How I Treat: Mixed Phenotype Acute Leukemia

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Abstract:

Mixed phenotype acute leukemia (MPAL) encompasses a heterogeneous group of rare leukemias in which assigning a single lineage of origin is not possible. A variety of different terms and classification systems have been used historically to describe this entity. MPAL is currently defined by a limited set of lineage-specific markers proposed in the 2008 WHO monograph on classification of tumors of hematopoietic and lymphoid tissues. In adult patients MPAL is characterized by relative therapeutic resistance that may be attributed in part to the high proportion of patients with adverse cytogenetic abnormalities. No prospective, controlled trials exist to guide therapy. The limited available data suggests that an ‘acute lymphoblastic leukemia-like’ regimen followed by allogeneic stem cell transplant may be advisable; addition of a tyrosine kinase inhibitor in patients with t(9;22) translocation is recommended. The role of immunophenotypic and genetic markers in guiding chemotherapy choice and post remission strategy as well as the utility of targeted therapies in non-Ph positive MPALs are unknown.
Case presentation- Part 1:

A 51-year-old physician complained of two weeks of exertional dyspnea. White blood cell count was 280X10^9/L (76% blasts), platelets 91X10^9/L, and Hemoglobin 9g/dL. Bone marrow aspirate (Figure 1a) revealed two distinct morphological /cytochemical populations of blasts that were positive for myeloperoxidase (MPO) and periodic acid-Schiff (PAS) stains, respectively. Flow cytometry (Figure 1b) also demonstrated two atypical blast populations with distinct CD45 expression associated with 1) uniform expression of B-lymphoid markers CD19 and CD10, uniform stem cell marker CD34 and variable myeloid marker CD33 and 2) uniform expression of CD33, small subset CD34, variable CD19 and lack of CD10 expression.

All 20 metaphases assessed contained a t(9;22)(q34;q11.2) translocation associated with loss of the 7p and 16q arms. Molecular studies demonstrated the e1a2 BCR-ABL transcript (p190) but no additional recurrent mutations associated with acute leukemia (96 gene panel).

Introduction

The absence of essentially any useful prospectively collected data on how to treat mixed phenotypic acute leukemia (MPAL) in adults both simplifies and complicates any discussion of this topic. Given little truly useful information, we have derived an approach that is based on data in the literature, makes logical sense, and can be adhered to: once MPAL is definitely identified patients should be treated according to an ‘acute lymphoid leukemia’ type induction regimen followed by allogeneic stem cell transplant (alloSCT) in responding patients if feasible.
What is Mixed Phenotype Acute Leukemia?

Patients who are diagnosed with acute leukemia (greater than 20% blasts in blood or marrow, or fewer in the case of certain chromosomal translocations or an extra-medullary presentation) can generally be classified as having either myeloid lineage-derived disease (AML) or lymphoid lineage-derived disease (ALL). Occasionally the immature cells display cytochemical and/or immunophenotypic features of both lineages (biphenotypic) or there are different populations of leukemia cells (bilineal). The distinction between bilineal and biphenotypic leukemias is often blurred, especially given the fact that ‘two populations’ of cells perhaps represent subclones derived from a unique stem cell. Accordingly, this distinction does not generally affect our diagnostic or therapeutic approach.

Two important recent algorithms have been used to define this entity. In the first of these (1995), the European Group for Immunological Characterization of Acute Leukemias (EGIL) developed a scoring algorithm in which a point system determined whether or not a patient had enough immunophenotypic variety to qualify as biphenotypic (see Figure 2A)\(^1\)\(^2\). The second and most recent 2008 WHO monograph on classification of tumors of hematopoietic and lymphoid tissues includes a helpful chapter on acute leukemias of ambiguous lineage, “leukemias that show no clear evidence of differentiation along a single lineage”\(^3\). These encompass both MPAL, the primary topic of this review, but also acute undifferentiated leukemia (AUL) wherein the malignant cells do not express lineage-specific antigens. This classification (Figure 2B) tries to minimize the difficult distinction between bilineal and biphenotypic leukemia and subclassifies these promiscuously derived cells as usually either B-myeloid or T-myeloid. MPAL that harbor Philadelphia chromosome (Ph+) or MLL rearrangements are considered a distinct diagnostic subgroup (Figure 2B). An important point is that
AML-defining balanced translocations such as t(8;21), a type of favorable prognosis AML which frequently expresses multiple B cell markers\(^4\), are not considered biphenotypic. It also excludes secondary leukemias (arising after prior cancer therapy or myelodysplasia), leukemias with FGFR1 mutations which have features of both T lymphoid and myeloid differentiation, and chronic myeloid leukemia (CML) in blast crisis which can present with a variety of lineages. The latter is sometimes difficult to separate from Ph+ MPAL (that may actually represent transformation from a previously undiagnosed chronic phase CML).

