CD44 expression in T-ALL and its association with *RAS* mutations

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INTRODUCTION

CD44 is a multifunctional adhesion glycoprotein which helps with the homing of precursor cells from the bone marrow to the thymus in the development of T lymphocytes. CD44 expression was associated with tumor progression, influencing the survival and organ infiltration of murine T-ALL models. *CD44* gene is a target of the RAS pathway, which promotes its alternative splicing, throughout a positive feedback loop. Several genomic similarities were observed between Early T-cell precursor-ALL (ETP-ALL, a T-ALL subset) and acute myeloid leukemia (AML), where CD44 is known to be a stem cell marker, and expression of its variant proteins has been associated with poor prognosis.

OBJECTIVES

- To evaluate the profile of the cellular expression of CD44 among different maturational subtypes of pediatric T-ALL, as a possible predictor to *RAS* mutations;
- To compare the CD44 expression with the patient's clinical characteristics.

METHODS

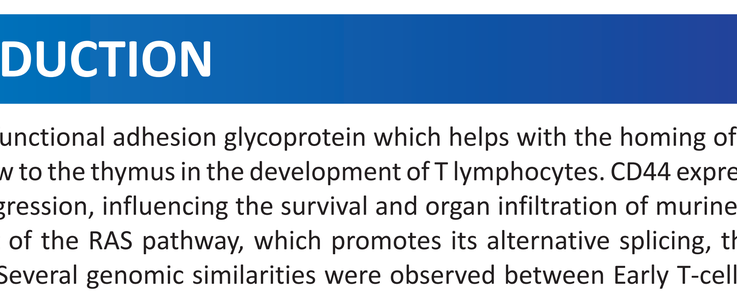

Figure 1: Methodology flowchart of T-cell Acute Lymphoid Leukemia and Acute Myeloid Leukemia cases.

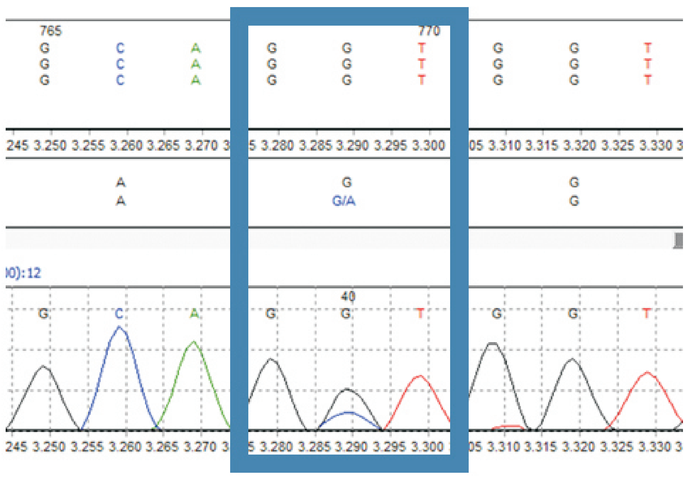

Figure 2: Electropherogram of a codon 12 mutated *NRAS* case detected by Sanger sequencing, with a simple substitution of a Guanine (G) for a Cytosine (C), resulting in an amino acid change from Glycine to Alanin.

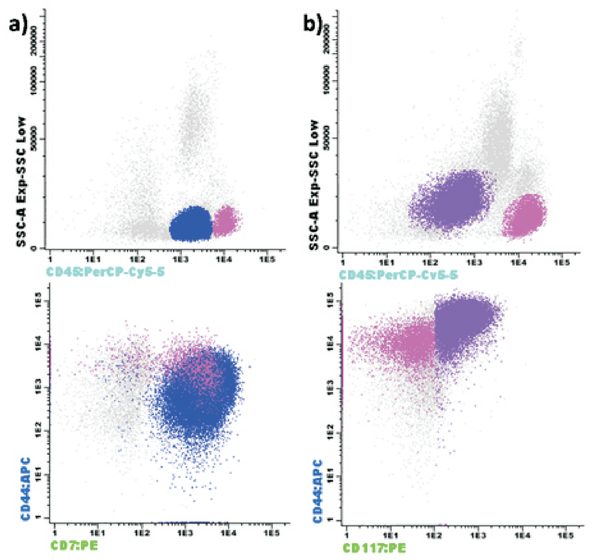

Figure 3: CD44 expression evaluated by multiparameter flow cytometry in a) T-cell Acute Lymphoid Leukemia, where the lymphoblasts are depicted in blue and the lymphocytes in pink and b) Acute Myeloid Leukemia, where the myeloblasts are in violet.

The Fisher's exact test or chi-square test were used to evaluate the distribution of categorical variables, whereas Mann-Whitney (two groups) or Kruskal Wallis (more than two groups) tests were used to evaluate the distribution of non parametric continuous variables. t-test and one-way ANOVA were used for parametric variables. p values of < 0.05 were considered statistically significant.

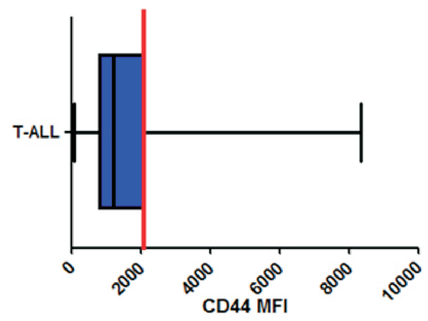

Figure 4: Box Plot showing the CD44 expression in the T-ALL. Vertical lines represent the minimum, maximum, quartiles and median values. The Median Fluorescence Intensity (MFI) in the 75th percentile was used as a cutoff to discriminate between high or low expression of CD44 in T-ALL.

