GENOMIC PROFILE OF *RUNX1-ETV6*$^{+}$ PAEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA

CAROLINE BARBIERI BLUNCK[1], BRUNO DE ALMEIDA LOPES[1], THAYANA DA CONCEIÇÃO BARBOSA[1,] JULIO CESAR SANTORO[1], ELDA PEREIRA NORONHA[2], EUGÊNIA TERRA GRANADO[2], GABRIELA VERA-LOZADA[3], ROCIO HASSAN[3], MARCELA BRAGA MANSUR[1], MARIA DO SOCORRO POMBO DE OLIVEIRA[2] AND MARIANA EMERENCIANO[1]

[1]MOLECULAR CANCER STUDY GROUP, DIVISION OF CLINICAL RESEARCH, RESEARCH CENTRE, INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO, RJ, BRAZIL [2]PROGRAMA DE HEMATOLOGIA-ONCOLOGIA PEDIÁTRICO – INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO, RJ, DIVISÃO DE PESQUISA CLÍNICA E DESENVOLVIMENTO TECNOLÓGICO – INCA, CPQ, BRAZIL [3]LABORATÓRIO DE ONCOVIROLOGIA - CENTRO DE TRANSPLANTE DE MEDULA ÓSSEA, INSTITUTO NACIONAL DE CÂNCER - INCA, RIO DE JANEIRO, RJ, DIVISÃO DE PESQUISA CLÍNICA E DESENVOLVIMENTO TECNOLÓGICO – INCA, CPQ, BRAZIL

INTRODUCTION

- B-cell precursor acute lymphoblastic leukaemias (BCP-ALL) are characterized by recurrent translocations, with t(12;21)(p13;q22) being the most common. This translocation generates the *ETV6-RUNX1(ER)* and the reciprocal *RUNX1-ETV6 (RE)* gene fusions in 100% and 76% of cases, respectively.
- Studies have shown that *RE* high expression confers worse prognosis. Moreover, other genetic changes have been described as important risk stratification markers.
- We aimed to characterize the genomic profile of patients harboring *RUNX1-ETV6*.

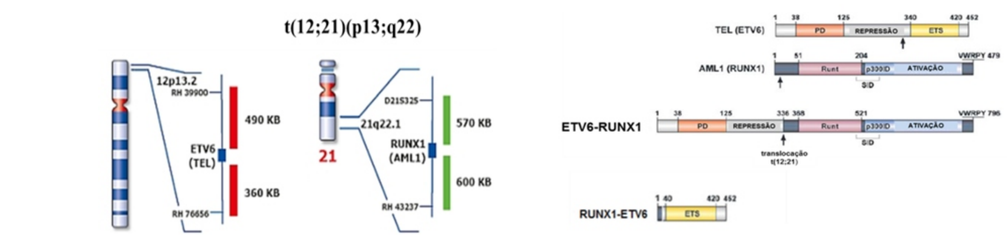

Figure 1. Schematic representation of the fusion *ETV6-RUNX1* and *RUNX1-ETV6*. B-cell precursor acute lymphoblastic leukaemias (BCP-ALL) are characterized by recurrent translocations, with t(12;21)(p13;q22) being the most common. This translocation generates the ETV6-RUNX1(ER) and the reciprocal RUNX1-ETV6 (RE) gene fusions in 100% and 76% of cases, respectively.

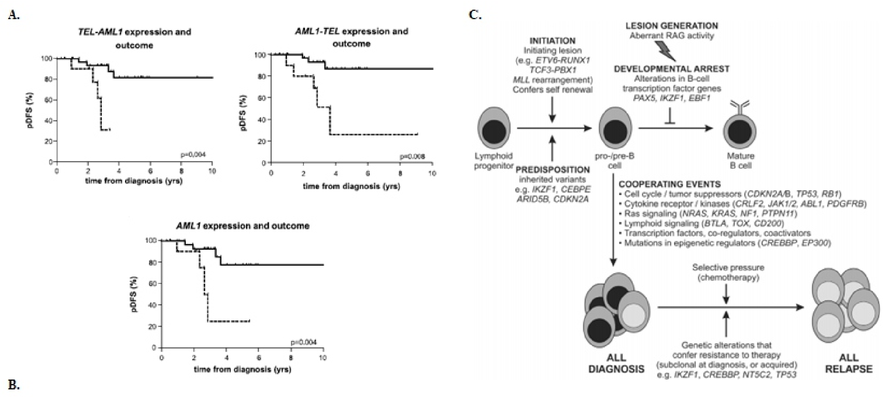

Table 2. Multivariate analysis of risk factors in t(12;21)-positive ALL

Variable	Unfavorable feature	Hazard ratio*	95% confidence interval	*P*
Age at diagnosis (y)	>10	9.05	0.47-173.31	0.14
WBC at diagnosis (10^9 cells/L)	>25	4.70	0.53-41.90	0.17
TEL-AML1 expression		1.23	0.79-1.93	0.36
AML1-TEL expression		7.02	2.01-24.52	0.002
AML1 expression		1.10	0.84-1.43	0.49

*Cox proportional hazard analysis using mRNA expression levels as continuous variable.

Figure 2. Patients were divided into two groups by the 75thpercentile for the expression of *ETV6-RUNX1* , *RUNX1-ETV6* , and *RUNX1* . Dashedline, high expression (p>75); solid line, lowexpression (p<75). B) High expression is associatedwith an unfavorable prognosis. C) Schematic for the nature and time of acquisition of genetic alterations in the pathogenesis of B-ALL.

METHODS AND RESULTS

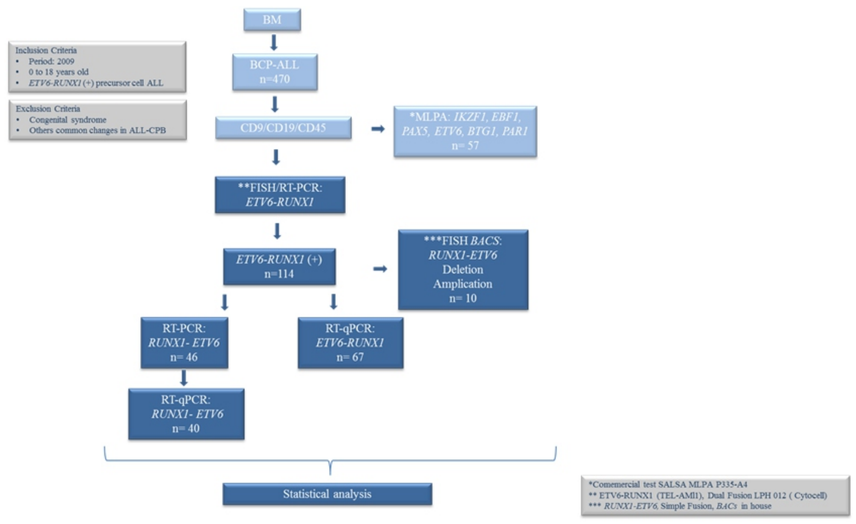

Figure 3. Test flowchart

Demographic and laboratory characteristics

Table 1. Demographic and laboratory characteristics of the patients in relation to the presence of the direct and reciprocal transcript found by RT-PCR

Variables	*ETV6-RUNX1*		*P* valor
	RUNX1-ETV6^{-} n (%)	*RUNX1-ETV6*$^{+}$ n (%)*	
Age (months)			
0-12	02(2.85)	0	
13-120	20(28.5)	41(58.5)	*P* = 0.236
>120	02(2.85)	04(5.71)	
Sex			
Femino	09(12.8)	18(25.7)	*P* = 0.122
Masculino	15(21.4)	28(40.0)	
Subtype BCP-ALL			
pro-B	01(1.4)	0	
Common	21(30.0)	39(55.7)	*P*= 0.631
pre-B	02(2.85)	05(7.1)	
WBC x 10^9 /L			
<50.000	19(27.1)	39(55.7)	
>50.000	05(7.1)	07(10.0)	*P*= 0.603
CD9			
≤ 64%	14(20.0)	10(1.4)	
> 64%	09(12.8)	36(51.4)	*P* = 0.001

* RT-PCR for detection of *RUNX1-ETV6* was performed in 70 cases.

Detection of additional changes by MLPA

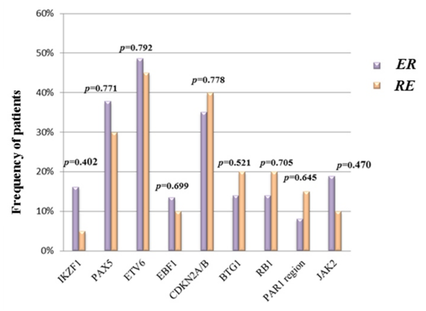

Figure 5. Frequency of deletions found in patients with direct transcript (n=37) and patients with direct and reciprocal transcript (n=20).
The most frequently deletions found in *RE* and *ER* patients affecting *ETV6* (*RE*=45%/*RE*=49%), *CDKN2A/B* (*RE*=40%/*ER*=33%) and *PAX5* (*RE*=29%/*ER*=38%), genes.

Evaluation of transcripts by RT-qPCR

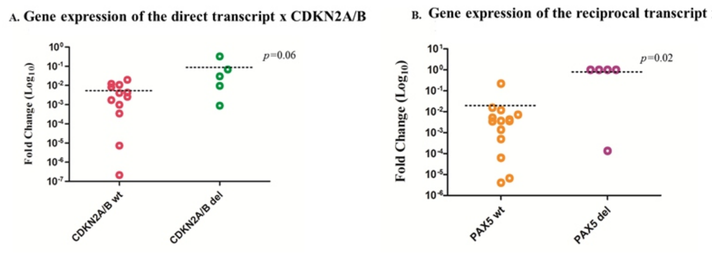

Figure 6. Quantification of direct and reciprocal transcristal expression levels in patients with *CDKN2A/B* and *PAX5* status. A) Expression of the direct transcript in patients with wild type *CDKN2A/B* and deleted *CDKN2A/B*. B) Expression of the reciprocal transcript in patients with *PAX5* wild type and *PAX5* deleted

