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Immunophenotype characterization of hematopoietic stem cells, progenitor cells restricted to myeloid lineage and their leukemia counterparts

Minireview

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The aim of this review is to evaluate recent immunophenotype knowledge of hematopoietic stem cells and their restricted progenies committed to myeloid lineage – common myeloid progenitors, myelo-monocytic progenitors, megakaryo-erythroid progenitors and granulocyte progenitors up to mature neutrophil granulocyte. This study evaluates also recent knowledge of immunophenotype of leukemic stem cells and their more differentiated progeny committed to myeloid lineage – acute myeloid leukemia blast cells with regard to their phenotypic similarity to normal stem and granulocyte committed progenitor cells. Improved knowledge of normal stem and progenitor cells phenotypes, identifying new leukemia-specific markers, searching for aberrant marker expression and evaluation of aberrant intensity or combination of various marker expressions is important for distinguishing normal cells from their malignant counterpart in view of the diagnostics of leukemias or follow-up of minimal residual disease.

Key words: Immunophenotype, CD markers, hematopoietic stem cells, lineage-restricted progenitors, leukemic stem cells, AML blast cells, multiparameter flow cytometry

Bone marrow is a mesenchyme-derived complex structure consisting of hematopoietic precursors, hematopoietic mature cells and stromal cells which provide a complex microenvironment that facilitates the maintenance of hematopoietic stem cells (HSCs) and supports the differentiation and maturation of the progenitors. In general, hematopoietic stem cells are defined as a clonogenic cells with self-renewing and multilineage differentiation capacity *in vivo*. HSCs give rise to nonself-renewing oligolineage-restricted progenitors which in turn give rise to progeny that is more restricted in its differentiation potential and finally to functionally mature cells [1, 2].

Normal stem and progenitor cells. The normal hematopoietic process of HSCs differentiation and maturation is characterized by gradually increasing or decreasing expression of specific surface markers which allows recognition of various stages of cell differentiation and assessment of commitment to different lineages (e.g. lymphoid, myeloid and erythroid) [3, 4]. Flow cytometry method enables studying hematopoiesis according to the cell-surface expression of specific CD molecules and their expression patterns are used to characterize the differentiation stages of cells belonging to various hematopoietic lineages. The development of multiparameter fluorescence-activated cell sorting (FACS) has enabled purification and high enrichment of HSCs and their progeny cells. HSCs and their more mature progeny cells reside within $CD34^+$ cell fraction and comprise ~ 0,2-3% of the nucleated cells in bone marrow, cord blood and mobilized peripheral blood [5, 6, 7]. Therefore, $CD34^+$ cell population is heterogeneous. Only a small fraction (1-10%) of $CD34^+$ cells does not express CD38 and lineage specific markers (Lin) [8] and contains cells with lymphoid and myeloid differentiation potential [9, 10, 11, 12]. The majority of $CD34^+$ cells (90-99%) coexpress CD38 antigen and this subset represents most of the lineage-restricted progenitors [13].

Assays for the determination of progenitor cells maturation. Various forms of *in vitro* clonogenic assays (colony forming assay, long-term culture initiating cell assay, LTC-IC) were developed for the determination of maturation and differentiation of HSCs and their more mature hematopoietic progenitor cells. These assays are very useful to define a variety

of progenitors and provide the proof of self-renewal capacity or multilineage differentiation potential [11, 12, 14, 15]. However, major functional properties of the HSCs i.e. bone marrow repopulating capacity and ability to maintain long-term hematopoiesis, cannot be studied in *in vitro* clonogenic assays. To analyze the functional properties of HSCs *in vivo* xenotransplant animal models were developed. The most suitable models are mice with non-obese diabetes and severe combined immunodeficiency (NOD/SCID mice). In such mice, human HSCs can repopulate the entire hematopoietic system (except T cells) [16] and can undergo unlimited self-renewal [17, 18, 19, 20]. Limited life-span of NOD/SCID mice in particular and tendency towards human B-cell development also resulted in creation of alternative xenotransplantation models [13]. Srour et al. [21] and Zanjani et al. [22] established a large animal transplantation model in which human HSCs are transplanted intraperitoneally into unconditioned, early gestational sheep fetuses.

Hematopoietic stem cells

All normal hematopoietic cells are continually generated from a small pool of pluripotent HSCs residing in bone marrow. The first experimental evidence indicated HSCs discovery in 1961 by Till and McCulloch [23] who described a population of clonogenic bone marrow cells capable of generating myelo-erythroid colonies in the spleen of lethally irradiated hosts. HSCs can be divided into a long-term subset (LT-HSC), capable of self-renewal for the whole life of the host, and a short-term subset (ST-HSC) that retain self-renewal capacity for approximately 8 weeks [24]. LT-HSCs are able to maintain in NOD/SCID mice for prolonged period and their further retransplantation into secondary lethally irradiated host can reconstitute the immune system again [23]. Majority of studies define HSCs as a most immature progenitor cells with $CD34^+CD38^-Lin^-$ immunophenotype [26, 17].

Phenotype of hematopoietic stem cells. First phenotype analyses described the most immature HSCs as cells with $CD34^+Lin^-$ ($CD38^-$, $CD2^-$, $CD3^-$, $CD14^-$, $CD15^-$, $CD16^-$, $CD19^-$, $CD24^-$, $CD33^-$, $CD56^-$, $CD66b^-$, $CD41^-$, $CD235a^-$, $HLA\text{-}DR^-$) immunophenotype [17, 19, 26, 27]. $CD34^+CD38^-Lin^-$ HSCs were able to self-renew and differentiate *in vitro* in LTC-IC, contained NOD-SCID-repopulating cells [14, 17] and some of them were able to detect even in secondary NOD-SCID transplants [28, 20]. However, $CD34^+CD38^-Lin^-$ cell fraction are still very heterogeneous with regard to surface marker expression. Recent immunophenotype studies of HSCs have showed various (negative, low or medium) expression of additional progenitor CD markers CD90 (Thy-1), CD117 (c-Kit), CD123 (IL-3Rα), CD135 (Flt3), medium expression of CD45, HLA-DR, CD133 (Prominin); low forward scatter (FSC) and side scatter (SSC) characteristics [9, 29, 30, 25].

