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RESEARCH ARTICLE

Gene expression profiling identifies FLT3 mutation-like cases in wild-type FLT3 acute myeloid leukemia

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Abstract

Background

FLT3 mutation is present in 25–30% of all acute myeloid leukemias (AML), and it is associated with adverse outcome. FLT3 inhibitors have shown improved survival results in AML both as upfront treatment and in relapsed/refractory disease. Curiously, a variable proportion of wild-type *FLT3* patients also responded to these drugs.

Methods

We analyzed 6 different transcriptomic datasets of AML cases. Differential expression between mutated and wild-type *FLT3* AMLs was performed with the Wilcoxon-rank sum test. Hierarchical clustering was used to identify *FLT3*-mutation like AMLs. Finally, enrichment in recurrent mutations was performed with the Fisher's test.

Results

A *FLT3* mutation-like gene expression pattern was identified among wild-type *FLT3* AMLs. This pattern was highly enriched in *NPM1* and *DNMT3A* mutants, and particularly in combined *NPM1*/*DNMT3A* mutants.

Conclusions

We identified a *FLT3* mutation-like gene expression pattern in AML which was highly enriched in *NPM1* and *DNMT3A* mutations. Future analysis about the predictive role of this biomarker among wild-type *FLT3* patients treated with FLT3 inhibitors is envisaged.

1. Introduction

FMS-like tyrosine kinase-3 (FLT3) is a receptor tyrosine kinase commonly mutated in acute myeloid leukemia (AML) [1]. *FLT3 internal tandem duplication* (ITD) is the most common mutation, affecting 25% of de novo AML cases [2], and additional tyrosine kinase mutations (*FLT3*-TKD) in codons D385 and I386 have been observed in 5–10% of cases [3]. Both types of mutations induce constitutive activation of FLT3 kinase activity, inducing pro-survival and proliferative signals [4]. *FLT3*-ITD mutation confers a poor outcome in de novo and relapsed AML, and it is currently suggested to include these patients in clinical trials [5, 6].

A number of promising FLT3 inhibitors are under development for AML treatment. The addition of the multi-kinase inhibitor midostaurin [7] to standard chemotherapy in newly diagnosed *FLT3*-mutated ($FLT3^{mut}$) AML significantly improved event-free survival and overall survival in all *FLT3* mutation subtypes [8]. Several novel FLT3 inhibitors with increased specificity for FLT3 are under study, such as gilteritinib, crenolanib and quizartinib [3], with encouraging results from phase 2 and 3 trials in the relapsed & refractory AML setting [9–11]. Curiously, overall responses among wild-type *FLT3* AML patients were reported for both gilteritinib (12% of composite overall responses) and quizartinib (30–36% of composite complete remissions) [9, 11]. It has been hypothesized that this effect could be due to nonspecific inhibition of other kinases, cryptic FLT3 activation by other mutations or hyperactivation of FLT3 by its ligand [9]. Not surprisingly, 2 clinical trials are currently underway in order to study the activity of FLT3 inhibitors in wild-type FLT3 patients [12, 13], but there is still no biomarker to predict which patients will respond to these drugs. Therefore, an interesting point would be to identify gene expression changes associated with *FLT3* mutation in order to search for wild-type *FLT3* cases that resemble $FLT3^{mut}$ AMLs at the transcriptomic level.

In this study we analyzed the transcriptomic pattern of 6 different AML cohorts, which enabled the identification of a *FLT3* mutation-like gene expression pattern highly enriched in *NPM1* and *DNMT3A* mutants. Our results indicate common deregulated pathways among these leukemias, opening the way to study the role of this biomarker in order to predict responses to FLT3 inhibitors in wild-type *FLT3* AML.

2. Materials and methods

We analyzed five AML gene expression datasets available in the *Gene Expression Omnibus* (GEO): GSE14468 (461 cases of de novo cytogenetically normal AML), GSE10358 (188 cases of de novo AML), GSE61804 (279 de novo AML cases), GSE17855 (237 pediatric AML cases) and GSE15434 (251 cases of normal karyotype AML). In the case of GSE14468 and GSE10358, both *FLT3*-ITD and D385 mutation status were reported, whereas in the remaining datasets only the *FLT3*-ITD status was informed. All datasets used the same type of gene expression arrays: *Affymetrix Human Genome U133 Plus 2.0 Array*. Signal intensities were rank-normalized, and differential expression between $FLT3^{mut}$ (either ITD or TKD) and wild-type samples was performed in the largest cohort (GSE14468) using the two-sided Wilcoxon-rank sum test. P-values were adjusted for multiple testing using the Bonferroni method. Hierarchical clusterization and heatmap plots were created using the *heatmaps3* package with default parameters [14], and enrichment analysis was performed with the two-sided exact Fisher's test. Gene ontology analysis was performed on *WebGestalt* with default parameters (FDR significance threshold, 0.05) [15].

