Inside the Biology of Acute Leukemias of Ambiguous Lineage: Diagnostic Work-Up, Genomic and Clinical Characterization

Binsah George*, Anneliese Gonzalez, Adan Rios

Abstract

Mixed-phenotype acute leukemia (MPAL) is rare subtype of leukemia characterized by blasts with both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) markers. MPAL is a high-risk disease which represents only 2%–3% of acute leukemias and involves a genetically and immunophenotypically diverse group of patients with poor clinical outcomes. The limited incidence and lack of prospective data on therapeutic outcomes poses uncertainty about the best approach for patients with MPAL. The modest evidence on therapeutic decisions is based on uncontrolled studies and retrospective data suggesting higher remission rates with an ALL-like induction approach than with an AML-like regimen followed by allogeneic stem cell transplant during the complete remission.

Advances in understanding the genetic landscape of MPAL demonstrates that most cases are associated with somatic mutations in tumor suppressors, transcription factors, and epigenetic regulators. Recent studies showed that MPALs derive from multipotent primitive cells with considerable genetic diversity, which may promote treatment with targeted therapy. Prospective studies should be prioritized to provide answers about this innately heterogeneous disease.

Keywords: Mixed-phenotype acute leukemia; Ambiguous leukemia; Genomics; Allogeneic stem cell transplant; Leukemia; Immunophenotype

Introduction

Acute leukemia is a clonal hematopoietic malignancy characterized by increased proliferation and disorganized differentiation of hematopoietic cells. Although acute leukemia usually presents with a lymphoid or myeloid lineage, in rare cases it shows no clear evidence of differentiation along a single lineage of myeloid, B, or T lymphoid and is classified as acute leukemia of ambiguous lineage (ALAL). In most cases, acute leukemia can be delineated into a myeloid or lymphoid lineage based on cytochemistry or immunophenotype. However, 2008, the World Health Organization (WHO) subclassified ALAL to include acute leukemia with multi-lineage immunophenotype to be called “mixed-phenotype acute leukemia” (MPAL) and acute leukemia with no lineage defining features “acute undifferentiated or acute unclassifiable leukemia” (AUL)[1, 2].

This classification proposed a simpler algorithm which relies on fewer lineage-specific markers to define MPAL [3]. ALAL comprises around 1%–5% of acute leukemias, the majority of which are MPAL. Per the Surveillance, Epidemiology and End Results (SEER) registry, the incidence of MPAL is around 0.35/1,000,000 persons per year [2, 4-6] and comprises various subgroups: B/myeloid (~59%), T/myeloid (~35%), B/T (~4%), and trilineage (~2%) [7-9]. MPAL has a bimodal age distribution at 19 and ≥60 years [5]. In SEER data, compared with acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), patients with MPAL have a worse prognosis [5]. Other prognostic factors include older age, adverse cytogenetics, extramedullary disease at diagnosis, and initial response to induction therapy [5]. AUL is distinct from and rarer than MPAL, with far less data to provide insights about genomics, hence, molecular characterization has no defined incidence rates for these rare leukemias.
Diagnostic Approach to MPAL

Morphological analysis

Blast morphology in MPAL is heterogeneous and may include monoblastic, myeloblastic and/or lymphoblastic features, a high nuclear-to-cytoplasmic ratio, prominent nucleoli, and scant cytoplasm. Sometimes it may lack classic myeloid features (e.g., Auer rods or azurophobic cytoplasmic granules) [7, 10].