Heterogeneous CD38 marker expression was observed by Ziegler et al. [28] when they demonstrated the highest NOD-SCID mice and fetal sheep repopulating ability in a $CD34^+KDR^+$ ($VEGFR2^+$) fraction in bone marrow, cord blood, and mobilized peripheral blood; whereas most of $CD34^+KDR^+$ cells were $CD38^+$ and only ~ 30% $CD34^+KDR^+$ were $CD38^-$.

Interestingly, another study suggests that the majority of cells within the $CD34^+CD38^-Lin^-$ compartment express the myeloid-associated lineage markers CD13, CD33 and progenitor marker CD123. Hu et al. [31] have noted that genes associated with specific lineages are expressed in mice cells with a stem cell phenotype. Taussig et al. [32] showed that CD13, CD33, well-established myeloid markers, are expressed on human long-term repopulating cells from bone marrow and cord blood. In addition, $CD34^+CD38^-Lin^-CD33^+$ cell fraction transplanted into NOD-SCID mice showed ability multilineage engraftment and repopulate the recipient bone marrow more effectively than $CD34^+CD38^-Lin^-CD33^-$ cell fraction. However, the data published by Taussig et al. [32] are in contrast to later *in vitro* results of Andrews et al. [33, 34] who found that long-term bone marrow culture initiating cells are $CD34^+CD33^-$. Therefore, more studies are needed to verify expression of lineage-specific markers on HSCs.

$CD34^-$ hematopoietic stem cells. Recent studies have described existence of human $CD34^-$ HSCs. These astonishing observations challenge a concept that HSCs necessarily and exclusively express the CD34 antigen. Osawa et al. [35] first time reported the presence of rare subpopulation $CD34^-$ cells with HSCs properties in murine bone marrow. Transplantation of murine single $CD34^-$ cells into host resulted in bone marrow repopulation and sustained multilineage hematopoietic reconstitution. Later the presence of $CD34^-CD38^-Lin^-$ cells was reported also in human bone marrow. Bhatia et al. [18] and Zanjani et al. [36] observed *in vitro* clonogenic activity and *in vivo* NOD-SCID engraftment with human $CD34^-CD38^-Lin^-$ cells. Nakamura et al. [37] reported low *in vitro* colony-forming activity and *in vivo* repopulating activity of $CD34^-$ cells, but highly purified human $CD34^-CD38^-Lin^-$ cells from normal bone marrow differentiate into $CD34^+$ cells and further rapidly proliferate and differentiate *in vitro* into erythrocytes, granulocytes, and megakaryocytes in serum-free culture, turn into $CD34^+$ cells after 10 days cultivation, and significantly increase their colony-forming potential [38]. Human $CD34^+$ cells have also been detected in animals transplanted with human $CD34^-$ cells, again suggesting that $CD34^-$ HSCs can differentiate to $CD34^+$ cells *in vivo* [36]. These observations indicate that $CD34^-CD38^-Lin^-$ cells might be upstream of $CD34^+CD38^-Lin^-$ cells in the hematopoietic hierarchy.

Types of lineage-restricted progenitors according to classical model of lymphohematopoetic differentiation

HSCs undergo cell division, become lineage-restricted and ultimately turn into unipotent progenitor cells. Differentiation of HSCs to mature cells of lymphohematopoietic system involves progressive loss of self-renewal capacity, proliferation ability and multilineage potential. Classical (dichotomy)

model of the lymphohematopoietic differentiation have been elucidated mainly in studies of the murine hematopoiesis. According to this model LT-HSCs differentiate into ST-HSCs retained self-renewal capacity for ~ 8 weeks [9] and give rise to shortly self-renewing multipotent progenitors (MPPs) [39], which then differentiate into a common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). CLPs are restricted to give rise to T lymphocytes, B lymphocytes, and natural killer cells [40]. CMPs give rise to myelo-monocytic progenitors (GMPs), which in turn produce monocytes/macrophages and granulocytes, and to megakaryo-erythroid progenitors (MEPs), which differentiate into megakaryocytes/platelets and erythrocytes [41]. Interestingly, both CMPs and CLPs can give rise to dendritic cells [41, 42, 43]. All lineage-restricted progenitors reside within $CD34^+CD38^{low/+}$ cell fraction [17, 44].

Phenotype of common lymphoid progenitors. Galy et al. [45] showed that fetal and adult bone marrow $CD34^+/CD38^+/CD10^+/Lin^-$ cells generate no myeloid progeny and contain clonal progenitors of B, NK, and dendritic cells.

Hao et al. [46] also described a $CD34^+/CD38^-/CD7^+$ lymphoid progenitor from human cord blood that give rise to B, NK and dendritic cells. Because the described $CD34^+/CD38^-/CD7^+$ progenitors reside in the $CD38^-$ fraction and ~30% of them are $CD10^-$, it was suggested, that these cells are developmentally upstream of the $CD34^+/CD38^+/CD10^+/Lin^-$ cells. Interestingly, the existence of $CD34^+/CD38^-/CD7^+$ cell population have not been observed in adult bone marrow.

Phenotype of myeloid progenitors and their more mature progeny. Manz et al. [47] identified the human counterparts of the CMPs, GMPs, and MEPs from both adult bone marrow from healthy volunteers and cord blood within the $CD34^+CD38^+$ cell fraction by the differential expression of CD45RA and IL3Ra. *In vitro* clonogenic experiments showed that candidate CMP cell fraction $CD34^+CD38^+CD123^{low}CD45RA^-$ gave rise to all types of myeloid colonies (including mixed colonies, burst-forming units erythroid, CFU-megakaryocyte, CFU-megakaryocyte-erythroid, CFU-granulocyte-macrophage, CFU-granulocyte, and CFU-macrophage). In contrast, candidate GMP cell fraction $CD34^+CD38^+CD123^{low}CD45RA^+$ gave rise exclusively to CFU-granulocyte, CFU-macrophage, and CFU-granulocyte-macrophage, whereas candidate cells of MEP fraction $CD34^+CD38^+CD123^-CD45RA^-$ produced mainly burst-forming units erythroid, CFU-megakaryocyte, CFU-megakaryocyte-erythroid. CMPs, GMPs, and MEPs cell populations displayed relatively uniform levels of CD117 (c-Kit), CD13, CD33, and HLA-DR and were negative for CD90 (Thy-1).

However, the separation of the CMPs and MEPs populations based on their moderately different CD123 (IL-3Ra) expression is difficult to achieve. Therefore, Edvardsson et al. [48] found a complementary MEP surface marker – thrombopoietin receptor (TpoR, CD110) and obtained a more effective separation of these two progenitor populations for their further characterization. *In vitro* experiments showed that $CD34^+CD123^{low}CD45RA^-TroR^-$ cell population, representing putative CMPs, showed a mixed clonogenic potential with the whole spectrum of colony-forming cells. Population of the $CD34^+CD123^{low/-}CD45RA^-TroR^+$ cells, the putative MEPs, displayed a purely megakaryocyte-erythroid clonogenicity. Edvardsson et al. [48] also found that labeling of the TpoR and the assessment of its expression offers a way to separate early and late MEPs. $CD34^+CD45RA^-CD123^{low/-}TroR^{low}$ population gave rise to CFU-megakaryocyte-erythroid, but with the higher proportion comprised CFU-megakaryocyte and on the contrary, $CD34^+CD45RA^-CD123^{low/-}TroR^{high}$ population gave rise to CFU-megakaryocyte-erythroid with a relatively large proportion of CFU-erythroid.

Multiple studies examine expression of the surface specific markers for GMPs and MEPs. When comparing this studies show that $CD34^+$ cells that are either $CD45RA^+$ or $CD45RA^-$, $CD64^+$, $CD123^+$ (IL-3Rα^+), $CD135^+$ ($Flt3^+$), $CCR1^+$ are enriched for CFU-granulocyte-macrophage, whereas $CD34^+$ cells that are $CD45RO^+$, $CD123^-$ (IL-3Rα^-), $CD135^-$ ($Flt3^-$) or $CCR1^-$ are enriched for erythroid-colony forming cells [13].

Other models of lymphohematopoietic differentiation

Some recent studies have indicated discrepancies in classical model of the lymphohematopoietic differentiation. Mori et al. [49] published study of human eosinophil differentiation *in vitro*. They showed that HSCs and CMPs generate CFU-granulocyte-macrophage and CFU-megakaryocyte-erythroid, whereas GMPs and MEPs give rise mainly to neutrophils, monocyte/macrophage and megakaryocyte-erythroid cell colonies, but without developing into the eosinophil lineage. Therefore they proposed that the eosinophil progenitor (EoP) develops from the CMP or its upstream MPP independent of the GMP and the MEP. Mori et al. [49] distinguished CMPs and EoPs according to different IL-5Rα expression. IL-5Rα is not expressed in the neutrophil and basophil lineage but is expressed on the eosinophils and EoPs. The EoP population ($CD34^+CD38^+CD123^{low}CD45RA^-IL\text{-}5R\alpha^+$), in contrast to CMPs ($CD34^+CD38^+CD123^{low}CD45RA^-IL\text{-}5R\alpha^-$), give rise only to eosinophils and they do not differentiate into basophils or neutrophils.

Alternative, so-called 'myeloid-based model' of the lymphohematopoietic differentiation was proposed by Katsura and Kawamoto [50]. This model postulates that the HSC first diverges into the common myeloid-erythroid progenitor (CMEP) and a common myelo-lymphoid progenitor (CMLP). The CMLP then generates T and B cell progenitors through a bipotential myeloid-T progenitor and a myeloid-B progenitor stage, respectively. The major difference between the myeloid based model and the classical model is that the potential to generate myeloid cells is retained in all erythroid, T, and B lineage branches in the myeloid-based model [51]. This alternative model is supported by studies using mice that showed retaining of myeloid potential in the early lymphoid progenitors in adult murine bone marrow [52].

Kawamoto et al. [53, 54, 55] also analyzed developmental potential of individual progenitors towards T, B, and myeloid lineages in the murine fetal liver by *in vitro* clonal assay. They showed consistent detection of progenitors generating myeloid and T cells and progenitors generating myeloid and B cells in the face of the complete absence of progenitors generating T and B cells without myeloid cells. Furthermore, the application of the myeloid-based model seems to explain origin of bi-phenotypic leukemia cases when myeloid-B and myeloid-T types are commonly seen, but the T–B type is rare [56, 57].

Phenotype of granulocyte progenitors

Common myeloid progenitor gives rise to 7 various cell lineages – neutrophil granulocytes, basophil granulocytes, eosinophil granulocytes, monocytes/macrophages, erythrocytes, megakaryocytes/platelets and mastocytes. Differentiation of the neutrophil progenitors is better characterized because eosinophil and basophil progenitors comprise only minor subpopulations in normal BM.

Neutrophils mature from myelo-monoblasts through a linear process, historically divided into morphological stages termed promyelocytes, myelocytes, metamyelocyte band cells, and neutrophils. Myeloblasts cannot be distinguished immunophenotypically from the most immature monocytic precursor cell. Myelo-monoblasts express $CD34^+$, $CD117^+$, $HLA\text{-}DR^+$, $CD13^+$, $CD33^+$, intermediate CD45 antigens, have intermediate FSC and low SSC characteristics. When the myeloblasts progress to the stage of promyelocytes, loss of CD34 and CD117 and subsequent expression of mature antigens CD15 and CD66b is seen; increased amounts of cytoplasm vacuoles and granules resulted in a dramatic increase in SSC. Promyelocytes are defined by acquisition of CD15 and CD66b; acquisition of CD11b, CD11c, CD24, and CD66a is characteristic for myelocytes; gain of CD55, CD16, CD35 and CD87 for metamyelocyte band cells and gain of CD10 for mature neutrophil granulocytes.

CD13 is a unique marker in the granulocytic differentiation as it is dynamically expressed during granulocytic differentiation. It is expressed at high levels on myeloblasts and promyelocytes. CD13 is downregulated and dimly expressed on myelocytes and is gradually upregulated again as the granulocytic cells develop into segmented neutrophils. Similarly, CD44 and CD55 are highly expressed in myelo-monoblast stage, then exhibit a decrease at an intermediate stage of maturation and increase again with terminal differentiation. A less common pattern of antigen expression, exemplified by CD64, is characterized by an absence of expression on myeloblasts, an increase at intermediate stages of maturation, and a loss with terminal differentiation [58, 59, 60, 61].

