



Bone marrow findings in blast phase of polycythemia vera

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Abstract

Approximately 10% of patients with polycythemia vera (PV) transform to acute leukemia (blast phase) at 10 years after initial diagnosis of PV. The bone marrow pathologic, cytogenetic, and molecular features of blast phase have not been well characterized. In this study, we reviewed 422 PV patients over a period of 11 years and identified 58 patients who developed acute myeloid leukemia (blast phase) during the course of disease. We found that blast phase of PV was characterized by overt myelodysplasia ($n = 51$, 88%); moderate to severe myelofibrosis (33 of 45, 73%); an abnormal karyotype ($n = 51$, 88%) that was often complex karyotype ($n = 42$, 72%); and gene mutations involving *TP53* (55%), *TET2* (27%), and *DNMT3A* (25%). Patients with blast phase of PV had an aggressive clinical course, with a median overall survival of 4 months after onset of blast phase. Eleven patients had close follow-up from polycythemic phase to blast phase: Four patients showed dysplastic changes in the polycythemic phase, and three of them transformed to blast phase without a “middle phase” of post-PV myelofibrosis. We conclude that blast phase of PV is characterized by myelodysplasia, moderate to severe fibrosis, a high frequency of an abnormal and often complex karyotype, and frequent *TP53* mutation.

Keywords Polycythemia vera · Blast phase · Clinicopathologic features · Cytogenetics · Molecular mutation

Introduction

Polycythemia vera (PV) is the second most common myeloproliferative neoplasm (MPN) [1] characterized by increased red blood cell (RBC) production, somatic gain-of-function mutation of *JAK2*, and panmyelosis in the bone marrow (BM) [1–3]. The natural course of PV usually includes prepolycythemic phase, polycythemic phase (PP), and postpolycythemic myelofibrosis (post-PV MF) phase. The natural progression of PV also includes a low incidence of evolution to a myelodysplastic and/or blast phase (BP) [3]. In earlier reports, within 10 years after initial diagnosis of PV, 2.3–14.4% of patients have transformed to BP or acute myeloid leukemia (AML) with blasts $\geq 20\%$ in peripheral blood

(PB) and/or BM [4–8]. Risk factors for BP transformation reported by others include longer disease duration, older age, treatments with P^{32} or alkylating agents, high leukocyte count, BM myelofibrosis, splenomegaly, an abnormal karyotype, and somatic mutations involving *TP53*, *SRSF2*, *RUNX1*, and *IKZF1* [4, 5, 7–14]. Progression to BP is invariably associated with a poor response to treatment and shorter overall survival; the median survival after onset of BP is 3 to 6 months [15, 16].

In the revised World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissue [3], the diagnostic criteria for PV were modified substantially. These changes include lowering the hemoglobin or hematocrit thresholds of diagnosis for PV (> 16.5 g/dL or $> 49\%$ for men and > 16.0 g/dL or $> 48\%$ for women) and upgrading BM morphology (hypercellularity for age with trilineage growth) from a minor to major criterion. Until now, the pathologic features of the blast phase of PV have been reported rarely [13, 17]. Others have shown that patients with higher grade myelofibrosis (\geq MF-1) in the polycythemic phase (PP) have a higher risk of disease progression and transformation to AML [14, 18]. Morphologic features of a myelodysplasia also have been observed in a subset of PV patients during the

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course of disease [8], and morphologically, these changes may resemble a myelodysplastic syndrome (MDS) or MDS/MPN. However, little is known regarding the potential prognostic impact or biology of myelodysplasia or myelofibrosis in blast phase of PV. It is not clear whether development of morphologic evidence of dysplasia is associated with transformation of PV to BP.

Most cases of acute leukemia that arise in patients with PV are AML. In the current WHO classification [3], though blast phase was used to describe this stage, various terms have been used in BM diagnosis, such as blast phase of PV, AML evolving from PV, secondary AML, or simply AML, not otherwise specified. It is clinically important to render a consistent and biologically accurate diagnosis for the BP of PV, and having a better understanding of the clinicopathologic, cytogenetic, and molecular genetic features of this neoplasm is therefore required for this purpose [19].

In this study, we retrospectively reviewed 422 PV patients and identified 58 patients who developed AML (blast phase) during the course of disease. We summarize the clinicopathologic, cytogenetic, and molecular genetic features of blast phase of PV. In addition, 11 patients underwent multiple bone marrow examinations from the polycythemic phase to blast phase. This subset of patients allowed us to better study the dynamic changes during the course of PV and analyze for possible factors that contribute to transformation into blast phase.

Materials and methods

Patients

We searched the archives of The University of Texas MD Anderson Cancer Center for patients with PV who were diagnosed and/or managed at this institution from January 1, 2005 through June 30, 2016. A detailed chart review was conducted for all patients and the following data were collected: clinical presentation, treatment history, clinical outcomes, laboratory data, and pathological findings. Blasts were counted on PB smears based on a 200-cell differential count and on BM smears based on a 500-cell differential count. Blast phase was defined as $\geq 20\%$ myeloblasts in PB or BM or both. This study was approved by the Institutional Review Board of MDACC.

