haematologica
Journal of The Ferrata Storti Foundation

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Received: August 28, 2022.
Accepted: December 5, 2022.

Citation: Naseema Gangat, Rimal Ilyas, Kristen McCullough, Kebede H. Begna, Aref Al-Kali, Mrinal M. Patnaik, Mark R. Litzow, William J. Hogan, Abhishek Mangaonkar, Hassan Alkhateeb, Mithun V. Shah, Michelle A. Elliott, James M. Foran, Talha Badar, Jeanne M. Palmer, Curtis A. Hanson, Animesh Pardanani, and Ayalew Tefferi. Predictors of response to venetoclax plus hypomethylating agent therapy and survival in blast-phase myeloproliferative neoplasm. Haematologica. 2022 Dec 15. doi: 10.3324/haematol.2022.282019 [Epub ahead of print]

Predictors of response to venetoclax plus hypomethylating agent therapy and survival in blast-phase myeloproliferative neoplasm

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Running title: Venetoclax + HMA in blast phase MPN

Key words: Hypomethylating, Myelofibrosis, Polycythemia, Thrombocythemia, *JAK2*

Text: 1781 words

Tables: 2

Figures: 1

Data sharing: please email the corresponding author

References: 16

Disclosures: None of the authors have any conflicts of interest

Author contributions: NG and AT designed the study, collected data, performed analyses and wrote the paper. RI collected and analyzed data. CH reviewed bone marrow morphology. KM, KHB, AA, MMP, MRL, WJH, AM, HA, MVS, MAE, JMF, TB, JMP, AP provided study patients. CAH reviewed bone marrow morphology. All authors reviewed the final draft of the paper.

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Myeloproliferative neoplasms (MPN) with blast phase (BP) transformation (MPN-BP) are associated with a dismal prognosis with median overall survival of 3.6 months.[1] The majority of patients are elderly and unfit for intensive chemotherapy. Venetoclax (Ven) in combination with hypomethylating agent (HMA) is FDA approved for elderly/unfit acute myeloid leukemia (AML), however MPN-BP patients were excluded from Ven+HMA clinical trials.[2] Nonetheless, therapeutic efficacy of Ven+HMA in MPN-BP has been established through retrospective studies,[3,4] with complete remission with (CR) or without count recovery (CRi) rate of 44% in a multicenter series of 32 treatment-naïve and relapsed patients with MPN-BP that received Ven plus either azacitidine or decitabine.[4] In that particular study, response was superior in the absence of polycythemia vera (PV)/post-PV myelofibrosis phenotype, complex karyotype, and *RAS* mutations.[4] Accordingly, in the current study, our main objective was to examine Ven+HMA treatment outcomes including the impact of karyotype and mutations on response and survival in an expanded cohort of MPN-BP patients treated at the Mayo Clinic outside the clinical trial setting.

The current study comprises of 47 consecutive patients with MPN-BP treated with Ven+HMA at the Mayo Clinic (Rochester MN, Arizona, Florida) between July 2018 and May 2022 and includes 27 patients from a previously published cohort with additional follow-up.[4] Study patients were retrospectively recruited after institutional review board approval. Diagnosis of MPN-BP required the presence of ≥20% blasts in either the peripheral blood or bone marrow; patients with isolated extramedullary accumulation of blasts (myeloid sarcoma) were excluded.[5] Cytogenetic and molecular studies were performed by conventional karyotype, and next-generation sequencing (NGS) of a 42-gene panel, respectively. All patients received at least one cycle of Ven+HMA, with Ven dose adjusted based on drug interactions particularly with azole antifungal prophylaxis. Azacitidine 75 mg/m2 days 1-7 or decitabine 20 mg/m2 days 1-5 was administered as part of the combination therapy. Bone marrow biopsy was obtained after either cycle 1 or 2 in the majority of cases based on treating physician discretion with response assessed according to the 2017 European Leukemia Net (ELN) criteria.[6] Determinants of treatment response were assessed by Chi-square 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 16.0.0 software package, SAS Institute, Cary, NC.

