COMMENTARY

blood advances

TO THE EDITOR:

13q12.2 deletions and *FLT3* overexpression in acute leukemias

Caroline Pires Poubel,[1,2] Mariana Boroni,[2,*] and Mariana Emerenciano[1,*]

[1]Division of Clinical Research and [2]Bioinformatics and Computational Biology Laboratory, Research Center, Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil

It was with great enthusiasm that we read the recently published work by Yang and colleagues entitled "13q12.2 deletions in acute lymphoblastic leukemia lead to upregulation of *FLT3* through enhancer hijacking."[1] In their very well-conducted study, the authors show a novel mechanism of *FLT3* disruption in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). In 2019, we had anticipated that unknown mechanisms were to be discovered in acute leukemias with *FLT3* overexpression.[2] With this in mind, we highlighted that a substantial proportion of either BCP-ALL, T-cell acute lymphoblastic leukemia (T-ALL), or acute myeloid leukemia (AML) cases with *FLT3* overexpression lacked a known mechanism leading to this upregulation. Yang et al described in their article that somatic 13q12.2 deletions were present in approximately 2% of all BCP-ALL cases included in the study (5 different cohorts have been evaluated), and they discovered that these deletions lead to high expression levels of *FLT3* through chromatin remodeling and enhancer hijacking. In brief, the 13q12.2 microdeletion results in cis interactions between the *FLT3* promoter and an enhancer element in intron 8 of *PAN3*, which ultimately leads to *FLT3* upregulation. Although a population-based cohort study is still needed to define the actual frequency of these deletions in BCP-ALL, it is important to note that Yang and colleagues also reported that the 13q12.2 deletions are more frequent in high hyperdiploid BCP-ALL cases. However, other acute leukemia subtypes have not yet been evaluated.

Dr. Spencer has wisely commented on Yang and colleagues' work, adding that one of the reasons why their findings is significant is that detection of 13q12.2 deletions could have clinical implications for BCP-ALL patients because FLT3 inhibitors are available for targeted therapy. Based on this, Dr. Spencer highlighted that it would be important to investigate whether 13q12.2 deletions also occur in other acute leukemia subtypes in which *FLT3* gene alterations are relevant, such as AML.[3] In the meantime, we sought to check if this novel mechanism could also explain the *FLT3* overexpression recurrently observed in a fraction of T-ALL and AML patients.

Taking advantage of the genomic data sets available, we analyzed a total of 264 T-ALL and 292 AML samples from the Therapeutically Applicable Research to Generate Effective Treatments program (TARGET) ALL phase 2 cohort and TARGET AML cohort, respectively (Figure 1A). The T-ALL samples consisted of 264 primary samples and the AML samples included 102 primary and 95 matched primary and recurrent samples. The T-ALL samples were selected based on the availability of public data to evaluate copy number variations (CNVs) by Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix), which has been analyzed according to the intern pipeline developed at St. Jude Children's Research Hospital. In summary, to generate copy number data, Affymetrix intensity (.CEL) and the single-nucleotide protein call files have been analyzed using the birdseed (version 2) algorithm into dChip, and probe-level values were summarized.[4] After data normalization using the reference normalization algorithm,[5] the paired circular binary segmentation has been applied[6] with thresholds set to detect copy number segments >2.3 or <1.7 copies covering at least 8 probes (https://ocg.cancer.gov/programs/target/target-methods). Thus, based on these preanalyzed data, we evaluated the presence of 13q12.2 deletions in T-ALL samples. The AML patients were selected based on the availability of whole genome sequencing data that have been analyzed according to the Complete Genomics Assembly Pipeline (version 1.12). In this case, the determination of somatic CNVs was carried out through a coverage sequence computation followed by the estimation and correction of the guanine-cytosine (GC) content bias in coverage of the tumor and matched normal samples. Then, each tumor GC-corrected coverage estimation was normalized by comparison with matched normal

Submitted 19 October 2020; accepted 19 March 2021; published online 20 April 2021. DOI 10.1182/bloodadvances.2020003643.

*M.B. and M.E. contributed equally to this work.

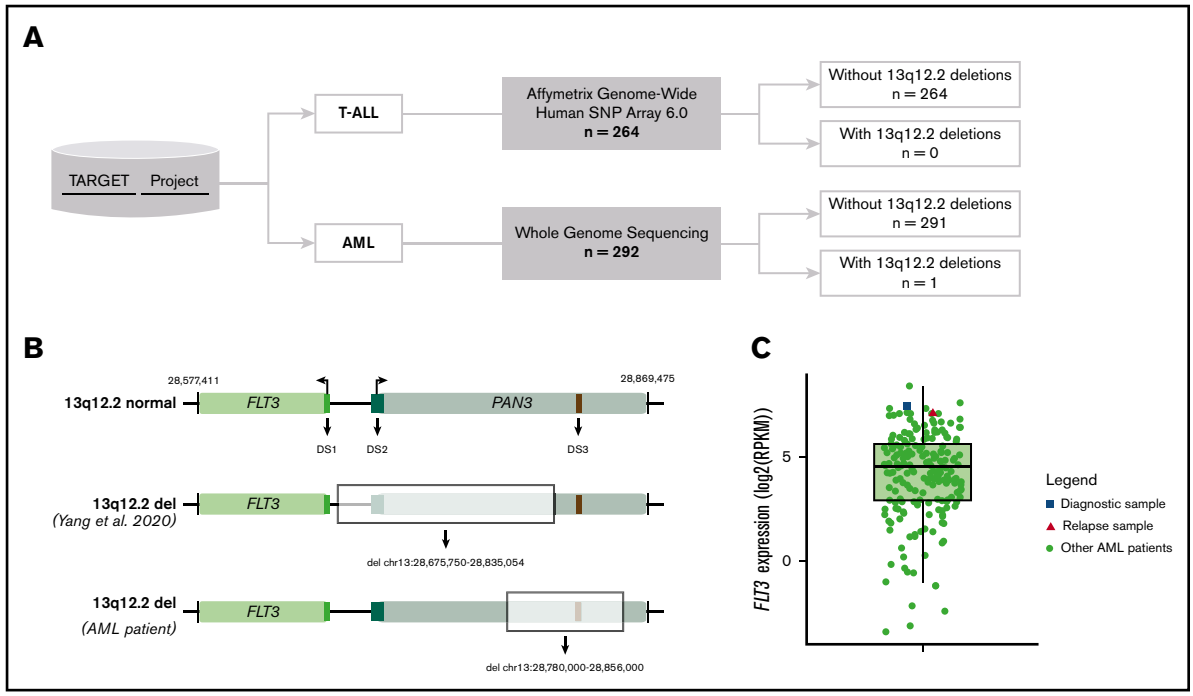

Figure 1. Study overview. (A) TARGET data used for copy number variants assessment in the 13q12.2 region in either T-ALL or AML patients. (B) Schematic figure of the region where *FLT3* (light green rectangle) and *PAN3* (light blue rectangle) genes are located. (Upper) Wild-type scenario. (Middle) Largest 13p12.2 deletion found by Yang et al. (Lower) Location of 13q12.2 deletion found in the AML patient (highlighted rectangle). DS1 (*FLT3* promoter, chr13:28 674,500-28 675,000; dark green rectangle), DS2 (chr13:28 710,000-28 715,000; dark blue rectangle) and DS3 (*PAN3* intron 8; chr13:28 843,000-28 843,500; red rectangle). (C) Dispersion of *FLT3* expression among AML patients demonstrated by boxplot. The dot plot shows the *FLT3* expression of each AML patient (green circle), highlighting the diagnostic (blue square) and relapse samples (red triangle) from the female patient who presented a 13q12.2 deletion. These expression data were obtained according to the availability of RNA-sequencing data from patients assessed for copy-number abnormality (n = 203).

