MOLECULAR MECHANISM ACCOUNTING FOR CRLF2 OVEREXPRESSION IN ACUTE LYMPHOBLASTIC LEUKAEMIA

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INTRODUCTION

CRLF2 overexpression (*CRLF2* -over) has been associated with unfavourable prognosis in acute lymphoblastic leukaemia (ALL) cases. In B-cell ALL, the presence of *CRLF2* rearrangements *(CRLF2-r*) can explain half of the cases with this gene overexpression. Nonetheless, the mechanism accounting for the other 50% of cases lacking *CRLF2-r* is still unknown. For T-ALL, specific *NOTCH1* mutations were associated with *CRLF2-over,* however, this is not fully elucidated.

Recent discoveries have shown that somatically acquired mutations in non-coding regulatory regions of the DNA can create new transcription factor binding sites (TFBS) leading to an aberrant overexpression of critical oncogenes (neomorphic super-enhancers) in human cancers (including ALL).

In this scenario, we hypothesise that the occurrence of these neomorphic super-enhancer mutations might be a potential mechanism behind oncogene overexpression in ALL patients lacking other somatic abnormalities. Hence our aim is to identify the mechanism responsible for CRLF2 -over in those cases.

MATERIAL AND METHODS

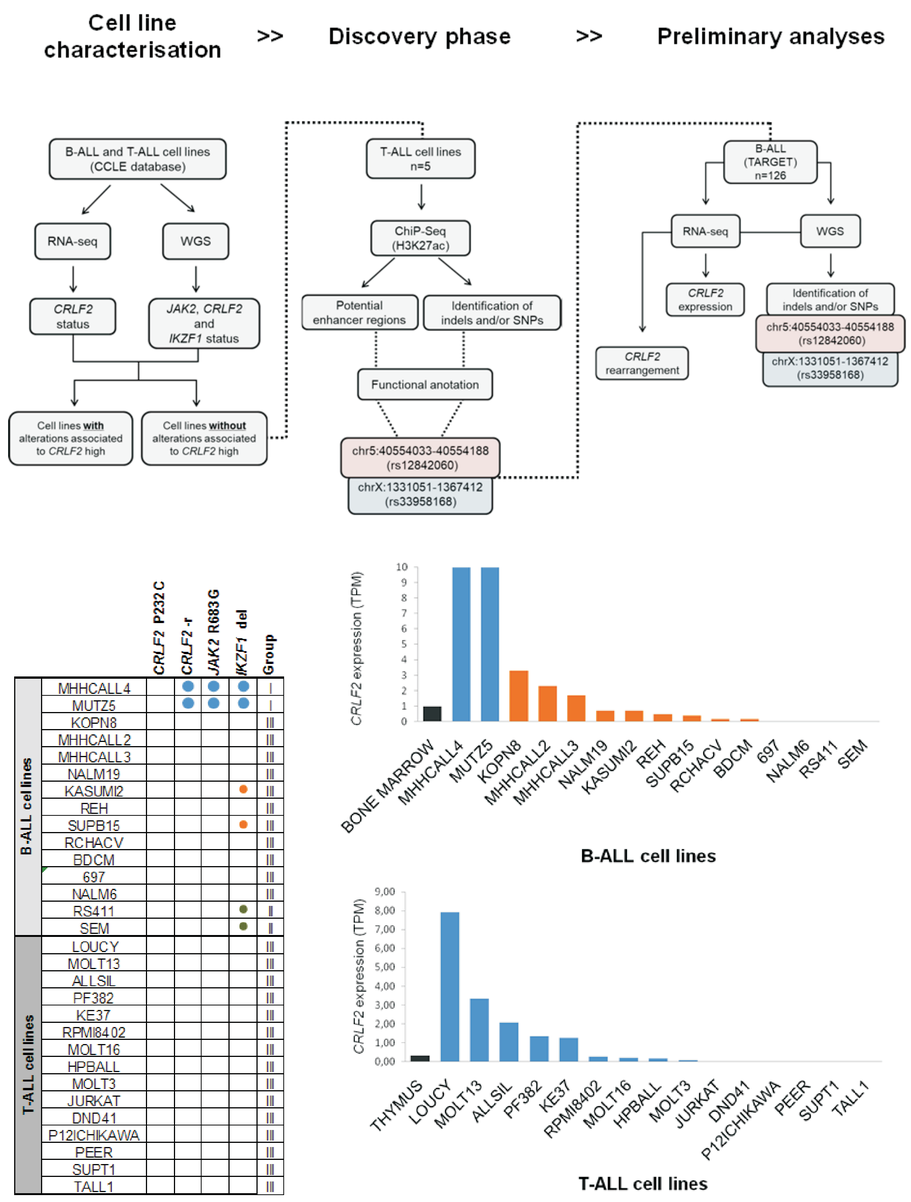

