Analysis of Molecular Mechanisms and Predictive Biomarkers of Disease Transformation in Polycythemia Vera

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Background

- Progression to myelofibrosis (MF) represents a major cause of morbidity and mortality for patients with polycythemia vera (PV); however, predictors of progression remain incompletely understood
- REVEAL (NCT02252159) is a multicenter, prospective, observational study that followed patients with PV for a median of 4 years, with optional peripheral blood collection every 12 months[1,2]
- Of the 2510 patients enrolled in REVEAL, 135 progressed to MF during the study period, of whom 117 had an enrollment biospecimen

Objective

- Clinical and genomic data from REVEAL were utilized to investigate the molecular mechanisms that contribute to PV transformation to MF

Methods

Table 1. PV Transformation Criteria*

Criterion 1	Death due to MF/MDS/AML
Criterion 2	New/worsening splenomegaly and ≥2 of: WBC count >11×10^9/L, Hb <10 g/dL, and platelet count <100×10^9/L
Criterion 3	Bone marrow biopsy and fibrosis grade ≥2 or pathologic diagnosis of MF
Criterion 4	Circulating blasts >1% and new/worsening splenomegaly

*Laboratory values were required from ≥1 time point. Progression criteria defined for use in this analysis have not been validated across other studies.
AML, acute myeloid leukemia; Hb, hemoglobin; MDS, myelodysplastic syndrome; WBC, white blood cell.

- Transformation to MF was determined using modified World Health Organization criteria as previously defined[3] **(Table 1)**
- Whole exome sequencing (WES) was performed on 20 patients who transformed to MF during the study period and 16 nontransformed controls
- Pretransformation biospecimens collected at study enrollment were sequenced using the Illumina (paired-end) platform and processed using Genome Analysis Toolkit (GATK) best practices, followed by variant calling via Mutect2 (Broad Institute, Cambridge, MA, USA)
- Variants were annotated using GoldenHelix (VarSeq) and were required to meet the following criteria: ≥120× coverage, ≥3 reads support, and <0.05 minor allele frequency in the Genome Aggregation Database or the 1000 Genomes project
- We focused on 25 genes with an established role in the pathogenesis of myeloid malignancy[4]

Results

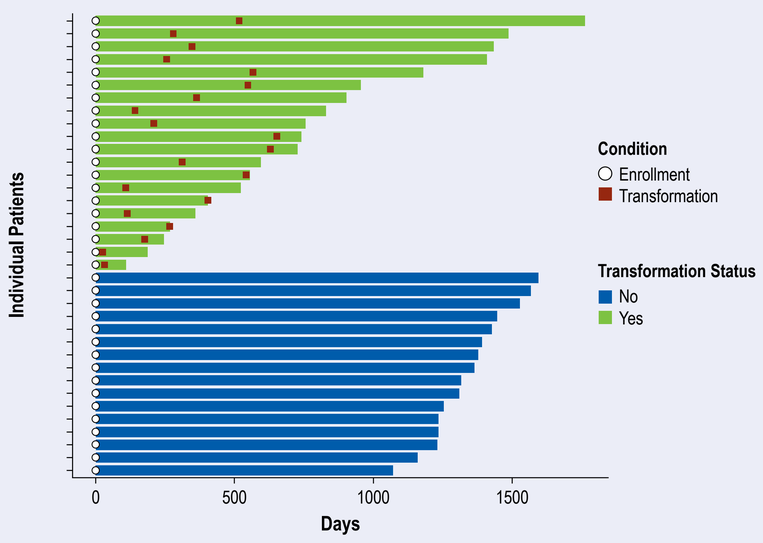
Table 2. Characteristics of Patients With and Without Transformed PV

Characteristic	Transformed PV (n=20)	Nontransformed PV (n=16)	Total (n=36)
Age at enrollment, median (range), years	68.0 (49.0-87.0)	70.5 (52.0-82.0)	69.5 (49.0-87.0)
Female, n (%)	8 (40.0)	9 (56.3)	17 (47.2)
High-risk PV at diagnosis, n (%)	10 (50.0)	14 (87.5)	24 (66.7)
High-risk PV at enrollment, n (%)	16 (80.0)	15 (93.8)	31 (86.1)
Time from PV diagnosis to enrollment, median (range), years	8.7 (0.1-25.7)	6.2 (0.1-13.5)	7.7 (0.1-25.7)
<5 years, n (%)	5 (25.0)	4 (25.0)	9 (25.0)
≥5 years, n (%)	15 (75.0)	12 (75.0)	27 (75.0)
PV treatment before enrollment, n (%)			
Watchful waiting only	1 (5.0)	2 (12.5)	3 (8.3)
Phlebotomy	2 (10.0)	2 (12.5)	4 (11.1)
Hydroxyurea	13 (65.0)	6 (37.5)	19 (52.8)
Hydroxyurea and phlebotomy in combination	2 (10.0)	4 (25.0)	6 (16.7)
Treatment with other agents	2 (10.0)	2 (12.5)	4 (11.1)
Time from enrollment to MF transformation, median (range), months	9.75 (0.95-21.50)	N/A	
JAK2 mutation, n/N (%)	19/20 (95.0)	15/16 (93.8)	34/36 (94.4)

JAK2, Janus kinase 2; N/A, not applicable.

