Article type: Short Research article

EXPRESSION OF CD123 IN B-ACUTE LYMPHOBLASTIC LEUKEMIA AS A PREDICTOR OF BCR/ABL REARRANGEMENT AND DISEASE RELAPSE

ABSTRACT

Introduction: CD123 is the alpha chain of the interleukin 3 receptor (IL-3R) andis normally expressed on hematopoietic progenitor cells, monocytes, B lymphocytes and endothelial cells. Leukemic stem cells can be detected using CD123, and its usefulness for measuring residual disease and potential involvement in disease relapse is being evaluated. It also regulates the growth, proliferation, survival, and differentiation of hematopoietic cells, along with immunity and inflammatory response.
Materials and Methods: Bone marrow or peripheral blood from 50 B-Acute Lymphoblastic Leukemia (B-ALL) patients were enrolled in the study. CD123 expression was studied by flow cytometry technique and correlated with clinical and hematological parameters as well as BCR-ABL status, MRD status and disease status.
Results: CD123 expression was found positive in 38% ofpatients. No significant correlation of CD123 expression with clinical and hematological parameters was observed. A significantly higher incidence of CD123 expression was noted in patients with BCR-ABL fusion (70%), relapse patients (67%) and MRD patients (67%).
Conclusion: CD123 can be used to predict BCR-ABL status in B-ALL patients and it has potential role to recognize high risk of relapse and helps to scrutinize high risk B-ALL patients who benefited with aggressive chemotherapy. Further, higher expression of CD123 in MRD patients can be used to evaluate minimal residual disease in follow-up B-ALL patients.

Keywords: B-Acute Lymphoblastic Leukemia, Flow cytometry, CD123

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disease marked by the clonal growth of leukemic cells in the bone marrow (BM), lymph nodes, thymus, and spleen. This is a diverse disease classified into multiple subtypes [1]. Acute lymphoblastic leukemia (ALL), which accounts for 75% of all leukemias in children under the age of 15, affects persons of all ages but is the most prevalent disease in youngsters. Among adults, it is more common in people older than 45. B acute lymphoblastic leukemia/lymphoma (B-ALL) is a hematologic malignancy derived from B-cell progenitors [2].

CD123 is the alpha chain of the interleukin 3 receptor (IL-3R) and isnormally expressed on hematopoietic progenitor cells, monocytes, B lymphocytes and endothelial cells. Several hematologic neoplasms, including B-ALL, express CD123, but normal

hematopoietic stem cells either express it less or don't express it at all [3]. Importantly, it has been reported that leukemic stem cells and more differentiated leukemic blast cells both express CD123 positively [4, 5]. Leukemic stem cells can be detected using CD123, and its usefulness for measuring residual disease and potential involvement in disease relapse is being evaluated [6]. It also regulates the growth, proliferation, survival, and differentiation of hematopoietic cells, along with immunity and inflammatory response [7,8]. This study aimed to assess the pattern of CD123 expression in B-ALL patients.

2. MATERIAL AND METHODS

2.1 PATIENT CHARACTERISTICS

In this prospective study, 50 B-cell Acute Lymphoblastic Leukemia (B-ALL) patient samples were collected at The Gujarat Cancer & Research Institute (G.C.R.I.) from of January 2023 to April 2023. First differential counts at diagnosis and clinicopathological data such as age, gender, karyotype and bone marrow were recorded from available hospital records files maintained at the Institutional Medical Record Department. Patients provided general consent to use their sample for the study. This study was approved by the Institutional Scientific Review Board and Ethics Committee.

2.2 SAMPLE COLLECTION

Bone marrow or peripheral blood samples of 50 patients (25 newly diagnosed + 25 follow-up patients) were collected in Ethylenediamine Tetra Acetic Acid (EDTA) vacuette.