The essential feature of MPAL (figure 2B) is that cells express lineage specific myeloid markers as well as lineage specific T or B lymphoid markers. Although there are caveats (see legend to figure 2B), CD3 expression equals T-lymphoid development and CD19 plus one or two other markers suggest B-lymphoid origin. Myeloid origin can be determined with a set of monocytic markers or more commonly by MPO expression. Although various thresholds for flow-based MPO positivity were introduced over the years (e.g. 10% of blast population\(^1,5\)), no specific threshold has been acknowledged in the 2008 WHO monograph\(^3,6\).

As compared to the EGIL classification, the 2008 WHO classification utilizes a more limited set of lineage markers that can be more consistently applied. In 2015, the 2008 WHO classification still remains the most practical means to define and sub-classify MPAL, but hopefully advances in deciphering the molecular pathogenesis of acute leukemia will soon lead to a more robust approach to the diagnosis of these entities.
What drives biphenotypic expression in leukemia?

During the 1980’s, two leading hypotheses were raised to explain biphenotypic expression in leukemia. Greaves’ hypothesis suggested ‘lineage promiscuity’ in which hematopoietic progenitor cells possess multi-lineage potential that is preserved as a relic if leukemic transformation occurs at that stage. The term ‘lineage infidelity’ denoted an alternative hypothesis involving oncogenetically-driven misprogramming of the leukemic cell resulting in multi-lineage expressing blasts. Significant strides have been made since in our understanding of the normal and pathologic pathways that drive lineage fate.

Maturation and differentiation of blood cells during the process of hematopoiesis is associated with the expression of specific sets of markers that define lineage. This is a tightly regulated, multi-step, hierarchical process that is driven by a network of transcription factors. Although some transcription factors are thought to have primary roles in driving hematopoietic progenitors toward a specific lineage (for example, C/EBPα in myeloid cells or PAX-5 in B lymphocytes), this relationship in vivo is far more complex, context-dependent and regulated at multiple cellular levels. Early hematopoietic multipotential progenitors were previously shown to express markers of multiple lines with the specific fate selection relying on complex interactions that both promote a specific lineage phenotype but also suppress alternative programming (so called ‘lineage priming’). The timing and level of expression of a specific transcription factor may affect lineage determination. Competing transcription factors interact to antagonize each other’s functions to promote the expression of one lineage over the other. For example, a high level of PAX5 expression is critical for development of common lymphoid progenitors along the B-cell pathway whereas low levels result in a mixed phenotype; C/EBPα suppression of PAX-5 drives common lymphoid progenitor
cells toward myeloid phenotypes. In zebra fish PU.1 and GATA-1 exert mutually antagonistic effects with the balance driving myeloid vs. erythroid differentiation. The fate of early T cell lineage progenitors is dependent on the Notch receptor signaling pathway, without which myeloid differentiation may occur.

Dysregulation and aberrant expression of transcription factors that govern cell differentiation occur on the basis of the genomic and epigenetic alterations seen in acute leukemia. Gene expression profiling in a large group of patients with AML correlated T/myeloid phenotype with a distinct expression profile that included C/EBPα promoter hypermethylation /gene silencing and up regulation of T cell lineage pathways, via aberrantly activated NOTCH1 signaling. Activating mutations in NOTCH1 were previously described in the context of lineage switch from AML to T-ALL, suggesting the potential role of mutations in transcription factors on lineage specific cell reprogramming. Indeed mutations may trump phenotype. Early T cell precursor (ETP) ALL is associated with recurrent mutations typically associated with myeloid tumors such as DNMT3A, IDH1 and IDH2 and is transcriptionally related to myeloid progenitors.

