MOLECULAR ALTERATIONS IN RARE SUBSETS OF PEDIATRIC ACUTE MYELOID LEUKEMIA

Francianne Gomes Andrade, Ingrid Sardou Cezar, Filipe Vicente dos Santos Bueno, Luísa Vieira Codeço Marques, 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

- Recurrent fusion genes are the genetic events associated with pediatric acute myeloid leukemia (AML) and have been used for prognostic risk group stratification.
- Rare genetic alterations (<2% of cases), including *DEK-NUP214, NUP98* rearrangements (*NUP98-r*), *RBM15-MKL1, MYST3-CREBBP*, and *CBFA2T3-GLIS2* demand to be investigated in large cohorts.
- For many years, the accepted model of leukemogenesis was the "two-hit hypothesis," which suggested that two different types of genetic mutation (type I and II) are required for malignant transformation of a myeloid precursor.
- Type I and II mutations are only one part of a more complex scenario. There is also a temporal component to leukemogenesis; mutations are associated with age and occur at a particular stage in cell development (Figure 1).

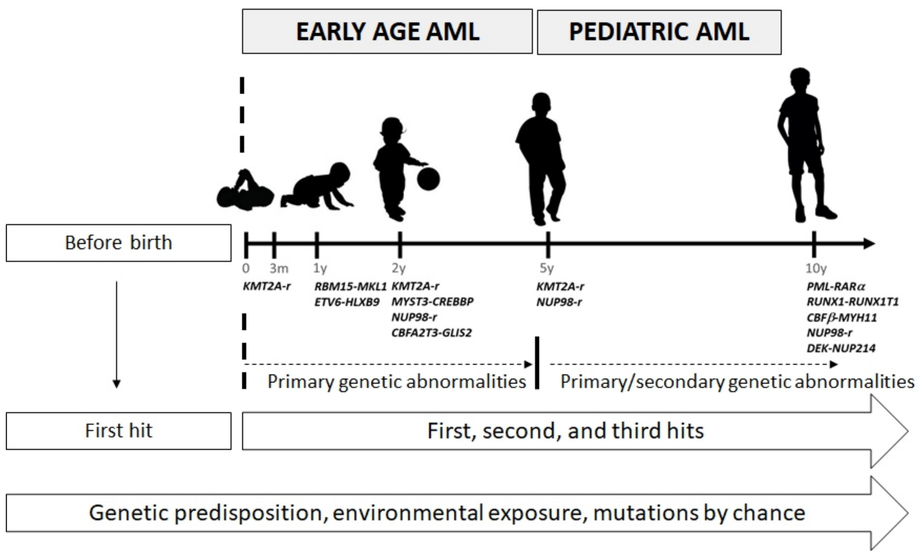

Figure 1. Timeline with the most frequent fusion genes of acute myeloid leukemia in children and adolescents. Fusion genes (first hit) are associated with age and may occur before birth or during the first years of life. Secondary mutations are required to overt leukemia.

AIM

Our aim was to evaluate the association between molecular alterations and clinical-demographic features assessing the probability of overall survival (pOS). We also aimed to explore the genetic susceptibility associated with leukemogenesis.

MATERIAL AND METHODS

Patients. We analyzed a cohort of 804 cases of AML (de novo and therapy related cases) <19 years-old referred to the Pediatric Hematology-Oncology Research Program, INCA, Rio de Janeiro, between January 1, 2000, and May 31, 2017. Cases included were forwarded from Brazilian medical institutions that are reference in oncological care for children with leukemia for diagnostic purpose.

Molecular alterations. Mutations in hotspot regions of RAS pathway affecting genes (class I mutations; *FLT3, NRAS, KRAS, PTPN11*, and *KIT*) were analyzed by direct sequencing. Briefly, *FLT3* mutations were examined at the tyrosine kinase domain (TKD) in codon 835 and 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 screening in exon 3, and *KIT* mutations were identified in exons 8/17.

Fusion genes (class II mutations) associated with pediatric AML were screened by RT-PCR and/or FISH [*MLL/KMT2A* rearrangements (*KMT2A-r*), *RUNX1-RUNX1T1*, *CBFb-MYH11*, *PML-RARa*, *NUP98-r*, *CBFA2T3-GLIS2*, *MYST3-CREBBP*, and *RBM15-MKL1*].