Histological assessment

Wright-Giemsa stained PB and BM aspirate smears and H&E stained bone marrow aspirate clot and core biopsy tissue sections were reviewed. The PB smear blast percentage and BM cellularity, blast percentage, and

morphologic dysplasia were evaluated. Granulocytic dysplasia includes hypogranulation, nuclear hypolobation, or hypersegmentation; erythroid dysplasia includes nuclear budding or hyperlobation, ring sideroblasts, or cytoplasmic vacuolization; and megakaryocytic dysplasia includes micromegakaryocytes, nuclear hypolobation, widely separated nuclear lobes, or atypical clusters. In this study, only “overt dysplasia” was considered if dysplasia involved two or more lineages or $> 50\%$ cells if only one lineage was involved. Myelofibrosis was evaluated by reticulin and trichrome stains performed on the BM core biopsy in all cases with core biopsy available. The grade of myelofibrosis was based on the European Consensus on grading of BM fibrosis [20].

Cytogenetics

Conventional chromosomal analysis was performed on all patients, and G-banded metaphases were prepared from unstimulated 24- and 48-h BM aspirate cultures using standard techniques. Twentymetaphases were analyzed, and the results were reported using the 2016 International System for Human Cytogenetics Nomenclature (ISCN 2016) [21]. A complex karyotype was defined as ≥ 3 chromosomal abnormalities.

Mutational analysis

Molecular analysis was performed in most patients as a part of the routine clinical work-up. *JAK2 V617F* and codon 12 mutations were evaluated in all patients at time of initial diagnosis and in most patients in BP. In addition, *NPM1* (exon 12), *KIT* (exons 8 and 17), *CEBPA*, *FLT3* (internal tandem duplication and *D835* point mutation), and *IDH1* and *IDH2* mutations were analyzed at the time of BP transformation. Next-generation sequencing (NGS) for somatic mutations was performed in 11 patients at the time of BP progression as described previously [22]. To identify pathways involved in BP progression, genes were grouped by functional classification as defined by The Cancer Genome Atlas Research Network [23].

Statistical analysis

Frequencies and percentages were calculated for categorical variables, and medians (range) were calculated for continuous variables. Overall survival was calculated from the date of transformation to BP to the date of death or censored at the date of last follow-up for surviving patients. *p* value of < 0.05 was considered statistically significant.

Results

Patients and clinical features

From a group of 422 patients with PV, 58 (14%) patients progressed to AML and formed the study group. There were 30 men and 28 women, with a median age 67 years (range, 32–82 years) at the time of onset of BP. The median interval from initial diagnosis of PV to onset of BP was 109 months (range, 9–283 months). The first BM biopsy was performed at a median of 95 months (range, 0–167 months) after initial diagnosis of PV. At the time of first BM evaluation, 11 patients had PP, 13 had post-PV MF, 6 had accelerated phase (AP), and 28 had BP (Fig. 1a). For the 28 patients who showed BP in the first BM specimen available for our review, all these patients had an established diagnosis of PV at another hospital before they were referred to our institution. For the other 30 patients, the median interval from first BM evaluation to BP was 53 months (range, 4–137) in patients with PP, 12.5 months (range, 1–48) in patients with post-PV MF, and 7 months (range, 1–24) in patients with AP.

Prior to development of BP, 30 patients had splenomegaly, 26 had constitutional symptoms (weight loss, night sweats, and/or fever), and 10 patients had a history of thrombotic events. Prior to onset of BP, 3 patients were treated by phlebotomy only and 53 patients received various therapeutic agents, including hydroxyurea ($n = 44$), ruxolitinib ($n = 5$), interferon alpha ($n = 3$), aspirin or coumadin or Plavix ($n = 4$), and P^{32} ($n = 1$). The therapeutic history was unclear in 3 patients.

Laboratory and pathological findings

At time of onset of BP, the complete blood count showed a median hemoglobin level of 9.4 g/dL (range, 6.8–11.1; normal 14–18 g/dL for men and 12–16 g/dL for women), leukocyte count $9.7 \times 10^9/L$ (range, 4.8–118.3; normal $4–11 \times 10^9/L$), platelet count $44 \times 10^9/L$ (range, 7–724; normal, $150–440 \times 10^9/L$), and blast percentage of 18% (range, 8–78%) (Table 1).