**Natural History of MPAL**

**Incidence:**

The frequency, clinical features and outcome of patients with ambiguous lineage expression are largely dependent on the classification system used at the time of report. The WHO 2008 classification is less inclusive than the preceding EGIL system resulting in a lower reported prevalence. Weinberg and Arber retrospectively reviewed series encompassing 7627 pediatric and adult patients with acute leukemia and determined that 2.8% had biphenotypic leukemia (BAL) and 1.6% had MPAL using the EGIL and WHO 2008 systems, respectively.
more recent Chinese study reported MPAL in 2.4% of 4780 patients with acute leukemia (ages 14-81 years)\textsuperscript{27}. In 517 pediatric and adult Dutch patients with acute leukemia, 30 patients (5.8%) would be considered as having BAL based on EGIL criteria, 8 cases (1.5%) were consistent with MPAL using the WHO 2008 classification; only 6 patients (1.1%) would qualify as both BAL and MPAL suggesting that these classification systems may select different patients\textsuperscript{28}.

**Characteristics of MPAL:**

Matutes et al\textsuperscript{29} presented a review of 100 patients, mostly from the UK and Austria, with MPAL based on the WHO definition. Of the 62 men and 38 women (32% under the age of 16), 39 displayed ALL, 38 AML, and 13 cases were defined as acute undifferentiated leukemia by morphological assessment (10 were not analyzed). Immunophenotyping showed that 58% of the cases had a B-myeloid and 36% had a T-myeloid phenotype. Combined B+T and trilineage (myeloid+B+T) immunophenotypes were rare (n=6) and all of these had ALL morphology. Expression of stem-cell like markers was common and included terminal deoxynucleotidyl transferase (TdT) in 89% of the cases, HLA-DR in 92% and CD34 in 74%. Among cases with myeloid commitment, MPO was expressed in at least in 5% of the blasts in 98% of cases and in more than 20% of the blasts in 76% of the cases. All except nine cases expressed both MPO as well as CD33 and/or CD13. By definition, cytoplasmic CD3 was expressed in all 35 T-myeloid and CD19 was present in 93% of cases with the B-myeloid phenotype and was always associated with CD10, cytCD22 and/or cytCD79a expression. In the 76 patients with cytogenetic information, 20% had a Ph+ and 8% had MLL gene (11q23) rearrangements. Thirty two percent had complex karyotype (CK) that was
commonly associated with deletion of the long arm of chromosome 6, abnormalities involving the long arm of chromosome 7 or abnormalities in long arm of chromosome 5. Normal karyotype was demonstrated in 13%. While early deaths were seen, most of the patients died of their disease, with an overall median survival of 18 months and a 37% overall 5-year survival. Age, Ph+ and the type of induction therapy were significant predictors for survival with children surviving 139 months vs. 11 months for adults, 8 months for Ph+ vs. 139 months for those with normal karyotype and 28 months for those with other abnormalities. Yan et al\textsuperscript{27} reported on 117 patients with WHO 2008 defined MPAL. Median patient age was 35 (range 14-81 years) with slight male predominance (51.3%) and a median white blood cell (WBC) count of 5.4×10\textsuperscript{9}/L (range 0.8-278.7) at diagnosis. Thirty four percent of patients demonstrated AML morphology (mostly FAB M1 and M5), 44% were felt to have ALL (FAB L1) and 22% were unclassifiable. B-myeloid immunophenotype was seen in 55% and T-myeloid in 33%. Of 92 patients assessed, 64% presented with cytogenetic abnormalities; CK was the most prevalent aberration found in 24% of patients followed by Ph+ chromosome in 15% (all B-myeloid) and translocations involving MLL gene at 11q23 in 4.3% of patients. Monosomy 7, polysomy 21 and trisomy 8 were also noted in a significant minority of patients.

