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Myeloid Malignancies: Myelodysplastic Syndromes, Myeloproliferative Disorders, and Acute Myeloid Leukemia

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As hematopoietic cells proceed in differentiation from stem cells to committed progenitors through to later stage mature forms, they undergo a characteristic and tightly regulated sequence of morphologic, immunophenotypic, and functional changes. The process is a consequence of interaction between the underlying cellular genetic program and environmental cues, is essentially linear for each cell lineage, and results in a pattern of antigenic expression in each cell that is related to its lineage and stage of maturation. Over the past 2 decades, the antigenic patterns characteristic of normal maturation have been elucidated systematically and found essentially invariant between individuals [1–5]. Consequently, using the appropriate set of reagents and routine immunophenotyping techniques, any individual cell can be classified into its lineage and maturational stage with a high degree of specificity. Deviation from this normal pattern of antigen expression is a hallmark of hematopoietic neoplasia and allows for the diagnosis, classification, and post-therapeutic monitoring of these disorders.

Immunophenotypic detection of hematopoietic neoplasia

Neoplastic cells frequently show nonrandom expression of antigens in a manner that deviates from the tightly regulated patterns of antigen expression seen in normal maturation. This is the basic principle that allows for the detection of hematopoietic neoplasia by immunophenotyping, whether or

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not it is lymphoma, myelodysplasia (MDS), or acute leukemia. Abnormal antigenic expression in neoplasia can be grouped into four basic categories:

- Abnormally increased or decreased levels of expression (intensities) of antigens normally expressed by cell type or lineage at a particular stage of maturation, including the complete loss of normal antigens in some instances
- Asynchronous antigen expression (ie, expression of antigens normally expressed by the cell type or lineage but at an inappropriate time during maturation)
- Abnormally homogeneous expression of one or more antigens by a population that normally exhibits more heterogeneous expression
- Gain of antigens not normally expressed by cell type or lineage

Under this paradigm, the reference point for the recognition of aberrant antigenic change is normal maturation; consequently, a detailed knowledge of normal maturational antigenic expression is the single most important prerequisite for this type of analysis. Perturbation of normal antigenic expression also may occur during non-neoplastic disease states and secondary to therapeutic maneuvers, however, and understanding these changes is important to providing specificity for the diagnosis of neoplasia. Finally, knowledge of the types of antigenic abnormalities commonly seen in specific disease states can assist in the recognition and classification of neoplasia.

Population identification

A complete description of normal patterns of antigenic expression during differentiation is beyond the scope of this article and readers are referred to more comprehensive articles on this subject [1–7]. Simple antigenic combinations useful for the identification of specific maturational subpopulations in a clinical laboratory setting are provided in Table 1. The identification of these subpopulations produces groups having relatively homogeneous immunophenotypes and is the prerequisite to understanding and assessing antigenic expression in a complex cell mixture, such as bone marrow. If specific subpopulations are not identified first, then it becomes difficult to attribute changes in antigen expression to specific cell types, obscuring some antigenic changes that diagnostically are useful and resulting in a phenomenologic approach to immunophenotyping that reduces its potential power. Although most of the populations outlined in Table 1 are assessed in most clinical laboratories, there are a few that are used less commonly and deserve special comment.

The early progenitor or hematopoeitic stem cell population is a relatively discrete CD34-positive subpopulation commonly identified by its low to absent expression of CD38 and having an immunophenotype different from the majority of the CD34-positive committed progenitor population. This

Table 1
Antigenic combinations suitable for the identification of hematopoietic populations by flow cytometry

Population	Antigenic combination
Early progenitors	CD34 high, CD38 low to absent
Committed progenitors	CD34+, CD38+
B cells	CD19+
Myelomonocytic	CD123+
Erythroid	CD71 high
Proerythroblasts	CD71 high, CD117+
B-cell progenitors	CD19+, CD38 int, CD45 low to int
Promonocytes	CD64 high, HLA-DR high, CD14 low to absent
Monocytes	CD14 high
CD16+ monocytes	CD14 int, CD16+
Neutrophilic	CD45+, SS high
Promyelocytes	CD11b−, CD13+
Myelocytes	CD13 low, CD16−
Metamyelocytes	CD13 low, CD16 low
Bands	CD13 int, CD16 int
Neutrophils	CD13 high, CD16 high
Basophils	CD123 high, HLA-DR−
Eosinophils	CD45 high, CD16−, SS high
Plasmacytoid dendritic	CD123 high, HLA-DR+
Lymphocytes	CD45 high, SS low
Plasma cells	CD38 high or CD138+

Abbreviations: SS, side scatter; int, intermediate.

population essentially is uncommitted to differentiation along any particular lineage and so lacks the expression of early antigens characteristic of more differentiated stages. It also demonstrates uniformly lower level expression of a variety of antigens than is seen on the lineage-committed, CD34-positive progenitors, including low CD13, low CD33, low to absent CD38, low CD117, and low HLA-DR [8]. In contrast, CD45 is expressed at a slightly higher level than that seen on committed progenitors. This population also expresses the early progenitor antigen CD133 that is lost on differentiation to later stage forms and CD123 [8], an important growth factor receptor for early progenitors. As the early progenitors begin to differentiate, CD38 expression increases to a uniformly intermediate level, regardless of cell lineage, and the cells begin to express antigens characteristic of early lineage commitment. Early B cells begin to express CD10 and CD19 with the rapid loss of CD117 and CD123. Early erythroid cells acquire high-level expression of CD71 with rapid loss of CD13 and CD123 and initial retention of CD117 as proerythroblasts. Early myelomonocytic cells retain expression of CD123, acquire increased CD33, and subsequently acquire increasing CD15 and CD64. In the author's experience, immunophenotypic abnormalities of the early progenitor population are common in myeloid disorders of all types and, given the increasing literature on the importance of populations with stem cell properties in the pathogenesis and

maintenance of these disorders, specific identification and analysis of this subpopulation seems particularly relevant. The author finds the incorporation CD38 and CD123 alongside antigens assessed more commonly in a routine clinical setting (CD13, CD19, CD33, CD34, CD45, CD71, CD117, HLA-DR, and so forth) to be helpful in this regard.

Promonocytes are an infrequent component of normal bone marrow characterized by the expression of high CD64, low CD13, intermediate CD15 and CD36, and high HLA-DR without significant CD34, minimal CD117, and, importantly, with low to absent CD14. The population is distinguished from more mature monocytes by its more uniformly high expression of HLA-DR, lower CD13 and CD36, higher CD15, and low to absent CD14. CD64 is expressed at a high and invariant level throughout monocytic maturation and serves as an excellent marker for that lineage, with the caveats that promyelocytes and myelocytes also express CD64 at a slightly lower level and that it is an activation antigen for mature neutrophils. Additionally, the detection of CD14 expression is epitope dependent, with some reagents demonstrating positivity at earlier stages of maturation than others [9], so care should be taken in defining the performance characteristics of this class of reagents before use. The importance of the identification of promonocytes is their frequent expansion in myeloid disorders, particularly in acute myeloid leukemia (AML), having myelomonocytic or monocytic differentiation where this population may form a majority of the infiltrate and have an immunophenotype that may be mistaken as aberrant.