Wood [61] characterized one immature stage of eosinophil maturation - eosinophilic myelocyte. Eosinophilic myelocyte showing high value of SSC, intermediate expression of CD45 at a level slightly higher than neutrophilic myelocytes, low to intermediate CD11b, intermediate CD13, and low CD33 with bright CD66b without CD16. Differentiation to the mature eosinophil is accompanied by an increased level of CD45, a mild decrease in SSC, and an increase in CD11b with a decrease in CD33.

Malignant stem and progenitor cells

High proliferative potential, capacity for self-renewal and developmental plasticity make HSCs and progenitor cells ideal target for malignant transformation. Several lines of experimental evidence suggest that leukemias might contain some leukemic stem cells (LSCs), which are rare cells with indefinite proliferation potential that drive the formation leukemic cells [62]. Transformation of normal hematopoietic cells have origin in genetic mutations and the signaling pathways that have been shown to be involved in the regulation of HSCs self-renewal, are also hypothesized to be associated with oncogenesis [63, 64, 62, 65]. It is not currently known for certain whether the mutations occur in the normal stem cells or in more differentiated cell types, which then acquire stem cell-like features [66, 67, 68]. Blair et al. [69] and Bonnet and Dick [70] published studies with a clear demonstration that most of the leukemic cells are unable to proliferate extensively and only a small, defined subset of cells – leukemic stem cells were consistently clonogenic. Bonnet and Dick [70] showed that the immature LSCs reside within only a small fraction of the $CD34^+CD38^-$ subpopulation and are able to transmit acute myeloid leukemia (AML) to NOD-SCID mice and repopulate the bone marrow of the irradiated recipients. In contrast, $CD34^+CD38^+$ AML cells are not able to engraft and repopulate NOD-SCID mice.

Acute myeloid leukemia (AML) is characterized by the uncontrolled overproduction of progenitor cells arrested in maturation and differentiation, and hierarchically organized similar to normal hematopoietic cells. On the top of this hierarchy are LSCs acting as AML-initiating and maintaining cells, whereas their more mature progeny AML blast cells are unable to maintain the long-term growth of leukemias [27, 71, 72]. Although LSCs have the potential for self-renewal, they spend the majority of time in the G_0 phase of the cell cycle. Chemotherapeutic drugs, which act on cell-cycle active cell populations, are less effective on LSCs than on leukemic blasts [73]. Chemotherapy-resistant leukemogenic LSCs, even if present in low numbers (minimal residual disease, MRD), may be responsible for the relapses in AML and are therefore considered to contribute essentially to the pathology and clinical outcome in these patients [27].

Phenotype of leukemic stem cells. First immunophenotypic studies of LSCs showed phenotype pattern similar to HSCs - $CD34^+CD38^-Lin^-$ [70, 71]. Recent phenotypic studies revealed the expression of additional LSCs surface antigens and despite functional and phenotypic similarities to HSCs, novel LSCs-specific antigens were found.

Taussig et al. [32] showed that myeloid markers CD13, CD33 are expressed not only on HSCs but also on LSCs and

just $CD34^+CD38^-Lin^-CD33^+$ LSC fraction, in contrast to $CD34^+CD38^-Lin^-CD33^-$ LSC fraction, showed ability multilineage engraftment and repopulation of NOD-SCID mice. Hauswirth et al. [74] also demonstrated that the vast majority of the $CD34^+CD38^-$ AML stem cells express CD33 and CD13 but the level of these lineage markers expression on $CD34^+CD38^-$ LSCs were found to vary among donors.

Heterogeneous expression, often in dependence on donors, was observed also for markers CD116 (GM-CSFRα), CD117 (SCFR), CD71, CD133 (AC133), HLA-DR [74, 75]. In contrast to previous studies, Blair et al. [76, 77] and van Gosliga et al. [78] showed no expression of CD71, HLA-DR, and CD117 on LSCs.

Numerous investigations have provided evidence that LSCs express considerable amounts of the CD123 (IL-3 receptor α chain) [32, 72, 74, 79, 80], compared to lower level of CD123 expression on HSCs in normal and regenerating bone marrow [75, 81]. Florian et al. [75] observed increased expression of CD44 on LSCs. van Rhenen et al. [31, 32] have described recently that C-type lectin-like molecule-1 (CLL-1) is present on AML $CD34^+CD38^-$ cells in the majority of patients and absent on $CD34^+CD38^-$ cells in normal and regenerating bone marrow. Hosen et al. [83] demonstrated that CD96 is selectively expressed on the majority of $CD34^+CD38^-$ AML cells in many cases, whereas only a few cells in the normal HSC-enriched population expressed CD96 weakly. $CD34^+CD38^-CD96^+$ AML cells also showed ability to engraft bone marrow of the recipient mice.

Phenotype of AML blast cells. The progeny of LSCs – AML blasts are due to their arrest in maturation and differentiation phenotypically partially similar to normal granulocyte progenitors, but they often possess imunophenotype abnormalities not typical for normal myeloid development. Multiple studies have been done to assess phenotypic abnormalities in AML blasts (leukemia-associated immunophenotypes, LAPs) which are totally absent or present at very low levels in normal bone marrow and bone marrow recovering from induction or consolidation chemotherapy [84, 85]. LAPs include expression of markers not normally expressed on myeloid cells, asynchronous coexpression of markers normally expressed at different stages of maturation as well as overexpression or underexpression of myeloid markers [86]. These unusual phenotypes are detected on leukemic cells at diagnosis and can be used to monitor MRD after induction or consolidation therapy [85].

There are several classification systems of AML. French-American-British (FAB) classification from 1976 is based on the type, morphology and degree of maturity of the cells from which is leukemia comprised. FAB divides AML into 8 subtypes (M0 – M7) [87]. Immunophenotype of AML-M0, AML-M1, AML-M2, AML-M3 and AML-M4 blast subtypes is similar to granulocyte progenitors, whereas immunophenotype of AML-M5, AML-M6 and AML-M7 subtypes is more similar to monocyte, erythrocyte or megakaryocyte progenitors. However, FAB classification doesn't reflect biologic features, genetic characteristics and prognostic factors of leukemic blasts. The World Health Organization (WHO) classification from 2001 reflects present knowledge on morphology, immunophenotyping, cytogenetics, molecular genetics, prognoses and responses to therapy [88]. Although the WHO classification is more specific, the FAB classification is still in use.

