251095	115258874	9	100	4
	KRAS	12	25362621	25398385	10	83	5
Transcription factors	***ETV6***	12	11802955	12044078	20	94	8
	RUNX1	21	36164534	36421235	18	69	10
Tumor suppressor	***VHL***	3	10183314	10195319	27	55	3
	TP53	17	7572847	7579960	21	93	13
	PTEN	10	89624161	89725315	21	93	9

Supplemental Table S2. Variants detected

List of detected allelic variants by NGS pipelines. Indicated in the table is the name of the gene (Gene), the chromosome number where the variant is located (Chr), the location in chromosomal coordinates (Location), the nomenclature of the variant in DNA sequence according to HGVS criteria (HGVS cDNA), the nomenclature of the variant in protein sequence according to HGVS criteria (HGVS Protein), the effect it causes **(**Effect), type of the variant (Type: SNV or InDel), level according to custom pipeline categorized from 1 to 5 (see **Supplemental Figure S2)** and ACMG classification (Benign, Likely benign,VUS, Likely Pathogenic, Pathogenic). (1)

Gene	Chr	Location	HGVS cDNA	HGVS Protein	Effect	Type	Level	ACMG Clasification
ASXL1	20	31023403	c.2888C>T	p.Pro963Leu	missense	SNV	4	Likely Benign
ASXL1	20	31023821	c.3306G>T	p.Glu1102Asp	missense	SNV	1	Benign Likely Benign
ASXL1	20	31023408	c.2894del	p.Gly966del	Inframe deletion	InDel	5	Likely pathogenic
CALR	19	13054627	c.1154insTTGTC	p.Lys385fs	Frameshift insertion	InDel	5	Likely pathogenic
CBL	11	119103319	c.357G>A	p.Met119Ile	missense	SNV	3	VUS
CBL	11	119077179	c.56dup	p.Ser20LeufsTer61	Frameshift insertion	InDel	4	Pathogenic Likely pathogenic
DNMT3A	2	25457243	c.2644C>T	p.Arg882Cys	missense	SNV	1	Pathogenic
DNMT3A	2	25457252	c.2635A>G	p.Asn879Asp	missense	SNV	1	Pathogenic Likely pathogenic
DNMT3A	2	25470497	c.977G>T	p.Arg326Leu	missense	SNV	3	Likely pathogenic
DNMT3A	2	25469945	c.1096ins	p.Arg366fs	Frameshift insertion	InDel	5	VUS
DNMT3A	2	25463290	c.2202_2203del	p.Phe734LeufsTer6	Frameshift deletion	InDel	5	Pathogenic Likely pathogenic
EPOR	19	11488844	c.1343C>A	p.Thr448Asn	missense	SNV	3	Likely Benign
EPOR	19	11494811	c.73dup	p.Ala25GlyfsTer5	Frameshift insertion	InDel	5	VUS
EPOR	19	11494835	c.49_50insG	p.Leu17ArgfsTer13	Frameshift insertion	InDel	5	VUS
ETV6	12	12038908	c.1201T>G	p.Tyr401Asp	missense	SNV	3	Likely pathogenic
ETV6	12	12038918	c.1212del	p.Asn405fs	Frameshift deletion	InDel	5	Pathogenic Likely pathogenic
EZH2	7	148506462	c.2050C>T	p.Arg684Cys	missense	SNV	1	Likely pathogenic
EZH2	7	148512096	c.1582T>C	p.Cys528Arg	missense	SNV	1	Likely pathogenic
EZH2	7	148516756	c.931T>A	p.Tyr311Asn	missense	SNV	3	Likely pathogenic