Figure 1. Characterisation of haematopoietic cell lines. Ten of cell lines evaluated (5 B-ALL and 5 T-ALL) presented *CRLF2*-over compared to *CRLF2* expression in normal bone marrow and thymus, respectively. B an T cell lines were grouped according to the presence of alterations associated with *CRLF2* overexpression.

Figure 2. CRLF2 high cells presents particular H3K27ac marks. Based on the differential peak analysis, we identified 22,988 potential peaks in active chromatin regions exclusively observed in *CRLF2*-over cell lines, not annotated in enhancer data bases neither in normal thymus. By overlapping these peaks with the variants found in *CRLF2*-over cell lines, we identified two SNPs located at chrX (rs12842060) and chr5 (rs33958168). The SNP rs12842060 significantly disrupts the TFBS AREB6.03 and KLF12.01 and creates new binding sites for TWIST.01 and TCFE2A.01. On the other hand, the rs33958168, which results in an insAC, disrupts ZNF771.01 and AML3.01 sites and creates new TFBS for AML1.02.

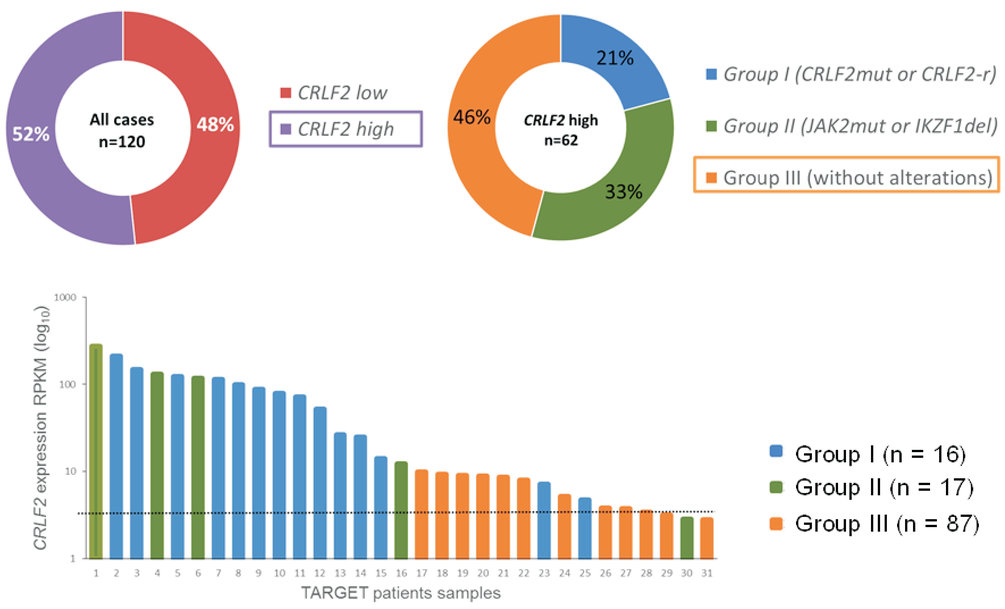

Figure 3. Characterisation of TARGET patients' samples. Fifty-two percent of B-ALL cases included having *CRLF2* high. Seventy-nine percent of these lacked *CRLF2*-r and/or CRLF2 P232C mutation and were used to test the reproducibility of our initial findings.