BM blast counts ranged from 14 to 70% (median 30%). In the cases with blast counts $< 20\%$ in BM, blast counts were $\geq 20\%$ in the PB. Based on WHO classification

Fig. 1 a Disease stage of polycythemia vera at the first bone marrow evaluation and the number of patients who progressed to blast phases by the end of follow-up. **b** The risk of acute leukemia transformation is significantly higher in patients with myelodysplasia comparing to that in patients without myelodysplasia at polycythemic phase (20 vs. 5.6%, $p = 0.0453$)

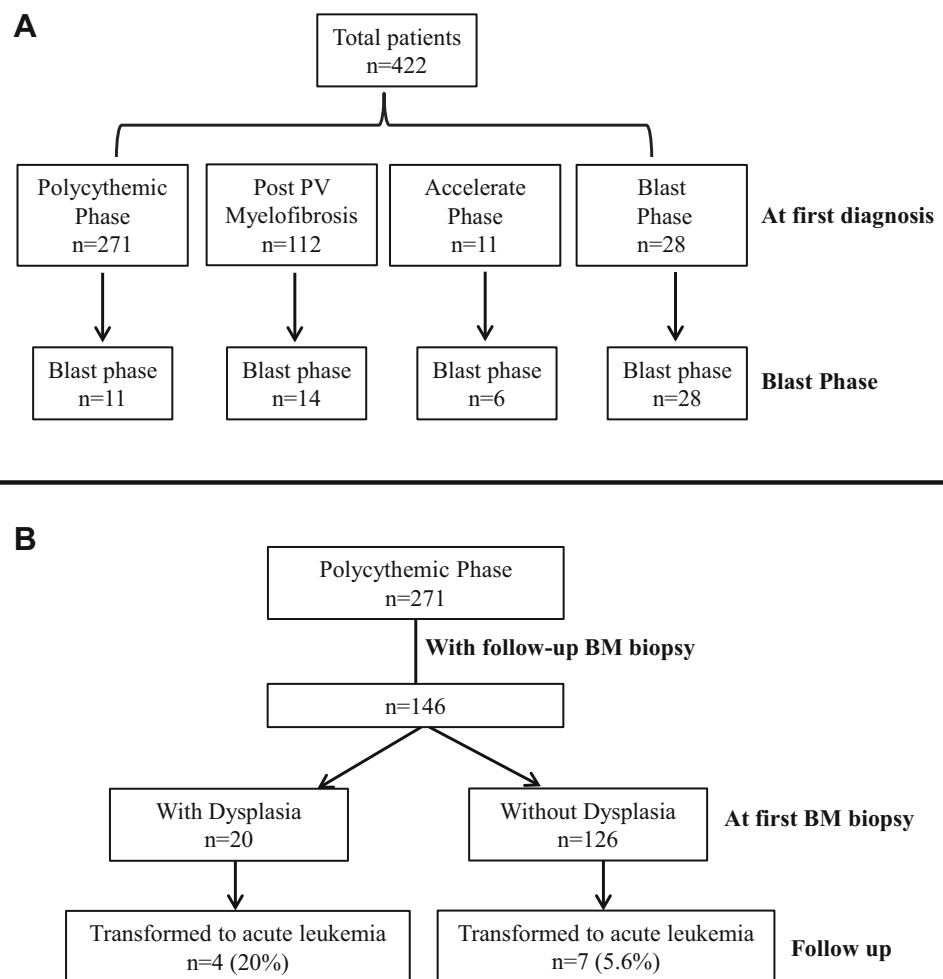


Table 1 Clinical and pathological features of patients with polycythemia vera in blast phase

Characteristic	Number (range or %)
Age (years)*	67 (32–82)
Male/female	30/28
Constitutional symptoms	26 (44%)
Pruritus	4 (7%)
Prior history of thrombosis	10 (17%)
Splenomegaly	30 (51%)
Hemoglobin (g/dL)*	9.4 (6.8–11.1)
Leukocyte ($\times 10^9/L$)*	9.7 (4.8–118.3)
Platelets ($\times 10^9/L$)*	44 (7–724)
Peripheral blood blast count (%)*	18 (8–78)
Bone marrow blast count (%)*	30 (14–70)
Myelofibrosis	(<i>N</i> = 45)
• MF-0	5 (11%)
• MF-1	7 (16%)
• MF-2/3	33 (73%)
Dysplastic changes	51 (88%)
Karyotype	
• Diploid karyotype	6 (10%)
• Abnormal karyotype	52 (90%)
- Complex	42 (71%)
- Non-complex	10 (19%)
AML subtype (WHO)	
• AML with MRC	48
• AML with inv(3)	3
• AML with maturation	3
• AML with t(9;11)	1
• AML with minimal differentiation	1
• Acute monocytic leukemia	1
• Acute erythroid leukemia	1

AML, acute myeloid leukemia; MRC myelodysplasia-related changes

*Presented as median (range)

criteria [3], the acute leukemia was classified as follows: AML with myelodysplasia-related changes (AML-MRC) (*n* = 48), AML with inv(3)(q21.3q26.2)/*MECOM* rearrangement (*n* = 3), AML with maturation (*n* = 3), AML with t(9;11)(p22;q23)/*MLLT3-MLL* rearrangement (*n* = 1), AML with minimal differentiation (*n* = 1), acute myelomonocytic leukemia (AMML, *n* = 1), and acute erythroid leukemia (*n* = 1).

