# Pediatric Mixed-Phenotype Acute Leukemia

Subjects: Oncology | Hematology

Contributor: Sandeep Batra

Mixed phenotypic acute leukemias (MPAL) are rare hematological malignancies in children, accounting for less than 5% of pediatric acute leukemias. MPAL are heterogeneous and can exhibit cross-lineage myeloid, B-lymphoid, or T-lymphoid antigen expression on a single blast population (biphenotypic) or have distinct single-lineage blast populations (bilineal). Due to phenotypic and genetic diversity, lack of well-defined diagnostic criteria, treatment resistance, and lineage switch, MPAL often present a diagnostic dilemma, and prove difficult to treat.

mixed-phenotype acute leukemia MPAL + lineage switch minimal residual disease treatment

## 1. Introduction

Biphenotypic MPAL are more common than bilineal MPAL. However, the true prevalence and survival can be difficult to determine given the various diagnostic criteria utilized, lack of a centralized review of cases, and treatment protocols that are based on results from retrospective studies. The Children's Oncology Group Acute Leukemia of Ambiguous Lineage Task Force reported that routine institutional flow cytometry was insufficient for the diagnosis of MPAL in about 15% of children, which further highlights the diagnostic challenge faced by oncologists [1].

Currently, MPAL are classified based on lineage-specific immunophenotypic markers determined by flow cytometry, immunohistochemistry, or cytochemistry and primary molecular alteration, and are considered by most co-operative groups to be high-risk leukemias [1]. The majority of MPAL present as B-lymphoid/myeloid (in about 2/3 cases), with a T-lymphoid/myeloid immunophenotype being the second most common presentation. Rarely, it can present as B-lymphoid/T-lymphoid or B-lymphoid/T-lymphoid/myeloid subtypes [2][3][4]. Myeloid-surface antigen co-expression does not appear to be prognostic [5]. The classification of MPAL also includes two distinct entities: MPAL with *KMT2A* (mixed-lineage leukemia or *MLL*) rearrangement and MPAL with t(9;22)(q34.1;q11.2); *BCR-ABL1*(Philadelphia chromosome positive or *Ph*+).

After MPAL were recognized as a distinct entity, numerous sets of diagnostic criteria were established, including the European Group for the Immunological Characterization of Leukemias (EGIL), and most recently the World Health Organization (WHO) 2016 system (updated from previous WHO 2008 classification), which is being increasingly utilized for MPAL diagnosis [2][6][7]. A hallmark of MPAL in the WHO classification scheme rests on the fact that other leukemia subtypes (i.e., AML-defining balanced translocations such as t(8;21) that frequently

expresses multiple B-cell markers) must be excluded prior to the MPAL designation [8]. Given its more widespread adaption in clinical practice, the World Health organization (WHO) 2016 criteria are presented in **Table 1** and **Table 2**. There are distinct differences between the classification schema, with the European Group for the Immunological Classification of Leukemias (EGIL) scheme generally considered to be more inclusive, which often leads to more acute leukemias being classified as MPAL, and a higher incidence of MPAL compared to the WHO classification. Weinberg et al. published a review looking at 7627 patients (both pediatrics and adults) with leukemia showing a mixed phenotype incidence of 2.8% using EGIL compared with 1.6% when using WHO 2008 criteria [5][8].

**Table 1.** Criteria for lineage assignment in mixed phenotypic acute leukemia.

| **Lineage Assignment Criteria** |
|---|
| Myeloid Lineage |
| MPO+ (Flow cytometry, immunohistochemistry, or cytochemistry)<br>or<br>Monocytic differentiation (at least two of the following: nonspecific esterase cytochemistry, CD11c, CD14, CD64, lysozyme) |
| **T-Lymphoid Lineage** |
| Strong * cytoplasmic CD3 (with antibodies to CD3 ε chain)<br>or<br>Surface CD3 |
| B-Lymphoid Lineage |
| Strong * CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10<br>or<br>Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10 |

* Strong is defined by equal or brighter expression than normal B or T cells in the sample.