One acute promyelocytic leukemia (APL) patient with a healthy twin sibling, was select for exome sequencing using SureSelect^{QTX} Target Enrichment kit (Agilent Technologies) to assess genetic variants associated with predisposition to leukemia development.

Statistical Analysis. Statistical analyses were performed taking into account descriptive frequencies of variables in order to measure central tendency and/or dispersion. Fisher's test was applied to compare proportions between subgroups. Mann-Whitney U test was used for continuous variables. Age strata were considered a categorical variable as three groups: ≤2 years-old; >2-10 years old and ≥11 years old. The Kaplan-Meier survival analysis was used to calculate the 5-year pOS and estimated survival values were compared using the log-rank test. Cox proportional-hazard regression model with an estimated hazard ratio (HR) and 95% confidence intervals (CI) were presented.

Treatment. Patients were not formally enrolled in treatment protocols but received homogeneous treatment following international consensus guidelines on AML treatment, with two different induction regimens using cytarabine, idarubicin, and etoposide as the BFM-AML protocol.

RESULTS

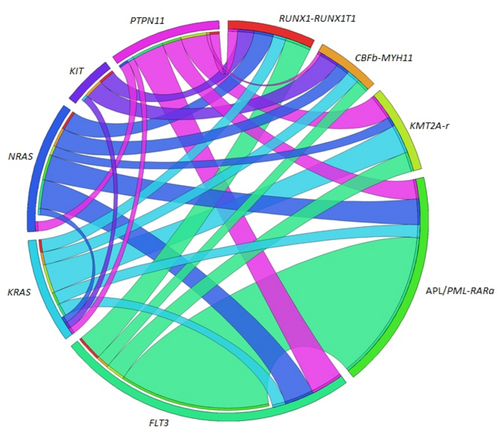

Figure 2. Cooperating of type I and II abnormalities in pediatric AML. The circos plot depicts the frequency of the Type II mutations (*RUNX1-RUNX1T1, CBFb-MYH11, KMT2A-r, PML-RARa*) and co-occurrence of type I mutations (*FLT3, KRAS, NRAS, KIT, PTPN11*) in patients with AML. The length of the arch corresponds to the frequency of the type II mutation and the width of the ribbon with the percentage of patients with a specific type I mutation or a combination of type I mutations.

Table 1. Distribution of molecular alterations in pediatric AML, Brazil, 2000-2017

		Age (years)					Sex			WBC count (x10^9/l)			
Molecular alteration [a]	Frequency n/total (%)	Median (range)	≤2 n (%)	>2-10 n (%)	≥11 n (%)	p	Males n (%)	Females n (%)	p	Median (range)	≤10 n (%)	>10 n (%)	p
Fusion genes (class II) [b]													
RUNX1-RUNX1T1	85/469 (18.1)	9.3 (0.2-18.3)	9(10.6)	36 (42.4)	40 (47.1)	**<0.001**	35 (41.2)	50 (58.8)	0.17	20.1 (1.7-136)	23 (27.7)	60 (72.3)	0.36
CBFβ-MYH11	30/457 (5.5)	9.3 (0.3-17.8)	5 (16.7)	10 (33.3)	15 (50.0)	0.07	17 (56.7)	13 (43.3)	0.32	96.9 (2.2-373)	2 (6.9)	27 (93.1)	**0.02**
KMT2A-r	94/436 (21.6)	1.7 (0-18.2)	55 (58.5)	26 (27.7)	13 (13.8)	**<0.001**	46 (48.9)	48 (51.1)	0.53	42 (0.9-451)	17 (18.5)	75 (81.5)	0.09
APL/*PML-RARα* [c]	157/788 (19.9)	9.8 (0.8 -18.7)	6 (3.8)	73 (46.5)	78 (49.7)	**<0.001**	68 (43.3)	89 (56.7)	0.58	10.6 (0.2-800)	75 (48.4)	80 (51.6)	**<0.001**
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
MYST3-CREBBP [d]	3/22 (13.6)	1.0 (0.0-1.8)	na	na	na	na	1 (7.7)	22 (22.2)	0.33	8.6 (5.7-111)	2 (13.3)	1 (14.3)	0.95
CBFA2T3-GLIS2	1/62 (1.6)	na	1 (2.0)	0 (0.0)	0 (0.0)	0.89	1 (3.3)	0 (0.0)	0.30	na	1 (2.2)	0 (0.0)	0.57
RBM15-MKL1 [e]	2/37 (5.4)	0.5 (0.2-0.7)	2 (11.1)	0 (0.0)	0 (0.0)	0.33	2 (11.8)	0 (0.0)	0.12	14 (7.2-20.8)	2 (6.7)	0 (0.0)	0.55
RAS pathway mutations (class I)													
FLT3 (ITD or TKD)	125/549 (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	35/568 (6.2)	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	51/510 (10.0)	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	18/265 (6.8)	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	40/445 (9.0)	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
Outcome (%)													
5y-pOS (SE) [f]	469/613 (76.5)	na	33 (4.8)	43.1 (4.5)	43.7 (4.1)	0.10	39.2 (3.6)	42.2 (3.7)	0.73	na	44.6 (3.2)	32.2 (4.3)	**0.003**