2.3 SAMPLE PREPARATION

100 µl BM/PB samples were stained with 5 µl CD45 (V500c) and 10 µl CD123 (FITC) monoclonal antibodies, then RBCs were lysed with 2 ml RBC lysing solution (1:10 Dilution), further, washed with 2 ml Phosphate Buffer Saline (PBS) and then resuspended with 500 µl PBS.The stained samples wereanalyzed in Flow Cytometer within 72 hours.

2.4 SAMPLE ACQUISITION

Sample acquisition is done in BDFACSCanto Ⅱ Flow Cytometerusing FACSCDiva software and total 1,00,000 cells or events were acquired for each sample.

2.5 DATA ANALYSIS

CD123 positive cells werecalculatedondim CD45 blast population. The percentage of each positive population is noted from the “population hierarchy” table. Histogram is plotted and gated to find the CD123 MFI (median fluorescence intensity) value of leukemic blasts.

2.6 STATISTICAL ANALYSIS

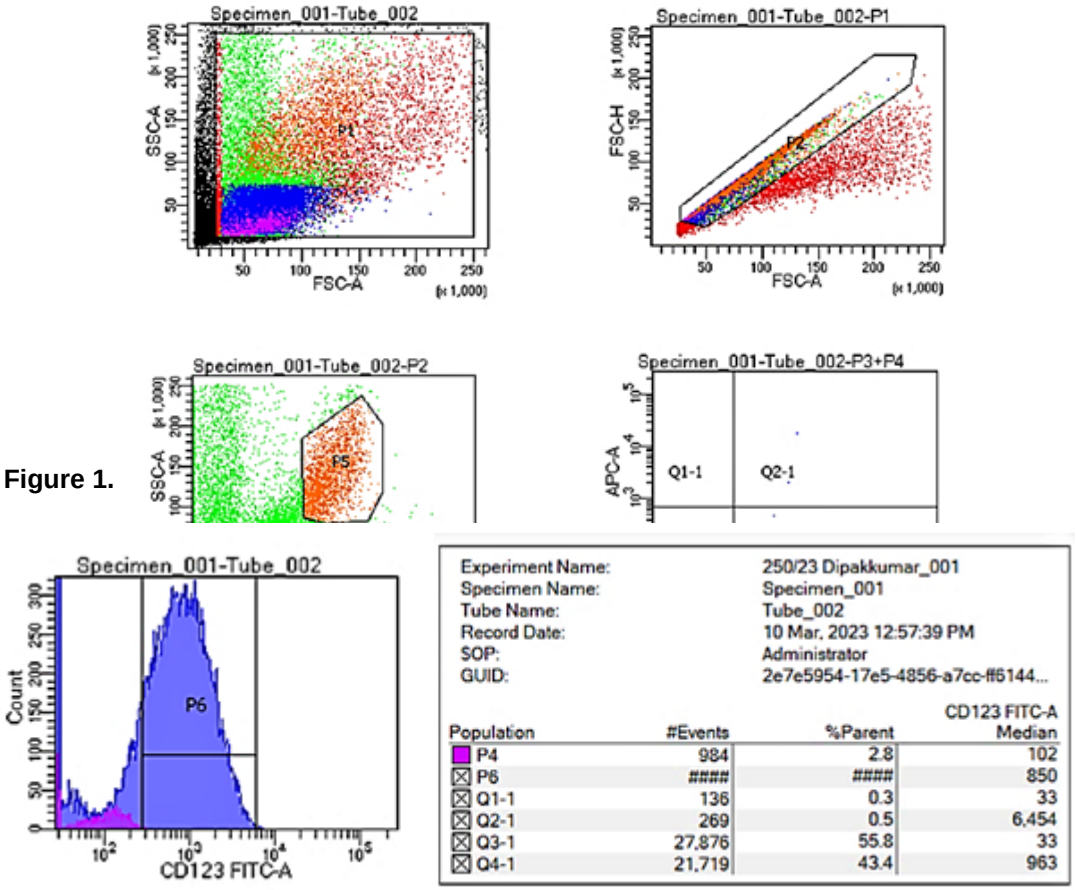
Statistical analysis was carried out using SPSS statistical software version 27. Receiver operating characteristic (ROC) curve was generated to know the sensitivity and specificity of the marker. Pearson’s chi-square test with Pearson’s correlation coefficient (r) was used to assess correlation and significance between two parameters. p values ≤ 0.05 were considered to be statistically significant.