A total of 47 patients with intramedullary MPN-BP (median age 71 years, range 46-84; 60% males) received Ven+HMA either upfront or following relapse, of which 32 patients were treatment naïve and 15 were relapsed/refractory, with 8 patients having received prior HMA. Patients with relapsed/refractory disease had received either one (*n*=15), or two (*n*=4) prior therapies which included liposomal daunorubicin/cytarabine (*n*=6), "7cytarabine + 3idarubicin" (*n*=3), "5cytarabine + 2idarubicin" (n=1), cladribine (*n*=1), gemtuzumab (*n*=1), decitabine (n=1), venetoclax + cytarabine (n=1), azacitidine+ ivosidenib (n=1); second line therapies comprised of enasidenib in two patients, and FLAG-IDA, and gemtuzumab, in one patient each. Of note, two patients had relapsed following allogeneic hematopoietic stem cell transplantation (AHSCT). Antecedent MPN included ET/post-ET MF in 18 (38%), PV/post-PV MF in 16 (34%), and PMF in 13 (28%) patients. Driver mutation profile included *JAK2* in 76% of the patients and *CALR* in 18%; other mutations included *TP53* in 17 patients (39%), *TET2* in 10 (23%), *ASXL1* in 15 (34%), *IDH1/2* in 12 (27%), *EZH2* in 6 (14%), *RUNX1* in 6 (14%), *N/KRAS*, *SRSF2* and *U2AF1* in 5 (11%) each. ELN cytogenetic risk distribution was favorable (2%), intermediate (34%) or adverse (64%); among the latter, 55% were classified as complex. Table 1 lists characteristics of 47 patients with intramedullary MPN-BP, with treatment details, response rates, and overall outcome.

Thirty-one (66%) patients received decitabine and the remainder azacitidine with median Ven dose of 200 mg (range, 100-400 mg) administered for a median of 3 cycles (range, 1-9 cycles). 21 (45%) patients experienced cycle delays/interruptions, with Ven and HMA dose reductions instituted in 27(57%) and 10 (21%) patients, respectively. Pancytopenia related to therapy was noted in 29 (62%) patients and complicated by neutropenic fever in 22 (47%) cases, major hemorrhage in 1 (2%), tumor lysis syndrome in 1 (2%), while gastrointestinal toxicity and hepatic dysfunction was documented in 5 (11%) and 4 (9%) patients, respectively. Treatment was discontinued due to toxicity in 6 (13%) patients. Eleven (23%) deaths occurred within 90 days, majority (*n*=8, 73%) were unrelated to therapy.

Response was evaluable in all patients with CR and CRi documented in 20 (43%) patients; 12 (26%) patients with CR and 8 (17%) with CRi, partial response in 5 (11%) patients, resulting in an overall response rate of 53%. Residual morphological features of MPN were noted in a total of 12 patients which included 10 with CR/CRi. Among complete responders, median time to response was 1.7 months (range; 1-7 months), with median response duration of 5 months (range, 0.4-35 months). Of the 10 patients achieving CR/CRi with residual morphological features of MPN, measurable residual disease (MRD) by flow cytometry was present in 2 of 3 patients that were assessed. Presence of morphological features of MPN did not significantly impact duration of response (median 6 vs 2 months in its presence vs absence; p=0.75). Subsequent relapse was documented in 9 (45%) of responding patients. Importantly, 7 of 13 (54%) transplant-eligible patients that achieved CR/CRi, were bridged to AHSCT.

CR/CRi rates were similar between patients who received Ven+HMA upfront or in the relapsed setting (47% vs 33%; p=0.38), with azacitidine or decitabine (50% vs 39%; p=0.46) or prior HMA exposure (25% vs 46%; p=0.26). Similarly, presence or absence of *JAK2* (37% vs 55%; p=0.31), *TP53* (41% vs 44%; p=0.83*), ASXL1* (47% vs 41%, p=0.74), *IDH1/2* (50% vs 41%; p=0.58), and *K*/*NRAS* mutations (20% vs 46%; p=0.25) did not significantly impact achievement of CR/CRi. On the other hand, CR/CRi was superior among patients without vs with antecedent PV (55% vs 19%, p=0.01), with thrombocytopenia (p=0.10), presence vs absence of *TET2* mutation (70% vs 35%, p=0.05), and absence of complex including monosomal karyotype (60%, vs 29%, p=0.04). Antecedent PV clustered with complex karyotype in 11 of 15 (83%) vs 45% without antecedent PV (p=0.07). Multivariable analysis confirmed the favorable impact of *TET2* mutation (p=0.02), and absence of antecedent PV (p=0.009) on CR/CRi (Table 2). Moreover, CR/CRi rates were significantly higher in *TET2* mutated vs unmutated patients without antecedent PV (83% vs 48%) and with antecedent PV (50% vs 9%) (p=0.01).