FLT3	13	28592623	c.2522A>T	p.Asn841Ile	missense	SNV	1	Pathogenic Likely pathogenic
FLT3	13	28592642	c.2503G>T	p.Asp835Tyr	missense	SNV	1	Likely pathogenic
FLT3	13	28609724	c.1505A>T	p.Asn502Ile	missense	SNV	4	VUS
FLT3	13	2861015	c.1337C>T	p.Ser446Leu	missense	SNV	1	VUS
IDH1	2	209113113	c.394C>T	p.Arg132Cys	missense	SNV	1	Pathogenic
IDH2	15	90631838	c.515G>A	p.Arg172Lys	missense	SNV	1	Likely pathogenic
IDH2	15	90631934	c.419G>A	p.Arg140Gln	missense	SNV	1	Pathogenic
IDH2	15	90631935	c.418C>T	p.Arg140Trp	missense	SNV	1	Likely pathogenic
JAK2	9	5073770	c.1849G>T	p.Val617Phe	missense	SNV	1	Pathogenic
JAK2	9	5126715	c.3323A>G	p.Asn1108Ser	missense	SNV	1	Benign Likely Benign
JAK2	9	5080558	c.2308_2309insT	p.His770LeufsTer17	Frameshift insertion	InDel	1	Pathogenic Likely pathogenic
KDM6A	X	44733223	c.215T>G	p.Leu72Arg	missense	SNV	2	VUS
KIT	4	55604640	c.2848G>A	p.Val950Met	missense	SNV	4	Benign Likely Benign
KIT	4	5558977	c.1253_1255delACG	p.Asp419del	Frameshift Deletion	InDel	5	Likely pathogenic
KIT	4	55602762	c.2583dup	p.Leu862AlafsTer17	Frameshift insertion	InDel	1	Pathogenic Likely pathogenic
KMT2A	11	118343378	c.1505A>T	p.Asn502Ile	missense	SNV	4	Likely Benign
KMT2A	11	118344081	c.2207G>T	p.Arg736Met	missense	SNV	2	VUS
KMT2A	11	118352769	c.3974G>A	p.Ser1325Asn	missense	SNV	4	Benign Likely Benign
KMT2A	11	118374758	c.8142C>G	p.Ile2714Met	missense	SNV	3	Likely Benign
KMT2A	11	118377003	c.10396A>G	p.Thr3466Ala	missense	SNV	4	Benign Likely Benign
KRAS	12	25398285	c.34G>A	p.Gly12Ser	missense	SNV	1	Pathogenic
KRAS	12	25378603	c.395dup	p.Asp132GlufsTer12	Frameshift insertion	InDel	5	Pathogenic Likely pathogenic
MPL	1	43818306	c.1771T>G	p.Tyr591Asp	missense	SNV	2	VUS
NRAS	1	115256528	c.183A>C	p.Gln61His	missense	SNV	1	Pathogenic
NRAS	1	115258744	c.38G>A	p.Gly13Asp	missense	SNV	1	Pathogenic
NRAS	1	115258745	c.37G>T	p.Gly13Cys	missense	SNV	1	Pathogenic
NRAS	1	115258747	c.35G>A	p.Gly12Asp	missense	SNV	1	Pathogenic
PHF6	X	133549140	c.824G>A	p.Gly275Glu	missense	SNV	3	Pathogenic
PRPF40B	12	50031516	c.1676G>C	p.Gly559Ala	missense	SNV	3	VUS
PTEN	10	89624271	c.45dup	p.Tyr16IlefsTer28	Frameshift insertion	InDel	5	Likely pathogenic
RUNX1	21	36164627	c.1247ins	p.Phe416LeufsTer185	Frameshift insertion	InDel	2	Pathogenic Likely pathogenic
RUNX1	21	36231773	c.611G>A	p.Arg204Gln	missense	SNV	1	Pathogenic

RUNX1	21	36231791	c.593A>G	p.Asp198Gly	missense	SNV	1	Likely pathogenic
SF3A1	22	30733135	c.1985insC	p.Ala663fs	Frameshift insertion	InDel	5	VUS
SF3B1	2	198267303	c.2054G>A	p.Ser685Asn	missense	SNV	1	VUS
SF3B1	2	198266834	c.2098A>G	p.Lys700Glu	missense	SNV	1	Pathogenic
SF3B1	2	198270040	c.1396T>A	p.Phe466Ile	missense	SNV	4	VUS
SRSF2	17	74733073	c.170T>A	p.Phe57Tyr	missense	SNV	1	VUS
TET2	4	106157845	c.2746C>T	p.Gln916Ter	Stop gained	SNV	1	Pathogenic
TET2	4	106164767	c.3635T>C	p.Leu1212Ser	missense	SNV	1	VUS
TET2	4	106164913	c.3781C>T	p.Arg1261Cys	missense	SNV	1	Likely pathogenic
TET2	4	106164032	c.3543del	p.Tyr1182fs	Frameshift insertion	InDel	5	Pathogenic Likely pathogenic
TP53	17	7573931	c.1096T>G	p.Ser366Ala	missense	SNV	4	Benign Likely Benign
TP53	17	7577097	c.841G>T	p.Asp281Tyr	missense	SNV	1	Likely pathogenic
TP53	17	7577099	c.839G>A	p.Arg280Lys	missense	SNV	1	Likely pathogenic
TP53	17	7578413	c.517G>A	p.Val173Met	missense	SNV	1	Pathogenic
TP53	17	7578398	c.532dup	p.His178ProfsTer3	Frameshift insertion	InDel	1	Pathogenic Likely pathogenic
TP53	17	7577112	c.825del	p.Cys275fs	Frameshift deletion	InDel	5	Pathogenic Likely pathogenic
U2AF1	21	44514777	c.470A>G	p.Gln157Arg	missense	SNV	5	Likely pathogenic
VHL	3	10188307	c.450dup	p.Ile151TyrfsTer23	Frameshift insertion	InDel	5	Pathogenic Likely pathogenic
ZRSR2	X	15841230	c.1314insAGCCGG	p.Gly438_Ser439insSerArg	Non-frameshift Insertion	InDel	5	VUS

Supplemental Table S3. Samples evaluated.

Table summary of the samples included in the study of therapeutic failure. Are detailed the samples included per patient listed from Patient 1 to Patient 23 (P1-P23), the type of sample evaluated (BM=bone marrow, PB=peripheral blood), the moment evaluated (dx=diagnosis, Rf=refractoriness, R=relapse, PR=partial remission, CR=complete remission; s indicate sample following the evaluated sample number). It also details the time elapsed since the diagnosis, expressed in days and in months.

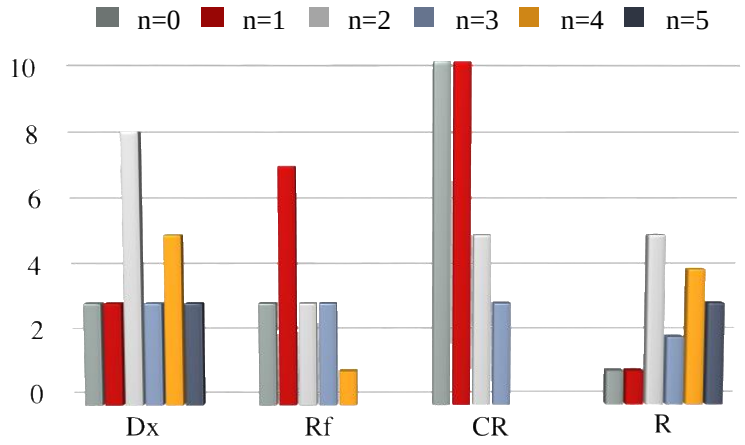
Patient	Type of sample	Time evaluated	Days elapsed	Months elapsed
P1	BM	Dx		
	BM	Rf1	70	2.3
P2	BM	Dx		
	BM	Rf1	104	3.4
P3	BM	Dx		
	BM	Rf1	64	2.1
P4	PB	Dx		
	BM	Rf1_s1	35	1.15
	BM	Rf1_s2	102	3.34
P5	BM	Dx		
	BM	Rf1_s1	42	1.38
	BM	Rf1_s2	85	2.79
P6	BM	Dx		
	BM	PR_s1	36	1.18
	BM	PR_s2	70	2.3
	BM	CR1_s1	118	3.87
	BM	CR1_s2	115	4.95
	BM	CR1_s3	166	5.44
P7	BM	Dx		
	PB	PR1	12	0.39
P8	PB	Dx		
	BM	Rf1_s1	38	1.25
	BM	Rf1_s2	65	2.13
P9	BM	Dx		
	BM	Rf1	25	0.82
	BM	CR1	65	2.13
	BM	R	357	11.70
	BM	CR2_s1	399	13.08
	BM	CR2_s2	1885	61.8
	BM	CR2_s3	2058	67.48
P10	BM	Dx		
	BM	Rf1	38	1.25
	BM	CR1_s1	85	2.79
	BM	CR1_s2	155	5.08
	BM	CR1_s3	255	8.36
	BM	R1	328	10.75

