Molecular predictors of response and survival in patients with relapsed/refractory acute myeloid leukemia following venetoclax plus hypomethylating agent therapy

Venetoclax (Ven) in combination with hypomethylating agents (HMA) is used for treatment of relapsed/refractory acute myeloid leukemia (AML) in routine practice.[1-3] However, limited and inconsistent data exists on molecular predictors of response and survival following Ven + HMA therapy in the relapsed/refractory setting. Available literature suggests that outcomes are poor; one study on 43 patients with relapsed/refractory myeloid neoplasms, including AML found complete response (CR) or complete response with incomplete hematologic recovery (CRi) in only 12% of patients treated with Ven in combination with either HMA or low-dose cytarabine (LDAC), with median overall survival (OS) of 3 months.[4] In a separate study on 86 patients with relapsed/refractory AML receiving Ven + HMA or LDAC, CR/CRi was documented in 24% and *NPM1* mutations were associated with higher remission rates (CR/CRi 46%); median OS was 6.1 months for the entire cohort, while adverse cytogenetics, *TP53, K/NRAS*, and *SF3B1* mutations predicted inferior OS.[5] For example, patients with adverse genetic risk had a median OS of only 5.62 months, compared to 15.02 months in those with intermediate or favorable risk per 2017 European Leukemia Net (ELN) criteria. On the other hand, a retrospective study on 90 patients with Ven + HMA-treated relapsed/refractory AML, demonstrated CR/CRi in 46% with superior response rates observed in patients with *TET2* and *ASXL1* mutations.[6] A recent study has shown CR/CRi rate of only 8% in patients with *KMT2A*-rearranged relapsed/refractory AML, treated with Ven + HMA.[7] Given these varying results, our primary objective was to determine the impact of mutations on response and survival in relapsed/refractory AML patients receiving Ven + HMA therapy at our center.

Patients with relapsed or refractory AML, excluding post-transplant relapse, receiving Ven + HMA outside of clinical trials between November 2018 and April 2022 at the Mayo Clinic were retrospectively recruited after institutional review board approval. All methods were performed in accordance with the Declaration of Helsinki, the relevant guidelines, and regulations.

Follow-up information was updated in October 2024. The treatment regimen was chosen by the treating physician with judgment based on disease status and patient fitness. Cytogenetic and molecular studies were performed at the time of AML diagnosis by conventional karyotype, and next-generation sequencing (42-gene panel), respectively. Reverse transcriptase polymerase chain reaction (PCR) was performed to assess for *FLT3* and *NPM1* mutations. Patients received either azacitidine 75 mg/m^2 days 1-7 or decitabine 20 mg/m^2 days 1-5 with Ven dose adjusted based on azole antifungal prophylaxis. None of the patients had received prior Ven. Response was assessed via bone marrow biopsy, completed after cycle 1 or 2 of treatment based on physician discretion, according to the 2022 ELN criteria.[8] Minimal residual disease (MRD) was assessed via multiparametric flow cytometry (sensitivity =0.01%). Response determinants were assessed by χ^2 or Fisher's exact test for nominal data and Wilcoxon rank sum test for continuous variables. Overall survival was evaluated by the Kaplan-Meier method with differences compared by the log-rank test. Analyses were performed using JMP Pro 18.1.0 software package, SAS Institute, Cary, NC.

Eighty-six patients with relapsed (N=56) or refractory AML (N=30) (median age 64 years, 62% male, 50% *de novo*, 38% secondary, 12% therapy-related, 21% with prior HMA exposure) received Ven + HMA. Sixty-eight (79%) patients received decitabine and the remainder azacitidine with a median Ven dose of 100 mg for a median of two cycles. ELN cytogenetic risk (N=86) included favorable (1%, N=1), intermediate (56%, N=48) or adverse (43%, N=37). Mutations involved *TP53* in 20 patients (23%), *ASXL1* in 18 (21%), *IDH1/2* in 11 (13%), *TET2* in 11 (13%), *K/NRAS* in 11 (13%), *NPM1* in nine (10%), and *FLT3*-internal tandem duplications (ITD) in seven (8%). Table 1 provides patient demographics and outcomes. Ven + HMA was used as second-line therapy in 36% of cases (N=30), third-line in 28% (N=24), and as fourth-line or later in 37% (N=32). Frequently administered prior therapies included 7 (cytarabine) +3 (daunorubicin/idarubicin) (N=51), CPX-351 (N=17), enasidenib (N=5), ivosidenib (N=1), midostaurin (N=2), and gilteritinib (N=3).

