GMR
The Original

PROGNOSTIC IMPACT OF CD244 EXPRESSION IN PATIENTS WITH ACUTE LEUKEMIA

Mai Shalaby[1], Doaa A. Aladle[2], ElMenshawy N.[3], Shaimaa El-Ashwah[4], Omnia Khaled[5]

[1]Assistant lecturer, Haematology Unit, Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. maishalaby@mans.edu.eg , https://orcid.org/0000-0002-6509-8868
[2]Professor, Haematology Unit, Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. profdoaa@mans.edu.eg , https://orcid.org/0000-0003-0908-0118
[3]Professor, Haematology Unit, Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. n_elmenshaway@mans.edu.eg , https://orcid.org/0000-0002-6558-2131
[4]Assistant professor, Haematology Unit, Internal Medicine Department, Faculty of Medicine, Oncology center, Mansoura University, Mansoura, Egypt. Shaimaasaber@mans.edu.eg , https://orcid.org/0000-0003-2210-1534
[5]Lecturer, Haematology Unit, Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. omniakhaled@mans.edu.eg , Ohttps://orcid.org/0000-0002-6959-106X

ABSTRACT

Background: Acute leukemia is a heterogeneous clonal hematological malignancy. Despite advances in diagnosis, prognosis, risk stratification, and treatment, cure rate remains modest, and relapse-related mortality remains high. CD244 has been implicated in the maintenance of stemness of leukemia initiating cells, but not in hematopoietic stem cells, suggesting its potential as a novel therapeutic target against leukemia initiating cells for cure of acute leukemia (AL).

Methods: This prospective longitudinal study included assessment of CD244 expression using multiparameter flow cytometry in 141 newly diagnosed acute leukemia patients (85 AML and 56 ALL) and sixteen age and sex matched controls. Bone marrow samples were analyzed at diagnosis, after induction in patients achieved complete remission and at relapse at any point of time. Associations between CD244 expression and clinicopathological characteristics, treatment response, and survival outcomes were investigated.

Results: CD244 expression was significantly elevated in acute leukemia patients, with noticeably higher expression and MFI in AML compared to both ALL and controls (both P<0.001). Although CD244 expression was also elevated in ALL compared to controls (P=0.030), suggesting CD244 can serve as a lineage discriminator differentiating between both types of leukemia. Also, in both AML and ALL patients, CD244 expression and MFI were significantly elevated at diagnosis and relapse compared to remission. These dynamic changes in CD244 can help in monitoring disease progression. Furthermore, higher CD244 expression was associated with adverse risk stratification and poorer clinical outcomes in AML and ALL. Cox regression analysis revealed that elevated CD244 MFI was independent predictor of inferior overall survival (OS) (adjusted HR=1.322, 95% CI: 1.154–2.673, P=0.037) together with non-complete remission (adjusted HR=11.690, P<0.001) and relapse (adjusted HR=3.962, P=0.010). However, in ALL higher CD244 MFI was associated with shorter disease-free survival (DFS) in univariate analysis (HR=5.364, 95% CI: 1.034–27.832, P=0.046).

Conclusion: CD244 represents promising differentiating biomarker in acute leukemia. Its dynamic changes throughout the disease course and its association with adverse clinicopathological features support its potential role in disease monitoring and highlight its potential as therapeutic target.

KEYWORDS: Acute leukemia, Acute myeloid leukemia**,** Acute lymphoblastic leukemia, Mean fluorescence intensity, CD244, flow cytometry.

BACKGROUND

CD244 (also known as 2B4 or Signaling Lymphocytic Activation Molecule Family member 4(SLAMF-4)) is an important member of the CD2 subset of the immunoglobulin superfamily molecules, which expressed on natural killer (NK) cells subset of CD8 T cells and monocytes. It plays a role in immune regulation via interaction with CD48 representing a dual-functional immune modulator whose regulatory switch depends on the intracellular adaptor protein SLAM-Associated Protein (SAP). Upon binding its ligand CD48, CD244 recruits SAP and (Fyn Proto-Oncogene, Src Family Tyrosine Kinase) Fyn kinase to propagate activating signals that drive NK cell cytotoxicity and cytokine release. Conversely, in the absence or mutation of SAP, CD244 recruits alternative adaptors like Ewing's Sarcoma-Associated Transcript 2 (EAT-2) or inhibitory phosphatases such as Src Homology region 2 domain-containing

protein tyrosine Phosphatase (SHP-1/SHP-2) effectively switching to an inhibitory receptor that suppresses immune cell activation [1].

In benign conditions, the CD244 (2B4) signaling axis acts as a homeostatic regulator of self-tolerance and immunopathology. Persistent antigen exposure in chronic viral states, including Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV), induces aberrant CD244 upregulation coupled with a deficiency in the intracellular adaptor SAP; this switches CD244 to an inhibitory phenotype that drives T-cell and NK-cell exhaustion, minimizing inflammatory tissue injury while allowing viral evasion. Similarly, CD244–CD48 interactions are critical for suppressing autoreactive immune populations in systemic lupus erythematosus and rheumatoid arthritis, ensuring maternal–fetal immunotolerance, and modulating the hyper-inflammatory and immune-paralytic phases of bacterial sepsis [2].

Emerging evidence suggests that CD244 contributes to the maintenance, proliferation and survival of both human leukemic cell lines and leukemic stem cells (LSCs); notably, CD244 deletion dramatically delays leukemogenesis. Moreover, targeted blockade of the immune checkpoints CD244 and Leukocyte Immunoglobulin-Like Receptor B2 (LILRB2) offers a dual-action therapeutic strategy to eradicate LSCs and reverse microenvironmental immune evasion. While CD244 inhibition directly destabilizes downstream pro-survival oncogenic networks (e.g., c-Kit/SHP-2) within leukemic blasts, LILRB2 blockade intercepts dominant inhibitory pathways in tumor-infiltrating myeloid cells. Together, this combinatorial blockade suppresses the cell-intrinsic stemness machinery of AML clones while simultaneously transforming the immunosuppressive tumor niche into an active, immunocompetent environment capable of robust cytolytic clearance [3].

These findings highlight the potential of CD244 as a novel therapeutic target, particularly in the treatment of AML [4]. Furthermore, since 2B4 is already being used as transmembrane domains for Chimeric Antigen Receptor Natural Killer cells (CAR-NK) structuring, it has shown highly promising efficacy, paving the way for advanced Chimeric Antigen Receptor T-cells (CAR-T and CAR-NK) immunotherapeutic strategies in acute leukemia [5]. However, despite prior studies, the precise clinical significance of CD244 across both acute leukemia types remains poorly defined. Specifically, its utility as a lineage-specific discriminator, its diagnostic value in measurable residual disease (MRD) monitoring, and its relationship with disease progression, therapeutic response, and long-term patient outcome warrant deeper investigation.

Despite substantial advances in acute leukemia regarding diagnostic techniques, risk stratification and therapeutic strategies, the overall prognosis of acute leukemia remains suboptimal, particularly in adult patients, with high rates of relapse and treatment-related mortality. However, decisions on consolidation chemotherapy, timing of hematopoietic stem cell transplantation, are evaluated on each patients' likelihood of attaining complete remission (CR), detailed analysis of MRD which facilitates the rational use of molecular targeted therapy and immunotherapy as new treatment strategies against LSCs [6]. Consequently, extensive researches are currently focused on exploiting novel surface biomarkers, such as CD244, as therapeutic targets in patients with acute leukemia, in addition to establishing their utility in diagnosis and prognosis.

METHODS

Study aim, design and setting

This prospective longitudinal study aimed to evaluate the clinical utility of CD244 expression as a diagnostic and prognostic biomarker in Egyptian patients with newly diagnosed acute leukemia utilizing multiparameter flowcytometric analysis and to determine its role as lineage specific discriminator differentiating between ALL and AML, evaluate its dynamic fluctuations throughout successive phases of disease progression to elucidate its role in monitoring clinical outcome and to explore the correlation between CD244 expression levels and established various clinicopathological prognostic factors, therapeutic response and its impact on patients' outcome and survival.

Study participants

This study conducted on 141 newly diagnosed patients with acute leukemia who attended the Oncology Center Mansoura University (OCMU) between May 2024 to December 2025, of whom 85 patients were diagnosed with AML and rest 56 patients were diagnosed with ALL. The diagnosis was based on FAB and WHO classification and was confirmed through morphological, immunophenotypic, molecular, and cytogenetic analyses. Patients with secondary acute leukemia, other hematological malignancies and pediatric acute leukemia patients were excluded from the study.

Sample collection

Bone marrow (BM) samples were collected from all 141 patients at initial diagnosis. In addition, follow-up samples were obtained from patients who achieved CR after induction therapy (71 samples), as well as from patients who developed relapse during the study period (26 samples). Sixteen age and sex-matched apparently healthy individuals who underwent bone marrow examination and were confirmed to be free of hematological malignancy served as the control group.

Immunophenotypic assessment of CD244 expression and analysis strategy:
Bone marrow aspirate samples were processed for both routine immunophenotypic evaluation and assessment of CD244 expression by multiparameter flow cytometry.
Washed cells were incubated with the fluorescein isothiocyanate (FITC) labeled Anti-Human CD244 monoclonal antibody (BD Pharmingen™, San Diego, CA, USA; Catalog No. 550815). 100 µL of sample was added to each test tube needed, followed by 10 µL of anti CD45 (ECD), immaturity markers as (CD34, CD117, CD99 and CD1a), B-lineage markers (CD19, CD10, CD20, CD79a CD81, CD58 and CD38), T-lineage markers (CD7, CD5, CD3, CD4, CD8 and CD2) together with anti-CD244 FITC-conjugated antibody. Tubes were vortexed and incubated in the dark at room temperature for 15 minutes. Then after incubation, lysing solution were added followed by incubation in the dark at room temperature for 15 minutes. After that, cells were then washed twice with phosphate buffered saline (PBS) and centrifuged at 2000 rpm for 5 minutes and the supernatant was removed leaving the cellular pellet at the bottom that was resuspended in 1 ml of Flow Cytometry Staining Buffer and analyzed by Navios EX Flow Cytometer using 488 nm wavelength laser excitation and 525/40 nm bandpass filter.