The most AML blast cells reside within $CD34^+CD38^+$ cell fraction [70]. Early myeloblasts of undifferentiated AML (AML-M0), AML with minimal maturation (AML-M1), AML with maturation (AML-M2) and acute myelomonocytic leukemia (AML-M4) express CD34 and HLA-DR but these are lost by the pathologic promyelocytes which are typical for acute promyelocytic leukemia (APL or AML-M3) [89]. Similar to normal myelo-monoblasts, leukemic blasts have intermediate FSC, low SSC and low or intermediate CD45 [90].

The leukemic cells in all cases of AML-M1 to AML-M4 commonly express various combinations of CD13, CD33, CD65, CD117 and myeloperoxidase (MPO), whereas blasts of AML-M0 express only CD34 and TdT without expression of CD13,CD33, CD117, MPO [91]. Expression of monocyte-associated markers CD4, CD14, CD64 is related to monocyte progenitors of acute myelomonocytic leukemia (AML-M4). Other myeloid associated markers CD11b, CD11c, CD13, CD33, CD15, CD65, CD66, and CD117 are also frequently expressed in various combinations on leukemic blasts but are not useful for distinguishing the different subtypes of AML [89]. The AML-M1 subtype is usually associated with asynchronous coexpression of T-cell markers – CD7 and CD4 [92, 93]. Zeleznikova et al. [90] showed the most frequent coexpression of CD7 mainly in subtypes M0-M2. Leukemic blasts in case of AML-M2 weakly express CD19, CD2 and less commonly CD56. Asynchronous coexpression of CD2 and CD56 is also associated with AML-M3 [94, 95].

In future, synthesis of immunophenotype, cytogenetics and molecular genetics knowledge may elucidate of LAPs genesis and clarify the effect of LAPs and specific genetics changes in view of AML treatment and prognostic outcomes and may be important in design a more aggressive therapeutic approach.

In conclusion

Acute myeloid leukemia comprises heterogeneous group of malignant hematopoietic diseases that is characterized by proliferation and accumulation of immature myelopoietic progenitor cells in bone marrow. AML originates in transformation of hematopoietic stem cells or their progeny cells. Multiparameter flow cytometry method (FCM) analysis of human bone marrow became a major tool for a rapid and consistent diagnosis of AML. FCM provides exact determination of lineage specificity, stage of differentiation, aberrant intensity of leukemic cell gene expression and aberrant expression of lineage-specific or differential antigens. Detected leukemia associated phenotype is then used not only to ascertain exact diagnosis but is also used for determination of minimal residual disease during therapy and for post-therapeutic monitoring.

In some cases malignant transformation of leukemic cells doesn't result into formation of leukemia associated phenotype

and leukemic cells possess identical phenotype like granulocyte progenitor cells. Absence of leukemia specific markers on leukemic cells and their small numbers make the follow up of minimal residual disease more difficult. Therefore it is still important to improve knowledge about normal bone marrow stem and progenitor cells phenotypes, identify new leukemia-specific markers (CD96, CLL-1), search for aberrant marker expression and evaluate aberrant intensity or combination of various marker expressions for distinguishing normal cells from their malignant counterpart, in view of the diagnosis of leukemias or follow-up of minimal residual disease (MRD). Novel leukemia-specific markers also represent potential targets for example as a therapeutic agents based on monoclonal antibodies conjugated with cytostatic drugs.

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References

[1] DOMEN J and WEISSMAN IL Self-renewal, differentiation or death: regulation and manipulation of hematopoietic stem cell fate. Molecular Medicine Today 1999; 5: 201–208. doi:10.1016/S1357-4310(99)01464-1

[2] WEISSMAN IL Stem cells: units of development, units of regeneration, and units in evolution. Cell 2000; 100: 157–68. doi:10.1016/S0092-8674(00)81692-X

[3] BABUSIKOVA O, ZELEZNIKOVA T Normal maturation sequence of immunoglobulin light and heavy chains in hematogone stages 1, 2 and 3 in acute leukemia after treatment. Neoplasma 2008; 55: 501–506.

[4] BABUSIKOVA O, ZELEZNIKOVA T, KIRSCHNEROVA G, KANKURI E Hematogones in acute leukemia during and after therapy. Leuk Lymphoma 2008; 49: 1935–44. doi:10.1080/10428190701817274

[5] CIVIN CI, STRAUSS LC, BROVAL C, FACKLER MJ, SCHWARTZ JF, et al. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J. Immunol. 1984; 133: 157–165.

[6] KRAUSE DS, FACLER MJ, CIVIN CI, MAY WD CD34: structure, biology and clinical utility. Blood 1996; 87: 1–13.

[7] SUTHERLAND DR, KEATING A The CD34 antigen: structure, biology, and potential clinical applications. J. Hematother 1992; 1: 115–29.

[8] DEAGLIO S, MEHTA K, MALAVASI F. 2001. Human CD38: a (r)evolutionary story of enzymes and receptors. Leukoc. Res. 2001; 25: 1–12. doi:10.1016/S0145-2126(00)00093-X

[9] BAUM CM, WEISSMAN IL, TSUKAMOTO AS, BUCKLE AM, PEAULT B Isolation of a candidate human hematopoietic stem-cell population. Proc Natl Acad Sci USA. 1992; 89: 2804–2808. doi:10.1073/pnas.89.7.

[10] HUANG S, TERSTAPPEN LW Lymphoid and myeloid differentiation of single human CD34+, HLA-DR+, CD38- hematopoietic stem cells. Blood 1994; 83: 1515–26.

[11] HAO QL, SMOGORZEWSKA EM, BARSKY LW, CROOKS GM In vitro identification of single CD34+CD38ĂˇÂÂ' cells with both lymphoid and myeloid potential. Blood 1998; 91: 4145– 51.

[12] MILLER JS, MCCULLAR V, PUNZEL M, LEMISCHKA IR, MOORE KA Single adult human CD34+/LinĂˇÂÂ'/CD38– progenitors give rise to natural killer cells, B-lineage cells, dendritic cells, and myeloid cells. Blood 1999; 93: 96–106.

[13] KONDO M, WAGERS AJ, MANZ MG, PROHASKA SS, SCHERER DC, et al. Biology of hematopoietic stem cells and progenitors: implications for clinical application. Annual Review of Immunology 2003; 21: 759–806.

[14] HAO QL, THIEMANN FT, PETERSEN D, SMOGORZEWSKA EM, CROOKS GM Extended long-term culture reveals a highly quiescent and primitive human hematopoietic progenitor population. Blood 1996; 88: 3306–13.