After a median follow up of 6 months (range, 1-37 months) from initiation of Ven+HMA, 31 (66%) patients have died from progressive disease (*n*=18), infection (*n*=11), and major hemorrhage (*n*=2). Overall median survival was 7 months (range; 1-37 months) with 1/2/3-year survival rates of

28%/15%/15% and longer in transplanted patients vs those not transplanted (11 vs 6 months; p=0.04, 1 /2/3-year survival, 46%/30%/30%, vs 25%/16%/0%) (Figure 1a and b).

On univariate analysis, overall survival was superior in the absence of complex including monosomal karyotype (10 vs 5 months, p=0.003), *N/KRAS* mutations (8 vs 4 months; p=0.02), and *P53* mutations (8 vs 7 months; p= 0.08), in the presence of *IDH1/2* mutations (19 vs 7 months; p=0.07), achievement of CR/CRi (10 vs 6 months; p=0.02) and AHSCT (11 vs 6 months, p=0.04). Multivariable analysis confirmed the favorable impact on survival of absence of complex karyotype and *N/KRAS* mutations (p=0.003 and 0.03, respectively) (Table 2). Figures 1c and d highlight the superior survival observed in patients without complex karyotype, irrespective of AHSCT.

The current series, which is the largest compilation of Ven+HMA treated patients with MPN-BP, serves to expand and refine our prior observations,[3,4] and differs from other reports in terms of exclusion of venetoclax based regimens with cytarabine or cladribine and patients with MPN in accelerated phase.[7,8] The high complete response rate (43%) observed with Ven+HMA was comparable to response following intensive AML induction chemotherapy (CR/CRi 59%).[1] In a phase 2 study of ruxolitinib plus decitabine in patients with either MPN-BP or accelerated phase MPN, overall response rate was 44% (CR/CRi/partial remission (PR) of 0%, 8% and 36%, respectively) per the modified Cheson criteria.[9,10] In our study, CR/CRi rate was higher in relapsed MPN-BP than a prior MD Anderson series in which none of the patients with relapsed disease achieved CR for reasons that are not entirely clear.[7] In the particular study, treatment related adverse events (infections in 83%and intracranial hemorrhage in 19%) were also much higher likely because of the utilization of venetoclax in combination with intensive chemotherapy including cytarabine $\geq$1 g/m2 or CPX-351 in 19% of patients.[7] In another multicentre series of MPN-BP treated with venetoclax based regimens, 28% had documented infections and 19% grade 3 hemorrhage.[8] The differences in adverse event rates between our study and others are possibly a result of differences in treatment regimens. In the current study, response was superior in *TET2* mutated patients without antecedent PV. The sensitivity of *TET2* mutations to Ven+HMA is novel in the context of MPN-BP, although previously reported in a series of Ven+HMA treated relapsed/refractory AML

(*n*=90), inclusive of a small minority with MPN-BP (*n*=7).[11] Whether the aforementioned finding is a reflection of *TET2* mutations and superior response to HMA as in myelodysplastic syndromes (MDS) is unclear,[12] since historically response to HMA alone in MPN-BP has been inferior with CR/CRi rate as low as 4%.[1] The clustering of antecedent PV with complex karyotype likely accounts for the lower CR/CRi rates observed in patients with antecedent PV. The longer follow-up in our study enabled an accurate estimation of survival which was expectedly longer in patients that underwent AHSCT (median survival; 11 months, 3-year survival, 30%). In addition, survival was prolonged in patients without complex karyotype and *N/KRAS* mutations. The current study highlights the divergent effect of tumor genetics on Ven+HMA treatment response in MPN-BP and underscores the significant differences in molecular patterns of response to therapy in comparison with *de novo* AML in which responses were favorable with *NPM1*, *IDH1/2*, and *DNMT3A* mutations.[13] In addition, *ASXL1* mutations have been shown to confer sensitivity to Ven+HMA in both AML and MDS with excess blasts, unlike the case in MPN-BP.[14,15] The prognostic impact of *ASXL1* mutations in blast phase MPN differs from that in MDS and *de novo* AML as shown in our prior work in which the presence of *RUNX1* mutations but not *ASXL1* predicted inferior survival in MPN-BP.[16] In an analysis of paired chronic and blast phase samples, *ASXL1* mutations were detected only during blast phase disease in 33%,[16] which might explain the discrepancy in response rates to Ven+HMA.

Taken together, our findings which require validation, serve to identify novel subsets of patients with MPN-BP with a higher likelihood of response (*TET2* mutated without antecedent PV) and prolonged survival (absence of complex karyotype and *N/RAS* mutations) following treatment with Ven+HMA.

