# MOLECULAR CHARACTERIZATION OF PEDIATRIC ACUTE MYELOID LEUKEMIA: RESULTS OF A DIAGNOSTIC AND NATIONAL MULTICENTRIC STUDY IN BRAZIL

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## BACKGROUND

❖ In Brazil, childhood acute myeloid leukemia (c-AML) accounts for 18-24% of all diagnosed cases <19 years of age and the disease characterization represents an important challenge in pediatric hematology.

❖The two major types of genetic events in AML associated with outcome are mutations that enhance self-renewal and proliferation potential of the myeloid progenitor, conventionally known as type I and II mutations, respectively.

❖ We performed a comprehensive analysis of the main type I (*FLT3, KIT, NRAS, KRAS,* and *PTPN11*) and type II [*PML-RARα, RUNX1-RUNX1T1, CBFb-MYH11, KMT2A* and *NUP98* rearrangements *(KMT2A-r* and *NUP98-r,* respectively)] mutations in c-AML providing an overview of the molecular profile of the Brazil patients, as recommended by WHO for classification of myeloid neoplasms. We determined the frequencies of somatic alterations and investigate the potential contribution of these markers with clinical outcome.

## MATERIAL AND METHODS

**Patients.** This is a retrospective and multicentric study of 760 de novo c-AML referred to the Pediatric Hematology-Oncology Research Program, INCA, Rio de Janeiro, between January 1, 2000 and December 31, 2016. Cases included were forwarded from 49 Brazilian medical institutions that are reference in oncological care for children with leukemia for diagnostic purpose (Figure 1).