P11	PB	Dx		
	BM	R1	2923	95.84
P12	PB	Dx		
	BM	R1	672	22.03
P13	BM	Dx		
	BM	R1	1234	40.46
	BM	CR1	1304	42.75
P14	PB	Dx		
	BM	CR1	305	10
	BM	R1	553	18.13
P15	BM	Dx		
	BM	CR1	47	1.54
	BM	R1	186	6.1
	BM	CR2	333	10.92
P16	BM	Dx		
	BM	CR1	143	4.69
	BM	R1	298	9.77
P17	BM	Dx		
	BM	R1	203	6.66
	BM	R2	258	8.46
P18	BM	Dx		
	BM	CR1_s1	34	1.11
	BM	CR1_s2	78	2.56
	BM	CR1_s3	578	18.95
	BM	R1	1324	43.41
P19	BM	Dx		
	PB	R1	317	10.39
P20	PB	Dx		
	BM	CR1_s1	99	3.25
	BM	CR1_s2	146	4.79
	BM	R1	1144	37.51
	BM	CR2	1316	43.15
P21	BM	Dx		
	BM	CR1_s1	92	3.02
	BM	CR1_s2	134	4.39
	BM	CR1_s3	269	8.82
	BM	R1	336	11.02
	BM	CR2_s1	399	13.08
	BM	CR2_s2	521	17.08
	BM	R2	577	18.92
P22	BM	Dx		
	BM	CR1_s1	30	0.98
	BM	CR1_s2	130	4.26
	BM	PR1	228	7.48
	BM	R1	323	10.59
	BM	PR2	359	11.77
	BM	Rf2	423	13.87
P23	BM	Dx		
	BM	CR1_s1	140	4.59

	BM	CR1_s2	225	7.38
	BM	CR1_s3	276	9.05
	BM	R1	350	11.48
	PB	Rf1_s1	389	12.75
	BM	Rf1_s2	426	13.97
	BM	Rf1_s3	464	15.21
N=23		N=91		

Supplemental Figure S1. Number of variants per patient.

The figure represents the number of samples (vertical axis) in which no variant has been detected (dark grey), one variant has been detected (red), two variants (grey), three variants (blue), four variants (yellow) and five variants (dark blue). They are represented grouped by different clinical states (Dx=diagnosis; Rf= refractoriness, CR=complete remission, R=Relapse).



Supplemental Figure S2. Filtering and prioritization of variants.

Once the massive sequencing data was obtained, in *.fastq* format, a series of concatenated processes including:

- Annotation of allelic variants, using the RUbioseq bioinformatics tool (2). Which includes technical filtering that discards first and automatically variants with a depth of coverage less than 15 genomic sequences or readings and in second place discards sequences with quality values less than Q_{30}. As a result, a file in .xls format is obtained, where 39 parameters relative to the information obtained from each detected allelic variant.

- Filtering and prioritization of the variants, through a pipeline of our own design developed in R environment (v3.4.4), starting from each of the files annotated with the variants corresponding to each of the patients, each annotated variant is labeled with a patient identification code (HUCN), as well as sequencing data (RUN, BARCODE); which allows us to unify all the files in a single file, and incorporate an internal counter of concurrences of the same variant in the studied cohort.

Variant prioritization starts from a single file containing all non-recurring variants, those variants located in the group of control samples were eliminated, enhancing the selection of somatic variants. The third criterion selects those variants that affect coding regions depending on the effect of the variant at the transcript level: stop gained, frameshift variant, stop lost, start lost, inframe insertion, inframe deletion, missense variant, protein altering variant and coding sequence variant.

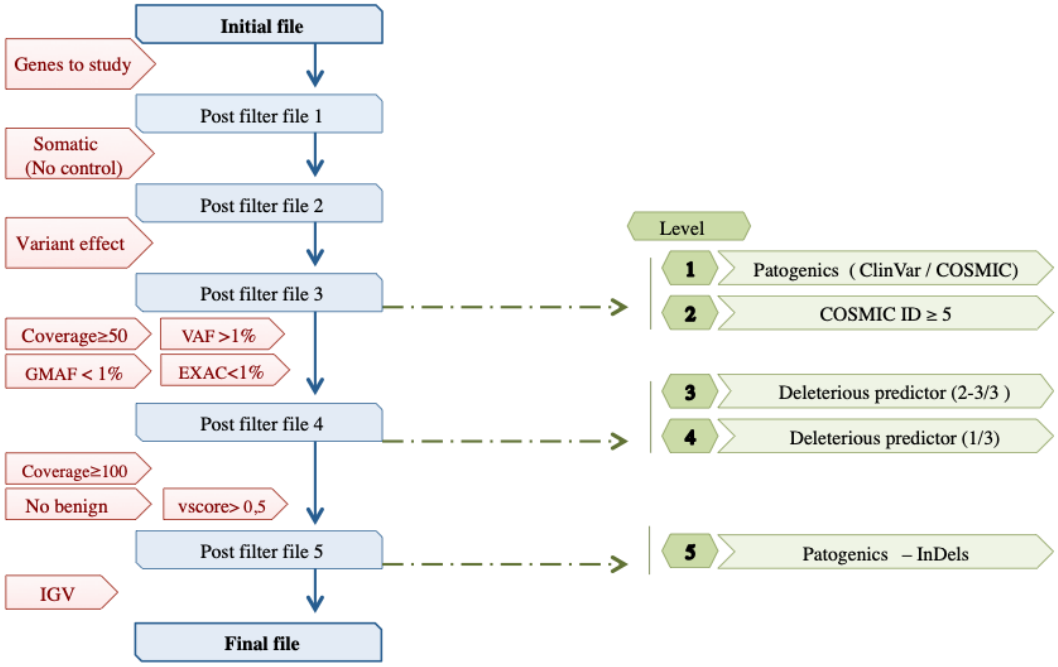
Level 1 variants are labeled at this point, variants described as pathogenic at the base from ClinVar (3) or COSMIC data (4); and variants of **level 2**, variants identified in COSMIC with a described prevalence of at least 5 evidences bibliographic.

