# Molecular characterization of pediatric acute myeloid leukemia in Brazil: perspectives of a multicentric study

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## BACKGROUND

- The improvement of acute myeloid leukemia (AML) characterization in children represents an important challenge in pediatric hematology.
- Some founder leukemogenic effect is largely described as been somatic translocations and fusion genes. These recurrent genetic aberrations are important prognostic factors in pediatric AML and an increasing number of study groups are using them for risk group stratification.
- The two major types of genetic events in AML are type I and II aberrations that, in general, enhance the self-renewal and proliferation potential of the myeloid progenitor.

We performed a comprehensive analyses of the main type I (*FLT3, c-KIT, NRAS, KRAS* and *PTPN11)* and II [*PML-RARα, RUNX1-RUNX1T1, CBFb-MYH11* and *MLL(KMT2A)*-rearrangements (*MLL-r*)] mutations in pediatric AML cases providing an overview of the largest case series in Brazil as recommended by World Health Organization (WHO) for classification of myeloid neoplasms. We determined the distribution frequencies of pediatric AMLs according to somatic alterations and investigate the potential contribution of these markers with the clinical outcome association, enabling appropriate oncological risk group stratification.

## MATERIAL AND METHODS

This is a retrospective analysis from a multicentric study of 702 de novo childhood AML cases (2000-2015). We analyzed hotspot regions of *FLT3* (exon 11/12 for internal tandem duplication, ITD; exon 17 for punctual mutations in tyrosine kinase domain, TKD), *NRAS* (exon 1, codons 12/13), *KRAS* (exon 1, codons 12/13), *PTPN11* (exon 3), and c-*KIT* (exon 8/17) genes. The four most frequent fusion genes in overall pediatric AML including *RUNX1-RUNX1T1, CBFb-MYH11, MLL-r* and *PML-RARα* were directly performed. Patients were treated out of a specific protocol, but following the BFM-AML2004 treatment regimens. Categorical variables were compared using $\chi 2$ test analysis or Fisher's exact test. An estimate of overall survival (OS) and event free survival (EFS) was determined using the Kaplan–Meier and log rank tests in order to verify the association of one genetic alteration in the patients' outcome. OS was defined as time from study entry to death from any cause and events for EFS were defined as study enrollment to the date of death or early/late realpse. Patients lost to follow up were censored at their date of last known contact.

## RESULTS

**Table 1.** Distribution of demographic and clinical characteristics according to molecular alterations in pediatric AML cases, Brazil, 2000-2015

| | | Age (years) | | | | | Gender | | | WBC (x10$^9$/l) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Molecular alteration** | **Frequency n/total (%)** | **≤2 n (%)** | **>2-10 n (%)** | **>10 n (%)** | **Median (range)** | ***p*** | **Male n (%)** | **Female n (%)** | ***p*** | **≤50 n (%)** | **>50 n (%)** | **Median (range)** | ***p*** |
| **Type II mutations** | | | | | | | | | | | | | |
| *RUNX1-RUNX1T1* | 74/370 (20.0) | 9 (12.2) | 29 (39.2) | 36 (48.6) | 9.3 (0.2-18.3) | **<0.001** | 44 (59.5) | 30 (40.5) | 0.25 | 58 (80.6) | | 20.1 (6-136) | **0.002** |
| *CBFb-MYH11* | 23/350 (6.6) | 5 (21.7) | 5 (21.7) | 13 (56.5) | 13.3 (0.8-19.3) | 0.05 | 10 (43.5) | 13 (56.5) | 0.35 | 6 (27.3) | 16 (72.7) | 111.0 (7.2-268) | **<0.001** |
| *MLL* rearrangements | 73/288 (25.4) | 50 (65.8) | 18 (23.7) | 8 (10.5) | 1.3 (0.0-21.1) | **<0.001** | 35 (46.1) | 41 (53.9) | 0.10 | 38 (52.1) | 35 (47.9) | 49.0 (66-451) | **0.008** |
| *PML-RARα* | 63/127 (49.6) | 2 (3.2) | 25 (39.7) | 36 (57.1) | 11.2 (1.3-18.0) | 0.08 | 31 (49.2) | 32 (50.8) | 0.93 | 48 (77.4) | 14 (22.6) | 10.8 (2-800) | 0.57 |
| **Type I mutations** | | | | | | | | | | | | | |
| *FLT3* | 110/473 (23.3) | 5 (4.5) | 42 (37.8) | 64 (57.7) | 11.1 (1.0-21.3) | **<0.001** | 60 (54.5) | 50 (45.5) | 0.88 | 66 (60.0) | 44 (40.0) | 31.4 (0.1-800) | 0.147 |
| *FLT3*-ITD | 86/473 (18.2) | 3 (3.5) | 35 (40.7) | 48 (55.8) | 10.9 (1.0-21.3) | **<0.001** | 47 (54.7) | 39 (45.3) | 0.88 | 51 (59.3) | 35 (40.7) | 34.7 (0.1-540) | 0.15 |
| *FLT3*-TKD | 24/473 (5.1) | 2 (8.3) | 6 (25.0) | 16 (66.7) | 11.9 (1.8-19.3) | **0.001** | 13 (54.2) | 11 (45.8) | 0.97 | 15 (62.5) | 9 (37.5) | 25.8 (2.5-800) | 0.61 |
| *KRAS* | 30/464 (6.5) | 9 (30.0) | 12 (40.0) | 9 (30.0) | 4.7 (0.5-18.3) | 0.77 | 20 (66.7) | 10 (33.3) | 0.14 | 16 (53.3) | 14 (46.7) | 40.4 (1-700) | 0.15 |
| *NRAS* | 44/409 (10.8) | 8 (18.2) | 13 (29.5) | 23 (52.3) | 10.2 (0.7-18.0) | 0.24 | 24 (54.5) | 20 (45.5) | 0.91 | 23 (52.3) | 21 (47.7) | 48.5 (5.1-800) | 0.06 |
| c-*KIT* | 21/193 (10.9) | 5 (23.8) | 12 (57.1) | 4 (19.0) | 4.5 (0.6-19.3) | 0.20 | 12 (57.1) | 9 (42.9) | 0.43 | 12 (60.0) | 8 (40.0) | 42.7 (4.5-168) | 0.66 |
| *PTPN11* | 14/189 (7.4) | 3 (21.4) | 6 (42.9) | 5 (35.7) | 7.4 (0.5-17.1) | 0.78 | 11 (78.6) | 3 (21.4) | 0.07 | 9 (64.3) | 5 (35.7) | 38.8 (1.0-200) | 0.60 |