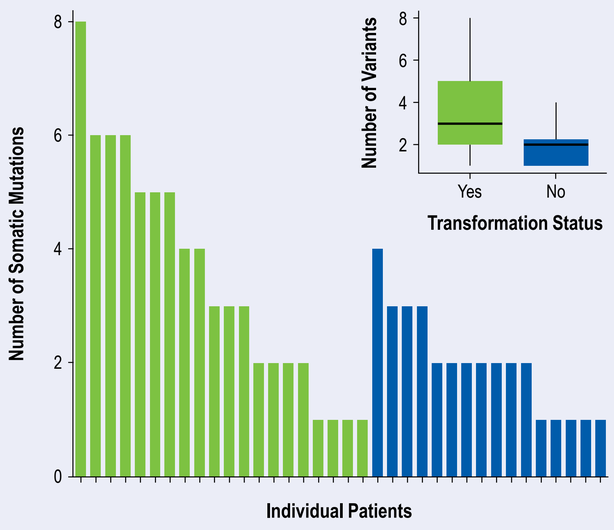
- The percentage of patients characterized as high-risk PV at diagnosis due to age ≥60 years or history of thrombosis was significantly higher in the nontransformed vs the transformed group (**Table 2**; *P*=0.0177)
- WES identified a *Janus kinase 2* (*JAK2*) p.V617F mutation in 33 patients and a *JAK2* exon 12 mutation in 1 patient; 2 patients without a *JAK2* mutation had evidence of a p.V617F variant below the quality thresholds used in this study
- Mean time from PV diagnosis to study enrollment was slightly longer in the transformed group (**Table 2**; 8.7 vs 6.2 years)

Figure 1. Overview of Patients and Biospecimen Collection



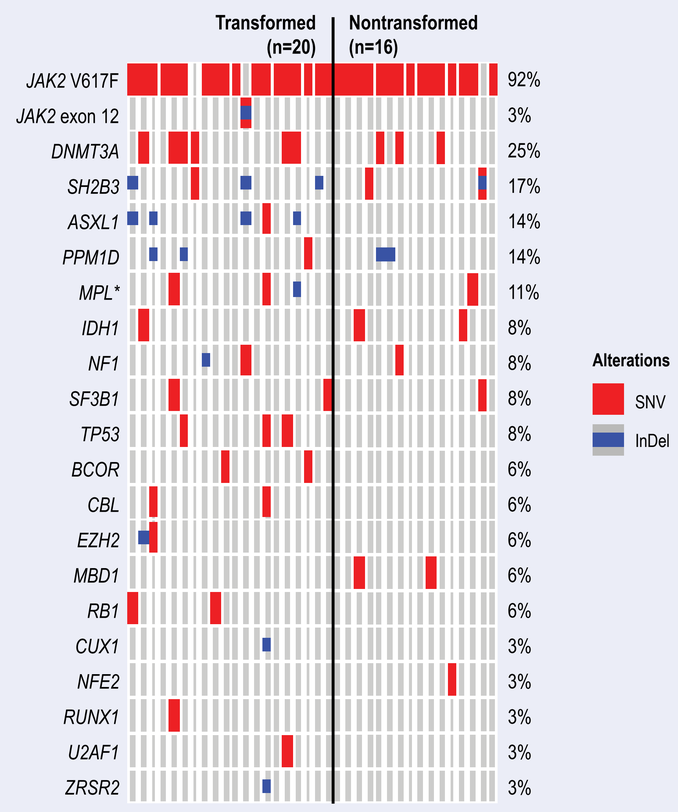
- Pretransformation biospecimens collected at study enrollment (circle) were analyzed for patients who transformed (green) and patients who did not transform (blue) **(Figure 1)**
- Transformation time (red box) compared with enrollment varied between patients who transformed

Figure 2. Patients With Transformed PV Had a Higher Number of Somatic Mutations Before Transformation



- Analysis of commonly mutated genes in myeloid malignancy identified more somatic mutations in the enrollment samples of the transformed cohort compared with the nontransformed cohort **(Figure 2)**
- The median number of somatic mutations per patient was significantly greater in the transformed cohort (3; interquartile range [IQR], 2-5) compared with the nontransformed cohort (2; IQR, 1-2.25; *P*=0.029; Wilcoxon test) **(INSET)**

