MUTATION OF GENES AFFECTING THE RAS PATHWAY ASSOCIATED WITH *PTPN11* IN PEDIATRIC ACUTE MYELOID LEUKEMIA

Francianne Gomes Andrade, Filipe Vicente dos Santos Bueno, Ingrid Sardou Cezar, Gisele Dallapicola Brisson, Elda Pereira Noronha, Eugênia Terra-Granado, 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

- Deregulation of the *RAS* signaling cascade is often caused by somatic mutations in genes encoding proteins that influence the activity of this pathway including *NRAS, KRAS, FLT3, PTPN11*, and *KIT*.
- Somatic mutations in RAS pathway genes are found in myeloproliferative disorders (MD) and acute myeloid leukemia (AML) in different frequencies. To distinguish between MD and AML in childhood is a challenge and should consider variables such as age, blast percentage, the absence of *BCR-ABL1*, *PTPN11* mutations, and chromosomal abnormalities.
- We performed a comprehensive mutational screening in hotspot regions of *RAS* pathway genes in pediatric AML (p-AML) at diagnosis to evaluate their prognostic value in the probability of overall survival (pOS).

MATERIAL AND METHODS

Patients. This is a retrospective and multicentric study of 342 de novo p-AML referred to the Pediatric Hematology-Oncology Research Program, INCA, Rio de Janeiro, between Jan/2010 and May/2017. 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 p-AML were screened by RT-PCR and/or FISH [*PML-RARα, RUNX1-RUNX1T1, CBFb-MYH11,* and *KMT2A-r*]. Mutations in hotspot regions of *RAS* pathway genes (*FLT3, NRAS, KRAS, PTPN11*, and *KIT*) were analyzed by direct sequencing. 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.

RESULTS

Table 1. Distribution of molecular alterations in p-AML cases according to demographic and laboratorial features

		Age (years)					Sex			WBC count (x10^9/L)		
***RAS* pathway mutations [a]**	**Frequency n/total (%)**	**Median (min-max)**	**≤2 n (%)**	**>2-10 n (%)**	**≥11 n (%)**	**p**	**Males n (%)**	**Females n (%)**	**p**	**≤50 n (%)**	**>50 n (%)**	**p**
***FLT3* (ITD or TKD)**	58/278 (20.9)	10.9 (1.0-21.0)	1 (1.9)	22 (19.8)	35 (30.4)	<0.001	28 (20.6)	30 (21.1)	0.91	34 (18.1)	24 (27.0)	0.09
KRAS	8/271 (3.0)	11.0 (1.7-15.8)	1 (1.9)	3 (2.8)	4 (3.6)	0.82	4 (3.1)	4 (2.9)	0.92	4 (2.2)	4 (4.6)	0.28
NRAS	22/256 (8.6)	9.2 (0.7-17.5)	5 (10.9)	7 (6.9)	10 (9.3)	0.69	10 (8.1)	12 (9.0)	0.80	11 (6.4)	11 (13.3)	0.07
KIT	15/208 (7.2)	5.4 (1.3-12.3)	2 (4.2)	9 (10.8)	4 (5.2)	0.25	9 (9.3)	6 (5.4)	0.28	11 (7.8)	4 (6.1)	0.65
PTPN11	10/136 (7.4)	7.7 (1.7-17.1)	1 (3.3)	5 (9.4)	4 (7.5)	0.59	7 (10.4)	3 (4.3)	0.20	9 (9.5)	1 (2.5)	0.28

[a] Total number of analyzed cases reflects the availability of biological material for molecular tests. ITD, internal tandem duplication; n, number; TKD, tyrosine kinase domain; WBC, white blood cell count at diagnosis.

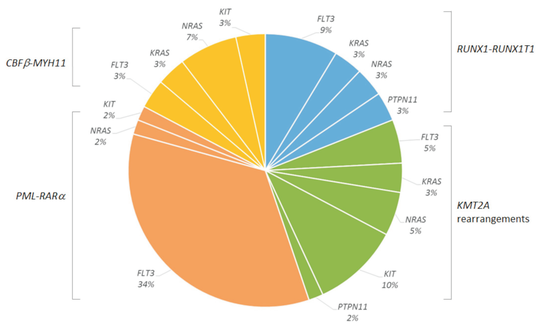

Figure 1. Frequency of *RAS* pathway mutations among fusion genes subgroups in p-AML