WBC, white blood cell count at diagnosis. Bold *p* values are statistically significant.

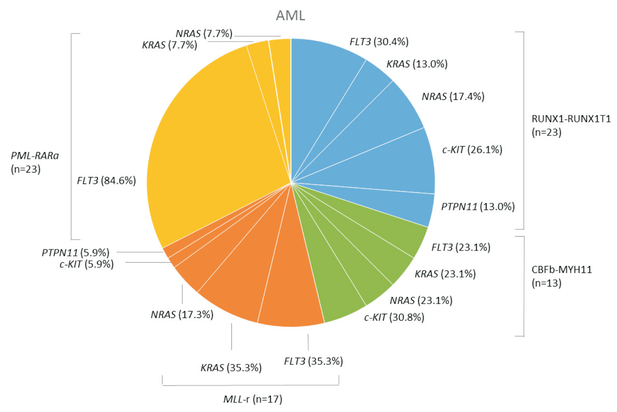


**Figure 1.** Description of molecular aberrations as well as their nonrandom associations in pediatric AML (*p*=0.001).

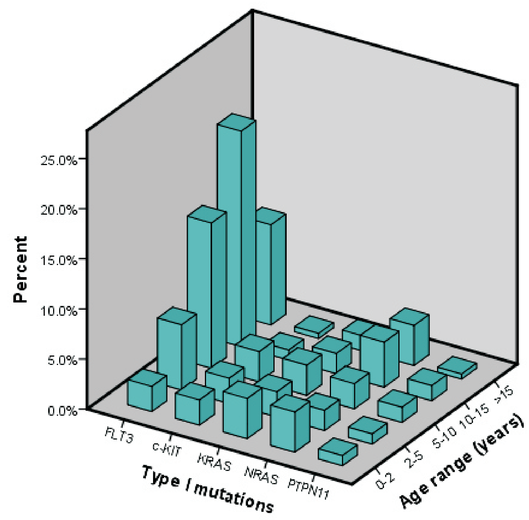


**Figure 2.** Distribution of mutations according to age range.

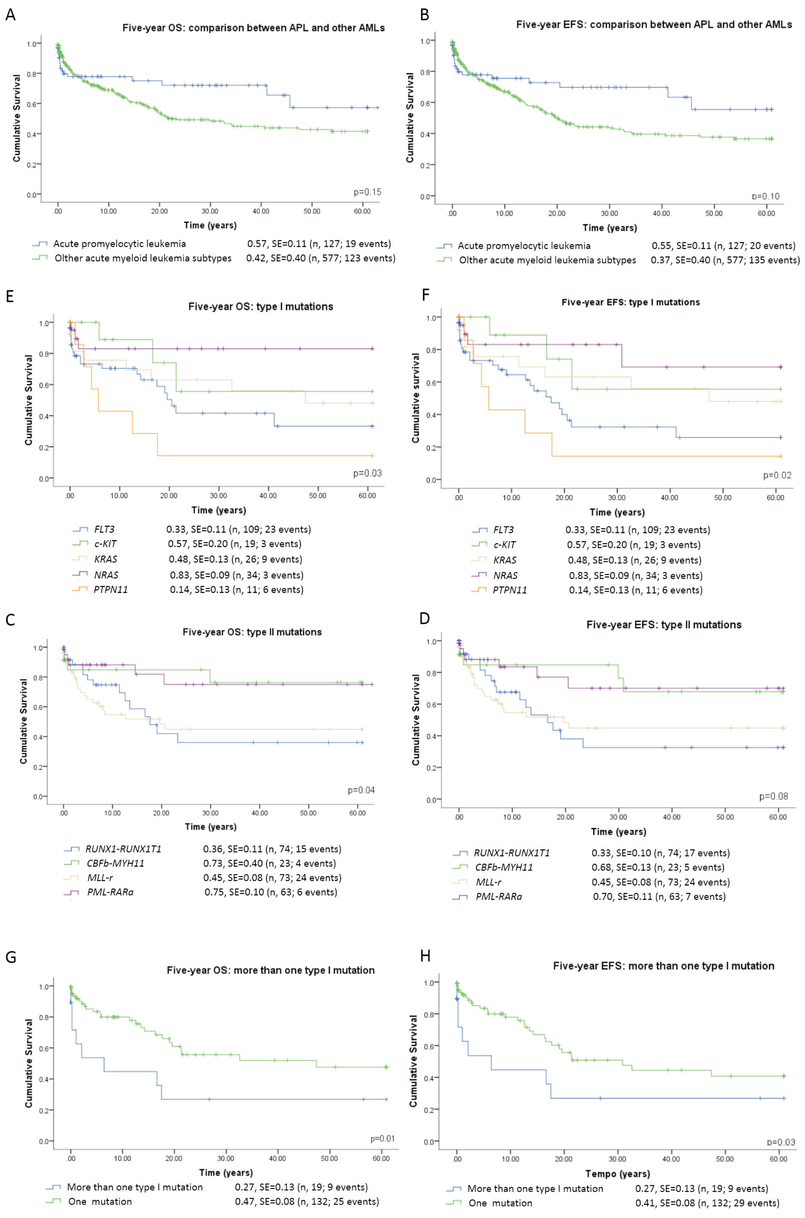


**Figure 3.** Survival analysis of the type I and type II mutations in pediatric AML cases. Kaplan-Meier estimates for (A, C, E, G) cumulative overall survival (OS) and (B, D, F, H) cumulative event free survival (EFS) for different subtypes and genetic aberrations showed. A-B, Comparison of estimate survival curves between acute promyelocytic leukemia (APL) and other AMLs. C-D/E-F, Comparison of estimate survival curves among type II/I mutations, respectively. G-H, Comparison of estimate survival curves between pediatric AML cases presenting concomitant type I mutations, excluding APL cases. P values were calculated using log rank test.

## CONCLUSION

This is the larger study of morphological-immunophenotypic and molecular characterization of pediatric AML cases in Brazil, reflecting the main genetic profile of cases. The identification of these genetic subgroups may assist to improve the molecular-epidemiology, risk stratification and biology of AML worldwide. As the recommendations from WHO experts, the molecular-cytogenetic screening is important for childhood AML and contributes to growing knowledge on different frequencies of mutations in Brazilian AMLs.