3. RESULTS

Out of 50 B-ALL patients, CD123 expression was found in 19(38%) of all B Acute Lymphoblastic Leukemia patientswhereas 31(62%) of patients did not express CD123 (Figures. 1 & 2). The expression was further correlated with clinical and hematological parametersusing the median as cut off. In correlation with clinical parameters, a trend of higher incidence of CD123 expression was found in male, absence of hepatomegaly and lymphadenopathy as compared to their counterparts. In correlation with hematological

parameters, a trend of higher incidence of CD123 expression was found in B-ALL patients with high blast cells, high lymphocytes and high platelets as compared to their counterparts. No significant correlation of CD123 expression with other clinical and hematological parameters was observed (Table.1).

Figure 1.



Expression of CD123 in B-ALL

Figure 2. Median fluorescence intensity of CD123 in B-lymphoblasts

Table 1: correlation of CD123 expression with clinical and hematological parameters

Parameters	**Total Patients**	**CD123 Negative**	**CD123 Positive**	**□²**	**R**	***p***

	N (%)	N (%)	N (%)			
Age	**50(100)**	**31(62)**	**19(38)**			
Adult	25(50)	15(60)	10(40)	0.08	0.04	0.77
Pediatric	25(50)	16(64)	9(36)			
Gender	**50(100)**	**31(62)**	**19(38)**			
Male	32(64)	18(56)	14(44)	1.24	0.16	0.26
Female	18(36)	13(72)	5(28)			
Splenomegaly	**40(100)**	**24(60)**	**16(40)**			
Positive	21(52)	13(62)	8(38)	0.07	-0.04	0.79
Negative	19(48)	11(58)	8(42)			
Hepatomegaly	**40(100)**	**24(60)**	**16(40)**			
Positive	23(58)	16(70)	7(30)	2.06	-0.23	0.15
Negative	17(42)	8(47)	9(53)			
Lymphadenopathy	**21(100)**	**10(48)**	**11(52)**			
Positive	5(24)	3(60)	2(40)	0.40	-0.14	0.52
Negative	16(76)	7(44)	9(56)			
Hemoglobin (gm/dl)	**50(100)**	**31(62)**	**19(38)**			
>8.9	23(46)	15(65)	8(35)	0.19	-0.06	0.66
≤8.9	27(54)	16(59)	11(41)			
RBC (cells/µl)	**50(100)**	**31(62)**	**19(38)**			
$>3.27^{x}10^{6}$	24(48)	14(58)	10(42)	0.26	0.07	0.61
$\leq 3.27^{x}10^{6}$	26(52)	17(65)	9(35)			
WBC (cells/µl)	**50(100)**	**31(62)**	**19(38)**			
$>7.295^{x}10^{3}$	25(50)	16(64)	9(36)	0.08	-0.04	0.77
$\leq 7.295^{x}10^{3}$	25(50)	15(60)	10(40)			
Platelets (cells/µl)	**50(100)**	**31(62)**	**19(38)**			
>34x103	24(48)	13(54)	11(46)	1.20	0.15	0.27
≤34x103	26(52)	18(69)	8(31)			
Lymphocytes (%)	**50(100)**	**31(62)**	**19(38)**			
>30	24(48)	18(75)	6(25)	3.31	-0.26	0.07
≤30	26(52)	13(50)	13(50)			
Polymorphs (%)	**49(100)**	**30(61)**	**19(39)**			
>25	23(47)	14(61)	9(39)	0.002	0.007	0.96
≤25	26(53)	16(61)	10(39)			
Blast cells (%)	**31(100)**	**22(71)**	**9(29)**			
>52	15(48)	9(60)	6(40)	1.70	0.23	0.19
≤52	16(52)	13(81)	3(19)			