Overall, 30-day mortality was 9% (N=8) (7% *vs*. 13% with Ven + HMA as second-line therapy *versus* third-line and beyond; *P*=0.67), and treatment-emergent complications included cytopenias (N=28), febrile neutropenia (N=16), pericardial effusions (N=3), supraventricular tachycardia (N=1), heart failure (N=2), acute kidney injury (N=2), tumor lysis syndrome (N=1), and infections (N=7).

Eighteen (21%) patients achieved CR, and 18 (21%) CRi, resulting in CR/CRi in 36 (42%). Median time to response was 1 month with median response duration of 3 months (range, 1-19 months). MRD was assessed in 53% of patients achieving CR/CRi (N=19) and was negative in 63% (N=12).

Response rates were lower with prior HMA exposure (CR/CRi 23% *vs*. 45% with or without prior HMA; *P*=0.12). On the other hand, response rates were similar with azacitidine

(CR/CRi 44%) or decitabine (CR/CRi 44% *vs*.42%; *P*=0.8), in relapsed *versus* refractory AML (CR/CRi 39% *vs*. 47%; *P*=0.51), in patients receiving Ven + HMA as second line *versus* beyond (CR/CRi 46% *vs*. 40%; *P*=0.64). In univariate analysis, age above 65 years (CR/CRi, 61% *vs*. 28%; *P*=0.002), presence of *IDH1/2* (73% *vs*. 37%; *P*=0.02) and *ASXL1* mutations (67% *vs*. 35%; *P*=0.02) were associated with favorable response; adverse karyotype (28% *vs*. 53%; *P*=0.02) and presence of *TP53* mutations (25% *vs*. 47%; *P*=0.06) predicted inferior response. Presence of *FLT3*-ITD (71% *vs*. 39%; *P*=0.09) *NPM1* (67% *vs*. 39%; *P*=0.11) and *TET2* mutations (64% *vs*. 39%; *P*=0.12) were borderline significant. In multivariable analysis, age >65 years (odds ratio [OR] =3.2) presence of *ASXL1* mutations (OR=3.3) and absence of adverse karyotype (OR=3.2) remained independent predictors of favorable response, while *TP53* mutation was no longer significant (*P*=0.58). Remainder of the mutations, outlined in Table 1, did not impact response.

At a median follow-up of 6 months (range, 0.1-64 months), 21 (24%) patients relapsed, 76 (88%) patients have died and 22 (26%) underwent allogeneic stem cell transplant (ASCT). Of the 22 who underwent transplant, 18 patients were trans-

Table 1. Clinical characteristics at time of treatment with venetoclax and hypomethylating agent for 86 patients with relapsed/refractory acute myeloid leukemia stratified by achievement of complete response or complete response with incomplete count recovery.

Variables	All patients N=86	Patients in CR/CRi N=36	Patients not in CR/CRi N=50	Univariate *P*
Age in years, median (interquartile range)	64 (15)	67 (13)	61 (19)	0.02*
Male, N (%)	55 (63)	26 (72)	29 (58)	0.17
AML type, N (%) *De novo* Secondary or therapy-related	 43 (50) 43 (50)	 18 (50) 18 (50)	 25 (50) 25 (50)	1.0
Hemoglobin g/dL, median (range)	8.3 (4.8-17.1)	8.5 (6.7-12.3)	8.1 (4.8-17.1)	0.59
Leukocyte count x10^9/L, median (range)	2.03 (0.1-165)	2.1 (0.4-51.4)	1.84 (0.1-165)	0.38
Platelet count x10^9/L, median (range)	37 (2-391)	55 (2-380)	29 (3-391)	0.48
Circulating blasts %, median (range)	3 (0-85)	1 (0-71)	7 (0-85)	0.12
Bone marrow blasts %, median (range)	25 (1-98)	20 (1-80)	30 (0-98)	0.75
European LeukemiaNet. 2022 cytogenetic risk stratification, N (%) Favorable/intermediate Adverse	 47 (55) 39 (45)	 25 (31) 11 (69)	 22 (44) 28 (56)	0.02*
Mutations, N (%) *TP53* *TET2* *SRSF2* *ASXL1* *RUNX1* *IDH1/2* *NPM1* *K/NRAS* *DNMT3A* *FLT3*-ITD *BCOR* *CEBPA bZIP* *SF3B1* *EZH2* *WT1* *JAK2* *SETBP1* *CBL*	 20 (23) 11 (13) 9 (10) 18 (21) 16 (18) 11 (13) 9(10) 11 (13) 9 (10) 7 (8) 5 (6) 1 (1) 3 (4) 6 (7) 6 (7) 8 (9) 3 (4) 3 (4)	 5 (14) 7 (19) 6 (17) 12 (33) 7 (19) 8 6 (17) 4 (11) 6 (17) 5 (14) 4 (11) 1 (3) 1 (3) 4 (11) 3 (8) 6 (17) 2 (6) 0 (0)	 15 (30) 4 (8) 3 (6) 6 (12) 9 (18) 3 (6) 3 (6) 7 (14) 3 (6) 2 (4) 1 (2) 0 (0) 2 (4) 2 3 2 (4) 1 (2) 3 (6)	 0.06 0.12 0.11 0.02* 0.86 0.02* 0.11 0.69 0.11 0.09 0.07 0.74 0.75 0.2 0.68 0.05* 0.38 0.07
HMA used, N (%) Azacitidine Decitabine	 18 (21) 68 (79)	 8 (22) 28 (78)	 10 (20) 40 (80	0.8
Final dose of venetoclax in mg, median (range)	100 (100-400)	100 (100-400)	100 (100-400)	0.43
Allogeneic transplant, N (%)	22 (25)	18 (50)	4 (8)	<0.002*