**Table 2.** WHO 2016 criteria for acute leukemia of ambiguous lineage.

| **Acute Undifferentiated Leukemia** |
|---|
| Mixed-phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); BCR-ABL1 |
| MPAL with t(v;11q23.3); KMT2A rearranged |
| MPAL, B/myeloid, NOS |
| MPAL, T/myeloid, NOS |

Further classification of MPAL can be implemented utilizing the mutational status of the leukemia. Most MPAL have an abnormal and often complex karyotype, with t(9;22) mostly identified in adult or older patients, whereas t(v;11q23) (*AFF1* is the most common fusion partner of *MLL*) is primarily seen in infant B/myeloid MPAL [8]. Matutes et al. looked at 100 MPAL patients diagnosed using the WHO 2008 criteria and found that the most common abnormality was a complex karyotype in 32% of patients, t(9;22) in 20%, and normal karyotype in only 13% [4]. Less commonly, chromosome 1, 6, and 12 deletions; trisomy 4; and near-tetraploidy have been reported [1][2][4]. These mutations aid in subclassification but the therapeutic and prognostic importance is still under appreciated.

## 2. Treatment of MPAL

Historically, MPAL have inferior outcomes and a high risk of induction failure, compared with ALL/AML, and are treated per high-risk leukemias protocols [9]. Poor prognostic factors include: an older age at diagnosis, higher white blood cell (WBC) count at presentation, T-lymphoid/myeloid phenotype, adverse cytogenetics (such as a *KMT2A*/*AFF1* rearrangement), extramedullary disease at diagnosis, and MRD positivity [8][10][11]. There have been various chemotherapy approaches for the treatment of MPAL including acute ALL, AML, and hybrid ALL/AML (such as FLAG (fludrabine, cytarabine, granulocyte-stimulating factor)-IDA (idarubicin) with vincristine and prednisone (VCR-PRED) or hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone) regimens) [4][12][13]. Optimal therapy remains a subject of controversy and differences between adult and pediatric treatment approaches are often striking [4][14].

St. Jude Children's Research Hospital reviewed outcomes of 35 pediatric MPAL patients (treated from 1985 to 2006) and reported that the majority (65%) of the patients received AML induction chemotherapy with cytarabine, daunomycin, and etoposide, while 35% of the patients received ALL four-drug induction [15]. In this review, of the patients treated with upfront AML therapy, 12/23 (52%) achieved complete remission compared with 10/12 (83%) with ALL therapy. Interestingly, 8/10 (80%) of patients who did not achieve complete remission (CR) with AML therapy went into remission after being switched to ALL-like induction therapy [15]. Long-term survival was achieved in 17 out of 35 patients (5 patients treated with AML therapy and 12 patients treated with ALL therapy either upfront or after initial AML failure). The same study compared historical survival rates, comparing MPAL with standard ALL therapy to AML therapy. The 5-year survival estimates for MPAL (combined B/myeloid and T/myeloid) were 47.8% ± 11.5%, similar to that of AML (56.5% ± 3.5%), but were significantly less than patients receiving ALL therapy at 84.6% ± 1.1% [15].

Previous studies on adults have shown that the historical 4-year survival for adult MPAL is less than 10% [4]. A case series of 100 MPAL patients by Matutes et al. further showed that older patients had inferior survival compared with patients less than 15 years of age [4][16]. In this case, series median survival in adults was 11.8 months compared with 139 months in children. A similar pattern was seen in patients treated with ALL therapy, with a median survival of 139 months compared with 11 months with AML therapy and 3 months with a hybrid approach [4].

However, more recently, due to improved diagnostic criteria and genomic techniques, and the observation that ALL-like regimens in both pediatric and adult populations are associated with superior treatment response, the

treatment landscape has clearly changed [1][12][17][18][19].

