Clinical and genetic characteristics of *CRLF2*-rearranged pediatric acute lymphoblastic leukemia

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Background Acute lymphoblastic leukemia (ALL) with *CRLF2* rearrangements (*CRLF2*r) is one of the most common subtype of *BCR::ABL1*-like ALL.

Objective To characterize pediatric ALL patients carrying *CRLF2*r.

Methods Nine hundred and fifty eight children with B-lineage ALL enrolled into the national multicenter ALL-MB 2015 trial were included in the current study. The median age was 4.2 years (range 1.02-17.9). All initial patients' samples were screened by flow cytometry (FC) using TSLP receptor monoclonal antibody (clone 1A6) and split-signal FISH. Positive by each method cases were further investigated by multiplex ligation-dependent probe amplification (MLPA) P335 and P327 kits (both MRC-Holland, Netherlands) and customized next-generation sequencing panels. Hybridization-based capture targeted RNA panel (Nanodigmbio, Singapore), that covered all coding regions and sites of splicing in 69 genes, previously described in in ALL and *BCR::ABL1*-like ALL, was applied. Informed consents of parents or legal guardians were obtained in all cases.

Results

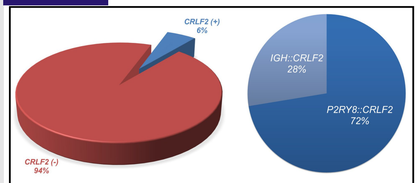


Figure 1. *CRLF2*r were detected in 58 pts (6.1%). Among them there were 42 *P2RY8::CRLF2*-positive (72%) and 16 *IGH::CRLF2*-positive cases (28%).

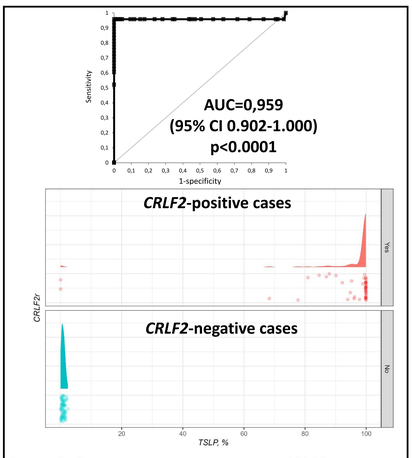


Figure 2. Overall correct prediction of *CRLF2r* by flow cytometry of TSLP receptor detection was 97.7% with only 2 false-negative cases. All cases where *CRLF2* gains were revealed (n=256) by MLPA or FISH (mainly high hyperdiploid pts) were correctly interpreted as *CRLF2*-negative by flow cytometry.

Table 1. Initial parameters and early response criteria in *CRLF2*r patients.

Parameters	All *CRLF2*-rearranged (n=58)	*P2RY8::CRLF2*-positive (n=42)	*IGH::CRLF2*-positive (n=16)	P
Male / Female, n (%)	24 (41.4) / 34 (58.6)	17 (40.1) / 25 (59.9)	7 (43.8) / 9 (56.2)	0.943
Median age (range), years	5.6 (1.3-17.6)	4.5 (1.3-16.0)	8.6 (1.4-17.6)	**0.001**
Down syndrome, n (%)	10 (17.2)	10 (23.8)	0 (0.0)	**0.048**
CNS disease, n (%)	8 (13.8)	5 (11.9)	3 (18.8)	0.686
Initial WBC count ≥50*10^9/L	21 (36.2)	8 (19.0)	13 (81.2)	**<0.001**
NCI-Rome high risk, n (%)	23 (39.7)	9 (21.4)	14 (87.5)	**<0.001**
≥1000 blast cells at day 8, n (%)	2 (3.4)	0 (0)	2 (12.5)	0.073
M2+M3 BM status at day 15, n (%)	8 (13.8)	6 (14.3)	2 (12.5)	0.328
Any EOI Flow-MRD-positivity, n (%)	20 of 47 (42.6)	14 of 35 (40.0)	6 of 12 (50.0)	0.736
Slow EOI Flow-MRD response, n (%)	14 of 47 (29.8)	8 of 35 (22.9)	6 of 12 (50.0)	0.141
IKZF1 deletion, n (%)	17 of 45 (37.7)	10 of 32 (31.2)	7 of 13 (53.8)	0.188
IKZF1^{plus} profile, n (%)	10 of 45 (22.2)	7 of 32 (21.8)	3 of 13 (23.1)	0.999
JAK1/JAK2 point mutations, n (%)	6 of 43 (14.0)	6 of 28 (21.4)	0 of 15 (0)	0.076
RAS point mutations, n (%)	10 of 43 (23.3)	6 of 28 (21.4)	4 of 15 (26.7)	0.268
PAX5 alterations, n (%)	8 of 43 (18.6)	6 of 28 (21.4)	2 of 15 (13.3)	0.272

