# MOLECULAR FINDINGS OF INFANT ACUTE MYELOID LEUKEMIA

**Francianne Gomes Andrade, Ingrid Sardou Cezar, Filipe Vicente dos Santos Bueno, Gisele Dallapicola Brisson, Eugênia Terra-Granado, Elda Pereira Noronha, Maria S. Pombo-de-Oliveira and Brazilian Study Group of Childhood Acute Myeloid Leukemia (IMol-AMLBSG) as co-authors**

Pediatric Hematology-Oncology Program, Instituto Nacional de Câncer, Rio de Janeiro, Brazil

## BACKGROUND

❖Acute myeloid leukemia (AML) diagnosed in the first two years of life displays unique features that have provided important insights into the mechanisms of leukemogenesis in the fetus and young children, once the first genetic hit arises prenatally.

❖Recurrent genetic abnormalities are observed in the majority of infant AML (i-AML, ≤ 2 years-old) (~80%) and large differences in distribution of frequencies are detected in children ≤ 2 and >2 years of age.

❖Our aim was to describe the molecular characterization of i-AML cases and investigate the contribution of gene mutations in the probability of overall survival (pOS).

## MATERIAL AND METHODS

This is a retrospective analysis of 179 de novo non-Down Syndrome i-AML cases (2000-2016) assessed throughout a multicentric network study that includes 760 cases of pediatric AML (≤21 years-old). 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 *KIT* (exon 8/17) genes. The most frequent fusion genes in pediatric AML including *MLL/KMT2A* and *NUP98* rearrangements (*KMT2A-r* and *NUP98-r*, respectively), *MYST3-CREBBP*, *RUNX1-RUNX1T1*, *CBFß-MYH11*, and P*ML-RARα* were directly performed by FISH and/or RT-PCR.

Patients were treated out of a specific protocol, but following the BFM-AML treatment regimens. Categorical variables were compared using $\chi^2$ test analysis or Fisher's exact test. An estimate of overall survival (OS) 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 the time from study entry to death from any cause. Patients lost to follow-up were censored at their date of last known contact.

## RESULTS

**Table 1.** Demographic characteristics and classification of AML cases, Brazil, 2000-2016

| | Infant AML, n (%) | Pediatric AML, n (%) | p |
|---|---|---|---|
| **Brazilian geographic regions** | | | <0.0001 |
| **Northeast** | 64 (35.8) | 305 (52.5) | |
| **South** | 21 (11.7) | 10 (1.7) | |
| **Southeast** | 51 (28.5) | 146 (25.1) | |
| **Midwest** | 43 (24.0) | 120 (20.7) | |
| **Sex** | | | 0.52 |
| **Males** | 94 (52.5) | 321 (55.2) | |
| **Females** | 85 (47.5) | 260 (44.8) | |
| **WHO classification** | | | <0.0001 |
| **AML with *RUNX1-RUNX1T1*** | 9 (5.0) | 68 (11.7) | |
| **AML with *CBFb-MYH11*** | 5 (2.8) | 23 (4.0) | |
| **APL with *PML-RARα*** | 4 (2.2) | 142 (24.4) | |
| **AML (megakaryoblastic) with *RBM15-MKL1*** | 1 (0.6) | 0 (0.0) | |
| **AML with *KMT2A* rearrangements** | 54 (30.2) | 33 (5.7) | |
| **AML with minimal differentiation** | 8 (4.5) | 23 (4.0) | |
| **AML without maturation** | 4 (2.2) | 34 (5.9) | |
| **AML with maturation** | 3 (1.7) | 48 (8.3) | |
| **Acute myelomonocytic leukemia** | 23 (12.8) | 89 (15.3) | |
| **Acute monoblastic/monocytic leukemia** | 26 (14.5) | 37 (6.4) | |
| **Pure erythroid leukemia** | 4 (2.2) | 12 (2.1) | |
| **Acute megakaryoblastic leukemia** | 30 (16.8) | 24 (4.1) | |
| **Total** | 179 (23.6) | 581 (76.4) | |

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; Infant AML, children aged ≤ 2 years-old with acute myeloid leukemia; Pediatric AML, children and young adults aged >2 years-old with acute myeloid leukemia; n, number of cases; WHO, World Health Organization.