Overt dysplasia was observed in 51 (88%) patients including 16 with trilineage, 24 with bilineage, and 11 with unilineage dysplasia. Granulocytic dysplasia was observed in 34 patients, erythroid dysplasia in 34 patients, and megakaryocytic dysplasia in 39 patients. There were 7 patients without overt dysplasia; 2 had a normal karyotype, 3 had single karyotypic abnormality (+13, +11, t(5;10)(q33;q21), respectively), and 2 had trisomy 1q in a complex karyotype. None of

these 7 patients showed abnormalities involving chromosomes of 5, 7, or 17.

Reticulin and trichrome stains were performed on BM biopsy specimens of 45 (76%) patients to evaluate the grade of myelofibrosis. Moderate to severe myelofibrosis (MF-2 or MF-3) was detected in 33 (73%) patients, MF-1 in 7 (16%) patients, and MF-0 in 5 (11%) patients. Among the group of 12 patients who had MF-0/1, 8 patients had BP involving the first BM specimen reviewed by us at our institution; 2 patients showed 3 distinct stages including PP, post-PV MF (with MF-2), and BP; and 2 patients transformed to BP directly from PP stage. All 12 patients showed overt dysplasia at time of BP.

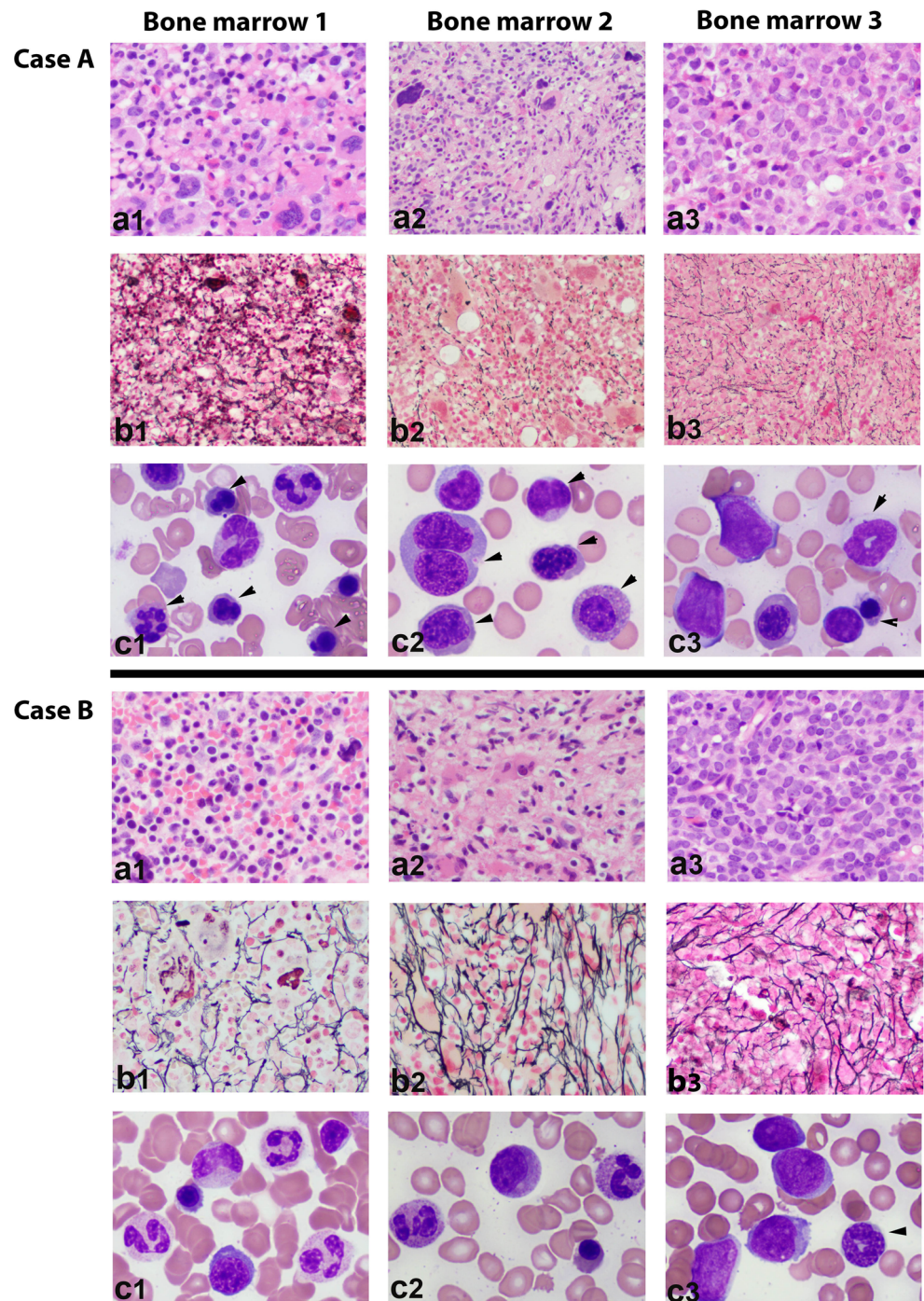
We evaluated the association between myelodysplasia developing in polycythemic phase and the risk of transformation to BP. As shown in Fig. 1b, of 271 patients who had a BM specimen that showed the PP stage of PV, 146 patients had follow-up BM evaluation. Twenty of these 146 (14%) patients showed dysplastic changes and 4 (20%) subsequently transformed to BP. Of note, none of these 4 patients had blast counts $\geq 5\%$ in PB or BM at the time when morphologic dysplasia was detected. By contrast, of the other 126 patients in whom the BM did not show dysplastic changes, 7 (5.6%) transformed to BP. The difference between these two groups was significant ($p = 0.0453$). In addition, these 20 patients with dysplasia showed a higher frequency of abnormal karyotype (9/20 vs. 28/126, $p = 0.0492$), an inferior overall survival (median 126 vs. 169 months from the first BM diagnosis, $p = 0.0332$), but comparable value in hemoglobin level, white blood cell, and platelet counts compared to the 126 patients without dysplasia.

