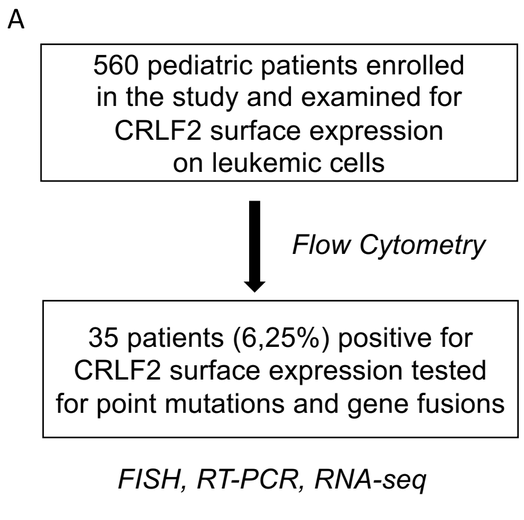
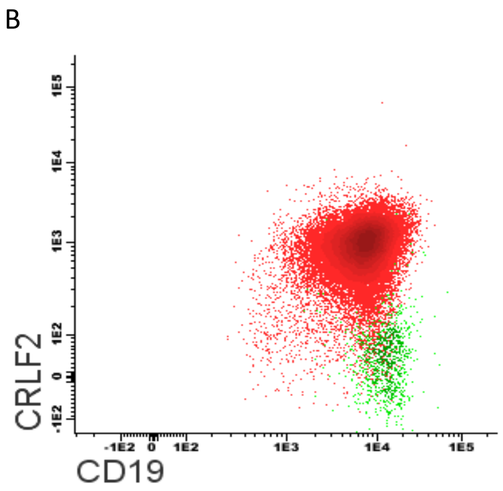
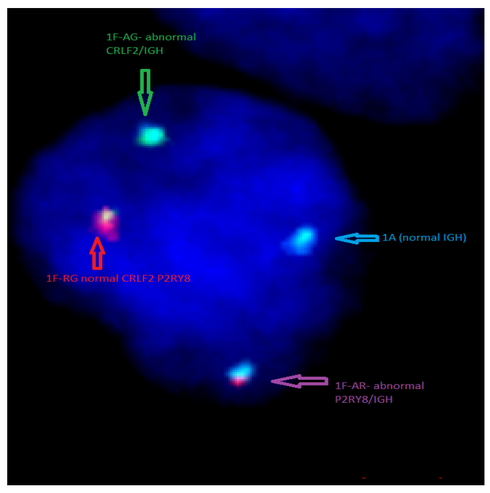
Julia Kołodrubiec, MD
Department of Pediatrics, Oncology, and Hematology, Medical University of Lodz

Activation of JAK-STAT pathway in childhood acute lymphoblastic leukemia – clinical and molecular aspects

Julia Kołodrubiec MD; Department of Pediatrics, Oncology, and Hematology, Medical University of Lodz, Lodz, Poland, julia.kolodrubiec@stud.umed.lodz.pl







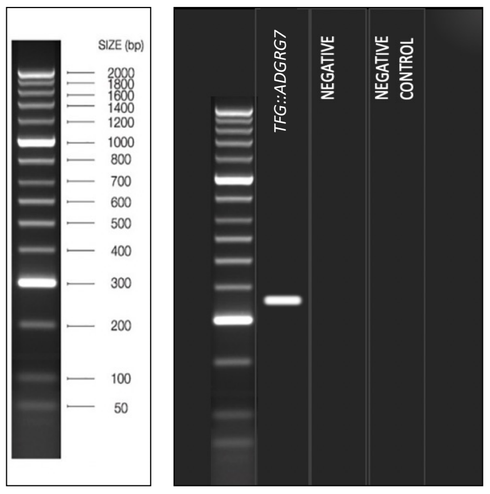


Table 1.

