Childhood Myeloid Neoplasms With *PTPN11* Mutations in Brazil

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To The Editor:

Recently, Hamdly et al reported the high frequency of gene mutation for RAS pathways, driving attention to the concept of mutually exclusive gene mutations observed in a series of juvenile myelomonocytic leukemia (JMML).[1] We have focused on the *PTPN11* somatic mutations in the molecular characterization of patients with myeloid neoplasms (MN) because they are scarcely explored in the context of a predictive marker. *PTPN11* mutations are associated with myeloproliferative disorders, JMML, and acute myeloid leukemia (AML). However, when used as a biomarker, *PTPN11* mutations may help to distinguish the MN subtypes. The *PTPN11* gene encodes a cytoplasmic protein tyrosine phosphatase 2 (SHP-2) that is an effector of the RAS/MAPK pathway.[2,3] Germline mutations are described in approximately 50% of individuals with Noonan syndrome (NS), a disorder associated with a predisposition for childhood hematologic malignancies and bleeding disorders.[4] Residues D61, A72, and E76 codified by exon 3 and S502 and G503 codified by exon 13 are the most affected regions. Mutations in exons 3 and 13 lead to constitutive activation of the pathway as a consequence of increasing the catalytic activity of SHP-2 by reducing or eliminating the interaction of the N-SH2 and PTP domains that maintain SHP-2 in its inactive state.[2] Our study aimed to explore genetic mutations in the RAS/MAPK pathway, to identify mutations in children and adolescents with MN with an emphasis on *PTPN11* as a predictive marker.

The Brazilian series includes pediatric patients with MN diagnosed between 2010 and 2018. The details of study design, inclusion and exclusion criteria, and methods are described in the Supplemental Appendix (in the online version). A clinical cohort of 385 patients was assessed, including patients with AML (n = 364) and myelodysplastic syndromes (MDS)/JMML (n = 21). There were no statistical differences according to age groups, gender, and ethnicity among patients with AML and patients with MDS/JMML (shown in Supplemental Table 1 in the online version). Overall, *RAS* mutations were found in 10.4% of patients with AML and 16.6% of patients with JMML, whereas *PTPN11* mutations were found in 4.6% (18/385) patients, and were prevalent in the MN series of cases; there were 12 (3.3%) patients with AML and 6 (28.6%) patients with MDS/JMML. The highest rate of mutations was observed in males (77.8%; $P = .021$), whereas there was no significant statistical association according to age strata ($P = .516$) or white blood cell count ($P = .276$). We observed concomitant mutations of *PTPN11* and *KMT2A-r* in patients with AML and/or monosomy 7 in patients with MDS/JMML. The main findings of patients with MN with *PTPN11* mutations (Table 1) demonstrate that mutations were predominantly found in patients with MN with monocytic differentiation (AML-M4, M5, and JMML). In 1 patient with AML-M7, the mutation c.216C > T p.A72V in *PTPN11* was found concomitant with *NRAS* mutation and monosomy 7. Two patients with JMML presented a *PTPN11* mutation and monosomy 7. Figure 1 shows the schematic structure of *PTPN11* SHP-2 tyrosine-phosphatase protein and the location of *PTPN11* mutations found in Brazilian patients with MN. All missense mutations in *PTPN11* were located at amino acid residues G60, D61, A72, and E76 in the N-SH2 domain of the protein tyrosine phosphatase. *PTPN11* mutations were found mostly in the

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Table 1 Clinical and Laboratory Characteristics of *PTPN11* Mutations in a Series of Myeloid Neoplasms in Brazil

