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Insights in Blood Disorders

Cytogenetic and Molecular Features of Mixed Phenotype Acute Leukemia and the Impacts of Targeted Therapy

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ABSTRACT

Background: *Currently, there is no treatment consensus on the optimal treatment regimen for mixed-phenotype acute leukemia (MPAL).*

Aims: *To analyze the cytogenetic and molecular characteristics of U.S. veterans diagnosed with MPAL as well as examine the effects of targeted therapy on their overall survival.*

Methods: *Retrospective study between the years 2000-2023*

Setting: *U.S. Department of Veterans Affairs*

Participants: *United States veterans in the Veterans Affairs Informatics and Computing Infrastructure (VINCI) database diagnosed with MPAL between 2000-2023. The text utilization integration feature was used to query all notes which included the phrases "mixed phenotype acute leukemia", "MPAL", and "biphenotypic leukemia" and identified 320 patients. All patient charts were reviewed manually. 94 patients were included in the final analysis.*

Exposure: Treatment for MPAL.

Main outcomes and measures: *The primary outcome was 5-year overall survival (OS), KaplanMeier and Cox regression analyses were used to compare this between different patient groups.*

Results: *Of the 74 patients treated with curative intent, 33 patients had potentially targetable mutations and 20 patients received targeted therapy. The 5-year OS for the entire cohort was 26%. Mutations noted in more than one patient included RUNX1 (n=10), NOTCH1 (n=4), DNMT3A (n=4). For the purposes of the study, TP53, TET2, and inv(16) were removed from the model due to small number of positive patients. Patients with Ph, RUNX1, and FLT3 mutations had improved OS with HR of 0.21 (p=<0.001), 0.43 (p=0.15), and 0.65 (p=0.5) respectively.*

Patients older than 60 years in age had poorer outcomes, HR 1.06 (p>0.09) with patients older than 80 years in age, with the worst outcomes, HR 11.9 (p=0.008).

Patients who were sent to transplant in their first clinical remission (CR1) had better outcomes with HR 0.14 (p=<0.001), while patients who required salvage chemotherapy had worse outcomes, as expected, with a HR of 7.59 (p=<0.001).

Conclusions and Relevance: *Molecular and cytogenetic testing may be beneficial for use in the treatment of MPAL; sending patients to transplant in addition to using targeted therapy has the most beneficial outcome.*

Keywords
Mixed phenotype acute leukemia, Targeted therapy, Molecular profiling, Cytogenetics, Survival outcomes.

Key Points
Question: How can precision medicine improve overall survival (OS) in patients with mixed phenotype acute leukemia (MPAL)?

Findings: In this retrospective study of 94 patients diagnosed with MPAL, the 5-year OS improved from 26% for the entire cohort to 100% for patients who received bone marrow transplant in first clinical remission (CR1) with targeted therapy. Patients with Ph, RUNX1, and FLT3 mutations had improved OS with HR of 0.21 (p=<0.001), 0.43 (p=0.15), and 0.65 (p=0.5) respectively.

Meaning: Use of targeted therapy in patients with MPAL may improve 5-year OS, and use of targeted therapy in conjunction with CR1 transplant may be the most beneficial.

Introduction
The integration of upfront precision medicine based on cytogenetic and mutational data has been shown to improve overall survival in acute myeloid leukemia (AML) [1] and acute lymphoblastic leukemia (ALL) [2], and has become the standard of care [3]. Mixed phenotype acute leukemia (MPAL) is a heterogeneous subtype of leukemia in which blasts express both ALL and AML markers, and accounts for 1-3% of acute leukemias in adults [4]. Given its rarity, prospective studies are lacking and questions regarding both the clonal origin and standard treatment approaches for MPAL remain unanswered. There is work being done to better understand the genomic landscape of MPAL [5], however, the role of targeted therapy in the treatment of MPAL has not yet been established.

We conducted a retrospective study of all veterans with MPAL diagnosed between 2000-2023 to examine the molecular landscape and impact of targeted treatment strategies on overall survival.

We believe that our work builds on findings from previous studies which have examined the genetic landscape of MPAL and further supports the integration of precision medicine into front line acute leukemia treatments.

Methods
Study Population and Data Collection
This study looked at all patients with MPAL from the Veterans Affairs Informatics and Computing Infrastructure (VINCI) database diagnosed between 2000-2023. Retrospective data collection and review was approved by Washington DC VA IRB Committee. The text utilization integration feature was used to query all notes which included the phrases “mixed phenotype acute leukemia”, “MPAL”, and “biphenotypic leukemia” and identified 320 patients. All patient charts were reviewed manually. Pathology reports and clinical notes were reviewed to collect data on diagnosis, treatment, and outcome. Patients were included if they had a correct diagnosis of MPAL per the 2022 ICC as well as pathology and treatment information documented. 94 patients were included in our final analysis. The most common reasons for excluding patients were an incorrect diagnosis and incomplete records (Figure 1).