**Table 2.** Characteristics of genetic subgroups identified in i-AML, Brazil, 2000-2016

| | *KMT2A-r*, n (%) | *RUNX1RUNX1T1*, n (%) | *CBFß-MYH11*, n (%) | *PML-RARα*, n (%) | *NUP98-r*, n (%) | *MYST3-CREBBP*, n (%) | *CBFA2T3-GLIS2*, n (%) | *RBM15-MKL1*, n (%) |
|---|---|---|---|---|---|---|---|---|
| **Brazilian geographic regions** | | | | | | | | |
| Northeast | 18 (34.0) | 5 (55.6) | - | - | 2 (28.6) | - | 1 (100.0) | 1 (100.0) |
| South | 8 (15.1) | 1 (11.1) | - | 1 (50.0) | 1 (14.3) | 1 (33.3) | - | - |
| Southeast | 13 (24.5) | 2 (22.2) | 2 (50.0) | - | 1 (14.3) | - | - | - |
| Midwest | 14 (26.4) | 1 (11.1) | 2 (50.0) | 1 (50.0) | 3 (42.9) | 2 (66.7) | - | - |
| **Age groups (months)** | | | | | | | | |
| ≤1 | 10 (18.9) | 2 (22.2) | 1 (25.0) | - | - | 1 (33.3) | | 1 (100.0) |
| >1-10 | 18 (34.0) | 2 (22.2) | 1 (25.0) | - | 3 (42.9) | - | | - |
| ≥11 | 25 (47.2) | 5 (55.6) | 2 (50.0) | 2 (100.0) | 4 (57.1) | 2 (66.7) | 1 (100.0) | - |
| **Race** | | | | | | | | |
| Blacks | 28 (54.9) | 4 (44.4) | - | - | 5 (71.4) | 2 (66.7) | - | - |
| Non-Blacks | 23 (45.1) | 5 (55.6) | 3 (100.0) | 2 (100.0) | 2 (28.6) | 1 (33.3) | 1 (100.0) | 1 (100.0) |
| **Sex** | | | | | | | | |
| Males | 24 (45.3) | 7 (77.8) | 2 (50.0) | 1 (50.0) | 4 (57.1) | 1 (33.3) | | |
| Females | 29 (54.7) | 2 (22.2) | 2 (50.0) | 1 (50.0) | 3 (42.9) | 2 (66.7) | 1 (100.0) | 1 (100.0) |
| **WBC count ($x10^9$/l)** | | | | | | | | |
| ≤50 | 26 (51.0) | 90 (64.3) | 1 (33.3) | 2 (100.0) | 4 (57.1) | 2 (66.7) | 1 (100.0) | 1 (100.0) |
| >50 | 25 (49.0) | 50 (35.7) | 2 (66.7) | | 3 (42.9) | 1 (33.3) | | |
| **Type I mutations** | | | | | | | | |
| *FLT3* (ITD or TKD) | 2 (4.5) | - | - | - | - | - | - | - |
| *FLT3-ITD* | 1 (2.3) | - | - | - | - | - | - | - |
| *FLT3-TKD* | 1 (2.3) | - | - | - | - | - | - | - |
| *KRAS* | 6 (13.0) | - | 3 (100.0) | - | - | - | - | - |
| *NRAS* | 3 (9.7) | - | 2 (66.7) | - | 1 (20.0) | - | - | - |
| *KIT* | 1 (3.8) | 1 (50.0) | 1 (33.3) | - | - | - | - | - |
| *PTPN11* | 3 (10.0) | 1 (20.0) | - | - | - | - | - | - |

*KMT2A-r*, *KMT2A* rearrangements; ITD, internal tandem duplication; *NUP98-r*, *NUP98* rearrangements; n, number of cases; TKD, tyrosine kinase domain; WBC, white blood cell count.

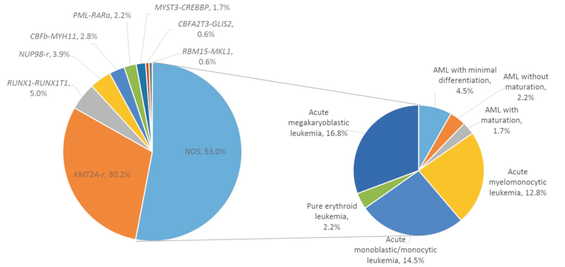


**Figure 1.** Distribution of i-AML cases according to genetic subtypes and morphological classification. NOS, not otherwise specified by molecular alterations.