Immunophenotypic assessment of CD244:
Initial gating was performed using time on Y-axis versus side scatter (SS) peak on X-axis to ensure continuity of cell flow. Doublets were excluded by plotting forward scatter (FS) peak versus FS integral. Following this another gate was performed using CD45 on Y-axis versus FS integral on X-axis to exclude debris and dead cells, then blast population were gated as low side scatter (SCC) on X-axis in dim CD45 on Y-axis and calculated as a percentage of the total number of gated events [8]. CD244 expression was determined with in blast gate in plot displayed CD244 on X-axis versus side scatter (SS) on Y-axis and by measurement of mean fluorescence intensity (MFI) using histogram plot, the marker (CD244) was displayed versus cell count. B-Lymphocytes were used as negative control in bone marrow samples. While monocytes were used as a positive control [9]. In addition, CD244 MFI was assayed on blast cells using histogram plot, the marker (CD244) was displayed versus cell count.
Follow up was done particularly after induction therapy (1st cycle chemotherapy) for assaying CD244 after achieving CR. Also, CD244 expression level and CD244 MFI were reevaluated in case of relapse that occurred at any time during follow-up to evaluate kinetic changes during disease progression and treatment response.

Treatment Regimen and follow up
Treatment protocols were tailored according to patients' diagnosis, age and performance status (PS), for AML cases induction therapy either a standard 7+3 regimen, azacitidine, or low dose cytarabine, each administered ± venetoclax, while ALL patients were treated by either pediatric inspired protocol or Hyper-CVAD or Steroid/ vincristine ± tyrosine kinase inhibitors. Following the first cycle, BM examination was performed to assess residual blasts and evaluate treatment response according to the ELN guidelines [7,8]. Patients who achieved CR underwent consolidation therapy, while refractory/relapsed cases received salvage therapy. Allogeneic hematopoietic stem cell transplantation was considered for eligible patients based on risk stratification and the availability of a fully HLA-matched related sibling donor.

Statistical analysis
Data were entered and analyzed using IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Qualitative data were expressed as N and percentage (%). The level of antigen expression was identified as MFI values. Quantitative data were initially tested for normality using Kolmogorov-Smirnov test. Quantitative data were expressed as median (min-max) if abnormally distributed and compared using Mann-Whitney U-test for two group or Kruskal-Wallis test for more than two groups. Cox regression analysis performed to identify which independent factors might have a significant influence on OS and DFS. P value is considered significant if <0.05 at confidence interval 95%.

RESULTS
Basic descriptive data for ALL and AML:
This study included 141 newly diagnosed patients with acute leukemia (63 females and 78 males). Eighty-five patients were diagnosed as AML aged from (18 to 85 years) and reminder 56 patients were ALL aged from (18 to 70 years). Complete remission (CR) following induction therapy was achieved in 69.4% of AML patients and 78.6% of ALL patients (Table 1).

Table 1: **Descriptive data of studied groups.**

Variables	AML patients (N=85)	ALL patients (N=56)
Median age	55.0 (18-85)	47.0 (18-70)

Gender	**Male, (N (%))**	46 (54.1%)		32 (57.1%)	
	Female, (N (%))	39 (45.9%)		24 (42.9%)	
WBCS at diagnosis, median (range) $\times 10^9$ /L		28.6 (0.8-345.5)		23.0 (1.2-533.2)	
Hemoglobin at diagnosis, median (range) g/dL		7.8 (4.4-12.3)		8.5 (5.0-13.4)	
PLT at diagnosis, median (range) $\times 10^9$/L		37.0 (5-317)		52.5 (5-442)	
Bone marrow blast (%), median (range)		80.0 (23-95)		90.0 (30-98)	
Molecular and cytogenetics (No positive/No tested (%))		**t(8;21)**	18/81 (23.5%)	**t(9;22)**	5/22 (22.7%)
		Inver16	23/81 (28.4%)	**t(1;19)**	0/10 (0.0%)
		t(9;22)	0/35 (0.0%)	**t(12;21)**	2/24 (8.3%)
		t(4;11)	0/12 (0.0%)	**11q23**	0/7 (0.0%)
		t(11;19)	0/32 (0.0%)	**14q11 rearrangement**	4/9 (44.4%)
		11q23	1/23 (4.3%)	**7q34 rearrangement**	1/12 (8.3%)
		NPM	4/14 (28.6%)		
		FLT3	16/63 (25.4%)	**BCR-ABL190**	5/22 (22.7%)
Risk stratification		**Favorable**	38 (45.2%)	**Standard risk**	32 (57.1%)
		Intermediate	29 (34.5%)		
		Adverse	17 (20.3%)	**High risk**	24 (42.9%)
Induction response	**Complete Remission (CR), (N (%))**	59 (69.4%)		44 (78.6%)	
	Refractory disease (RD), (N (%))	14 (16.5%)		7 (12.5%)	
	Induction death (ID), (N (%))	12 (14.1%)		5 (8.9%)	
Relapse	**No relapse, (N (%))**	40 (67.8%)		37 (84.1%)	
	Relapse, (N (%))	19 (32.2%)		7 (15.9%)	

CD244 as a lineage discriminator marker:

Despite that the percentage of CD244-positive blasts was significantly higher in blast cells compared to controls ($P<0.05$), the CD244 MFI on blasts was significantly increased in AML compared to both ALL and controls ($P < 0.001$ each), and no significant difference was observed between ALL and controls suggesting the CD244 could be used as a marker for clarifying unclear cases (Table 2) (Figure 1-4).

Table 2: Comparison of baseline percent and MFI of CD244 and LSCs among studied groups.

Parameter	Control (N=16)	ALL (N=56)	AML (N=85)	P^1	P^2	P^3	P^4
CD244 % on blast	0.165 (0.09-2.90)	1.15 (0.10-10.0)	8.40 (0.20-86.40)	**0.030**	**<0.001**	**<0.001**	**<0.001**
CD244 MFI on blast	0.538 (0.232-0.856)	0.539 (0.122-1.70)	1.55 (0.655-3.24)	1.00	**<0.001**	**<0.001**	**<0.001**
CD244% on monocyte	35.2 (17.6-79.2)	34.0 (5.0-88.0)	36.4 (4.6-98.1)	0.978	0.692	0.468	0.753
CD244 MFI on monocyte	0.942 (0.526-1.82)	0.986 (0.480-2.90)	0.988 (0.822-2.59)	0.253	0.056	0.225	0.095
CD244 MFI on B-lymphocyte	0.378 (0.285-0.948)	0.447 (0.180-0.990)	0.453 (0.301-0.995)	0.319	0.090	0.423	0.237
LSC %	--------	0.08 (0.01-0.26)	0.24 (0.04-6.8)	----	-----	**<0.001**	------

Continuous variables are expressed median (min-max). data are compared using kruskal wallis. **P^1** between control and ALL group. **P^2** between control and AML group. **P^3** between ALL and AML group. **P^4** between 3groups. **significant (P value < 0.05)

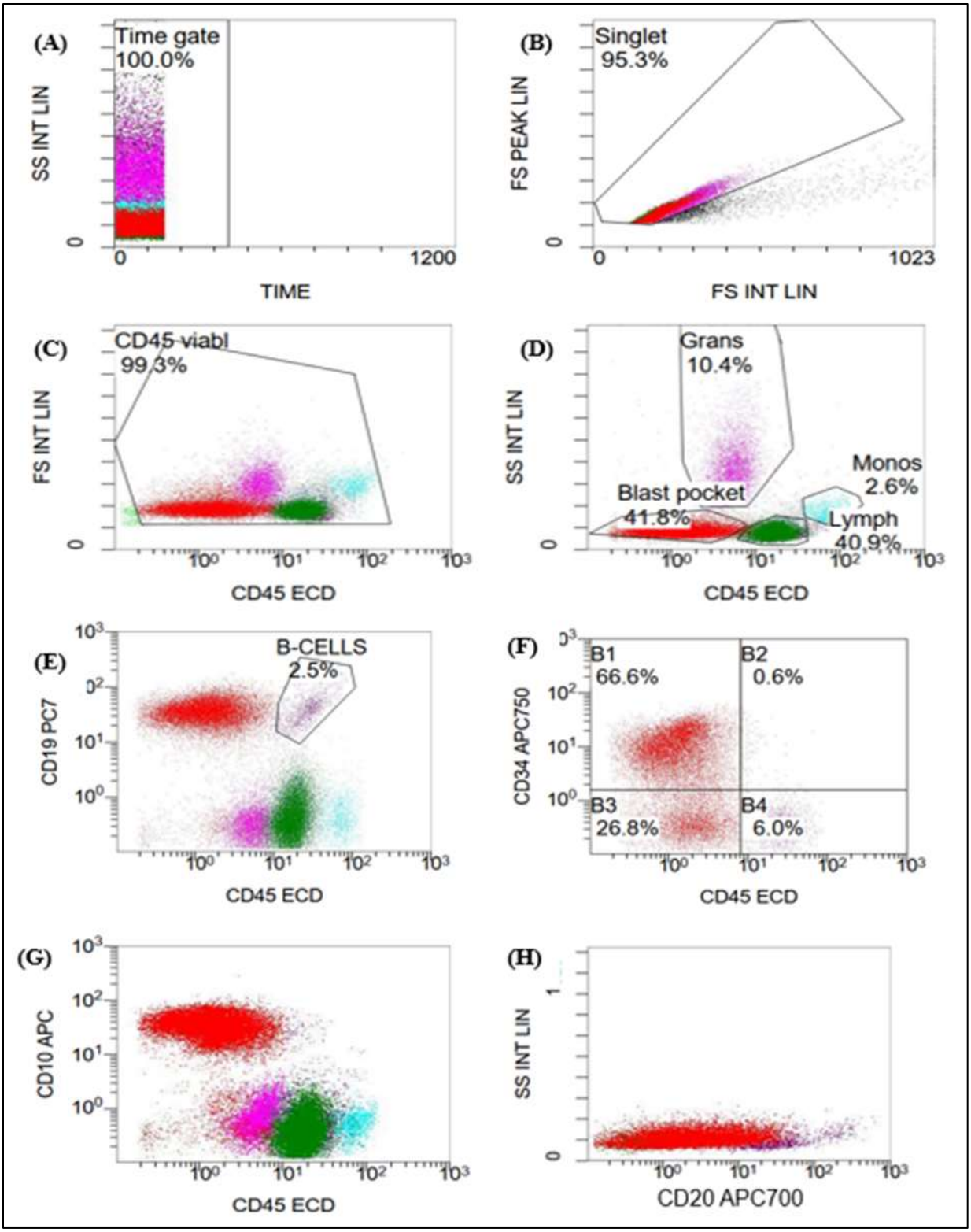

Figure 1: Initial immunophenotyping of the B-cell acute lymphoblastic leukemia (B-ALL) case. Figure (A) Time versus SS to ensure continuity of cell flow.