Nr	Age	Sex	CRLF2	Expression pattern	MRD TP1	MRD TP2	RG	JAK/STAT	Point mutation	Gene fusion	Other
1	2	F	99%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	*JAK2*	*CRLF2::P2RY8*	
2	3	M	11%	heterogenous low	< 5x10^{-4}	≤ 10^{-4}	MR	negative	-	-	*PAX5::CBFA2T3*
3	4	F	99%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	*JAK2*	*CRLF2::P2RY8*	
4	**17**	**F**	**100%**	**homogenous strong**	**≥ 5x10^{-4}**	**≤ 10^{-4}**	**HR**	**positive**	***CRLF2***	***CRLF2::IGH***	
5	2	M	91%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	-	*CRLF2::P2RY8*	*KMT2A::ZC3H7B*
6	10	M	100%	homogenous strong	≥ 5x10^{-4}	< 5x10^{-4}	HR	positive	*JAK2*	*CRLF2::P2RY8*	
7	2	F	15%	bimodal dim	< 5x10^{-4}	≤ 10^{-4}	MR	positive	-	*NUP214::ABL1*	*TFG::ADGRG7*
8	3	M	10%	heterogenous low	≤ 10^{-4}	≤ 10^{-4}	SR	negative	-	*TFG::ADGRG7*	
9	2	M	100%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	*JAK2*	*CRLF2::P2RY8*	
10	3	M	98%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	*CRLF2*	*CRLF2::P2RY8*	
11	**15**	**M**	**97%**	**homogenous strong**	**≥ 5x10^{-4}**	**< 5x10^{-4}**	**HR**	**positive**	***JAK2***	***CRLF2::IGH***	
12	5	M	100%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	-	*CRLF2::P2RY8*	
13	**5**	**F**	**100%**	**homogenous strong**	**≥ 5x10^{-4}**	**≤ 10^{-4}**	**MR**	**positive**	**-**	***CRLF2::P2RY8***	
14	11	F	24%	heterogenous low	inconclusive	inconclusive	HR	negative	-	*KMT2A::MLLT3*	
15	2	M	100%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	*JAK2*	*CRLF2::P2RY8*	
16	17	F	7%	heterogenous low	≤ 10^{-4}	≤ 10^{-4}	SR	negative	-	-	
17	2	F	98%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	*CRLF2*	*CRLF2::P2RY8*	
18	10	F	99%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	-	*CRLF2::P2RY8*	
19	4	M	99%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	*JAK2*	*CRLF2::IGH*	
20	**14**	**M**	**96%**	**homogenous strong**	**≥ 5x10^{-4}**	**≥ 5x10^{-4}**	**HR**	**positive**	**-**	***CRLF2::IGH***	
21	3	F	97%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	*JAK2*	*CRLF2::P2RY8*	
22	**3**	**F**	**99%**	**homogenous strong**	**≥ 5x10^{-4}**	**< 5x10^{-4}**	**HR**	**positive**	**-**	***CRLF2::P2RY8***	
23	3	M	99%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	*JAK2*	*CRLF2::P2RY8*	
24	**4**	**M**	**20%**	**heterogenous low**	**< 5x10^{-4}**	**≤ 10^{-4}**	**HR**	**positive**	**-**	***CRLF2::P2RY8***	
25	2	M	9%	heterogenous low	≤ 10^{-4}	≤ 10^{-4}	SR	negative	-	-	
26	4	F	99%	homogenous strong	< 5x10^{-4}	< 5x10^{-4}	MR	positive	-	*CRLF2::P2RY8*	
27	**3**	**F**	**99%**	**homogenous strong**	**≥ 5x10^{-4}**	**≤ 10^{-4}**	**MR**	**positive**	**-**	***CRLF2::P2RY8***	
28	12	M	81%	homogenous dim	≤ 10^{-4}	≤ 10^{-4}	SR	positive	-	*CRLF2::P2RY8*	
29	8	F	100%	homogenous strong	< 5x10^{-4}	-	-	positive	*JAK2*	*CRLF2::IGH*	
30	3	M	100%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	-	*CRLF2::P2RY8*	
31	2	F	100%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	*JAK2*	*CRLF2::P2RY8*	
32	2	F	100%	homogenous strong	≤ 10^{-4}	≤ 10^{-4}	SR	positive	-	*CRLF2::P2RY8*	
33	2	F	100%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	positive	-	*CRLF2::P2RY8*	
34	3	M	100%	homogenous strong	< 5x10^{-4}	≤ 10^{-4}	MR	ND	-	-	
35	14	F	18%	homogenous dim	≤ 10^{-4}	≤ 10^{-}	SR	negative	-	-	

Methods and molecular diagnostic

Figure 1. The diagram of the study group's selection strategy and diagnostic process for pediatric BCP-ALL patients (A). Scatter plot from multicolor flow cytometry analysis in a patient with the strong homogenous expression of CRLF2 surface protein (n=26; 91-100% positive); blasts (red) lymphocytes (green) (B). FISH analysis of the patient's diagnostic bone marrow aspirates showing *CRLF2::IGH* gene fusion activating JAK/STAT signaling pathway (Leica) (C). RT-PCR results of *TFG::ADGRG7* germline fusion diagnosed in two patients with dim expression of CRLF2 (D).