Another monocyte-related population that may be mistaken as aberrant is a subset of monocyte-derived cells having the expression of CD16 [10]. This population is prominent particularly in peripheral blood and less frequently in bone marrow. It is characterized by the decreased expression of a variety of antigens in comparison to mature monocytes, including CD14, CD64, and HLA-DR, and characteristically has an increased level of CD45 with decreased side scatter that overlaps the lymphocyte population on CD45 versus side scatter projections. This population can be expanded dramatically in inflammation and some myeloid neoplasms, indicating a skewed maturational progression, and should not be mistaken for an intrinsically aberrant immunophenotype.

Lymphoplasmacytoid dendritic cells are a discrete subpopulation found in peripheral blood and bone marrow that can be identified easily by their expression of high CD123 [11], similar to that of a basophil, with high HLA-DR and intermediate CD4. The level of CD45 expression is slightly lower than that of a lymphocyte but slightly higher than that of a committed CD34-positive progenitor, such that it lies between the two populations on a CD45 versus side scatter projection. This population also expresses a variably low level of CD34, such that portions of the population often are included in blast populations defined by CD34 and CD45 expression. Expansions of this subpopulation occasionally accompany myeloid neoplasms, in some cases being a major component of abnormal monocytic expansions, and should

not be mistaken for an aberrant immunophenotype. Awareness of this population also allows correct identification of the infrequent cases of primary neoplasms derived from this population (eg, CD4+CD56+ hematodermic neoplasm, formerly "blastic natural killer–cell lymphoma").

Basophils are identified easily by their expression of high CD123 without HLA-DR or by their expression of intermediate CD33 without CD15 or HLA-DR. This population has a similar CD45 and side scatter profile to lymphoplasmacytoid dendritic cells, lying between mature lymphocytes and committed CD34-positive progenitors on a CD45 versus side scatter projection. Basophils are more common in peripheral blood than bone marrow, where they are infrequent. In a variety of myeloid neoplasms, however, in particular myeloproliferative disorders (MPD) and a subset of acute myeloid leukemias, the number of lymphoplasmacytoid dendrite cells can be increased markedly in number. These cells also can be difficult to appreciate morphologically, as they often demonstrate poor granularity on standard morphologic preparations because of the water-soluble nature of the granules and may be mistaken for dysplastic neutrophils or other forms.

Early proerythroblasts may be identified by their expression of high CD71 in combination with CD117. Although the level of CD71 expression generally is not as high as that seen on later stages of erythroid maturation, it is higher than that seen normally on myeloid blasts. CD34 often is retained to some degree on this population at a variably low level, as are CD33 and HLA-DR, but CD13 uniformly is absent. CD45 is expressed at a level similar to or slightly below that of committed myelomonocytic progenitors but with mildly increased side scatter, causing them to appear to the right and slightly below CD34-positive progenitors on a CD45 versus side scatter projection. The proerythroblast population rarely may be expanded dramatically in myelodysplastic syndromes, particularly in AML having prominent erythroid differentiation.

Technical considerations

Consistency

The ability to recognize deviation from normal patterns of antigenic expression assumes a degree of consistency in the ability to evaluate antigenic expression. Consistency is achieved by the use of standardized instrument set-up, standardized and validated reagent combinations, and standardized sample preparation. In all these activities, quality control is key.

Collect enough events

During sample acquisition, sufficient cells must be analyzed to allow a statistically accurate representation of the smallest population to be evaluated. In the case of myeloid disorders, the smallest populations of interest

generally are progenitor subpopulations, in particular the most immature progenitor subpopulation. Given a frequency in normal marrow of approximately 0.1%, and the suggestion from Poisson counting statistics for 100 events to provide a coefficient of variation of 10%, a minimum of 100,000 total white cell events should be acquired.

Cell aggregates

The use of lysing reagents, fixatives, and centrifugation during specimen processing provides an opportunity for cell aggregation to occur and is an issue particularly for myelomonocytic populations. Careful attention should be paid to optimization of specimen processing to minimize this artifact. Additionally, on modern instruments, area, height, and width measurements in some combination on one or more parameters can be used to provide doublet discrimination and exclude many aggregates.

Informative antibody panels

The panels of reagents used for analysis should be capable of demonstrating normal patterns of maturation and antigen expression for the populations listed in Table 1. The reagents also need to be capable of identifying common abnormalities seen in myeloid neoplasia. Suitable panels using three- and four-color flow cytometry are published [12–15]. The use of an increased number of simultaneous fluorochromes facilitates specific subpopulation identification and cross-correlation of antigenic expression, and the author has published a panel suitable for the identification of myeloid neoplasia using 9- and 10-color flow cytometry [16].

Blast quantitation

The enumeration of blasts is of central importance in the diagnosis and classification of myeloid neoplasia. The concept of a ''blast'' is derived from and defined by morphologic criteria, however, not by immunophenotyping. From an immunophenotypic perspective, morphologic blasts or their equivalents generally consist of a combination of early CD34-positive progenitors, committed CD34-positive myelomonocytic progenitors, promyelocytes, promonocytes, and B-cell progenitors in some proportion. Consequently, although flow cytometry can define and quantify subpopulations with a high degree of accuracy, making the correspondence between morphologic blasts and immunophenotypically defined populations can be problematic. In practice, CD34-positive cells commonly are referred to as blasts. An additional complication is that the denominator for morphologic blast counts includes nucleated erythroid cells, a population that variably is lost with the use of lysing reagents during specimen processing. This situation is not improved with the use of density gradient methods, such as Ficoll

separation. To provide a consistent denominator, the reporting of population percentages derived from flow cytometry, including blast enumeration, should use CD45-positive events as their denominator. The net result is that current definitive classification of myeloid neoplasia under the World Health Organization classification [17] still requires a morphologic blast count.

Common abnormalities in myeloid neoplasia

The distinction between the basic classes of myeloid disorders (ie, MDS, MPD, and AML) relies in large part on clinical information, blood cell counts, or morphologic information, such as blast counts. In addition, the flow cytometric findings seen in these disorders have a great degree of overlap and do not allow for definitive classification when viewed in isolation. Consequently, the basic types of abnormalities that can be identified by flow cytometry are reviewed and then put in context for each of the classes of myeloid neoplasia.