RNAseq and mutation data from 246 AML patients treated with intensive chemotherapy was retrieved from *Bamopoulos et al*. (GEO identification GSE146173) [16]. Raw count data was transformed to normalized transcripts per million using the *fpkm* function implemented in the *DESEQ2* package [17]. Afterwards, rank-transformation was applied. 595 genes

matching genes with the *FLT3*-like pattern were selected, followed by standard hierarchical clusterization. Differential mutation between the *FLT3*-like and no-*FLT3*-like group was performed with Fisher's test.

All computations except gene ontology analysis were performed in R. All data used for this analysis is readily accessible from public repositories.

3. Results

FLT3-like gene expression pattern

We chose the largest database (GSE14468) as the discovery set, and we identified 911 probes mapping to 649 different genes which were differentially expressed between $FLT3^{mut}$ (ITD and/or TKD) and wild-type *FLT3* samples (Bonferroni p-value <0.05, S1 Table). None of the probes mapped to *FLT3*. These probes were significantly enriched in 52 gene ontology terms, among which 17 terms were associated with hemopoiesis & immunology, and 3 terms were specifically linked to myeloid differentiation, namely *myeloid cell homeostasis*, q-value 5.01 x 10^{-3}; *myeloid cell differentiation*, q-value 4.86 x 10^{-3}; and *negative regulation of myeloid cell differentiation*, q-value 0.03 (S2 Table). Hierarchical clusterization revealed two broad groups. A cluster of 46.20% of patients was substantially enriched in *FLT3* mutants (p-value $< 1 \times 10^{-4}$), since it contained 81.67% of all *FLT3*-ITD cases, 61.36% of all *FLT3*-TKD cases and 83.33 of composed mutants (*FLT3*-ITD plus *FLT3*-TKD; Fig 1A). 28.52% of wild-type *FLT3* cases were also clustered within this group. A similar finding was identified in the independent GSE10358 database, where a cluster of 51.06% of patients contained 81.08% of all *FLT3*-ITD mutants and 70.00% of all *FLT3*-TKD mutants (p-value $< 1 \times 10^{-4}$). Furthermore, 42.15% of wild-type *FLT3* cases were also clustered within this group (Fig 1B).

The same clustering was repeated in 3 different datasets that only reported *FLT3*-ITD mutation status. In GSE61804 a cluster comprising 58.06% of all patients harbored 78% of all *FLT3*-ITD cases (p-value 1.50×10^{-3}), and 53.71% of all non *FLT3*-ITD cases were grouped within this cluster (Fig 1C). In GSE17855, a cluster of 47.67% of patients contained 81.25% of all *FLT3*-ITD patients (p-value $< 1 \times 10^{-4}$), but it also included 39.15% of all non *FLT3*-ITD cases (Fig 1D). Finally, in GSE15434, a cluster of 64.14% of patients was enriched in *FLT3*-ITD cases, comprising 86.67% of the whole cohort (p-value $< 1 \times 10^{-4}$); and additionally 49.11% of all non *FLT3*-ITD cases co-clustered within this group (Fig 1E). We replicated the *FLT3*-like pattern in GSE146173 using RNAseq data, identifying 28.49% of wild-type *FLT3* AMLs as *FLT3*-like (Fig 2).

Mutation landscape of *FLT3*-like leukemias

Four of the microarray databases provided data about driver mutations in a few genes (S3 Table). The most significant finding was the enrichment of *FLT3*-like AMLs in *NPM1* mutants, which was observed in the 4 cohorts. This proportion was variable, ranging from 80.72% in karyotype normal AML (GSE15434) to just 12.16% in pediatric AML (GSE17855), indicating that the existence of the *FLT3*-like pattern is not explained by co-occurring *NPM1* mutations. Additionally, a relative enrichment in *IDH1* mutants within *FLT3*-like leukemias was detected in the discovery set GSE14468 (p-value 2.07×10^{-4}). Furthermore, depletion of *CEBPA* mutants in *FLT3*-like AML was a common phenomenon in all 3 cohorts that reported mutations in this gene, and a reduced frequency of *KIT* mutations was also discovered in the pediatric AML dataset (GSE17855).