Figure (B) showed FS peak versus FS integral to exclude doublets. **Figure (C)** CD45 versus FS to remove debris and non-viable cells. **Figure (D)** Identification of the blast population on a dim CD45 versus low SSC. **Figure (E)** D19 positive expression within the gated blast population. **Figure (F)** Partial expression of CD34 on blast cells. **Figure (G)** Positive expression of CD10on the blast population. **Figure (H**) Heterogenous expression of CD20 on blast cells.

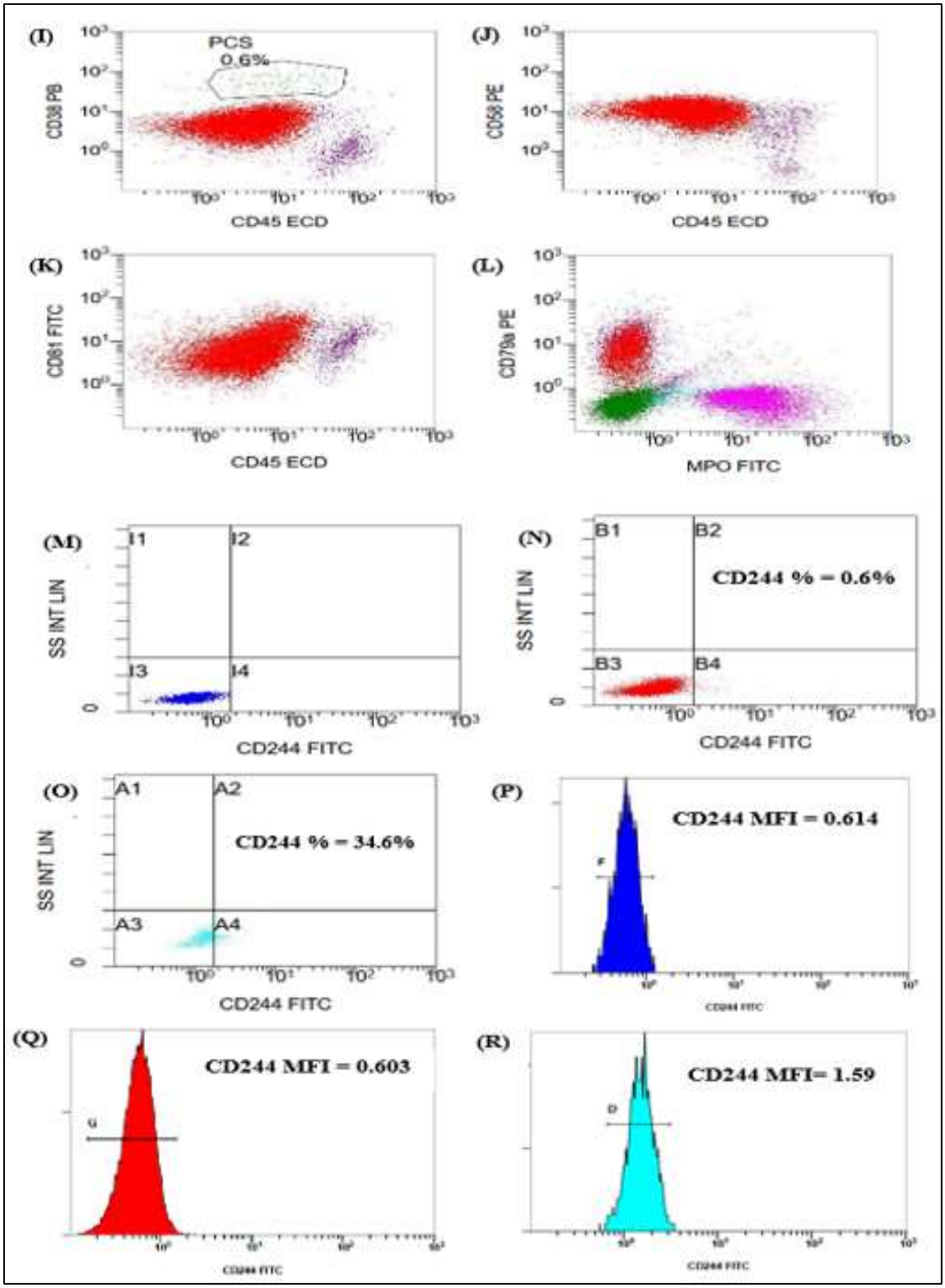

Figure 1: Immunophenotyping of B-ALL case.

Figure (I) showed dim CD38 expression on blast cells. **Figure (J)** showed positive CD58 expression on blast cells. **Figure (K)** showed positive CD81 expression on blast cells. **Figure (L)** showed positive cytoCD79a and negative MPO on blast cells. **Figure (M)** showed negative CD244 expression on B-cells. **Figure (N & O)** showed CD244 expression on Blast (0.6%) and monocytes (34.6%). **Figure (P, Q & R)** showed CD244 MFI on B-lymphocytes (0.614), Blast (0.603) and monocytes (1.59).

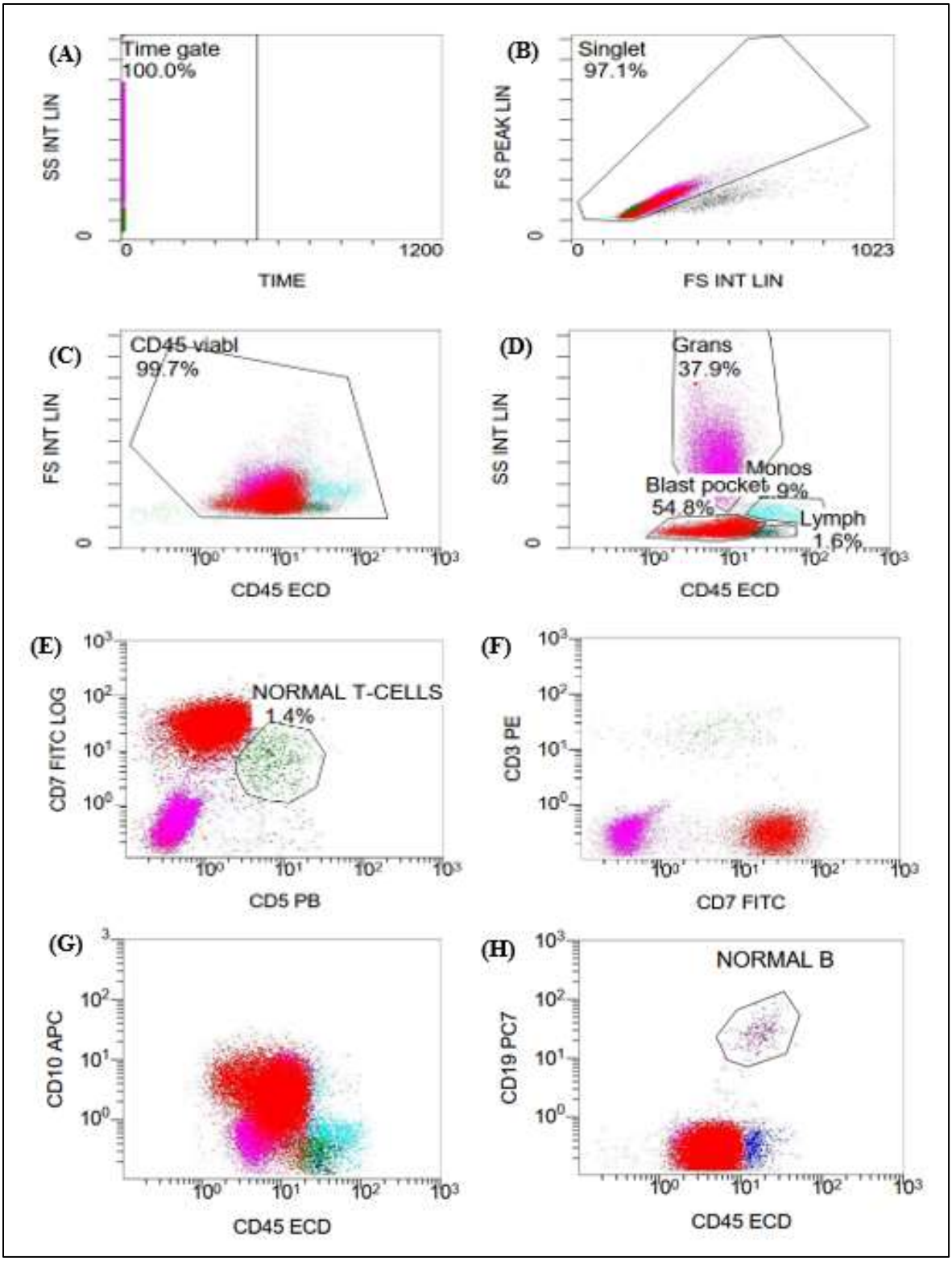

Figure 2 Initial immunophenotyping of T-cell acute lymphoblastic leukemia (T-ALL) case.

Figure (A) Time versus SS to ensure continuity of cell flow. **Figure (B)** showed FS peak versus FS integral to exclude doublets. **Figure (C)** CD45 versus FS to remove debris and non-viable cells. **Figure (D)** Identification of the blast population on a dim CD45 versus low SSC. **Figure (E)** showed CD7 versus CD5 (positive CD7 and CD5 on blast cells). **Figure (F)** showed negative surface CD3 expression on blast cells. **Figure (G)** Aberrant CD10 expression with in blast population. **Figure (H)** showed normal B-cells.

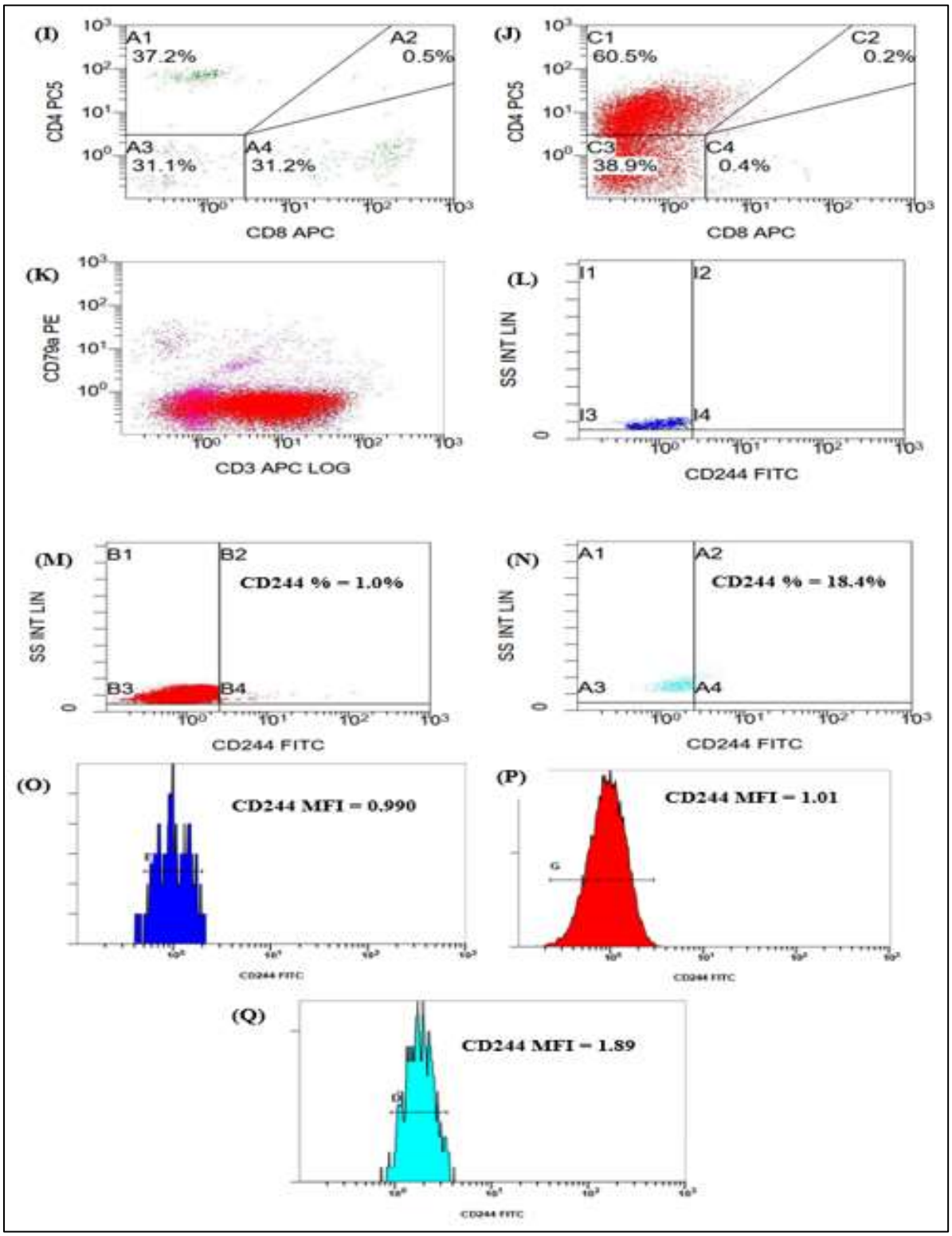

Figure 3 Immunophenotyping of T-ALL case.

Figure (I) showed normal CD4 and CD8 expressions on T-cells. **Figure (J)** showed positive CD4 and negative CD8 expression on blast cells. **Figure (K)** showed positive cyto CD3 expression on blast cells. **Figure (L)** showed negative CD244 expression on B-cells. **Figure (M & N)** showed CD244 expression on Blast (1.0%) and monocytes (18.4%). **Figure (O, P & Q)** showed CD244 MFI on B-lymphocytes (0.990), Blast (1.01) and monocytes (1.89).

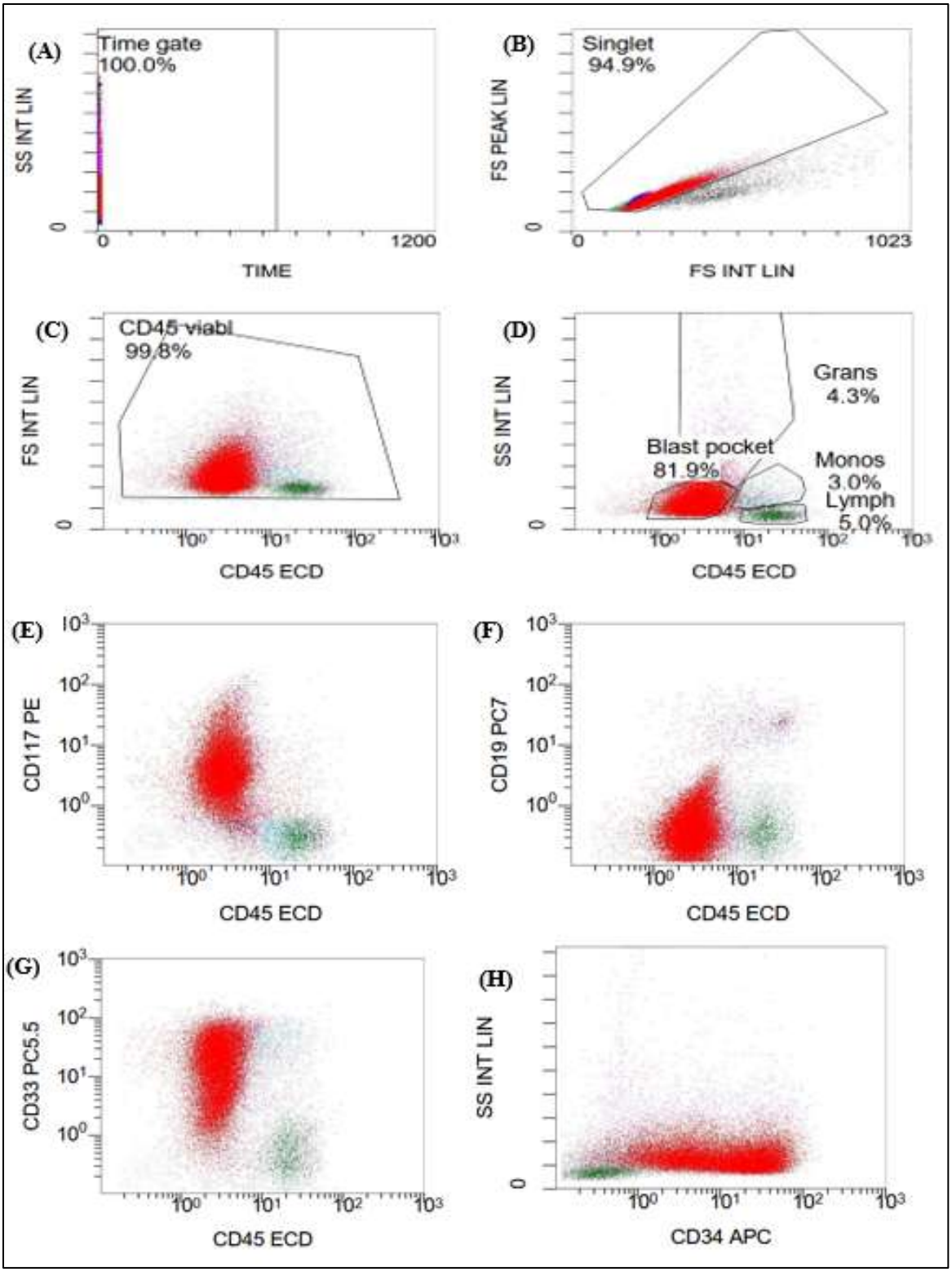

Figure 4 Initial immunophenotyping of AML case.

Figure (A) Time versus SS to ensure continuity of cell flow. **Figure (B)** showed FS peak versus FS integral to exclude doublets. **Figure (C)** CD45 versus FS to remove debris and non-viable cells. **Figure (D)** Identification of the blast population on a dim CD45 versus low SSC. **Figure (E)** showed CD117 versus CD45 (positive CD117 on blast cells). **Figure (F)** showed negative CD19 expression on blast population. **Figure (G)** showed positive CD33 expression on blast population. **Figure (H)** showed positive CD34 on blast population.

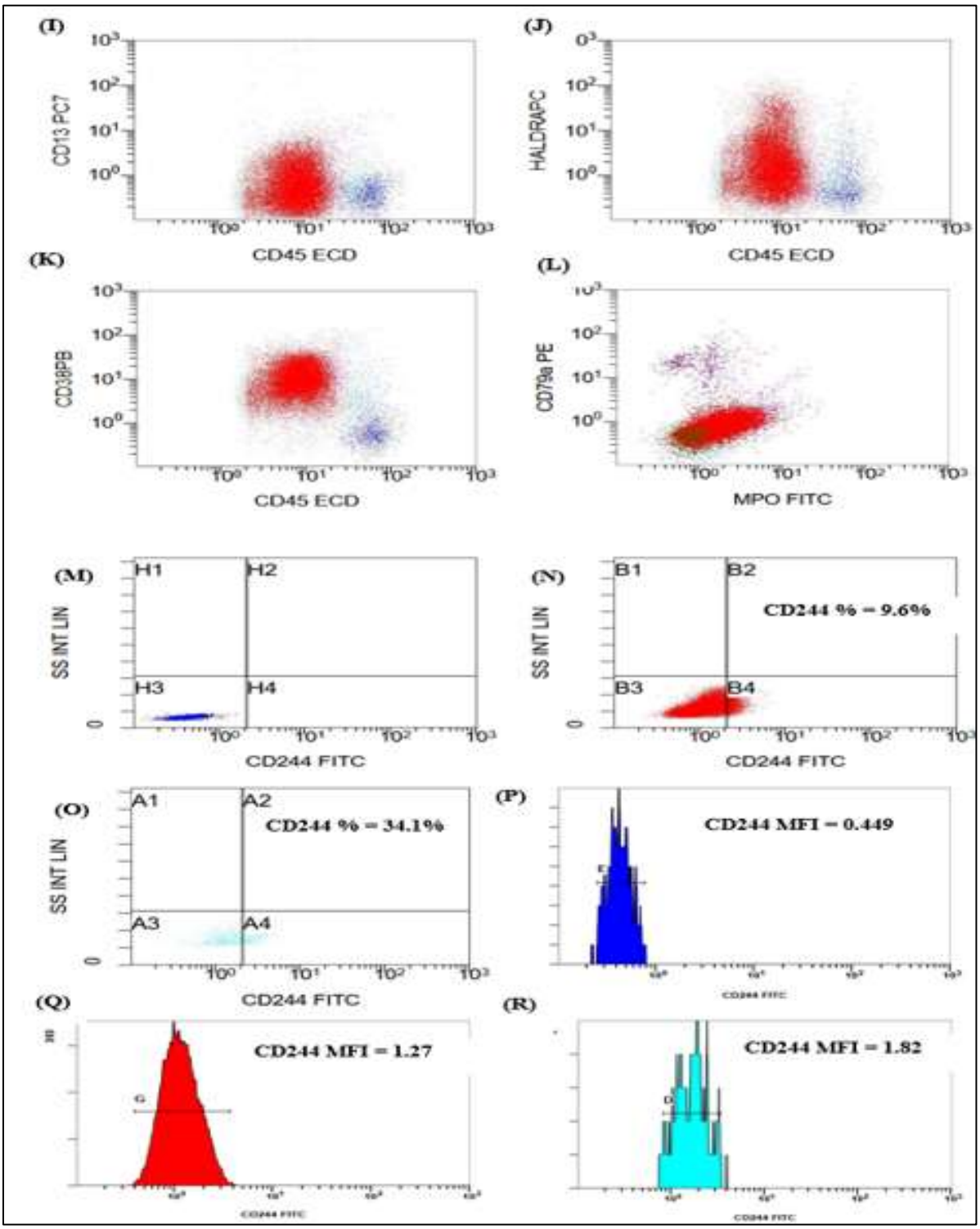

Figure 4 Immunophenotyping of AML case.

Figure (I) showed dim CD13 on blast cells. **Figure (J)** showed positive HLADR on blast cells. **Figure (K)** showed positive CD38 expression on blast cells. **Figure (L)** showed negative CD79a and dim MPO expression on blast cells. **Figure (M)** showed negative CD244 on B-cells. **Figure (N & O)** showed CD244 expression on Blast (9.6%) and monocytes (34.1%). **Figure (P, Q & R)** showed CD244 MFI on B-lymphocytes (0.449), Blast (1.27) and monocytes (1.82).

CD244 Expression Dynamics

In ALL and **AML** there was significant elevation of CD244 % and CD244 MFI at diagnosis and relapse compared to remission and In **AML** there was significant elevation of CD244 % and CD244 MFI at diagnosis compared to relapse (figure 5-7).

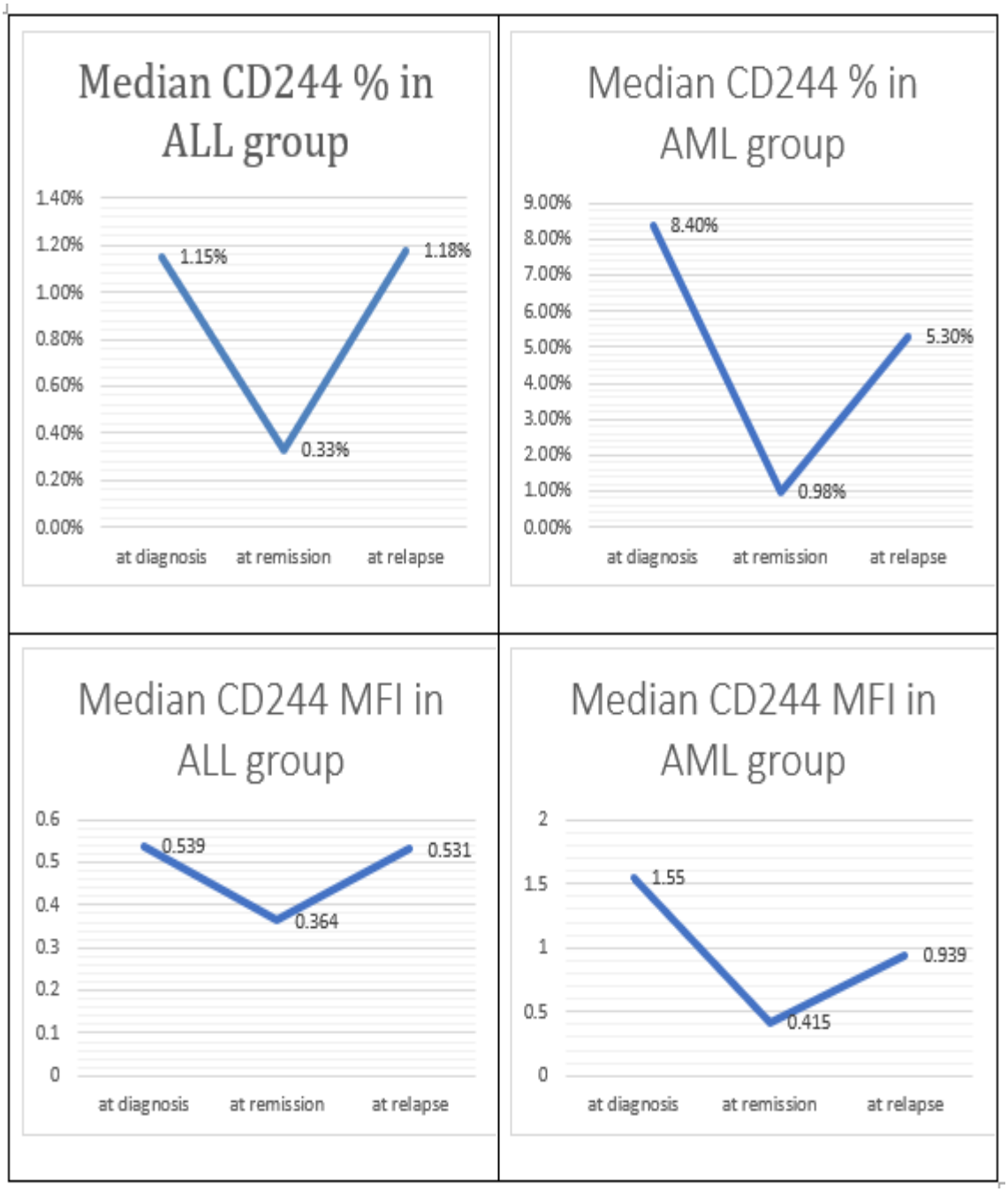

Figure 5: Kinetic expression of CD244 in ALL and AML groups. ALL patients achieved CR showed significant lower levels of CD244 compared to levels that were detected at diagnosis

(P= <0.001) and relapse **(P= 0.043)**. AML patients achieved CR showed significant lower levels of CD244 compared to levels that were detected at diagnosis **(P= <0.001)** and relapse **(P= 0.003)**. Kinetic expression of CD244 MFI in ALL group and AML groups. ALL patients achieved CR showed significant lower levels of CD244 compared to levels that were detected at diagnosis **(P= 0.019)** and relapse **(P= 0.018)**. AML patients achieved CR showed significant lower levels of CD244 compared to levels that were detected at diagnosis **(P= <0.001)** and relapse **(P= 0.001)**.

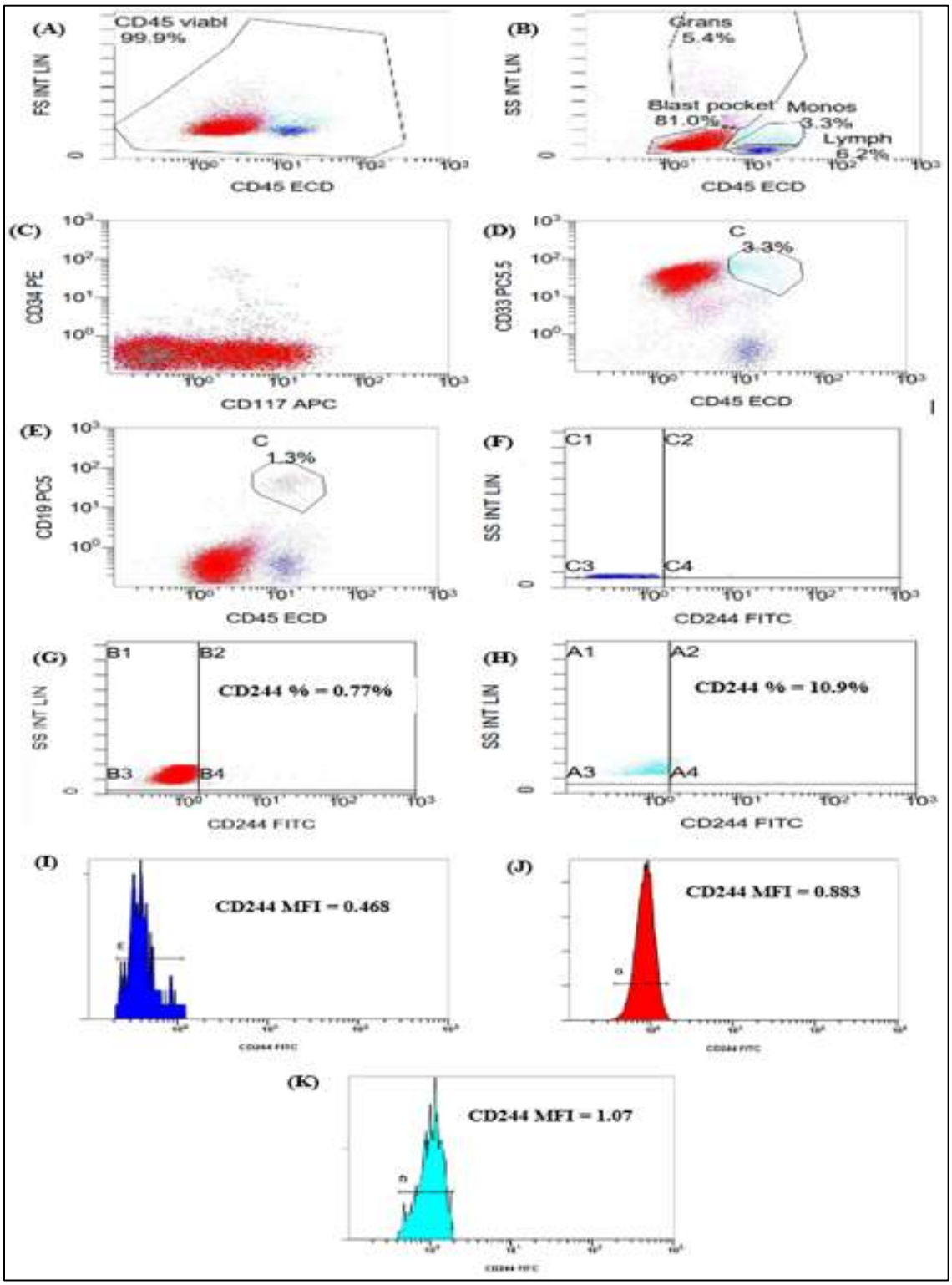

Figure 6: CD244 in AML patient in relapse.

Figure (A) showed CD45 versus FS to FS to remove debris and non-viable cells. **Figure (B)** Identification of the blast population on a dim CD45 versus low SSC**. Figure (C)** showed positive CD117 and negative CD34 on blast cells. **Figure (D)** showed positive CD33 on blast cells and monocytes. **Figure (E)** showed positive CD19 on B-cells. **Figure (F)** showed negative CD244 on B-cells. **Figure (G & H)** showed CD244 expression on Blast (0.77%) and monocytes (10.9%). **Figure (I, J & K)** showed CD244 MFI on B-lymphocytes (0.468), Blast (0.883) and monocytes (1.07).

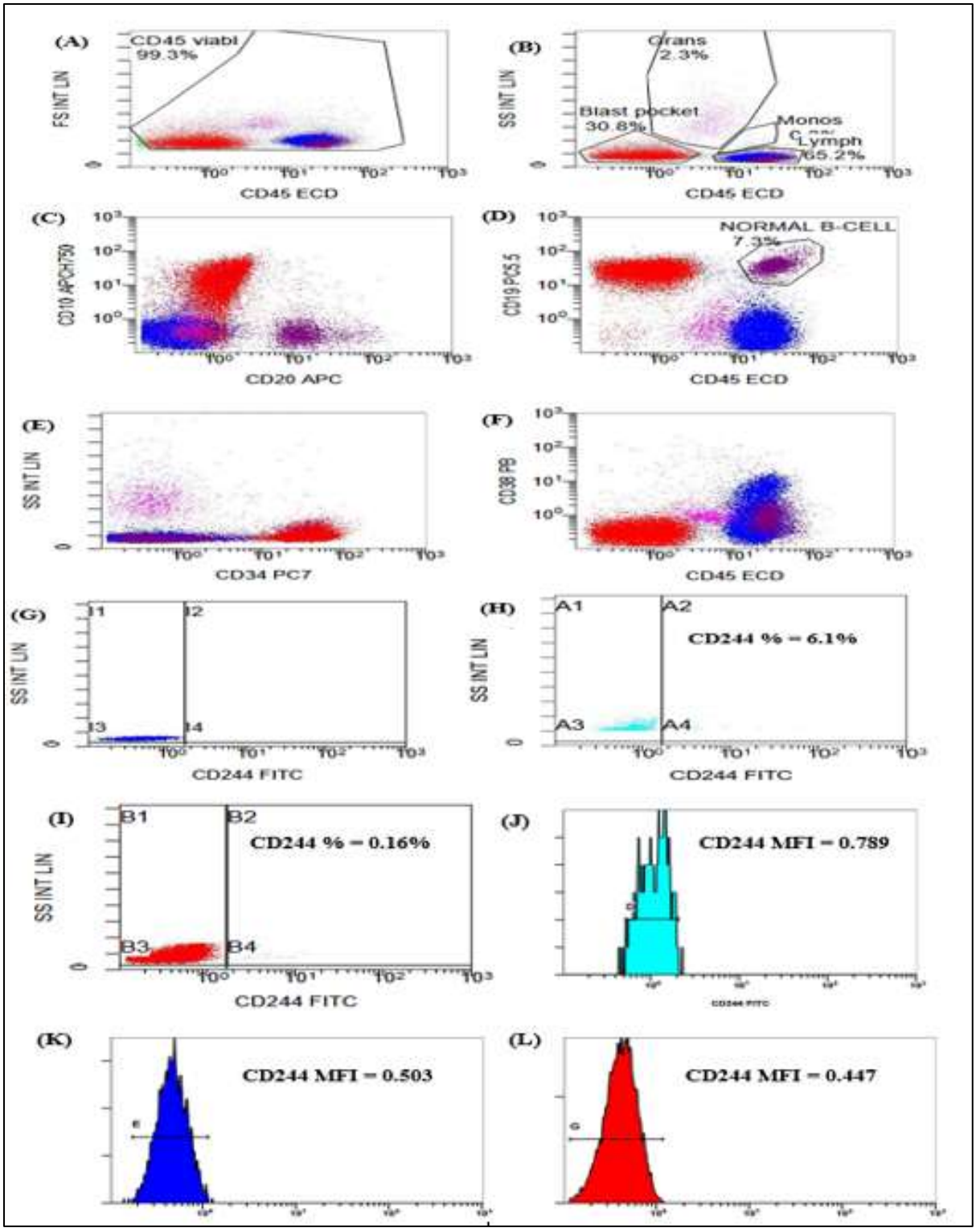

Figure 7: CD244 in ALL patient in relapse.

Figure (A) showed CD45 versus FS to FS to remove debris and non-viable cells. **Figure (B)** Identification of the blast population on a dim CD45 versus low SSC. **Figure (C)** showed positive CD10 and negative CD20 on blast cells. **Figure (D)** showed positive CD19 on blast cells. **Figure (E)** showed positive CD34 on Blast cells. **Figure (F)** showed negative CD38 on Blast cells. **Figure (G)** showed negative CD244 on B-cells. **Figure (H & I)** showed CD244 expression on Monocytes (6.1%) and Blast cells (0.16%). **Figure (J, K & L)** showed CD244 MFI on Monocytes (0.789), B-lymphocytes (0.503) and Blast cells (0.447).

The prognostic impact of CD244 expression and survival outcome

In ALL patients, CD244% was significantly higher in T-ALL compared to B-ALL (P = 0.027) and in high-risk patients compared to standard-risk patients (P = 0.032). CD244 MFI was significantly higher in high-risk patients (P = 0.001) and in patients who failed to achieve CR (P = 0.04). In AML, both CD244% and CD244 MFI were significantly higher in intermediate and poor risk groups (P = 0.006 and < 0.001, respectively) and in patients who failed to achieve CR (P = 0.011 and < 0.001, respectively) (table 3).

Table 3: Comparison of CD244 % and CD244 MFI as regards demographic, clinical and laboratory data of ALL and AML patients.