[a] The total number of analyzed cases reflect the availability of biological material for molecular tests. [b] *DEK-NUP214* was not found in any of the 59 cases screened. [c] Acute promyelocytic leukemia (APL) according to Worlds Health Organization classification. [d] *MYST3-CREBBP* was analyzed among cases aged ≤2 years old with hemophagocytosis. [e] *RBM15-MKL1* was analyzed among cases of acute megakaryoblastic leukemia. [f] Excluding acute promyelocytic leukemia. ITD. internal tandem duplication; na, not applicable; TKD, tyrosine kinase domain; WBC, white blood cell count at diagnosis; 5y-pOS, estimated probability of overall survival in 5 years.

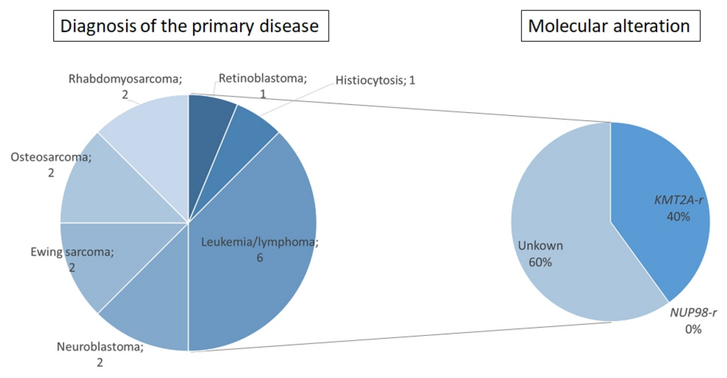

Figure 3. Characterization of therapy-related AML.

Table 2. Univariate analysis for overall survival parameters of pediatric AML cases, 2000-2017, Brazil

		Univariate analysis[a]		
	N (N of events)	5y-pOS, (SD)	Median [b], (CI 95%)	p
Periods of diagnosis				0.146
First period (2000-2008)	221 (94)	39.4 (4.2)	19.2 (8.3-30.1)	
Second period (2009-2017)	212 (109)	32.9 (4.3)	14.8 (10.0-19.5)	
Locality of treatment				**0.009**
North/Northeast	206 (88)	33.0 (4.9)	16.3 (10.0-23.3)	
South/Southeast	121 (52)	48.6 (5.2)	47.4 [c]	
Midwest	106 (63)	24.6 (5.1)	7.3 (0.1-14.5)	
Age (years)				0.449
≤2	140 (76)	33.1 (5.1)	13.1 (7.1-19.1)	
>2-10	146 (64)	35.5 (5.4)	19.3 (13.3-25.4)	
≥11	147 (63)	39.0 (5.0)	15.2 (8.9-21.6)	
Sex				0.631
Females	183 (90)	38.6 (4.2)	15.3 (10.1-20.5)	
Males	250 (113)	34.5 (4.1)	19.1 (13.7-24.5)	
White blood cell count (x10^9/L)				0.821
≤50	273 (126)	36.6 (3.7)	16.3 (12.4-20.3)	
>50	145 (72)	33.8 (5.1)	13.8 (5.5-22.1)	
Type II mutations				
RUNX1-RUNX1T1	55 (26)	32.8 (8.2)	13.5 (6.8-20.2)	0.813
CBFβ-MYH11	19 (5)	71.5 (10.9)	[c,d]	**0.010**
KMT2A-r	74 (39)	36.7 (6.7)	11.5 (3.6-19.4)	0.646
Type I mutations				
FLT3	54 (33)	18.0 (7.4)	11.8 (8.4-15.2)	0.056
K/N-RAS	54 (23)	45.0 (8.3)	17.5 [c]	0.333
PTPN11	26 (16)	13.0 (8.4)	3.3 (1.6-5.1)	**0.005**
Concomitant type I mutations				0.052
Single mutation	114 (55)	37.2 (5.8)	17.6 (10.1-25.2)	
More than one mutation	18 (11)	16.0 (10.3)	6.4 (0.0-19.7)	