## CO- AUTHORS ACKNOWLEDGMENTS

Brazilian Collaborative Study Group of Infant Leukemia that contributed to the study as co-authors: Adriana V. S. Deyl[2], Ana F. Winn[3], Alejandro M. Arancibia[4], Bruno M. R. Freire[5], Claudia T. Oliveira[4], Denise B. Silva[3], Eloisa Cartaxo[6], Eny G. Carvalho[7], Everaldo R. Junior[8], Fernando A. Werneck[9], Gustavo R. Neves[10], Imarui Costa[3], Isis Maria Q. Magalhães[11], Maria D. Dorea[12], Marcelo S. Souza[13], Maura R. V. Ikoma[4], Nilma Pimentel[14], Patricia C. Brito[15], Renata S. C. Gurgel[16], Teresa C. C. Fonseca[17], Terezinha J. M. Salles[18]. Affiliations: [2]Hospital de Clínicas de Porto Alegre, RS; [3]Hospital Infantil Joana de Gusmão, SC; [4]Hospital Amaral Carvalho, SP; [5]Hospital Santa Isabel, BA; [6]Hospital Napoleão Laureano, PB; [7]Hospital Martagão Gesteira, BA; [8]Hospital Santa Casa de Misericórdia de Goiânia; [9]Hospital dos Servidores do Estado, RJ; [10]Hospital Sarina Rolin Caracante, SP; [11]Hospital da Criança, DF; [12]Hospital São Rafael, BA; [13]Hospital Regional de Mato Grosso do Sul, MS; [14]Hospital Aristides Maltez, BA; [15]Hospital Araújo Jorge, GO; [16]Hospital Universitário Alcides Carneiro, PB; [17]Santa Casa de Itabuna, BA; [18]Hospital Universitário Oswaldo Cruz, PE.

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