Cd44 expression in T cell Acute Lymphoblastic Leukemia and Acute Myeloid Leukemia associated with *RAS* mutations

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INTRODUCTION

Cd44 is an adhesion glycoprotein which helps with the homing of hematopoietic precursor cells. In acute myeloid leukemia (AML), CD44 is been investigated as a stem cell marker, and expression of its variant proteins has been associated with poor prognosis. Only in murine T-cell acute lymphoblastic leukemia (T-ALL) models, CD44 expression was associated with tumor progression, organ infiltration and influencing survival. In early T-cell precursor-ALL (ETP-ALL, a T-ALL subset) several similarities were observed with AML genomic aberrations. Taking the *CD44* gene that is a target of the RAS pathway, which promotes its alternative splicing, throughout a positive feedback loop, we have investigated whether the cellular expression of CD44 in different maturational subtypes of pediatric T-ALL and AML would predict *RAS* mutations.

METHODS

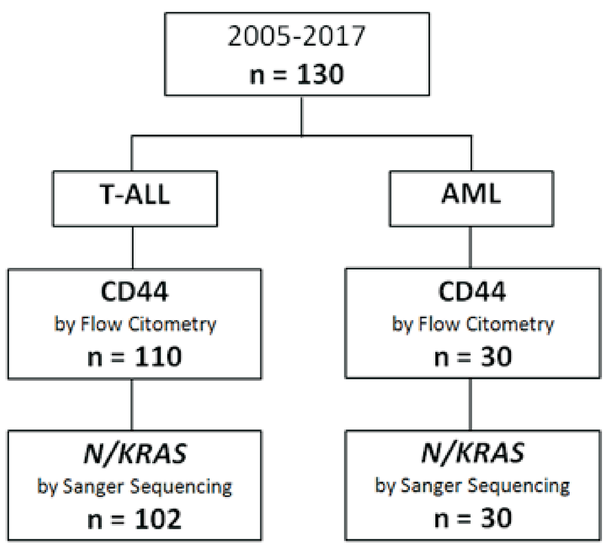

Figure 1: Methodology flowchart of T-cell Acute Lymphoid Leukemia and Acute Myeloid Leukemia cases.

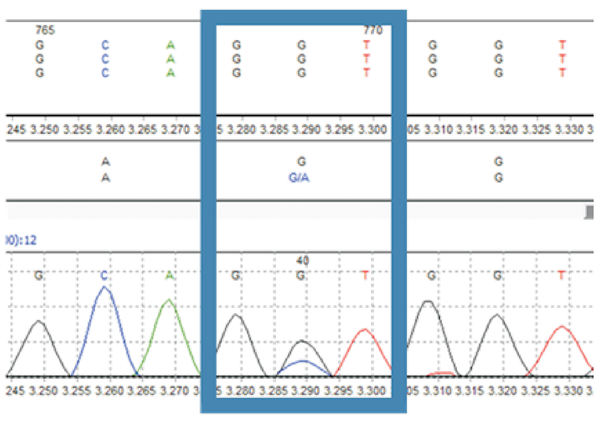

Figure 2: Electropherogram of a codon 12 mutated *NRAS* case detected by Sanger sequencing, with a simple substitution of a Guanine (G) for a Cytosine (C), resulting in an amino acid change from Glycine to Alanin.