[a] Excluding acute promyelocytic leukemia subtype, which presented 5y-pOS of 68.2±6.2% and average of 45.1 months (95%CI 39.3-50.9). [b] Median in months. [c] Confidence intervals undefined. [d] Median not reached. CI, confidence interval; N, number; pOS, probability of overall survival; SE, Standard error; y, years.

FUTURE DIRECTIONS

We will perform exome analyses in order to access germline and somatic variants associated with leukemogenesis in rare cases of APL. The identification of genetic subgroups contributes to the molecular epidemiology and biology of AML worldwide, reflecting the profile of pediatric AML cases in Brazil. Survival data of pediatric AML subtype in countries of Latin America are found rarely in the literature. The inclusion of cytogenetic-molecular markers in the characterization of AML is of great predictive value for pOS.

Co-authors
Alejandro Mauricio Arancibia[a], Rosania Maria Basegio[b], Patricia Carneiro de Brito[c], Eny Guimarães Carvalho[d], José Carlos Martins Cordoba[e], Imarui Costa[f], Virginia Maria Cóser[g], Adriana Vanessa Santini Deyl[h], Anna Carolina Silva Dias[c], Eloisa Cartaxo Fialho[i], Bruno Marcelo Rocha Freire[j], Renata Silva de Carvalho Gurgel[k], Maura Rosane Valério Ikoma[a], Ingrid Koster[l], Isis Maria Quezado Magalhães[m], Ana Maria Marinho[c], Renato Melaragno[m], Suellen Valadares Moura[l], Gustavo Ribeiro Neves[n], Claudia Teresa Oliveira[a], Ilana Zalcberg Renault[l], Terezinha de Jesus Marques Salles[o], Denise Browsfield Silva[f], Marcelo Santos Souza[b], Fernando de Almeida Werneck[p], Ana Freund Winn[f], and Gustavo Zamperlini[q].
[a]Hospital Amaral Carvalho, Jaú, São Paulo, Brazil; [b]Hospital Regional de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil; [c]Hospital Araújo Jorge, Goiânia, Goiás, Brazil; [d]Hospital Martagão Gesteira, Salvador, Bahia, Brazil; [e]Hospital da Criança, Brasília, Distrito Federal, Brazil; [f]Hospital Infantil Joana de Gusmão, Florianópolis, Santa Catarina, Brazil; [g]Hospital Universitário de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil; [h]Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil; [i]Hospital Napoleão Laureano, João Pessoa, Paraíba, Brazil; [j]Hospital Santa Isabel, Salvador, Bahia, Brazil; [k]Hospital Universitario Alcides Carneiro, Campina Grande, Paraíba, Brazil; [l]Instituto Nacional de Câncer, Rio de Janeiro, Rio de Janeiro, Brazil; [m]Hospital Santa Marcelina, São Paulo, São Paulo, Brazil; [n]Hospital Sarina Rolin Caracante, Sorocaba, São Paulo, Brazil; [o]Hospital Universitário Oswaldo Cruz, Recife, Pernambuco, Brazil; [p]Hospital dos Servidores do Estado do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; [q]Hospital Infantil Darcy Vargas, São Paulo, São Paulo, Brazil.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

MINISTÉRIO DA SAÚDE