CD45 versus side scatter

The display of flow cytometric data using CD45 versus side scatter provides a consistent starting point for the identification of basic hematopoietic cell populations and has enjoyed widespread use in routine clinical laboratoris [18,19]. In particular, this method allows for the more ready identification of progenitor populations based on their differential expression of CD45 and side scatter from mature lymphocyte, monocyte, and neutrophilic populations (Fig. 1). Myelomonocytic and B-lymphoid progenitors also display differences in side scatter that facilitate their discrimination. Additional information is provided regarding maturation for each cell lineage, and exclusion of erythroid precursors may be accomplished. Although this projection is useful, it must be recognized that populations identified in this manner rarely are pure and additional antigens often must be used for definitive population identification.

Increased blasts

An increased number of myelomonocytic progenitors (blasts) defines higher-grade MDS, accelerated phase or blast crisis of MPD, and AML. Even in low-grade MDS, where blasts are less than 5% of the total nucleated cells, they still may be increased above the normal level of 1% to 2% and assist in the recognition of myeloid neoplasia. Increases in blasts also occur during marrow regeneration, however, such as after marrow insult, and a simple elevation in number is not as informative as the identification of immunophenotypic abnormalities on the blasts.

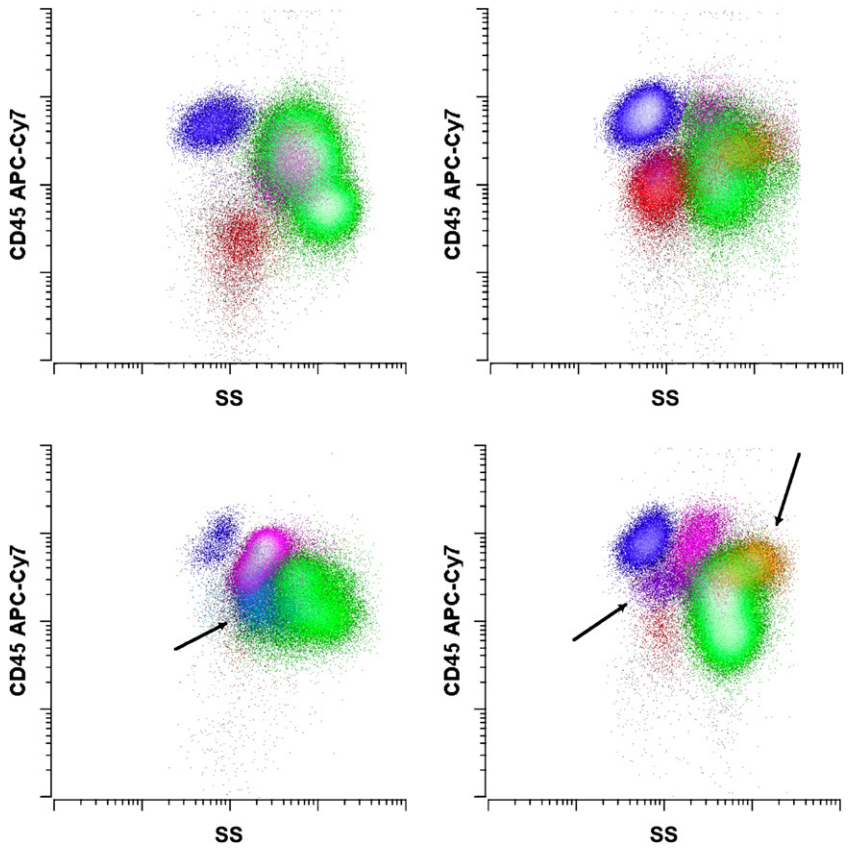

Fig. 1. Abnormalities of CD45 versus side scatter. Progenitors with decreased CD45 and maturing neutrophils with decreased side scatter (*top left*). Increased progenitors and maturing neutrophils with decreased side scatter (*top right*). Monocytosis with increased immature forms (*arrow, bottom left*). Basophilia (*lower arrow*) and eosinophilia (*upper arrow, bottom right*).

Decreased CD45 on blasts

Decreased expression of CD45 on myelomonocytic progenitors occurs in MDS and MPD [13] and when present is a reliable indicator of neoplasia. The finding may be made more apparent by using a less intense fluorochrome for CD45 (see Fig. 1).

Decreased B-cell progenitors

A decrease in normal B-cell progenitors having decreased side scatter compared with myelomonocytic progenitors is reported as a finding characteristic of low-grade MDS [20,21]. Given the normal decline in the number of B-cell progenitors with age and after certain therapies, additional

validation of this parameter using age-matched controls and larger patient populations is required.

Hypogranularity of neutrophils

A decrease in side scatter on mature neutrophils, the flow cytometric equivalent of morphologic hypogranularity, is seen in a subset of patients who have MDS (see Fig. 1) [12,13,22]. In the authors' experience, hypogranularity of neutrophils also may be seen occasionally with aged samples and infrequently may be seen during marrow recovery after marrow transplantation.

Monocytosis

Monocytosis in the marrow may be seen in a wide variety of infectious and inflammatory disorders but also is characteristic of chronic myelomonocytic leukemia and AML having monocytic differentiation, where it may be accompanied by an increase in immature monocytic forms or promonocytes (see Fig. 1).

Basophilia

Basophils normally are present in lower in numbers in normal bone marrow in comparison with peripheral blood and characteristically are increased in MPD, in particular chronic myeloid leukemia and myelofibrosis [23]. Occasionally, basophilia also is seen in association with MDS [24] or AML and infrequently in the absence of neoplasia. The presence of basophilia, with few exceptions, is associated with myeloid neoplasia and requires careful evaluation (see Fig. 1).

Eosinophilia

The presence of marrow eosinophilia may be seen in association with MDS [24], MPD [25], and a subset of AML, in particular those containing abnormalities of the core-binding factor genes (eg, inv[16]) (see Fig. 1). This finding may be seen in many other non-neoplastic conditions [26], however, and lacks specificity for the diagnosis of myeloid neoplasia.

CD34-positive progenitors

Alterations in the pattern of antigenic expression on CD34-positive early and committed progenitors are a frequent occurrence in all forms of myeloid neoplasia [13–15,27,28], and recognition of abnormalities of these stages of maturation is fundamental to the recognition of these disorders. As low-grade MDS and chronic phase MPD progress to a more aggressive phase of disease, progenitors commonly acquire a block in maturation that

manifests as an accumulation in immature forms, often with an associated increase in the degree of antigenic abnormalities. Increasing evidence implicates populations having stem cell characteristics as the proximal cause for these disorders [29–31], and future developments are likely to focus on these populations. The wide variety of immunophenotypic abnormalities described on CD34-positive progenitor populations can be grouped into the basic categories (described previously).