Sequential BM biopsies from PP to BP were available in 11 patients. In polycythemic phase, overt dysplasia was detected in three patients, moderate fibrosis (MF-2) in two patients, overt dysplasia plus MF-2 in one patient, and the remaining five patients did not show dysplasia or myelofibrosis. Of the four patients (including the one with MF-2) who showed overt dysplasia, all had an abnormal karyotype at the first karyotyping analysis; three of four did not show a distinct stage of post-PV MF and progressed to BP from PP stage directly (Fig. 2, patient A). Of the two patients with MF-2 without dysplasia at first BM biopsy, both cases progressed to post-PV MF and then BP (Fig. 2, patient B).

Cytogenetic analysis

For the 58 patients in the study, 6 (10%) had a normal karyotype and 52 (90%) had an abnormal karyotype at the onset of BP. The latter included a complex karyotype (*n* = 42, 72%), double abnormalities (*n* = 4), and a single abnormality (*n* = 6). The most common abnormalities included $-7/del7q$ (*n* = 26), $-5/del5q$ (*n* = 21), $-17/del17p$ (*n* = 14), $+21$ (*n* = 10), trisomy 1q (*n* = 10), and -18 (*n* = 9); all of these abnormalities were parts of complex karyotype. Three patients showed

Fig.2 Sequential bone marrow morphologic evolution from polycythemic phase to blast phase. **a** Bone marrow core biopsy ($\times 40$), **b** reticulum stain ($\times 40$), and **c** bone marrow aspirate smear ($\times 100$). Case A showed overt myelodysplasia but no or mild myelofibrosis in polycythemic phase and all follow-up bone marrow biopsies. Bone marrow 1: polycythemic phase (the first biopsy, 0 months); bone marrow 2: accelerated phase with 12% blasts (biopsy at 2 years); bone marrow 3: blast phase with 38% blasts (biopsy at 2 years plus 4 months). Case B showed moderate to marked myelofibrosis in the polycythemic phase and all follow-up bone marrow biopsies, and myelodysplasia was only observed at blast phase. Bone marrow 1: polycythemic phase (the first biopsy, 0 months); bone marrow 2: post-polycythemic myelofibrosis (biopsy at 5 years plus 2 months); bone marrow 3: blast phase with 66% of blasts (biopsy at 6 years plus 6 months). Cells with morphologic dysplasia are marked with arrow head



inv(3)(q21q26), one as sole abnormality and the other two as part of a complex karyotype. One patient showed t(9;11)(p22;q23).

For 19 patients with available data, the karyotypes before and after onset of BP were compared (Table 2). Prior to BP, 11 patients had a normal karyotype, 1 had isolated del(20q), and 7 had a complex karyotype. At the onset of BP, 10 patients

gained new clones; 4 showed clonal evolution; and in 5 patients, the karyotype was unchanged.

Molecular mutations

All 58 patients showed a *JAK2* mutation prior to BP transformation. At the time of BP, 54 patients underwent molecular studies to assess for gene mutations, either by the next-generation sequencing (NGS, $n = 11$) or quantitative PCR of

Table 2 Comparison of the cytogenetic abnormalities at pre-blast phase and blast phase