(□ ² = Chi-square; R = Pearson's correlation coefficient; *p* = p-value)

Table 2: correlation of CD123 expression with BCR-ABL status

	CD123 Expression (N%)	□²	R	*p*

	Positive N (%)	Negative N (%)			
BCR-ABL Status N (%)					
Positive	7(70)	3(30)	5.68	0.35	0.01
Negative	10(29)	25(71)			
Total	17(38)	28(62)			
MRD Status N (%)					
Positive	6(67)	3(33)	7.21	0.60	0.007
Negative	1(9)	10(91)			
Total	7(35)	13(65)			
Disease Status N (%)					
Relapse	4(67)	2(33)	3.75	0.35	0.05
Remission	6(25)	18(75)			
Total	10(33)	20(67)			

(□ ² = Chi-square; R = Pearson's correlation coefficient; *p* = p-value)

3.1 CD123 expression in relation to BCR-ABL status

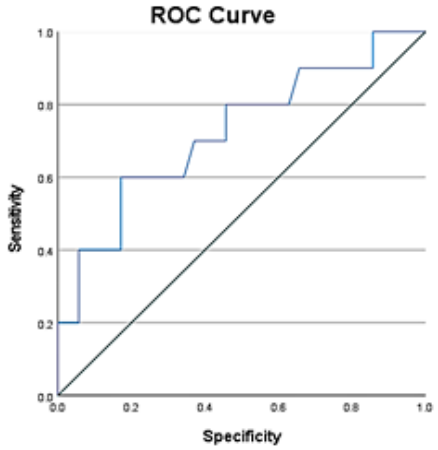


Figure 3. ROC Curve showing CD123 expression with BCR-ABL

Correlation of CD123 expression was evaluated with BCR-ABL status as shown in Table 2. BCR-ABL fusion was detected in 10 out of 45 B-ALL patients. A higher incidence of CD123expression (7/10, 70%) was seen in BCR-ABL positive group (*p = 0.01*) as compared to low expression of CD123 expression (3/10, 30%). (Table.2). ROC curve analysis (AUC 0.723) suggests patients with CD123 MFI more than 130 have a higher chance of having BCR-ABL fusion genes (Figure.3).

3.2 CD123 expression in relation to MRD status

Correlation of CD123 expression was evaluated with MRD status as shown in Table 2. MRD Status of 20 patients was evaluated of which in 9 B-ALL patients shoed residual disease cells. In correlation with MRD status, higher incidence of CD123 positive expression was found in MRD positive group (67%, 6/9) (*p* = 0.007) as compared to MRD negative group (9%, 1/11) (*p* = 0.007) (Table.2) (Figure 4).