GC-corrected coverage. After normalization, the ploidy inference, segmentation, and scoring of samples were performed using hidden Markov models (for more details: https://www.completegenomics.com/documents/CNV+Methods.pdf). These preanalyzed data were deposited in a specific file for each AML patient containing the estimated ploidy for each genome segment. Although the microarray CNV analysis allows the detection of alterations ranging from 40 bp to 8 Mbp,[7] the whole genome sequencing makes it possible to detect all types of genomic variation, especially small deletions and duplications, even smaller than 1 kbp.[8,9] Once the 13q12.2 microdeletions found by Yang et al varied from 39 to 160 kbp (median size, 129 kbp), we were able to accurately assess the copy number status within the 13q12.2 region in our study cohort.

Based on the CNV analysis for T-ALL samples, the specific 13q12.2 deletion has not been found in any patient. Among 292 AML samples, only 1 patient was identified with a 13q12.2 deletion of 76 kb (chr13:28 780,000-28 856,000). The leukemic sample presented a remarkably high level of *FLT3* expression (~3.6 times greater than the mean). This sample belongs to a female patient who was first diagnosed at 4 months old and treated according to the AAML0531 protocol. Unfortunately, she relapsed at 8 months old and died about 1 year after. However, unlike what has been described by Yang et al, we observed that the deletion found in this AML case affects exclusively the *PAN3* gene region, including the enhancer element in intron 8 (Figure 1B). It is remarkable that this alteration was identified in the recurrent but not in the primary sample of this case, especially because Yang et al showed that the 13q12.2 deletions they described were highly enriched in cases that subsequently relapsed. Besides, they also identified 1 BCP-ALL case harboring the 13q12.2 deletion at relapse but not at diagnosis, suggesting a later occurrence. However, we cannot exclude the possibility that this *PAN3* deletion is present in a small percentage of cells in the diagnostic sample. Nonetheless, by analyzing expression data in these 2 time points, we noticed that there was no difference in *FLT3* messenger RNA expression levels between both samples, indicating that this deletion is probably not the mechanism behind *FLT3* overexpression in this patient (Figure 1C). Moreover, there was no evidence of allele-specific expression for the *FLT3* gene by analyzing the aligned RNA-sequencing data. It is important to mention that none of the samples (diagnostic and relapsed) had any *FLT3* amplification or known activating mutations. On the other hand, both had a t(4;11)(q21;q23) translocation, which results in the *KMT2A-AFF1* (or *MLL-AF4*) fusion gene. The presence of this gene rearrangement is known to be directly associated with *FLT3* overexpression.[10] It is important to note that this patient was diagnosed with the AML-M5 subtype according to the French-American-British classification, which has also been associated with *FLT3* overexpression.[11,12]

The most common alteration associated with *FLT3* overexpression is *FLT3* activating mutations, mainly in AML,[2,13,14] and in some ALL subtypes such as high hyperdiploid BCP-ALL, *BCR-ABL1*-like (Ph-like) BCP-ALL, and "early T-cell precursor" ALL.[15] However, other genetic alterations have also been associated with *FLT3*

