Biphenotypic, bilineal, ambiguous or mixed lineage: strange leukemias!

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The diagnosis of acute leukemias (AL) relies both on clinical features and an array of multidisciplinary approaches including morphology, immunophenotyping, cytogenetic and molecular investigations. In the vast majority of cases, congruent data is obtained from these various sources and allow us to assign the malignant cells to a given lineage and differentiation stage. Further prognostic information is provided by the chromosomal studies, allowing us to refine the choice of therapeutic options in order to best preserve the patient's chances for prompt remission while limiting the toxicity of chemotherapy.

In rare cases, however, it is very difficult to classify the blasts. In some instances, the very first stage of morphological examination identifies what seems to be different blast cell populations. In others, blasts cells of homogeneous morphology display an aberrant immunophenotype. These bilineal/biphenotypic cases have been regularly reported in the literature, usually as small series of poor prognosis. One such report from Xu *et al*. appears in this issue of the journal,[1] describing 21 new cases identified in a Shanghaï hospital over seven years among 452 AL patients, confirming the poor outcome of these patients. These authors also provide an interesting comparison of 9 other published series of between 19 and 63 cases that appeared in the literature between 1996 and 2007. This compilation shows that the most frequent type of biphenotypic AL (BAL) involves the co-expression of markers of myeloid- and B-lineage, between 47 and 72% depending on the series. BAL with myeloid- and T-lineage markers are next in frequency, around 24%, while both B/T and triple myeloid/B/T BAL are exceptional.

Traditional definition of biphenotypic acute leukemia

The first published reports mentioning the entity of BAL can be found in the 80s, soon after the appearance of the first monoclonal antibodies and their application to the definition of leukemic cells. One of the earliest[2] interestingly noted the simple coexpression, in 3 patients with acute myeloblastic leukemia (AML), of myeloperoxidase and terminal deoxynucleotidyl transferase; the latter having been shown at the time as being strongly correlated to acute lymphoblastic leukemia, both as enzymatic activity[3] or as a protein identified in immunofluorescence.[4] In such very early publications, allusion was already made to the possible existence of a *stem cell leukemia capable of differentiating into myeloid and lymphoid cells*.[5] The scoring systems proposed by Catovsky *et al*.[6] and by the EGIL[7] allowed for a better definition of BAL, clearly distinguishing them from classical AL expressing aberrantly one or two markers of another lineage. Typical examples of such aberrations are the expression of CD15 on B-ALL[8] or of CD2 on acute promyelocytic -AML.[9] In 1998, the EGIL further refined this scoring system by attributing one point for the expression of CD117, after showing the strong relationship of this marker with engagement in the myeloid lineage.[10] To identify BAL, it is therefore necessary to consider aberrant co-expression of markers usually associated to different lineages, with a score higher than 2 in more than one lineage[7] (Table 1). This implies that a sufficiently exhaustive immunophenotype of the blast cells has been performed. For many reasons, including the cost of reagents and of manpower, often a minimal orienting immunophenotyping panel is performed to assess the cells' lineage, and then further characterize these blasts for that lineage. For instance, and although this was largely debated, the recommendations of the 2006 Bethesda consensus[11] do not fulfill the requirements of EGIL for the detection of BAL as, for instance, CD79a or cMPO do not appear in the orientation panel suggested. For this reason, the mandatory panel proposed by the European LeukemiaNet is more comprehensive and would allow better detection of BAL.[12]

The key publication about BAL identification, however, came from Catovsky's group, under the supervision of Estella Matutes.[13] In this publication reporting on 26 cases, the main characteristics of this disease were reviewed, including the variety of chromosomal anomalies referred to later.

A new definition

In the most recent issue of the WHO classification of tumors of hematopoietic and lymphoid tissues,[14] the intriguing group of AL where blast cells exhibit features from more than one lineage has been revisited. These entities have been associated to some other types of non-conventional AL in the chapter dubbing them *of ambiguous lineage* (Table 2). BAL thus are now better called *mixed phenotype acute leukemias* or MPAL. They are further partitioned according to the major cytogenetic anomalies reported in such patients, namely Philadelphia chromosome, or translocations involving 11q23 and the *MLL* gene. Caution is provided, in the case of t(9;22), not to include as MPAL blast crises of patients formerly known to have chronic myelogenous leukemia (CML). As for MLL involvement, it can only be used to classify such cases if the other criteria of MPALL are met. Besides these cytogenetic aberrations, MPAL have been partitioned according to the lineage mix they display, i.e. as B/myeloid, T/myeloid and *rare types* including triple lineage or B/T co-expression. Another novelty is that a distinction is no longer made between bilineal cases where two types of blasts of different lineage co-exist and truly aberrant cells co-expressing normally exclusive markers. In this group of acute leukemias of ambiguous lineage, MPAL are accompanied by acute undifferentiated leukemias and other ambiguous lineage leukemias. This last group encom-

Table 1. EGIL scoring system for biphenotypic acute leukemia.

	B-lineage	T-lineage	Myeloid lineage
2 points	CD79	CD3	MPO
	cμ	TCR	(lisozyme)
	cCD22		
1 point	CD19	CD2	CD13
	CD10	CD5	CD33
	CD20	CD8	CDw65
		CD10	CD117
0.5 point	TdT	TdT	CD14
	CD24	CD17	CD15
		CD1a	CD64

passes natural killer cell leukemias/lymphomas and unclassifiable leukemias. In the latter case are rare disorders exhibiting T-lineage markers but no cytoplasmic CD3 or cases with myeloid markers without MPO as reported by Casasnovas *et al.*[15] as *ME*-AML. Undifferentiated leukemias are exceptional cases where no lineage can be asserted, care having been taken to exclude blastic plasmacytoid dendritic cell neoplasms (BPDC) which can display no lineage marker but co-express CD4 and CD56 as well as, most often, CD123 and CD303 (BDCA2),[16] or other rare types such as basophilic of NK precursor leukemias.

