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Original Contribution

Ring sideroblasts in chronic phase of polycythemia vera identifies a subset of patients with an increased risk of progression to blast phase

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ABSTRACT

Blast phase of PV is often associated with a complex karyotype (CK) and bilineage dysplasia. We hypothesized that BM morphologic abnormalities detected in the Chronic phase (CP) can identify patients with an increased risk of developing blast phase (BP). We also compared cases of BP PV to a group of acute myeloid leukemia cases with *JAK2* mutation (AML-JAK2mut).

We collected morphological, cytogenetics (CG), and molecular information at the time of diagnosis and at time of diagnosis of BP. We evaluate the presence of splicing factor mutations at BP.

A total of 60/477 (12.5%) patients with diagnosis of BP of PV were identified, 17 of them had BM sample available during CP. Ten patients with PV CP were used as control group. We found that dyserythropoiesis during evolution were more frequent in patients who develop BP than in patients who remain in CP (13/17 vs. 3/10; $P = .0402$). Similarly, ring sideroblast (RS) increase during CP were more frequent in patients who develop BP (8/16 vs. 0/10. $P = .0095$). By ELN risk stratification for CG risk in BP all patients had adverse or intermediate risk; in AML-JAK2mut 2/11 patients (18%) had favorable as risk category. *TP53* mutations were significantly more frequent in BP than in AML-JAK2mut (7/14 vs. 1/11, $P = .0421$). Mutation analysis for splicing factor at BP was performed on 13 patients. Only 2 patients with > 15% RS had *SRSF2* (2 patients) and *SF3B1* (1 patient) mutations. The other patients were wild type.

Dyserythropoiesis and the acquisition of RS precede other markers of disease progression to BP. CK and *TP53* mutation are more frequent in BP than in AML-JAK2mut. *SF3B1* mutations are rare in BP.

1. Background

Patients with myeloproliferative neoplasms (MPN) have an increased risk of progression to blast phase/acute leukemia. In 2013, Tefferi and colleagues developed a risk score for MPN patients based on patient age, leukocyte count, and the presence of venous thrombosis [1]. More recently, a personalized prognosis model of MPN was proposed that integrated 63 clinical and genomic variables and identified 8 genomic subgroups with different risks of leukemic transformation and event-free survival [2]. However, only 17% of patients included in this study had polycythemia vera (PV), and in this model extensive molecular studies are required to classify PV patients into different risk categories. Interestingly, the study also described patients with spliceosome mutations in the second-highest risk group [2]. In 2017, we reported that blast phase is often associated with a complex karyotype and bilineage dysplasia, the latter usually including dyserythropoiesis [3]. We also showed that presence of clonal evolution or acquisition of new cytogenetic clone(s) in CP plays a role in disease progression [4]. The aim of this study is to address the hypothesis that bone marrow (BM) morphologic abnormalities and cytogenetic/molecular changes detected in the CP can identify patients with an increased risk of developing blast phase (BP). We also compared cases of BP PV to a group of acute myeloid leukemia (AML) cases with JAK2 mutation.

2. Materials and methods

2.1. Study group

We searched the database of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center from 2004 to 2018

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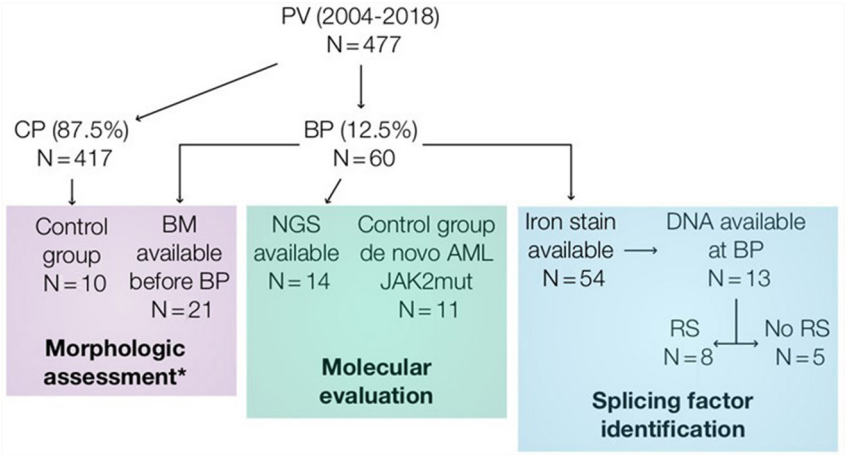

Fig. 1. Patients distribution and control groups.
AML: Acute Myeloid Leukemia, BP: Blastic Phase, BM: bone Marrow, CP: Chronic Phase, NGS: Next Generation Sequencing, PV: Polycythemia Vera, SF: splicing factors. *Della Porta Score was used for morphologic assessment.

for cases of Polycythemia Vera. The study was conducted under an Institutional Review Board-approved protocol. All patients signed a consent form before enrollment in accordance with the Declaration of Helsinki.

From a group of 477 patients with PV diagnosis who came to our center we identified 60 patients who developed BP. Based on samples availability, we performed different analysis (Fig. 1). Morphologic assessment in purple was performed in 21 of 60 patients who developed BP and had BM samples available before the BP. For statistical analysis we selected a control group of 10 patients matched by age, sex and time in follow up that remained in CP. Molecular evaluation, in green, was performed in 14 of 60 patients who developed BP and had next generation sequencing (NGS) panel done in BP BM samples. Eleven patients diagnosed with de novo AML with *JAK2* mutation were used as control group. Splicing factor identification in blue, was performed in patients with iron stain available at BP evolution (54 patients). Thirteen patients had DNA available for SF identification at BP, 8 patients had increased RS and an internal control group of 5 patients without RS. We collected demographic, clinical, morphological, conventional cytogenetics (CG), and NGS information at the time of diagnosis and at time of diagnosis of blast phase. We used Della Porta Score to quantify the degree of dysplasia [5].

2.2. Cytogenetic studies

Conventional chromosomal analysis was performed on G-banded metaphases prepared from unstimulated 24-h and 48-h BM aspirate cultures using standard techniques described previously [6]. The results were reported using the 2016 International System for Human Cytogenetics Nomenclature as described previously [7].

2.3. Molecular studies

We performed targeted next-generation sequencing (NGS)-based somatic mutation analysis using a 28-gene panel as described previously [8]. We also analyzed *CEBPA* using PCR followed by Sanger sequencing. Additional genes including splicing factors (*SF3B1, SRSF2* and *U2AF1*) were assessed as described previously [9,10]. Internal tandem duplication (ITD) of the *FLT3* gene was assessed by PCR followed by capillary electrophoresis. All molecular tests were performed on genomic DNA extracted from BM as has been described previously [8,11,12].

2.4. Statistical analyses

Frequencies and percentages were calculated for categorical variables, and means (range) were calculated for continuous variables. All statistical analyses were performed using GraphPad Prism 6. *P*-values ≤ .05 was considered significant.

3. Results

3.1. Study group

The study cohort included 60 patients with CP PV who subsequently progressed to BP, representing 12.5% of all PV patients at our institution. There were 33 men and 27 women with a median age of 53 years at time of diagnosis of PV. The median time between diagnosis of CP and progression to BP was 9 years. The median BM blast count at BP presentation was 28%; dysplasia was present in 88% of patients and dyserythropoiesis in 71%. Conventional cytogenetics showed that 43 (72%) patients had a complex karyotype, 8 (13%) had a normal karyotype, and 4 (7%) and 5 (8%) had abnormal karyotypes with 2 or 1 abnormalities, respectively.

3.2. Morphologic assessment in CP PV identifies patients with increased risk of blast phase progression

For morphologic assessment of PV over time, we selected 21 patients who had at least one BM sample before diagnosis of BP. For these patients, the median time from diagnosis of CP to progression to BP was 12.5 years (range, 1–28 years). Seventeen patients had CP ("CP baseline"), and 4 patients had accelerated phase PV. Twelve patients with CP had a second BM sample ("CP 2nd BM").

For the "CP baseline" group, 10 of 17 patients (59%) had mild dysplasia in any cellular lineage (Della Porta score: 3; range: 3–6) [5]. From this group, the erythroid lineage was the most common affected cell line (8/10 patients). Cytogenetic data were available for 11 of 17 patients (6 with normal and 5 with a complex karyotype). High-grade (grade 2 or 3) myelofibrosis was present in 10 of 13 patients with available reticulin and trichrome stains.

For the "CP 2nd BM" group, the median time from PV diagnosis to second BM evaluation was 15 years (range, 1–28 years). Mild to moderate dysplasia was identified in 10 of 12 patients (83%) (Della Porta score: 3, range 3–10) [5]. Of these 10 patients, 9 had dyserythropoiesis and 7 had unilineage dysplasia. Cytogenetic data were available in 9 of 12 patients (3 with a normal karyotype, 1 with a del(20q) karyotype, and 5 with a complex karyotype). High grade MF (MF-2/3) was found

in 8 of 11 patients with MF with available reticulin and trichrome stains. A comparison of morphologic findings in the second BM samples versus the initial BM samples showed that 5 patients had dyserythropoiesis that was not previously identified; 3 had the same score and cell lines affected; 1 had worsening dysplasia (score increased from 6 to 9, with the appearance of 15% RS and megakaryocyte dysplasia); and 1 had new onset dysplasia in in the granulocytic line.

Eight patients (4 with and 4 without BM samples obtained at CP diagnosis) had accelerated phase. The median blast count was 14%, (range 11–17%) and the median time from original PV diagnosis to AP diagnosis was 8 years (range, 7–24 years). All patients showed mild to severe dysplasia in any cellular lineage (Della Porta Score: 10, range: 3–19) [5]. All patients had a complex karyotype. Seven patients had high-grade myelofibrosis, and 1 patient had low-grade myelofibrosis. A comparison of the morphologic findings in the AP BM samples to the second BM samples available in 4 patients showed that 3 patients went from having mild to having severe dysplasia, with scores increasing from 3 to 15, 3 to 14, and 6 to 19, respectively. One patient went from having no dysplasia to having moderate unilineage dysplasia (in the granulocytic lineage), with the score increasing from 0 to 7.

The morphologic assessment of dyserythropoiesis and acquisition of ring sideroblasts (RS) during PV evolution are shown in Fig. 2A. Dyserythropoiesis was significantly more frequent in CP patients whose disease evolved to BP than in CP patients whose disease did not evolve to BP (13/17 vs. 3/10; $P = .0402$). Similarly, RS acquisition was significantly more frequent in patients who dyserythropoiesis developed BP than in patients whose disease remained in CP (8/16 vs. 0/10. $P = .0095$).

3.3. Complex karyotypes and TP53 mutations are more frequent in BP of PV than in other JAK2-mutated neoplasms

The molecular evaluation of PV BP compared to de novo acute myeloid leukemia (AML) with JAK2 mutation (AML-JAK2mut) is shown in Fig. 2B. European LeukemiaNet risk stratification for cytogenetics [13] showed that all PV BP patients were adverse or intermediate risk compared to AML-JAK2mut more homogeneous distribution over risk categories. Mutations in activating signaling genes were more frequent in BP than in AML-JAK2mut (4/14 vs. 0/11). Epigenetic modifier and spliceosome mutations were less frequent in BP than in AML-JAK2mut (1/14 vs. 4/11 and 2/9 vs. 4/8, respectively). *TP53* mutations were significantly more frequent in BP than in AML-JAK2mut (7/14 vs. 1/11, $P = .0421$).

3.4. Splicing factor mutations are infrequent in BP PV

To characterize splicing factors (SF) at BP, we performed RS counts in all BM samples with Prussian blue iron staining available (54 BM samples). We found RS percentages of 0% in 24 patients (44%), 1–14% in 19 patients (35%), and > 15% in 11 patients (20%). DNA for SF analysis was available for 13 patients (8 with and 5 without RS). Only 3 patients had SF gene mutations: 1 patient with 54% RS had an *SF3B1* mutation, and 2 patients with 73% and 15% RS, respectively, had *SRSF2* mutations. The remaining 10 patients (2 with > 15% RS, 3 with 1–14% RS, and 5 with 0% RS) had wild type SF genes.

4. Discussion

Our findings demonstrate that dyserythropoiesis and RS are important in PV evolution and support the results of earlier studies that suggested that disordered iron metabolism is an important component of PV pathobiology [14]. Recently, we described the morphologic and cytogenetic features of BM in BP PV [3]. In the present study, we found evidence that the evolution to BP PV can be identified early in patients who develop dyserythropoiesis and RS. Thus, morphologic assessment can identify CP PV patients who will likely develop BP PV and who might benefit from early intervention.

