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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Received: April 4, 2020.
Accepted: July 20, 2020.
Pre-published: July 30, 2020.
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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 persistence 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).
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 + daunorubicine (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, achieving 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, achieving 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   | 14850463  | 148544423  | 44        | 99           | 21    |
|                                   | PHF6 | X   | 13351157  | 133559416  | 22        | 98           | 11    |
| Transcriptional regulation        | DNMT3A| 2   | 25457019  | 25523119   | 51        | 91           | 25    |
| Epigenetic regulation             | 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    |
| Epigenetic regulation             | KMT2A| 11  | 118339409 | 118392930  | 145       | 96           | 37    |
|                                   | SF1  | 11  | 64532722  | 64545911   | 30        | 80           | 19    |
| Splicing                          | 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    |
| Splicing                          | 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 Classification               |
|-------|-----|------------|-----------------|--------------|------------|----------|-------|-----------------------------------|
| 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  | 13056267   | c.1154insTTGTC  | p.Lys385fs   | Frameshift insertion | InDel | 5     | Likely pathogenic                 |
| CBL   | 11  | 11903319   | c.357G>A        | p.Met119Ile  | missense   | SNV      | 3     | Pathogenic Likely pathogenic      |
| CBL   | 11  | 11907179   | 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.As879Asp   | 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.Leu17Argf5fsTer13 | Frameshift insertion | InDel | 5     | VUS                               |
| ETV6  | 12  | 12038908   | c.1201T>G       | p.Tyr401Asp  | missense   | SNV      | 3     | Likely pathogenic                 |
| ETV6  | 12  | 12038918   | c.1212del       | p.As405fs    | Frameshift deletion | InDel | 5     | Pathogenic Likely pathogenic      |
| EZH2  | 7   | 14850642   | 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                 |
| Gene | Chromosome | Position | cDNA Change | Amino Acid Change | Mutation Type | SNP Type | Pathogenicity |
|------|------------|----------|-------------|-------------------|---------------|----------|--------------|
| FLT3 | 13         | 28592623 | c.2522A>T   | p.Asn841Ile       | missense      | SNV      | Pathogenic   |
| FLT3 | 13         | 28592642 | c.2503G>T   | p.Asp835Tyr       | missense      | SNV      | Likely pathogenic |
| FLT3 | 13         | 28609724 | c.1505A>T   | p.Asn502Ile       | missense      | SNV      | VUS          |
| FLT3 | 13         | 2861015  | c.1337C>T   | p.Ser446Leu       | missense      | SNV      | VUS          |
| IDH1 | 2          | 20911311 | c.394C>T    | p.Arg132Cys       | missense      | SNV      | Pathogenic   |
| IDH2 | 15         | 90631838 | c.515G>A    | p.Arg172Lys       | missense      | SNV      | Likely pathogenic |
| IDH2 | 15         | 90631934 | c.418C>T    | p.Arg140Trp       | missense      | SNV      | Likely pathogenic |
| IDH2 | 15         | 90631935 | c.419G>A    | p.Arg140Gln       | missense      | SNV      | Pathogenic   |
| JAK2 | 9          | 5073770  | c.1849G>T   | p.Val617Phe       | missense      | SNV      | Pathogenic   |
| JAK2 | 9          | 5080558  | c.3208_2309insT | p.His770LeufsTer17 | Frameshift insertion | InDel | Pathogenic | Likely pathogenic |
| KDM6A | X          | 44733223 | c.215T>G    | p.Leu72Arg        | missense      | SNV      | VUS          |
| KIT  | 4          | 55604640 | c.2848G>A   | p.Val950Met       | missense      | SNV      | Benign       |
| KIT  | 4          | 5558977  | c.1253_1255delACG | p.Asnp419del     | Frameshift Deletion | InDel | Likely pathogenic |
| KIT  | 4          | 55602762 | c.2583dup   | p.Leu862AAlafsTer17 | Frameshift insertion | InDel | Pathogenic | Likely pathogenic |
| KMT2A | 11         | 118343378| c.1505A>T   | p.Asn502Ile       | missense      | SNV      | Likely pathogenic |
| KMT2A | 11         | 118344081| c.2207G>T   | p.Arg736Met       | missense      | SNV      | VUS          |
| KMT2A | 11         | 118352769| c.3974G>A   | p.Arg1325Asn      | missense      | SNV      | Benign       |
| KMT2A | 11         | 118374758| c.8142C>G   | p.Ile2714Met      | missense      | SNV      | Likely Benign |
| KMT2A | 11         | 118377003| c.10396A>G  | p.Thr3466Ala      | missense      | SNV      | Benign       |
| KRAS | 12         | 25398285 | c.34G>A     | p.Gly125Ser       | missense      | SNV      | Pathogenic   |
| KRAS | 12         | 25378603 | c.395dup    | p.Asnp132GlufsTer12 | Frameshift insertion | InDel | Pathogenic | Likely pathogenic |
| MPL  | 1          | 43818306 | c.1771T>G   | p.Tyr591Asp       | missense      | SNV      | VUS          |