Immunophenotypic evaluation

In the 1980s, it was identified that some leukemias can exhibit characteristics of more than one hematopoietic lineage. Morphologically, MPAL is indistinguishable from AML or ALL, and diagnosis relies on immunophenotyping [7, 11]. A diagnosis of AUL is by definition the lack of immunophenotypically lineage-specific markers—usually MPO, cCD3, CD19, cCD22, and CD79a. When surface markers are expressed in AUL, such expression is usually weak or partial. Generally, AUL expresses no more than one surface marker (e.g., CD7, CD13, or CD33), often expresses HLA-DR or CD34, and may or may not express terminal deoxynucleotidyl transferase (Tdt) [3]. The challenge in classifying MPAL, which includes both biphenotypic acute leukemia (BAL) and bilineal leukemia, is identifying between subgroups. Biphenotypic-type MPAL has multilineage immunophenotypic markers in a single blast and requires the use of the lineage assignment criteria described in Table 1, whereas the bilineal type has two distinct lineage blasts populations [3] [12]. There is no clinical significance assigned by the WHO classification in differentiating between bilineal and biphenotypic MPAL [3, 13].

It is not uncommon to observe the aberrant expression of CD2, CD7, or CD19 lymphoid antigens in AML or myeloid markers CD13 or CD33 in ALL [14, 15]. In NPM1-mutated and t(8;21) AML, CD19 is commonly expressed [16, 17]. Similarly, after exposure to chemoradiation, myeloid leukemia can express both lymphoid and myeloid markers, as in the case with early T cell precursor lymphoblastic leukemia (ETP), which can express myeloid markers (CD13 and CD33).

In terms of diagnosing MPAL, the WHO classification is restrictive and specific, with a limited set of markers that define lineage (Table 2). With T lineage MPAL, exclusive expression of cytoplasmic CD3 is relied on, as surface CD3 is uncommon on MPAL. Other T lymphoid markers such as CD2, CD5, CD7, and CD8 are commonly expressed in B-ALL and even AML and hence are not assigned to T lineage leukemia in MPAL. The expression of CD19 in addition to one or two markers (e.g., CD10, CD22, or CD79a) is required to assign B lineage MPAL. Leukemic blasts for myeloid lineage assignment require MPO expression or monocytic differentiation with two or more of CD11c, CD14, CD64, non-specific esterase, or lysozyme. Supplemental information on lineage with immunohistochemistry of PAX5, MPO, and CD3 can be helpful. MPAL can also be diagnosed without an objective threshold for antigen positivity or a specific percentage cutoff of positive cells to assign lineage [3, 13, 18]. In the case of bilineal leukemia, with two distinct blast populations, it is not necessary to meet specific lineage criteria as described above, but rather each distinct population should meet criteria for B/T lymphoid or myeloid leukemia.

The identification of two distinct populations of cell immunophenotypes meeting criteria for AML or ALL can be classified as MPAL without meeting the specific lineage criteria described above. However, leukemia with AML-defined translocations such as PML-RARA, core binding factor leukemia, FGFR1 rearranged leukemia, therapy-related leukemia, myelodysplasia related changes, or blast-phase chronic myeloid leukemia, which can present with multilineage expression, cannot be classified as MPAL. The most recent updated WHO guidelines in 2016 identified specific genetic-driven rearrangement involving BCR-ABL and MLL as specific diagnostic subgroups.

Cytogenetic aberrations

Most cases of MPAL are associated with an abnormal karyotype: 64%—85% of cases [4, 5, 7, 19, 20]. In two large retrospective studies, 15%—20% of patients with MPAL had BCR-ABL translocations, one-third with complex karyotype, and 3%—8% of cases with MLL rearrangement (MLLr) [4, 7]. Monosomy 5 or 7 is frequently reported in MPAL with complex cytogenetics in the background of myelodysplasia-related AML; however, because this entertains an alternative WHO diagnosis.
of AML with myelodysplasia-related changes, these cases are controversial and are often dropped from the MPAL group [21].

Due to the common occurrence of t(9;22) and t(v;11q23) in MPAL, it is a specific diagnostic entity in the WHO classification of MPAL [3, 22, 23]. Usually associated with B/myeloid phenotypes, KMT2A (MLLr) rearrangement is often seen in infants and compromises around 10% of MPAL cases, while t(9;22) is often seen in adults and constitutes around 15%–20% of cases. Near tetraploidy, trisomy 4, del (6q), del (1) (p32), and 12p11.2 aberrancies are the other abnormalities seen in the B/myeloid MPAL phenotype [7, 20, 24, 25].