At the molecular level, our findings corroborate differences between BP PV and AML JAK2mut. Compared with patients with AML JAK2mut, patients with BP PV more frequently present with a complex karyotype and *TP53* mutations. Another group reported similar results for a more heterogeneous group of MPN patients who developed BP [15]. Recently, we described the morphologic, cytogenetic and molecular findings of AML JAK2mut when compared to *JAK2* wild-type AML. We found that AML JAK2mut frequently present with mutations in DNA methylation and epigenetic-modifying genes, absence of gene mutations in activating signaling pathways and no differences in the frequency of tumor suppressor or splicing factor genes [16]. Different from

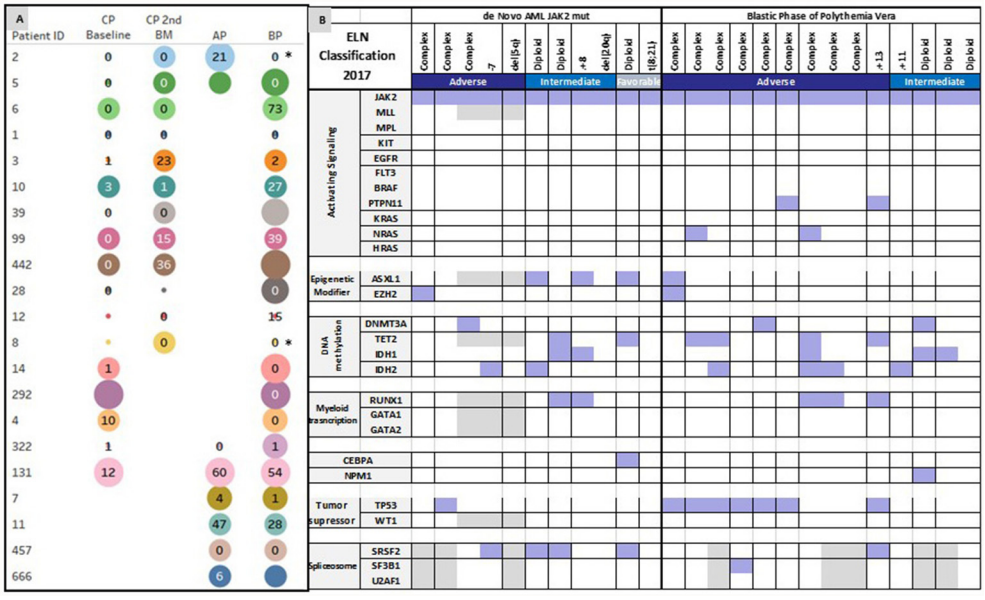

Fig. 2. Morphologic assessment and molecular evaluation.
(A) Twenty-one patients had BM samples available for morphologic evaluation over time. In this figure we show the changes in the degree of dyserythropoiesis (DE) and ring sideroblast (RS) count over time. Each row is a patient, and each column is a time point, chronic phase at baseline (CP Baseline), at follow up (CP 2nd BM), at accelerated phase (AP), and at blast phase (BP). We used Della Porta score to quantify the degree of dyserythropoiesis identified as the circle size. RS count is the number. Patients with no sample available for morphologic assessment appear as blank. (B) Cytogenetic and molecular profile of 11 patients with de novo AML with JAK2 mutations compared with 14 patients with BP. The first row describes cytogenetics and European LeukemiaNet (ELN) classification. Each mutated gene is highlighted in blue; genes not mutated are shown in white. Gray selections are not tested. *Markedly decreased erythropoiesis, dysplasia not evaluable. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

these neoplasms, BP of PV have low frequency of mutations in epigenetic-modifying and splicing factor genes and increased frequency of *TP53* mutations.

Our findings also provide a resource that physicians worldwide can use for the early identification of PV patients with increased risk of BP progression. Given the link between RS and SF mutations, our results support the recently published personalized prognosis model of MPN, in which patients with *SRSF2* mutations are identified as a high-risk group [2]. Our findings showed that *SF3B1* mutations in BP PV are rare despite the presence of dyserythropoiesis and RS. These findings suggest that mutations in genes other than SF genes help trigger the increase in RS.

5. Conclusions

This study demonstrates that dyserythropoiesis is and the acquisition of RS are early events in PV that precede other markers of disease progression to BP. These morphologic features can help identify patients with increased risk of BP evolution who will benefit from more aggressive therapy. At the molecular level, we found that complex karyotypes and *TP53* mutations are more frequent in BP of PV than in other JAK2-mutated neoplasms. Therefore BP has a distinctive molecular profile and adverse genetics in the European LeukemiaNet risk stratification system. Lastly, we show that *SF3B1* mutations are rare in BP and that other genes are responsible for increased RS in BP. Dysregulation of pathways other than SF3B1 should be explored as a cause of dyserythropoiesis and RS in this population.

Disclosure of conflicts of interest

The authors have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors.

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