Cytogenetic abnormalities in MPAL and BAL were reported in a recent systematic review to be present in 59-91% of patients\textsuperscript{30}. The prevalence of Ph+ and complex karyotype increases with age. The frequency of translocations involving 11q23 (usually MLL-AF4 or MLL-ENL fusions) decreases with age and are quite uncommon in adults with MPAL\textsuperscript{30}. One could argue that CK and other myelodysplasia specific changes should be classified as AML with MDS-related changes rather than MPAL.
Genetic alterations in MPAL:

Rubnitz 31 analyzed gene expression patterns in 13 pediatric patients with EGIL defined BAL and found that while 5 patients clustered with known AML expression patterns, 8 patients displayed gene expression patterns that were different from AML and ALL suggesting that some cases of BAL may be a biologically distinct entity. In contrast, microRNA profiling studies suggest that MPAL does not appear to be a distinct entity. de Leeuw 32 analyzed 16 cases of acute leukemia of ambiguous lineage and demonstrated that all cases had microRNA expression profiles that clustered with AML or ALL. Heesch33 noted a higher expression of BAALC and ERG, adverse prognostic characteristics in AML, in 26 cases of EGIL-defined BAL compared to other cases of AML.

Information regarding the mutational landscape of MPAL is based on small patient numbers. Yan et al27. analyzed 31 patients with MPAL for 18 leukemia-related mutations and reported that 12 patients (39%) were found to harbor a mutation, including IKZF1 deletion in 4 patients (all B-myeloid phenotype), EZH2 in 3 (B or T-myeloid), ASXL1 in 2 (both B-myeloid), TET2 in one (B -myeloid) and ETV6 and NOTCH1 in one patient each (both T-myeloid). A high rate of DNMT3A mutations was reported in adults with T-myeloid MPAL (10/18 patients; mostly biallelic mutations)34. Whole exome sequencing in 19 adult patients with MPAL (12 T-myeloid, 6 B-myeloid and 1 B/T) demonstrated that 63% of patients had mutations in epigenetic regulatory genes. DNMT3A was the most common mutation (n=6) followed by EZH2, IDH1/2, TET1 and TET3. Other recurrent mutations included PRPF40B (n=6), TP53 (n=5), BRAF (n=4) and NOTCH1 (n=4)35. Some of these mutations (e.g. IDH1/2, BRAF, NOTCH1 and FLT3) could be theoretically targeted by available agents or those in current clinical trials. 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-positive\textsuperscript{36}. Array-based comparative genomic hybridization analysis in 12 patients with MPAL demonstrated that all patients had at least one abnormality including deletions of CDKN2A, IKZF1, MEF2C, BCOR, EBF1, KRAS, LEF1, MBNL1, PBX3 and RUNX1\textsuperscript{27}.

**Risk factors and outcomes:**

The reasons underlying resistance to therapy in this heterogeneous group are not clear but may be related to the high prevalence of drug efflux pump expression\textsuperscript{37-39} and the high proportion with cytogenetic abnormalities\textsuperscript{30}. Whatever the classification employed, there appears to be a uniformly poor outcome in (BAL or) MPAL which is inferior to that of more typical AML or ALL. Based on adult and mixed pediatric/adult series patients with EGIL defined BAL have CR rates of 30-80.6\%\textsuperscript{33,37,40-46} with median DFS and OS of 5-12 months\textsuperscript{37,44-46} and 6.5-30.3 months\textsuperscript{33,37,40,41,44-46}, respectively. In the few larger retrospective series of WHO 2008 defined MPAL CR rates are reported at 61.5-85.2\%\textsuperscript{27,29,47,48} and median OS is at 14.8-18 months\textsuperscript{29,47}. Factors that are associated with outcome in these analysis include age\textsuperscript{29,31,37,40,44,48-50}, WBC count at diagnosis\textsuperscript{41,44}, Ph\textsuperscript{+29,40}, CK or MLL rearrangement\textsuperscript{45}, baseline creatinine and uric acid levels\textsuperscript{47}, extra medullary involvement at diagnosis\textsuperscript{44}, immunophenotype (T-myeloid being worse)\textsuperscript{41}, failing to respond to induction therapy\textsuperscript{37,44,48}, type of induction therapy (favoring non-AML)\textsuperscript{29,44,48} and type of post remission therapy (favoring transplant\textsuperscript{27,33} and more intensive conditioning\textsuperscript{48}).
Treatment of MPAL