*Statistically significant value; AML: acute myeloid leukemia; CR: complete response; CRi: CR with incomplete count recovery; HMA: hypomethylating agent.

planted after response to Ven + HMA. The remainder received either cladribine, cytosine arabinoside, G-CSF, mitoxantrone (CLAG-M) (N=2), 7+3 (N=1) or azacitidine + ivosidenib (N=1) as bridging therapy prior to transplant. Nine of the ten patients alive at the end of the study had received an ASCT. Subsequent therapies following Ven + HMA included enasidenib (N=3) and gilteritinib (N=2). Median OS following Ven + HMA was 6 months (1-year/3-year survival rate; 27%/11%) and longer in transplanted patients *versus* those that were not transplanted (18 *vs*. 4 months; *P*<0.01). Univariate analysis identified CR/CRi (median OS 12 *vs*. 3 months; *P*<0.01) and presence of *IDH1/2* mutations (17 *vs*. 5 months; *P*<0.01) as favorable risk factors for survival, and *TP53* (3.5 *vs*. 7.5 months; *P*<0.01) as an unfavorable risk factor for survival; ELN-defined adverse karyotype (5 *vs*. 8 months; *P*=0.08) was borderline significant (Table 2). Despite higher CR/CRi rate, presence of *ASXL1* mutation did not appear to significantly impact survival (10 *vs*. 6 months; *P*=0.25). Multivariable analysis confirmed the negative survival impact of not achieving CR/CRi (hazard ratio [HR] =3.1; 95% confidence interval [CI]: 1.9-3.5; *P*<0.01), absence of *IDH 1/2* (HR=3.3; 95% CI: 1.5-7.4; *P*<0.01) and presence of *TP53* mutations (HR=2.1; 95% CI: 1.2-3.5; *P*=0.01) (Table 2). Accordingly, a three-tiered survival model was generated by allocating 1 adverse point each for absence of CR/CRi, absence of *IDH 1/2* and presence of *TP53* mutations, resulting in low (0-1 point, N=33, median OS 13 months), intermediate (2 points, N=39, median OS 4 months) and high risk (3 points, N=14, median OS 1.5 months) categories (*P*<0.001) (Figure 1A). The proposed model remained applicable when survival was censored for ASCT (9 *vs*. 4 *vs*. 1.5 months; *P*<0.001) (Figure 1B).

The current study showed a CR/CRi rate of 42% in relapsed or refractory AML receiving Ven + HMA, which was superior compared to the MD Anderson Cancer Center (MDACC) (CR/CRi in 12%)[4] and Memorial Sloan Kettering Cancer Center (MSKCC) experience (CR/CRi in 24%),[5] but similar to that previously reported by investigators from the City of Hope (CR/CRi in 46%).[2] It should be brought to attention that

Table 2. Predictors of complete response or complete response with incomplete count recovery and overall survival for 86 patients with relapsed/refractory acute myeloid leukemia treated with venetoclax plus hypomethylating agent therapy.