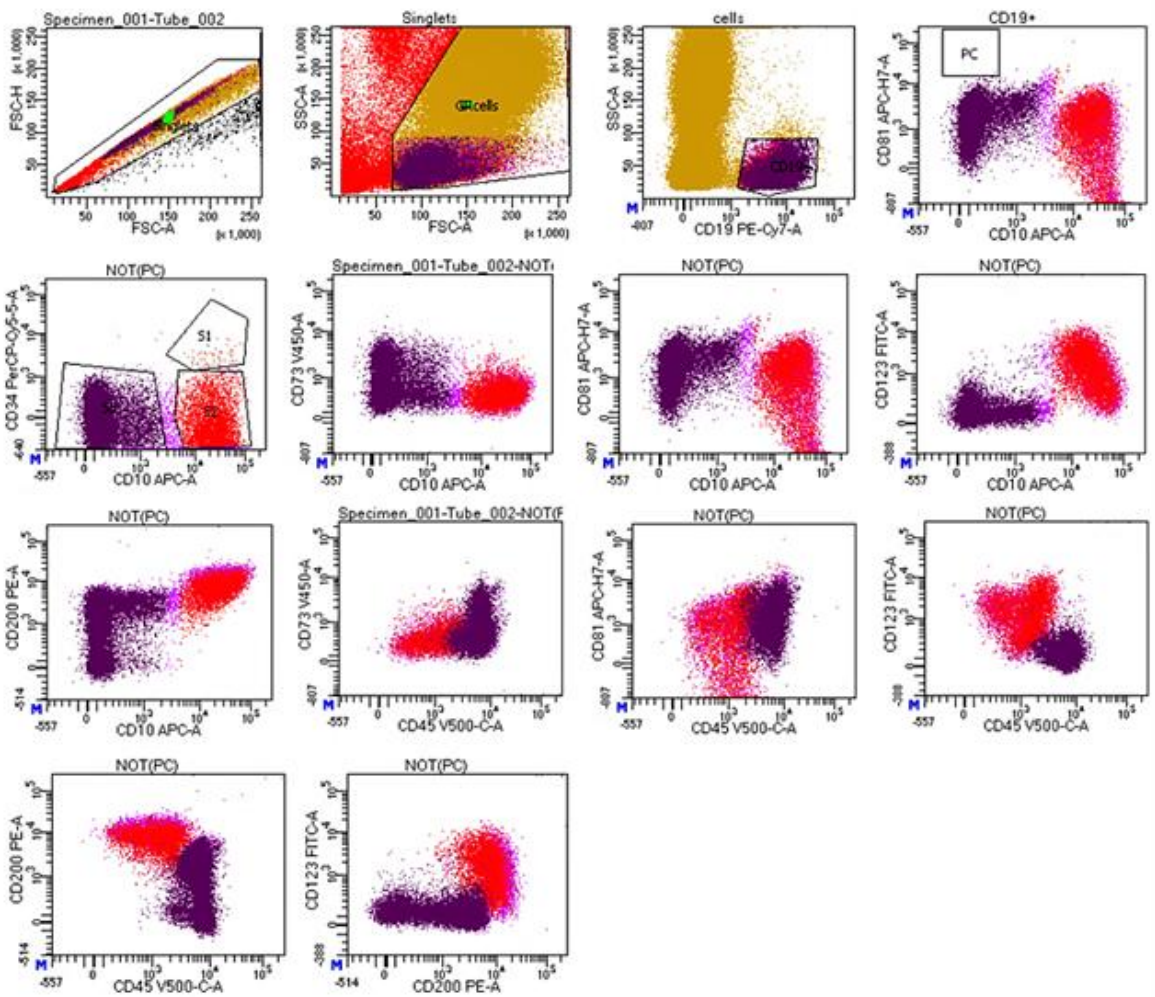


Figure 4. Aberrant CD123 expression in B-ALL useful in MRD detection along with other markers

3.3 CD123 expression in relation to Disease status

Disease status of 30 patients was available out of which 20% (6/30) showed disease relapse and 80% (24/30) showed disease remission. CD123 expression was found positive in 33% (10/30) with 4 relapse patients and 6 remission patients. (p = 0.05). CD123 expression was found negative in 67% (20) with 2 relapse patients and 18 remission patients (p = 0.05) (Table.2). ROC curve analysis (AUC 0.820) suggests patients with CD123 MFI more than 108 have higher chance of having disease relapse (Figure.5)