Alterations in the intensity of antigens normally expressed by early CD34-positive progenitor cells, as defined by low to absent expression of CD38 and intermediate to high CD34, include increased or decreased expression of CD13, CD33, CD34, CD117, CD123, or HLA-DR (Fig. 2). Similar alterations also may be seen on committed progenitors, in addition to

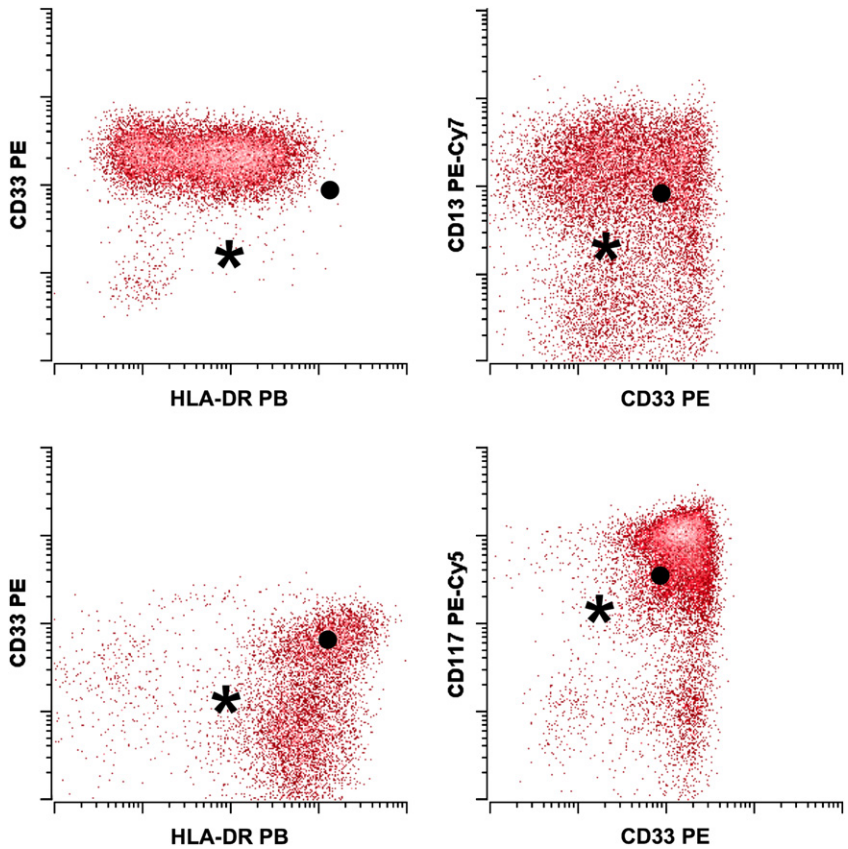

Fig. 2. Abnormalities of antigenic intensity on progenitors. The normal locations of early (*) and committed (•) progenitors are indicated for the blast population gated by CD45 and side scatter. Increased CD33 and decreased HLA-DR (*top left*). Increased CD13 on early progenitors (*top right*). Increased HLA-DR on early progenitors (*bottom left*). Increased CD117 (*bottom right*).

increased or decreased expression of CD38, and may not necessarily correlate with changes seen on early progenitors. Abnormal increased expression of lymphoid antigens on CD34-positive progenitors may include CD2, CD4, CD5, CD7, CD19, and CD56 [13,14,32]. A subpopulation of progenitors normally expresses CD7, however, generally at a low level and on a small subset, so aberrant expression requires either an unusually increased level of expression or expression on a significantly increased proportion of progenitors (Fig. 3). Similarly, CD4, CD5, and CD56 expression may be seen on progenitors in the setting of active marrow regeneration, but the expression generally is at a low level and only present on a small subset. Antigenic homogeneity, often as a consequence of poor maturational progression, is

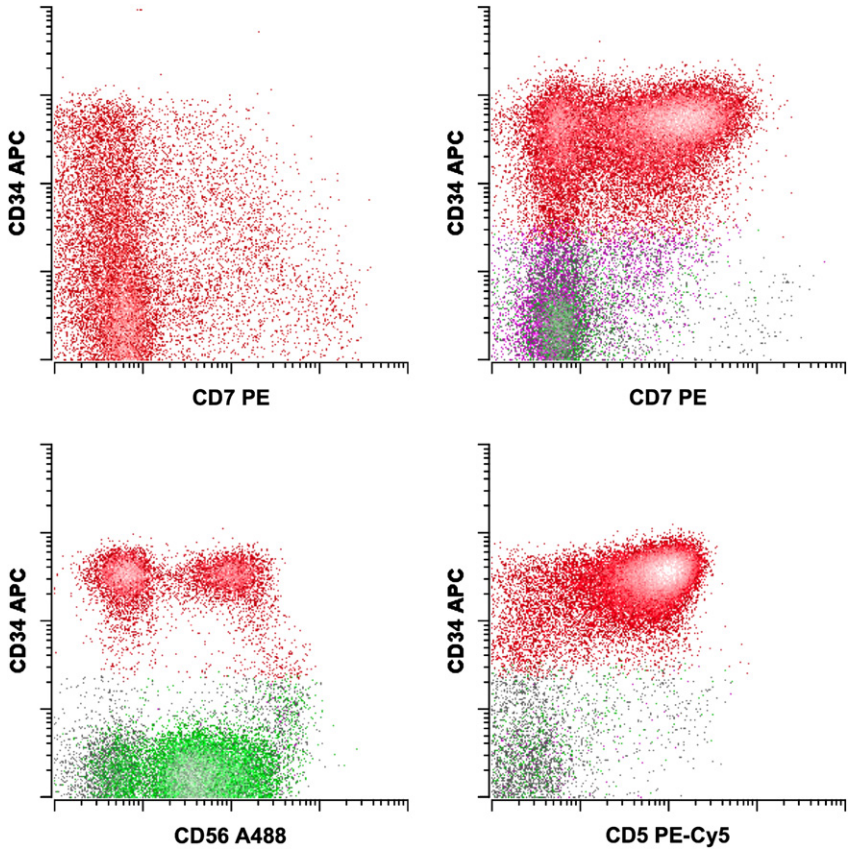

Fig. 3. Abnormal lymphoid antigen expression on progenitors. Normal CD7 expression on progenitors gated by CD45 and side scatter; note that most CD7 expression is low and on low CD34-positive forms (*top left*). Abnormal expression of CD7 on progenitors; note the higher intensity and greater percentage of CD7 expression (*top right*). Abnormal CD56 expression on progenitors; also note CD56 expression on CD34 negative maturing neutrophils (*bottom left*). Abnormal CD5 expression on progenitors (*bottom right*).

a common abnormality and suggests neoplasia even in the absence of alterations in the level of expression of the antigens involved (Fig. 4). Occasionally, progenitors may show the asynchronous expression of more mature antigens, including CD11b, CD15, or CD64. As normal myelomonocytic maturation occurs, however, a small subset of CD34-positive progenitors show a coordinated gain of CD15 and CD64 as CD34 and CD117 decline, albeit at different rates. Consequently, coexpression of these antigenic combinations is not strictly aberrant, it is the degree of deviation from this normal maturation that should be considered abnormal. A decrease in side scatter on myelomonocytic progenitors also may be observed in some cases and is a useful aberrancy when present.