Variables	CR/CRi		Overall survival	
	Univariate *P* CR/CRi rates	Multivariate *P*/OR	Univariate *P* HR (95% CI)	Multivariate *P* HR (95% CI)
Age >65 years	<0.01 61% *vs*. 28%	0.02/3.2	0.97	-
De novo vs. secondary/therapy-related AML	1.0 42% *vs*. 42%	-	0.12	-
European LeukemiaNet 2022 adverse karyotype	0.02 28% *vs*. 53% Presence *vs*. absence	0.02/0.31	0.10 1.5 (0.9-2.3) Presence *vs*. absence	0.67
TP53 mutation	0.07 25% *vs*. 47% Presence *vs*. absence	-	<0.01 2.2 (1.3-3.7) Presence *vs*. absence	0.02 2.1 (1.2-3.5)
IDH1/2 mutation	0.03 73% *vs*. 37% Presence *vs*. absence	0.14/3.1	<0.01 2.9 (1.3-6.5) Absence *vs*. presence	<0.01 3.0 (1.4-6.9)
NPM1 mutation	0.11 67% *vs*. 39% Presence *vs*. absence	-	0.79	-
FLT3-ITD mutation	0.09 71% *vs*. 39% Presence *vs*. absence	-	0.38	-
ASXL1 mutation	0.02 67% *vs*. 35% Presence *vs*. absence	-	0.26	-
Absence of CR/CRi	-	-	<0.01 3.1 (1.9-5.1)	<0.01 3.4 (2.0-5.7)
Allogeneic transplantation (not transplanted)	-	-	<0.01 4.3 (2.3-8.1)	<0.01 3.4 (1.7-6.8)

AML: acute myeloid leukemia; CR: complete response; CRi: CR with incomplete count recovery; HMA: hypomethylating agent; OR: odds ratio; HR: hazard ratio; CI: confidence interval.

the MDACC and MSKCC studies considered all Ven-based therapies including Ven + LDAC. Suprisingly, in our study, response rates were found to be significantly higher in patients >65 years, which might have been due to a higher proportion of older patients harboring *ASXL1* mutations (33% *vs*. 12%; *P*=0.02), which positively influenced response rates. A preclinical study showed that *ASXL1*-mutated cells demonstrate increased dependence on *BCL2* and increased gene-body methylation, which may increase vulnerability to treatment with Ven and HMA, respectively, and may explain improved response rates to Ven + HMA.[9] Notably, in the current study, 18 (20%) of patients were bridged to ASCT following response to Ven + HMA. A recent study underlined the value of Ven-based therapy as an effective bridge to ASCT in relapsed/refractory AML; in the particular study, survival was numerically longer in Ven-treated patients in comparison to non-Ven-based regimens (15.8 *vs*. 10.5 months; *P*=0.15).[10] Noteworthy findings from our study include favorable treatment response in the presence of *IDH1/2* and *ASXL1* mutations, and age older than 65 years; this is in keeping with the 2024 ELN recommendations which classify *IDH1* and *IDH2* as favorable risk in patients receiving less-intensive therapies.[11] Not surprisingly, adverse karyotype and *TP53* mutations were associated with inferior response to Ven + HMA.[12,13] Furthermore, survival following Ven + HMA therapy in the relapsed/refractory setting was positively influenced by achievement of CR/CRi, presence of *IDH1/2*, and absence of *TP53* mutations. Among 11 patients harboring *IDH1/2* mutations, only three (27%) received an IDH inhibitor following Ven + HMA, which is unlikely to have accounted for the observed survival advantage. The findings from the current study are in line with those reported in newly diagnosed AML patients receiving Ven + HMA; in a Mayo Clinic study of 301 patients, survival was superior with achievement of CR/CRi, presence of *IDH2* mutations, absence of *TP53* mutations and adverse karyotype.[14] Our prior work demonstrates that *NPM1, IDH2* and *DDX41* are favorable predictors of response in the frontline setting; molecular predictors appear to vary somewhat between upfront and relapsed/refractory settings.[15] Also, we have previously demonstrated that *ASXL1* mutation positively influences response in AML patients receiving Ven + HMA frontline therapy, however, as in the current study, the superior response did not translate into a survival advantage.[16] Our findings require validation in prospective series, which should also take into consideration the survival impact of subsequent targeted therapies.