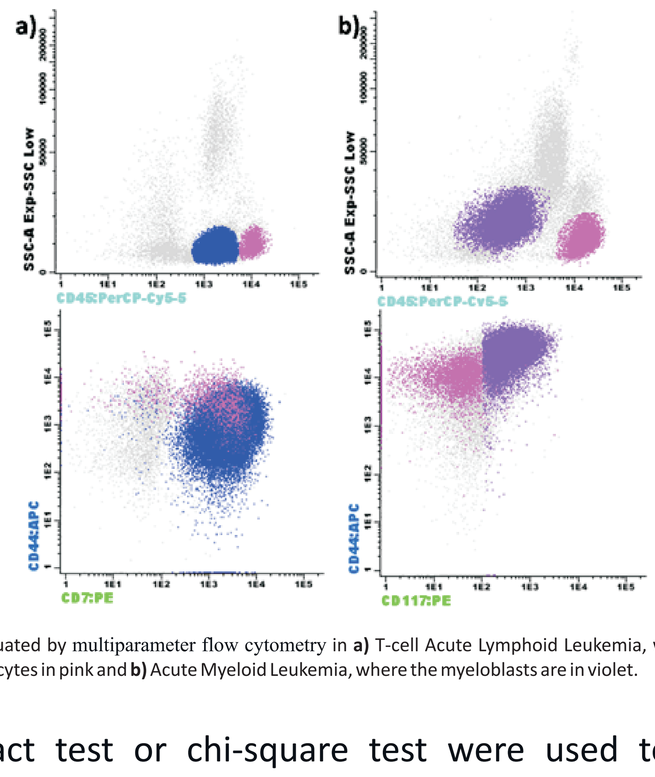

Figure 3: CD44 expression evaluated by multiparameter flow cytometry in **a)** T-cell Acute Lymphoid Leukemia, where the lymphoblasts are depicted in blue and the lymphocytes in pink and **b)** Acute Myeloid Leukemia, where the myeloblasts are in violet.

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.

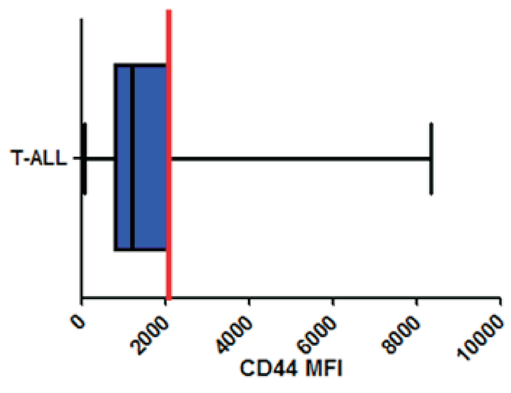

Figure 4: Box Plot showing the CD44 expression in the T-ALL. Vertical lines represent the minimum, maximum, quartiles and median values. The Median Fluorescence Intensity (MFI) in the 75th percentile was used as a cutoff to discriminate between high or low expression of CD44 in T-ALL.

RESULTS

Only two cases were $CD44^{neg}$ (<20% positive). There was no association between high expression of CD44 and organomegaly in T-ALL **(Table 1)** while for chloroma in AML the evaluation impaired due to small series. There was no association between the expression of CD44 and T-ALL subtypes and patients with AML have a higher cellular expression of CD44 (MFI: 18890 [1019-44720]) than T-ALL (MFI: 1211 [68-8325]) (p<0,0001) **(Figure 5)**. The CD44 expression in ETP-ALL were lower than in AML (MFI: 1504 [855-3296], p<0,0001). 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 (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)**.

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.