Age, y/Gender/Ethnicity	Myeloid Neoplasms	PTPN11 Mutations	Chromosomal Aberrations	Other RAS/MAPK Pathway
4/F/White	JMML	c.181 G > T p.D61Y	Absent	Absent
4/M/White	AML-M4	c.181G > T p.D61Y	Absent	Absent
16/M/White	AML-M4	c.182A > C p.D61A	Absent	Absent
7/F/White	AML-M5	c.182A > T p.D61V	Absent	Absent
1/M/White	JMML	c.214 G > A p.A72T	Absent	Absent
9/M/Others	AML-M0	c.214 G > A p.A72T	Absent	Absent
4/M/Others	JMML	c.214 G > A p.A72T	Monosomy 7	Absent
18/F/Black	AML-M2	c.215C > A p.A72D	Absent	Absent
7/M/Others	AML-M4	c.216C > T p.A72V	Absent	Absent
7/M/Others	AML-M7	c.216C > T p.A72V	Monosomy 7	*NRAS* c.35 G > T p.G12V
14/M/White	AML-M1	c.223 G > A p.E76K	*KMT2A-r*	Absent
11/M/White	AML-M1	c.226 G > A p.E76K	Absent	Absent
3/M/Others	AML-M4	c.226 G > A p.E76K	Absent	Absent
0/M/White	JMML	c.226 G > A p.E76K	Absent	Absent
1/M/White	JMML	c.226 G > A p.E76K	Monosomy 7	Absent
17/M/White	AML-M4	c.227A > C p.E76A	Absent	Absent
14/M/Black	AML-M4	c.227A > C p.E76A	Absent	Absent
2/F/White	JMML	c.277 G > A p.G60R	Absent	Absent

Abbreviations: AML = acute myeloid leukemia; F = female; ID = identification; JMML = juvenile myelomonocytic leukemia; M = male.

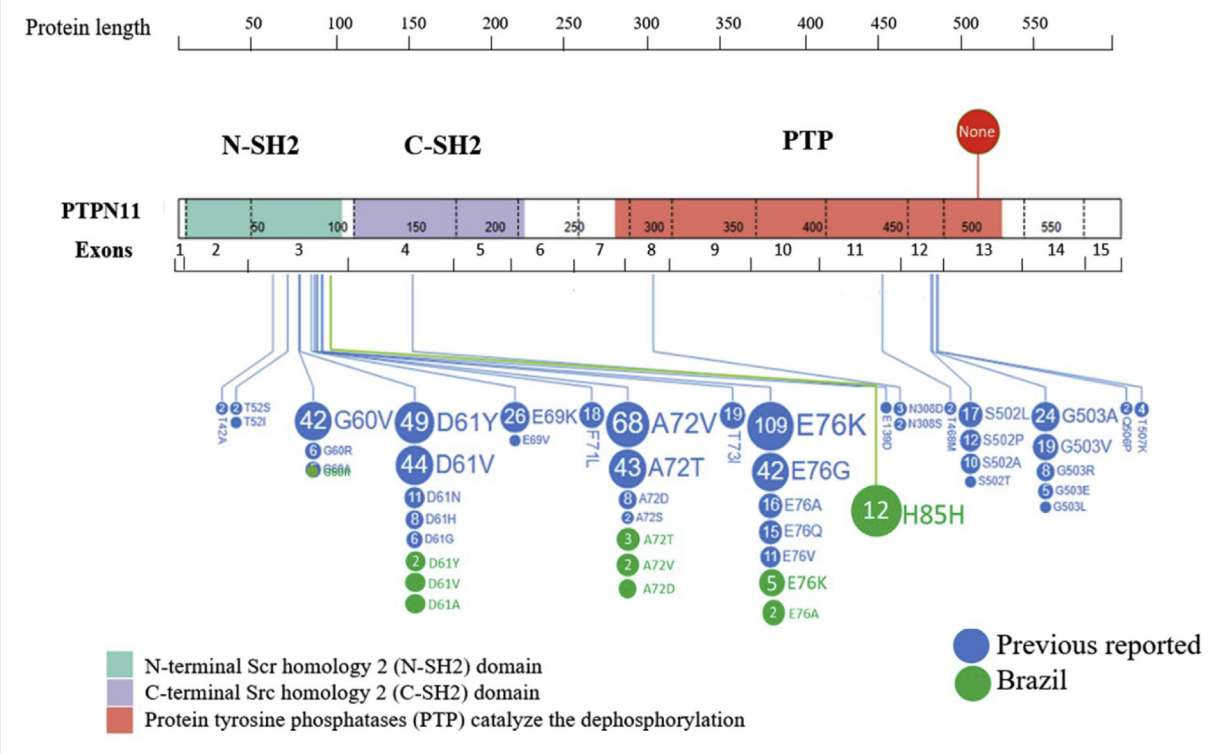
myelomonocytic lineage. We found a patient diagnosed with AML with megakaryocyte differentiation (AML-M7) who presented a concomitant mutation in *PTPN11* (c.216C > T A72V), *NRAS* (c.G> T p.G12V), and monosomy of chromosome 7. Additionally, the polymorphism in *PTPN11* (c.255C>T p.H85H) was found in 3.1% (12/385) of patients with MN, including patients with APL (n = 3). Univariate analysis of 48-month probability of overall survival (pOS) of MN cases and the impact of mutations in the clinical outcome are shown in Supplemental Table 2 (in the online version). Patients with MDS/JMML presented better outcome (48-month pOS, 56.3% ± 18.4%; $P = .041$) compared with patients with AML (48-month pOS, 32.3% ± 3.9%). Age, gender, ethnicity, and leukocytosis were not significantly associated with differences in pOS. The Kaplan-Meier test for patients with AML was assessed according to *PTPN11* status, with pOS of 12.5% ± 10.8% and 33.2% ± 4.0% for patients with AML with and without mutations in *PTPN11*, respectively (Figure 2). Patients with APL were excluded.