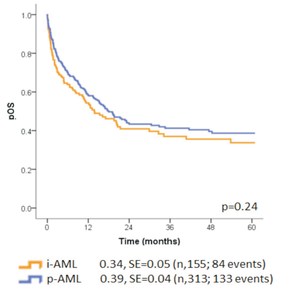


**Figure 2.** Survival analysis of the AML cases*. Comparison of survival curves among i-AML (≤ 2 years-old) and p-AML (>2 years-old). P values were calculated using log rank test. n, number of cases; SE, standard error. *Excluding acute promyelocytic cases.

**Table 3.** Univariate analysis for overall survival according to molecular alterations of i-AML cases, Brazil, 2000-2016

| | Univariate analysis | | |
|---|---|---|---|
| **Molecular alteration** | **N (N of events)** | **5-year pOS, (SE)** | **p value** |
| ***KMT2A-r*** | 50 (27) | 0.36 (0.08) | 0.82 |
| ***RUNX1-RUNX1T1*** | 8 (4) | 0.47 (0.19) | |
| ***CBFß-MYH11*** | 5 (2) | 0.53 (0.25) | |
| ***PML-RARα*** | 4 (1) | 0.75 (0.22) | |
| ***NUP98-r*** | 6 (2) | 0.67 (0.18) | |
| ***RBM15-MKL1*** | 1 (0.0) | - | |

N, number of cases; pOS, probability of overall survival; SE, standard error.

## CONCLUSION

Acute leukemia in early childhood gathers groups of different biological subtypes characterized by somatic mutations associated with specific age strata. Recurrent genetic abnormalities were observed in the half of i-AML; the detection of the different, prognostically relevant *KMT2A* fusions genes is also important, although other alterations should be investigated. The disease-related death rate was high in i-AML.

**Co-authors**

Alejandro Mauricio Arancibia[1], Rosania Maria Basegio[1], Patricia Carneiro de Brito[1], Eny Guimarães Carvalho[1], José Carlos Martins Cordoba[1], Imaru Costa[1], Virginia Maria Cóser[1], Adriana Vanessa Santini Deyl[1], Anna Carolina Silva Dias[1], Eloisa Cartaxo Fialho[1], Bruno Marcelo Rocha Freire[1], Renata Silva de Carvalho Gurgel[1], Maura Rosane Valério Ikoma[1], Ingrid Koster[1], Isis Maria Quezado Magalhães[1], Ana Maria Marinho[1], Renato Melaragno[1], Suellen Valadares Moura[1], Gustavo Ribeiro Neves[1], Claudia Teresa Oliveira[1], Ilana Zalcberg Renault[1], Terezinha de Jesus Marques Salles[1], Denise Browsfield Silva[1], Marcelo Santos Souza[1], Tállita Meciany Farias Vieira[1], Fernando de Almeida Werneck[1], Ana Freund Winn[1], and Gustavo Zamperlini[1].

[1]Hospital Amaral Carvalho, Jaú, São Paulo, Brazil; [2]Hospital Regional de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil; [3]Hospital Araújo Jorge, Goiânia, Goiás, Brazil; [4]Hospital Martagão Gesteira, Salvador, Bahia, Brazil; [5]Hospital da Criança, Brasília, Distrito Federal, Brazil; [6]Hospital Infantil Joana de Gusmão, Florianópolis, Santa Catarina, Brazil; [7]Hospital Universitário de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil; [8]Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; [9]Hospital Napoleão Laureano, João Pessoa, Paraíba, Brazil; [10]Hospital Santa Isabel, Salvador, Bahia, Brazil; [11]Hospital Universitário Alcides Carneiro, Campina Grande, Paraíba, Brazil; [12]Instituto Nacional de Câncer, Rio de Janeiro, Rio de Janeiro, Brazil; [13]Hospital Santa Marcelina, São Paulo, São Paulo, Brazil; [14]Hospital Sarina Rolin Caracante, Sorocaba, São Paulo, Brazil; [15]Hospital Universitário Oswaldo Cruz, Recife, Pernambuco, Brazil; [16]Hospital dos Servidores do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; [17]Hospital Infantil Darcy Vargas, São Paulo, São Paulo, Brazil.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA