Exp Oncol 2022
44, 3, 198–207

Experimental ONCOLOGY

PROFILE OF CD150 EXPRESSION IN BONE MARROW CELLS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

L. Shlapatska, I. Gordiienko, A. Polishchuk, D. Gluzman*

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, 03022 Kyiv, Ukraine

Background: **Acute myeloid leukemia (AML) is a highly heterogeneous disease accompanied by the arrest of myeloid cell lineage differentiation due to accumulation of genetic abnormalities and clonal proliferation of myeloid blasts. Finding the differentially expressed molecules and studying their function within AML subgroups may help to improve diagnosis and prognosis with the aim of developing selected therapies for AML subsets.** ***The aim*** **of this study was to reveal the profile of CD150 cell surface expression on bone marrow (BM) cells of AML patients.** ***Materials and Methods:*** **The study was performed on samples of BM aspirates from 55 patients with primarily diagnosed AML. Flow cytometry analysis was applied for the evaluation of immunophenotype profile and CD150 cell surface expression on BM cells from AML patients.** ***Results:*** **Four AML subtypes (M1, M2, M3 and M5) were identified. The CD150 expression was found in 14 (25.5%) cases predominantly of AML M3 subtype. CD150 expression was detected on 43.2–83.8% of leukemia cells in AML M3. The frequency of CD150 positive cases of non-M3 AML subtypes was low: all AML M1 cases were CD150-negative, while only 1 (10.0%) of 10 patients with AML M2 and 6 (19.4%) of 31 patients with AML M5 were CD150 positive. The median percentage of CD150 positive leukemia cells and the index of mean fluorescence intensity in AML M3 cases were significantly higher than in non-M3 AML cases ($p < 0.05$). The CD150 expression was significantly associated with CD11c, CD11b, CD14, CD34, CD36, CD56 and HLA-DR negative expression and CD33, CD38, CD117 positive expression among the examined cohort of patients with AML M3.** ***Conclusions:*** **High level of CD150 expression is a unique feature of AML M3 subtype and may serve as additional phenotype marker for the identification of blast cells with impaired maturation at the promyelocyte stage and the development of AML M3. At the same time, the revealed negative association of CD150 expression with poor prognostic factor CD56 in AML M3 subtype also allows us to suggest potential prognostic value of CD150 examination in AML patients.**

Key Words: **acute myeloid leukemia, CD150, bone marrow cells, immunophenotype.**

DOI: 10.32471/exp-oncology.2312-8852.vol-44-no-3.18307

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy characterized by the proliferation of myeloid blasts that fail to undergo normal differentiation due to the accumulation of genetic lesions in hematopoietic stem cells [1]. Based on morphological, cytogenetic, immunophenotype and molecular features of malignant AML cells, different diagnostic classification systems exist. French — American — British (FAB) classification system based on the morphological and cytochemical method categorized AML subtypes as M0 to M7 [2, 3]. More recent classification systems such as WHO classification include genetic abnormalities as key factors of AML prognostication [4]. Differentially expressed signaling molecules including cell surface receptors could be valuable markers for precise diagnosis and prognosis. Moreover, the pattern of cell surface receptor expression partially reflects the status of the signaling network of neoplastic cells and could be involved in regulation of their biological properties. That is why more accurate evaluation of immunophenotype and molecular profiles of AML subtypes could help to improve prognostic and predictive power leading to the development of efficient targeted therapies for AML subsets.

The CD150 cell surface receptor is a known marker of activated B and T lymphocytes [5]. In hematologic malignancies, CD150 cell surface expression is restricted to cutaneous T-cell lymphomas, few types of B-cell non-Hodgkin's lymphoma, near half of cases of chronic lymphocytic leukemia, Hodgkin's lymphoma, and multiple myeloma. Differential expression among various types of hematological malignancies allows considering CD150 as a putative diagnostic and potential prognostic marker [6]. However, CD150 expression in neoplasms of myeloid origin is unclear.

To elucidate the expression profile of CD150 in AML, a bioinformatics search of the Oncomine™ Platform database was applied. Oncomine™ is a public database and web platform where the results of DNA microarrays of tumors of different histogenesis are collected and presented in order to facilitate research using whole-genome analysis of gene expression (http://www.oncomine.com). Haferlach Leukemia 2 Statistic with the largest number of analyzed samples (1152) and genes (910) was chosen for analysis of CD150 expression among the large number of DNA microarray datasets presented. According to Haferlach Leukemia 2 Statistic, CD150 mRNA is expressed in AML in approximately 20% of cases. In contrast, in chronic lymphocytic leukemia, CD150 mRNA at various levels is detected in 80% of cases. Bioinformatics analysis suggests that the expression of CD150 is not widely represented among AML cases, however, the detection of CD150 mRNA in a small cohort of cases may indicate the feasibility of further screening of CD150 expression on clinical samples. Therefore, we focused on evaluation of CD150 cell surface expression in AML subtypes with the aim of finding any association with clinical and immunophenotype parameters.

Submitted: July 16, 2022.

*Correspondence: E-mail: larisash70@ukr.net

Abbreviation used: AML – acute myeloid leukemia; BM – bone marrow; ECD – electron coupled dye; FAB – French – American – British; FITC – fluorescein isothiocyanate; FSC – forward scatter; iMFI – the index of mean fluorescence intensity; mAbs – monoclonal antibodies; PC5 – phycoerythrin-cyanin 5; PC7 – phycoerythrin-cyanine 7; PE – phycoerythrin; SSC – side scatter.

MATERIALS AND METHODS

AML patients. Bone marrow (BM) aspirates from patients with newly diagnosed acute leukemias were obtained from Kyiv City Clinical Hospital No. 9, Dnipro Clinical Hospital No. 4 and Ivano-Frankivsk regional clinical hospital. Lineage differentiation of acute leukemias and subtypes of AML were verified in 55 patients at the Department of Oncohematology of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Science of Ukraine (IEPOR NASU) according to the criteria proposed by the FAB Cooperative Group based on morphological and cytochemical examination of BM smears and supplemented by immunophenotypic analysis of BM leukemia (blast) cells. All patients provided an informed consent; the study was carried out in accordance with the Declaration of Helsinki, and Research Ethics Committee of IEPOR NASU. The clinicopathological details of AML patients are summarized in Table 1.

Table 1. Clinico-pathological characteristics of AML patients

Patients characteristics	n = 55
Sex	
Male, n (% of total)	19 (34.5)
Female, n (% of total)	36 (65.5)
Age, years	
Male, median (range)	70 (30–81)
Female, median (range)	58 (30–76)
Leukocyte count · 10^9/L (median; range)	48 (1–450)
Thrombocytes count · 10^9/L (median; range)	75.5 (11–149)
Hemoglobin, g/L (median; range)	68 (9–214)
FAB classification, n (% of total)	
M1	5 (9.1)
M2	10 (18.2)
M3	9 (16.3)
M5	31 (56.4)

Flow cytometry. Immunophenotyping of BM cells from AML patients was performed using a standard direct immunofluorescent method with a panel of monoclonal antibodies (mAbs) against myeloid- and lymphoid-associated antigens recommended by Bethesda International Consensus [7] and EuroFlow Consortium [8] for the diagnosis and classification of hematological malignancies. The list of mAbs labeled by fluorescein isothiocyanate (FITC), phycoerythrin (PE), electron coupled dye (ECD), PE-cyanin 5 (PC5) and PE-cyanine 7 (PC7) used in our study is shown in Table 2. All mAbs except anti-CD150 FITC and anti-CD45 PC5.5 (IEPOR NASU) were obtained from Becton Dickinson (USA). Labeling of BM cells with mAbs was performed in 2–4 color combinations. The BM samples for flow cytometry analysis were prepared as follows: 30 µl EDTA anticoagulated BM sample containing up to 5 x 10^5 leukocytes was added into each 12 × 75 mm polystyrene test tube (Beckman Coulter, USA) with various combinations of FITC/PE/ECD/PC7 conjugated mAbs at a working concentration. Samples were immediately vortexed for 3 s and incubated for 20 min at 17–22 °C in the dark. Then 200 µl lysing solution OptiLyse C (Beckman Coulter, USA) was added to the tube, and vortexed for 3 s. Probes were incubated for 10 min at room temperature in the dark. Samples were washed twice in 2 ml PBS using centrifugation at 400 g for 5 min. At the final stage, BM cells from the AML patients were resuspended in 0.5 ml of PBS containing 0.1% paraformaldehyde. For cytoplasmic detection of MPO, CD3, CD22, CD79a, µ-immunoglobulin heavy chain the samples were permeabilized before staining with 50 µl IntraPrep (Beckman Coulter, USA) for 15 min. Measurements were conducted using Beckman Coulter DxFLEX (Beckman Coulter, USA) and data analysis was performed with CytExpert for DxFLEX software. The results were obtained by gating the cells populations with side scatter (SSC) *vs* forward scatter (FSC) followed by SSC *vs* CD45 gating. It total, 10,000 CD45 positive events per tube was acquired into FSC *vs* SSC. The BM cells were considered positive on cell surface antigen expression when > 20% of the gated leukemic cells reacted with a specific mAbs and > 10% cell positivity was considered for cytoplasmic antigen. The CD150 antigen results are presented as the percentage of positive cells and the index of mean fluorescence intensity (iMFI) — MFI ratio of antigen to isotype control, which were calculated on the histograms. iMFI was calculated to determine the expression level of CD150.

Table 2. Fluorochrome-labeled monoclonal antibodies used for immunophenotyping of BM cells

Fluorochrome	Marker
FITC	CD10, CD11b, CD13, CD36, CD38, CD150, MPO, µ
PE	CD11c, CD14, CD22, CD33, CD79a, CD117
ECD	CD3, CD19, CD56
PC5.5	CD3, CD 15, CD34, CD45, HLA-DR
PC7	CD45

Statistical analysis. To analyze statistical significance of the data, Student's *t*-test was used. *p*-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Based on the FAB classification, we identified four AML subtypes (Table 1). The prevalent AML type among analyzed patients was acute monocytic/monoblastic leukemia (AML M5, 56.4% of cases) while acute myeloblastic leukemia with maturation (AML M2), acute promyelocytic leukemia (AML M3) and the acute myeloblastic leukemia with minimal maturation (AML M1) were classified in 18.2%, 16.3%, and 9.1% of cases respectively. The immunophenotype features of BM blast cells of AML

Table 3. Immunophenotype of BM leukemia cells in AML patients

FAB subtype	Marker, n/%, range										
	CD11b	CD11c	CD13	CD14	CD15	CD33	CD34	CD36	CD38	CD117	HLA-DR
M1	0	0	3/60	0	0	4/80	2/40	1/20	4/80	4/80	1/20
(n = 5)			35–76			64–96	51–77	27	50–93	49–84	78
M2	7/70	3/30	6/60	0	3/30	10/100	4/40	2/20	10/100	10/100	6/60
(n = 10)	24–54	22–67	36–57		22–46	40–99	65–86	21–37	25–95	35–91	22–80
M3	0	0	7/77.8	2/22.2	4/44.4	9/100	1/11.1	0	9/100	9/100	0
(n = 9)			21–61	25–33	28–38	77–99	27		30–95	28–99	
M5	24/77.4	25/79.6	16/51.6	9/29.0	10/32.3	27/87.1	18/58.1	21/67.7	27/87.1	20/61.3	23/74.2
(n = 31)	22–87	26–94	24–89	22–64	23–60	21–99	23–73	24–95	21–97	21–88	28–56

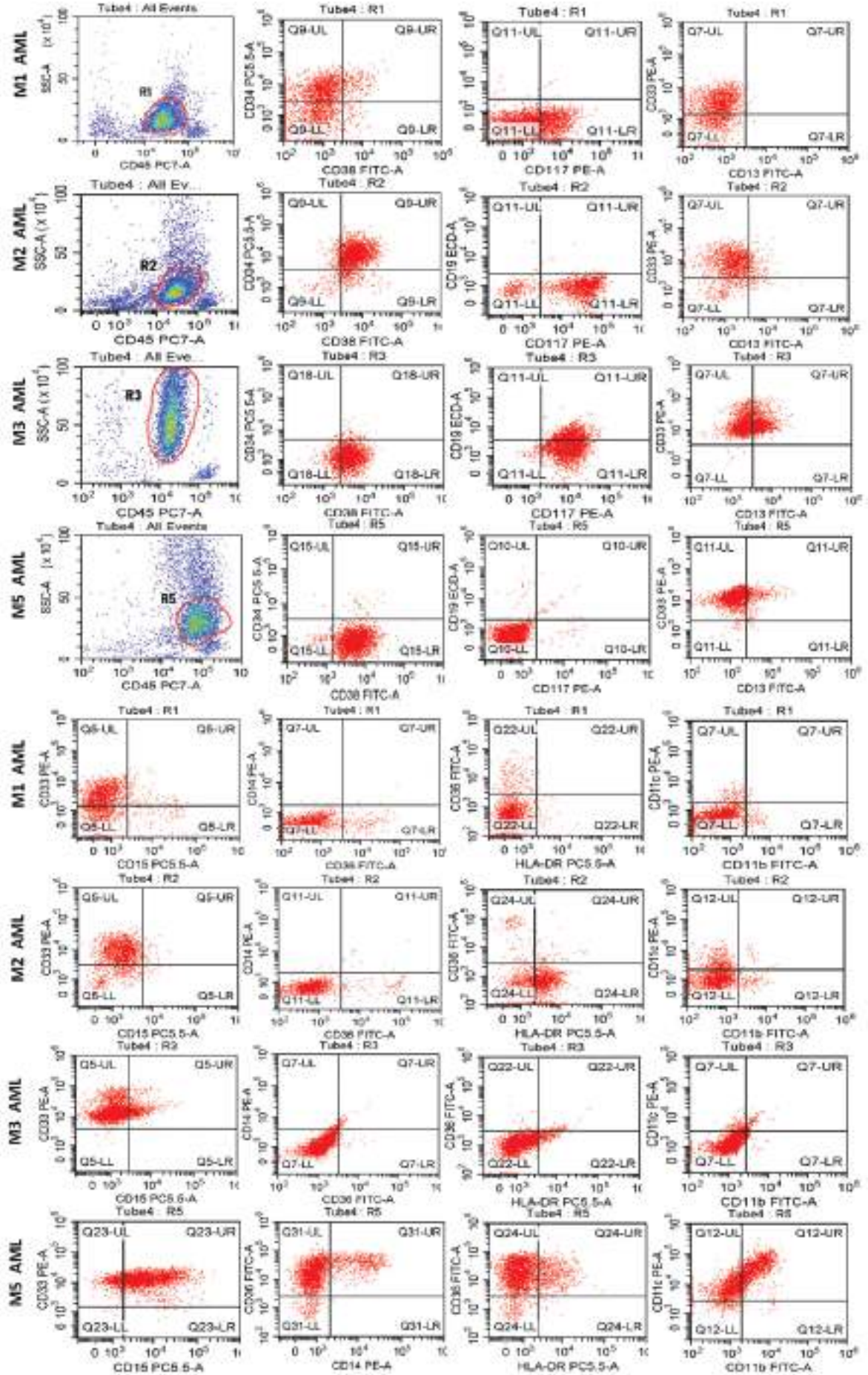

Fig. 1. Representative results of immunophenotyping of BM leukemic cells in AML M1, M2, M3 and M5 patients by flow cytometry. Leukemic cells were gated on CD45 *vs* SSC dot plots

patients taking into account different FAB AML subtypes are presented in Table 3 and Fig. 1.

The CD45 *vs* SSC gated blast population of AML M1 has moderate CD45 expression and low (basic cell population located below 30 of the SSC axis) or moderate (basic cell population located between 30 and 50 of the SSC axis) SSC signals (Fig. 1). The AML M1 blast population showed its inherent expression of CD13, CD33, CD38 and CD117 in most analyzed cases (Fig. 1, Table 3). The CD34 expression was observed in 40% of patients. The percentage of HLA-DR positive cases among AML M1 was low, which is not typical for this AML subtype and can be explained by the small number of cases included in this study. It should be noted that previously HLA-DR negative cases among AML M1 were also described, in association with FLT3 mutations (ITD and D835) of blast cells [4, 9, 10]. In general, loss of HLA-DR expression is a feature of malignant promyelocytes in AML M3 [4, 11]. The HLA-DR negative BM blasts of AML M3 patients have a number of biological features that distinguish them from those in other AML subtypes (non-M3 AML) [12, 13]. There were found the differences between some clinical characteristics of patients in HLA-DR negative AML M3 and non-M3 AML groups, in particular in leukocytes and platelet count [14, 15]. According to our study, in patients with HLA-DR negative non-M3 AML (n = 27) median leukocyte counts (88 • 10^9/L) and median platelet counts (77.5 • 10^9/L) were significantly higher compared with the HLA-DR negative AML M3 group (n = 6) (16 • 10^9/L and 37 • 10^9/L, respectively), which confirms the data of previous studies. It should be noted that median leukocyte count (76 • 10^9/L) in HLA-DR negative AML M1 subgroup (n = 4) was also higher than that in HLA-DR negative AML M3 subgroup, but median platelet count (21 • 10^9/L) was lower ($p < 0.05$). In patients with HLA-DR negative AML M2 (n = 4) and AML M5 (n = 19), both the median leukocyte counts (152 × 10^9/L and 67 • 10^9/L, respectively) and platelet counts (83 • 10^9/L and 92 • 10^9/L, respectively) were higher compared to those of AML M3. Median hemoglobin concentration in HLA-DR negative AML subtypes differed insignificantly ($p > 0.05$), which was also reported by other authors [15, 16].

According to the criteria of FAB classification, blasts of AML M2 as well as AML M1 possess characteristics of immature myeloid cells. Myeloid blasts of AML M1 and AML M2 on CD45 *vs* SSC gating dot plots have moderate CD45, however they differ in SSC signal. AML M2 blasts showed slightly higher SSC signal than AML M1 blasts (Fig. 1). Differences in immunophenotype were also found (Table 3). The most significant differences were detected in the profile of CD11a, CD11b and CD15 expression. Negative expression of CD11a, CD11b and CD15 was a specific feature of AML M1 blasts, while 30% of AML M2 cases expressed CD11b and CD15, 70% of AML M2 — CD11a. The AML M2 immunophenotype was characterized by the expression of CD33, CD38 and CD117 in 100% cases. The CD13 and HLA-DR expression was detected in the majority of AML M2 patients.

Also we studied 9 cases of AML M3. AML M3 subtype is characterized by clonal proliferation of promyelocytes in the BM as well as in the peripheral blood. These leukemic cells on CD45 *vs* SSC gating dot plot have moderate CD45 with low to high (> 50 of the SSC axis) SSC profile (Fig. 1). The profile of CD34 and HLA-DR expression are used for the differentiation of AML subtypes. Usually, AML M3 is characterized by the lack or weak expression of CD34 and absent HLA-DR [17, 18]. In our study, 8 (88.9%) from 9 cases of AML M3 were negative for both CD34 and HLA-DR. Expression of CD34 was observed only in one case, which corresponded to the hypogranular variant of AML M3 with moderate SSC. In addition, immunophenotype analysis of AML M3 leukemia cells revealed expression of CD33, CD38 and CD117 in all examined patients and CD13 in 77.8% of patients. The expression frequencies of CD14 and CD15 were low. We did not detect expression of CD11a, CD11b and CD36 in AML M3 subtype similar to that in published literature [19]. In addition, the expression of CD56 was not detected in any of the studied AML M3 cases.

The BM leukemia cells of AML M5 were located in the monocytic area of CD45 *vs* SSC dot plot. Given that AML M5 subtype included both acute monoblastic leukemia and acute monocytic leukemia cells, CD45 gated leukemic cells had different MFI of CD45 and SSC signal varied from moderate to high (Fig. 1). Immunophenotype of AML M5 leukemic cells (n = 31) showed myeloid and monocytic lineage markers CD11b, CD11c, CD13, CD33, CD34, CD36, CD38, and CD117 expression in most cases. Expression of CD14, which is a marker of cells of monocytic origin, was revealed only in 29% of AML M5 patients (Table 3).

Therefore, based on the established complex of leukemia cells characteristics, M1, M2, M3 and M5 subtypes of AML were identified for further study of CD150 expression.

Using a 20% cut-off point to determine the positive or negative status of the antigen, CD150 expression was found in 14 (25.5%) of 55 patients with AML (Fig. 2, *a*). Half of all CD150 positive cases were identified among patients with AML M3. Analysis of AML M3 cases showed heterogeneity of CD150 expression on promyelocytes (Fig. 3). The BM blast immunophenotype profile of 77.8% AML M3 patients (7 of 9 cases) included CD150 expression on 43.2-83.8% of leukemia cells (Table 4). Only two cases of AML M3 were classified as CD150-negative since the percentage of CD150 positive cells was 3.4% and 15.6%. It should be noted that CD150-negative cases were found among the hypogranular variant of AML M3.

As mentioned earlier, CD34 and HLA-DR expression patterns with different lineage-specific markers are used for immunophenotype characterization of the blast cell lineage, stage of maturation that provides differential diagnosis lineage-associated acute leukemias. The CD150 expression was significantly

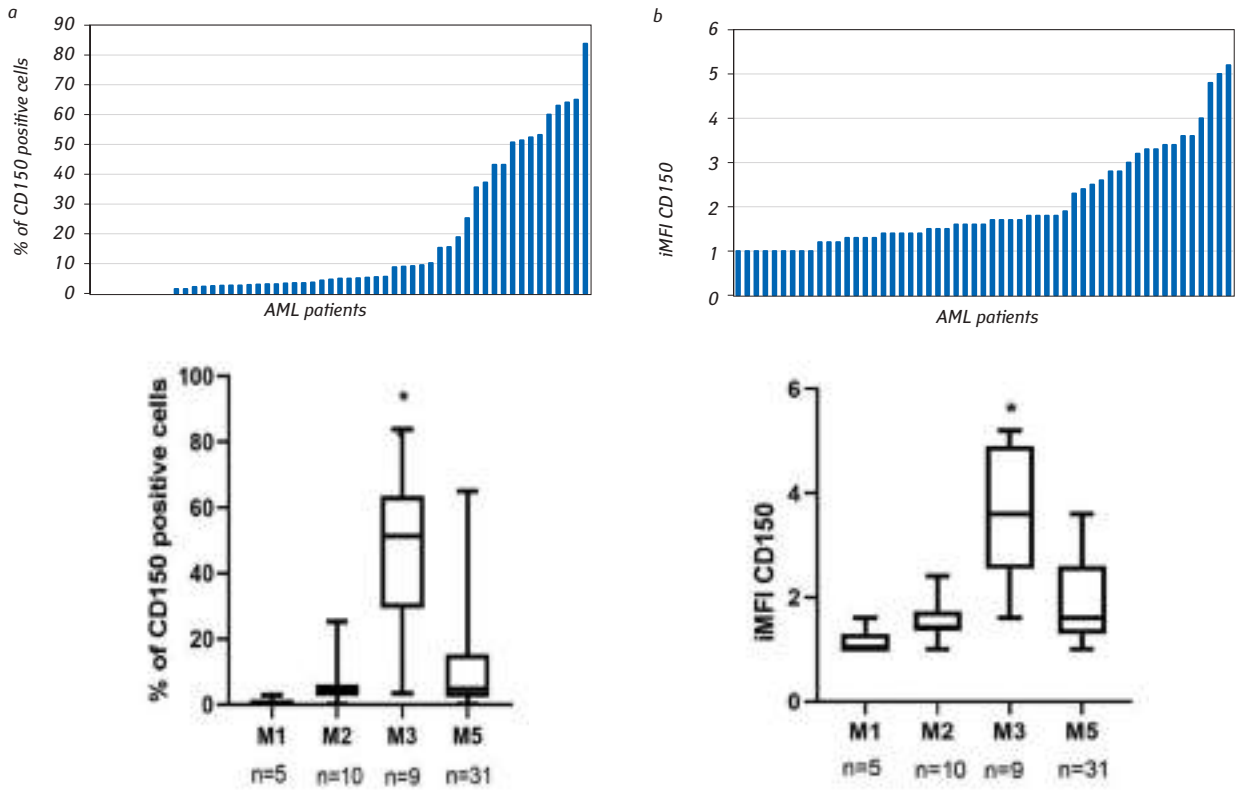

Fig. 2. Cell surface expression of CD150 in acute myeloid leukemia. Percent of CD150 positive leukemia cells (*a*) and CD150 expression level (*b*) in 55 primary bone marrow samples from AML patients. Mean fluorescence intensity (MFI) of CD150 were normalized to the MFI of isotype control of each sample. The median of CD150 expression and iMFI CD150 of M3 AML cases were significantly higher compared to that in the non-M3 AML cases ($p < 0.01$)

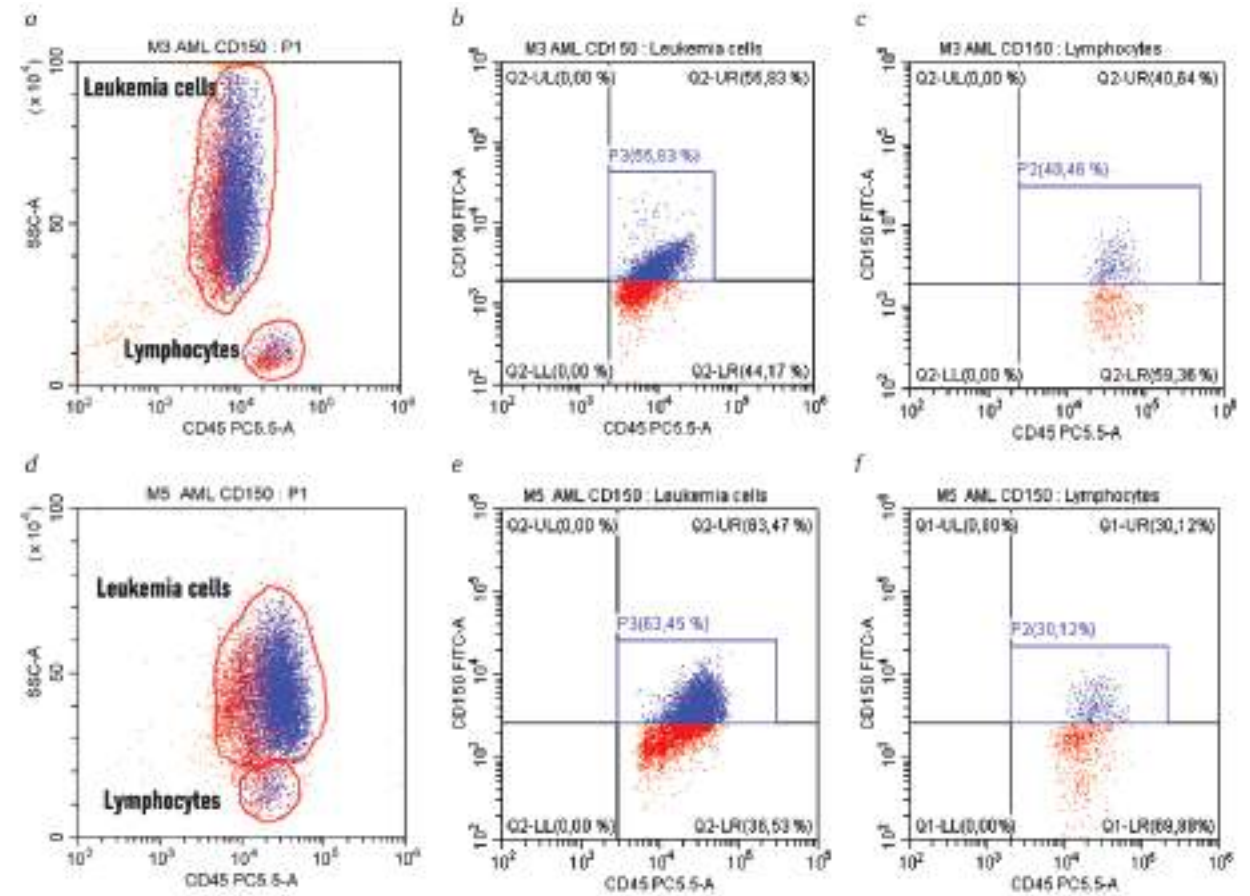

Fig. 3. CD150 expression on bone marrow cells of patients with acute myeloid leukemia. Distribution of CD150 positive cells (blue plots) among bone marrow cells patients with AML M3 (*a*) and AML M5 (*d*) on CD45 *vs* SSC dot plots. CD150 positive expression on both leukemia cells and lymphocytes in AML M3 (*b*, *c*) and AML M5 (*e*, *f*)

Table 4. Features of CD150 expression on bone marrow cells of patients with different AML subtypes

FAB subtypes	Leukemia cells			Lymphocytes	
	CD150 positive cases, n/%	Median of CD150 expression, %, range	Median of iMFI CD150, range	Median of CD150 expression, %, range	Median of iMFI CD150, range
M1	0	–	–	18.0	3.3
(n = 5)				16.0–22.0	1.9–4.1
M2	1/10.0	25.3	2.4	19.8	3.7
(n = 10)				7.0–31.0	1.2–5.6
M3	7/77.8	52.4	4.1	27.0	5.7
(n = 9)		43.2–83.8	2.8–5.2	9.0–57.0	2.9–10.8
M5	6/19.4	48.2	3.2	17.0	3.4
(n = 31)		35.6–63.5	2.8–3.6	5.0–52.3	1.2–7.4

associated with CD11c, CD11b, CD14, CD34, CD36, CD56 and HLA-DR negative expression and CD33, CD38, CD117 positive expression among the examined cohort of patients with AML M3 ($p < 0.05$).

The frequency of CD150 positive cases in non-M3 AML subtypes was low. We did not detect CD150 positive cases among AML M1. The CD150 positive leukemia cells (> 20%) were detected only in 1 (10.0%) of 10 patients with AML M2 and 6 (19.4%) of 31 patients with AML M5 (Table 4).

The CD150 positive leukemia cells of a patient with AML M2 were detected in CD45 moderate/SSC low area, i.e. location of the main population of blast cells with $CD33^+$, $CD34^-$, $CD117^+$, $CD38^+$, $CD11b^+$, $CD11c^-$, $CD13^-$, $CD14^-$, $CD15^-$, $HLA\text{-}DR^-$ immunophenotype profile was atypical for this AML subtype. It is possible that clonal changes in leukemia cells supported with atypical immunophenotype for AML M2 lead to the expression of CD150.

The immunophenotype of AML M5 leukemia cells was characterized by negative CD150 expression in the majority of studied cases. The CD150 expression in the range of 35.6–63.5% positive leukemia cells was found in 19.4% of AML M5 subtype cases. CD150 positive cells are usually evenly located within the leukemia cell population with $CD11b^+$, $CD11c^+$, $CD13^-$, $CD14^-$, $CD15^-$, $CD33^+$, $CD34^-$, $CD36^-$, $CD38^+$, $CD117^+$, $HLA\text{-}DR^-$ immunophenotype profile. The exception was 1 of 6 AML M5 cases because CD150 positive cells were located in the area of more mature monocyte cells (Fig. 3). In this case, leukemia cells had several immunophenotype alterations that are distinguished from other CD150 positive cases. In particular, we identified CD14, CD36, HLA-DR expression together with the lack of CD38 and CD117. Usually, such immunophenotype is observed in maturing promonocytes and monocyte cells. The median percentage of CD150 positive leukemia cells in patients with AML M3 is significantly higher than that in non-M3 AML patients ($p < 0.05$) (Fig. 2, *b*).

Besides the percentage of CD150 positive cells, the expression intensity of this marker was also evaluated. Among all CD150 positive AML cases, iMFI CD150 was highly variable and ranged from 2.4 to 5.2 (Table 4). Level of CD150 expression significantly differed between AML M3 and non-M3 AML subtypes (Fig. 2, *b*). Analysis of CD150 expression level on BM blast cells in the general cohort of patients showed that median iMFI of CD150 was generally higher in patients with AML M3 (3.6) than that in patients with AML M1 (1.0), AML M2 (1.4) and AML M5 (1.6). Individual values of iMFI CD150 among CD150 positive cases of AML in most patients with AML M3 were statistically higher than those in other subtypes ($p < 0.05$). No significant differences were found in the expression level of CD150 between non-M3 AML subtypes.

The expression of CD150 was evaluated also on BM lymphocytes from AML patients, since these cells were distinctively segregated from the blast cell population on the scattergram SSC *vs* CD45. In all AML patients, BM lymphocytes were CD150 positive. The percentage of CD150 positive lymphocytes ranged from 5.0 to 57.0% and iMFI CD150 was 1.2–10.8 (Table 4). The median of both the percentage of CD150 positive lymphocytes and the iMFI CD150 in patients with AML M3 was higher than in patients with non-M3 AML ($p < 0.05$) (Table 4).

In the general cohort of patients with AML, no significant difference was found between clinical characteristics of patients and the expression profile of CD150 on leukemic cells.

Thus, in this study for the first time CD150 expression profile and CD150 expression level were evaluated in BM cells of patients with different AML subtypes. How important is the determination of CD150 expression in the differential diagnosis of AML subtypes?

The modern WHO classification of AML takes into account a complex of morphological, immunophenotypic, cytogenetic and molecular characteristics of leukemic cells. However, the FAB classification of AML based on the complex of morphological, cytochemical and immunophenotypic features of blasts is used when cytogenetic and molecular genetic studies could not be provided [20].

To date, AML blasts immunophenotype characterization by flow cytometry is the standard method of diagnostic and monitoring of AML. Moreover, results of flow cytometry immunophenotype are usually available before the results of molecular studies, which minimize the risk of wasting time for the patient [21, 22].

A panel of mAbs to leukocyte differentiation antigens, makes it possible to determine the lineage of differentiation, the stage of the differentiation block of leukemia blasts, and hence identify the AML subtype [7, 23, 24]. In the differential diagnostic of AML, it is mandatory to use a panel of mAbs to lineage-associated antigens (granulocyte and monocyte lineage) and maturation stage-associated antigens. In addition to the above, the mAbs against the markers

of lymphoid cells CD2, CD3, CD5, CD7, CD19, CD22, CD79a and natural killer cells CD56 are also important and some of them have prognostic value [25–28].

The CD150 was initially identified as a marker of activated T and B lymphocytes [5, 29], but later its expression was detected at different stages of development of both normal B-lymphocytes (from pro-B to plasma cells) [30] and T-lymphocytes (from immature $CD4^-CD8^-$ thymocytes to effector T cells) [31]. It should be noted that the expression level of CD150 on B- and T-lymphocytes of peripheral blood and tonsils, as a rule, increases with their maturation [32, 33]. The expression profile of CD150 on cells of the monocyte and granulocyte differentiation lineages has not been studied enough. There are only a few data that demonstrated CD150 expression in neutrophils and eosinophils [31]. The CD150 is not expressed on the cell surface membrane of monocytes, but its expression appears on activated macrophages. Importantly, cell surface membrane CD150-negative monocytes have a positive cytoplasmic reaction for CD150, where it colocalizes with early recirculating endosomal compartments [34].

It is well known that CD150 is aberrantly expressed on malignant B-lymphocytes compared to that on their developmentally related normal B-cell subsets. Thus, CD150 negative B-cell malignancies include pre-B acute lymphoblastic leukemia, small lymphocytic lymphoma, sporadic Burkitt lymphoma, germinal center subtype of diffuse large B-cell lymphoma, lymphoplasmacytic lymphoma, and primary cutaneous marginal zone B-cell lymphoma [6]. However, there have been no previous attempts to determine CD150 expression on malignant myeloid cells.

In this study, we have examined CD150 expression on BM cells of patients with AML. Within FAB subtypes, we observed a high frequency of CD150 expression in AML M3 cases, and significantly lower in AML M5, AML M2, and all BM blast cells from patients with AML M1 were CD150 negative. What does this indicate? The appearance of both M0 and M1 AML is a result of myeloid lineage differentiation arrest leading to the accumulation of aberrant immature myeloid precursors with the corresponding phenotype. The AML M2 blasts exert more pronounced signs of granulocyte differentiation [35]. Only in one case of AML M2, CD150 expression was found on 25.3% of blasts. The majority of patients with AML M2 had the typical for this AML subtype phenotype of blasts: $CD11b^+$, $CD11c^-$, $CD13^+$, $CD14^-$, $CD15^-$, $CD33^+$, $CD34^+$, $CD36^-$, $CD38^+$, $CD117^+$, $HLA\text{-}DR^+$. However, the leukemia cells immunophenotype profile in patient with CD150 positive blasts differed by the lack of CD13, CD34, HLA-DR expression. HLA-DR and CD34 negativity has been observed in some M1 and M2 AML cases [18, 36], which was associated with *FLT3* and *NPM1* mutations [9, 37, 38]. It is possible that the presence of CD150 positive cases among AML M2 is also associated with individual genetic disorders, but this requires further research.

Mutation accumulation that leads to the myeloid differentiation block at the promyelocyte stage results in AML M3 [39]. The AML M3 subtype is a more differentiated form of AML compared to AML M0–M2. The flow cytometry strategy for diagnosing AML M3 is constantly being improved. The expression status of CD11a, CD11b, CD11c, CD13, CD18, CD33, CD34, CD117 and HLA-DR is used most often for the flow cytometry based identification of AML M3 [27, 39–42]. However, a number of studies have shown that estimating only the expression profile of CD34, CD117 and HLA-DR can distinguish AML M3 from non-M3 AML with high accuracy. The combination of $CD34^-$, $HLA\text{-}DR^-$ and $CD117^+$ can specifically identify an AML M3 case [12, 43]. In our series of 9 cases, the immunophenotype of AML M3 leukemic cells showed classical profile and was associated with the expression of CD13, CD33, CD38, CD117 and negative expression of CD11b, CD11c, CD34, HLA-DR.

Most AML M3 (77.8% patients) cases were characterized by CD150 expression, which was detected in 43.2–83.8% of blasts. In the other two cases, CD150 expression was found on 3.4% and 15.6% of leukemic cells. It should be noted that in some works had been used cutoff > 10% to determine the positivity of the marker expression [44]. Under such conditions, the percentage of CD150 positive cases may be higher. We also established a relationship between CD150 expression and some cell markers used in the diagnosis of AML M3. Some of these markers, CD56, CD34 and HLA-DR, have a negative impact on AML M3 prognosis [28, 42, 44–46]. Based on differential CD150 expression in hematological neoplasms, prognostic value of CD150 could also be considered. Moreover, positive expression of CD150 on chronic lymphocytic leukemia B-cells is associated with favorable clinical outcome and longer overall survival of patients [47, 48]. We found that CD150 expression on BM blasts of AML M3 was accompanied by negative CD56, CD34 and HLA-DR expression. Therefore, evaluation of the CD150 expression on leukemia cells of patients with AML M3 may have prognostic significance and could be associated with favorable clinical outcome, but large-scale clinical trials are needed to determine in more detail the possible association between CD150 expression and clinical prognosis.

The BM cells in AML M5 are characterized by delayed maturation of monocyte cell lineage at the stage of monoblasts or more mature promonocytes. These cells can be easily distinguished by light scattering and phenotype profile by flow cytometry [49, 50]. AML M5 BM cells often express CD4, CD11b, CD11c, CD13, CD14, CD15, CD33, CD36, CD38, CD65, HLA-DR, less often CD2, CD7, CD34, CD117 [25, 26, 51]. The expression frequency of some of these markers is associated with the maturity degree of the main population of leukemia cells, which was clearly identified in our studies. A small subset of AML M5 cases showed CD150 expression which was detected on leukemia cells with immunophenotype features of monoblasts,

promonocytes and monocytes. Conditions under which CD150 expression is observed at the early stages of monocyte maturation require further study. Although the percentage of CD150-positive leukemia cells in patients with AML M5 was similarly high in patients with AML M3, the median iMFI CD150 was significantly higher in cases of AML M3 compared to AML M5 CD150 positive cases (4.1 *vs* 3.2, $p < 0.05$). In addition, the expression level of CD150 on BM lymphocytes in patients with AML M3 was also significantly higher than that in patients with non-M3 AML ($p < 0.05$). Since level of antigen expression is one of the features used to characterize the cell population, iMFI of CD150 may be an informative indicator for BM leukemic cells of AML patients [52]. In addition, comprehensive consideration of CD150 antigen distribution within cell population and expression intensity may help to determine the stage of myeloid differentiation block, and hence the AML subtype.

In summary, we have shown for the first time the profile of CD150 cell surface expression in BM leukemia cells of patients with AML. The CD150 expression at the high level is primarily associated with the AML M3 subtype. The CD150 positive reaction on BM cells was low and not frequent in AML M5 cases and absent in AML M1 and 90% of AML M2 cases.

The CD150 expression evaluation among other phenotype markers included in the AML diagnostic panel may improve the identification of impaired maturation of blast cells at the promyelocyte stage and the development of AML M3 subtype. In addition, CD150 is rarely detected on monoblasts and promonocytes in patients with AML M5 making CD150 an unreliable marker for differential diagnosis of this AML subtype. At the same time, revealed association of CD150 expression with negative CD56 expression in AML M3 subtype also allow to propose potential prognostic value of CD150 examination in AML patients. The facts of aberrant CD150 expression in B-cells malignancies, its functional significance, and differential expression of CD150 isoforms allow us to assume the perspective of determining biological function of CD150 in AML positive subtypes.

ACKNOWLEDGEMENTS

The work was supported by scientific grant № 0120U100412 “To Develop Modern Precision Methods of Acute Myeloid Leukemia Diagnosis Using Immunophenotypic, Epigenetic and Molecular Markers of Leukemic Stem Cells” from research program of the NAS of Ukraine.

REFERENCES

1. **Newell LF, Cook RJ.** Advances in acute myeloid leukemia. BMJ 2021; **375:** n2026. doi: 10.1136/bmj.n2026

2. **Bennett JM, Catovsky D, Daniel MT, *et al*.** Proposals for the classification of the acute leukaemias French-American-British (FAB) co-operative group. Br J Haematol 1976; **33:** 451–8. doi: 10.1111/j.1365-2141.1976.tb03563.x

3. **Bennett JM**, **Catovsky D, Daniel MT, *et al*.** Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985; **103:** 620–5. doi: 10.7326/0003-4819-103-4-620

4. **Arber DA**, **Orazi A, Hasserjian R, *et al*.** The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016; **127:** 2391–405. doi: 10.1182/blood- 2016-03-643544

5. **Sidorenko SP, Clark EA.** Characterization of a cell surface glycoprotein IPO-3, expressed on activated human B and T lymphocytes. J Immunol 1993; **151:** 4614–24.

6. **Gordiienko I**, **Shlapatska L, Kovalevska L, *et al*.** SLAMF1/CD150 in hematologic malignancies: Silent marker or active player? Clin Immunol 2019; **204:** 14–22. doi: 10.1016/j.clim.2018.10.015

7. **Wood BL**, **Arroz M, Barnett JM, *et al*.** 2006 Bethesda International Consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. Cytometry B Clin Cytom 2007; **72:** 14–22. doi: 10.1002/cyto.b.20363

8. **van Dongen JJ**, **Orfao A.** EuroFlow: Resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics and personalized medicine. Leukemia 2012; **26:** 1899–907. doi: 10.1038/leu.2012.121

9. **Syampurnawati M**, **Tatsumi E, Furuta K, *et al*.** HLA-DR-negative AML (M1 and M2): FLT3 mutations (ITD and D835) and cell-surface antigen expression. Leuk Res 2007; **31:** 921–9. doi: 10.1016/j.leukres.2007.11.017

10. **Kussick SJ**, **Yi HS, Sheets KM, *et al*.** A distinctive nuclear morphology in acute myeloid leukemia is strongly associated with loss of HLA-DR expression and FLT3 internal tandem duplication. Leukemia 2004; **18:** 1591–8. doi: 10.1038/sj.leu.2403458

11. **Devi K**, **Ali N.** The curious case of HLA-DR-positive APL. Clin Case Rep 2021; **9:** 825–9. doi: 10.1002/ccr3.3683

12. **Dong HY**, **Kung JX, Bhardwaj V, *et al*.** Flow cytometry rapidly identifies all acute promyelocytic leukemias with high specificity independent of underlying cytogenetic abnormalities. Am J Clin Pathol 2011; **135:** 76–84. doi: 10.1309/AJCPW9TSLQNCZAVT

13. **Noguera NI**, **Catalano G, Banella C, *et al*.** Acute promyelocytic leukemia: update on the mechanisms of leukemogenesis, resistance and on innovative treatment strategies. Cancers (Basel) 2019; **11:** 1591. doi: 10.3390/cancers11101591

14. **Moon H**, **Lee S, Huh J, *et al*.** Characteristics of acute myeloid leukemia without HLA-DR expression. Korean J Lab Med 2007; **27:** 313–7. doi: 10.3343/kjlm.2007.27.5.313

15. **El-Meligui YM**, **Abd Elrhman HE, Salahuddin A, *et al*.** Correlation study on HLA-DR and CD117 (c-kit) expressions: its prognosis and treatment response in acute myeloid leukemia patients. Pharmgenomics Pers Med 2021; **14:** 381–93. doi: 10.2147/PGPM.S268986

16. **Oelschlaegel U**, **Mohr B, Schaich M, *et al*.** HLA-DRneg patients without acute promyelocytic leukemia show distinct immunophenotypic, genetic, molecular, and cytomorphologic characteristics compared to acute promyelocytic leukemia. Cytometry B Clin Cytom 2009; **76:** 321–7. doi: 10.1002/cyto.b.20475

17. **Paietta E**. Expression of cell-surface antigens in acute promyelocytic leukemia. Best Pract Res Clin Haematol 2003; **16:** 369–85. doi: 10.1016/s1521-6926(03)00042-2

18. **Kaleem Z, Crawford E, Pathan H, *et al*.** Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. Arch Pathol Lab Med 2003; **127:** 42–8. doi: 10.5858/2003-127-42-FCAOA

19. **Gorczyca W.** Acute promyelocytic leukemia: four distinct patterns by flow cytometry immunophenotyping. Pol J Pathol 2012; **63:** 8–17.

20. **Gluzman DF, Sklyarenko LM, Telegeev GD, *et al*.** Diagnosis of myeloid neoplasms and acute leukemias. Scientific and methodical guide. Kyiv: DIA, 2016. 124 p. (in Ukrainian)

21. **Rastogi P**, **Sachdeva MU.** Flow cytometric minimal residual disease analysis in acute leukemia: current status. Indian J Hematol Blood Transfus 2020; **36:** 3–15. doi: 10.1007/s12288-019-01118-5

22. **Bain BJ**, **Béné MC.** Morphological and immunophenotypic clues to the WHO categories of acute myeloid leukaemia. Acta Haematol 2019; **141:** 232–44. doi: 10.1159/000496097

23. **Stubbins RJ**, **Karsan A.** Differentiation therapy for myeloid malignancies: beyond cytotoxicity. Blood Cancer J 2021; **11:** 193. doi: 10.1038/s41408-021-00584-3

24. **Aanei CM**, **Veyrat-Masson R, Rigollet L, *et al*.** Advanced flow cytometry analysis algorithms for optimizing the detection of "different from normal" immunophenotypes in acute myeloid blasts. Front Cell Dev Biol 2021; **9:** 735518. doi: 10.3389/fcell.2021.735518

25. **Thalhammer-Scherrer R**, **Mitterbauer G, Simonitsch I, *et al*.** The immunophenotype of 325 adult acute leukemias: relationship to morphologic and molecular classification and proposal for a minimal screening program highly predictive for lineage discrimination. Am J Clin Pathol 2002; **117:** 380–9. doi: 10.1309/C38D-D8J3-JU3E-V6EE

26. **Klobusicka M**, **Kusenda J, Babusikova O.** Myeloid enzymes profile related to the immunophenotypic characteristics of blast cells from patients with acute myeloid leukemia (AML) at diagnosis. Neoplasma 2005; **52:** 211–8.

27. **Hamed EO**, **El-Deen AF.** Flow cytometric diagnosis of acute leukemia and aberrant antigen: sohag university experience. Open J Blood Diseases 2018; **8:** 37-48. doi: 10.4236/ojbd.2018.82005

28. **Al-Mawali A**, **To LB, Gillis D, *et al*.** The presence of leukaemia-associated phenotypes is an independent predictor of induction failure in acute myeloid leukaemia. Int J Lab Hematol 2009; **31:** 61–8. doi: 10.1111/j.1751-553X.2007.01003.x

29. **Pinchouk VG**, **Sidorenko SP, Gluzman DF, *et al*.** Monoclonal antibodies IPO-3 and IPO-10 against human B-cell differentiation antigens. Anticancer Res 1988; **8:** 1377–80.

30. **De Salort J**, **Sintes J, Llinàs L, *et al*.** Expression of SLAM (CD150) cell-surface receptors on human B-cell subsets: from pro-B to plasma cells. Immunol Lett 2011; **134:** 129–36. doi: 10.1016/j.imlet.2010.09.021

31. **Romero X**, **Benitez D, March S, *et al*.** Differential expression of SAP and EAT-2-binding leukocyte cell-surface molecules CD84, CD150 (SLAM), CD229 (Ly9) and CD244 (2B4). Tissue antigens 2004; **64:** 132–44. doi: 10.1111/j.1399-0039.2004.00247.x

32. **Laksono BM**, **Grosserichter-Wagener C, de Vries RD, *et al*.** *In vitro* measles virus infection of human lymphocyte subsets demonstrates high susceptibility and permissiveness of both naive and memory B-cells. J Virol 2018; **92:** e00131–18. doi: 10.1128/JVI.00131-18

33. **Gordiienko IM**, **Shlapatska LM, Kovalevska LM, *et al*.** Differential expression of CD150/SLAMF1 in normal and malignant B-cells on the different stages of maturation. Exp Oncol 2016; **38:** 101–7.

34. **Yurchenko M**, **Skjesol A, Ryan L, *et al*.** SLAMF1 is required for TLR4-mediated TRAM-TRIF-dependent signaling in human macrophages. J Cell Biol 2018; **217:** 1411–29. doi: 10.1083/jcb.201707027

35. **Basso G**, **Buldini B, de Zen L, *et al*.** New methodologic approaches for immunophenotyping acute leukemias. Haematologica 2001; **86:** 675–92.

36. **Wetzler M**, **McElwain BK, Stewart CC, *et al*.** HLA-DR antigen-negative acute myeloid leukemia. Leukemia 2003; **17:** 707–15.