Several other studies have also investigated optimal upfront therapy for patients with MPAL, and support that notion that ALL treatment regimens tend to lead to better overall survival than AML-based regimens [20][3][8][21][16][17][22][23][24]. The BFM group international pediatric cooperative study (AMBL2012) demonstrated a superior outcome when patients were treated with ALL primary therapy with a 5-year event-free survival (EFS) of 80% ± 4% compared with AML therapy (36% ± 7.2%) or hybrid ALL/AML therapy (50% ± 12%) [13]. In particular, ALL/AML hybrid approach in *KMT2A*-rearranged patients resulted in a subpar 5-year EFS of only 28% [13]. A 2018 systematic review from Maruffi et al. looked at over 1300 adults and children diagnosed with MPAL and showed that ALL induction regimen was more likely to lead to remission (OR = 0.33) and improved overall survival (OR = 0.45) compared to AML-like treatment protocols, or an even worse outcome associated with hybrid regimens [20]. Additionally, a recent multi-center analysis showed that ALL induction therapy was able to achieve MRD negative remission by the end of induction in a majority of patients (70%) [18]. Even in studies showing similar survival benefits between ALL and AML/hybrid regimens, ALL regimens tend to lead to overall decreased morbidity, due to less toxicity, compared with AML or hybrid-based treatments [12]. Lumbar puncture is recommended at diagnosis to determine central nervous system (CNS) status for all MPAL patients, as frequency of CNS involvement is higher [3][25]. CNS-directed intrathecal chemotherapy is administered, similar to treatment protocols for acute leukemia with CNS involvement.

A recent review from Children's Healthcare of Atlanta, highlights that patients with B/myeloid MPAL with isolated MPO expression might in fact be a unique entity, and have a more favorable response to ALL therapy [26]. In this review, patients with B/myeloid with isolated MPO had an overall survival rate at 3 years of 100% compared with 63.1% with other MPAL, and interestingly, the degree of MPO positivity was not prognostically relevant [26]. This highlights that the detection of a B/MPAL phenotype with isolated MPO expression is important, and may allow for better prognostic information and treatment decisions.

MPAL with *KMT2A* (*MLL*) rearrangement, are more common in children than adults, are typically bilineal (lymphoblasts and monoblasts) but rarely biphenotypic, and are prone to lineage switch [27][28][29][14]. The fusion partner of MLL1 is a determinant of the leukemic phenotype [30]. For example, *MLL-AF4* is predominantly associated with a lymphoid phenotype and *MLL-AF9* with a myeloid phenotype [30]; however, the role of fusion partners in determining MPAL phenotype or lineage switch remains unclear [31]. Compared with *Ph*+ MPAL, the *MLL*+ MPAL patients have significantly inferior survival odds (HR = 10.2, $p < 0.001$) [32], and are transplanted, upfront, if induction failure occurs, or at relapse [8][33][34]. New emerging targets blocking the *MLL* fusion complex are under evaluation, include *menin* [35], disruptor of telomeric silencing 1-like (*DOTL1*) inhibitors [36], and spleen tyrosine kinase (*SYK*) inhibitors [37].

## 3. Conclusions and Future Directions

MPAL are a heterogeneous group of leukemias that often have a complex phenotype/genetic basis and historically have been difficult to diagnose and treat. Despite recent advances in the diagnosis criteria and treatment

landscape of MPAL, there is still much to learn about this unique subset of acute leukemias. As most current treatment recommendations are based on retrospective studies, prospective clinical trials standardizing the treatment regimens and utilizing MRD for assessing treatment response, such as the ongoing COG trial AALL1732, are urgently needed to solidify a uniform approach for the management of MPAL.