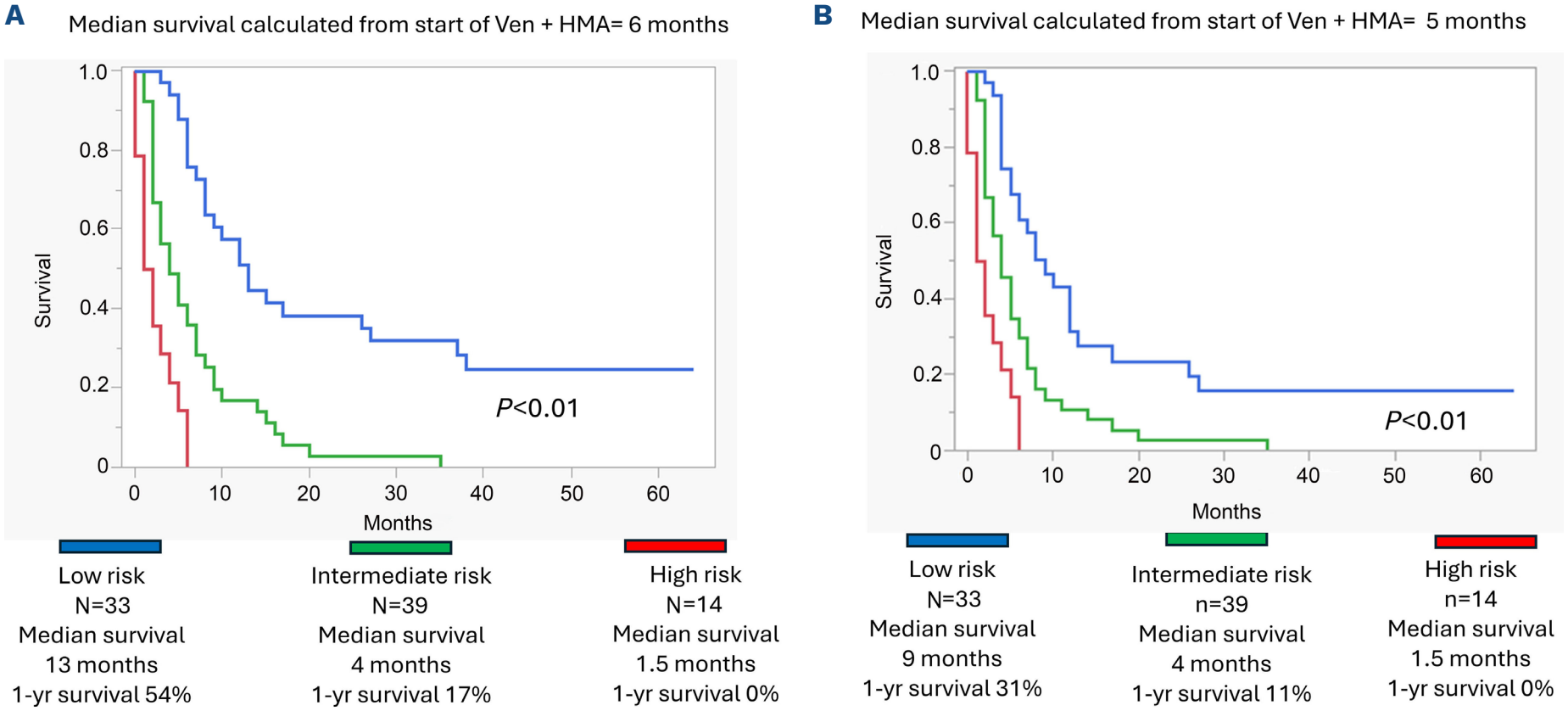

Figure 1. Survival of 86 relapsed/refractory acute myeloid leukemia patients receiving venetoclax and hypomethylating agent therapy. (A) Survival of 86 relapsed/refractory acute myeloid leukemia (AML) patients receiving venetoclax and hypomethylating agent (Ven + HMA) therapy, stratified by hazard ratio (HR)-weighted scoring system, HR in the absence of complete response or compete response with incomplete count recovery (CR/CRi) (HR=3.4; 95% confidence interval [CI]: 2.0-5.7), absence of *IDH1/2* mutations (HR=3.0; 95% CI: 1.4-6.9) and presence of *TP53* mutations (HR=2.1; 95% CI: 1.2-3.5), allocating 1 point for not achieving CR/CRi, 1 adverse point for absence of *IDH1/2* mutations, and 1 adverse point for *TP53* mutation. Median overall survival stratified by low risk (0-1 points), intermediate risk (2 points) and high risk (3 points) is shown. (B) Transplant-censored survival of 86 relapsed/refractory AML patients receiving Ven + HMA therapy, stratified by HR-weighted scoring system, HR in the absence of CR/CRi (HR=4.5; 95% CI: 2.5-8.3), absence of *IDH1/2* mutations (HR=3.0; 95% CI: 1.3-7.4) and presence of *TP53* mutations (HR=2.1; 95% CI: 1.2-3.8), allocating 1 point for not achieving CR/CRi, 1 adverse point for absence of *IDH1/2* mutations, and 1 adverse point for *TP53* mutation. Median overall survival stratified by low risk (0-1 points), intermediate risk (2 points) and high risk (3 points) is shown. yr: year.