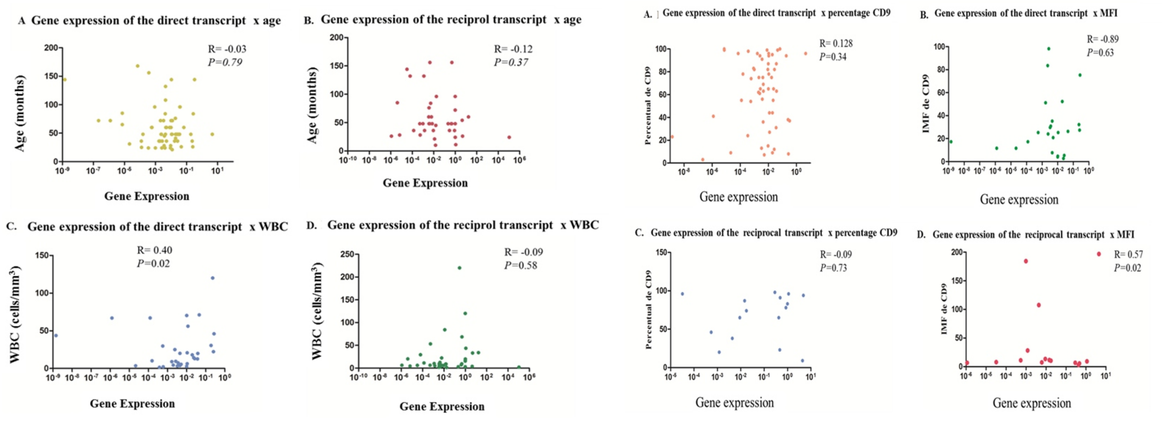

Figure 7. Correlation analysis between levels of the direct / reciprocal transcripts and age and leucometria.
P-value calculated through the Pearson test (Pearson's R measures the degree of linear correlation between two quantitative variables).

Figure 8. Correlation analysis between the levels of the direct/reciprocal transcripts and CD9 cell expression (Percentual and IMF).
P-value calculated through the Pearson test (Pearson's R measures the degree of linear correlation between two quantitative variables).

Detection of direct and reciprocal transcripts and additional changes by FISH

• FISH analysis using commercial probe

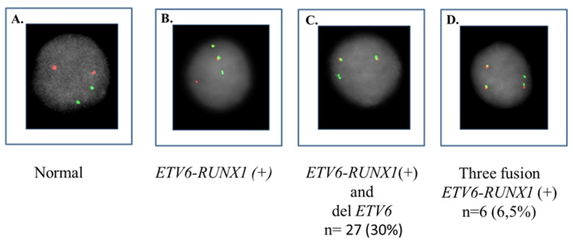

Figure 9. Interphase nucleus FISH using commercial double-dip probe.
Analysis using commercial probe showed 114 with *ER*$^{+}$ and 30% (26/114) of these patients had both the gene fusion and a non-rearranged *ETV6* allele del.

• FISH analysis using in-house probes

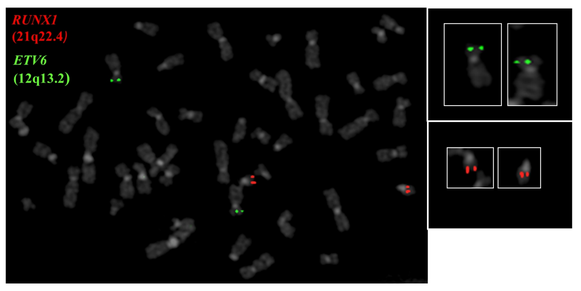

Figure 10. Localization of FISH probes developed in-house on metaphase chromosomes. A) Location of *RUNX1* and *ETV6* bacs on healthy donor metaphase chromosomes in their respective chromosomes

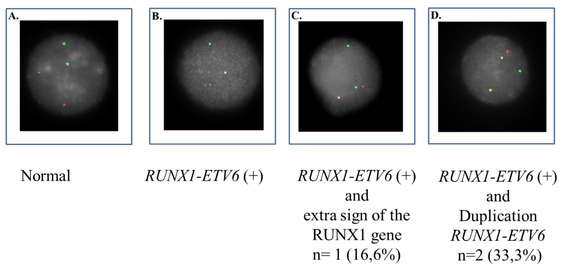

Figure 11. FISH of interphase nuclei using probe type *BACs*. Analysis using probe in-house developed (*BACs*) probes revealed a *RUNX1-ETV6* amplification pattern in 2% of cases. Interestingly, we observed that 3 of 10 *RE* patients showed a duplication pattern of the *RE* fusion.

CONCLUSION

The correlation between *RE* expression and *PAX5* del is still unclear and is worthy of further investigation. In addition, all cases with *RE* duplication had a concomitant del of the non-rearranged *ETV6* allele, which suggests that this loss may contribute to the formation of the duplication. Our results suggest that patients with *RE* have a heterogeneous pattern and the characterization of this subgroup may provide new insights for risk stratification and treatment strategies.

Development agency: Ministério da Saúde, FAPERJ, CNPq

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

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