There are no prospective trials that point to an optimal strategy. Beyond one’s own experience, we are left with heterogeneous case series which describe outcomes retrospectively. Further complicating data interpretation is the inclusion of patients with well-defined AML syndromes in previous classifications (such as core binding factor leukemias) that may bias those reports towards the use of AML type therapy. Case studies from individual centers or countries tend to examine all cases of acute leukemia and describe MPAL in 2-3%. While these studies probably fairly accurately reflect the true incidence of the entity, treatment decisions are haphazard and are subject to unknown bias regarding individual physicians, since there was no widespread treatment ‘policy’. The few studies which retrospectively garnered MPAL cases from cooperative group trials use more homogeneous treatments, but inevitably have excluded cases from eligibility based on ambiguity. One won’t find much guidance in the NCCN guidelines either. The precise definition of which cases should be treated according to the ALL vs. AML guidelines is sidestepped. The reader is advised to consult a center or individual with experience in diagnosing these entities. As someone who serves on the AML NCCN guidelines committee (RMS); I readily admit personal uncertainly which can be blamed on the lack of evidence.

Caveats aside, the dilemma when considering a patient who is a bona fide case of MPAL can be lessened by a) information gathering, b) considering pathophysiology, and c) consulting literature. The optimum information would include the patient’s age, past medical history/comorbidities, blast morphology (including cytochemistry), a complete immunophenotype, cytogenetics, and molecular studies. Older infirm patients with multiple comorbidities are not good candidates for standard ALL or AML induction therapy. The presence of Auer
rods, degree of MPO-positivity on flow cytometry and cytochemistry could be the basis for a therapeutic decision, albeit without any supporting data.

Cytogenetic studies if rapidly available could categorize the patient. Patients with MPAL and 11q23 rearrangement are considered a separate entity in the 2008 WHO schema, although their initial treatment may not be different than for most MPAL patients. However, it is critical to define the Ph+ patient as rapidly as possible since such patients should have a tyrosine kinase inhibitor (TKI) added to their treatment. Finally, although the molecular biology has not been studied at any depth in MPAL, it makes sense to assess for the presence of mutations with prognostic and/or therapeutic relevance in leukemia and perhaps to rule out the presence of Ph-like signature which could have eventual therapeutic implications in ALL\textsuperscript{52}. If nothing else, collecting the data may be retrospectively useful in learning about MPAL.

As this disease is believed to emanate from a proximal, presumed long-lived stem cell in the hematopoietic hierarchy (high level of CD34 expression, capable of lineage switch or infidelity), one could surmise that chemotherapy alone would be insufficient to eradicate the disease. Ph+ ALL and MDS-associated and/or adverse chromosome AML are historically incurable without a stem cell transplant; the same likely applies to MPAL. Moreover, the inclusion of more chemotherapeutic agents in upfront therapy used in an ALL or combined regimen would seem more logical than an AML regimen, especially latter’s use of ara-C, less useful against a slowly dividing primitive stem cell. Ideally one could inhibit the gene product of a ‘founder’ mutation present early in disease development and throughout the course. This serendipitous situation appears to be the case for Ph+ MPAL.
**Ph+ and MLL rearranged MPAL**

The only ‘special’ cases within the MPAL WHO framework are patients with (9;22) or 11q23 cytogenetic abnormalities. At present, treatment considerations for those with cytogenetic rearrangements at 11q23 are not different than those for MPAL with any non-Philadelphia cytogenetic abnormal or normal karyotype. However, the 11q23 rearranged patients should be considered for a pathophysiologically-based clinical trial if chemotherapy and alloSCT fails or if the patient is not a candidate for aggressive chemotherapy. Such therapy could include a histone modifying-enzyme inhibitor or a bromodomain inhibitor based on the primary molecular abnormality\textsuperscript{53,54} or could target downstream activation of Hox genes via glycogen synthase kinase 3 or β-catenin inhibitors\textsuperscript{55}.

Ph+ MPAL demand a specific approach involving the use of a TKI. This entity is usually a combination of B-lymphoid and myeloid markers; it accounts for about 25% of all MPAL\textsuperscript{30}. Essentially all case series describing this entity mention the adverse prognosis engendered by this molecular lesion\textsuperscript{29,40}. However, in the TKI era the situation may be changing.

One can reasonably look to the Ph+ ALL literature for guidance. Ph+ALL was historically considered a poor prognostic entity but prospective studies in which imatinib\textsuperscript{56,57} or dasatinib\textsuperscript{58} have been combined with standard multi-agent chemotherapy depict a long-term disease free survival of 40%-60%, approaching that seen with Ph-negative ALL in adults. While Ph+ ALL patients achieving remission with TKI plus chemotherapy-based therapy conventionally should be consolidated with alloSCT if feasible, emerging data suggests that autologous transplant for patients with Ph+ ALL in remission\textsuperscript{59} and/or ongoing TKI as maintenance\textsuperscript{56} may be associated with long term remissions, calling into question
the obligate need for alloSCT in this MPAL subtype. For older adults, excellent short-term results have been obtained with dasatinib plus steroids and intrathecal chemotherapy\(^6^0\); one wonders about the need for aggressive chemotherapy even in younger patients given the potent anti-leukemic efficacy of TKIs. Therefore, we treat all MPAL t(9;22) patients with age-specific ALL chemotherapy in combination with a TKI (CALGB 9111\(^6^1\), hyperCVAD\(^5^6\), MRC-ECOG 2993\(^6^2\) for middle-aged patients and the Foa regimen\(^6^0\) for older patients) followed by alloSCT if feasible. In some pediatric and adult series biphenotypic expression was reported to be associated with high frequency of central nervous system (CNS) involvement at presentation\(^4^4,4^9,5^0,6^3\), thus we try to adhere to CNS directed therapy according to the specific ALL protocol that is chosen. Whether to use highly effective pediatric-like chemo\(^6^4\) plus TKI for patients under 40 with t(9;22) MPAL is unclear, although limited pediatric experience suggests this may be possible. A recent retrospective analysis compared characteristics and outcomes of 13 Ph+ MPAL patients to 27 patients with Ph+ ALL and demonstrated comparable CR rates among the 2 groups (100% vs. 85%) as well as similar 5 year OS (55% vs. 53%) and DFS (46% vs. 42%)\(^6^5\).

**Non-Ph+ MPAL**

What is the best approach for the non-t(9;22) MPAL patient? We treat with an ALL-regimen and consolidate with an alloSCT if a donor is available. Most of the retrospective case series suggest that the complete remission rate is higher with ALL therapy or ALL/AML combined regimen than with AML-type therapy. Matutes et al\(^2^9\) noted a CR rate of 85% compared 41% for AML-type therapy. Presumptively many of the patients who had morphological AML (42%) received AML-type therapy; the inferior CR rate with this therapy may have been a manifestation of intrinsic resistance in this subset. Whether these ‘AML-like’
patients would fare better with ‘ALL-type’ therapy is unknown. Other studies, albeit with smaller patient numbers showed similar findings regarding ALL type vs. AML-type CR rates: 75% vs. 28%46; 64% ‘vs’ 33%45.

While the preponderant thought has been to use ALL type therapy, the situation is far from straightforward. Are there any patients in whom it makes sense to use AML-type therapy? While we have tended to use 3+7 in MPAL patients who express MPO cytochemically, there is little support for this common sense approach. One prospective clinical trial used AML therapy in 7 MPAL patients who had > 20% expression of MPO by flow but noted only 2 CRs46. We could find no data applying the use in MPO or Sudan black positivity to assign therapy. The use of combination AML+ALL regimens has some appeal (e.g. the VAPA 10 approach66). These ‘combined’ regimens vary among studies but generally add ALL-active agents such as steroids and vincristine to the AML anthracycline/cytarabine backbone. Remission rates with these combined regimens are largely comparable to those achieved with established ALL protocols27,40,41,44-46 and some reports found these regimens to be rather toxic40,44. In a few reports from adult and pediatric BAL series, high rates of remission with ALL directed salvage therapy were reported after AML induction failure31,46 and vice versa27.

