CRLF2 EXPRESSION ASSOCIATES WITH COMBINED *NOTCH1/IKZF1* STATUS IN CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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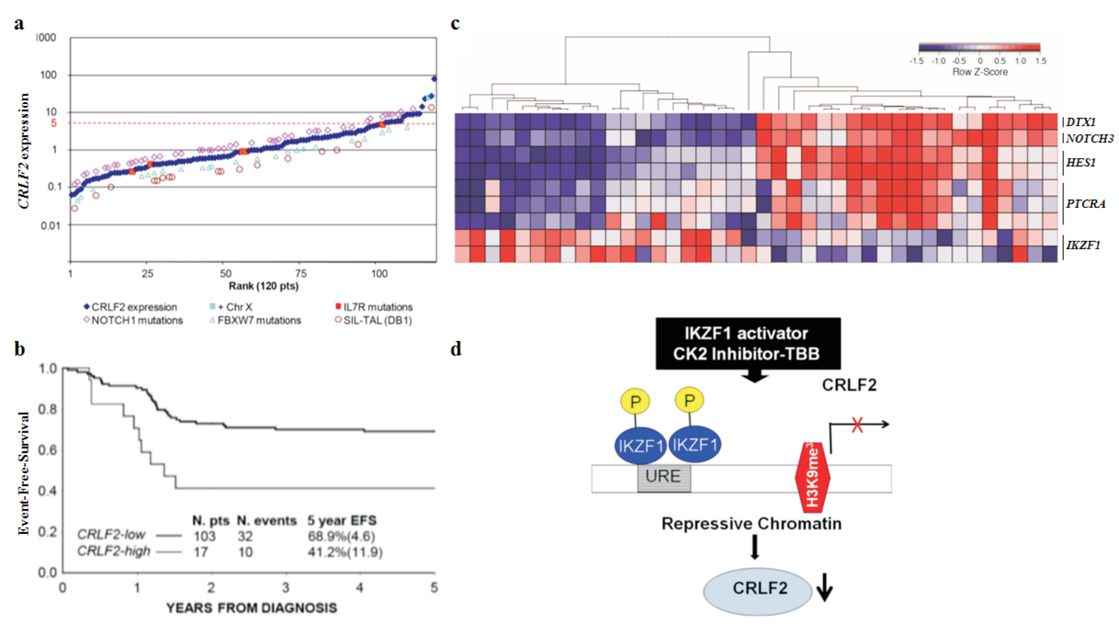
BACKGROUND

❖ T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive haematopoietic disorder that represents 15% of paediatric ALL. Although numerous relevant molecular alterations have already been identified, few have a consensual prognostic value;

❖ *CRLF2* overexpression was recently identified in high-risk T-ALL patients., for these cases no genomic abnormality was found to be associated with *CRLF2* overexpression (figure 1a and b).

❖ *IKZF1* has been recently shown as a direct transcriptional regulator of *CRLF2* expression. Moreover, it is known that NOTCH1 controls *IKZF1* expression in T-ALL (figure 1c and d).

❖ We hypothesized that *NOTCH1/IKZF1* status could be one mechanism responsible for *CRLF2* deregulation in T-ALL.



Palmi *et al, 2016;* Witkowski *et al, 2015;* Ge *et al*, 2016.

Figure 1 . a. *CRLF2* expression and genomic alterations. Red line represented the cut off for *CRLF2* overexpression patients. ***b.*** Association of *CRLF2* overexpression to event free survival (Palmi *et al*, 2016. **c.** Heatmap of microarray-based differential gene expression (*IKZF1* ang Notch1 target genes) following treatment of human T-ALL cell lines with activating *NOTCH1* mutations (Witkowski *et al*, 2015*)* **d.** Model of *CRLF2* expression regulation by *IKZF1* (Ge *et al*, 2016).