		Parameter	CD244 % Median (Min-Max)	CD244 MFI Median (Min-Max)	P[1]	P[2]
ALL group	**Gender**	**Male**	1.50 (0.10-10.0)	0.518 (0.153-1.630)	0.371	0.625
		Female	0.77 (0.10-7.20)	0.539 (0.122-1.700)		
	WHO	**B ALL**	0.80 (0.10-7.20)	0.488 (0.217-0.829)	**0.027**	0.081
		T ALL	1.95 (0.30-10.0)	0.646 (0.122-1.700)		
	Risk stratification	**Standard risk**	0.80 (0.10-10.0)	0.375 (0.122-0.920)	**0.032**	**0.001**
		High risk	1.95 (0.20-7.20)	0.626 (0.312-1.700)		
	Response	**CR**	0.95 (0.10-10.0)	0.497 (0.122-1.630)	0.150	**0.04**
		Non-CR	2.05 (0.20-7.20)	0.626 (0.378-1.700)		
	Relapse	**Non-relapsed**	0.80 (0.10-10.0)	0.480 (0.122-1.630)	0.062	0.152
		Relapsed	2.9 (0.50-6.0)	0.652 (0.312-1.270)		
AML group	**Gender**	**Male**	6.6 (0.2-71.3)	1.45 (0.655-2.840)	0.056	0.066
		Female	11.4 (0.2-86.4)	1.60 (0.939-3.240)		
	FAB	**M1-M2**	6.2 (0.2-71.3)	1.32 (0.655-2.600)	0.119	**0.009**
		M4-M5	10.4 (0.4-86.4)	1.62 (0.879-3.240)		
	Risk stratification	**Favorable**	5.3 (0.2-68.5)	1.24 (0.808-2.840)	**0.006**	**<0.001**
		Intermediate	14.4 (0.5-75.6)	1.71 (1.037-3.240)		
		Adverse	14.5 (1.9-86.4)	1.76 (0.655-2.590)		
	Response	**CR**	5.6 (0.2-75.6)	1.29 (0.655-2.840)	**0.011**	**<0.001**
		Non-CR	15.6 (1.1-86.4)	1.76 (0.927-3.240)		
	Relapse	**Non-relapsed**	5.3 (0.2-75.6)	1.24 (0.655-2.610)	0.347	0.110
		Relapsed	6.0 (0.7-52.0)	1.56 (1.005-2.840)		

Mann-Whitney test, Kruskal Wallis. **P[1]** between groups as regard CD244 %, **P[2]** between groups as regard CD244 MFI.
**significant (P value < 0.05)

For ALL, COX Regression analysis conducted for predicting factors affecting OS and DFS showed that the significant independent factors for shorter OS in multivariate analysis were non-CR and relapse (P 0.002, and 0.047 respectively). While the significant independent factors for shorter DFS in univariate analysis are high risk stratification (P=0.030) and CD244 MFI (P=0.046) (Table 4).

Table (4): Cox regression analysis to predict the hazardous factor that could predict OS and DFS in ALL patients

Parameter	Overall survival	Disease free survival

	Univariable			Multivariable			Univariable			Multivariable		
	P value	**HR**	**95 % CI**	**P value**	**HR**	**95 % CI**	**P value**	**HR**	**95 % CI**	**P value**	**HR**	**95 % CI**
Age	**0.022**	**1.047**	**1.007 - 1.089**	0.175	1.054	0.977 - 1.136	0.187	1.043	0.980-1.110			
Gender (female vs male)	0.233	0.530	0.187 - 1.506				0.708	0.750	0.167-3.375			
WBCs	0.826	1.000	0.996 - 1.005				0.136	1.004	0.999-1.009			
Hemoglobin	0.194	0.847	0.659 - 1.088				0.314	0.821	0.560-1.204			
Platelets count	0.886	1.000	0.995 - 1.004				0.667	0.998	0.990-1.006			
BM blast cells %	0.457	1.013	0.980 - 1.047				0.427	1.023	0.968-1.080			
LDH	0.153	1.0000	0.999 - 1.001				0.336	1.001	0.999-1.002			
Risk stratification (high risk vs standard risk)	**0.004**	**4.772**	**1.670 - 13.638**	0.423	1.416	0.949 - 3.553	**0.034**	**5.111**	**1.129-23.130**	0.266	2.871	0.521-15.821
Response (non-CR vs CR)	**< 0.001**	**11.5744**	**4.299 - 31.166**	**0.002**	**11.293**	**2.410 - 52.912**						
Relapse (relapsed vs non-relapsed)	**0.027**	**5.4800**	**1.219 - 24.623**	**0.047**	**4.966**	**1.022 - 24.133**						
CD244 %	0.096	2.3333	0.859 - 6.337				0.175	3.111	0.602-16.068			
CD244 MFI	0.101	2.2666	0.852 - 6.023				**0.046**	**5.364**	**1.034-27.832**	0.204	3.323	0.520-21.232
LSC %	0.397	1.0	0.992 - 3.393				0.691	5.135	0.991-6.216			

		41										

HR: Hazard ratio, **CI:** Confidence interval.

For AML, COX Regression analysis conducted for predicting factors affecting OS showed that the significant independent factors for shorter OS in multivariate analysis were non-CR, relapse and high CD244 MFI in AML patients (P <0.001, 0.010, and 0.037 respectively). While the significant independent factors for shorter DFS in both univariate and multivariate analysis were higher WBCS (P=0.041 and 0.006 respectively) and adverse risk stratification (P=0.045 and 0.029 respectively) (Table 5).

Table (5): Cox regression analysis to predict the hazardous factor that could predict OS and DFS in AML patients

Parameter	OS						DFS					
	Univariable			Multivariable			Univariable			Multivariable		
	P value	HR	95 % CI	P value	HR	95 % CI	P value	HR	95 % CI	P value	HR	95 % CI
Age	0.409	1.008	0.990 - 1.026				0.712	1.005	0.978 - 1.033			
Gender (female vs male)	0.233	0.956	0.785 - 1.699				0.670	0.995	0.973 - 1.018			
WBCs	0.992	1.000	0.996 - 1.004				**0.041**	**1.005**	**0.999 - 1.011**	**0.006**	**1.008**	**1.002 - 1.013**
Hemoglobin	0.419	0.994	0.978 - 1.009				0.165	0.805	0.593 - 1.094			
Platelets count	0.567	0.998	0.992 - 1.005				0.321	0.995	0.984 - 1.005			
BM blast cells %	0.759	1.031	0.849 - 1.251				0.803	1.122	0.855 - 2.764			
LDH	0.999	1.000	0.999 - 1.001				0.691	1.001	0.999 - 1.002			
Risk stratification (Intermediate vs favorable) (Adverse vs favorable)	**0.003** **0.022**	**3.074** **2.605**	**1.480 - 6.387** **1.149 - 5.906**	0.358 0.395	1.584 1.501	0.186 - 1.836 0.589 - 3.821	0.057 **0.045**	2.969 **2.985**	0.968 - 9.106 **1.025 - 8.690**	------ **0.029**	------ **3.394**	---------- **1.132 - 10.175**
Response (non-CR vs CR)	**<0.001**	**8.779**	**4.475 - 17.221**	**<0.001**	**11.690**	**4.276 - 31.959**						
Relapse (relapsed vs non-relapsed)	**0.003**	**3.739**	**1.547 - 9.035**	**0.010**	**3.962**	**1.387 - 11.320**						

CD244 %	0.145	1.573	0.855 - 2.893				0.357	1.528	0.620 - 3.764			
CD244 MFI	**0.016**	**2.142**	**1.151 - 3.987**	**0.037**	**1.322**	**1.154 - 2.673**	0.065	2.341	0.948 - 5.783			
LSC %	0.809	1.056	0.963 - 1.378				0.298	1.213	0.843 - 1.744			

HR: Hazard ratio, **CI:** Confidence interval.

DISCUSSION

Our study represents the diagnostic and prognostic significance of CD244 expression in adult patients with acute leukemia. The present study demonstrated that CD244 was significantly overexpressed in leukemic blast, particularly in AML followed by ALL compared with controls. Moreover, the dynamic change of CD244 expression throughout disease course and association between CD244 expression with adverse clinicopathological characteristics, poor treatment response and adverse survival outcomes especially in AML, all of these finding represent CD244 not only as lineage associated marker, but also as relevant biomarker affecting disease progression and prognosis.

CD244 expression was significantly higher in AML compared to ALL and normal controls, similarly CD244 MFI on blasts was significantly higher in AML, whereas no significant difference in CD244 MFI was observed between ALL and controls. These findings agreed with previous reports supporting the diagnostic role of CD244 in acute leukemia particularly AML [9,10]. The overexpression of CD244 in AML may be biologically clarified by developmental origin of leukemic cells. ALL blasts retain an early lymphoid progenitor phenotype that normally exhibits minimal or absent CD244 receptor, resulting in relatively lower expression of CD244. Contrary, AML originates from myeloid progenitors that are more closely linked to innate immune lineages and therefore, can readily express immune-regulatory receptors. Furthermore, during leukemogenesis, dysregulation of transcriptional programs promotes aberrant expression of molecules such as CD244 that not only serve as a surface marker but an active signaling receptor that can transmit signals promoting leukemic blast survival, proliferation, and leukemic stem cell maintenance [3]. Consequently, these distinct expression profiles allow CD244 to serve as a potential effective lineage discriminator that differentiate between acute lymphoblastic and myeloid leukemia. In routine flowcytometry, the lineage assignment depends on comprehensive immunophenotypic panels. However, additional markers may help in improving accuracy in diagnosis, particularly in leukemias with ambiguous immunophenotypic features.

A major strength of the present study is longitudinal assessment of CD244 expression throughout the disease course that revealed both CD244 expression and MFI exhibited significant reduction in patients achieved complete remission following induction therapy compared with levels reported at diagnosis and relapse in both ALL and AML patients. Levels documented at relapse approached diagnosis level. These dynamic changes support the potential role of CD244 for monitoring treatment response and disease progression. Although CD244 show low or dim expression in ALL patients, it has a prognostic value in those patients. As far as we know, no published studies directly report longitudinal changes in CD244 % or CD244 MFI across diagnosis, remission, and relapse in ALL patients making this one of the novel aspects of the present study. In AML, our observations consistent with Haubner et al. [10], who reported significant reduction of CD244 expression in AML patients who achieve CR consistently with blast clearance. Similarly, Ung et al., [11] demonstrated that elevated CD244 expression in AML blasts and LSCs by flowcytometry from newly diagnosed and relapsed patients, suggesting its role in disease persistence and recurrence. These findings support the utility of CD244 as a robust immunophenotypic marker for AML blast identification and potentially as an indicator of disease activity and relapse. Also, these dynamic changes along disease progression indicate that CD244 could be used as a valuable marker for MRD monitoring.

