

# Impact of *JAK2(V617F)* mutation status on treatment response to anagrelide in essential thrombocythemia: an observational, hypothesis-generating study

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**Abstract:** A *JAK2(V617F)* mutation is found in approximately 55% of patients with essential thrombocythemia (ET), and represents a key World Health Organization diagnostic criterion. This hypothesis-generating study (NCT01352585) explored the impact of *JAK2(V617F)* mutation status on treatment response to anagrelide in patients with ET who were intolerant/refractory to their current cytoreductive therapy. The primary objective was to compare the proportion of *JAK2*-positive versus *JAK2*-negative patients who achieved at least a partial platelet response ( $\leq 600 \times 10^9/L$ ) after anagrelide therapy. Of the 47 patients enrolled, 46 were included in the full analysis set (*JAK2*-positive,  $n=22$ ; *JAK2*-negative,  $n=24$ ). At 12 months, 35 patients ( $n=14$  and  $n=21$ , respectively) had a suitable platelet sample; of these, 74.3% ( $n=26$ ) achieved at least a partial response. The response rate was higher in *JAK2*-positive (85.7%,  $n=12$ ) versus *JAK2*-negative patients (66.7%,  $n=14$ ) (odds ratio [OR] 3.00; 95% confidence interval [CI] 0.44, 33.97). By using the last observation carried forward approach in the sensitivity analysis, which considered the imbalance in patients with suitable samples between groups, the overall response rate was 71.7% ( $n=33/46$ ), with 77.3% ( $n=17/22$ ) of *JAK2*-positive and 66.7% ( $n=16/24$ ) of *JAK2*-negative patients achieving at least a partial response (OR 1.70; 95% CI 0.39, 8.02). There was no significant change in median allele burden over 12 months in the 12 patients who achieved a response. In conclusion, the overall platelet response rate was high in both *JAK2*-positive and *JAK2*-negative patients; however, a larger study would be required to confirm the differences observed according to *JAK2(V617F)* mutation status.

**Keywords:** essential thrombocythemia, mutation, *JAK2*, anagrelide, treatment response, allele burden

## Introduction

Essential thrombocythemia (ET) is a clonal myeloproliferative neoplasm (MPN) characterized by an overproduction of platelets and an increased risk of thrombohemorrhagic complications.<sup>1,2</sup> A *JAK2(V617F)* gain-of-function mutation is found in approximately 55% of patients with ET.<sup>3–11</sup> *JAK2* encodes a cytoplasmic tyrosine kinase involved in normal hematopoiesis. Available data show the *JAK2* mutation to be associated with an increased risk of arterial thrombosis in patients with ET.<sup>12,13</sup> The risk of evolution to myelofibrosis may also be increased by the *JAK2* mutation and appears to vary according to allele burden,<sup>9,13</sup> although conflicting data are available.<sup>4</sup> In 2008, the World Health Organization (WHO) identified the *JAK2* mutation as a key diagnostic criterion for Philadelphia-negative MPNs.<sup>14</sup> The *JAK2* mutation does not differentiate between ET and other clonal MPNs (such as polycythemia vera and

primary myelofibrosis), but is a molecular marker that distinguishes clonal MPNs from reactive thrombocytosis.<sup>14,15</sup>

Somatic mutations in the thrombopoietin receptor (*MPL*) and calreticulin (*CALR*) genes have also been reported in patients with ET. As with *JAK2(V617F)*, the *MPL* mutation appears to have a phenotype-modifying effect in ET; however, this mutation is infrequent, occurring in only around 3% of patients.<sup>10,16,17</sup> Most patients with ET who do not harbor a *JAK2* or *MPL* alteration carry a *CALR* mutation,<sup>18</sup> with an overall *CALR* mutational frequency in patients with ET up to 32% as reported in one recent study.<sup>10</sup> The clinical course of ET in patients with mutated *CALR* appears more indolent than that in patients with mutated *JAK2*.<sup>19–21</sup>