METHODS AND RESULTS

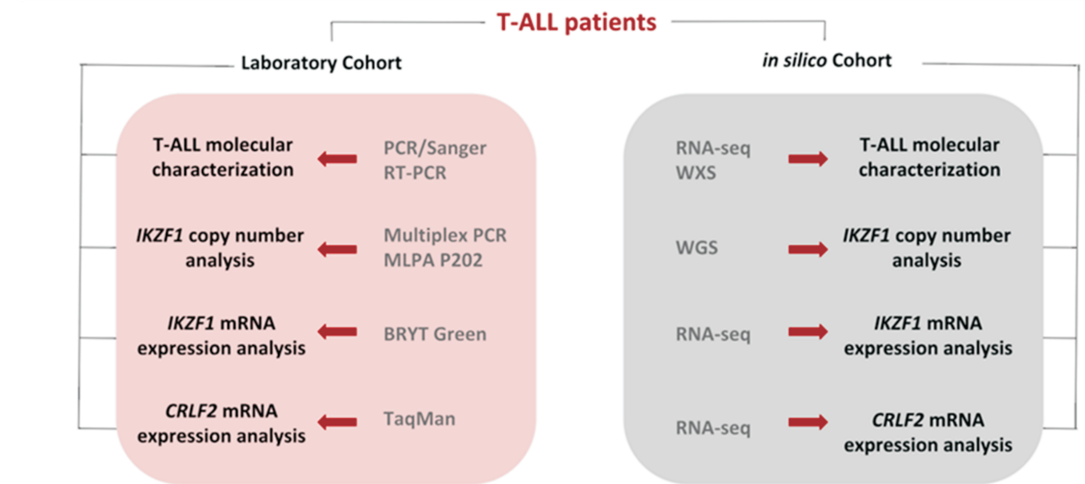


Figure 2 . Flowchart representing the study design developed for investigate *CRLF2* overexpression*, IKZF1* loss and *NOTCH1* activating mutations in T-ALL. Two independent cohorts were investigated and *in silico* tests were realizes with TARGET database.