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Contributions

NG, IJ and AT designed the study, collected data, performed analysis and co-wrote the paper. RI, KM, FF, AA, HBA, KHB, AM, AS, MRL, WH, MS, MMP and AP contributed patients. All authors reviewed and approved the final draft of the paper.

Data-sharing statement

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References

1. Bataller A, Kantarjian H, Bazinet A, et al. Outcomes and genetic dynamics of acute myeloid leukemia at first relapse. Haematologica. 2024;109(11):3543-3556.
2. Aldoss I, Yang D, Aribi A, et al. Efficacy of the combination of venetoclax and hypomethylating agents in relapsed/refractory acute myeloid leukemia. Haematologica. 2018;103(9):e404-e407.
3. Thol F, Döhner H, Ganser A. How I treat refractory and relapsed acute myeloid leukemia. Blood. 2024;143(1):11-20.
4. DiNardo CD, Rausch CR, Benton C, et al. Clinical experience with the BCL2-inhibitor venetoclax in combination therapy for relapsed and refractory acute myeloid leukemia and related myeloid malignancies. Am J Hematol. 2018;93(3):401-407.
5. Stahl M, Menghrajani K, Derkach A, et al. Clinical and molecular predictors of response and survival following venetoclax therapy in relapsed/refractory AML. Blood Adv. 2021;5(5):1552-1564.
6. Aldoss I, Yang D, Pillai R, et al. Association of leukemia genetics with response to venetoclax and hypomethylating agents in relapsed/refractory acute myeloid leukemia. Am J Hematol. 2019;94(10):E253-e255.
7. Ball BJ, Arslan S, Koller P, et al. Clinical experience with venetoclax and hypomethylating agents (HMA) in patients with newly diagnosed and relapsed or refractory KMT2A-rearranged acute myeloid leukemia (AML). Leuk Lymphoma. 2022;63(13):3232-3236.
8. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
9. Rahmani NE, Ramachandra N, Sahu S, et al. ASXL1 mutations are associated with distinct epigenomic alterations that lead to sensitivity to venetoclax and azacytidine. Blood Cancer J. 2021;11(9):157.
10. Unglaub JM, Schlenk RF, Middeke JM, et al. Venetoclax-based salvage therapy as bridge-to-transplant is feasible and effective in patients with relapsed/refractory AML. Blood Advances. 2025;9(2):375-385.
11. Döhner H, DiNardo CD, Appelbaum FR, et al. Genetic risk classification for adults with AML receiving less-intensive therapies: the 2024 ELN recommendations. Blood. 2024;144(21):2169-2173.
12. Patel SA, Cerny J, Gerber WK, et al. Prognostic heterogeneity and clonal dynamics within distinct subgroups of myelodysplastic syndrome and acute myeloid leukemia with TP53 disruptions. EJHaem. 2023;4(4):1059-1070.
13. Patel SA, Lloyd MR, Cerny J, et al. Clinico-genomic profiling and clonal dynamic modeling of TP53-aberrant myelodysplastic syndrome and acute myeloid leukemia. Leuk Lymphoma. 2021;62(14):3348-3360.
14. Gangat N, Karrar O, Iftikhar M, et al. Venetoclax and hypomethylating agent combination therapy in newly diagnosed acute myeloid leukemia: genotype signatures for response and survival among 301 consecutive patients. Am J Hematol. 2024;99(2):193-202.
15. Gangat N, Elbeih A, Ghosoun N, et al. Mayo genetic risk models for newly diagnosed acute myeloid leukemia treated with venetoclax + hypomethylating agent. Am J Hematol. 2025;100(2):260-271.
16. Gangat N, Johnson I, McCullough K, et al. Molecular predictors of response to venetoclax plus hypomethylating agent in treatment-naïve acute myeloid leukemia. Haematologica. 2022;107(10):2501-2505.