Discussion

In this study, we have explored the aspects of childhood MN with *PTPN11* mutations in a Brazilian cohort. *PTPN11* mutations in AMLs were found in a similar frequency (4%) as reported by Loh et al and Tartaglia et al.[5,6] In JMML, we have found *PTPN11* mutations in 28.6% compared with 40% and 29% described by Tartaglia et al and Hamdy et al, respectively.[1,2] *PTPN11* mutations were predominant in males (77.8%) and were not associated with age nor elevated leukocyte counts, differently from cases reported by Loh et al.[5] We have included a smaller number of patients with JMML compared with the study from Hamdy et al, and the similarity of our findings corroborate that our cohort is representative.[1] *PTPN11* mutations occur exclusively in childhood MN and are rarely reported in adult patients with MDS/MPN or AML.[7] Although Brazil has a population with a mixed genetic background, we have found the same frequencies of the studies performed in homogeneous genetic populations reported in case series and cohort studies,[2,5-11] demonstrating that somatic mutations in *PTPN11* are related to any ethnicity. We have identified 12 patients (AML, n = 10 and JMML, n = 2) with a silent alteration in H85 residue, which was previously reported by Tartaglia et al as a neutral polymorphism.[4] Ensembl database describes this alteration as a synonymous variant, being the "T" allele observed in 9.0% of the African population. In the current version of the gnomAD population database, the *PTPN11* c.255C> T p.H85H variant is described in 2.3% of the global population and 0.4% in Latinos, reaching a frequency as high as 7.7% in the African population. The COSMIC database describes c.255C> T p.H85H as a somatic alteration present in hematopoietic and lymphoid tissues. This variant is found in ClinVar with a classification of benign with a 3-star rating. Therefore, the interpretation of our findings is compatible with the general Brazilian genetic ancestrality of African descendants.

Similarly to Loh et al and Tartaglia et al, somatic *PTPN11* mutations were predominant in AML with myelomonocytic/monoblastic differentiation (AML-M4/M5) in our cohort.[5,6] These findings corroborate with the hypothesis that *PTPN11* mutations have a fundamental contribution to the clonal expansion caused by the deregulation of transduction pathways that selectively control the proliferation or survival of precursor cells of the myelomonocytic lineage.[11] Molecular similarities between AML and JMML with mutations in *PTPN11* and genes of the RAS/MAPK pathway corroborate to the premise that secondary events cooperate with a genetic injury initiation spectrum. Although previous reports

Figure 1 SHP-2 Tyrosine-Phosphatase Protein Structure and the Exons of the *PTPN11* Gene