Using the most recent RNAseq data published by *Bamopoulos et al.* [16] we observed that *FLT3*-like leukemias were highly enriched in concurrent *NPM1* and *DNMT3A* mutants, whereas 19.60% of *FLT3*-like AMLs were *NPM1* and *DNMT3A* wild-type. Additionally, there

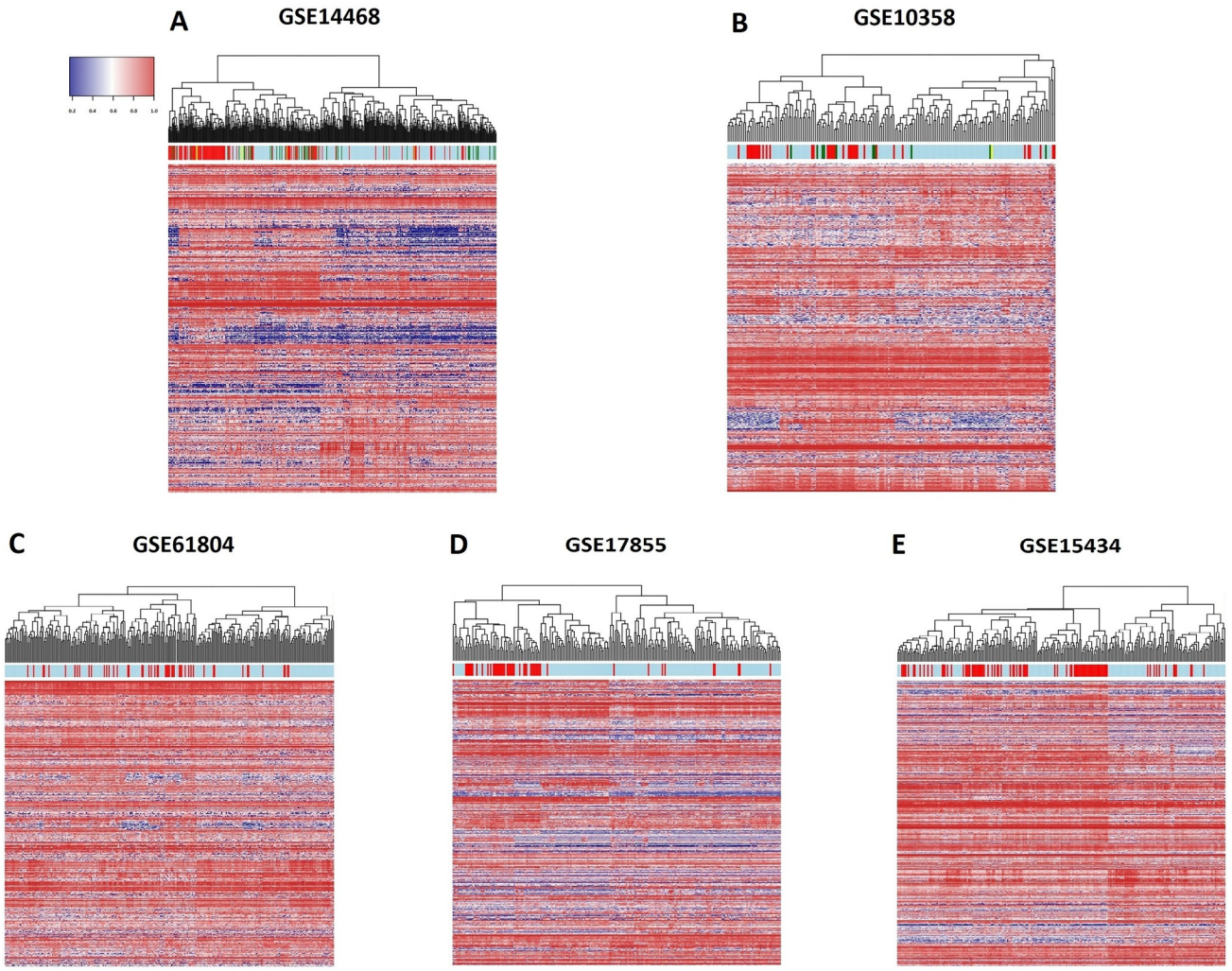

Fig 1. A and B) Heatmaps representing the hierarchical clustering of AML cases according to the expression of the 911 probes associated with *FLT3* ITD and TKD mutation status. The color coding of the bar indicates if a patient harbos a *FLT3*-ITD mutation (red bars), a *FLT3*-TKD mutation (green bars), both mutations (yellow bars) or no *FLT3* mutation (light blue bars). **C-E)** Heatmaps representing the hierarchical clustering of AML cases according to the expression of the 911 probes associated with *FLT3*-ITD mutation status. No information about *FLT3*-TKD mutations was reported for patients in these databases. Red bars indicate *FLT3*-ITD cases and light blue bars indicate lack of *FLT3*-ITD mutation.

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was a tendency for an enrichment in *IDH1* mutations (p-value 0.09). On the contrary, *FLT3*-like AMLs were depleted in *ASXL1*, *CEBPA*, *RUNX1*, *TP53*, *SF3B1* and *U2AF1* mutations (Table 1).

4. Discussion

In this report we describe the existence of a *FLT3* mutation-like gene expression pattern in wild-type *FLT3* AMLs. The *FLT3*-mutation like pattern was enriched in *NPM1* and *DNMT3A* mutant leukemias. This is probably related to the