Neutrophilic maturation

A variety of antigenic abnormalities is described in maturing neutrophils, particularly with regard to MDS where they were the initial focus in early studies [33]. In the author's experience, abnormalities in this lineage are common but not as frequent as in progenitor populations where they almost invariably are present. In the evaluation of samples from patients who have cytopenia, it is common to observe a preferential increase in immature forms (ie, myelocytes or metamyelocytes) at the expense of mature neutrophils. In its extreme form, there may be a maturational arrest without the presence of mature neutrophils. Although abnormal and generally indicative of a prior marrow insult, left-shifted maturation in the absence of immunophenotypic

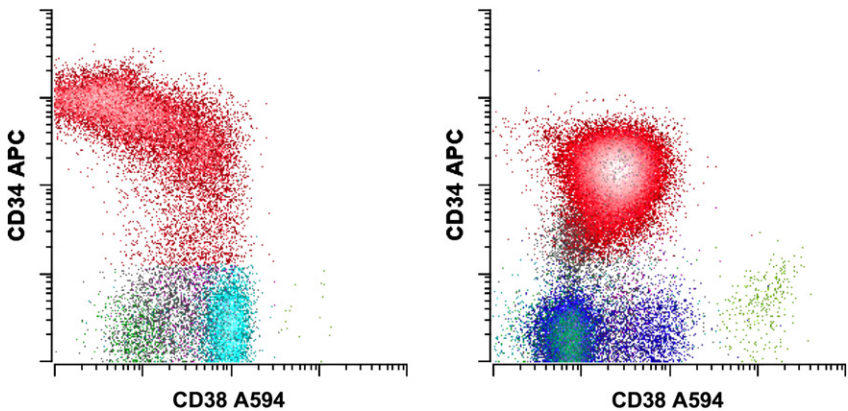

Fig. 4. Antigenic homogeneity and poor maturational progression of progenitors. Progenitors gated by CD45 and side scatter show markedly left shifted maturation as evidence by low CD38 and high CD34 with minimal progression to later stage mature forms (*left*). Progenitors with homogeneous expression of CD34 and CD38 (low) and other antigens (not pictured) with poor maturational progression (*right*).

deviancy does not indicate myeloid neoplasia. Nevertheless, there are several publications reporting what seems to be in large part left-shifted maturation in MDS, and the significance of some of these findings has been challenged [34]. In particular, the reports of abnormal patterns of CD11b and CD16 expression [12,35] and absence of CD10 on neutrophils [36] may fall into this category. Additional reports of abnormalities, such as CD64 negative granulocytes [12], are difficult to correlate with knowledge of normal maturation, as CD64 normally is expressed at low to absent levels on mature granulocytes and is considered an activation antigen [37]. Similarly, the reported frequent aberrant expression of lymphoid antigens, such as CD7 and CD22, on maturing neutrophils [12], is uncommon in the author's experience and that of others [13]. The exception here is CD56, which is expressed frequently on maturing myelomonocytic cells in a variety of myeloid disorders [12–15,38] but also may be seen at a low level on a subset of immature neutrophilic precursors in an actively regenerating marrow or after granulocyte colony-stimulating factor (G-CSF) therapy, although this is not well documented in the literature.

Abnormalities on maturing neutrophilic cells, which seem to have diagnostic usefulness, include decreased side scatter (discussed previously), which is synonymous with hypogranularity. Alterations in the intensity of normally expressed antigens are frequent, such as abnormalities in the relationship between CD13 and CD16 expression [12–14] in which decreased and inappropriate expression of CD16 is a major component (Fig. 5). Increased expression of CD14 and CD64 on maturing neutrophils may be seen, likely indicating neutrophil activation as can be seen after G-CSF administration [39]. Decreased expression of CD45, low expression of CD34, and loss of CD11b also are reported [13] but are infrequent. Abnormalities in CD66 expression have been suggested [22,40] but are not well characterized. An aberrant lack of CD33 expression also may be observed; however, the level of CD33 expression normally varies between individuals with some having quite low expression. Consequently, the author routinely uses the monocyte population, normally the brightest CD33-positive population, as an internal control to gauge whether or not a disproportionate reduction in CD33 expression is present on blasts and neutrophilic forms. A significant subset of cases shows aberrancy in the coordinated expression of antigens during maturation, also termed asynchronous shift to the left by some, which in certain cases can give rise to different visible simultaneous pathways of maturation (see Fig. 5).

Monocytic maturation

Antigenic abnormalities during monocytic maturation are described but are recognized less frequently than those seen on CD34-positive progenitors or maturing neutrophils [13,14]. Alterations in the intensity of normally expressed antigens include CD11b, CD13, CD14, CD15, CD33, and

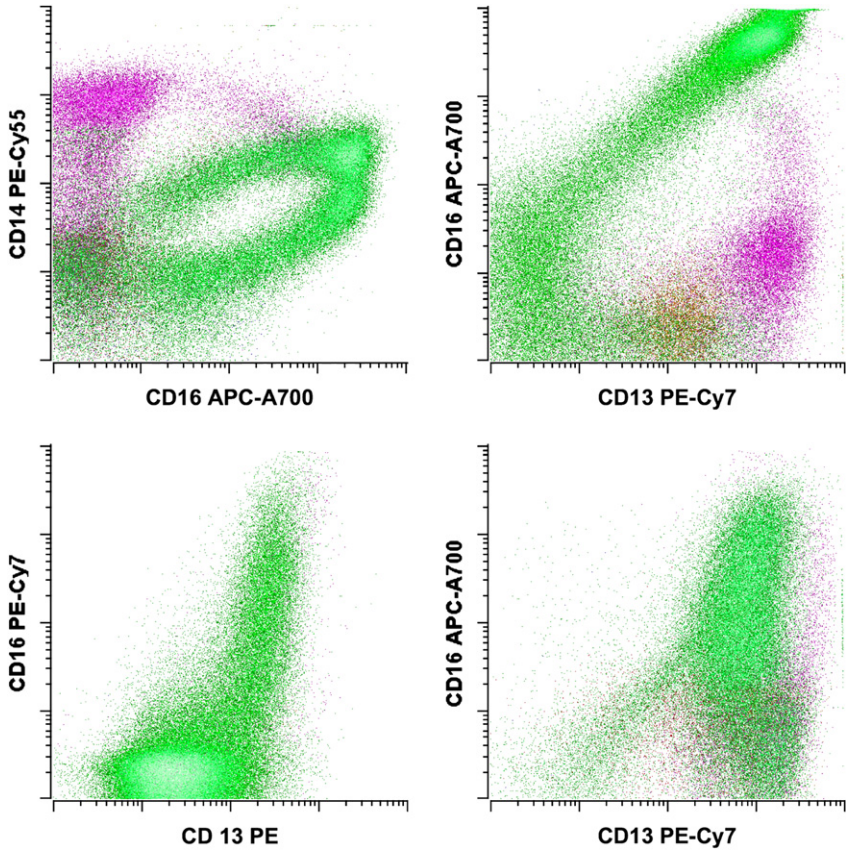

Fig. 5. Abnormal antigen expression on maturing neutrophils: maturing neutrophils gated by CD45 and side scatter show parallel pathways of maturation (*top left*) one normal (*lower*) and the other abnormal with premature and elevated acquisition of CD14 (*upper*). Normal correlated expression of CD13 and CD16 (*upper right*) is contrasted with abnormal maturation (*lower panels*).