The possibility of non-hematopoietic tumors must also have been excluded before arriving at a conclusion of acute undifferentiated leukemia (AUL).

The new definition of MPAL is also more stringent than the EGIL proposal. Major differentiation antigens have been retained for all three major lineages. Myeloid engagement is therefore defined by the expression of myeloperoxidase or, for cells already showing differentiation towards the monocytic lineage, by the expression of at least two of the monocyte-associated antigens CD11c, CD14, CD64, lysozyme and/or non-specific esterase activity demonstrated in cytochemistry. T-lineage commitment is characterized by cytoplasmic or surface (rare) expression of the epsilon chain of CD3.

B-lineage engagement requires several markers. Strong CD19 expression must be concomitant to at least one of the following: cytoplasmic or membrane expression of CD79a, CD22, and/or CD10 surface labeling. If CD19 is weakly expressed, two of the latter markers must be present to confirm B-lineage features.

By requiring less, yet stronger, markers than the former scoring proposals, this WHO classification might lead to a better identification of MPAL cases, assuming that the suggested markers are incorporated in primary panels. Interestingly, re-analyzing the series reported in this issue by Xu *et al.*[1] according to these criteria, as far as is possible with the information provided, changes the series quite drastically since, for instance, MPO is only expressed in 11 cases, usually at low percentages, 3 cases finally displaying a majority of MPO+ blasts and enough of other lineages' markers to qualify as MPAL.

Table 2. Acute leukemias of ambiguous lineage according to the WHO classification of tumors of hematopoietic and lymphoid tissues.[14]

Condition	Definition
Acute undifferentiated leukemia	Acute leukemia that does not express any marker considered specific for either lymphoid or myeloid lineage
Mixed phenotype acute leukemia with t(9,22) (q34;q11.2); *BCR-ABL1*	Acute leukemia meeting the diagnostic criteria for mixed phenotype acute leukemia in which the blasts also have the (9,22) translocation or the *BCR-ABL1* rearrangement
Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged	Acute leukemia meeting the diagnostic criteria for mixed phenotype acute leukemia in which the blasts also have a translocation involving the *MLL* gene
Mixed phenotype acute leukemia, B/myeloid, NOS	Acute leukemia meeting the diagnostic criteria for assignment to both B and myeloid lineage, in which the blasts lack genetic abnormalities involving *BCR-ABL1* or *MLL*
Mixed phenotype acute leukemia, T/myeloid, NOS	Acute leukemia meeting the diagnostic criteria for assignment to both T and myeloid lineage, in which the blasts lack genetic abnormalities involving *BCR-ABL1* or *MLL*
Mixed phenotype acute leukemia, B/myeloid, NOS – rare types	Acute leukemia meeting the diagnostic criteria for assignment to both B- and T- lineage
Other ambiguous lineage leukemias	Natural killer cell lymphoblastic leukemia/lymphoma

Molecular explanations?

It is interesting to note that most reports on BAL describe the frequent involvement of two types of translocations, namely the Philadelphia chromosome t(9;22) and translocations related to chromosome 11q23. In the former case, the intrinsic tyrosine kinase activity related to the loss of the myristylation regulatory domain of ABL can certainly be involved in disrupting a number of differentiation pathways, mixing up cell programs and leading in some cases to vast aberrations of gene expression.[17]

For 11q23, the story is stranger, but has recently become largely unraveled, as is explained also in this issue by RK Slany.[18] Interestingly, the *MLL* gene was initially reported by Ziemin-van der Poel *et al.*[19] in a yeast artificial chromosome bearing the CD3D and CD3G gene loci and constructed because of the frequent involvement of this region in both lymphoblastic and myeloblastic AL. The first name proposed was in fact *myeloid/lymphoid, or mixed-lineage, leukemia* without reference at that time to BAL or MPAL. The frequency of MLL involvement is estimated to be about 20% of ALL and 5% of ALL,[20] a much higher frequency than the incidence of MPAL. The complex functions of the wild MLL

and the large number of its fusion partners described in leukemias suggest that in some specific cases, through a disrupted function of *HOX* or other factors, the leukemic event may lead to a profound dysregulation of differentiation patterns and therefore MPAL.

A different future?

The first definitions of BAL used single labelings and co-expression could only be certified when the percentages of positive cells clearly overlapped. For instance, the B/T case reported by Xu *et al.*[1] is disputable as presented, as there could well be both a major contingent of T cells and a smaller independent one of B cells. These ambiguities became easier to identify with the development of multiparameter flow cytometry. Co-expression can thus be more readily defined and varying patterns can be observed, as shown in the relevant chapter of the 2008 WHO classification.[14] The definition of MPAL will thus possibly change again with the application of increasingly sophisticated labelings and software. The use of CD45 to gate at best the blastic population might not be sufficient to properly discriminate between abnormal cells and residual normal hematopoiesis. This would account for the patterns of partial co-expression mentioned above,[14] in fact analyzing different subsets gated together for CD45 low expression. Even CD34 expression, highly frequent in BAL (15 of 21 cases over 60% in the series of Xu *et al.*),[1] but also a key feature of hematopoietic progenitors could mistakenly be considered homogeneous on such mixed populations. A better knowledge of normal bone marrow flow cytometric features, adequate marker combinations and forthcoming progress in flow cytometry may further refine the definition of these rare diseases, hopefully in the near future.

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