[15] MILLER JS, MCCULLAR V, VERFAILLIE CM Ex vivo culture of CD34+/LinĂˇÂÂ'/DRĂˇÂÂ' cells in stroma-derived soluble factors, interleukin-3, and macrophage inflammatory protein-1ĂŽÂ± maintains not only myeloid but also lymphoid progenitors in a novel switch culture assay. Blood 1998; 91: 4516–22.

[16] BABUSIKOVA O, ZELEZNIKOVA T Normal maturation sequence of immunoglobulin light and heavy chains in hematogone stages 1, 2 and 3 in acute leukemia after treatment. Neoplasma 2008; 55: 501–506.

[17] BHATIA M, WANG JC, KAPP U, BONNET D, DICK JE Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci USA. 1997; 94: 5320–5325. doi:10.1073/pnas.94.10.5320

[18] BHATIA M, BONNET D, MURDOCH B, GAN OI, DICK JE A newly discovered class of human hematopoietic cells with SCID-repopulating activity. Nat. Med. 1998; 4 :1038–45. doi:10.1038/2023

[19] BONNET D, BHATIA M, WANG JCY, KAPP U, DICK JE Cytokine treatment or accessory cells are required to initiateengraftment of purified primitive human hematopoietic cells transplanted at limiting doses into NOD/SCID mice. Bone Marrow Transplantation 1999; 23: 203–9. doi:10.1038/sj.bmt.1701564

[20] GUENECHEA G, GAN OI, DORRELL C, DICK JE Distinct classes of human stem cells that differ in proliferative and self-renewal potential. Nat Immunol 2001; 2: 75–82. doi:10.1038/83199

[21] SROUR EF, ZANJANI ED, BRANDT JE, LEEMHUIS T, BRIDDELL RA, et al. Sustained human hematopoiesis in sheep transplanted in utero during early gestation with fractionated adult human bone marrow cells. Blood 1992; 79: 1404–12.

[22] ZANJANI ED, FLAKE AW, RICE H, HEDRICK M, TAVASSOLI M. Long-term repopulating ability of xenogeneic transplanted human fetal liver hematopoietic stem cells in sheep. J. Clin. Invest. 1994; 93: 1051–55 doi:10.1172/JCI117054

[23] TILL JE, MCCULLOCH EA A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiation Research 1961; 14: 213–22. doi:10.2307/3570892

[24] MORRISON SJ, WEISSMAN IL The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity 1994; 1: 661–673. doi:10.1016/1074-7613(94)90037-X

[25] LAROCHELLE A, VORMOOR J, HANENBERG H, WANG JCY, BHATIA M, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. Nat. Med. 1996; 2: 1329–1337.

[26] BONNET D Haematopoietic stem cells. J Pathol 2002; 197: 430–40. doi:10.1002/path.1153

[27] SPERR WR, HAUSWIRTH AW, FLORIAN S, OHLER L, GEISSLER K, et al. Human leukaemic stem cells: a novel target of therapy. Eur J Clin Invest. 2004; 34: 31–40.

[28] ZIEGLER BL, VALTIERI M, PORADA GA, De MARIA R, MULLER R, et al. KDR receptor: a key marker defining hematopoietic stem cells. Science 1999; 285: 1553–58

[29] GUNJI Y, NAKAMURA M, OSAWA H, NAGAYOSHI K, NAKAUCHI H, et al. Human primitive hematopoietic progenitor cells are more enriched in KITlow cells than in KIThigh cells. Blood 1993; 82: 3283-9.

[30] KAWASHIMA I, ZANJANI ED, ALMEIDA-PORADA G, FLAKE AW, ZENG H, et al. CD34+ human marrow cells that express low levels of Kit protein are enriched for long-term marrow-engrafting cells. Blood 1996; 87: 4136–4142.

[31] HU M, KRAUSE D, GREAVES M, SHARKIS S, DEXTER M, et al. Multilineage gene expression precedes commitment in the hemopoietic system. Genes Dev. 1997; 11: 774–785. doi:10.1101/gad.11.6.774

[32] TAUSSIG DC, PEARCE DJ, SIMPSON C, ROHATINER AZ, LISTER TA, et al. Hematopoietic stem cells express multiple myeloid markers: implications for the origin and targeted therapy of acute myeloid leukemia. Blood 2005; 106: 4086–4092.

[33] ANDREWS RG, TAKAHASHI M, SEGAL GM, POWELL JS, BERNSTEIN ID, et al. The L4F3 antigen is expressed by unipotent and multipotent colony-forming cells but not by their precursors. Blood 1986; 68: 1030–1035.

[34] ANDREWS RG, SINGER JW, BERNSTEIN ID Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of the CD33 and CD34 antigens and light scatter properties. J Exp Med 1989; 169: 1721–1731. doi:10.1084/jem.169.5.1721

[35] OSAWA M, HANADA K, HAMADA H, NAKAUCHI H Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science 1996; 273: 242–45. doi:10.1126/science.273.5272.242

[36] ZANJANI ED, ALMEIDA-PORADA G, LIVINGSTON AG, FLAKE AW, OGAWA M Human bone marrowCD34- cells engraft in vivo and undergo multilineage expression that includes giving rise to CD34+ cells. Exp. Hematol. 1998; 26: 353–60.

[37] NAKAMURA Y, ANDO K, CHARGUI J, KAWADA H, SATO T, et al. Ex vivo generation of CD34+ cells from CD34- hematopoietic cells. Blood 1999; 94: 4053–59.

[38] FUJISAKI T, BERGER MG, ROSE-JOHN S, EAVES CJ. Rapid differentiation of a rare subset of adult human Lin–CD34–CD38– cells stimulated by multiple growth factors in vitro. Blood 1999; 94: 1926–1932.

[39] MORRISON SJ, WRIGHT DE, WEISSMAN IL Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. Proceedings of the National Academy of Sciences of the USA 1997; 94: 1908–1913. doi:10.1073/pnas.94.5.1908

[40] KONDO M, WEISSMAN IL, AKASHI K Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 1997; 91: 661–72. doi:10.1016/S0092-8674(00)80453-5

[41] AKASHI K, TRAVER D, MIYAMOTO T, WEISSMAN IL A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 2000; 404: 193–197. doi:10.1038/35004599

[42] TRAVER D, AKASH K, MANZ M, MERAD M, MIYAMOTO T, et al. Development of CD8ĂŽÂ±-positive dendritic cells from a common myeloid progenitor. Science 2000; 290: 2152–2154. doi:10.1126/science.290.5499.2152

[43] MANZ MG, TRAVER D, MIYAMOTO T, WEISSMAN IL, AKASHI K Dendritic cell potentials of early lymphoid and myeloid progenitors. Blood 2001; 97: 3333–3341. doi:10.1182/blood.V97.11.3333

[44] GLIMM H, EISTERER W, LEE K, CASHMAN J, HOLYOAKE TL, et al. Previously undetected human haemopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID-beta2 microglobulin-null mice. Journal of Clinical Investigation 2001; 107: 199–206.

[45] GALY A, TRAVIS M, CEN D, CHEN B Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. Immunity 1995; 3: 459–73. doi:10.1016/1074-7613(95)90175-2

[46] HAO QL, ZHU J, PRICE MA, PAYNE KJ, BARSKY LW, et al. Identification of a novel, human multilymphoid progenitor in cord blood. Blood 2001; 97: 3683–90.

[47] MANZ MG, MIYAMOTO T, AKASHI K, WEISSMAN IL Prospective isolation of human clonogenic common myeloid progenitors. Proc Natl Acad Sci USA 2002; 99: 11872–7. doi:10.1073/pnas.172384399

[48] EDVARDSSON L, DYKES J, OLOFSSON T Isolation and characterization of human myeloid progenitor populations – TpoR as discriminator between common myeloid and megakaryocyte/erythroid progenitors. Exp Hematol. 2006; 34: 599–609. doi:10.1016/j.exphem.2006.01.017

[49] MORI Y, IWASAKI H, KOHNO K, YOSHIMOTO G, KIKUSHIGE Y, et al. Identification of the human eosinophil lineage-committed progenitor: revision of phenotypic definition of the human common myeloid progenitor. The Journal of Experimental Medicine 2008; 206: 183–193. doi:10.1084/jem.20081756

[50] KATSURA Y and KAWAMOTO H Stepwise lineage restriction of progenitors in lympho-myelopoiesis. Int. Rev. Immunol. 2001; 20: 1–20

[51] KAWAMOTO H, KATSURA Y A new paradigm for hematopoietic cell lineages: revision of the classical concept of the myeloid-lymphoid dichotomy. Trends Immunol 2009; 30: 193–200. doi:10.1016/j.it.2009.03.001

[52] IGARASHI H, GREGORY SC, YOKOTA T, SAKAGUCHI N, KINCADE PW Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. Immunity 2002; 17, 117–130. doi:10.1016/S1074-7613(02)00366-7

[53] KAWAMOTO H, OHMURA K, KATSURA Y Direct evidence for the commitment of hematopoietic stem cells to T, B and myeloid lineages in murine fetal liver. Int. Immunol. 1997; 9: 1011–1019. doi:10.1093/intimm/9.7.1011

[54] KAWAMOTO H, OHMURA K, FUJIMOTO S, KATSURA Y Emergence of T cell progenitors without B cell or myeloid differentiation potential at the earliest stage of hematopoiesis in the murine fetal liver. J. Immunol. 1999; 162: 2725–2731.

[55] KAWAMOTO H, IKAWA T, OHMURA K, FUJIMOTO S, KATSURA Y T cell progenitors emerge earlier than B cell progenitors in the murine fetal liver. Immunity 2000; 12: 441–450. doi:10.1016/S1074-7613(00)80196-X

[56] MATUTES E, MORILLA R, FARAHAT N, CARBONELL F, SWANSBURY J, et al. Definition of acute biphenotypic leukemia. Haematologica 1997; 82: 64–66.

[57] CHAPIRO E, DELABESSE E, ASNAFI V, MILLIEN C, DAVI F et al. Expression of T-lineage-affiliated transcripts and TCR rearrangements in acute promyelocytic leukemia: implications for the cellular target of t(15;17). Blood 2006; 108: 3484–3493. doi:10.1182/blood-2005-09-009977

[58] TERSTAPPEN LW, LOKEN MR Myeloid cell differentiation in normal bone marrow and acute myeloid leukemia assessed by multi-dimensional flow cytometry. Anal Cell Pathol 1990; 2: 229–40.

[59] TERSTAPPEN LW, HOLLANDER Z, MEINERS H, LOKEN MR Quantitative comparison of myeloid antigens on five lineages of mature peripheral blood cells. J Leukoc Biol 1990; 48: 138–148.

[60] VAN LOCHEM EG, VAN DER VELDEN VHJ, WIND HK, TE MARVELDE JG, WESTERDAAL NAC, et al. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: Reference patterns for age-related changes and disease-induced shifts. Cytometry Part B: Clinical Cytometry Volume 60B 2004; 1: 1–13.

[61] WOOD B Multicolor immunophenotyping: human immune system hematopoiesis. In: Darzynkiewicz Z, Roederer M, Tanke HJ, editors. Cytometry, 4th Edition New Developments. Elsevier Academic Press. 2004; Methods in Cell Biology 75: 559–76.

[62] REYA T, MORRISON SJ, CLARKE MF, WEISSMAN IL Stem Cells, Cancer and Cancer stem cells. Nature 2001; 414: 105–111. doi:10.1038/35102167

[63] LAWRENCE HJ, SAUVAGEAU G, HUMPHRIES RK, LARGMAN C. The role of HOX homeobox genes in normal and leukemic hematopoiesis. Stem Cells 1996; 14: 281–91.

[64] TAIPALE J, BEACHY PA The Hedgehog and Wnt signalling pathways in cancer. Nature 2001; 411 (6835): 349–54. doi:10.1038/35077219

[65] SPRADLING A, DRUMMOND-BARBOSA D, KAI T Stem cells find their niche. Nature 2001; 414 (6859): 98–104. doi:10.1038/35102160

[66] COZZIO A, PASSEGUE E, AYTON PM, KARSUNKY H, CLEARY ML, et al. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes Dev 2003; 17: 3029–3035.

[67] SO CW, KARSUNKY H, PASSEGUE E, COZZIO A, WEISSMAN IL, et al. MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. Cancer Cell 2003; 3: 161–171.