HLA-DR, often representing decreases in expression (Fig. 6). In many cases, these abnormalities are related to an increase in immature forms or promonocytes and not necessarily are abnormal intensities for that population. The normal variability in CD33 expression between individuals should be kept in mind when assessing decreased CD33 for aberrancy, similar to neutrophilic maturation. Aberrant expression of lymphoid antigens, such as CD7, CD19, and CD56, are described; however, similar to neutrophilic maturation, CD56 expression commonly is seen at a low level during marrow regeneration or after G-CSF therapy and should be viewed with caution (see Fig. 6). Asynchronous expression of CD34 on monocytes also may be seen.

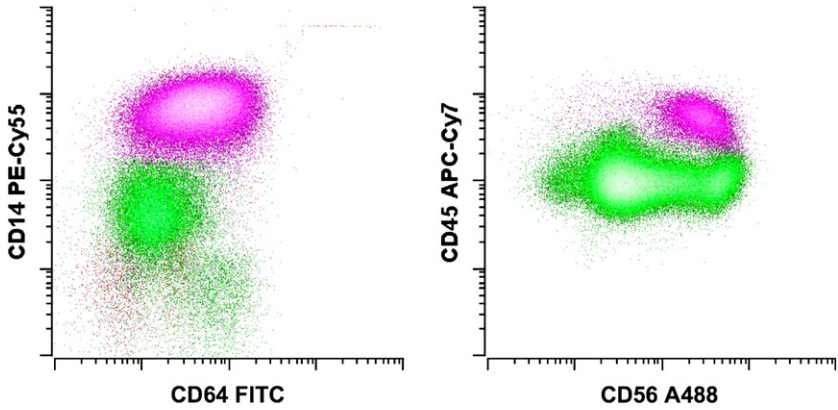

Fig. 6. Abnormal monocytes. Monocytes (CD14-positive) gated by CD45 and side scatter with abnormally decreased expression of CD64 (*left*). Monocytes (high CD45) with uniform expression of CD56; also note variable expression of CD56 on maturing neutrophils (lower CD45) (*right*).

Erythroid maturation

In comparison with myelomonocytic maturation, reagents to few antigens are available for assessing abnormalities in erythroid maturation. In addition, standard specimen-processing methods involve red blood cell lysis techniques and also compromise nucleated red cells with a variable degree of cell loss. In part, for these reasons, little has been published regarding erythroid maturation in myeloid neoplasia. Nevertheless, two groups [12,41,42] have reported a decreased level of CD71 expression as a common abnormality in MDS, where it seemed to correlate with megaloblastic erythropoiesis [12] and increased cytoplasmic and mitochondrial ferritin accumulation [42]. Changes in CD71 expression may be seen in other causes of anemia [43], however, and further study is required. An increase in immature erythroid forms expressing CD105 also is described in MDS [42]. Occasionally, in higher-grade myeloid neoplasia, such as MDS, and in a rare subset of AML, termed erythroleukemia, a marked predominance of early erythroid forms may be seen having antigenic abnormalities, often in conjunction with abnormalities on progenitor populations.

Megakaryocytic maturation

Although individual abnormalities in the expression of antigens on platelets are described in AML [44] and MPD, in particular essential thrombocytosis [45–48], no systematic study of their potential diagnostic usefulness has been performed. Megakaryocytes are difficult to identify by flow cytometry in bone marrow and essentially no literature has been published regarding

abnormalities in antigen expression in myeloid neoplasia for this cell population, although abnormalities in DNA ploidy are described [49,50]. A single publication has reported a normal number of megakaryocytes by flow cytometry in MDS [12], but technical concerns about megakaryocyte identification have been raised [34]. The expression of megakaryocytic or platelet antigens are described in a subset of AML, termed acute megakaryoblastic leukemia [51], in the transient MPD associated with Down syndrome [52], and infrequently in blast crisis of MPD [53]. The assessment of any platelet antigen expression is complicated by the frequent adherence of platelet to leukocytes [54], a problem that can be minimized but not eliminated by extensive washing before sample preparation.

Myelodysplasia

Currently, the diagnosis of MDS relies primarily on a combination of morphologic, clinical, and cytogenetic findings [17]; it is only recently that immunophenotyping has been demonstrated to have diagnostic potential for these disorders. Evidence now is provided by several studies that antigenic deviation from normal maturation is a common feature of MDS that has diagnostic usefulness [12–14,32,41,42,55,56]. For example, in our own study of an unselected group of patients having peripheral cytopenias, flow cytometric analysis yielded a sensitivity of 89% and specificity of 88% of the diagnosis of MDS when compared with the combination of morphology and cytogenetics [14]. The diagnostic usefulness of this approach includes MDS having less than 5% blasts, which is the more difficult diagnostic category. One common theme that emerges from these studies is that although the presence of a single immunophenotypic abnormality in general is insufficient for a diagnosis of MDS, it is the presence of two or more abnormalities that is required. In an attempt to quantitate the extent and degree of immunophenotypic abnormalities further, a scoring system has been proposed that incorporates blast percentage and immunophenotypic changes [13] and shows correlation with the International Prognostic Scoring System and outcome after bone marrow transplantation. The presence of immunophenotypic abnormalities also is shown to correlate independently with prognosis in patients who have MDS lacking cytogenetic abnormality [57]. Consequently, flow cytometry has the potential to allow for a more confident diagnosis of MDS through the identification of objective abnormalities in hematopoietic maturation and more precise blast identification and may provide prognostic information.