Results

Table.1 The study group consists of 35 patients with the expression of the CRLF2 protein on the leukemic cell's surface detected by FCM and gene fusions or mutations leading to JAK/STAT activation detected by FISH, SNP, and RNA-seq. Four patterns of CRLF2 expression were distinct, strong homogenous (n=26; 91-100% positive), homogenous low (n=2; 18-81% positive), bimodal dim (n=1; 15% positive), and heterogenous low (n=6; 7-24% positive). In 83% of patients (29/35), gene fusions as *CRLF2::P2RY8, CRLF2::IGH, TFG::ADGRG7, KMT2A::ZC3H,* and *NUP214::ABL1* were detected. The level of CRLF2 expression depends on the presence of *CRLF2::P2RY8*/ *CRLF::IGH*/ other fusion has statistical significance (p=0.0065) in the global test Kruskall- Wallis test. Point mutations in CRLF2 and JAK2 genes were present in 3 and 11 patients, respectively. In the case of 14 patients, more than one rearrangement involved in the JAK/STAT signaling pathway was present. In 8 patients the level of CRLF2 expression was lower than 25% and no lesions responsible for this phenomenon were observed. Patients were classified into standard (SR 38%), medium (MR 41%), and high (HR 21%), risk groups (RG). One patient passed away before the final stratification. The criteria of the Rux-cALL-Pol 2020 clinical trial were fulfilled by 7 patients who received targeted therapy with JAK1/JAK2 inhibitor, ruxolitinib (highlighted).

Conclusions

In the Polish pediatric population with ALL, around 6.25 % of patients comprise children with positive expression of CRLF2 and JAK/STAT molecular signature.
In CRLF2-positive patients, the most frequent mutations were JAK2 and CRLF2.
Patients with *CRLF2::IGH* fusion were stratified more often into the high-risk group (60%), and the median age of this group was 14 years.
Patients with CRLF2 expression should be subjected to advanced molecular diagnostics as they comprise a heterogeneous molecular subgroup.
In patients with poor response to the standard treatment and kinase-activating aberrations, targeted therapy (ruxolitinib) may be considered.

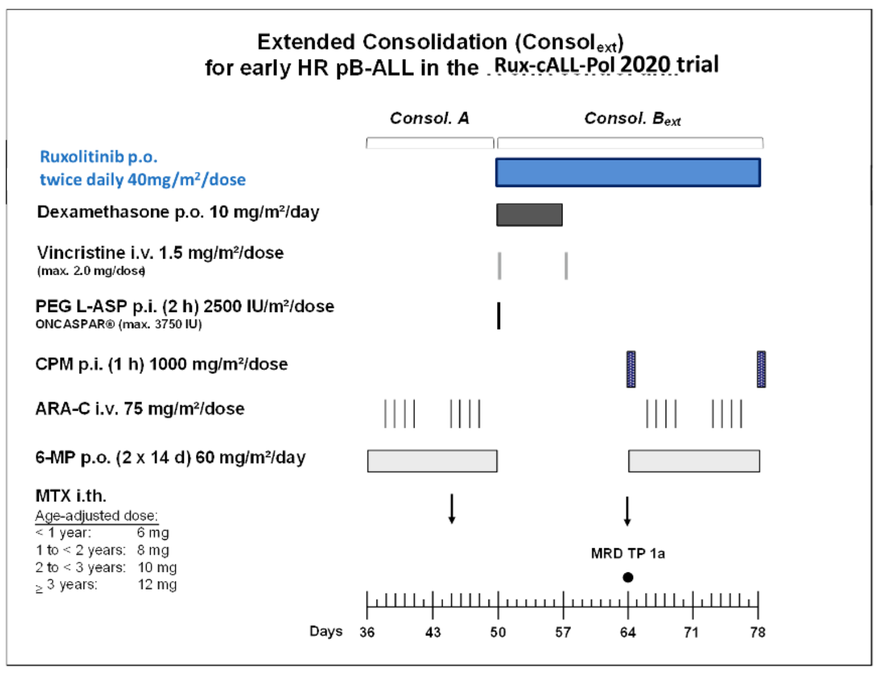


Figure 2. Single-arm interventional study with Ruxolitinib combined with AIOEP-BFM 2017 Poland therapy.