Chapter 17
Mixed Phenotype Acute Leukemia

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Introduction

Mixed phenotype acute leukemia is a rare disease and comprises 2–5% of all acute leukemias. These disorders have been historically labeled by a variety of names, such as mixed-lineage leukemia, bilineal leukemia, and biphenotypic leukemia [1]. Both the earlier 2008 and more recent 2016 World Health Organization (WHO) classifications have proposed a simpler diagnostic algorithm to define mixed phenotype acute leukemia (MPAL), which includes both biphenotypic and bilineal acute leukemias.

Clinical Presentation

Presenting clinical symptoms in MPAL are similar to other acute leukemias and include fatigue, infections, and bleeding disorders [2]. Usually, the white blood cell count is high and most patients will have a high number of circulating blasts [2].

Morphology and Immunophenotype

Morphologically, MPAL blasts appear most often as undifferentiated medium-sized blasts with fine chromatin and indistinct-to-prominent nucleoli; however, these blasts can show classical lymphoid features and appear smaller in size with variably condensed

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C.-C. Chang, R.S. Ohgami (eds.), *Precision Molecular Pathology of Myeloid Neoplasms*, Molecular Pathology Library, DOI 10.1007/978-3-319-62146-3_17

nuclear chromatin and very high nuclear-to-cytoplasmic ratios, or myeloid features with cytoplasmic granules, very fine nuclear chromatin, and large prominent nucleoli.

However, the diagnosis of MPAL rests on the immunophenotypic features of these blasts rather than morphology. Flow cytometry is the preferred method for recognizing MPAL. Even when there are not 2 distinctly separable populations, most cases of MPAL will show heterogeneity of expression of some antigens. For example, MPO expression will be expressed on the subset of blasts that show relatively brighter expression of myeloid markers and lower intensity of B-cell–associated markers. Figure 17.1 is an example of MPAL where the blasts are small with

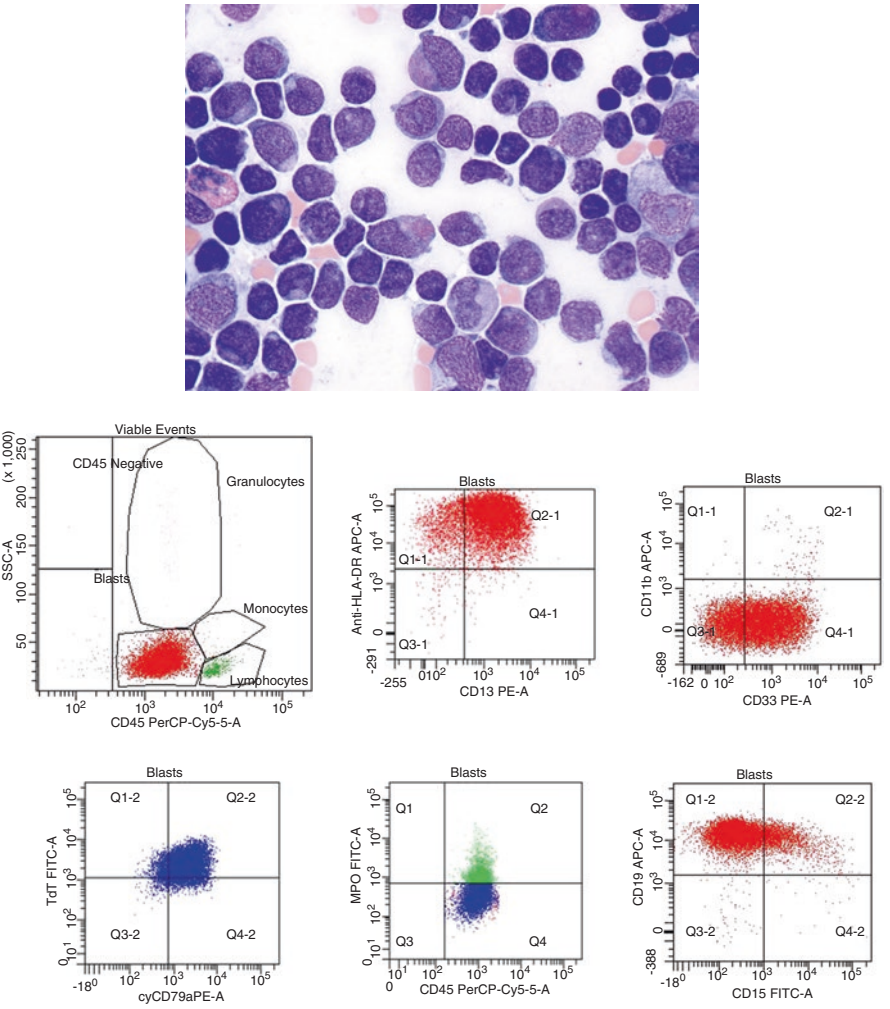

Fig. 17.1 Case of B/myeloid mixed phenotype acute leukemia. Blasts are mostly small with moderate cytoplasm (part **a**) and expressed CD34, CD13, CD33, CD19, CD79a, TdT, and MPO (part **b**)

moderate cytoplasm and express myeloid markers (CD13, CD33, MPO) as well as strong CD19 and CD79a.