Myeloproliferative disorders

Similar to MDS, the diagnosis of MPD relies primarily on a combination of morphologic, clinical, and cytogenetic/molecular findings [17]. Only

limited data have been produced regarding the presence of maturational immunophenotypic abnormalities in MPD that might be useful for diagnosis [15], and no study demonstrating their sensitivity and specificity has been conducted. In the author's experience, immunophenotypic abnormalities are observed infrequently on progenitors and maturing myelomonocytic forms in polycythemia vera and essential thrombocythemia. Whether or not identification of erythroid or platelet antigenic abnormalities has diagnostic usefulness is unexplored. Although immunophenotypic abnormalities are common on progenitors and maturing myelomonocytic forms in chronic myeloid leukemia in the author's experience, demonstration of t(9;22) remains the primary diagnostic modality for this disease. Alternatively, flow cytometry is useful for the enumeration of increased abnormal blasts as occurs in accelerated phase and blast crisis and the evaluation of progenitor lineage in blast crisis, assessments that have important therapeutic consequences in chronic myeloid leukemia. In the author's experience, the remaining MPD and overlap syndromes, including myelofibrosis, chronic myelomonocytic leukemia, and MPD unspecified, frequently display antigenic abnormalities on progenitors and maturing myelomonocytic forms, and it is likely that flow cytometry has diagnostic usefulness in these disorders, although further study is needed.

Acute myeloid leukemia

Flow cytometric immunophenotyping plays a well-established role in the diagnosis of acute leukemia [58,59], including AML, principally for blast enumeration, lineage assignment, and identification of immunophenotypic abnormalities suitable for post-therapeutic disease monitoring. In general, AML is characterized by a reduction in maturation with an accumulation of abnormal progenitors that demonstrate abnormalities similar to those seen in MDS and MPD, although more frank aberrancy is seen in a significant number of cases [27,58–63]. The assignment of myeloid lineage relies on identifying the expression of antigens characteristic of early myelomonocytic differentiation, including CD13, CD15, CD33, CD64, CD117, and cytoplasmic myeloperoxidase. Aberrant expression of lymphoid antigens, such as CD2, CD5, CD7, CD19, and CD56, is common [28] and generally does not indicate bilineal or mixed lineage differentiation. As a rule, the closer the level an antigen is expressed to that seen in normal maturation, the more likely it reflects differentiation along that lineage. In cases where lineage assignment is difficult, a scoring system has been suggested to provide more objective criteria [64].

Certain immunophenotypes seen in AML show a strong correlation with specific cytogenetic and molecular abnormalities [65]. AML with t(8;21) characteristically shows aberrant expression of CD19, high CD34, CD56, and TdT in some combination [66]. t(15;17) AML demonstrates an immunophenotype typical of promyelocytes, including a variable increase in

side scatter, lack of significant CD34, expression of variable CD13 and CD117, aberrantly high CD33, and aberrantly low to absent CD15 67 (Fig. 7) [67]. AML with inv(16) or t(16;16) does not have a distinctive immunophenotype but generally displays myelomonocytic differentiation as evidenced by a blast population, often expressing CD117 without CD34, which shows subsets with acquisition of CD15 and increased side scatter without increased CD45 (neutrophilic) and acquisition of CD64 or CD14 with increased CD45 and minimally increased side scatter (monocytic) (Fig. 8) [68]. A distinct subset of AML shows purely monocytic differentiation with an immunophenotype most similar to promonocytes having expression of CD4, CD33, high CD64, variable CD117, little to no CD13, or lack of CD34 and often with aberrant CD56. Some degree of maturation

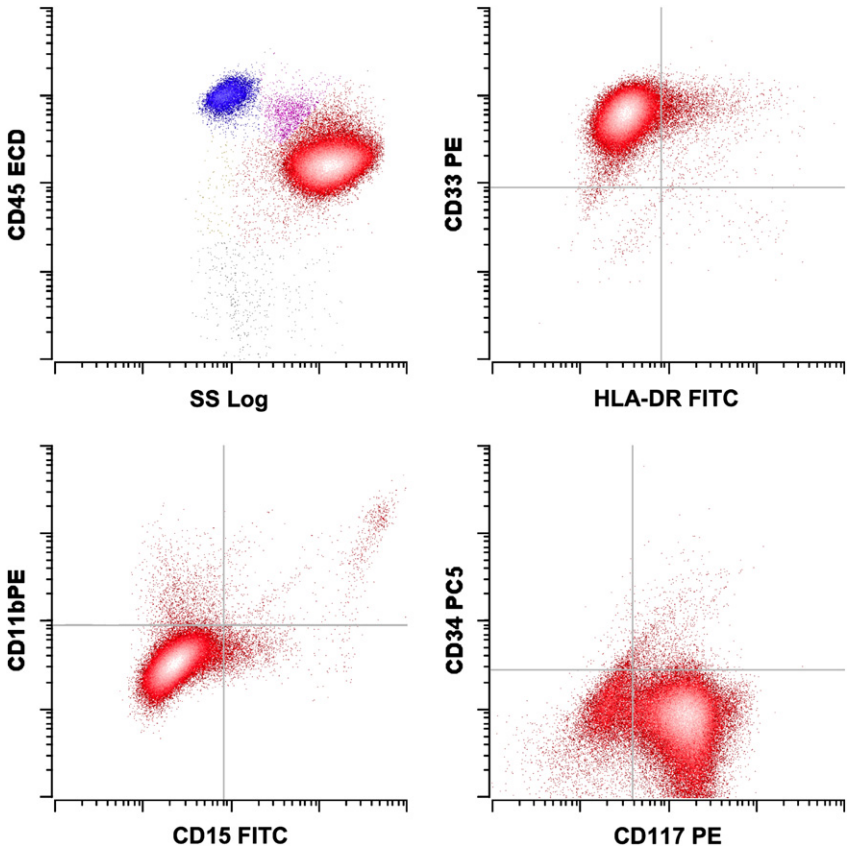

Fig. 7. Acute promyelocytic leukemia. Abnormal promyelocytes are seen without an apparent progenitor population. The abnormal promyelocytes have high side scatter (*top left*) with aberrantly high CD33 (*top right*) and aberrant absence of CD15 (*bottom left*). The absence of HLA-DR (*top right*), CD11b (*bottom left*), and CD34 (*bottom right*) with CD117 expression is normal for promyelocytes.