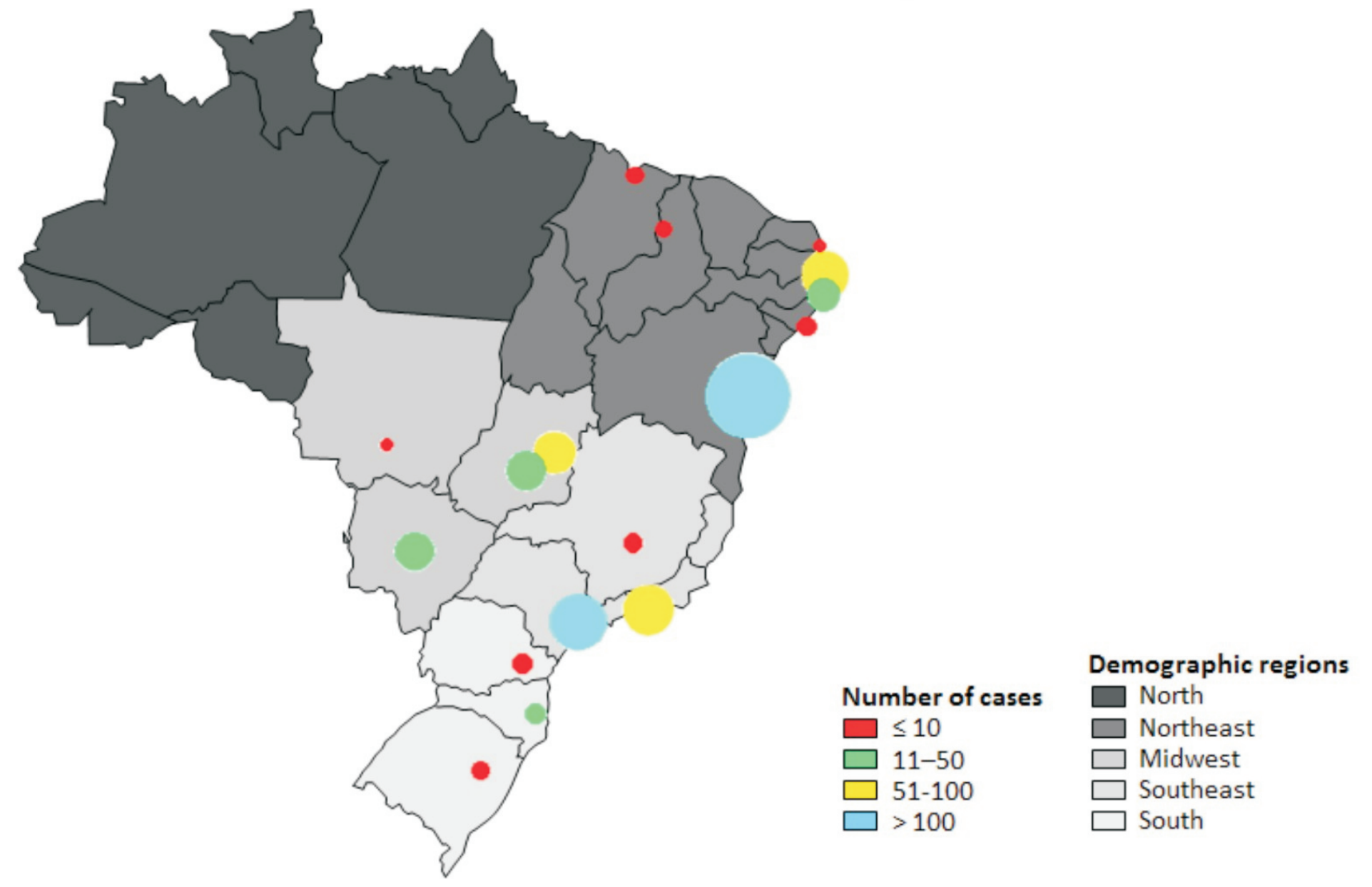

**Figure 1.** Brazilian map of the 760 c-AML cases sent from each geographic region. The number of cases from each collaborative institution is grouped by states and presented as proportional circles.

**Treatment.** Patients were treated out of a unique controlled clinical trial but received relatively homogeneous treatment following international consensus guidelines on AML treatment, with the BFM-AML2004 protocol after the year 2008. The institutional review board approved the treatment. Local agreements and approvals for this project were obtained by the Instituto Nacional de Câncer Research and Ethics Committee under the registry #186688.

**Molecular alterations.** Fusion genes associated with c-AML were screened by RT-PCR and/or FISH [*PML-RARα, RUNX1-RUNX1T1, CBFb-MYH11, KMT2A-r* and *NUP98-r*]. Mutations in hotspot regions of *RAS* pathway genes (*FLT3, NRAS, KRAS, PTPN11*, and *KIT*) were analyzed by direct sequencing. Briefly, *FLT3* mutations were screened at the tyrosine kinase domain (TKD) in codon 835 and at the juxtamembrane domain in exons 11/12 as internal tandem duplications (ITD). *NRAS/KRAS* status was determined by searching mutations in exon 1 (codons 12/13), *PTPN11* mutations were screened in exon 3, and *KIT* mutations in exons 8/17.

**Statistical Analysis.** Categorical variables were compared using qui-square or Fisher's exact tests. The Kaplan-Meier survival analysis was used to calculate the 5 year probability of overall survival (pOS) and estimated survival values were compared using the log-rank test. Overall survival was defined as time from study enrollment to death from any cause (event). Patients lost to follow up were censored at their date of last known contact. Cox proportional-hazard regression model with estimated hazard ratio (HR) and 95% confidence intervals (CI) were presented. Variables with p<0.20 were included in this model for multivariate analysis following the stepwise forward method.

## RESULTS

**Table 1.** Distribution of molecular alterations in c-AML cases according to demography and white blood cells count, Brazil, 2000-2016

| | | Age (years) | | | | | Sex | | | WBC count (x10⁹/l) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Molecular alteration [a] | Frequency n/total (%) | Median (min-max) | ≤2 n (%) | >2-10 n (%) | ≥11 n (%) | p | Males n (%) | Females n (%) | p | Median (min-max) | ≤50 n (%) | >50 n (%) | p |
| **Fusion genes** | | | | | | | | | | | | | |
| ***RUNX1-RUNX1T1*** | 80/547 (14.6) | 9.3 (0.1-20.5) | 9 (5.7) | 33 (18.1) | 38 (19.1) | **<0.001** | 48 (16.9) | 32 (12.5) | 0.16 | 21 (1.7-136) | 62 (18.0) | 16 (6.8) | **0.003** |
| ***CBFβ-MYH11*** | 29/532 (5.5) | 10.7 (0.3-19.3) | 5 (3.2) | 9 (5.2) | 15 (7.7) | 0.18 | 12 (4.3) | 17 (6.9) | 0.21 | 106 (2.2-373) | 8 (2.4) | 20 (11.1) | **<0.001** |
| ***KMT2A-r*** | 46/497 (9.3) | 1.6 (0.0-21) | 55 (34.0) | 23 (14.7) | 12 (7.2) | **<0.001** | 45 (17.3) | 45 (20.1) | 0.43 | 44.4 (1.0-451) | 49 (15.1) | 39 (25.7) | **0.006** |
| ***PML-RARα*** [b] | 109/137 (80.2) | 10.8 (1.3-19) | 2 (66.7) | 50 (76.9) | 57 (82.6) | 0.61 | 62 (80.5) | 47 (78.3) | 0.75 | 10.6 (15.8-228) | 79 (79.0) | 29 (80.6) | 0.84 |
| ***NUP98-r*** | 12/70 (17.1) | 1.7 (0.8-13.9) | 7 (30.4) | 3 (13.6) | 2 (10.5) | 0.19 | 5 (17.2) | 7 (20.0) | 0.78 | 36.9 (5.9-318.7) | 7 (14.9) | 5 (29.4) | 0.19 |
| **RAS pathway mutations** | | | | | | | | | | | | | |
| ***FLT3* (ITD or TKD)** | 125/544 (23.0) | 11.1 (1.0-21.0) | 5 (4.2) | 46 (22.1) | 73 (34.4) | **<0.001** | 67 (23.0) | 57 (22.9) | 0.97 | 34.7 (0.8-800) | 72 (20.7) | 52 (28.4) | **0.046** |
| ***KRAS*** | 34/535 (6.4) | 6.0 (0.5-18.3) | 10 (7.9) | 13 (6.5) | 11 (5.4) | 0.66 | 21 (7.4) | 13 (5.3) | 0.32 | 33.6 (1.0-700) | 19 (5.6) | 15 (8.2) | 0.25 |
| ***NRAS*** | 49/479 (10.2) | 10.2 (0.7-18.0) | 8 (8.7) | 15 (8.2) | 26 (13.0) | 0.26 | 27 (10.3) | 22 (10.3) | 0.98 | 50 (5.1-800) | 25 (8.3) | 24 (14.4) | **0.04** |
| ***KIT*** | 23/262 (8.8) | 5.4 (0.3-19.3) | 5 (6.9) | 12 (11.7) | 6 (7.1) | 0.44 | 13 (10.2) | 10 (7.6) | 0.46 | 42.7 (4.5-168) | 13 (7.8) | 81 (90.0) | 0.55 |
| ***PTPN11*** | 34/372 (9.1) | 8.2 (0.4-17.1) | 5 (7.2) | 15 (9.9) | 14 (9.4) | 0.81 | 23 (11.4) | 11 (6.5) | 0.11 | 40.2 (1.0-374) | 21 (8.8) | 13 (10.5) | 0.59 |

[a] The total number of analyzed cases reflects the availability of biological material for molecular tests. [b] Categories of *PML-RARα* include positive cases versus the remaining. ITD, internal tandem duplication; n, number; *KMT2A-r*, *KMT2A* rearrangements; *NUP98-r*, *NUP98* rearrangements; TKD, tyrosine kinase domain; WBC, white blood cell count at diagnosis. Bold p values are statistically significant. Percentage values are referent to cases presenting the molecular alteration

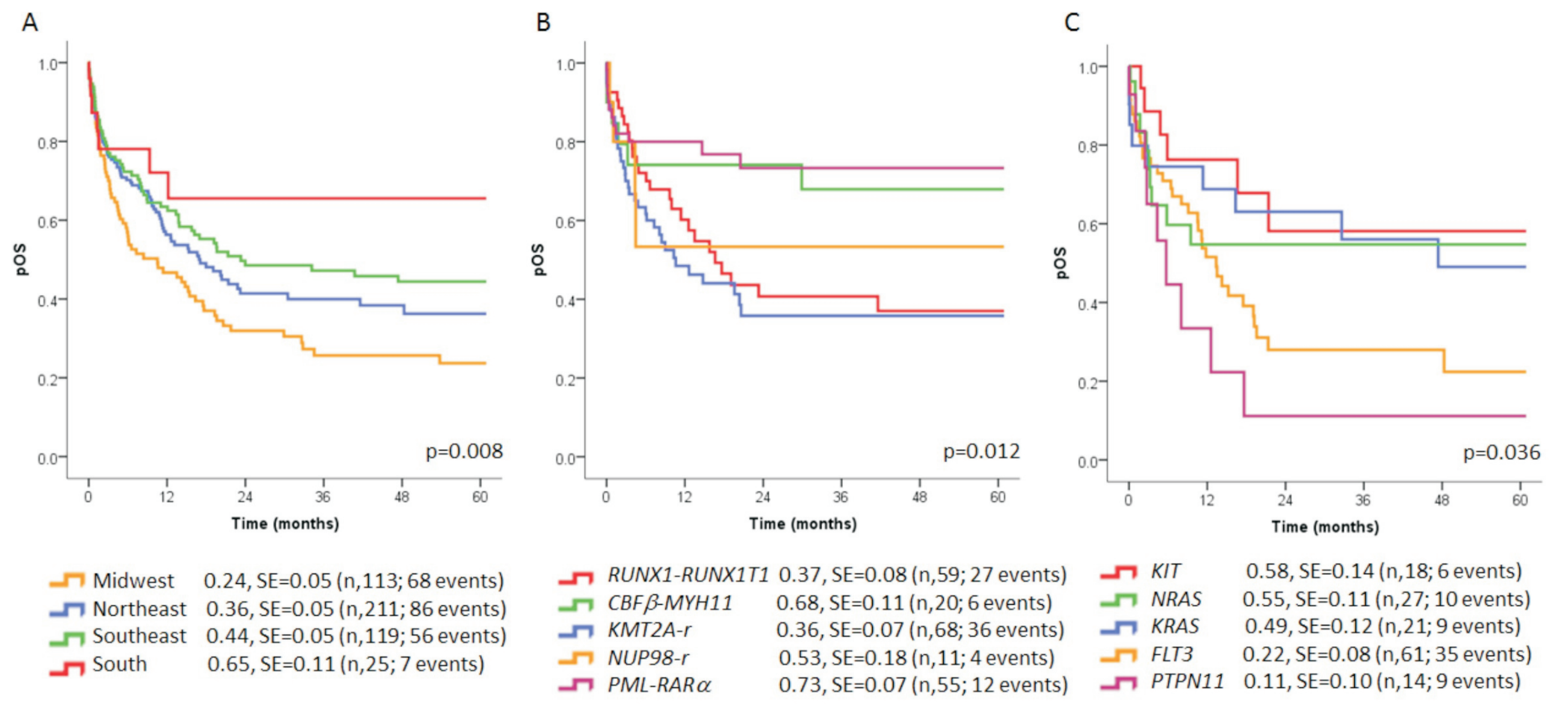

**Figure 3.** Survival analysis of the c-AML cases. Comparison of survival curves among geographic region* (A), type II (B), and type I mutations* (C). P values were calculated using log rank test. n, number of cases; SE, standard error. *Excluding acute promyelocytic leukemia cases.