significant co-occurrence of *FLT3* mutations with those of *NPM1* and *DNMT3A* [18], which probably leads to partially overlapping transcriptomic fingerprints. Not surprisingly, the *FLT3*-like pattern contains numerous *HOX* genes, which have been previously vinculated with the *NPM1*-transcriptional pattern [19]. Nevertheless, the heterogeneous frequency of *NPM1* mutations among *FLT3*-like leukemias,

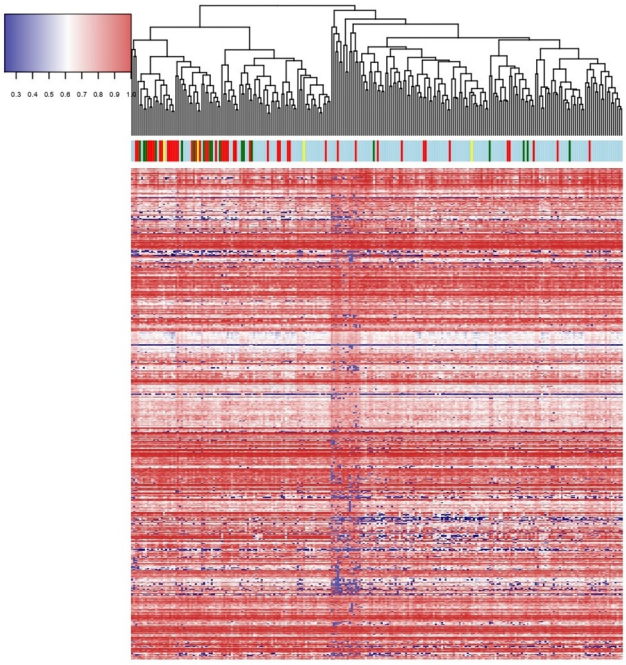

Fig 2. Heatmaps representing the hierarchical clustering of AML cases according to the expression of the 595 matching genes in the RNAseq cohort (GSE146173). The color coding of the bar follows the same pattern as that of Fig 1.

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and particularly its low frequency in pediatric AML, along with the reproducibility of the *FLT3*-like pattern in all cohorts, indicate that co-occurring *NPM1* mutations are insufficient to explain the existence of the *FLT3*-like pattern.

Our results suggest that a group of AMLs with *FLT3* plus *NPM1* and/or *DNMT3A* mutations share a similar transcriptomic background. Noteworthy, responses to the FLT3 inhibitor gilteritinib among relapsed $FLT3^{mut}$ AMLs are superior in those patients with mutations in *NPM1* or *DNMT3A*, and particularly in those with both genes mutated [20], and similar findings were

Table 1. Differential distribution of recurrently mutated genes in wild-type *FLT3* AML patients according to the presence or absence of the *FLT3*-like pattern (GSE146173).