Emerging data suggest that pediatric-like regimens lead to a 50-60% 3 year survival in adults with ALL age 18-4064,67, compared with 30%-40% rate with ‘legacy’ regimens such as CALGB 9111 and hyperCVAD61,68. While MPALs were not specifically included in the few reports, it does make sense to use such therapy in patients up to age 40. Children with MPAL seem to fare better with ALL regimens, although they do have inferior outcome compared with other children with non-MPAL ALL50. Although patients with MPAL who achieve CR and have an alloSCT are a selected group, every study suggests superior outcomes in adult
patients who receive alloSCT compared with those who receive only chemotherapy in the post-remission setting. Moreover, alloSCT is better than chemo in essentially all high risk leukemias. For example, 12 out of 34 patients with acute leukemia of ambiguous lineage (ALAL) underwent SCT. There was a 5 year OS rate of 70% compared with 19% for those who received chemotherapy only. Liu focused on 59 patients with ALAL who underwent alloSCT and noted a 5 year OS likelihood of 55% with an intensive preparative regimen and 24% with a ‘standard’ preparation. Although using multiparameter flow cytometry to define minimal residual disease (MRD) in MPAL cases can be challenging, one could speculate that such patients who are clearly MRD negative early in their course could be treated with consolidation chemotherapy rather than alloSCT in a similar fashion to Ph+ ALL that becomes molecularly negative after therapy.

**Summary**

As shown in Figure 3, a reasonable approach to a patient with MPAL is to first determine if the disease is driven by BCR-ABL1. If so, age appropriate ALL therapy plus a TKI followed by SCT is reasonable. If BCR-ABL1 negative, age appropriate ALL therapy followed by SCT after remission is an acceptable strategy. Important areas for further study are: 1) whether the degree of MPO positivity by immunophenotype/cytochemistry should influence the choice of therapy; 2) whether the presence of myeloid specific mutations or other genetic and molecular markers should be considered; 3) can the pathophysiology of 11q23 leukemia be successfully exploited and 4) will allo SCT be needed for MPAL t(9;22) patients who respond very well to chemotherapy plus TKI?
Case-Part 2

Based on immunophenotype and cytogenetic information, the diagnosis of MPAL with t(9;22)(q34;q11.2); BCR-ABL1 was made. The patient was treated with a CALGB9111-type induction regimen plus dasatinib and promptly entered complete remission; no immunophenotype-based MRD noted, but BCR-ABL1 transcripts remained detectable. The patient received dasatinib and CNS prophylaxis followed by sibling-matched allogeneic stem cell transplant with myeloablative conditioning. He is currently 3 months after transplant and remains in remission.

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Authorship:

Dr. Wolach wrote the paper and has no conflicts of interest.

Dr. Stone wrote the paper and has no conflicts of interest.
References


FIGURE LEGENDS

**Figure 1.** (A) Two atypical blast populations are seen on bone marrow aspirate smear. One population (arrowhead) is composed of small-sized cells with round nuclei, slightly condensed chromatin, distinct nucleoli and scant cytoplasm which shows cytoplasmic reactivity with periodic acid-Schiff (PAS) in a block-like pattern, and lacks reactivity with myeloperoxidase (MPO). The other population (arrow) is composed of large-sized cells with irregular nuclei, dispersed chromatin, variably-distinct nucleoli and small to moderate amounts of blue-gray cytoplasm which shows cytoplasmic reactivity with MPO and lacks reactivity with PAS. (B) Flow cytometric analysis of this bone marrow aspirate reveals two atypical blast populations (one highlighted in purple; one highlighted in red) with distinct CD45 expression and variable antigen expression profiles. The CD45(dim) purple population exhibits uniform expression of B-lymphoid markers CD19 and CD10, uniform stem cell marker CD34 and variable myeloid marker CD33. The red population shows brighter CD45 expression, exhibits uniform expression of CD33, small subset CD34, variable CD19 and lacks CD10 expression.

**Figure 2:** (A) EGIL criteria for the diagnosis of biphenotypic acute leukemia (BAL)a; (B) 2008 WHO criteria. Leukemias that fail to demonstrate differentiation along a single lineage are defined as acute leukemias of ambiguous lineage (ALAL) and are further subdivided into diagnostic subgroups. A practical approach for the diagnosis of mixed phenotype acute leukemia (MPAL) is presented.