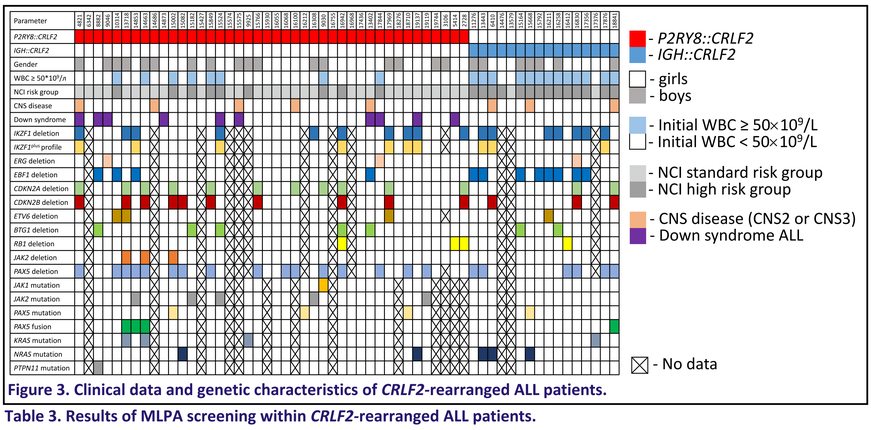


Figure 3. Clinical data and genetic characteristics of *CRLF2*-rearranged ALL patients.

Table 2. Revealed *PAX5* alterations.

***PAX5* fusions**	**Fusion exons**	**Additional mutations**
PAX5::CBFA2T3	ex5-ex7	*KRAS* G12D
PAX5::NOL4L	ex8-ex6	*KRAS* G12V
PAX5::ZCCHC7	ex2-ex2	*JAK2* R683G
PAX5::ZCCHC7	ex2-ex3	-

***PAX5* mutations**	**Tier AMP**	**Add. mut.**
c.911-2A>G (p.?)	III	
c.1012+2T>C (p.?)	III	
c.154T>C p.(Cys52Arg)	III	*NRAS* Q61H
c.833_841delAGGCCAATC insT (p.Lys278IlefsTer8)	III	

Table 3. Results of MLPA screening within *CRLF2*-rearranged ALL patients.

Parameters	All *CRLF2*-rearranged (n=46)	*P2RY8::CRLF2*-positive (n=33)	*IGH::CRLF2*-positive (n=13)	P
ERG deletion, n (%)	3 (6.5)	2 (6.0)	1 (7.7)	1
EBF1 deletion, n (%)	12 (26.1)*	4 (12.1)	8 (61.5)	**0.001**
IKZF1 deletion, n (%)	17 (37.7)*	10 (31.2)	7 (53.8)	0.188
JAK2 deletion, n (%)	3 (6.5)	3 (9.1)	0 (0.0)	0.547
CDKN2A deletion, n (%)	13 (28.2)	11 (33.3)	2 (15.4)	0.293
CDKN2B deletion, n (%)	13 (28.2)	10 (30.3)	3 (23.1)	0.729
PAX5 deletion, n (%)	22 (47.8)	16 (48.5)	6 (46.1)	0.998
ETV6 deletion, n (%)	4 (8.7)	3 (9.1)	1 (7.7)	0.974
BTG1 deletion, n (%)	7 (15.2)	5 (15.1)	2 (15.4)	0.999
RB1 deletion, n (%)	4 (8.7)	3 (9.1)	1 (7.7)	0.897
PAR1 deletion, n (%)	27 (58.7)	27 (81.8)	0 (0.0)	**0.001**
IKZF1^{plus}, n (%)	10 (22.2)*	7 (21.8)	3 (23.1)	0.982

