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

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Introduction: Philadelphia-like acute lymphoblastic leukemia (Ph-like ALL) is one of the high-risk subtypes with molecular alterations leading to abnormal JAK/STAT pathway activation. The most common causes of this phenomenon are gene fusions, point mutations, and the expression of the CRLF2 receptor.

Methods: Patients treated according to AIEOP BFM 2017 Protocol (2018- 2021) and CALL-POL clinical trial (since 2021) were analyzed. We distinguished patients (n=52) with the expression of the CRLF2 protein on the leukemic cell's surface detected by flow cytometry (FC). Expression of protein receptor CRLF2, in case of results < 10% was assessed as negative, 10-50% as a low (dim) expression, and >50% as a high expression, respectively. Furthermore, the complex molecular diagnostic by FISH, SNP, and RNA-seq is planned to detect gene fusions and point mutations leading to JAK/STAT activation (in the frame of the STRATEGMED3/304586/5/NCBR/2017 and CALL-POL 2019/ABM/01/00069-00 projects). Patients from the high-risk group with the molecular signature of JAK/STAT activation will be treated according to Rux-cALL-Pol 2020 clinical trial.

Results: From the study group of 52 patients, in 45 (86,5%) the expression of CRLF2 was found. In 34 (65,4%) patients the expression of CRLF2 was assessed as high and in 11 (21,1%), as low. In the 6 (11,5%) remaining cases, the expression of CRLF2 was negative. The analysis wasn't performed in one patient because of a lack of material. In 27 patients, in whom the complex molecular analysis was performed the gene fusions CRLF2- P2RY8; CRLF2- IGH; SSBP2-JAK2; PAX5-JAK2; NUP214-ABL1 and point mutations in JAK2; JAK1; CRLF2 were detected. The criteria of the Rux-cALL-Pol 2020 clinical trial were fulfilled by 5 patients. After 4 weeks of combined therapy, two patients achieved molecular remission (MRD TP1 >= 5x10-4 vs TP2 < 10-4). The other 3, are currently in the experimental phase.

Conclusion: Patients lacking the expression of CRLF2 protein on the leukemic cell surface in whom gene fusion with CRLF2 was found, compounding the interesting group. In this group of patients, a further diagnostic is indicated to determine the underlying causes of this phenomenon. Ruxolitinib can be beneficial in patients with the molecular signature of the JAK/STAT pathway, further recruitment of patients is needed.

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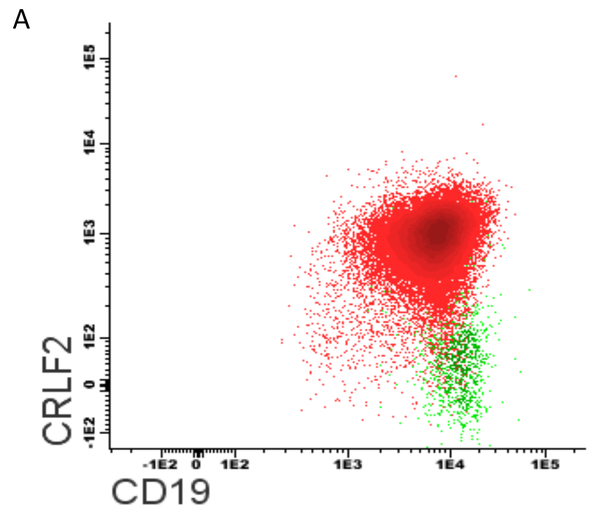

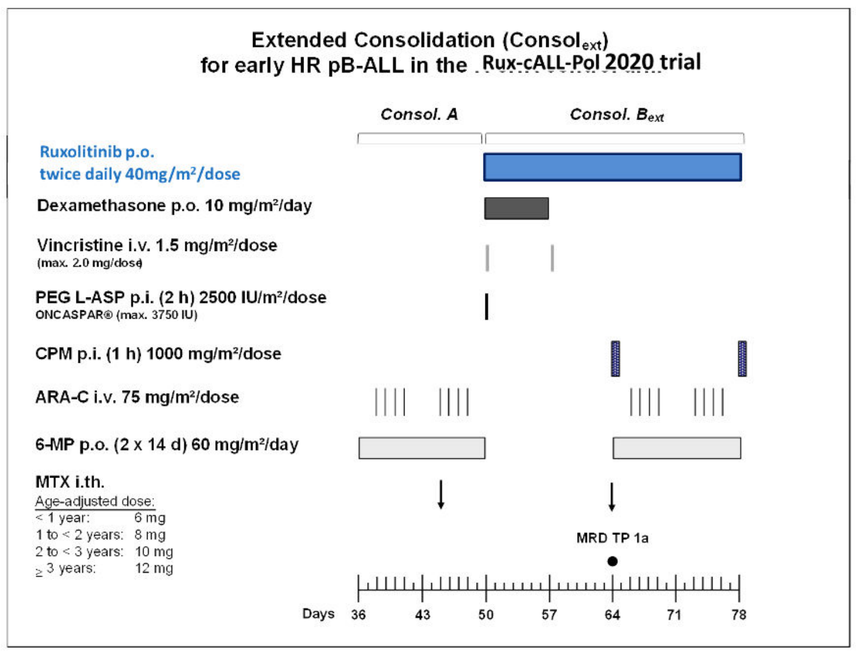

Figure1. Scatter plot from FC analysis; blasts (red) lymphocytes (green) (A). Risk group stratification; HR high risk; MR medium risk; SR standard risk (B). Single-arm interventional study with Ruxolitinib combined with AIOEP-BFM 2017 Poland therapy (C).

Ruxolitinib for childhood ALL in Poland (Rux-cALL-Pol 2020 trial)

Nr	Age	Sex	Initial WBC [1/µl]	Actual Risk Group	FC-MRD D15	CRLF2 [%]	TP1	TP2	IKZF1 deletion	JAK/STAT	Point Mutations	Gene fusions
1	17	F	549280	HR	≥10%	100.00	≥ $5x10^{-4}$	≤ 10^{-4}	negative	positive	CRLF2	CRLF2- IGH
2	15	M	107570	HR	0,1-9,999%	97.00	≥ $5x10^{-4}$	< $5x10^{-4}$	positive	positive	JAK2	CRLF2- IGH
3	5	F	13470	MR	0,1-9,999%	100.00	≥ $5x10^{-4}$	≤ 10^{-4}	negative	positive	-	CRLF2-P2RY8
4	14	M	120860	HR	≥10%	96.00	≥ $5x10^{-4}$	ND	positive	positive	-	CRLF2- IGH
5	3	F	24820	HR	≥10%	99.0	≥ $5x10^{-4}$	ND	negative	positive	-	CRLF2-P2RY8

Table 1. Clinical and molecular evaluation of patients from Rux-cALL-Pol 2020 clinical trial.