Table 1. Mechanisms of *FLT3* transcriptional deregulation in acute leukemias

Subtype	Experimental model	Genetic alteration	Mechanism	References
B-cell precursor acute lymphoblastic leukemia (BCP-ALL)	BCP-ALL patient samples (n = 1418); GM12878 and NALM-6 cell lines	13q12.2 deletion	13q12.2 deletions disrupt chromatin structure and change promoter-enhancer interactions in this region (enhancer hijacking), leading to increased expression of the *FLT3* gene	1
	Pro-B-cell lines; bone marrow progenitors; $Pax5^{-/-}$ mice; and case report	*PAX5* deletion	It was already demonstrated that Pax5 represses *Flt3* transcription in B-cell progenitors, and that Pax5-deficient pro-B cells express abundant *Flt3* levels. Based on that, it has been suggested that *PAX5* heterozygous deletion in B-cell acute leukemia could be related to *FLT3* overexpression	16,17
T-cell acute lymphoblastic leukemia (T-ALL)	T-ALL patient samples (n = 60); human leukemia xenograft model; and Jurkat cell line	Loss-of-function alterations of *SUZ12*, *EED*, and *EZH2*	PRC2 inactivation, due to loss-of-function alterations of *SUZ12*, *EDD*, and *EZH2*, leads to the loss of H3K27me3 in *FLT3* promoter region resulting in increased *FLT3* expression	18
Acute myeloid leukemia (AML)	AML patients (TCGA, n = 158); and leukemia stem cells from AML mouse model	*DNMT1* haploinsufficient	*DNMT1* haploinsufficiency may lead to hypomethylation of *FLT3* promoter region, which results in its increased expression	14
	MV4-11, K562, and THP1 human leukemia cell lines	Loss of *MSI2*	*FLT3* expression can also be regulated by a post-transcriptional mechanism through the MSI2 protein, which physically binds to *FLT3* messenger RNA transcripts. In case of genetic loss of *MSI2*, negative regulation of *FLT3* expression is observed, and impaired leukemic growth	19
	MV4-11 and THP-1 cell lines; THP-1 xenograft murine model	Inhibition of *PRMT5*	The PRMT5-Sp1 transcription repressor complex is capable of silencing miR-29b via dimethylation of histone 4 arginine residue H4R3. As a result, an increase in Sp1 is observed, as it is a bona fide target of miR-29b. This event in turn leads to the activation of *FLT3* transcription. Thus, the inhibition of *PRMT5* can result in a significant increase in the expression of miR-29b and consequent suppression of *Sp1* and *FLT3*	20
	Murine-cultured model and bone marrow samples from 104 AML patients	*CEBPA* biallelic mutations	Together, HOXA9, MEIS1, MYB, and C/EBPα are important elements in *FLT3* regulation. In this way, it was observed that *CEBPA* bi-allelic mutations, mainly in the absence of *FLT3* activating mutations, are associated with reduced levels of *FLT3* transcript	21

overexpression. These emerging mechanisms of *FLT3* transcriptional regulation are summarized in Table 1. In some cases, these alterations are crucial for leukemogenesis and, consequently, they constitute relevant biomarkers for those leukemias. These examples demonstrate the complexity of elucidating the mechanisms underlying *FLT3* regulation, especially when dealing with varied disease contexts.

In conclusion, our work has led us to conclude that 13q12.2 deletion is not a recurrent event in T-ALL nor AML cases. Therefore, our work adds novel and important data to Yang and colleagues' findings, indicating that this newly described mechanism is specifically associated with *FLT3* overexpression in BCP-ALL cases. Moreover, once we excluded the possibility that this mechanism plays a role in T-ALL or AML, an opportunity remains open for future studies that aim to elucidate the mechanisms behind *FLT3* overexpression in those leukemia subtypes.

Acknowledgments: The results published here are in whole based upon data generated by the Therapeutically Applicable Research to Generate Effective Treatments (TARGET, https://ocg.cancer.gov/programs/target) initiative, phs000218. The data used for this analysis are available at https://portal.gdc.cancer.gov/projects.

The authors thank the Bioinformatics Core Facility (INCA-RJ) for their support.

Contribution: C.P.P. performed data analyses, interpreted the results, and wrote the manuscript; M.B. and M.E. designed the study, supervised all analyses, and wrote the manuscript; and all authors critically reviewed and approved the final version of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: C.P.P., 0000-0003-3871-4749; M.B., 0000-0003-3495-9917; M.E., 0000-0003-2337-8420.

Correspondence: Mariana Emerenciano, Division of Clinical Research, Research Center–Instituto Nacional de Câncer, Rua André Cavalcanti, 37, 3° floor, Rio de Janeiro, RJ 20231-050, Brazil; e-mail: memerenciano@inca.gov.br.