One of the first major attempts to define MPAL was the scoring criteria proposed by the European Group for the Immunological Characterization of Leukemias (EGIL) (Table 17.1) [3]. A numerical value, ranging from 0.5 to 2, was assigned for individual myeloid-associated or lymphoid-associated markers expressed by the blasts, and a biphenotypic acute leukemia was defined when a score over 2 points was achieved for each lineage [3]. In later years, the 2001 World Health Organization (WHO) classification of hematopoietic and lymphoid neoplasms incorporated the EGIL scoring system when defining acute leukemias of ambiguous lineage [4].

Then, in 2008, the WHO classification proposed a simpler diagnostic algorithm to define MPAL, which relies on fewer, more lineage-specific markers [5] (Table 17.2). Myeloid lineage requires the presence of myeloperoxidase as detected by flow cytometry, immunohistochemistry or cytochemistry, or evidence of monocytic differentiation (with at least 2 of the following markers being positive: non-specific esterase cytochemistry, CD11c, CD14, and CD64). T-lineage can be shown with cytoplasmic or surface CD3, at least as intense as background reactive T-cells, and multiple antigens are required for B-lineage including CD19, CD79a, CD22, and CD10. All possible combinations of MPAL can be observed including B/myeloid,

Table 17.1 EGIL scoring system for biphenotypic acute leukemia

Points	Myeloid lineage	B lineage	T lineage
2	MPO	CD79a	CD3 (cyt/m)
	lysozyme	Cyt IgM	anti-TCR α/β
		Cyt CD22	anti-TCR γ/δ
1	CD13	CD19	CD2
	CD33	CD10	CD5
	CD65	CD20	CD8
	CD117		CD10
0.5	CD14	TdT	TdT
	CD15	CD24	CD7
	CD64		CD1a

Table 17.2 2008 WHO classification: acute leukemia of ambiguous lineage

Lineage	Markers
Myeloid	Myeloperoxidase OR Monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme
T lineage	Cytoplasmic CD3 OR Surface CD3
B lineage	Strong CD19 *and* at least 1 of the following with strong expression: CD79a, cytoplasmic CD22 or CD10 OR Weak CD *and* at least 2 of the following with strong expression: CD79a, cytoplasmic CD22 or CD10

T/myeloid, B/T, or even rarely B/T/Myeloid [5]. MPAL with t(9;22) and MLL rearrangement have been separated out as distinct subtypes. Acute leukemia of ambiguous lineage is reserved for cases of acute leukemia that show no clear evidence of differentiation along a single lineage.

In the 2016 revision to the WHO classification, no new entities were defined within this group of leukemias [6]. Although the list of lineage-specific markers is unchanged, it is now emphasized that in cases with 2 distinct blast populations, each population should meet criteria for B-lymphoblastic leukemia (B-ALL), T-ALL, or acute myeloid leukemia but it is not necessary that specific markers are present [6]. It is also now more specifically stated that cases of otherwise typical B-ALL with only low-level expression of MPO (without other evidence of myeloid differentiation) should not be classified as MPAL. Furthermore, a specific statement is now included that cases of otherwise typical ALL or AML do not need to meet the strict lineage defining criteria listed for MPAL.

MPAL with BCR-ABL Fusion Gene

Two genetic lesions are frequent enough in MPAL to now be considered as separate entities. The first is MPAL with t(9;22)(q34;q11.2) or *BCR-ABL1* rearrangement. The t(9;22)(q34;q11.2) translocation results in a *BCR-ABL1* fusion gene located on the Philadelphia chromosome (Ph), causing a constitutively active BCR-ABL1 tyrosine kinase. Acute leukemia with t(9;22) and blast phase of chronic myeloid leukemia (CML) have very similar clinical presentations and morphologic features. The 2008 WHO classification suggests caution when making the diagnosis of MPAL with t(9;22) [4]. Splenomegaly, peripheral leukocytosis due to maturing myeloid precursors and mature neutrophils, absolute basophilia, and a clinical history of CML may support the diagnosis of blast phase of CML with MPAL phenotype [4]. De novo MPAL with *BCR-ABL* rearrangement generally occurs more frequently in older patients. Although most studies found the frequency of MPAL with t(9;22) to be 28–35%, pediatric studies report it to be much lower at 3% [7]. Many of these cases show a dimorphic population of blasts, with most showing B and myeloid lineage [7]. Some studies suggest that this subtype of MPAL has a worse outcome [8].