demonstrated that concomitant alterations in other genes of the RAS/MAPK pathway are mutually exclusive[1,4,5]; herein, we found 1 patient with AML with the concomitant occurrence of *PTPN11* and *NRAS* mutations. *PTPN11* mutations are considered a late event in AML leukemogenesis and occur in varying sized subclones. Bolouri et al have also demonstrated the concurrent mutations in genes of the MAPK pathway in the same MN subtype.[11] Additionally, in this study, we have found that mutations in *PTPN11* have an impact on the survival of pediatric patients with AML. Similarly, Yoshida et al reported that patients with *PTPN11* mutations presented worse pOS (25.0%) than patients without mutations (64.0%).[12] We recognize that different factors, such as cytogenetic subtype, race, white blood cell count, treatment protocol, and clinical supportive care are associated with predictive risk of AML. The karyotypic aberrations allow the segregation of AML into 3 categories of favorable, adverse, and intermediate prognosis subgroups. Our previous results corroborate with the literature that *CBFβ-MYH11* is a predictor of a favorable outcome, although we did not evaluate other markers, such as *NMP1* mutations[9,10,13] Papaemmanuil et al have proposed a genomic classification of AML, which includes adult patients presenting *NPM1* and *PTPN11* mutations as a subgroup. Overall, the authors found about 9% of *PTPN11* mutations in adulthood AML. This report changed the role of the *PTPN11* gene in the AML outcomes context.[14] *NPM1* mutations often occur after mutations in the *RAS* pathway or epigenetic modifiers (*DNMT3A* and *IDH1)*, suggesting that the development of adulthood AML follows specific and ordered evolutionary trajectories, both being events leadership of cell proliferation and/or survival.[14,15] We did not evaluate these clonal relationships.

Conclusion

We found *PTPN11* mutations in MN mainly prevalent in JMML additionally to other RAS/MAPK pathway. *PTPN11* status should be investigated to distinguish JMML among the MNs because *PTPN11* mutations are also a predictive marker of outcome in childhood AML.

Disclosure

The authors have stated that they have no conflicts of interest.

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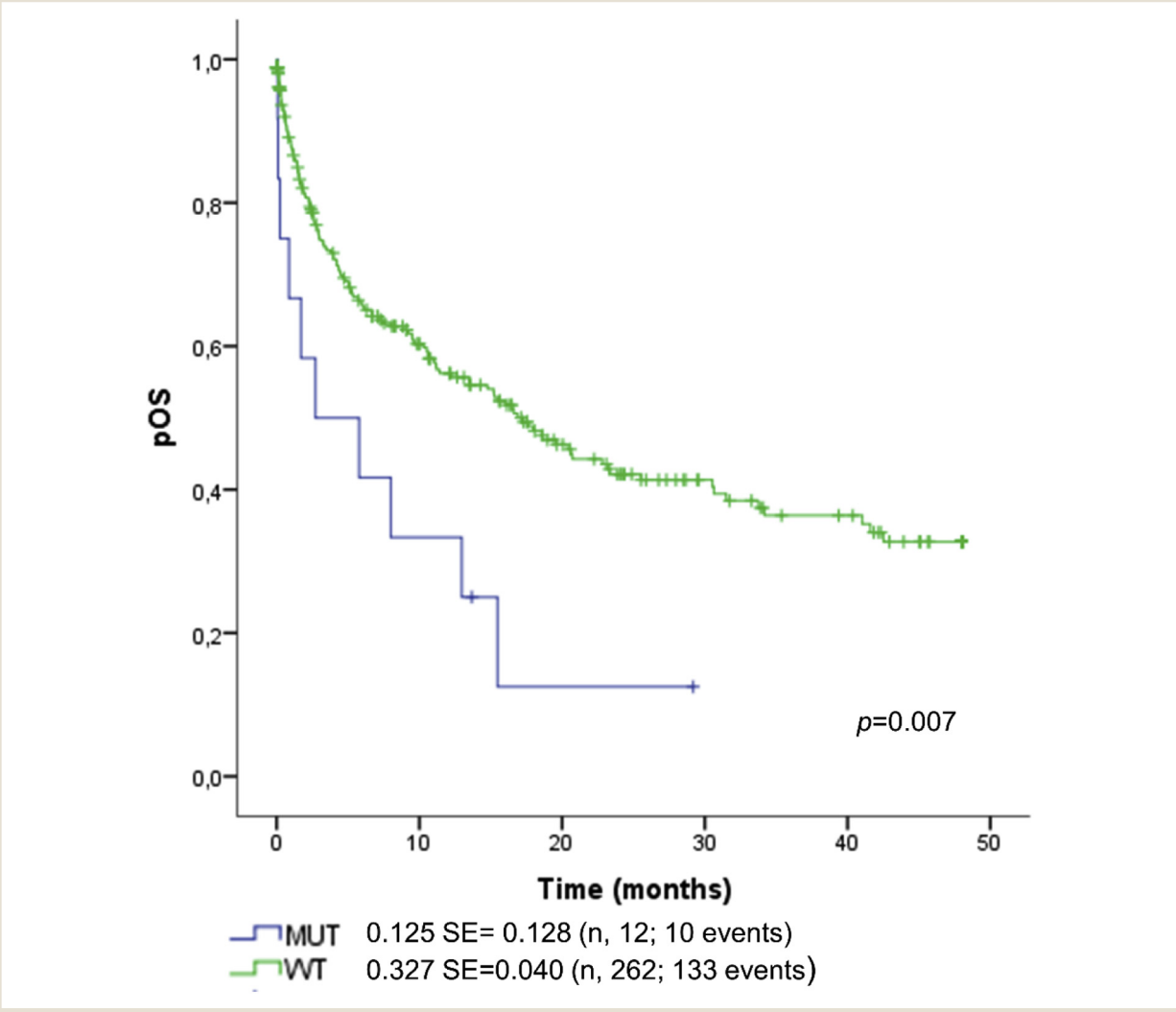
Figure 2 Overall Survival of Pediatric Acute Myeloid Leukemia according to *PTPN11* Status. The Kaplan-Meier Analysis was Performed in 48 Months. Acute Promyelocytic Leukemia Were Excluded From Survival Analysis. The Green Curve Represents the Overall Survival of Wild-type (WT) Cases and Blue Curve Represents Cases With *PTPN11* Mutations (MUT). *PTPN11* Mutations had a Significant Impact on the pOS in Childhood AML