| NRAS | 1          | 115256528| c.183A>C    | p.Gln61His        | missense      | SNV      | Pathogenic   |
| NRAS | 1          | 115258744| c.38G>A     | p.Gly13Asp        | missense      | SNV      | Pathogenic   |
| NRAS | 1          | 115258745| c.37G>T     | p.Gly13Cys        | missense      | SNV      | Pathogenic   |
| NRAS | 1          | 115258747| c.35G>A     | p.Gly12Asp        | missense      | SNV      | Pathogenic   |
| PHF6 | X          | 133549140| c.824G>A    | p.Gly275Glu       | missense      | SNV      | Pathogenic   |
| PRPF40B | 12      | 50031516 | c.1676G>C   | p.Gln559Ala       | missense      | SNV      | VUS          |
| PTEN | 10         | 89624271 | c.45dup     | p.Tyr161LeufsTer28 | Frameshift insertion | InDel | Likely pathogenic |
| RUNX1 | 21         | 36164627 | c.1247ins   | p.Phe416LeufsTer185 | Frameshift insertion | InDel | Pathogenic | Likely pathogenic |
| RUNX1 | 21         | 36231773 | c.611G>A    | p.Arg204Gln       | missense      | SNV      | Pathogenic   |
| Gene   | Chromosome | Position     | Mutation Details | Type     | HGVAS | Pathogenicity |
|--------|------------|--------------|------------------|----------|-------|---------------|
| RUNX1  | 21         | 36231791     | c.593A>G         | SNP      |       | Pathogenic    |
| SF3A1  | 22         | 30733135     | c.1985insC       | frameshift insertion | InDel | VUS           |
| SF3B1  | 2          | 198267303    | c.2054G>A        | missense | SNV   | VUS           |
| SF3B1  | 2          | 198266834    | c.2098A>G        | missense | SNV   | Pathogenic    |
| SF3B1  | 2          | 198270040    | c.1396T>A        | missense | SNV   | VUS           |
| SRSF2  | 17         | 74733073     | c.170T>A         | frameshift insertion | InDel | VUS           |
| TET2   | 4          | 106157845    | c.2746C>T        | stop gained | SNV   | Pathogenic    |
| TET2   | 4          | 106164767    | c.3635T>C        | missense | SNV   | VUS           |
| TET2   | 4          | 106164913    | c.3781C>T        | missense | SNV   | Likely pathogenic |
| TET2   | 4          | 106164032    | c.3543del        | frameshift insertion | InDel | Pathogenic Likely pathogenic |
| TP53   | 17         | 7573931      | c.1096T>G        | missense | SNV   | Benign Likely Benign |
| TP53   | 17         | 7577097      | c.841G>T         | missense | SNV   | Likely pathogenic |
| TP53   | 17         | 7577099      | c.839G>A         | missense | SNV   | Likely pathogenic |
| TP53   | 17         | 7578413      | c.517G>A         | missense | SNV   | Pathogenic    |
| TP53   | 17         | 7578398      | c.532dup         | frameshift insertion | InDel | Pathogenic Likely pathogenic |
| TP53   | 17         | 7577112      | c.825del         | frameshift deletion | InDel | Pathogenic Likely pathogenic |
| U2AF1  | 21         | 44514777     | c.470A>G         | missense | SNV   | Likely pathogenic |
| VHL    | 3          | 10188307     | c.450dup         | frameshift insertion | InDel | Pathogenic Likely pathogenic |
| ZRSR2  | X          | 15841230     | c.1314insAGCCGGG | non-frameshift insertion | InDel | 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             | 70           | 2.3            |
|         | BM             | Rf1            | 104          | 3.4            |
| P2      | BM             | Dx             | 64           | 2.1            |
| P3      | PB             | Dx             | 35           | 1.15           |
|         | BM             | Rf1_s1         | 102          | 3.34           |
| P4      | BM             | Dx             | 42           | 1.38           |
|         | BM             | Rf1_s2         | 85           | 2.79           |
| P5      | BM             | Dx             | 36           | 1.18           |
|         | BM             | PR_s1          | 70           | 2.3            |
|         | BM             | PR_s2          | 118          | 3.87           |
|         | BM             | CR1_s1         | 115          | 4.95           |
|         | BM             | CR1_s2         | 166          | 5.44           |
| P6      | BM             | Dx             | 12           | 0.39           |
| P7      | PB             | PR1            | 38           | 1.25           |
|         | BM             | Dx             | 65           | 2.13           |
| P8      | BM             | Dx             | 25           | 0.82           |
|         | BM             | Rf1            | 65           | 2.13           |
|         | BM             | R              | 357          | 11.70          |
|         | BM             | CR1            | 399          | 13.08          |
|         | BM             | CR2_s1         | 1885         | 61.8           |
|         | BM             | CR2_s2         | 2058         | 67.48          |
| P9      | BM             | Dx             | 38           | 1.25           |
|         | BM             | Rf1            | 85           | 2.79           |
|         | BM             | CR1_s1         | 155          | 5.08           |
|         | BM             | CR1_s2         | 255          | 8.36           |
| P10     | BM             | Dx             | 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 |
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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