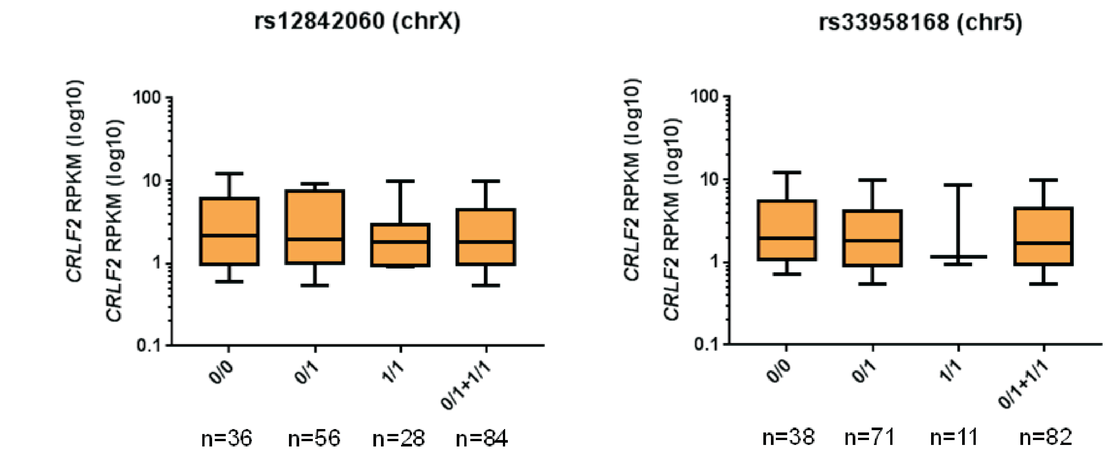

Figure 4. Association between genetic variants and *CRLF2* expression. The variants rs12842060 and rs33958168 were identified in 73% and 66% of cases, respectively.

Table 1. Characterisation of Brazilian patients' samples

Variables	*CRLF2* high n (%)	*CRLF2* low n (%)
Age (Years)		
Paediatric (<15y)	5 (26.3)	22 (51.2)
AYA (15-21y)	4 (21.0)	6 (13.9)
Adult (>21y)	10 (55.7)	15 (34.9)
WBC		
<50.000	12 (63.2)	30 (69.7)
≥50.000	7 (36.8)	13 (30.3)
Cytogenetic risk*		
Good risk	1 (6.2)	13 (33.3)
Intermediate risk	8 (50.0)	11 (28.2)
Poor risk	7 (43.8)	15 (38.5)
Follow-up status*		
Alive	8 (50.0)	15 (65.2)
Deceased	8 (50.0)	8 (34.8)
Total	19	43

*Contain missing informations

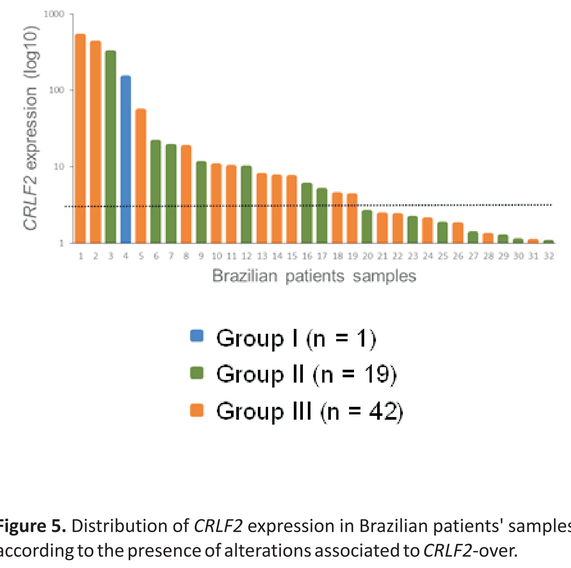

Figure 5. Distribution of *CRLF2* expression in Brazilian patients' samples according to the presence of alterations associated to *CRLF2*-over.

CONCLUSION

- Two potential enhancer neomorphic regions (chr5 and chrX) were identified;
- There are *CRLF2*-over patients with unknown alterations;
- Considering that these partial results are part of an ongoing investigation, our results revealed so far that other type of alterations, not only indels/mutations, can be the mechanism responsible for *CRLF2*-over in B-ALL cases lacking *CRLF2-r*;
- Of note, our validation cohort only includes patients at diagnosis and we consider extremely important to replicate these analyses in relapse samples. The discovery phase findings will be validated using patient's samples and T-ALL TARGET data.

Financial support: Ministério da Saúde, CNPq and FAPERJ

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