MPAL with MLL Rearrangement

The second most frequent genetic lesion in MPAL is translocations involving MLL gene. *MLL* rearrangement juxtaposes the amino-terminus of the histone methyltransferase MLL to a variety of fusion partners, with the most common partner gene being AF4 on chromosome 4 band q21.35 in MPAL [9]. This tends to occur more commonly in children and is more frequent in infancy [9]. One study showed frequency of 10% in adults to 12–18% in pediatric MPAL [1]. These cases also tend to

present with a dimorphic blast population, one resembling lymphoblasts and the other resembling monoblasts. By flow cytometry, the lymphoblasts usually have a CD19-positive, CD10-negative, B-precursor immunophenotype and are frequently positive for CD15. Usually, the flow cytometry identifies a separate population of myeloid blasts with monocytic differentiation. The prognosis of MPAL patients with MLL rearrangement is also poor [10].

Mixed Phenotype Acute Leukemia, Not Otherwise Specified

Cytogenetics and Molecular Findings

In a recent study, Yan et al., found that of 92 MAPL patients assessed, 64% presented with cytogenetic abnormalities [11]. The most prevalent aberration was the complex karyotype found in 24% of patients, followed by the t(9;22) chromosome in 15% (all B-myeloid phenotype) and translocations involving MLL gene at 11q23 in 4.3% of patients [11]. A specific reference was made in the 2008 WHO classification to exclude cases that can be classified in another category, either by genetic or clinical features. For instance, AML with t(8;21), t(15;17), and inv. [11] can express lymphoid-associated markers but should be classified as AML with recurrent genetic abnormalities. Cases of chronic myelogenous leukemia (CML) in blast crisis, AML with myelodysplasia-related changes, and therapy-related AML should be classified as their respective entities even if they happen to have a mixed phenotype.

In a study of 61 MPAL patients, Weinberg et al., found that 23 of 61 patients were under 21 years of age (38%), most showed a B/myeloid phenotype (67%), and had normal cytogenetics (44% of patients with cytogenetic information) [12]. Seven patients (or 22%) had t(9;22) or MLL rearrangement. This is a similar distribution to what Matutes et al., found in their study [13]. However, both Matutes et al., and Yan et al., included MPAL patients with complex karyotype (~24–32% of all their patients) in their series [11, 13]. In the 2008 WHO classification, the presence of a complex karyotype would be considered as AML with myelodysplasia-related changes if defined by cytogenetics alone, and such cases were excluded from the study by Weinberg et al.

Rubnitz et al., analyzed gene expression patterns in 13 pediatric patients with MPAL (as defined by EGIL) and found that 8 patients displayed gene expression patterns that were different from AML and ALL [14]. In contrast, using microRNA profiling studies, de Leeuw et al., demonstrated that 16 cases had microRNA expression profiles that clustered with AML or ALL [15]. Heesch et al., noted a higher expression of *BAALC* and *ERG* in 26 cases of MPAL as compared with other cases of AML [16]. Array-based comparative genomic hybridization analysis in 12 patients with MPAL demonstrated that all patients had at least 1 abnormality, including deletions of *CDKN2A, IKZF1, MEF2C, BCOR, EBF1, KRAS, LEF1, MBNL1, PBX3,* and *RUNX1* [14].

Information regarding the mutational landscape of MPAL is based on small patient numbers. Yan et al., analyzed 31 patients with MPAL and reported that 12 patients

(39%) were found to harbor a known mutation [11]. These included *IKZF1* deletion in 4 patients (all B-myeloid phenotype with evidence of BCR-ABL1 fusion gene), *EZH2* in 3 (B- or T-myeloid; one case showing complex karyotype and another showing loss of chromosome 7), *ASXL1* in 2 (both B-myeloid), *TET2* in one (B-myeloid), and *ETV6* and *NOTCH1* in 1 patient each (both T-myeloid) [11]. A high rate of mostly biallelic mutations *DNMT3A* mutations were reported in 10 of 18 adults with T-myeloid MPAL [14]. No evidence of mutations in C*BL, DNMT3A, FBXW7, FLT3, IDH1, IDH2, KIT, NPM1, PHF6, RUNX1,* and *WT1* were found in Yan's study [11].