Abbreviations: MUT = mutations; pOS = probability of overall survival; WT = wild-type.

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Supplemental Data

Supplemental Material, Tables and figure accompanying this article can be found in the online version at https://doi.org/10.1016/j.clml.2020.04.009.

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Supplemental Appendix

Patients and Methods

Subjects

This is a clinical cohort study of pediatric patients with myeloid neoplasm (MN) samples sent for diagnosis procedure at the Pediatric Hematology-Oncology Research Program, INCA, Brazil, between 2010 and 2018. Inclusion criteria were children and adolescents $\leq$ 19 years of age at diagnosis, before any oncologic treatment. Exclusion criteria consisted of patients with prior chemotherapy, phenotype syndrome, and genetic syndromes (eg, Down syndrome, Noonan syndrome, Bloom syndrome, others), unspecified leukemia subtype, and chronic myeloid leukemia with *BCR-ABL1*. Demographic and clinical data (age, gender, ethnicity, white blood cell count, and treatment protocol) were collected for each patient and are shown in Supplemental Figure 1 (in the online version).

MN *Categories*

The diagnosis of MN followed the World Health Organization criteria based on morphology, immunophenotyping, and genetic characterization of bone marrow and peripheral blood samples.[7] Based on the previously published data,[8] the routine evaluation of childhood acute myeloid leukemia (AML) included in this clinical cohort was performed with prognostically relevant genetic aberrations by cytogenetics/fluorescence in situ hybridization, such as *RUNX1-RUNX1T1*, *CBFB-MYH11*, *PML-RARA*, *MLL* rearrangements, and the potentially targetable selected set of molecular markers, such as *FLT3-ITD*, *c-KIT*, and *CEBPA* (data not included). Because *NPM1* mutations are rare events in very early age AML and less than 15% of cases were tested, *NMP1* gene status was excluded in our evaluation.[8] Karyotyping was not successfully available in 20.7% of the cases.

AMLs and myelodysplastic syndrome/myeloproliferative neoplasm cases were categorized according to the International Classification of Diseases for Oncology, third revision, by morphology codes M0-9872/3, M1-9873/3, M2-9874/3, M3-9866/3, M4-9867/3, M5-9891/3, M6-9840/3, M7-9910/3, not otherwise specified-9861/3, atypical chronic myeloid leukemia-9876/3 (*BCR-ABL1* negative), juvenile myelomonocytic leukemia-9868/3, and myelodysplastic syndromes-9989/1.

Molecular Analysis

Molecular tests were performed based on the quality of the diagnostic material. Genomic DNA was purified from bone marrow and/or peripheral blood samples with QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized following the addition of reverse transcriptase enzyme 8. The most frequent fusion genes in AML (*RUNX1-RUNX1T1*, *CBFβ-MYH11*, *KMT2A*-rearrangements, and *PML-RARα*) were identified as described elsewhere.[9]

Monosomy of chromosome 7 (−7/−7q) was analyzed by fluorescence in situ hybridization with commercial probes dual-color D7S486 [7q31] SpectrumOrange/CEP 7 and SpectrumGreen Probe Set (Vysis).