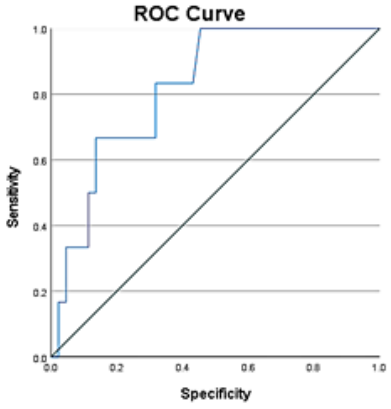


Figure 5. ROC curve showing CD123 expression with disease status

4. DISCUSSION

A clonal hematologic malignancy - acute lymphoblastic leukemia (ALL) develops from T- or B-lymphoid progenitor cells. In the present study, CD123 expression was found in 38% (19/50) of the studied B-ALL patients. This incidence is lower than that reported by Angelova E et al [3] (164/183, 89.6%); Djokic M et al [9] (98/119, 82%), Hassanein NM et al [10] (45/50, 89%); Bras AE et al. [11], who detected CD123 expression in 85% of B-cell precursor (BCP) ALL cases (224/ 262). In the present study, out of 50 B-ALL patients, CD123 expression was found in 44% male (14/32) and 28% female (5/18) patients. The study by Li Z et al. [12] has showed that out of 328 pediatric B-ALL patients, CD123 expression was found in 55% (105/190) male and 51% female (70/138) patients. In the current study, an expression of CD123 was not significantly correlated with hematological parameters such as hemoglobin, RBC, WBC, platelets, lymphocytes, polymorphs, and blast cells. Similarly, in the study by Aref S et al. [13], no significant correlation of CD123 expression was observed with hemoglobin, WBC, platelets, and blast cells count.

3-5% of children and 20-30% of adults have Philadelphia-positive (Ph+) B-ALL, and the frequency rises to roughly 50% in people over the age of 50. In the present study, thecorrelation of CD123 expression was evaluated with BCR-ABL fusion status. There was a strong association between CD123 positive expression and theBCR-ABL positive group (p = 0.017). BCR-ABL fusion was detected in 22% (10/45) of B-ALL cases, with 6% in children and 16% in adults. This finding is lower than that reported by Gadhia P et al. [14] (33.3%),Aref S et al. [13] (30%) and higher than that reported by Owaidah TM et al. [15] (17.5% (18/103).

In the present study, follow-up samples of 20 B-ALL patients were evaluated, and 9 patients showed minimal residual disease (MRD). Out of these 9 patients, 6 (67%) showed CD123 expression; this incidence is nearly similar to the study by Li Z et al. [12] (61.3%). Das N et al [6] reported that the presence of CD123 expression at baseline was substantially more frequently related to MRD-positive status (p< 0.001 for 10% or 20% and p = 0.005% for 5% of blasts expressing CD123).

In the present study, thedisease status of 30 B-ALL patients was evaluated and out of these, 6 patients showed relapse, and 24 patients showed remission. Out of 6 relapse patients, CD123 expression was seen in 4 (66.6%) relapse patients and out of 24 remission patients, CD123 expression was seen in 6 (25%) remission patients. In contrast to this finding, Li Z et al [12] reported that expression of CD123 was seen in 39.3%of relapse patients and 53.3% ofremission patients. This difference may be due to the fact that they were enrolled only pediatric B-ALL patients in their study. Thus, this study demonstrated that high expression of CD123 was correlated with BCR-ABL positivity, MRD positivity, and disease relapse.

5. Summary

CD123 can be used to predict BCR-ABL status in B-ALL patients, and it has the potential to recognize high-risk of relapse and help to scrutinize high-risk B-ALL patients who benefited from aggressive chemotherapy. Further, higher expression of CD123 in MRD patients can be used to evaluate minimal residual disease in follow-up B-ALL patients. However, inclusion of more number of patients is required for further conclusion.

CONSENT

Written informed consent was obtained from the patients and explained to them in their own language.

ETHICAL APPROVAL

The study was approved by the Institutional Scientific Review Board and Ethics Committee.

CONFLICT OF INTEREST

Authors have declared that no conflicts of interest exist.

REFERENCES

1) Faderl S, Jeha S, Kantarjian HM. The biology and therapy of adult acute lymphoblastic leukemia. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2003 Oct 1;98(7):1337-54.

2) Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. Swerdlow SH, editor. Lyon: International agency for research on cancer; 2008 Sep 20.