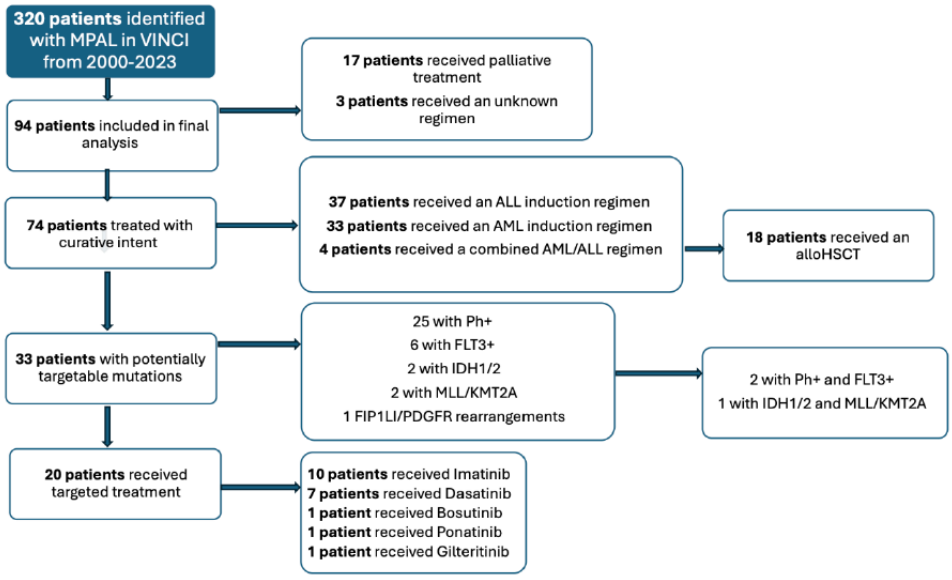

Figure 1: Flow Diagram of Patient Selection from VINCI. Depicted is the process by which patients were identified from the Veterans Affairs Informatics and Computing Infrastructure (VINCI) database for inclusion in the final study cohort.

Statistical Analysis

Overall survival analyses were done on the 74 patients treated with curative intent. Kaplan-Meier and Cox regression analyses were used to compare overall survival between different patient groups. Outcomes were time of diagnosis to death. In a univariate analysis, the effects of age (<40 vs >40), AML vs ALL induction regimens, and transplant status on overall survival were evaluated. In a multivariate analysis controlling for age, the prognostic effect of Ph, RUNX1, and FLT3 were evaluated. The prognostic significance of other mutations was not assessed given the small number of patients with each. Finally, the overall survival of the 33 patients who had potentially targetable mutations was evaluated with regards to whether or not they got targeted treatment, a transplant, or both.

Results

33 patients had potentially targetable mutations and 20 patients received targeted therapy (either as part of induction or maintenance). Other mutations noted in more than one patient included RUNX1 (n=10), NOTCH1 (n=4), DNMT3A (n=4).

Patients older than 60 years in age had poorer outcomes, HR 1.06 (p>0.09) with patients older than 80 years in age, with the worst outcomes of HR 11.9 (p=0.008). Furthermore, patients who were sent to transplant in their first clinical remission (CR1) had better outcomes with HR 0.14 (p=<0.001), while patients who required salvage chemotherapy had worse outcomes, as expected, with a HR of 7.59 (p=<0.001).

Using multivariate analysis, it was found that patients with Ph, RUNX1, and FLT3 mutations had improved OS with HR of 0.21 (p=<0.001), 0.43 (p=0.15), and 0.65 (p=0.5) respectively. Patients who received both transplant and targeted therapy did best in terms of survival, followed by transplanted patients alone. Patients who did not receive targeted therapy or a transplant were shown to have the worst survival outcomes (Figure 2).

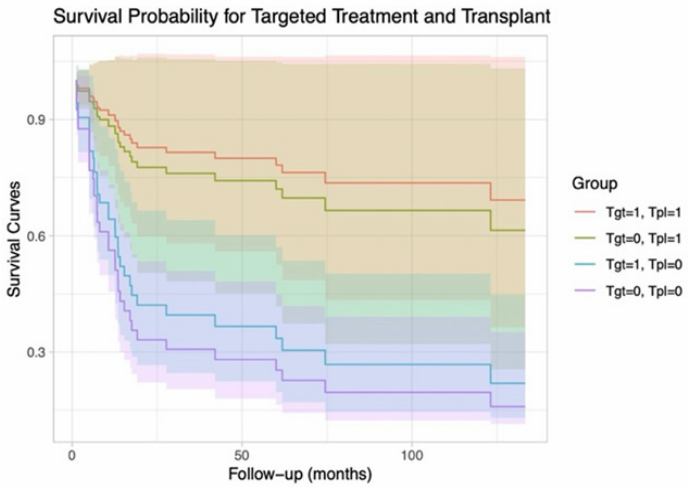

Figure 2: Survival Probability for Targeted Treatment and Transplant. Adjusted survival curves comparing targeted therapy and transplant, assuming treatment at diagnosis. Shaded areas represent 95% confidence intervals based on 200 bootstrap samples.