Mutations in the hotspot regions of genes involved in the RAS/MAPK pathway, including *FLT3*, *NRAS*, and *KRAS (N-KRAS)*, and *PTPN11*, were screened as previously described[19] or following primers and polymerase chain reaction conditions provided in Supplemental Table 1 (in the online version). Mutations in *FLT3* were detected as internal tandem duplication in exons 11/12 and point mutations in the tyrosine kinase domain (D835). *N-KRAS* mutational screening was performed in exon 1 (codons 12/13). *PTPN11* status was determined by screening of exons 3 and 13. Polymerase chain reaction products were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA) in a 3500 Genetic Analyzer (Applied Biosystems). Sequencing results were analyzed using the BioEdit 7.0.9 software (Carlsbad, CA), comparing electropherograms with the reference sequences accessed from the National Center for Biotechnology Information (see Supplemental Table 2 in the online version). A schematic structure of the SHP-2 protein and affected regions of the *PTPN11* gene was designed by the ProteinPaint data portal (https://pecan.stjude.cloud/), which provides interactive visualizations of pediatric cancer mutations.

Statistical Analysis

The descriptive analyses were performed through categorical variables to measure central tendency and dispersion, as well as to determine the frequency distribution. The χ^2 or Fisher exact tests were used to compare proportions between groups.

Overall survival was measured from the date of diagnosis to the date of the last follow-up or death from any cause. The survival analysis of patients with acute promyelocytic leukemia was performed separately, as acute promyelocytic leukemia is an AML subtype with unique characteristics and specific treatment. Patients who did not experience an event were censored at the time of the last follow-up, and those who did not attend a follow-up were censored at their date of last known contact. The Kaplan-Meier survival analysis method was used to calculate the 48-month survival probabilities, and estimated survival values were compared using the log-rank test to verify the association of *PTPN11* mutations and patients' outcome. All *P* values were considered significant if less than .05. All analyses were performed using SPSS 21.0 (SPSS, Chicago, IL).

Ethics

This research project has local agreements and approvals obtained by the National Cancer Institute of Brazil Research and Ethics Committee, under the registry number CAEE# 186688, as "Immuno-molecular alterations in pediatric AML: an interaction between somatic mutations and etiopathology risk factors." Patients were treated in accordance with the Declaration of Helsinki. The parents or legal guardians provided written consent.

Supplemental Table 1 Demographic, Clinical, and Laboratory Features of Myeloid Neoplasms According to PTPN11 Status in Brazil, 2010-2018

	Total (%)	*PTPN11*-MUT N = 18, n (%)	*PTPN11*-WT N = 356, n (%)	*P*
Age groups, y				.516
≤2	58/374 (15.5)	3 (16.7)	55 (15.4)	
>2-10	144/374 (38.5)	9 (50.0)	135 (37.9)	
≥11	172/374 (46.0)	6 (33.3)	166 (46.6)	
Ethnicity				.397
Black	28/374 (7.5)	2 (11.1)	26 (7.3)	
Non-black	346/374 (92.5)	16 (88.9)	330 (92.7)	
Gender				.021
Male	195/374 (52.1)	14 (77.8)	181 (50.8)	
Female	179/374 (47.9)	4 (22.2)	175 (49.2)	
WBC, $\times 10^9$/L				.276
≤50	242/374 (64.7)	10 (55.6)	232 (65.2)	
>50	132/374 (35.3)	8 (44.4)	124 (34.8)	
Myeloid neoplasms (ICD-O-3)				.0001
AML-M0/M1 (9872/3; 9873/3)	42/374 (11.2)	3 (16.6)	39 (10.7)	
AML-M2 (9874/3)	50/374 (13.4)	1 (5.6)	49 (13.8)	
AML-M3 (9866/3)	86/374 (23.0)	0 (0.0)	86 (24.2)	
AML- M4/M5 (9867/3; 9891/3)	143/374 (38.2)	7 (38.9)	136 (38.2)	
AML-M6/M7 (9840/3;9910/0)	30/374 (8.0)	1 (5.6)	29 (8.1)	
Not otherwise specified (9861/3)	3/374 (0.8)	0 (0.0)	3 (0.8)	
aCML (9868/3)	6/374 (1.6)	0 (0.0)	6 (1.7)	
MDS/JMML (9989/1; 9868/3)	14/374 (3.7)	6 (33.3)	8 (2.2)	
Chromosomal aberrations				
RUNX1-RUNX1T1	42/296 (14.2)	0 (0.0)	42 (14.8)	.153
CBFβ-MYH11	17/288 (5.9)	0 (0.0)	17 (6.2)	.475
KMT2A-r	45/235 (19.1)	1 (12.5)	44 (19.4)	.526
PML-RARα	64/107 (58.2)	0 (0.0)	61 (57.5)	.430
Monosomy 7	3/19 (15.8)	3 (18.8)	0 (0.0)	.578
RAS/MAPK pathway				
N-KRAS	39/385 (10.5)	1 (5.6)	38 (10.8)	.414
FLT3	63/310 (20.3)	0 (0.0)	63 (21.1)	.062