In a small study of 9 patients, recurrent cytogenetic aberration in AUL, demonstrated trisomy 14 in three, del(5q) in three, and complex karyotype and trisomy 12 in one each. Due to the rarity of AUL, cytogenetic aberrations in these diseases are not well defined [26].

**Genomic biology in MPAL**

The prevailing dogma on mutational ontogeny in MPAL appears to be derived from primitive hematopoietic pathogenic cells that maintain multipotent potential rather downstream mutational accumulation [6]. A previous hypothesis about the sequential acquisition of somatic mutations to development of multilineage blasts was disputed. On sequencing specific subclones of 50 MPAL patients, invariant founder genetic alterations, including similar methylation patterns and founder mutations, were present in multiple subpopulations [27].

One of the largest genetic profiles of MPLA was reported by St. Jude Children’s Hospital. A total of 115 pediatric patients with MPAL were analyzed by whole genome, exome, SNP array, or RNA sequencing, and 158 gene alterations were noted. Commonly associated somatic mutations in ALL, including ETV6, VPREB1, CDKN2A, and CDKN2B, and those frequently seen in AML, including FLT3, CEBPA, and RUNX1, were observed. Recurrent mutations in lymphoid and myeloid leukemia, including KMT2A and WT1, were also reported [6]. The genetic landscape in children with MPAL is different from that in adults. As seen in ALL, MLLr is more frequent in children and younger adults than in older patients, and BCR-ABL is more prevalent in older patients. KMT2A rearrangement (KMT2Ar) is observed in around 15% of childhood MPAL cases, and these patients usually have a B/myeloid immunophenotype [6]. Point mutations in IDH1, IDH2, and DNMT3A are regularly noted in adult cases of MPAL [19, 28, 29].

Genome patterns are different in B/myeloid and T/myeloid phenotypes of MPAL. RAS pathway mutations (63%) and gene fusion ZNF384 (48%) encoding transcription factor are commonly observed in B/myeloid type. ZNF384 rearrangement (ZNF384r) is a genomic subtype on chromosome 12. The epigenetic and transcriptional profile of MPAL with ZNF384r is similar to that of B-ALL with ZNF384r. Recent reports of patients treated with CD19-directed CAR-T cell therapy in B-ALL with ZNF384r or KMT2Ar showed that the disease can experience a lineage switch, suggesting that B-ALL with these genetic alterations can maintain multilineage capability [30, 31]. ZNF384r is not yet described in adult MPAL. ZNF384r expresses FLT3 more often in B/myeloid than T/myeloid MPAL [6]. Adult B/myeloid MPAL patients often have mutations in RUNX1, ASXL1, EZH2, and TET2 and deletion in IKZF1 (Ikars) [4].

T/myeloid MPAL is associated with higher mutational burden, and many cases exhibit a genetic profile similar to that of early T cell precursor ALL (ETP-ALL) with frequent mutations in FLT3ITD (43%) and the JAK-STAT pathway (57%) [6, 32]. Other frequently mutated genes similar to ETP-ALL in T/myeloid MPAL are ETv6, WT1, and EZH2, while MPAL often lacks genes frequently mutated in AML or T-ALL [33]. ETP is an immature hematopoietic phenotype that often expresses myeloid features as CD13 and CD33 but not MPO expression, which differentiates it from T/myeloid MPAL, which expresses MPO and, rarely, monocytic markers.[33, 34]. NOTCH1 mutations (10%–15%) are seen less frequently in T/myeloid MPAL than in T-ALL or ETP-ALL [9, 35].

Alexander et al. reported heterogeneity of MPALs in mouse models, demonstrating the capacity for blasts to switch immunophenotype even in the absence of selective pressure or treatment. The authors proposed a new entity in classification of MPAL for new therapeutic strategies. One entity would be mutation in WT1 in T/My MPAL, phenotypically similar to ETP, and the other involving ZNF384r characterized by expression of FLT3 in B/Myeloid MPALs, which could be a target for FLT3 inhibitors [6].