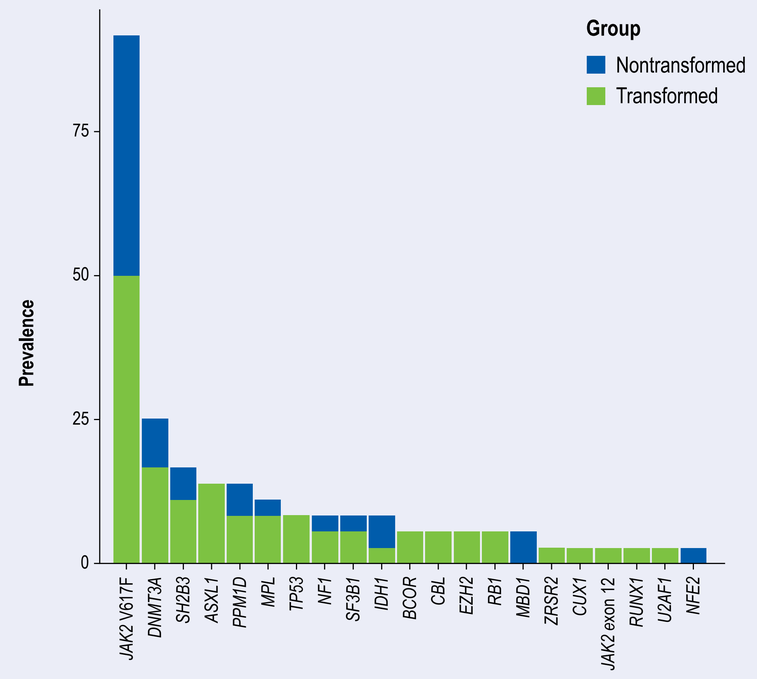
Figure 3. Summary of Mutations by Transformation Status



*One patient had an *MPL* p.W515L mutation; the other *MPL* mutations were not canonical MPN driver mutations. InDel, insertion-deletion; MPL, myeloproliferative leukemia; SNV, single nucleotide variant.

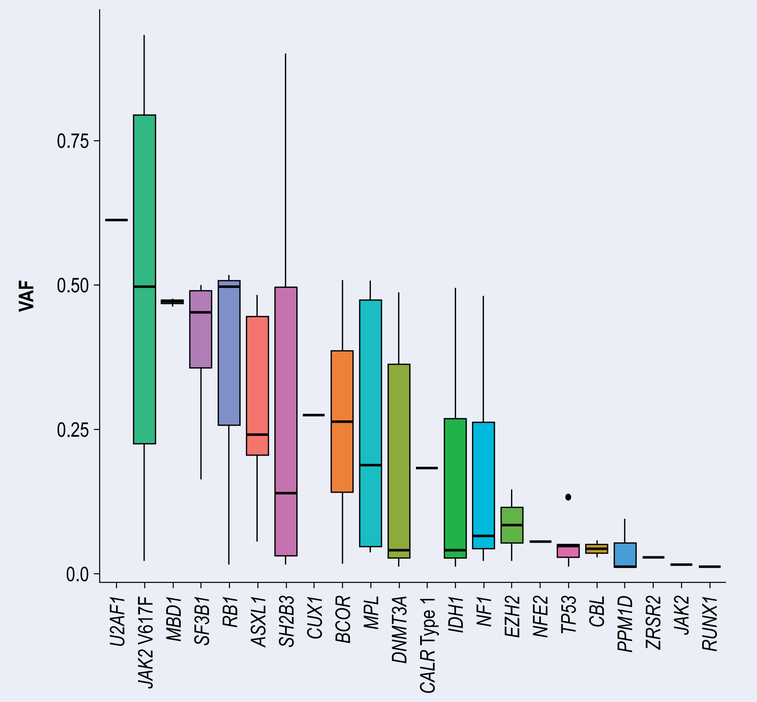
- Mutations in *additional sex combs-like 1* (*ASXL1*) and *tumor protein 53* (*TP53*) were not observed in the nontransformed cohort **(Figure 3)**

Figure 4. Patients With Transformed PV Had Increased Prevalence of Mutations



- The frequency of somatic mutations was greater for the transformed vs the nontransformed group **(Figure 4)**

Figure 5. Distribution of Variant Allele Frequency



VAF, variant allele frequency.

- For several genes, a wide VAF distribution was present **(Figure 5)**
- The VAFs of nondriver mutations within a single individual typically deviated from the VAF of the driver mutation, indicating the presence of multiple clones

Conclusions

- **There was no difference in median *JAK2* VAF between the transformed group and the nontransformed group, with a wide distribution present**
- **Analysis of 25 genes commonly mutated in myeloid malignancy identified that the number of somatic mutations per patient was significantly greater in the transformed group compared with the nontransformed group**
- **Presence of nondriver mutations before transformation may help to identify patients with PV at higher risk for transformation to MF**
- **Sequencing of additional biospecimens from patients enrolled in REVEAL, including longitudinal intrapatient biospecimens, is ongoing, and will enable the study of changes in clonal architecture over time**

Disclosures

Crowgey, Timmers, Xue, Alvarez Arias, Bhatt, Braunstein: Employment and stock ownership – *Incyte Corporation.* **Oh:** Consulting fees – *AbbVie, Bristol Myers Squibb, Cogent Biosciences, Constellation Pharmaceuticals/MorphoSys, CTI BioPharma, Geron, Incyte Corporation, Morphic Therapeutic, Protagonist Therapeutics, Sierra Oncology/GlaxoSmithKline.*

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