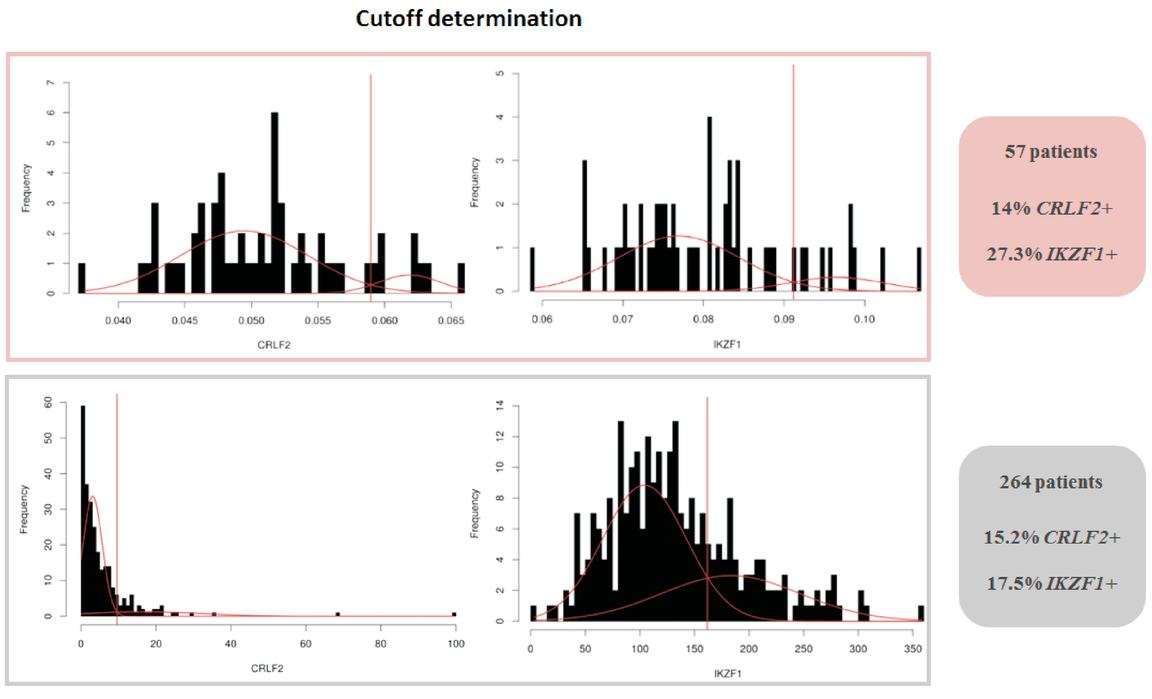


Figure 3. Cutoff for *CRLF2* and *IKZF1* determination by the web application Cutoff Finder. The highlight in pink represents laboratory cohort and in gray, *in silico* cohort.

Table 1. Clinical-demographic characteristics of T-ALL patients according to *CRLF2* and *IKZF1* expression

	CRLF2 n(%)			*IKZF1* n(%)		
	High	Low	*p*	High	Low	*p*
Age (Years)			0.446			0.166
>10	6 (75.00)	27 (55.10)		8 (80.00)	25 (53.19)	
10-21	2 (25.00)	22 (44.90)		2 (20.00)	22 (46.81)	
Sex			1.00			0.680
Female	2 (25.00)	11 (22.45)		3 (30.00)	10 (21.28)	
Male	6 (75.00)	38 (77.55)		7 (70.00)	37 (78.72)	
WBC (x10^{9}cels/L)			0.187			0.429
<50	4 (50.00)	11 (22.45)		4 (40.00)	11 (23.40)	
≥50	4 (50.00)	38 (77.55)		6 (60.00)	36 (76.59)	
Risk Classification (NCI)			0.004*			0.370
Standart	3 (37.50)	6 (12.24)		3 (30.00)	8 (17.02)	
High	5 (62.50)	43 (87.75)		7 (70.00)	41 (87.23)	
Molecular Characterization*						
NOTCH1/FBXW7 mut	2 (33.3)	16 (53.3)	0.658	2 (20.00)	16 (61.54)	0.059
STIL-TAL1+	1 (25.0)	8 (23.53)	0.616	2 (66.67)	7 (41.18)	0.566
TLX3 presence	0 (0)	3 (7.69)	1.000	1 (14.28)	2 (5.40)	0.436
IKZF1 alterations	0 (0)	1 (2.04)	1.000	0 (0)	1 (2.13)	1.000
CRLF2 alterations	NA	NA	-	NA	NA	-

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CRLF2 alterations	NA	NA	-	NA	NA	-

WBC, white cell count; NA, not analysed; CNA, copy number alterations; *IKZF1* alterations, *IKZF1* mutations or CNA. *The frequencies were calculated using the total number of analysed samples.

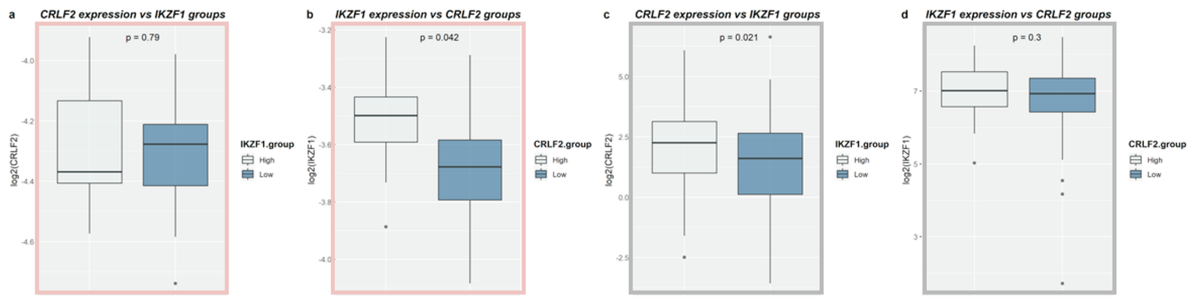

Figura 4. *IKZF1* expression (**a.** and **c.**) in *CRLF2* groups and *CRLF2* expression (**b.** and **d.**) in *IKZF1* groups.