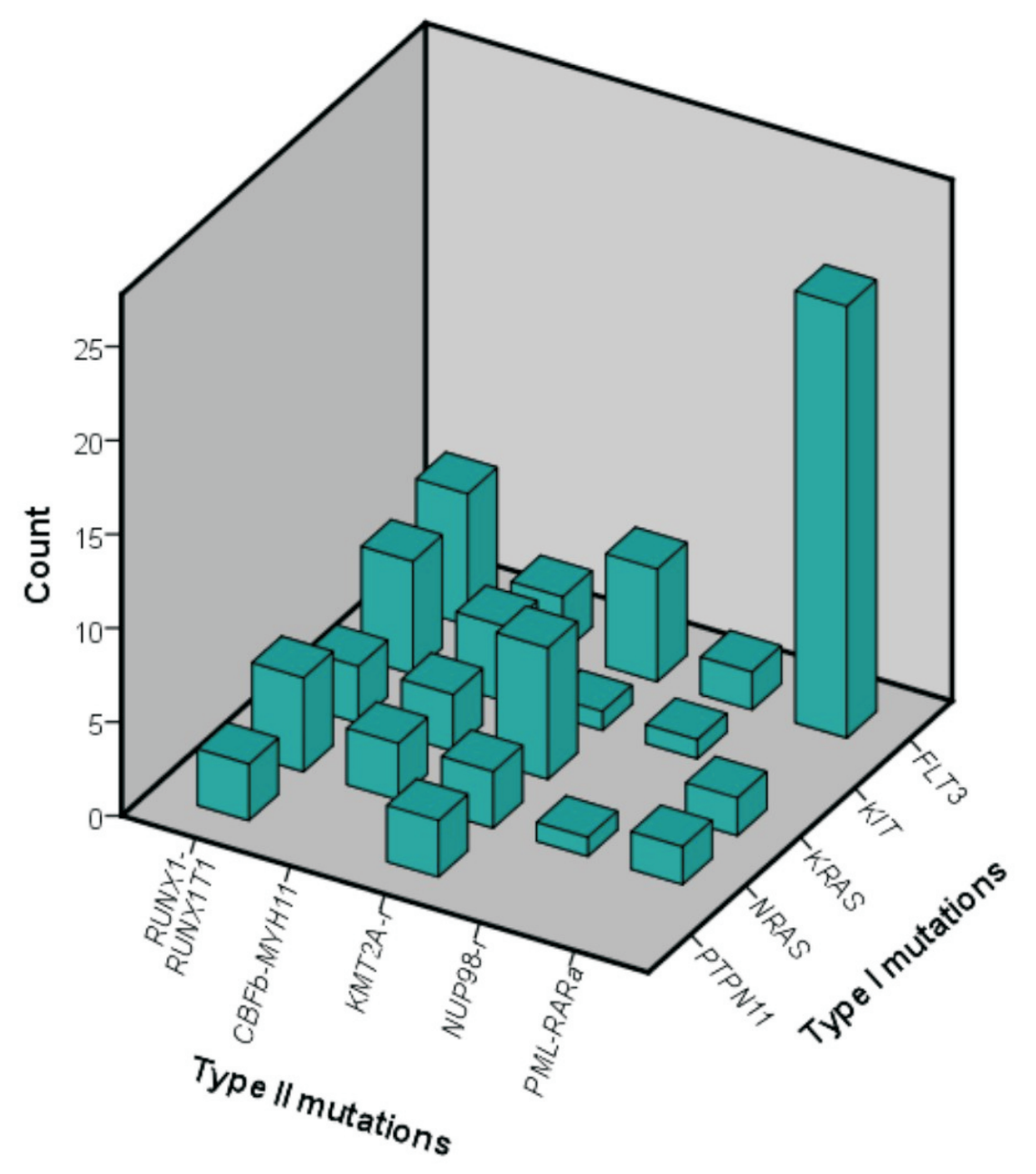

**Figure 2**. Association between fusion genes and *RAS* pathway mutations in c-AML

**Table 2.** Multivariate analysis of pOS in pediatric c-AML, Brazil, 2000–2016

| | Cox regression model [a] | |
|---|---|---|
| | HR (95% CI ) | p value |
| **Geographic regions of treatment** | | |
| **South** | Ref. | Ref. |
| **Midwest** | 4.7 (0.6-35.2) | 0.13 |
| **Northeast** | 4.4 (0.6-32.4) | 0.15 |
| **Southeast** | 1.8 (0.2-14.0) | 0.58 |
| **Type II mutation** | | |
| ***CBFß-MYH11*** | 0.4 (0.1-1.1) | 0.07 |
| **Type I mutation** | | |
| ***FLT3*** | 1.4 (0.7-2.8) | 0.30 |
| ***PTPN11*** | 2.0 (1.0-4.0) | 0.05 |

[a] Excluding acute promyelocytic leukemia cases. CI, confidence interval; HR, hazard ratio; pOS, probability of overall survival; Ref., reference; SE, standard error.

## CONCLUSION

The inclusion of cytogenetic-molecular markers in the characterization of AML is of great predictive value for pOS. Diagnosis and management of c-AML raise questions that should be considered for further action.

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