Table 2. Univariate analysis for overall survival of p-AML cases

		Univariate analysis		
	N (N of events)	**5-year pOS, (SE)**	**Median [a], (95% CI)**	**p [b]**
Geographic regions of treatment [c]				0.001
Northeast	82 (45)	30.9 (6.5)	15.0 (7.3-22.6)	
South	10 (2)	77.1 (14.4)	*	
Southeast	47 (18)	54.6 (8.4)	*	
Midwest	67 (43)	8.8 (6.9)	6.0 (4.1-7.8)	
Age range (years) [c]				0.08
≤2	54 (24)	33.1 (11.9)	34.0 (10.4-57.5)	
>2-10	70 (35)	34.0 (7.2)	15.0 (7.9-22.0)	
≥11	82 (49)	27.9 (5.7)	7.0 (2.7-11.2)	
Race [c]				0.009
Whites	87 (40)	38.4 (8.2)	21,0 (0.0-46,7)	
Non-Whites	119 (68)	25.4 (5.1)	8.0 (3.8-12.1)	
Sex [c]				0.40
Males	106 (59)	28.3 (5.8)	13.0 (8.1-17.8)	
Females	100 (49)	36.3 (6.4)	16.0 (7.1-24.8)	
WBC (x10^9/L) [c]				0.90
≤50	143 (72)	34.3 (5.1)	15.0 (7.8-22.1)	
>50	62 (36)	28.1 (7.4)	11.0 (6.2-15.7)	
Type II mutations [d]				
***RUNX1-RUNX1T1* [c]**	30 (18)	23.8 (10.0)	11.4 (0.0-23.8)	0.59
***CBFB-MYH11* [c]**	13 (4)	67.1 (13.5)	*	0.06
***KMT2A* rearrangements [c]**	31 (18)	17.6 (10.4)	10.6 (0.0- 21.7)	0.19
Type I mutations [c, d]				
FLT3	28 (17)	27.1 (10.0)	11.2 (7.1-15.2)	0.84
KRAS	7 (4)	42.9 (18.7)	16.6 (8.2-25.0)	0.79
NRAS	17 (6)	57.4 (13.4)	*	0.16
KIT	13 (5)	53.7 (16.2)	*	0.59
PTPN11	14 (12)	0.0 (0.0)	3.0 (0.0-6.4)	0.001
Concomitant type I mutations [c]				0.09
Single mutation	60 (30)	38.2 (7.4)	11.2 (5.78-16.6)	
More than one mutation	7 (6)	14.3 (13.2)	2.3 (0.0-5.78)	

[a] Median survival in months. [b] p values from log-rank test indicate whether the differences are significant between the subgroups. [c] Excluding acute promyelocytic leukemia subtype. [d] Analysis performed between groups positive and negative for the molecular alteration. * Median not reached. CI, confidence interval; N, number; pOS, the probability of overall survival; SE, Standard error.

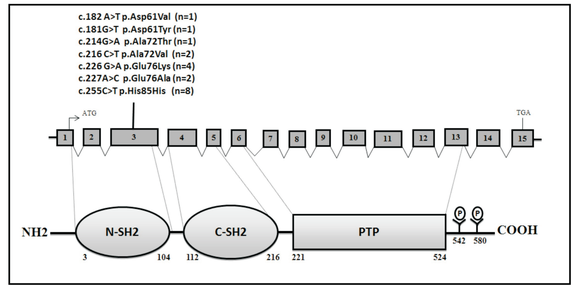

Figure 2. Structure of the protein tyrosine phosphatase 2 (SHP-2) encoded by *PTPN11* gene and mutations identified in exon 3. *PTPN11* has 15 exons: exons 2 and 3 encode the N-SH2 domain, exons 4 and 5, the C-SH2 domain, exons 6 to 13, domain tyrosine phosphatase (PTP), and exons 14 and 15 encode the carboxy-terminal region. Numbers of amino acid residues delineating various domains or corresponding to the phosphorylation sites are indicated in the figure. N, number of cases.

CONCLUSION

RAS pathway genes were found mutated in 47.1% of p-AML patients. Mutations were observed concomitantly in more than one gene in 10.4% of patients. *PTPN11* mutations in exon 3 were found in overall analysis to be associated with adverse prognosis, as well as, geographic region of diagnosis. Although there is a need of further action to improve the overall survival rates of p-AML patients, these aberrations are likely to guide risk stratification.

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[c], Bruno Marcelo Rocha Freire[c], Renata Silva de Carvalho Gurgel[i], Maura Rosane Valério Ikoma[a], Ingrid Koster[j], Isis Maria Quezado Magalhães[k], Ana Maria Marinho[l], Renato Melaragno[m], Suellen Valadares Moura[l], Gustavo Ribeiro Neves[n], Claudia Teresa Oliveira[l], Ilana Zalcberg Renault[l], Terezinha de Jesus Marques Salles[o], Denise Browsfield Silva[l], Marcelo Santos Souza[p], Tállita Meciany Farias Vieira[l], Fernando de Almeida Werneck[q], Ana Freund Winn[r], and Gustavo Zamperlini[s].
[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 Universit_ario 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: Serviço de Edição e Informação Técnico-Científica / INCA