Regarding the association between CD244 expression and adverse clinicopathological characteristics, in ALL patients, our findings revealed CD244 expression was significantly higher in T-ALL than in B-ALL. Moreover, increased CD244 expression and MFI were observed in high-risk patients relative to those classified as standard risk, while CD244 MFI was significantly elevated in patients who failed to achieve complete remission. These findings suggest that elevated CD244 may identify patients with more aggressive disease biology and could serve as a potential marker for risk stratification and treatment response in ALL. Previous reports reflecting similar lineage specific differences in CD244 expression between T-ALL and B-ALL [9, 12]. Previous study highlighted therapeutic importance of CD244 by incorporation of the 2B4 co-stimulatory domain, has demonstrated an improved antileukemic activity in xenograft T-ALL in preclinical models [13].

In the present, higher CD244 expression and MFI showed significant associations with disease subtypes, risk stratification, and clinical outcome in AML patients. CD244 MFI was significantly higher in monocytic subtypes (M4–M5) compared to less differentiated subtypes (M1–M2), reflecting the biological characteristics of monocytic lineage AML with lineage-related variation in expression. Moreover, both CD244 % and MFI were significantly elevated in patients classified as intermediate- and poor-risk compared with those in favorable-risk groups. Also, increased CD244 expression was observed in patients who failed to achieve complete remission, indicating a potential association with treatment resistance. Additionally, higher CD244 MFI was significantly associated with increased mortality. These findings suggest that CD244 expression correlates with more aggressive disease characteristics and adverse clinical outcomes, supporting its potential role as a prognostic biomarker in AML. In similarity to our results, [14,15] who stated that CD244 as an immune regulatory gene showed marked differential expressions between high- and low-risk AML groups with higher level in high-risk group AML patients. Although Zhang et al., [3] has found that CD244 expression was positive in different types of human AML (M2, M3, M4 and M5) but didn't compare its level between different AML subtypes. Moreover, Haubner et al. [10] found no significant difference in CD244 antigen expression in different molecularly defined subtypes of AML.

Notably, higher CD244 MFI independently predicted inferior overall survival in AML patients even after adjustment in multivariate analysis. This observation is one of the most important findings of the present study, suggests that the intensity of CD244 expression provides prognostic information beyond conventional clinicopathological factors. CD244 has been shown to promote leukemic stem-cell maintenance and survival through SHP-2-dependent signaling pathways, therefore contributing to treatment resistance and disease progression.

CONCLUSION

CD244 (SLAMF4) is significantly overexpressed in acute leukemia, acting as a lineage-specific biomarker that distinguishes **AML** from **ALL**. Beyond its utility in diagnostic immunophenotyping, the dynamic expression of CD244 suggests the association of CD244 with disease activity, which may have role in disease monitoring. Also, its expression is closely linked to adverse clinicopathological characteristics, treatment response and survival. Collectively, our study suggests that CD244 as a potential lineage specific immunophenotypic marker and a promising therapeutic target in acute leukemia.

Limitations and recommendations:

Despite the significant findings of this research, certain limitations should be considered when interpreting the results. Most notably, the study was conducted at a single center with a relatively small sample size, which may limit the generalizability of the findings. So, the present study recommends the further multicenter studies with larger sample size and longer period are necessary to confirm these findings and to clarify its diagnostic, prognostic, and therapeutic value before routine application.

List of abbreviations:

AL: Acute leukemia
ALL: Acute lymphoblastic leukemia
AML: Acute myeloid leukemia
BM: Bone Marrow
BMMC: Bone Marrow Mononuclear Cells
CAR-T and CAR-NK: Chimeric Antigen Receptor T-cells / Natural Killer cells
CD2: Cluster of differentiation
CITE-seq: Cellular Indexing of Transcriptomes and Epitopes by sequencing
c-Kit/SHP-2: Tyrosine-protein kinase KIT / CD117) & SHP-2 (Src Homology region 2 domain-containing protein tyrosine Phosphatase 2
CR: Complete Remission
EAT-2: Ewing's Sarcoma-Associated Transcript 2
FITC: Fluorescein Isothiocyanate
Fyn kinase: Fyn Proto-Oncogene, Src Family Tyrosine Kinase.
HBV: Hepatitis B Virus
HCV: Hepatitis C Virus
HIV: Human Immunodeficiency Virus
LILRB2: Leukocyte Immunoglobulin-Like Receptor B2
LSCs: Leukemia Stem Cells
MFI: mean fluorescence intensity
MRD: Minimal Residual Disease
NK: natural killer

PBS: Phosphate-Buffered Saline
ROC: Receiver Operating Characteristic
OCMU; Oncology Center Mansoura University
SAP: SLAM-Associated Protein
SHP-1/SHP-2 phosphatases: Src Homology region 2 domain-containing protein tyrosine Phosphatase 1 / 2
SLAMF-4: Signaling Lymphocytic Activation Molecule Family member 4.
WHO: World Health Organization

Declarations:

Ethics approval and consent to participate:

The study was approved by the local Ethical Committee: Institutional Research Board (IRB) of Mansoura Faculty of Medicine with Code Number: 24.03.841. All methods were performed in accordance with the ethical standards as laid down in the Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants from a parent and/or legal guardian.

Consent for publication

Not applicable.

Availability of data and materials:

The data underlying this article will be shared on reasonable request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding:

The authors would like to express their sincere gratitude to Mansoura University, represented by the Research Funding Unit, for their financial support of this research work, which greatly contributed to the successful completion of the study.

Authors' contributions

D.A., N.M., Sh.E., O.K., and M.SH. contributed to the conception and design of the study, as well as the analysis and interpretation of the data, and critically reviewed the article. M.SH. was responsible for data and sample collection. D.A., N.M., O.K., and M.SH. performed the experimental work. O.K. conducted the statistical analysis. D.A., Sh.A., O.K., and M.SH. were responsible for drafting the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

Not applicable.

REFERENCE

1. Li, W. (2022). The 5th edition of the World Health Organization classification of hematolymphoid tumors. Exon Publications, 1-21.
2. Sun, L., Gang, X., Li, Z., Zhao, X., Zhou, T., Zhang, S., & Wang, G. (2021). Advances in understanding the roles of CD244 (SLAMF4) in immune regulation and associated diseases. Frontiers in immunology, 12, 648182.
3. Zhang, F., Liu, X., Chen, C., Zhu, J., Yu, Z., Xie, J., ... & Zheng, J. (2017). CD244 maintains the proliferation ability of leukemia initiating cells through SHP-2/p27kip1 signaling. Haematologica, 102(4), 707.
4. Agresta, L., Lehn, M., Lampe, K., Cantrell, R., Hennies, C., Szabo, S., ... & Janssen, E. M. (2020). CD244 represents a new therapeutic target in head and neck squamous cell carcinoma. Journal for immunotherapy of cancer, 8(1), e000245.
5. Mathew, S. O., Ahmed, N. G., Powers, S., Jose, R., & Mathew, P. A. (2023). Differential expression of immune receptors in pediatric Acute Lymphoblastic Leukemia (ALL). The Journal of Immunology, 210(Supplement_1), 89-25.
6. Inaba, H., & Pui, C. H. (2021). Advances in the diagnosis and treatment of pediatric acute lymphoblastic leukemia. Journal of clinical medicine, 10(9), 1926.
7. Döhner, H., Wei, A. H., Appelbaum, F. R., Craddock, C., DiNardo, C. D., Dombret, H., ... & Löwenberg, B. (2022). Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood, The Journal of the American Society of Hematology, 140(12), 1345-1377.

8. Gökbuget, N., Boissel, N., Chiaretti, S., Dombret, H., Doubek, M., Fielding, A., ... & Bassan, R. (2024). Diagnosis, prognostic factors, and assessment of ALL in adults: 2024 ELN recommendations from a European expert panel. Blood, 143(19), 1891-1902
9. Anilkumar, A., Rajpal, S., Chatterjee, G., Shah, N., Girase, K., Ghogale, S., ... & Tembhare, P. (2022). Expression Pattern of CD244, a Novel SLAM Protein and Its Clinical Utility in the Diagnosis of Acute Leukemia.
10. Haubner, S., Perna, F., Köhnke, T., Schmidt, C., Berman, S., Augsberger, C., ... & Subklewe, M. (2019). Coexpression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. Leukemia, 33(1), 64-74.
11. Ung, M., Etchin, J., Halfond, A., DiFazio, J., Keschner, Y., Pyclik, A., ... & Lydeard, J. R. (2026). A multimodal atlas for immunotherapeutic targeting of AML surface heterogeneity. iScience.
12. Wang, M., Windgassen, D., & Papoutsakis, E. T. (2008). Comparative analysis of transcriptional profiling of CD3+, CD4+ and CD8+ T cells identifies novel immune response players in T-cell activation. BMC genomics, 9(1), 225.
13. Assi, R., & Salman, H. (2022). Harnessing the potential of chimeric antigen receptor T-cell therapy for the treatment of T-cell malignancies: a dare or double dare?. Cells, 11(24), 3971
14. Huang, X., Zhou, D., Ye, X., & Jin, J. (2021). A novel ferroptosis-related gene signature can predict prognosis and influence immune microenvironment in acute myeloid leukemia. Bosnian journal of basic medical sciences, 22(4), 608.
15. Chen, T., Zhang, Y., Zhang, D., & Zhou, H. (2024). Immune-based subgroups uncover diverse tumor immunogenicity and implications for prognosis and precision therapy in acute myeloid leukemia. Frontiers in Immunology, 15, 1451486.