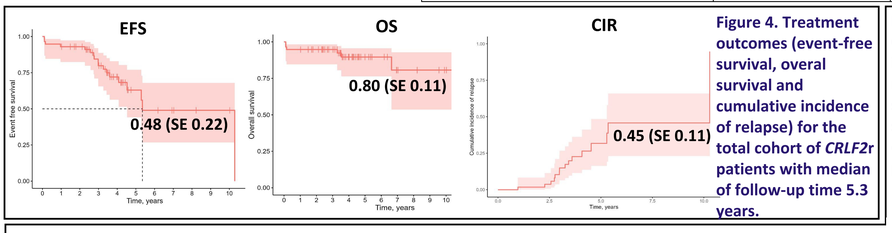
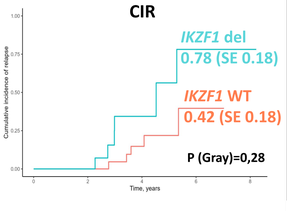


Figure 4. Treatment outcomes (event-free survival, overal survival and cumulative incidence of relapse) for the total cohort of *CRLF2*r patients with median of follow-up time 5.3 years.

Figure 5. Cumulative incidence of relapse in the selected subgroups of *CRLF2r* patients



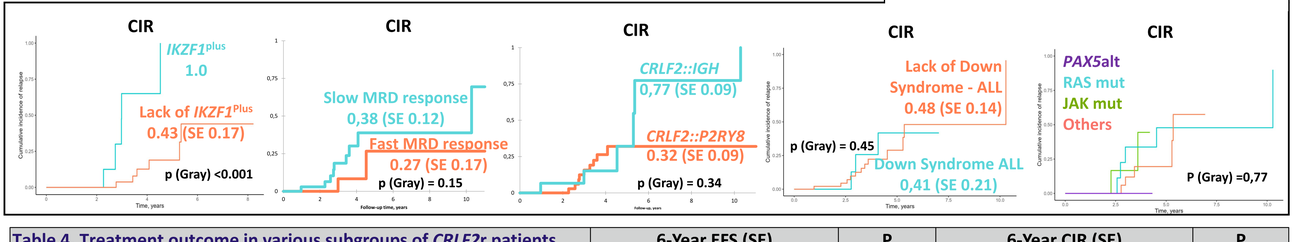


Table 4. Treatment outcome in various subgroups of *CRLF2*r patients	**6-Year EFS (SE)**	**P**	**6-Year CIR (SE)**	**P**
P2RY8::CRLF2(+) vs *IGH::CRLF2*(+)	0.64 (0.09) vs 0.21 (0.18)	0.35	0.32 (0.09) vs 0.77 (0.09)	0.34
Down syndrome: Yes vs No	0.48 (0.39) vs 0.47 (0.27)	0.54	0.41 (0.21) vs 0.48 (0.14)	0.45
Initial WBC count ≥50*10^9/L vs <50	0.18 (0.16) vs 0.44 (0.19)	0.56	0.81 (0.17) vs 0.53 (0.20)	0.52
NCI-Rome high risk vs standard risk	0.19 (0.16) vs 0.44 (0.19)	0.50	0.80 (0.17) vs 0.53 (0.20)	0.41
Any end-of-induction Flow-MRD-positivity: Yes vs No	0.56 (0.26) vs 0.79 (0.12)	0.64	0.43 (0.16) vs 0.20 (0.09)	0.63
End-of-induction Flow-MRD-response: Slow vs Fast	0.61 (0.12) vs 0.73 (0.18)	0.16	0.38 (0.12) vs 0.27 (0.17)	0.15
IKZF1 deletion: Yes vs No	0.22 (0.19) vs 0.53 (0.17)	0.22	0.78 (0.18) vs 0.42 (0.18)	0.07
IKZF1^{plus} profile: Yes vs No	0 vs 0.50 (0.16)	**0.003**	1.0 vs 0.47 (0.17)	**<0.001**
JAK mutation vs RAS mutation vs *PAX5*alterations @4 years	0.55 (0.24) / 0.42 (0,21) / 1.0	0.62	0.44 (0.10) / 0.47 (0.19) / 0,0	0.77

Conclusions The incidence of *CRLF2r* was 6.1% in our cohort. FC with TSLP receptor monoclonal antibody (clone 1A6) can efficiently predict the presence of *CRLF2r*. High genetic heterogeneity with relatively high rate of RAS mutation and PAX5alt was observed. All, except one, relapses occurred after the end of maintenance therapy and the majority of them were salvaged by HSCT in CR2. MRD did not distinguish patients with various treatment outcomes.

Questions? Comments? Please mail to: Grigory.Tsaur@gmail.com

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