[68] SATOH C, OGATA K Hypothesis: myeloid-restricted hematopoietic stem cells with self-renewal capacity may be the transformation site in acute myeloid leukemia. Leuk Res 2006; 30: 491–495. doi:10.1016/j.leukres.2005.08.017

[69] BLAIR A, HOGGE DE, AILLES LE, LANSDORP PM, SUTHERLAND HJ. Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. Blood 1997; 89: 3104–12.

[70] BONNET D, DICK JE Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730–7. doi:10.1038/nm0797-730

[71] SUTHERLAND HJ, BLAIR A, ZAPF RW Characterization of a hierarchy of human acute myeloid leukemia progenitor cells. Blood 1996; 87: 4754–61.

[72] APPELBAUM FR, ROWE JM, RADICH J, DICK JE Acute myeloid leukemia. Hematology (Am Soc Hematol Educ Program) 2001; 62–86. doi:10.1182/asheducation-2001.1.62

[73] RAVANDI F, ESTROV Z Eradication of leukemia stem cells as a new goal of therapy in leukemia. Clin Cancer Res 2006; 12: 340–344. doi:10.1158/1078-0432.CCR-05-1879

[74] HAUSWIRTH AW, FLORIAN S, PRINTZ D, SOTLAR K, KRAUTH MT, Expression of the target receptor CD33 in CD34+/CD38–/CD123+ AML stem cells. European Journal of Clinical Investigation 2007; 37: 73–82 (10).

[75] FLORIAN S, SONNECK K, HAUSWIRTH AW, KRAUTH MT, SCHERNTHANER GH, et al. Detection of molecular targets on the surface of CD34+/CD38- stem cells in various myeloid malignancies. Leukemia & Lymphoma 2006; 47: 207–222.

[76] BLAIR A, HOGGE DE, SUTHERLAND HJ Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34+/CD71Ă˘ÂÂ’/HLAĂ˘ÂÂ’DRĂ˘ÂÂ. Blood 1998; 92: 4325–35.

[77] BLAIR A, SUTHERLAND HJ Primitive acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo lack surface expression of c-kit (CD117). Exp Hematol 2000; 28: 660–71. doi:10.1016/S0301-472X(00)00155-7

[78] VAN GOSLIGA D, SCHEPERS H, RIZO A, VAN DER KOLK D, VELLENGA E, et al. Establishing long-term cultures with self-renewing acute myeloid leukemia stem/progenitor cells. Exp Hematol 2007; 35: 1538–1549. doi:10.1016/j.exphem.2007.07.001

[79] JORDAN CT, UPCHURCH D, SZILVASSY SJ, GUZMAN ML, HOWARD DS, et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia 2000; 14: 1777–1784.

[80] MUÑOZ L, NOMDEDÉU JF, LÓPEZ O, CARNICER MJ, BELLIDO M, et al. Interleukin-3 receptor α chain (CD123) is widely expresssed in hematologic malignancies. Haematologica 2001; 86: 1261-1269.

[81] VAN RHENEN A, MOSHAVER B, KELDER A, FELLER N, NIEUWINT AW, et al. Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission. Leukemia 2007; 21: 1700–1707.

[82] VAN RHENEN A, FELLER N, KELDER A, WESTRA AH, ROMBOUTS E, et al. High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin Cancer Res 2005; 11: 6520–6527.

[83] HOSEN N, PARK CY, TATSUMI N, OJI Y, SUGIYAMA H, et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc. Nat. Acad. Sci. USA 2007; 104: 11008–11013.

[84] ZELEZNIKOVA T, STEVULOVA A, KOVARIKOVA A, BABUSIKOVA O Increased myeloid precursors in regenerating bone marrow; implications for detection of minimal residual disease in acute myeloid leukemia. Neoplasma 2007; 54: 471–477.

[85] OLARU D, CAMPOS L, FLANDRIN P, NADAL N, DUVAL A, et al. Multiparametric analysis of normal and postchemotherapy bone marrow: Implication for the detection of leukemia-associated immunophenotypes. Cytometry B Clin Cytom. 2008; 74: 17–24. doi:10.1002/cyto.b.20371

[86] SAN MIGUEL JF, VIDRIALES MB, LOPEZ-BERGES C, DIAZ-MEDIAVILLA J, GUTIERREZ N, et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 2001; 98(6): 1746–51.

[87] BENNETT JM, CATOVSKY D, DANIEL MT, FLANDRIN G, GALTON DA, et al. Proposals for the classification of the acute leukaemias. French–American–British (FAB) co-operative group. Br J Haematol. 1976; 33: 451–458.

[88] VARDIMAN JW, HARRIS NL, Brunning RD The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002; 100: 2292–302. doi:10.1182/blood-2002-04-1199

[89] BEHM FG, CAMPANA D. Immunophenotyping. In: Pui CH editor. Childhood leukemias. UK: Cambridge University Press; 1999; 111–44.

[90] ZELEZNIKOVA T, BABUSIKOVA O The impact of cell heterogeneity and immunophenotypic changes on monitoring minimal residual disease in acute myeloid leukemia. Neoplasma 2005; 52: 517–22.

[91] GHOSH S, SHINDE SC, KUMARAN GS, SAPRE RS, DHOND SR, et al. Haematologic and immunophenotypic profile of acute myeloid leukemia: An experience of Tata memorial hospital. Indian J Cancer 2003; 40: 71–76.

[92] LAUNDER TM, BRAY RA, STEMPORA L, CHENGGIS ML, FARHI DC. Lymphoid- Associated antigen expression by acute myeloid leukemia. Am J Clin Pathol 1996; 106: 185–91.

[93] BALL ED, DAVIS RB, GRIFFITH JD, MAYER RJ, DAVEY FR, et al. Prognostic value of lymphocyte surface markers in acute myeloid leukemia. Blood 1991; 77: 2242–50.

[94] RASPADORI D, DAMIANI D, LENOCI M, RONDELLI D, TESTONI N, et al. CD56 antigenic expression in acute myeloid leukemia identifies patients with poor clinical prognosis. Leukemia 2001; 15: 1161–4. doi:10.1038/sj.leu.2402174

[95] BRADSTOCK K, MATTHEWS J, BENSON E, PAGE F, BISHOP J Prognostic value of immunophenotyping in acute myeloid leukemia. Australian Leukemia study group. Blood 1994; 84: 1220–5.