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Table 1. Clinical characteristics at time of leukemic transformation for 47 patients with intramedullary blast phase myeloproliferative neoplasm (MPN-BP) treated with hypomethylating agent (HMA) and venetoclax stratified by achievement of complete response (CR) or CR with incomplete count recovery (CRi).

Variables	All patients n=47	Patients in CR/CRi n=20 (43%)	Patients not in CR/CRi n=27 (57%)	*P-value*
Age in years, median (range)	71(46-84)	70 (53-81)	73(46-84)	0.35
Male, *n* (%)	28 (60)	12 (60)	16(60)	1.0
MPN type, *n* (%) - ET/ Post-ET MF - PV/ Post-PV MF - PMF	 18(38) 16(34) 13 (28)	 10(50) 3(15) 7(35)	 8(30) 13(48) 6(22)	 **0.05**
Driver mutation, *n* (%) -*JAK2* -*CALR* - *Triple negative* Mutations on NGS, *n* (%) -*TP53* -*TET2* -*ASXL1* -*IDH1/2* -*RUNX1* -*N/KRAS* -*SRSF2* -*EZH2* -*U2AF1* -*STAG2*	n=46 35(76) 8(18) 3(6) n=44 17(39) 10(23) 15(34) 12(27) 6(14) 5(11) 5(11) 6(14) 5(11) 4(9)	n=19 13(68) 4(21) 2(11) n=19 7(37) 7(37) 7(37) 6(32) 3(16) 1(5) 2(11) 4(21) 3(16) 3(16)	n=27 22(81) 4(15) 1(4) n=25 10(40) 3(12) 8(32) 6(24) 3(12) 4(16) 3(11) 2(8) 2(8) 1(4)	 0.53 0.83 **0.05** 0.74 0.58 0.72 0.24 0.88 0.21 0.42 0.17
Splenomegaly, *n* (%)	16(34)	6(30)	10(37)	0.61
Time to AML in months, median (range)	128 (1-468)	106(1-468)	133(4-404)	0.63
Hemoglobin, g/dl, median (range)	8.6(5.3-14.9)	8.5(5.3-14.9)	8.7(5.4-12.3)	0.90
Leukocyte count x 10^9/L, median (range)	6.3(1-82)	7.4(1.3-61.4)	6(1-82)	0.64
Platelet count x 10^9/L, median (range)	111(8-920)	78(8-357)	150 (15-920)	0.15
Circulating blasts %[#], median (range)	8(0-90)	4(0-49)	8(0-90)	0.78
Bone marrow blasts %[#], median (range)	31(5-90)	42(9-80)	30(5-90)	0.21
Karyotype available, *n* (%) -Normal karyotype -Complex including monosomal karyotype	n=44 12(27) 24(55)	n=19 7(37) 7(37)	n=25 5(20) 17(68)	 0.22 **0.04**
European LeukemiaNet (ELN) cytogenetic risk stratification, *n* (%) -Favorable -Intermediate -Adverse	n=44 1(2) 15(34) 28(64)	n=19 1(5) 8(42) 10(53)	n=28 0(0) 7(28) 18(72)	 0.28
Extramedullary involvement, *n* (%)	3(6)	2(10)	1(4)	0.38

ET: essential thrombocythemia, PV: polycythemia vera, PMF- primary myelofibrosis,

blast percentage was ≥20% either in the peripheral blood or bone marrow

Table 2. Predictors of complete response and survival in 47 patients with intramedullary blast phase myeloproliferative neoplasm (MPN-BP) treated with venetoclax (Ven) plus hypomethylating agent (HMA).

Variables	CR/CRi Univariate *P-value*	CR/CRi Multivariate *P-value* *Odds Ratio*	Overall survival Univariate *P-value*	Overall survival Multivariate *P-value* *HR (95% CI)*
Age	0.35		0.71	
Absence of antecedent PV	**0.01**	**0.009** 7.4	0.48	
Presence of thrombocytopenia	0.10		0.37	
Bone marrow blasts %	0.20		0.95	
Absence of Complex including monosomal karyotype	**0.04**	0.11	**0.003**	**0.003** 0.3 (0.1-0.7)
ELN adverse karyotype	0.19		**0.03**	
Presence of *TET2* mutation	**0.05**	**0.02** 7.0	0.78	
Absence of *RAS* mutation	0.25		**0.02**	**0.03** 0.3 (0.1-0.8)
Absence of *P53* mutation	0.83		**0.08**	0.75
ASXL1 mutation	0.74		0.98	
Presence of *IDH1/2* mutation	0.58		**0.07**	0.10
Presence of CR/CRi	n/a	n/a	**0.02**	0.33
Allogeneic transplantation	n/a	n/a	**0.08**	0.19

CR/CRi- complete response/CR with incomplete count recovery, PV- polycythemia vera, ELN- European LeukemiaNet, n/a- not applicable.