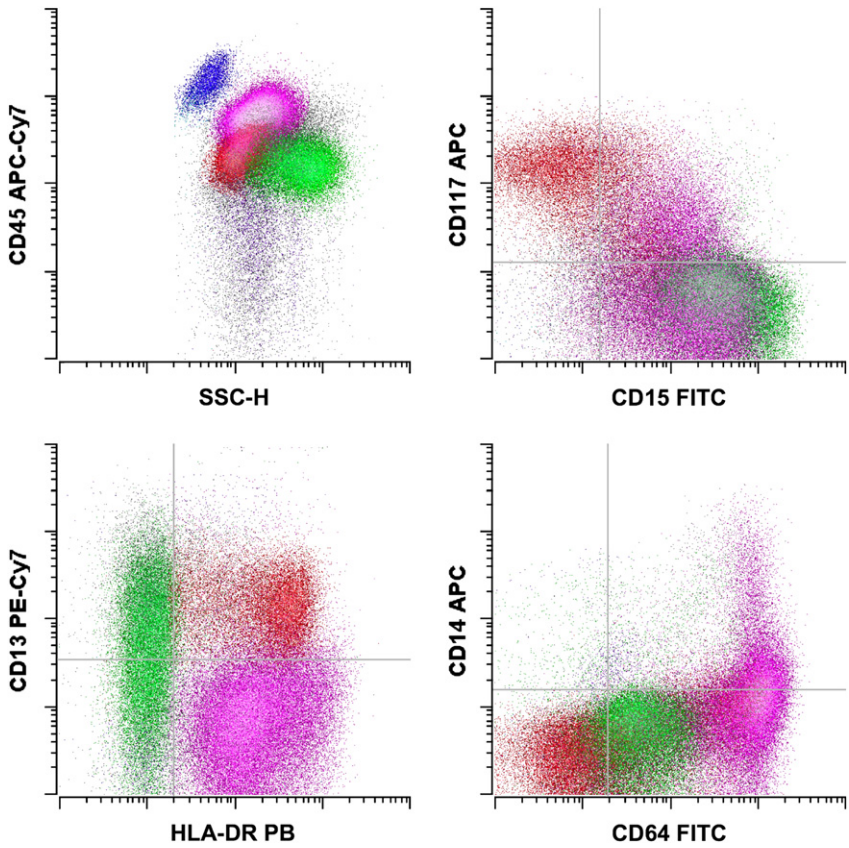

Fig. 8. Acute myelomonocytic leukemia. Progenitors show maturation to neutrophilic (increased side scatter with intermediate CD45) and monocytic (increased CD45) lineage by CD45 versus side scatter (*top left*). The progenitors express CD117 and acquire CD15 with maturation (*top right*) with differential expression of CD13 and HLA-DR, as is characteristic of immature forms of their respective lineages (*bottom left*). The monocyte subpopulation shows expression of CD64 (high) without significant CD14, consistent with promonocytes (*bottom right*).

to more mature monocytes also may be seen with acquisition of CD14 and CD45. Acute megakaryoblastic leukemia largely is defined by the expression of platelet-associated antigens CD41, CD42b, or CD61 on progenitors [54] and is associated with t(1;22) in children. Aside from facilitating the recognition of these specific cytogenetic abnormalities, subclassification by lineage of differentiation has uncertain clinical usefulness.

Minimal residual disease

The methodology for the detection of residual disease after therapy is a logical extension of the principles (outlined previously) to a situation

where the number of neoplastic cells hopefully is small in number and present in a background of more abundant normally maturing forms. The usefulness and sensitivity of the technique depend on the degree to which the immunophenotype of the neoplastic population deviates from normal maturation and on the relative proportions of the normal and abnormal populations. The frequencies at which many immunophenotypic aberrancies seen in AML occur in normal marrow has been estimated [69], although similar information for regenerating marrow is more rudimentary. Published literature suggests routine detection of involvement by AML at levels of 0.1% is consistently achievable, with detection down to 0.01% possible in many cases [69–78], and this is consistent with the author's experience (Fig. 9).

Evaluation of post-therapy specimens for a leukemia-associated immunophenotype, one or more aberrancies in immunophenotype identified at the time of diagnosis, is a common approach used successfully to stratify patients into prognostic groups [70–76]. Although this method has its merits, it generally relies on preset regions in multiparameter space, defined at the time of diagnosis, to identify the persistence of immunophenotypic aberrancy and, as such, is not focused on the recognition of discrete abnormal populations. As a result, there is little assurance that what is measured represents the entire neoplastic population or in many cases that it does not also represent a portion of a normal population that occurs at a low frequency in the setting of marrow regeneration. This potentially can result in errors in recognition and quantitation that become more evident at low levels of involvement. An inherit assumption also made in this approach is that the immunophenotype of the neoplastic population remains essentially unchanged after therapy. This assumption is not entirely correct, as

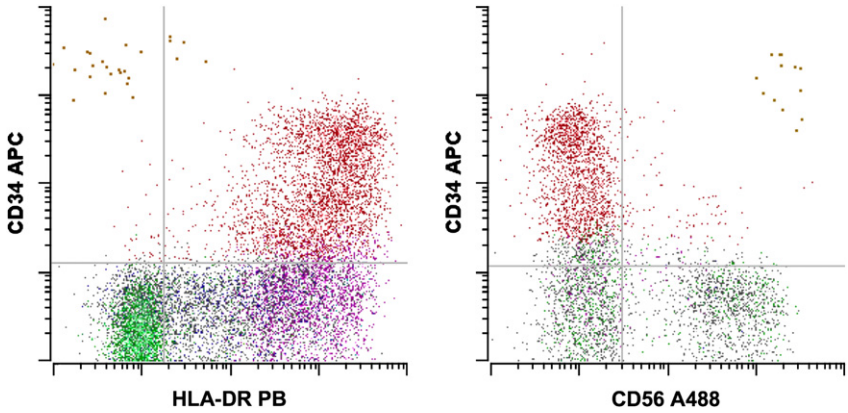

Fig. 9. Detection of minimal residual disease in AML. Progenitors gated by CD45 and side scatter show a small subpopulation having abnormal expression of CD34 (high), HLA-DR (very low to absent) (*left*) and CD56 (very high) (*right*). The population is present at 0.007% of the white cells but is recognized easily by its frankly aberrant immunophenotype.

changes in AML immunophenotype between diagnosis and relapse are reported in up to 88% of cases [79–81]. At least one of the aberrancies seen at diagnosis, however, is retained frequently and immunophenotypic shift is not believed to prevent residual disease evaluation if more than one aberrancy is used. Many of these problems, in part, can be mitigated by requiring recognition of discrete abnormal populations having immunophenotypic deviation from normal maturation, using leukemia-associated immunophenotyping largely as a starting point for analysis.

Nearly all of the studies evaluating the significance of AML residual disease detection conclude that the presence of residual disease after therapy is associated with a poor prognosis or clinical outcome [69–78,82], with one exception, where it was found not to be an independent risk factor [83]. Residual disease after therapy is associated with apoptosis-resistant and stem cell immunophenotypes [31,84]. The appropriate time point for evaluation and the level of involvement associated with increased poor outcome are a matter of debate. In general, the presence of detectable disease at end of induction chemotherapy in the range of greater than 0.045% to 0.1% [70,71,76] and at end of consolidation greater than 0.035% to 0.5% [72,74,77] is associated with poor outcome. One group has advocated use of the logarithmic difference in blast percentage between diagnosis and evaluation time point as a measure of residual disease and showed that separation at the 75th percentile correlates with overall and relapse-free survival at end of consolidation. Early response to therapy at day 16 in bone marrow also correlated with a poor outcome [85]. As a result of these studies, evaluation for residual disease in AML by flow cytometry is becoming a standard part of post-therapeutic monitoring in some institutions.

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