	Karyotype at pre-blast phase	Karyotype at blast phase	Summary
Polycythemic phase <i>N</i> = 8	Normal diploid	44,XX,-4,del(5)(q13q33),-7,-13,add(16)(p13.3),-19,-20,+22,+2mar[14]/ 44,X,add(X)(p22.3),-4,del(5)(q13q33),-7,-13,-20,+2mar[4]	New clone
	Normal diploid	47,XY,+1,der(1;7)(q10;p10),+21[19]/49,idem,+8,+15[1]	New clone
	Normal diploid	46,XY[20]	No change
	Normal diploid	47,XX,+1,add(1)(p11.2)[20]	New clone
	46,XY,del(20)(q11.2;q13.3)[8]/46,XY[12]	46,XY,del(20)(q11.2;q13.3)[1]/45,XY,-2,del(3)(p14),-5,del(7)(q22q36), add(10)(p11.2), add(12)(p13),-15,del(17)(p12p13),+21,+mar[19] 47,XY,+1,der(1;7)(q10;p10),+21[19]/46,XY[1]	New clone
Post-PV myelofibrosis <i>N</i> = 11	47,XY,+1,der(1;7)(q10;p10),+21[5]/46,XY[15]	add(12)(p13),-15,del(17)(p12p13),+21,+mar[19] 47,XY,+1,der(1;7)(q10;p10),+21[19]/46,XY[1]	No change
	45,XX,i(3;5)(q12;q33),-16,del(17)(p11.2), der(20)t(3;20)(q25;q11.2)[18]/46,XX[2]	44-45,XX,add(5)(q11.2),i(3;5)(q12;q33),-7,add(9)(p23),-10,-16, del(17)(p11.2),der(20)t(3;20)(q25;q11.2),+mar[cp15]/46,XX[5]	Clonal evolution
	43,X,-X,inv.(3)(q13.3q26.3),del(5)(q22q35),add(6)(p21.1),-7,-17,-19,+mar[8]/ mar[5]/46,XX[13]	43,X,-X,inv.(3)(q13.3q26.3),del(5)(q22q35),add(6)(p21.1),-7,-17,-19,+mar[8]/ 43, idem, del(11)(p11.2)[3]/44, idem, i(5)(q10), +mar[cp5]/46,XX[4]	Clonal evolution
	Normal diploid	44,XY,del(5)(q12q35),der(7;12)(p10;q10),-10,-17,add(17)(p13),+mar[17]/ 44, idem, add(18)(p11.3)[3]	New clone
	Normal diploid	46,XY,del(13)(q12q22)[2]/47,XY,+21,i(21)(q10)×2[12]/46,XY[6]	New clone
	Normal diploid	45,XX,del(2)(p13),i(3)(q10),-5,add(7)(q22), del(9)(q21), add(10)(q22),del(11)(p14), -18,-20,+2mar[6]	New clone
	Normal diploid	/45-46, idem, add(5)(q13),add(12)(p11.2),del(13)(q12q22),+2-4mar[cp11] /46,XX[3]	No change
	Normal diploid	46,XX[20]	No change
	Normal diploid	46,XX,+13[9]/46,XX[11]	New clone
	Normal diploid	46,XY,t(2;14)(q22;q12),del(9)(p22)[11]	New clone
47,XY,del(20)(q11.2;q13.3),+21[12]/ 48, idem,+8,+del(20)(q11.2;q13.3),-21[8]	47,XY,del(20)(q11.2;q13.3),+21[12]/48, idem,+8,+del(20)(q11.2;q13.3),-21[8]	Clonal evolution	
46,XX,+1,der(1;7)(q10;q10)[12]/ 47, idem,+21[5]/46,XX[3]	46,XX,+1,der(1;7)(q10;p10)[1]/46, idem, add(12)(p13)[17]/46-47, idem[cp2]	Clonal evolution	
46,XX,ins(12)(p13q24.1q24.3)[1]/ 46, idem,-4,+mar[cp2]/46,XX[16]	46,XX,der(6)t(1;6)(q23;p22),der(12)del(q22q24.1)inv.(12)(p13q22)[2]/49,XX,+ 6,der(6)t(1;6)(q23;p22)×2,+9,+21[18]	New clone	
43,XY,add(4)(p14),der(5;12)(p10;q10),-10, add(10)(q26),-12, del(13)(q12q22), der(14;17)(q10;q10),-18,+2mar[20]	43,XY,add(4)(p14),der(5;12)(p10;q10),-10,add(10)(q26),-12,del(13)(q12q22), der(14;17)(q10;q10),-18,+2mar[18]/46,XY[2]	No change	

selected genes ($n = 43$). *JAK2* mutation status at BP stage was available for 41 patients: 36 (88%) patients had *JAK2* mutation, including 35 with V617F (with a median allele burden of 64.1%) and 1 had exon 12 mutation; 2 (5%) patients did not have detectable *JAK2* mutation; and 3 patients had *JAK2* V617F mutations, but the mutant *JAK2* was not consistent with AML status; there was a high allelic burden of mutant *JAK2* when AML was in remission, or there was a low mutant *JAK2* allele burden when the blast count was high.

Other molecular mutations were also detected in a subset of patients, including *TP53* (6/11, 55%), *TET2* (3/11, 27%), *DNMT3A* (3/12, 25%), *IDH1* (4/20, 20%), *RUNX1* (2/11, 18%), *PTPN11* (2/11, 18%), *EZH2* (2/11, 18%), *NRAS* (2/42, 5%), *KIT* (1/23, 4.3%), *FLT3* (1/48, 2%), and *NPM1* (1/11, 9%) (Table 3).