References

1. Yang M, Safavi S, Woodward EL, et al. 13q12.2 deletions in acute lymphoblastic leukemia lead to upregulation of FLT3 through enhancer hijacking. *Blood*. 2020;136(8):946-956.
2. Poubel CP, Mansur MB, Boroni M, Emerenciano M. FLT3 overexpression in acute leukaemias: New insights into the search for molecular mechanisms. *Biochim Biophys Acta Rev Cancer*. 2019; 1872(1):80-88.
3. Spencer DH. Deletions in BCP-ALL result in a TAD more FLT3. *Blood*. 2020;136(8):919-920.
4. Lin M, Wei L-J, Sellers WR, Lieberfarb M, Wong WH, Li C. dChipSNP: significance curve and clustering of SNP-array-based loss-of-heterozygosity data. *Bioinformatics*. 2004;20(8):1233-1240.
5. Pounds S, Cheng C, Mullighan C, Raimondi SC, Shurtleff S, Downing JR. Reference alignment of SNP microarray signals for copy number analysis of tumors. *Bioinformatics*. 2009;25(3):315-321.
6. Venkatraman ES, Olshen AB. A faster circular binary segmentation algorithm for the analysis of array CGH data. *Bioinformatics*. 2007; 23(6):657-663.
7. Haraksingh RR, Abyzov A, Urban AE. Comprehensive performance comparison of high-resolution array platforms for genome-wide copy number variation (CNV) analysis in humans. *BMC Genomics*. 2017; 18(1):321.
8. Hehir-Kwa JY, Pfundt R, Veltman JA. Exome sequencing and whole genome sequencing for the detection of copy number variation. *Expert Rev Mol Diagn*. 2015;15(8):1023-1032.
9. Sudmant PH, Rausch T, Gardner EJ, et al; 1000 Genomes Project Consortium. An integrated map of structural variation in 2,504 human genomes. *Nature*. 2015;526(7571):75-81.
10. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet*. 2002;30(1):41-47.
11. Libura M, Asnafi V, Tu A, et al. FLT3 and MLL intragenic abnormalities in AML reflect a common category of genotoxic stress. *Blood*. 2003; 102(6):2198-2204.
12. Kuchenbauer F, Kern W, Schoch C, et al. Detailed analysis of FLT3 expression levels in acute myeloid leukemia. *Haematologica*. 2005; 90(12):1617-1625.
13. Ozeki K, Kiyoi H, Hirose Y, et al. Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia. *Blood*. 2004;103(5): 1901-1908.
14. Cheng J, Qu L, Wang J, Cheng L, Wang Y. High expression of FLT3 is a risk factor in leukemia. *Mol Med Rep*. 2018;17(2):2885-2892.
15. Mohseni M, Uludag H, Brandwein JM. Advances in biology of acute lymphoblastic leukemia (ALL) and therapeutic implications. *Am J Blood Res*. 2018;8(4):29-56.
16. Holmes ML, Carotta S, Corcoran LM, Nutt SL. Repression of Flt3 by Pax5 is crucial for B-cell lineage commitment. *Genes Dev*. 2006;20(8): 933-938.
17. Griffith M, Griffith OL, Krysiak K, et al. Comprehensive genomic analysis reveals FLT3 activation and a therapeutic strategy for a patient with relapsed adult B-lymphoblastic leukemia. *Exp Hematol*. 2016; 44(7):603-613.
18. Zhang J, Zhang Y, Zhang M, et al. FLT3 pathway is a potential therapeutic target for PRC2-mutated T-cell acute lymphoblastic leukemia. *Blood*. 2018;132(23):2520-2524.
19. Hattori A, McSkimming D, Kannan N, Ito T. RNA binding protein MSI2 positively regulates FLT3 expression in myeloid leukemia. *Leuk Res*. 2017;54:47-54.
20. Tarighat SS, Santhanam R, Frankhouser D, et al. The dual epigenetic role of PRMT5 in acute myeloid leukemia: gene activation and repression via histone arginine methylation. *Leukemia*. 2016;30(4): 789-799.
21. Volpe G, Walton DS, Del Pozzo W, et al. C/EBPα and MYB regulate FLT3 expression in AML. *Leukemia*. 2013;27(7):1487-1496.