The rest of the variants follow the filtering flow, in which the alternative variants that they do not reach a minimum depth (coverage) established in 50 readings, as well as the variants with an allelic frequency (VAF) less than 1%, since it is considered an artifact of sequencing indicating, in most cases, poor sample quality or errors in the sequencing. Furthermore, in order to discriminate polymorphisms, those variants are discarded. Whose allelic frequency in the global population (GMAF) is greater than 1%, or variants described in Exomas Aggregation Consortium (ExAC) (5) bases above 1%.

Next, based on 3 predictors of functional impact *in silico*: SIFT (6), CONDEL (7) and PolyPhen (8), the variants are classified into deleterious variants (according to SIFT or CONDEL criteria) or in harmful variants (according to PolyPhen criteria). **Level 3** variants contain variants with adverse prediction in 2-3 of the 3 predictors. And **level 4** variants contain variants with adverse prediction in 1 of them.

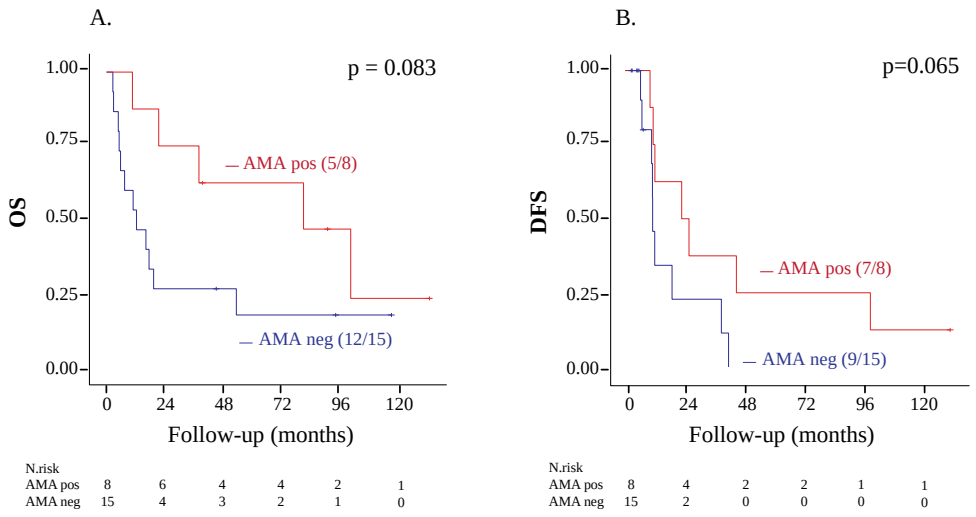
The rest of the variants, the vast majority of which are InDels, follow the filtering flow where the alternative variants that do not reach a minimum reading depth of 100, the variants described as benign according to PolyPhen criteria, and those with a custom score greater than 0.5. The variants obtained after this filtering is grouped at level 5.

After the computerized automated filtering, a manual screening of the variants was carried out by viewing them in The Integrative Genomics Viewer (IGV) software.



Supplemental Figure S3. Survival curves of additional molecular abnormalities features.

Kaplan-Meier survival curves of patients presenting with additional molecular abnormalities (AMA pos) *versus* absence of additional molecular abnormalities (AMA neg) for overall survival (OS, 3A) and for disease free survival (DFS, 3B). Follow-up is represented in months. Number of censored patients with respect to the stratified groups and the number at risk is indicated.



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