Gene ID	p-value	FLT3-like (%)	No FLT3-like (%)
ASXL1	5.29E-04	1.96	21.87
CEBPA	2.66E-15	0	5.47
DNMT3A	1.61E-03	41.17	17.19
EZH2	1	1.96	1.56
IDH1	0.09	15.69	7.03
IDH2	0.81	13.73	12.5
NPM1	3.40E-24	74.51	2.34
RUNX1	2.30E-05	1.96	28.12
TET2	0.29	23.53	16.41
TP53	0.02	1.96	14.06
SRSF2	0.62	9.8	13.28
U2AF1	7.95E-14	0	7.03
SF3B1	8.10E-18	0	3.12
Concurrent NPM1 & DNMT3A	7.11E-05	19.61	1.56

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reported with crenolanib [21]. Furthermore, *FLT3-ITD* leukemias with mutations in *NPM1* or *DNMT3A* exhibit a different drug response mechanism to the FLT3 inhibitor quizartinib, where the cell differentiation effect predominates over the cytotoxic mechanism [22].

The use of FLT3 inhibitors in wild-type *FLT3* AML has been tested in some trials. For example, the FLT3 inhibitor midostaurin evidenced blast reduction responses in 53% of relapsed & refractory AML cases [23]. Similarly, responses to novel FLT3 inhibitors in a variable proportion of wild-type *FLT3* cases have been observed in phase I & II trials [24–26]. This has motivated the development of new studies to specifically test the possible benefit of adding these drugs in the upfront treatment for AML [27, 28]. As it is expected that only a subgroup of patients might benefit from FLT3 inhibitors, it is imperative to develop and test new predictive biomarkers of response. Therefore, it becomes necessary to test the predictive value of the *FLT3*-like pattern in these clinical trials. Additionally, the enrichment of *FLT3*-like leukemias in *IDH1* mutations (which are correlated with *NPM1* mutation [29]) could set the basis for the development of new clinical trials testing the combination of different check-point inhibitors in AML [30].

This study has some limitations. Firstly, a variable proportion of *FLT3* mutation-like samples were clustered near *FLT3* mutants across the different cohorts, which probably reflects substantial heterogeneity between them. One of the possible explanations for this could be a differential distribution of *NPM1* and *DNMT3A* mutants, since these are drivers of cytogenetically-normal AMLs (such as in the case of GSE15434) [31]. Finally, some of the datasets had only *FLT3*-ITD annotation, and a minority of the *FLT3*-like cases might indeed have *FLT3*-TKD mutations.

5. Conclusions

Our results are concordant with the existence of a *FLT3* mutation-like transcriptomic pattern with a different mutational background which clusters a proportion of wild-type *FLT3* AMLs with $FLT3^{mut}$ samples. These leukemias were highly enriched in *NPM1*, and particularly in composed *NPM1*/*DNMT3A* mutants, but *NPM1* status alone was insufficient to explain the existence of the *FLT3*-like pattern. The analysis of wild-type *FLT3* AML patients treated with FLT3 inhibitors in clinical trials is envisaged in order to study its possible role as a drug response biomarker.

Supporting information

S1 Table. Annotation of all probes significantly associated with *FLT3* mutation status in AML.
(XLSX)

S2 Table. Significantly enriched gene ontology (biological process) terms from the list of genes associated with *FLT3* mutation status.
(XLSX)

S3 Table. Differential distribution of recurrently mutated genes in wild-type *FLT3* AML wild-type according to the presence or absence of the *FLT3*-like pattern. Data reported for mutations analyzed in GSE14468, GSE10358, GSE15434 and GSE17855.
(XLSX)

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All authors approved the final version of this manuscript.