**Figure 3:** The approach to therapy in patients with mixed phenotype acute leukemia (MPAL). Targeted therapy based on patient’s specific genetic profile
should be considered in refractory/relapse patients (e.g. MLL rearrangements, FLT3-ITD, IDH1, IDH2).
**Figure 2**

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According to the EGIL criteria, biphenotypic leukemia (BAL) is diagnosed when scores are >2 for the myeloid and one of the lymphoid lineages. A marker is considered positive if more than 20% of cells stain positive with a monoclonal antibody; a lower threshold of 10% was set for MPO, CD3, CD79a and TdT.

Abbreviations: cyt, cytoplasmatic; m, membrane; MPO, myeloperoxidase; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.
Mixed Phenotype Acute Leukemia (MPAL)

- Are t(9;22)(q34;q11.2); BCR-ABL1 rearrangement present?
  - Yes: MPAL with t(9;22)(q34;q11.2); BCR-ABL1
  - No: Is t(v;11q23) translocation present?
    - Yes: MPAL with t(v;11q23); MLL rearranged
    - No: Does leukemia meet criteria for assignment of both B and myeloid lineage?
      - Yes: MPAL, B/Myeloid, NOS
      - No: Does leukemia meet criteria for assignment of both T and myeloid lineage?
        - Yes: MPAL, T/Myeloid, NOS
        - No: MPAL, NOS rare types

Other entities within ALAL classification:
- Acute undifferentiated leukemia
- Other ambiguous lineage leukemia
The following lineage defining markers are required for assigning more than one lineage to a single blast population:

1) Myeloid lineage: myeloperoxidase (by flow cytometry, immunohistochemistry or cytochemistry) or monocytic differentiation (diffuse positivity for non-specific esterase or expression of at least 2 of the following: CD11c, CD14, CD36, CD64, lysozyme).

2) T-lineage: cytoplasmatic CD3 (flow cytometry with antibodies to CD3 epsilon chain; caution when using polyclonal anti-CD3 in immunohistochemistry as it may not be specific) or surface CD3.

3) B-lineage: strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmatic CD22, CD10 or weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmatic CD22, CD10.

For bilineal MPAL the myeloid component can be recognized when there are 2 or more distinct populations of blasts, one of which would meet the criteria for AML (need not comprise >20% of nucleated cells).

cThe following subgroups of leukemia should be defined primarily by their genetic or clinical features even if they satisfy the above requirements for mutli-lineage expression. A secondary notion regarding their mixed phenotype should be added: AML with recurrent cytogenetic abnormalities such as t(8;21), t(15;17) or inv(16), leukemia with FGFR1 mutations, chronic myeloid leukemia in blast crisis, myelodysplasia-related AML and therapy related AML.

dInclude rare cases where leukemic blasts show evidence of both T and B lineage, trilineage T, B and myeloid commitment or other rare combinations; CD79a and CD10 should not be considered as evidence for B cell differentiation in this setting as they lack specificity.

eThese diagnostic entities are associated with lack of definitive lineage commitment. ‘Acute undifferentiated leukemia’ include leukemias that express no lineage specific markers. ‘Other ambiguous lineage leukemia’ subgroup encompass the rare cases of leukemia that express combination of lineage associated markers that are suggestive but not sufficient for lineage assignment (so called ‘acute unclassifiable leukemias’); Natural killer cell lymphoblastic leukemia/lymphoma is regarded as a provisional entity in this category.
Figure 3

Mixed phenotype acute leukemia (MPAL)

MPAL with t(9;22)(q34;q11.2); BCR-ABL1?

Yes

Acute lymphoblastic leukemia – like chemotherapy + Tyrosine kinase inhibitor

CR?

No

- Reassess phenotype
- Assess for TKI resistance/change TKI as indicated
- Consider AML-like salvage

CR?

Yes

Allogeneic stem cell transplant

CR?

No

- Clinical trial/targeted therapy

CR?

No

- Clinical trial/targeted therapy

CR?

Yes

Relapse

Reasses phenotype
\textsuperscript{a}Consider pediatric inspired protocol if <40 years old (in which case transplant in first remission not generally indicated)

\textsuperscript{b}For example High-dose cytarabine and mitoxantrone (HAM).
How I treat mixed phenotype acute leukemia

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