Whole-exome sequencing in 23 adult and pediatric patients with MPAL demonstrated that 35% patients had mutations in epigenetic regulatory genes ([17], Table 17.3). *DNMT3A* was the most common mutation (23%) followed by *IDH2 (9%), TET3 (4%), and EZH2 (9%).* All of the *DNMT3A* mutations involved the methyltransferase domain, three of which were missense mutations at Arg882, the hotspot common in AML. *DNMT3A* occurred in all immunophenotypic subtypes examined. Similar to reports in AML, MPAL patients with mutation in *DNMT3A* trended toward being older and having a normal cytogenetics [17]. Tumor

Table 17.3 Summary of mutations from whole-exome sequencing of 23 MPAL samples

	Gene	Frequency (%)
Epigenetic	*DNMT3A*	6 (23%)
	IDH2	2 (9%)
	TET3	1 (4%)
	EZH2	2 (9%)
Activated signaling	*NRAS*	4 (17%)
	KRAS	3 (13%)
	NF1	2 (8%)
	FLT3	3 (13%)
	JAK2	1 (4%)
	JAK3	1 (4%)
Tumor suppressor	*TP53*	5 (22%)
	WT1	3 (13%)
	PHF6	2 (8%)
	PTCH11	2 (8%)
	CDKN2A	1 (4%)
Transcription factors	*NOTCH1*	5 (22%)
	RUNX1	4 (17%)
	GATA2	1 (4%)
	IKZF1	1 (4%)
Splicing	*SF3A1*	1 (4%)
Cohesin	*RAD21*	1 (4%)
	SMC1A	1 (4%)
Others	*CDKN2B*	1 (4%)
	LEF1	1 (4%)

Data from Eckstein et al. [17]

suppressors were also frequently mutated and 5 patients (22%) had *TP53* mutations ([17], Table 17.3). Mutations of DNMT3A and tumor suppressors showed high variant allele frequency (VAF), suggesting that these mutations arise early in the disease. Sixty-one percent of the patients also had mutually exclusive mutations of activating signaling genes including *NRAS, KRAS, and NF1* [18]. *NOTCH1* mutations were present in 5 of 16 (32%) with T-myeloid and B/T leukemia. Three samples (13%) also had *WT1* mutations. In another series, clustering of *FLT3 ITD* and *TKD* mutations was reported in patients with T-myeloid MPAL. Seven of 15 patients (47%) were positive for FLT3 mutations (mostly ITD), all of which were CD117+ [19].

Prognosis and Therapy

There is no set therapy for MPAL patients, which is a result of the absence of prospective trials. In the few larger retrospective series of MPAL, the median overall survival is reported to range from 14.8 to 18 months and the rate of achieving long-term survival in patients with adult MPAL is poor (<20%) [17, 19, 20]. Most of the retrospective case series suggest that the complete remission rates are higher with ALL therapy or an ALL/AML combined regimen than with AML-type therapy [21, 22]. Children with MPAL are suggested to do better, although they do have inferior outcome compared with those diagnostic with typical ALL [22]. A few studies compared outcome of MPAL patients with that of matched control ALL or AML groups and most found that MPAL patients did worse than AML or ALL [1]. In a study of 61 patients, Weinberg et al., found that when compared with 177 patients with acute myeloid leukemia (AML), MPAL patients had better overall survival (P = .0003) and progression-free survival (P = .0001). However, no difference in overall survival between MPAL and 387 patients with acute lymphoblastic leukemia was present (P = .599) [12]. For patients with t(9;22)-positive MPAL, a tyrosine kinase inhibitor (TKI) is usually added to treatment [23]. In his review, Wolach et al. suggested that the best approach for the non-t(9;22) MPAL patient is to treat with an ALL regimen and consolidate with an allogeneic stem cell transplant if a donor is available [23]. Shimizu H et al., have suggested that allogeneic hematopoietic stem cell transplantation may be an effective treatment for MPAL patients, especially early in the disease course [24].

Conclusion

Overall, acute leukemias with mixed phenotypes are uncommon and comprise 2–5% of all acute leukemias. Molecular studies showed frequent mutations in epigenetic regulatory genes and tumor suppressors in MPAL patients. The outcome of MPAL patients remains poor and mutations have been identified in this disease that are

potentially targetable by agents that are currently available or are being tested in clinical trials, including epigenetically targeted agents, tyrosine kinase pathway inhibitors, and NOTCH1 inhibitors. Studies suggest that the best treatment of non-t(9;22) MPAL patient is to treat with an ALL regimen and consolidate with an allogeneic stem cell transplant if a donor is available. More studies are needed to address the biology and treatment of MPAL patients.

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