Abbreviations: aCML = atypical chronic myeloid leukemia; AML = acute myeloid leukemia; ICD-O-3 = International Classification of Diseases for Oncology, third revision; JMML = juvenile myelomonocytic leukemia; MDS = myelodysplastic syndrome; MUT = mutant; WBC = white blood cell count; WT = wild-type.

Supplemental Table 2 Primers and PCR Conditions for Sequencing of Genes *RAS*, *PTPN11*, and *FLT3*

Gene[a]	Region	Primer Sequence Reverse 5'-3'	Primer Sequence Forward 5'-3'	PCR Conditions
KRAS	Exon 1	AACCTTATGTGTGACATGTTC	ATGGTCCTGCACCAGTAAT	Initial: 95°C 30 sec; annealing: 35 cycles of 94°C 30 sec, 60°C 1 min, 72°C 30 sec; final: 72°C 10 min[c]
NRAS	Exon 1	GACTGAGTACAAACTGGTGG	TGCATAACTGAATGTATACCC	Initial: 94°C 5 min ; annealing: 40 cycles of 94°C 1 min, 57°C 1 min, 72°C 1 min; final: 72°C 10 min[c]
PTPN11	Exon 3	CGACGTGGAAGATGAGATCTGA	CAGTCACAAGCCTTTGGAGTCAG	Initial: 94°C 8 min, annealing : 33 cycles of 94°C 45 sec, 58°C 30 sec, 72°C 45 sec; final: 72°C 10 min[c]
PTPN11	Exon 13	GGCTCTGCAGTTTCTCTTTATTC	CCAAGAGGCCTAGCAAGAG	Initial: 94°C 8 min, annealing: 33 cycles of 94°C 45 sec, 58°C 30 sec, 72°C 45 sec; final: 72°C 10 min[c]
FLT3 D835	Exon 20	CCGCCAGGAACGTGCTTG	CAGCCTCACATTGCCCC	Initial: 94°C 7 min, annealing: 35 cycles of 94°C 30 sec, 61°C 30 sec, 72°C 45 sec; final: 72°C 10 min[b]
FLT3 ITD	Exons 11/12	GCAATTTAGGTATGAAAGCCAGC	CTTTCAGCATTTTGACGGCAACC	Initial: 94°C 3 min, 35 cycles of 94°C 30 sec, 56°C 1 min, 72°C 2 min; final: 72°C 7 min

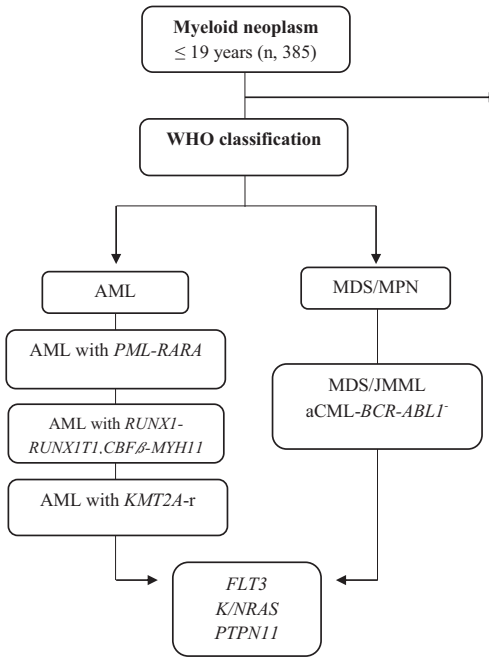
Abbreviation: PCR = polymerase chain reaction.
[a]Sequences reference National Center for Biotechnology Information: *KRAS*NG_007524.1; *NRAS* NG_007572.1; PTPN11 NG_00745; FLT3 NG_007066.1.
[b]PCR was followed by restriction fragment length polymorphism.
[c]PCR was followed by direct sequencing.