We were unable to compare the mutation profile before and after BP transformation in the same group of patients because there were only a few patients that underwent NGS analysis more than once during the course of disease. For this reason, we compared the mutation frequencies among the patients in BP and 83 patients in PP/post-PV MF stage and who never transformed to BP during the follow-up (Table 3). In general, mutations were more frequent in BP than those in the PP or post-PV MF phases, especially for *TP53* (55 vs. 1.2%, $p < 0.0001$), *DNMT3A* (25 vs. 4%, $p = 0.0410$), *PTPN11* (18 vs. 0%, $p = 0.0126$), *IDH1* (20 vs. 5%, $p = 0.0440$), *EZH2* (18 vs. 1.2%, $p = 0.0353$), and *RUNX1* (18 vs. 1.2%, $p = 0.0353$). Among 11 patients who had NGS at time of BP (28 gene

panel), all patients had at least one mutated gene in addition to *JAK2* mutation, and 5 patients showed more than 4 gene mutations.

Treatments and outcomes

After BP transformation, 22 patients were enrolled in clinical trials: 17 on a protocol that included hypomethylating agents and 5 on a protocol that included ruxolitinib. In addition, 12 patients received standard induction chemotherapy (“7 + 3 regimen”); 1 patient received FLAG-IDA; 10 patients had initial monotherapy with a hypomethylating agent; and 12 patients went to palliative care. Three patients also received allogeneic stem cell transplant. Two patients were lost to follow-up and their treatments are unknown. At time of the last follow-up, 53 (91%) patients were dead with a median overall survival of 4 months (Fig. 3). The overall survival was not significantly different among patients with MF 0/1 and patients with MF 2/3 (6.5 vs. 4 months, $p = 0.6237$).

Discussion

In this study, we have shown that patients with blast phase of PV have a high frequency of myelodysplasia, moderate to severe BM fibrosis, an abnormal and often complex karyotype, and gene mutations, particularly *TP53*.

During the polycythemic phase of PV, patients have an increased red blood cell (RBC) mass, and the BM is typically characterized by hypercellularity and panmyelosis. When the disease progresses to post-PV MF phase, BM cellularity and erythropoiesis decrease, leading to a decreased RBC mass. In the blast phase of PV, patients develop acute myeloid leukemia [17, 24, 25]. Based on the presence of overt dysplasia and a complex karyotype, most patients in this cohort belong to the category of AML-MRC as defined in the WHO classification. The high frequency of an abnormal karyotype or a complex karyotype, *TP53* mutation, and overt dysplasia indicates that the BP of PV is usually a high-grade AML similar to therapy-related AML [26]. In our cohort, only 1 of 58 patients developed acute erythroid leukemia, which is similar to the

Table 3 Comparison the frequency of molecular mutations among patients who at polycythemic phase/post-PV myelofibrosis and patients who at blast phase. Group A: patients at polycythemic phase/post-PV myelofibrosis ($n = 83$). Group B: patients with blast phase

Genes	Group A		Group B		<i>p</i>
	Total	Positive	Total	Positive	
<i>JAK2</i>	83	83 (100%)	42	36 (88%)	0.0005
<i>TP53</i>	83	1 (1.2%)	11	6 (55%)	<0.0001
<i>TET2</i>	83	21 (25%)	11	3 (27%)	1.0000
<i>DNMT3A</i>	83	4 (5%)	12	3 (25%)	0.0410
<i>IDH1</i>	83	4 (5%)	20	4 (20%)	0.0440
<i>PTPN11</i>	83	0	11	2 (18%)	0.0126
<i>ASXL1</i>	83	11 (13%)	11	2 (18%)	0.6463
<i>EZH2</i>	83	1 (1.2%)	11	2 (18%)	0.0353
<i>RUNX1</i>	83	1 (1.2%)	11	2 (18%)	0.0353
<i>NPM1</i>	83	0	11	1 (9%)	0.1170
<i>IDH2</i>	83	5 (6%)	11	1 (9%)	0.5364
<i>NRAS</i>	83	0	42	2 (5%)	0.1111
<i>KIT</i>	83	2 (2.4%)	23	1 (4.3%)	0.5237
<i>FLT3</i>	83	1 (1.2%)	48	1 (2%)	1.0000
<i>CEBPA</i>	83	1 (1.2%)	11	0 (0%)	1.0000

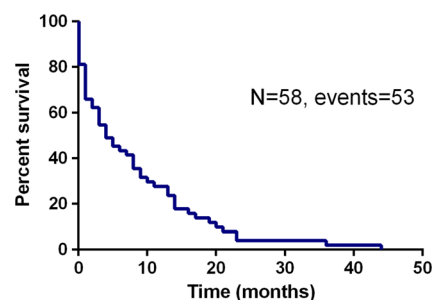


Fig. 3 Overall survival from blast phase transformation, with a median overall survival of 4 months

study by Passamonti et al. [17], in which only 1 out of 23 patients developed erythroid leukemia. However, in a study by Weinfeld et al. [13], 3 out of 8 patients developed erythroid leukemia.