## References


1. Orgel, E.; Alexander, T.B.; Wood, B.L.; Kahwash, S.; Devidas, M.; Dai, Y.; Alonzo, T.A.; Mullighan, C.G.; Inaba, H.; Hunger, S.P.; et al. Mixed-phenotype acute leukemia: A cohort and consensus research strategy from the Children's Oncology Group Acute Leukemia of Ambiguous Lineage Task Force. Cancer 2020, 126, 593–601.
2. Arber, D.A.; Orazi, A.; Hasserjian, R.; Thiele, J.; Borowitz, M.J.; Le Beau, M.M.; Bloomfield, C.D.; Cazzola, M.; Vardiman, J.W. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016, 127, 2391–2405.
3. Wolach, O.; Stone, R.M. Optimal therapeutic strategies for mixed phenotype acute leukemia. Curr. Opin. Hematol. 2020, 27, 95–102.
4. Matutes, E.; Pickl, W.F.; Van't Veer, M.V.; Morilla, R.; Swansbury, J.; Strobl, H.; Attarbaschi, A.; Hopfinger, G.; Ashley, S.; Bene, M.C.; et al. Mixed-phenotype acute leukemia: Clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood 2011, 117, 3163–3171.
5. Weinberg, O.K.; Arber, D.A. Mixed-phenotype acute leukemia: Historical overview and a new definition. Leukemia 2010, 24, 1844–1851.
6. Catovsky, D.; Matutes, E.; Buccheri, V.; Shetty, V.; Hanslip, J.; Yoshida, N.; Morilla, R. A classification of acute leukaemia for the 1990s. Ann. Hematol. 1991, 62, 16–21.
7. Bene, M.C.; Castoldi, G.; Knapp, W.; Ludwig, W.D.; Matutes, E.; Orfao, A.; Van't Veer, M.B. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 1995, 9, 1783–1786.
8. Wolach, O.; Stone, R.M. How I treat mixed-phenotype acute leukemia. Blood 2015, 125, 2477–2485.
9. Berry, D.A.; Zhou, S.; Higley, H.; Mukundan, L.; Fu, S.; Reaman, G.H.; Wood, B.L.; Kelloff, G.J.; Jessup, J.M.; Radich, J.P. Association of Minimal Residual Disease With Clinical Outcome in Pediatric and Adult Acute Lymphoblastic Leukemia: A Meta-analysis. JAMA Oncol. 2017, 3, e170580.