3) Angelova E, Audette C, Kovtun Y, Daver N, Wang SA, Pierce S, Konoplev SN, Khogeer H, Jorgensen JL, Konopleva M, Zweidler-McKay PA. CD123 expression patterns and selective targeting with a CD123-targeted antibody-drug conjugate (IMGN632) in acute lymphoblastic leukemia. Haematologica. 2019 Apr;104(4):749.

4) U. Testa, E. Pelosi, and G. Castelli, “CD123 as a therapeutic target in the treatment of hematological malignancies,” Cancers, vol. 11, no. 9, p. 1358, 2019.

5) Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, Klichinsky M, Aikawa V, Nazimuddin F, Kozlowski M, Scholler J. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. The Journal of clinical investigation. 2016 Oct 3;126(10):3814-26.

6) Das N, Gupta R, Gupta SK, Bakhshi S, Malhotra A, Rai S, Singh S, Prajapati VK, Sahoo RK, Gogia A, Sharma A. A real-world perspective of CD123 expression in acute leukemia as promising biomarker to predict treatment outcome in B-ALL and AML. Clinical Lymphoma Myeloma and Leukemia. 2020 Oct 1;20(10):e673-84.

7) Broughton SE, Dhagat U, Hercus TR, Nero TL, Grimbaldeston MA, Bonder CS, Lopez AF, Parker MW. The GM–CSF/IL-3/IL-5 cytokine receptor family: from ligand recognition to initiation of signaling. Immunological reviews. 2012 Nov;250(1):277-302.

8) Guthridge MA, Stomski FC, Thomas D, Woodcock JM, Bagley CJ, Berndt MC, Lopez AF. Mechanism of activation of the GM-CSF, IL-3, and IL-5 family of receptors. Stem cells. 1998 Sep;16(5):301-13.

9) Djokic M, Björklund E, Blennow E, Mazur J, Söderhäll S, Porwit A. Overexpression of CD123 correlates with the hyperdiploid genotype in

acute lymphoblastic leukemia. haematologica. 2009 Jul;94(7):1016.

10) Hassanein NM, Alcancia F, Perkinson KR, Buckley PJ, Lagoo AS. Distinct expression patterns of CD123 and CD34 on normal bone marrow B-cell precursors (“hematogones”) and B lymphoblastic leukemia blasts. American journal of clinical pathology. 2009 Oct 1;132(4):573-80.

11) Bras AE, de Haas V, van Stigt A, Jongen-Lavrencic M, Beverloo HB, TeMarvelde JG, Zwaan CM, van Dongen JJ, Leusen JH, van der Velden VH. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. Cytometry Part B: Clinical Cytometry. 2019 Mar;96(2):134-42.

12) Li Z, Chu X, Gao L, Ling J, Xiao P, Lu J, Wang Y, He H, Li J, Hu Y, Li J. Highexpression of interleukin-3 receptor alpha chain (CD123) predicts favorable outcome in pediatric B-cell acute lymphoblastic leukemia lacking prognosis-defining genomic aberrations. Frontiers in Oncology. 2021 Mar 16;11:614420.

13) Aref S, El Agdar M, Khaled N, Ibrahim L, El-Ghonemy MS. Clinical Impact of CD25/CD123 Coexpression in Adult B-Cell Acute Lymphoblastic Leukemia Patients. Advances in Hematology. 2020 May 20;2020.

14) Gadhia P, Parek N, Chavda P, Bhatia G, Vaniawala S. Cytogenetic findings of patients with acute lymphoblastic leukemia in west Indian region. International Journal of Advanced Research. 2018;6(2):2320-407.

15) Owaidah TM, Rawas FI, Elkum NB. Expression of CD66c and CD25 in acute lymphoblastic leukemia as a predictor of the presence of BCR/ABL rearrangement.
Hematology/Oncology and Stem Cell Therapy. 2008 Jan 1;1(1):34-7.