37. **Syampurnawati M**, **Tatsumi E, Ardianto B, *et al*.** DR negativity is a distinctive feature of M1/M2 AML cases with *NPM1* mutation. Leuk Res 2008; **32:** 1141–3. doi: 10.1016/j.leukres.2007.11.017

38. **Gupta A**, **Mishra D, Chandy M, *et al*.** Human leucocytic antigen-DR negative acute myeloid leukemia: A diagnostic dilemma for hematopathologist. Clin Cancer Invest J 2014; **3:** 383–7. doi: 10.4103/2278-0513.138055

39. **Horna P**, **Zhang L, Sotomayor EM, *et al*.** Diagnostic immunophenotype of acute promyelocytic leukemia before and early during therapy with all-trans retinoic acid. Am J Clin Pathol 2014; **142:** 546–52. doi: 10.1309/AJCPPOKEHBP53ZHV

40. **Liu M**, **Weng X, Gong S, *et al*.** Flow cytometric analysis of CD64 expression pattern and density in the diagnosis of acute promyelocytic leukemia: a multi-center study in Shanghai, China. Oncotarget 2017; **8:** 80625–37. doi: 10.18632/oncotarget.20814

41. **Mosleh M**, **Mehrpouri M, Ghaffari S, *et al*.** Report of a new six-panel flow cytometry marker for early differential diagnosis of APL from HLA-DR negative non-APL leukemia. Scand J Clin Lab Invest 2020; **80:** 87–92. doi: 10.1080/00365513.2019.1700427

42. **Siraj F**, **Tanwar P, Singh A, *et al*.** Analysing "tear-drop" prints of acute promyelocytic leukemia (APML): immunophenotypic prognostication of APML by FCM. Am J Blood Res 2021; **11:** 446–57.

43. **Zhou Y**, **Jorgensen JL, Wang SA, *et al*.** Usefulness of CD11a and CD18 in flow cytometric immunophenotypic analysis for diagnosis of acute promyelocytic leukemia. Am J Clin Pathol 2012; **138:** 744–50. doi: 10.1309/AJCPQU9R3FSLKFMI

44. **Xu F**, **Yin CX, Wang CL, *et al*.** Immunophenotypes and immune markers associated with acute promyelocytic leukemia prognosis. Dis Markers 2014; **2014:** 421906. doi: 10.1155/2014/421906

45. **Pinheiro L**, **Trindade L, Costa A, *et al*.** Aberrant phenotypes in acute myeloid leukemia and its relationship with prognosis and survival: A systematic review and meta-analysis. Int J Hematol Oncol Stem Cell Res 2020; **14:** 274–88. doi: 10.18502/ijhoscr.v14i4.4484

46. **Promsuwicha O**, **Auewarakul CU.** Positive and negative predictive values of HLA-DR and CD34 in the diagnosis of acute promyelocytic leukemia and other types of acute myeloid leukemia with recurrent chromosomal translocations. Asian Pac J Allergy Immunol 2009; **27:** 209–16.

47. **Bologna C**, **Buonincontri R, Serra S, *et al*.** SLAMF1 regulation of chemotaxis and autophagy determines CLL patient response. J Clin Invest 2016; **126:** 181–94. doi: 10.1172/JCI83013

48. **Schweighofer CD**, **Coombes KR, Barron LL, *et al*.** A two-gene signature, SKI and SLAMF1, predicts time-to-treatment in previously untreated patients with chronic lymphocytic leukemia. PLoS One 2011; **6:** e28277. doi: 10.1371/journal.pone.0028277

49. **van Lochem EG**, **van der Velden VHJ, Wind HK, *et al*.** Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Cytometry B Clin Cytom 2004; **60:** 1–13. doi: 10.1002/cyto.b.20008

50. **Matarraz S**, **Almeida J, Flores-Montero J**. Introduction to the diagnosis and classification of monocytic-lineage

leukemias by flow cytometry. Cytometry B Clin Cytom 2017; **92:** 218–27. doi: 10.1002/cyto.b.21219

51. **Zushi Y**, **Sasaki M, Mori A,** ***et al***. Acute monocytic leukemia diagnosed by flow cytometry includes acute myeloid leukemias with weakly or faintly positive nonspecific esterase staining. Hematol Rep 2018; **10:** 7435. doi: 10.4081/hr.2018.7435

52. **Ouyang G**, **Xu Z, Jiang D,** ***et al***. Clinically useful flow cytometry approach to identify immunophenotype in acute leukemia. J Int Med Res 2019; **47:** 1483–92. doi: 10.1177/0300060518819637

ПРОФІЛЬ ЕКСПРЕСІЇ CD150 У КЛІТИНАХ КІСТКОВОГО МОЗКУ ХВОРИХ НА ГОСТРИЙ МІЄЛОЇДНИЙ ЛЕЙКОЗ

Л. Шлапацька, І. Гордієнко, А. Поліщук, Д. Глузман

Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького НАН України, Київ 03022, Україна

Стан проблеми: Гострий мієлоїдний лейкоз (ГМЛ) — гетерогенне захворювання, що супроводжується блоком диференціювання клітин мієлоїдного ряду через накопичення в них генетичних аномалій і клональною проліферацією мієлоїдних бластів. Різні генетичні та епігенетичні ураження, відмінності у профілі фенотипу та функціональному статусі сигнальних молекул у злоякісно трансформованих клітинах лежать в основі відповіді на терапію, прогресування захворювання та виживаності пацієнта. Тому пошук диференційно експресованих молекул і дослідження їх функції в лейкемічних клітинах різних варіантів ГМЛ надалі може виокремити такі, які мають діагностичну, прогностичну та предиктивну значущість, що сприятиме удосконаленню індивідуалізованого підходу до лікування хворих на ГМЛ. ***Мета:*** З'ясувати профіль експресії CD150 у клітинах кісткового мозку (КМ) хворих на ГМЛ. ***Матеріали та методи***: Дослідження проведено на зразках аспіратів КМ 55 хворих із вперше встановленим діагнозом ГМЛ. Для аналізу імунофенотипового профілю та експресії CD150 на клітинах КМ хворих на ГМЛ було застосовано метод проточної цитометрії. ***Результати***: Під час виконання досліджень було виділено чотири варіанти ГМЛ (M1, M2, M3 і M5). Імунофенотиповий профіль лейкемічних клітин відповідав критеріям, визначеним для цих варіантів ГМЛ. Експресію CD150 було виявлено у 14 (25,5%) із 55 хворих на ГМЛ. Переважну більшість становили хворі на ГМЛ M3, 43,2–83,8% лейкемічних клітин яких були CD150-позитивними. У хворих на ГМЛ M1 експресію CD150 не було виявлено, тоді як лише у 1 (10.0%) з 10 пацієнтів з ГМЛ M2 і 6 (19,4%) з 31 хворого на ГМЛ M5 лейкемічні клітини були CD150-позитивними. Медіана відсотка CD150-позитивних лейкемічних клітин у хворих на ГМЛ M3 є значно вищою, ніж у пацієнтів з іншими варіантами ГМЛ ($p < 0,05$). Медіана рівня експресії CD150 також була достовірно вищою у хворих на ГМЛ M3 (3,6), ніж у пацієнтів з ГМЛ M1 (1,0), ГМЛ M2 (1,4) та ГМЛ M5 (1,6). Серед досліджуваної когорти хворих на ГМЛ M3 експресія CD150 асоційована з негативною експресією CD11c, CD11b, CD14, CD34, CD36, CD56 і HLA-DR і позитивною експресією CD33, CD38, CD117. ***Висновки:*** Експресія CD150 на високому рівні є унікальною ознакою лейкемічних клітин ГМЛ M3, що дозволяє вважати цей антиген додатковим імунофенотиповим маркером для ідентифікації бластних клітин, у яких відбувся блок диференціювання на стадії промієлоцитів, та, як наслідок, — розвиток ГМЛ M3. Водночас виявлений негативний зв'язок експресії CD150 з маркером негативного прогнозу перебігу захворювання CD56 при ГМЛ M3 дозволяє припустити потенційну прогностичну цінність дослідження CD150 у пацієнтів з ГМЛ M3.

Ключові слова: гострий мієлоїдний лейкоз, CD150, клітини кісткового мозку, імунофенотип.