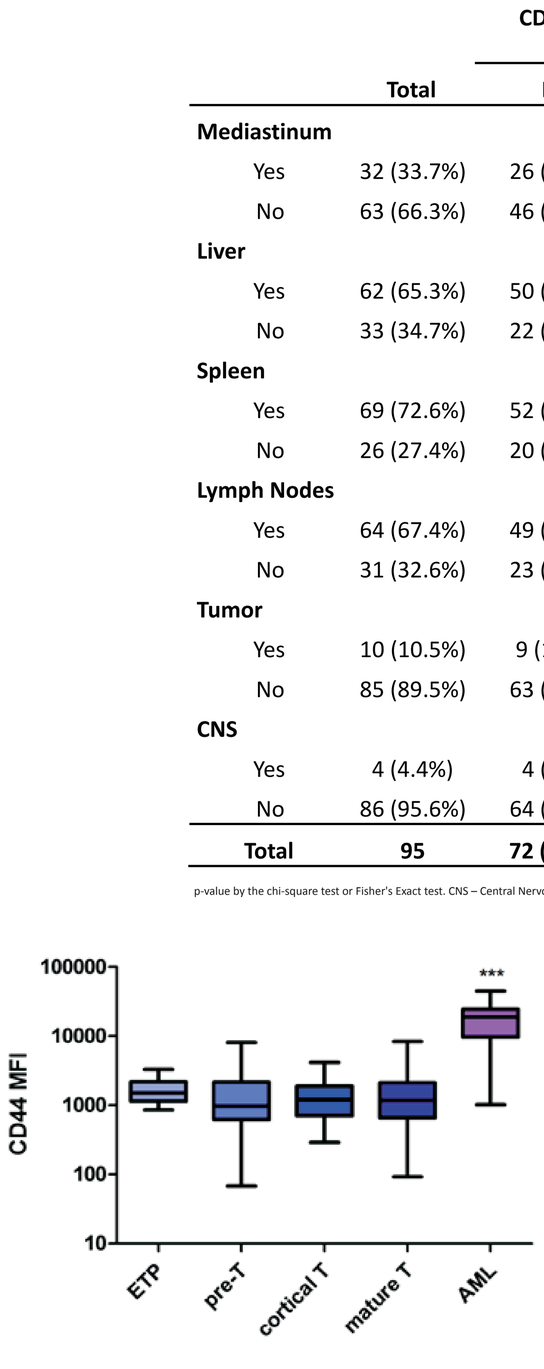

Figure 5: CD44 expression in the different T-ALL subtypes and AML. T-ALL subtypes, ETP-ALL, n = 13; Pre-T, n = 23; cortical T, n = 39; mature T, n = 35, AML, n = 28. p < 0.001 (Kruskal Wallis). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence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

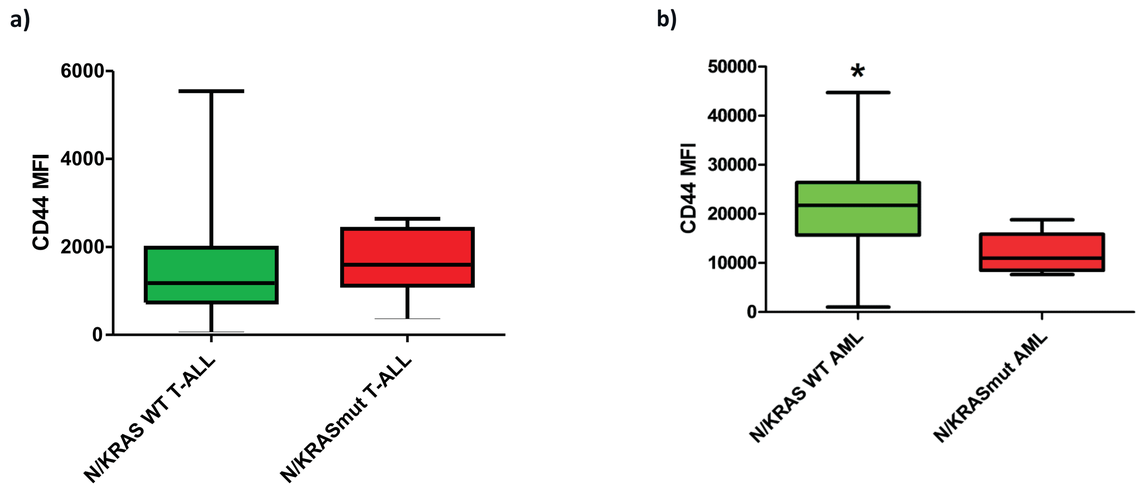

Figure 6: CD44 expression according to N/KRAS status. a) T-ALL *N/KRAS* wild type (WT) cases, n = 12, T-ALL *N/KRAS* mutated cases, n = 90. p = 0.14 (Mann-Whitney). b) AML *N/KRAS* wild type (WT) cases, n = 8, AML *N/KRAS* mutated cases, n = 20. p = 0.032 (t test). Box Plot with horizontal lines representing 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.

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