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References

1. Levis M, Small D. FLT3: ITDoes matter in leukemia. Leukemia. 2003 Sep; 17(9):1738–52. Review. https://doi.org/10.1038/sj.leu.2403099 PMID: 12970773.
2. Stirewalt DL, Radich JP. The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer. 2003 Sep; 3(9):650–65. Review. https://doi.org/10.1038/nrc1169 PMID: 12951584.
3. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia. 2019 Feb; 33(2):299–312. Epub 2019 Jan 16. Review. https://doi.org/10.1038/s41375-018-0357-9 PMID: 30651634.
4. Grafone T, Palmisano M, Nicci C, Storti S. An overview on the role of FLT3-tyrosine kinase receptor in acute myeloid leukemia: biology and treatment. Oncol Rev. 2012 Apr 17; 6(1):e8. eCollection 2012 Mar 5. Review. https://doi.org/10.4081/oncol.2012.e8 PMID: 25992210.
5. O'Donnell MR, Tallman MS, Abboud CN, Altman JK, Appelbaum FR, Arber DA, et al. Acute Myeloid Leukemia, Version 3.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2017 Jul; 15(7):926–957. https://doi.org/10.6004/jnccn.2017.0116 PMID: 28687581.
6. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 2016 Jun 9; 374(23):2209–2221. https://doi.org/10.1056/NEJMoa1516192 PMID: 27276561.
7. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med. 2017; 377(5):454–464. https://doi.org/10.1056/NEJMoa1614359 PMID: 28644114
8. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer SM, Bloomfield CD, et al. The Addition of Midostaurin to Standard Chemotherapy Decreases Cumulative Incidence of Relapse (CIR) in the International Prospective Randomized, Placebo-Controlled, Double-Blind Trial (CALGB 10603 / RATIFY [Alliance]) for Newly Diagnosed Acute Myeloid Leukemia (AML) Patients with FLT3 Mutations. Blood (2017) 130 (Supplement 1): 2580. https://doi.org/10.1182/blood.V130.Suppl_1.2580.2580
9. Perl AE, Martinelli G, Cortes JE, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory *FLT3*-Mutated AML. *N Engl J Med*. 2019; 381(18):1728–1740. https://doi.org/10.1056/NEJMoa1902688 PMID: 31665578

10. Randhawa JK, Kantarjian HM, Borthakur G, Thompson PA, Konopleva M, Daver N, et al. Results of a Phase II Study of Crenolanib in Relapsed/Refractory Acute Myeloid Leukemia Patients (Pts) with Activating FLT3 Mutations. Blood (2014) 124 (21): 389. https://doi.org/10.1182/blood.V124.21.389.389

11. Cortes JE, Khaled S, Martinelli G, et al. Quizartinib versus salvage chemotherapy in relapsed or refractory FLT3-ITD acute myeloid leukaemia (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial [published correction appears in Lancet Oncol. 2019 Jul;20(7):e346]. *Lancet Oncol*. 2019; 20(7):984–997. https://doi.org/10.1016/S1470-2045(19)30150-0 PMID: 31175001

12. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 -. Identifier NCT04107727 Trial to Compare Efficacy and Safety of Chemotherapy/Quizartinib vs Chemotherapy/Placebo in Adults FMS-like Tyrosine Kinase 3 (FLT3) Wild-type Acute Myeloid Leukemia (AML); 2017 Sept 27 [cited 2020 Feb 27]; [about 4 screens]. https://clinicaltrials.gov/ct2/show/NCT04107727

13. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 -. Identifier NCT03512197A Global Study of the Efficacy and Safety of Midostaurin + Chemotherapy in Newly Diagnosed Patients With FLT3 Mutation Negative (FLT3-MN) Acute Myeloid Leukemia (AML); 2019 Apr 30 [cited 2020 Feb 27]; [about 4 screens]. https://clinicaltrials.gov/ct2/show/NCT03512197

14. Zhao S, Guo Y, Sheng Q, Shyr Y. Advanced heat map and clustering analysis using heatmap3. Biomed Res Int. 2014; 2014:986048. Epub 2014 Jul 16. https://doi.org/10.1155/2014/986048 PMID: 25143956.

15. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res. 2019 Jul 2; 47(W1):W199–W205. https://doi.org/10.1093/nar/gkz401 PMID: 31114916.

16. Bamopoulos SA, Batcha AMN, Jurinovic V, et al. Clinical presentation and differential splicing of SRSF2, U2AF1 and SF3B1 mutations in patients with acute myeloid leukemia [published online ahead of print, 2020 May 1]. *Leukemia*. 2020; https://doi.org/10.1038/s41375-020-0839-4 PMID: 32358566

17. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15(12):550. https://doi.org/10.1186/s13059-014-0550-8 PMID: 25516281

18. Bezerra MF, Lima AS, Piqué-Borràs MR, et al. Co-occurrence of DNMT3A, NPM1, FLT3 mutations identifies a subset of acute myeloid leukemia with adverse prognosis. Blood. 2020; 135(11):870–875. https://doi.org/10.1182/blood.2019003339 PMID: 31977039