RESULTS

Only two cases were CD44^{neg} (<20% positive). There was no association between high expression of CD44 and the presence of organomegaly in T-ALL **(Table 1)** while for chloroma in AML the evaluation was impaired due to small series of cases. There was no association between the expression of CD44 and different T-ALL subtypes (**Figure 5)**. The frequency of *N/KRAS* mutations in T-ALL *cases* were 11,7% (12/102), whereas in AML cases were 16.7%. There was no significant difference in CD44 expression between cases with *N/KRAS* mutations (Median Fluorescence Intensity (MFI): 1715 [365-8325]) and without mutation (MFI: 1179 [68-5544]) in T-ALL **(Figure 6a)**, whereas in AML, *N/KRAS* mutated cases had a lower CD44 expression (MFI: 10979 [7650-18810]) than the cases without mutation (MFI: 21720 [1019-44720]) (p = 0,032) **(Figure 6b)**. AML patients have a higher expression of CD44 (MFI: 18890 [1019-44720]) than T-ALL patients (MFI: 1211 [68-8325]) (p < 0,0001) **(Figure 5)**. CD44 expression in ETP-ALL (MFI: 1504 [855-3296]) and other T-ALL subtypes (MFI: 1167 [68-8325]) were lower than in AML (p < 0,0001).

Table 1: Organomegaly presence according to CD44 status.

		CD44 Median Fluorescence Intensity (MFI)		
	Total	Low	High	p
Mediastinum				
Yes	32 (33.7%)	26 (36.1%)	6 (26.1%)	0.453
No	63 (66.3%)	46 (63.9%)	17 (73.9%)	
Liver				
Yes	62 (65.3%)	50 (69.4%)	12 (52.2%)	0.141
No	33 (34.7%)	22 (30.6%)	11 (47.8%)	
Spleen				
Yes	69 (72.6%)	52 (72.2%)	17 (73.9%)	1.0
No	26 (27.4%)	20 (27.8%)	6 (26.1%)	
Lymph Nodes				
Yes	64 (67.4%)	49 (68.1%)	15 (65.2%)	0.803
No	31 (32.6%)	23 (31.9%)	8 (34.8%)	
Tumor				
Yes	10 (10.5%)	9 (12.5%)	1 (4.3%)	0.442
No	85 (89.5%)	63 (87.5%)	22 (95.7%)	
CNS				
Yes	4 (4.4%)	4 (5.9%)	0 (0%)	1.0
No	86 (95.6%)	64 (94.1%)	22 (100%)	
Total	**95**	**72 (75.8%)**	**23 (24.2%)**	

p-value by the chi-square test or Fisher's Exact test. CNS – Central Nervous System.

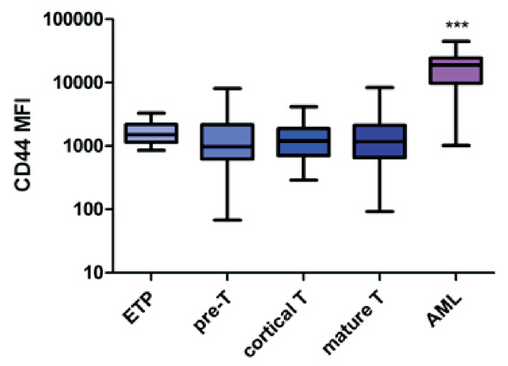

Figure 5: CD44 expression in the different T-ALL subtypes and AML. T-ALL subtypes, ETP-ALL, n = 13; Pre-T, n = 23; cortical T, n = 39; mature T, n = 35, AML, n = 28. p < 0.001 (Kruskal Wallis). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence Intensity (MFI) values. *** p < 0.001

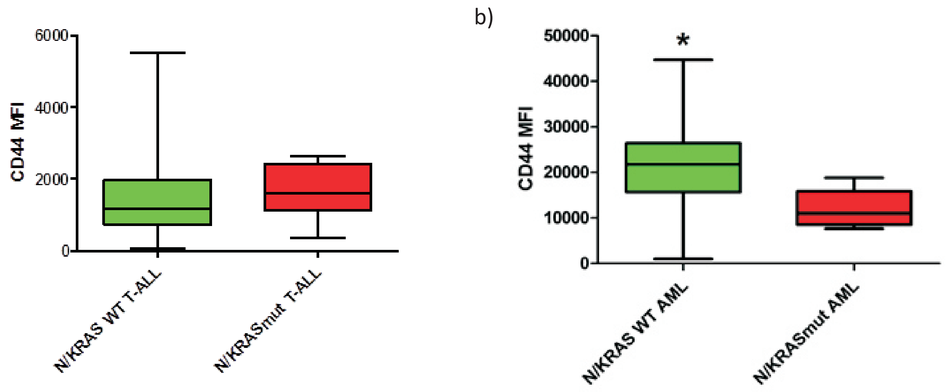

Figure 6: CD44 expression according to *N/KRAS* status. a) T-ALL *N/KRAS* wild type (WT) cases, n = 12, T-ALL *N/KRAS* mutated cases, n = 90. p = 0.14 (Mann-Whitney). b) AML *N/KRAS* wild type (WT) cases, n = 8, AML *N/KRAS* mutated cases, n = 20. p = 0.032 (t test). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence Intensity (MFI) values. * p<0.05

CONCLUSION

CD44 cellular status was not relevant for T-ALL tumoral profile and its expression was not associated with T-ALL subtype. CD44 is under expressed in T-ALL and ETP-ALL when compared with AML. *N/KRAS* mutation does not seems be associated with different expression of CD44 in pediatric T-ALL whereas further investigation is required in AML subsets, once the CD44 expression was lower in *N/KRAS* mutated cases.

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