10. Mejstrikova, E.; Volejnikova, J.; Fronkova, E.; Zdrahalova, K.; Kalina, T.; Sterba, J.; Jabali, Y.; Mihal, V.; Blazek, B.; Cerna, Z.; et al. Prognosis of children with mixed phenotype acute leukemia treated on the basis of consistent immunophenotypic criteria. Haematologica 2010, 95, 928–935.
11. Béné, M.C. Biphenotypic, bilineal, ambiguous or mixed lineage: Strange leukemias! Haematologica 2009, 94, 891–893.
12. Rasekh, E.O.; Osman, R.; Ibraheem, D.; Madney, Y.; Radwan, E.; Gameel, A.; Abdelhafiz, A.; Kamel, A.; Elfishawi, S. Acute lymphoblastic leukemia–like treatment regimen provides better response in mixed phenotype acute leukemia: A comparative study between adults and pediatric MPAL patients. Ann. Hematol. 2021, 100, 699–707.
13. Hrusak, O.; De Haas, V.; Stancikova, J.; Vakrmanova, B.; Janotova, I.; Mejstrikova, E.; Capek, V.; Trka, J.; Zaliova, M.; Luks, A.; et al. International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia. Blood 2018, 132, 264–276.
14. Brethon, B.; Lainey, E.; Caye-Eude, A.; Grain, A.; Fenneteau, O.; Yakouben, K.; Roupret-Serzec, J.; Le Mouel, L.; Cavé, H.; Baruchel, A. Case Report: Targeting 2 Antigens as a Promising Strategy in Mixed Phenotype Acute Leukemia: Combination of Blinatumomab With Gemtuzumab Ozogamicin in an Infant With a KMT2A-Rearranged Leukemia. Front. Oncol. 2021, 11, 637951.
15. Rubnitz, J.E.; Onciu, M.; Pounds, S.; Shurtleff, S.; Cao, X.; Raimondi, S.C.; Behm, F.G.; Campana, D.; Razzouk, B.; Ribeiro, R.C.; et al. Acute mixed lineage leukemia in children: The experience of St Jude Children’s Research Hospital. Blood 2009, 113, 5083–5089.
16. Shi, R.; Munker, R. Survival of patients with mixed phenotype acute leukemias: A large population-based study. Leuk. Res. 2015, 39, 606–616.
17. Seetharam, S.; Thankamony, P.; Gopakumar, K.G.; Nair, R.A.; Jacob, P.M.; Krishna, K.M.J.; Rajeswari, B.; Nair, M.; Guruprasad, C.S.; Prasanth, V.R. Outcomes of pediatric mixed phenotype acute leukemia treated with lymphoid directed therapy: Analysis of an institutional series from India. Pediatr. Hematol. Oncol. 2021, 38, 358–366.
18. Oberley, M.J.; Raikar, S.; Wertheim, G.B.; Malvar, J.; Sposto, R.; Rabin, K.R.; Punia, J.N.; Seif, A.E.; Cahen, V.C.; Schore, R.J.; et al. Significance of minimal residual disease in pediatric mixed phenotype acute leukemia: A multicenter cohort study. Leuk. 2020, 34, 1741–1750.
19. Duong, V.H.; Begna, K.H.; Kashanian, S.; Sweet, K.; Wang, E.S.; Caddell, R.; Shafer, D.A.; Singh, Z.N.; Baer, M.R.; Al-Kali, A. Favorable outcomes of acute leukemias of ambiguous lineage treated with hyperCVAD: A multi-center retrospective study. Ann. Hematol. 2020, 99, 2119–2124.
20. Maruffi, M.; Sposto, R.; Oberley, M.J.; Kysh, L.; Orgel, E. Therapy for children and adults with mixed phenotype acute leukemia: A systematic review and meta-analysis. Leukemia 2018, 32, 1515–1528.

21. Al-Seraihy, A.S.; Owaidah, T.M.; Ayas, M.; El-Solh, H.; Al-Mahr, M.; Al-Ahmari, A.; Belgaumi, A.F. Clinical characteristics and outcome of children with biphenotypic acute leukemia. Haematologica 2009, 94, 1682–1690.
22. Bachir, F.; Zerrouk, J.; Howard, S.C.; Graoui, O.; Lahjouji, A.; Hessissen, L.; Bennani, S.; Quessar, A.; El Aouad, R. Outcomes in Patients With Mixed Phenotype Acute Leukemia in Morocco. J. Pediatr. Hematol. 2014, 36, e392–e397.
23. Gerr, H.; Zimmermann, M.; Schrappe, M.; Dworzak, M.; Ludwig, W.-D.; Bradtke, J.; Moericke, A.; Schabath, R.; Creutzig, U.; Reinhardt, D. Acute leukaemias of ambiguous lineage in children: Characterization, prognosis and therapy recommendations. Br. J. Haematol. 2010, 149, 84–92.
24. Reid, J.H.; Perissinotti, A.J.; Benitez, L.L.; Boyer, D.; Lee, W.; Burke, P.W.; Pettit, K.; Bixby, D.L.; Marini, B.L. Hybrid chemotherapy regimen (FLAG-IDA-vincristine-prednisone) for acute leukemia with mixed-phenotype blasts. Leuk. Res. 2021, 103, 106539.
25. Lazarus, H.M.; Richards, S.M.; Chopra, R.; Litzow, M.R.; Burnett, A.K.; Wiernik, P.H.; Franklin, I.M.; Tallman, M.S.; Cook, L.; Buck, G.; et al. Central nervous system involvement in adult acute lymphoblastic leukemia at diagnosis: Results from the international ALL trial MRC UKALL XII/ECOG E2993. Blood 2006, 108, 465–472.
26. Raikar, S.S.; Park, S.I.; Leong, T.; Jaye, D.L.; Keller, F.G.; Horan, J.T.; Woods, W.G. Isolated myeloperoxidase expression in pediatric B/myeloid mixed phenotype acute leukemia is linked with better survival. Blood 2018, 131, 573–577.
27. Rayes, A.; McMasters, R.L.; O'Brien, M.M. Lineage Switch in MLL-Rearranged Infant Leukemia Following CD19-Directed Therapy. Pediatr. Blood Cancer 2016, 63, 1113–1115.
28. Monma, F.; Nishii, K.; Ezuki, S.; Miyazaki, T.; Yamamori, S.; Usui, E.; Sugimoto, Y.; Lorenzo, V.F.; Katayama, N.; Shiku, H. Molecular and phenotypic analysis of Philadelphia chromosome-positive bilineage leukemia: Possibility of a lineage switch from T-lymphoid leukemic progenitor to myeloid cells. Cancer Genet. Cytogenet. 2006, 164, 118–121.
29. Balducci, E.; Nivaggioni, V.; Boudjarane, J.; Bouriche, L.; Rahal, I.; Bernot, D.; Alazard, E.; Duployez, N.; Grardel, N.; Arnoux, I.; et al. Lineage switch from B acute lymphoblastic leukemia to acute monocytic leukemia with persistent t(4;11)(q21;q23) and cytogenetic evolution under CD19-targeted therapy. Ann. Hematol. 2017, 96, 1579–1581.
30. Winters, A.C.; Bernt, K.M. MLL-Rearranged Leukemias—An Update on Science and Clinical Approaches. Front. Pediatr. 2017, 5, 4.
31. Young, K.; Loberg, M.A.; Eudy, E.; Schwartz, L.S.; Mujica, K.D.; Trowbridge, J.J. Heritable genetic background alters survival and phenotype of Mll-AF9-induced leukemias. Exp. Hematol. 2020, 89, 61–67.e3.