19. Mullighan CG, Kennedy A, Zhou X, et al. Pediatric acute myeloid leukemia with NPM1 mutations is characterized by a gene expression profile with dysregulated HOX gene expression distinct from MLL-rearranged leukemias. Leukemia. 2007; 21(9):2000–2009. https://doi.org/10.1038/sj.leu.2404808 PMID: 17597811

20. Levis MJ, Perl AE, Martinelli G et al. Effect of gilteritinib on survival in patients with FLT3-mutated (FLT3mut+) relapsed/refractory (R/R) AML who have common AML co-mutations or a high FLT3-ITD allelic ratio. Journal of Clinical Oncology 2019 37:15_suppl, 7000–7000

21. Goldberg AD, Collins RH, Stone RM et al. Addition of Crenolanib to Induction Chemotherapy Overcomes the Poor Prognostic Impact of Co- Occurring Driver Mutations in Patients with Newly Diagnosed FLT3-Mutated AML. Blood (2018) 132 (Supplement 1): 1436. https://doi.org/10.1182/blood-2018-99-117016

22. Nybakken GE, Canaani J, Roy D, et al. Quizartinib elicits differential responses that correlate with karyotype and genotype of the leukemic clone. Leukemia. 2016; 30(6):1422–1425. https://doi.org/10.1038/leu.2015.320 PMID: 26585411

23. Fischer T, Stone RM, Deangelo DJ, et al. Phase IIB trial of oral Midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated FLT3. J Clin Oncol. 2010; 28(28):4339–4345. https://doi.org/10.1200/JCO.2010.28.9678 PMID: 20733134

24. Perl AE, Altman JK, Cortes J, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study. Lancet Oncol. 2017 Aug; 18(8):1061–1075. Epub 2017 Jun 20. Erratum in: Lancet Oncol. 2017 Dec;18(12): e711. Lancet Oncol. 2018 Jul;19(7):e335. Lancet Oncol. 2019 Jun;20(6):e293. https://doi.org/10.1016/S1470-2045(17)30416-3 PMID: 28645776.

25. Randhawa JK, Kantarjian HM, Borthakur G et al. Results of a Phase II Study of Crenolanib in Relapsed/Refractory Acute Myeloid Leukemia Patients (Pts) with Activating FLT3 Mutations. Blood (2014) 124 (21): 389. https://doi.org/10.1182/blood.V124.21.389.389

26. Cortes J, Perl AE, Döhner H, et al. Quizartinib, an FLT3 inhibitor, as monotherapy in patients with relapsed or refractory acute myeloid leukaemia: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol. 2018 Jul; 19(7):889–903. Epub 2018 May 31. https://doi.org/10.1016/S1470-2045(18)30240-7 PMID: 29859851.

27. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 -. Identifier NCT04107727 Trial to Compare Efficacy and Safety of Chemotherapy/Quizartinib vs Chemotherapy/Placebo in Adults FMS-like Tyrosine Kinase 3 (FLT3) Wild-type Acute Myeloid Leukemia (AML); 2017 Sept 27 [cited 2020 Feb 27]; [about 4 screens]. https://clinicaltrials.gov/ct2/show/NCT04107727

28. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000 Feb 29 -. Identifier NCT03512197A Global Study of the Efficacy and Safety of Midostaurin + Chemotherapy in Newly Diagnosed Patients With FLT3 Mutation Negative (FLT3-MN) Acute Myeloid Leukemia (AML); 2019 Apr 30 [cited 2020 Feb 27]; [about 4 screens]. https://clinicaltrials.gov/ct2/show/NCT03512197

29. Falini B, Spinelli O, Meggendorfer M, et al. IDH1-R132 changes vary according to NPM1 and other mutations status in AML. Leukemia. 2019; 33(4):1043–1047. https://doi.org/10.1038/s41375-018-0299-2 PMID: 30622284

30. DiNardo CD, Stein EM, de Botton S, et al. Durable Remissions with Ivosidenib in IDH1-Mutated Relapsed or Refractory AML. N Engl J Med. 2018; 378(25):2386–2398. https://doi.org/10.1056/NEJMoa1716984 PMID: 29860938

31. Wang M, Yang C, Zhang L, Schaar DG. Molecular Mutations and Their Cooccurrences in Cytogenetically Normal Acute Myeloid Leukemia. *Stem Cells Int.* 2017; 2017:6962379. https://doi.org/10.1155/2017/6962379 PMID: 28197208