Mutation in the RAS pathway is more prevalent in MPAL (30%) than AML (12%). Similarly, TP53 mutations are reported more frequently in MPAL (20%) than in de novo B-ALL (12%) or AML (8%). Recurrent somatic mutations in B-ALL, including IL7R (mutated in 7% cases) and PAX5 (mutated in 30%), which are involved in signaling and differentiation, are missing in MPALs, and NPM1, which is frequently seen in 30% of AML patients has not been reported in any MPAL series [36, 37]. Recent work by Takahashi et al. using RNA sequencing and methylation assays in 31 adult patients noted 86 driver mutations in 35 genes. Frequent mutations were DNMT3A, NOTCH1, RUNX1, and IDH2. T/My MPAL presented with NOTCH1 mutations, whereas B/Myeloid was enriched for RUX1 mutations. Intriguingly, methylation expression segregated T/My MPALs from T-ALL and B/Myeloid MPAL with an AML-like phenotype [19].

Due to the infrequency of AUL, thorough understanding of genetic profiling is scarce. However, a few studies have suggested the expression of AML mutations as MN1, ERG, and BAALC [3]. A thorough investigation of the mutational profile of B/T MPAL is challenging due to the rareness of the disease, but small series have described mutations in WT1, JAK3, NOTCH1, SF3B1, PTPN11, DNMT3A, and TP53 [9]. Although advances in genetic studies with next-generation sequencing (NGS), methylation assays, and RNA sequencing have enlarged our understanding of MPALs, larger multi-institutional efforts are
needed to determine the prognostic relevance and correlation between immunophenotype and specific mutations.

**Measurable residual disease (MRD) testing**

MRD testing is the most important prognostic factor in the therapeutic management of ALL. Newer sensitive techniques to monitor MRD via using real-time quantitative polymerase chain reaction or multiparametric flow cytometry (FCM) have largely changed the outlook in the prediction of relapse, disease risk stratification, and decision-making in the next steps for treatment, including changes in chemoinmunotherapy or consideration for allogeneic stem cell transplant. Recently, use of high-throughput NGS in laboratory routinely has upgraded the specificity and sensitivity of MRD detection. Use of MRD by FCM has yet to be standardized, and its influence on therapeutic decisions is controversial; however, in general an MRD of <0.01% at the end of consolidation (i.e., after 3 months of therapy) is an indicator to continue forward with treatment with no additional therapeutic intervention or change in treatment [38, 39].

Assessment of MRD is challenging in MPAL due to its variable immunophenotypes and the emergence of subclones during treatment. In the largest series of retrospective analysis, 233 patients in the international Berlin-Frankfurt-Munster study of leukemia of ambiguous lineage noted that patients with an MRD ≥5% at the end of induction therapy had a 5-year event-free survival of <50%. Current guidelines on the treatment of MPAL mainly suggest an ALL-focused regimen, unless the patient does not express CD19 and does not have expression of B cell markers according to immunophenotyping. Hence, the Children’s Cooperative Group has recommended using an MRD cutoff of <5% post-induction and <0.01% post-consolidation to carry on with any planned ALL-based regimen. Patients who do not reach these target goals would benefit from a switch in therapy to an alternative AML-type regimen or early intensification followed by suitable donor search for allogeneic stem cell transplant consolidation [40, 41].

**Conclusion**

MPAL is a challenging disease both clinically and diagnostically, owing to its heterogeneity and underlying lineage plasticity. The advent of newer and advanced genetics-based techniques have enhanced our knowledge of the molecular, immunophenotypic, and genetic complexity associated with MPAL. The rarity of this disease has made therapeutic standardization difficult, such as the treatment decision between ALL-based and AML-directed regimens, and the overall responses remain poor. With the possible implementation of genome-wide methylation analysis in the clinical setting, a more molecularly guided precision therapy approach could improve the overall response in MPAL [42].

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