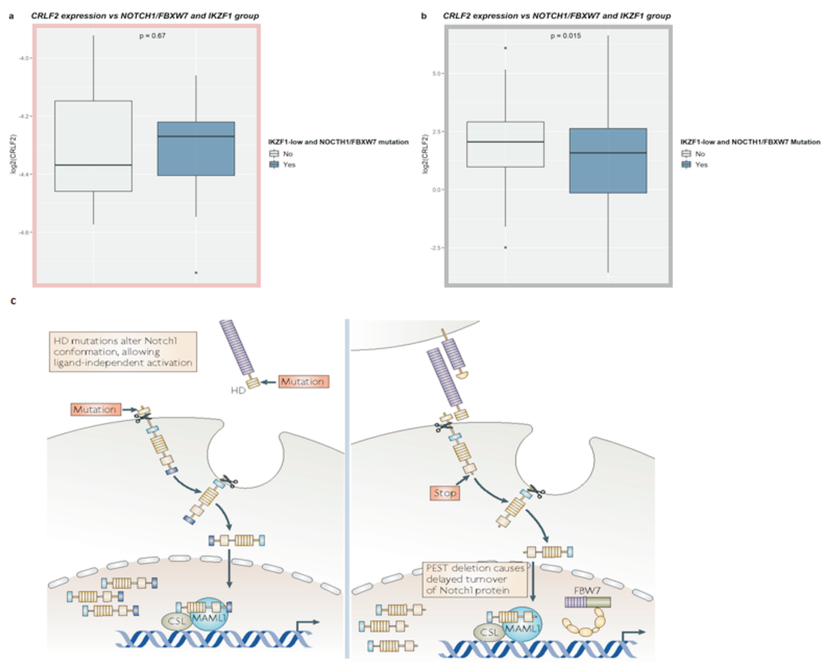

Figure 5. a. and b. CRLF2 expression in *NOTCH1/FBXW7* mutated and *IKZF1*-low group. c. Notch1 mutated (in PEST and HD domains) signalling pathway. Modified from Ainfants I et al, 2008.

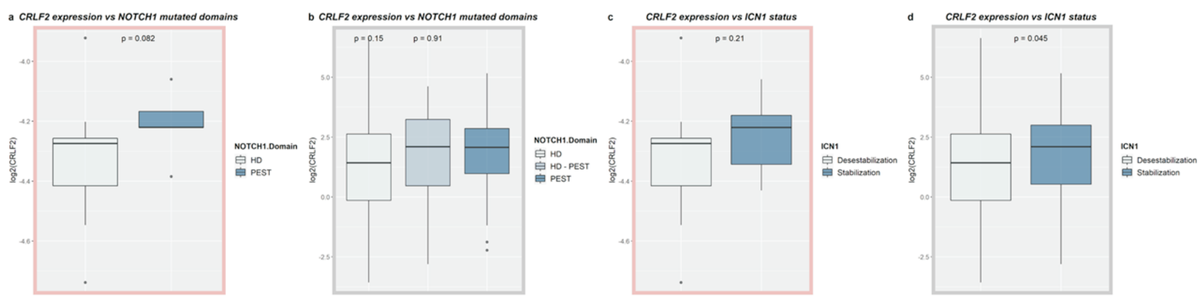

Figura 6. a and b. *CRLF2* expression and *NOTCH1* mutated domains. **c and d.** *CRLF2* expression and generating mutations of intracellular NOCTH1 stability and instability.

CONCLUSIONS

❖ Our data support the notion that aberrant IKZF1 low expression and NOTCH1/FBXW7 mutations, mainly those that lead to an increase the stabilization of ICN1, play a role in CRLF2 upregulation.

❖ However, it does not rule out that other mechanisms might also lead to this overexpression in those T-ALL cases.

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