32. Qasrawi, A.; Ramlal, R.; Munker, R.; Hildebrandt, G.C. Prognostic impact of Philadelphia chromosome in mixed phenotype acute leukemia (MPAL): A cancer registry analysis on real-world outcome. Am. J. Hematol. 2020, 95, 1015–1021.
33. Shimizu, H.; Saitoh, T.; Machida, S.; Kako, S.; Doki, N.; Mori, T.; Sakura, T.; Kanda, Y.; Kanamori, H.; Miyawaki, S.; et al. Allogeneic hematopoietic stem cell transplantation for adult patients with mixed phenotype acute leukemia: Results of a matched-pair analysis. Eur. J. Haematol. 2015, 95, 455–460.
34. Tian, H.; Xu, Y.; Liu, L.; Yan, L.; Jin, Z.; Tang, X.; Han, Y.; Fu, Z.; Qiu, H.; Sun, A.; et al. Comparison of outcomes in mixed phenotype acute leukemia patients treated with chemotherapy and stem cell transplantation versus chemotherapy alone. Leuk. Res. 2016, 45, 40–46.
35. Issa, G.C.; Ravandi, F.; DiNardo, C.D.; Jabbour, E.; Kantarjian, H.M.; Andreeff, M. Therapeutic implications of menin inhibition in acute leukemias. Leukemia 2021, 35, 2482–2495.
36. Stein, E.M.; Garcia-Manero, G.; Rizzieri, D.A.; Tibes, R.; Berdeja, J.G.; Savona, M.R.; Jongen-Lavrenic, M.; Altman, J.K.; Thomson, B.; Blakemore, S.J.; et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. Blood 2018, 131, 2661–2669.
37. Loftus, J.P.; Yahiaoui, A.; Brown, P.A.; Niswander, L.; Bagashev, A.; Wang, M.; Schauf, A.; Tannheimer, S.; Tasian, S.K. Combinatorial efficacy of entospletinib and chemotherapy in patient-derived xenograft models of infant acute lymphoblastic leukemia. Haematologica 2020, 106, 1067–1078.