In de novo AML, reticulin fibrosis is observed frequently, but usually is present to a mild or moderate degree, with the exception of acute megakaryoblastic leukemia in which fibrosis is often severe [27]. In this cohort, 33 (73%) patients showed moderate to severe fibrosis (MF-2/3). The interval from initial diagnosis to BP was slightly shorter in 12 (27%) patients with MF 0/1 compared with patients with MF 2/3 (median 94 vs. 129 months, $p = 0.1054$). Interestingly, all 12 patients with MF 0/1 showed dysplastic changes. In a previous study, we showed that a higher grade myelofibrosis (\geq MF-1) in PP stage was associated with a higher frequency of disease progression and inferior overall survival [14].

Morphologic dysplasia has been studied infrequently in PV patients. Hydroxyurea could cause erythrocytes megaloblastoid changes, which may mimic dysplastic changes. However, hydroxyurea often affects erythrocytes only, granulocytes and megakaryocytes are often spared. Besides, the effect of hydroxyurea is often transient, erythrocytes morphology restores to normal after stopping the use of hydroxyurea. Development of MDS-type PV (morphologically resembling MDS/MPN) has been reported to be related to exposure to high-dose P^{32} and alkylator agents [8], but only one patient in this study cohort received P^{32} treatment. In this cohort, of 11 patients who had PP phase at time of initial BM evaluation, 4 showed overt dysplasia and all 4 patients had an abnormal karyotype. Of interest, 3 of 4 patients progressed to BP without going through a “middle phase” of post-PV MF. We found that patients with myelodysplasia detected at polycythemic phase had a significantly higher risk for BP transformation, higher frequency of abnormal karyotype, and inferior survival, compared to patients without myelodysplasia. These findings indicate that overt dysplasia or MDS-like BM changes that develop during the course of PV are adverse features, similar to significant BM fibrosis and should be considered as one form of progression that may herald impending BP. Morphologic dysplasia was a prominent feature in most patients at time of BP, and development of myelodysplasia was closely correlated with chromosomal abnormalities, especially abnormalities involving chromosomes 5, 7, and 17.

In contrast to the low frequency of an abnormal karyotype in PP, 90% of patients in BP had an abnormal karyotype and about 70% of patients showed a complex karyotype, in line with previous studies [14, 17, 28]. The most commonly detected chromosomal abnormalities in BP were 7/del(7q), -5/del(5q), and -17/del(17p); these abnormalities were detected infrequently in PP of PV. Clonal evolution or acquisition of new clone(s) was very common in PV, in almost 75% of patients during BP

transformation in this cohort, which supports the concept that acquisition of cytogenetic abnormalities plays a critical role in progression of PV to BP [14]. Interestingly, 4 patients acquired recurrent translocations, 3 with inv(3)/MECOM rearrangement and 1 with t(9;11)/*MLL3-MLL* rearrangement; these abnormalities have not been reported in PV previously. A complex karyotype is a high risk factor in PV and AML [14, 29].

A higher mutant *JAK2* V617F allele burden has been associated with pruritus and post-PV MF in PV patients but does not appear to affect transformation to BP [30]. In this cohort, *JAK2* mutation was detected persistently at BP stage in 88% patients; the other 12% of patients had no detectable *JAK2* or a low percentage of mutated *JAK2* while blast counts were high. These findings suggest that the myeloblastic clone in BP is derived from the original PV clone in most patients. This is different from the report by Theocharides et al. that included 17 patients with *JAK2*-positive MPN (7 PV, 7 essential thrombocythemia, and 2 primary myelofibroses). In their study, 5 (29%) patients developed *JAK2*-positive AML, 9 (53%) developed *JAK2*-negative AML, and 3 (18%) were uninformative [31]. These results suggest that *JAK2*-negative BP may arise from a cell ancestral to the *JAK2* mutation or represents another independent clone arising from a different progenitor [31]. This phenomenon also has been observed in patients with chronic myeloid leukemia [32].

In this cohort, 55% of PV patients showed *TP53* mutation and 24% of patients showed del(17p)/-17 with *TP53* deletion. *TP53* mutation therefore seems to be detected more frequently in BP of PV than in patients with therapy related-AML (33.3%) or de novo AML (~14.5%) [33]. Loss of *TP53* is sufficient to induce leukemic transformation in *JAK2* V617F-driven PV in a murine model [34]. The presence of *TP53* mutation at time of diagnosis of PV has been associated with BP progression [35–37]. *TP53* mutation is also often associated with a poor response to treatment and shorter overall survival [38]. All of these data suggest that acquisition of *TP53* mutation plays an important role for BP transformation and an aggressive disease course in PV patients. Other than *TP53* mutation, *DNMT3A*, *IDH1*, *PTPN11*, *EZH2*, and *RUNX1* were also more frequently mutated in BP, and these mutated genes also may have prognostic significance [36].

In conclusion, the blast phase of PV is characterized by frequent morphologic evidence of dysplasia, myelofibrosis, an abnormal/complex karyotype, frequent gene mutations, and an aggressive clinical course.

Compliance with ethical standards This study was approved by the Institutional Review Board of MDACC.

Conflict of interest The authors declare that they have no conflict of interest.

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