Figure 1 Legend

Overall survival of patients with intramedullary blast phase myeloproliferative neoplasms (MPN-BP).

a. Overall survival of 47 patients with MPN-BP treated with venetoclax plus hypomethylating agent.
b. Overall survival of 47 patients with MPN-BP treated with venetoclax plus hypomethylating agent stratified by allogeneic transplantation.
c. Overall survival of 44 patients with MPN-BP treated with venetoclax plus hypomethylating agent stratified by presence or absence of complex including monosomal karyotype.
d. Overall survival of 20 patients with MPN-BP without complex including monosomal karyotype treated with venetoclax plus hypomethylating agent stratified by allogeneic transplantation

Figure 1a.

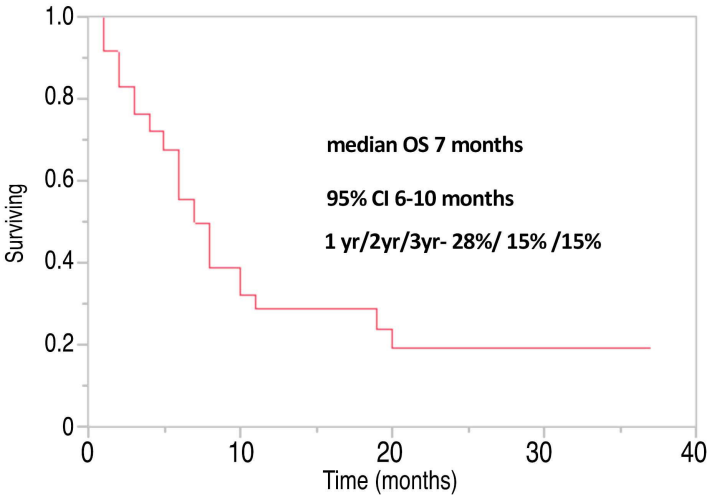

Figure 1b.

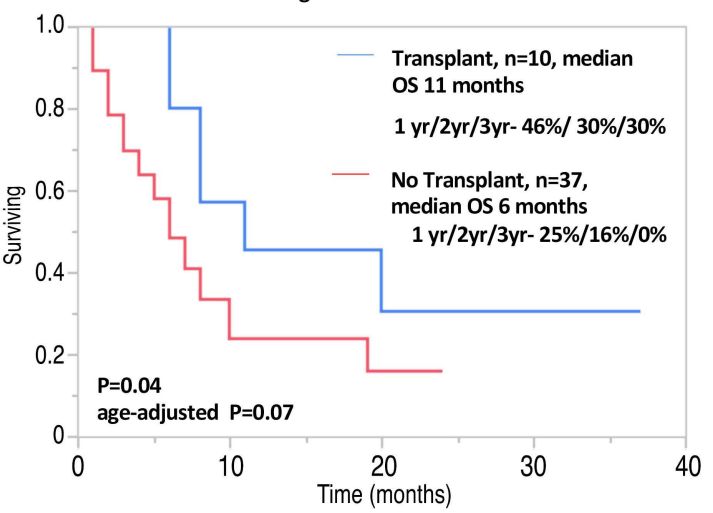

Figure 1c.

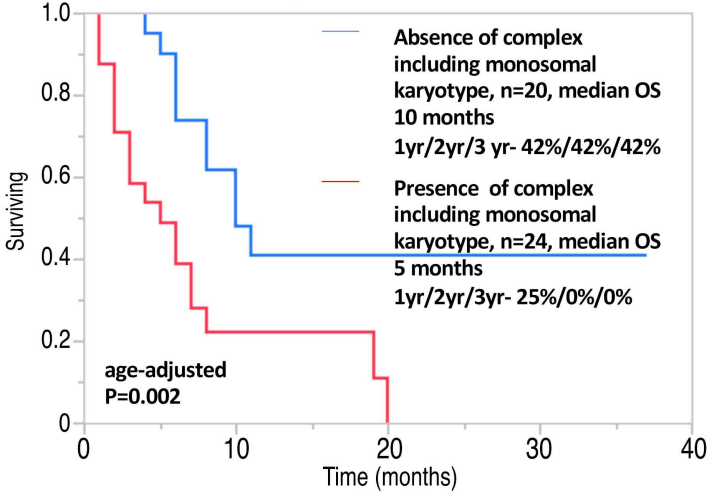

Figure 1d.