Supplemental Table 3 Univariate Analysis of Overall Survival in Myeloid Neoplasms in Brazil, 2010-2018

	No. Events	Univariate Analysis		
		48-month pOS, % (SE)	Median[a] (95% CI)	*P*
Myeloid neoplasms	289 (143)			.041
AML	269 (138)	32.3 (3.9)	16.6 (11.8-21.9)	
MDS/MPN	20 (5)	56.3 (18.4)	[b]	
Age range, y				.181
≤2	55 (23)	42.6 (9.3)	23.3 (6.5-40.0)	
>2-10	107 (51)	32.1 (6.3)	23.2 (9.3-37.1)	
≥11	127 (69)	31.8 (5.6)	11.4 (5.9-16.9)	
Gender				.949
Male	146 (74)	31.9 (5.5)	17.6 (9.6-25.5)	
Female	142 (69)	35.5 (5.4)	17.2 (10.8-23.7)	
Ethnicity				.540
Black	23 (13)	31.3 (11.4)	11.0 (0.0-32.1)	
Non-black	266 (130)	34.0 (4.1)	17.9 (12.5-23.3)	
WBC ($\times 10^9$/L)				.660
≤50	183 (93)	32.1 (4.7)	17.6 (13.4-21.9)	
>50	106 (50)	38.3 (6.5)	19.6 (0.0-41.3)	
Chromosomal aberrations				
RUNX1-RUNX1T1	43 (20)	33.5 (11.7)	17.6 (14.5-20.7)	.588
CBFβ-MYH11	17 (3)	79.0 (10.8)	[b]	.013
KMT2A-r	45 (25)	24.3 (9.3)	19.6 (7.9-31.2)	.275
Monosomy 7	3 (2)	0.0 (0.0)	[b]	.359
Mutations in RAS/MAPK pathway[c]				
FLT3	35 (24)	16.9 (7.8)	11.2 (7.4-14.9)	.274
N-KRAS	36 (18)	43.4 (9.0)	16.0 (7.2-24.8)	.987
PTPN11	18 (11)	17.3 (14.0)	13.0 (3.7-22.2)	.341

Abbreviations: AML = acute myeloid leukemia; CI = confidence interval; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasms; pOS = probability of overall survival; SE = standard error; WBC = white blood cell count.
[a]Median survival in months.
[b]Median not reached.
[c]Analysis performed between groups positive and negative for the molecular alteration.

Supplemental Figure 1 **Flowchart of the Study Design and the Demography of Myeloid Neoplasm in Brazil, 2010 to 2018. A Cohort of 385 Myeloid Neoplasms Cases (age, < 19 Years) is the Subject of this Study (AML [n = 364] and MDS/MPN [n = 21]). Cases Were Classified According to WHO Criteria Based on Morphology, Immunophenotyping, and Molecular Characterization**



		Total (%)	AML n, 364 (%)	MDS/MPN n, 21 (%)	*p*
Age (yrs)					0.654
	<2	60 (15.5)	56 (15.3)	4 (19.0)	
	>2-10	146 (37.9)	140 (38.4)	6 (28.5)	
	>11	179 (46.4)	164 (46.1)	11 (52.3)	
Sex:					0.406
	Males	201 (52.2)	189 (51.9)	12 (57.1)	
	Females	184 (47.7)	175 (48.0)	9 (42.8)	
Ethnicity					0.251
	Blacks	151 (39.2)	140 (38.5)	11 (52.4)	
	Non-Blacks	234 (60.8)	224 (61.5)	10 (47.6)	

Abbreviations: aCML = Atypical chronic myeloid leukemia, BCR-ABL1- AML = acute myeloid leukemia; MDS/MPN = myelodysplastic/myeloproliferative neoplasms; MDS/JMML = myelodysplastic syndromes/juvenile myelomonocytic leukemia; WHO = World Health Organization.