Discussion

In this retrospective cohort study of all veterans with MPAL, younger patients, those who got an ALL induction regimen, and received an allo-transplant in CR1 had significant survival gains, in line with prior research [6-8].

The prognostic significance of targetable and non-targetable cytogenetic and molecular abnormalities was also notable. Despite a small sample size, patients with Ph+, FLT3 and RUNX1 had improved OS. Variant allele frequency for TP53 mutations was not available, which is a limitation to this analysis [9]. Patients who received targeted therapy did better than those who did not. And for the few patients who received upfront targeted therapy with a transplant in CR1, they had an OS of 100% (n=3). It is important to note that the data in figure 2 is clinically but not statistically significant most likely due to small sample sizes.

Only 20 of 33 patients with targetable mutations in our cohort received targeted treatment. One important reason for why patients did not get targeted treatment may be that several of these agents were not available at the time of their diagnosis as we collected data on all patients diagnosed with MPAL from 2000-2023. While Imatinib, was first approved by the FDA in 2001 [10], midostaurin, the first FLT3 inhibitor, and enasidenib, the first IDH inhibitor, were not approved until 2017 [11,12]. Menin inhibitors such as revumenib, for patients with KMT2A re-arrangements, are still being studied but have showed significant promise in patients with relapsed and refractory leukemia in phase 1 and 2 studies [13].

Limitations

Limitations of this study include small sample size, retrospective design, lack of standardized genetic testing throughout the years studied, and lack of available targeted therapeutics earlier in the studied years.

Conclusions

Taken together, while the prognosis of MPAL has historically been poor [6], there are opportunities for improvements in outcomes. The 5-year OS for our entire cohort, including patients who received upfront palliative care, was 26%, yet the 5-year OS for patients who both got a transplant in CR1 and received targeted therapy was 100%. Because MPAL is so rare, prospective studies looking at MPAL patients alone are difficult - only five of the patients in this cohort were treated on a clinical trial. Including more patients with MPAL in clinical trials evaluating targeted therapy for other types of acute leukemia whenever feasible, would likely contribute to improved outcomes.

References

1. Burd A, Levine RL, Ruppert AS, et al. Precision medicine treatment in acute myeloid leukemia using prospective genomic profiling: Feasibility and preliminary efficacy of the Beat AML Master trial. Nat Med. 2020; 26: 1852-1858.
2. Rafei H, Kantarjian HM, Jabbour EJ. Recent advances in the treatment of acute lymphoblastic leukemia. Leuk Lymphoma. 2019; 60: 2606-2621.

3. National Comprehensive Cancer Network – Home. 2024.
4. Weinberg OK, Arber DA. Mixed-phenotype acute leukemia: Historical overview and a new definition. Leukemia. 2010; 24: 1844-1851.
5. Takahashi K, Wang F, Morita K, et al. Integrative genomic analysis of adult mixed phenotype acute leukemia delineates lineage associated molecular subtypes. Nat Commun. 2018; 9: 2670.
6. Shi R, Munker R. Survival of patients with mixed phenotype acute leukemias: A large population-based study. Leuk Res. 2015; 39: 606-616.
7. Orgel E, Alexander TB, Wood BL, 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.
8. Munker R, Brazauskas R, Wang HL, et al. Allogeneic hematopoietic cell transplantation for patients with mixed phenotype acute leukemia. Biol Blood Marrow Transplant. 2016; 22: 1024-1029.
9. Shah MV, Tran EN, Shah S, et al. TP53 mutation variant allele frequency of ≥10% is associated with poor prognosis in therapy-related myeloid neoplasms. Blood Cancer J. 2023; 13: 51.
10. Gleevec (imatinib mesylate) FDA approval history. 2024.
11. Center for Drug Evaluation and Research. Midostaurin. FDA. 2024.
12. Center for Drug Evaluation and Research. FDA granted regular approval to enasidenib for the treatment of relapsed or refractory AML. FDA. 2024.
13. Aldoss I, Issa GC, Thirman MJ, et al. Revumenib monotherapy in patients with relapsed/refractory KMT2Ar acute leukemias: Efficacy and safety results from the Augment-101 phase 1/2 study. Blood. 2023; 142: 2907-2907.