Anagrelide (Xagrid®; 0.5 mg hard capsules, Shire Pharmaceutical Contracts Limited, Basingstoke, United Kingdom) is an orally active, quinazalone-derived platelet-lowering agent indicated for second-line treatment of high-risk patients with ET in Europe.<sup>15,22,23</sup> Clinical studies in more than 4,000 patients have confirmed the safety and efficacy of anagrelide as a platelet-lowering agent in ET.<sup>22,24–26</sup>

Results from a previous study have suggested that anagrelide is similarly effective in controlling platelet levels in patients with ET irrespective of *JAK2* mutation status.<sup>27</sup> However, *JAK2*-positive patients have been reported to be more sensitive than *JAK2*-negative patients to hydroxycarbamide, as demonstrated by lower platelet counts at lower hydroxycarbamide dosages.<sup>27</sup> This hypothesis-generating study was undertaken to further explore the potential impact of *JAK2(V617F)* mutation status and allele burden on the response to anagrelide in patients with ET.

## Materials and methods

### Study design

This was an exploratory, observational, multicenter study (clinicaltrials.gov registration: NCT01352585) conducted across eleven centers in Italy from July 2011 to September 2013. Anagrelide 0.5 mg was administered at doses determined by the treating physician and in accordance with the European Union Summary of Product Characteristics (SPC).<sup>22</sup> Anti-aggregatory therapy was permitted at the discretion of the investigator. All evaluations were undertaken in accordance with routine clinical practice. No visits were imposed by the study outside of regularly scheduled visits for treatment purposes. Patients who discontinued anagrelide due to an adverse drug reaction (ADR) were followed throughout the study. All other patients who discontinued were followed according to local clinical practice. Patients were withdrawn

from the study if they switched from anagrelide to another ET therapy or combination therapy.

### Patients

Patients were eligible for enrollment if they had a confirmed diagnosis of ET, according to WHO 2008 diagnostic criteria,<sup>14</sup> and were intolerant or refractory to their first-line or previous cytoreductive therapy due to the lack of efficacy or intolerance. Patients had either started anagrelide treatment in the 7 days prior to study entry or a decision had been documented to commence anagrelide. Patients were excluded from the study if they had a known or suspected intolerance to anagrelide or any of the stated ingredients, or closely related compounds. Other exclusion criteria were contraindications to anagrelide as listed in the anagrelide SPC,<sup>22</sup> combination therapy with other cytoreductive agents, or participation in an interventional research study. Written informed consent was obtained from all patients prior to study entry. This observational study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice (GCP) standards, and local ethical and legal requirements.

### Assessments

Patient data were collected from medical records following routine visits at baseline and at 6±2 and 12±3 months after the initiation of anagrelide using electronic case report forms. As part of routine clinical practice, patients provided a 20 mL blood sample for hematological assessment at all visits. *JAK2* mutation status was measured in all patients at baseline. Allele burden was tested at baseline and at 6 and 12 months in *JAK2*-positive patients; allele burden was measured in both granulocyte DNA and platelet RNA. *JAK2* mutation status and allele burden were measured at a centralized laboratory (University of Florence, Italy) in order to standardize the assessments. *JAK2* mutation status was determined by real-time polymerase chain reaction, and *JAK2(V617F)* allele burden was quantified using the  $\Delta C_t$  method as previously described.<sup>8,28</sup> The safety of anagrelide was assessed by monitoring the incidence and severity of ADRs and changes in routine hematology parameters.

### Statistical analysis

The primary objective of this study was to compare the proportion of *JAK2*-positive versus *JAK2*-negative patients with ET who achieved at least a partial platelet response ( $\leq 600 \times 10^9/L$ ) after anagrelide therapy. Secondary objectives were to compare the proportion of *JAK2*-positive versus *JAK2*-negative patients with ET who achieved a complete

platelet response ( $\leq 400 \times 10^9/L$ ) after anagrelide therapy, to evaluate the relationship between platelet response and allele burden in the *JAK2*-positive group, and to observe the effect of anagrelide on routine hematological parameters.

The study was not statistically powered and was exploratory in nature. The target sample size was approximately 60 patients; enrollment was monitored to ensure that a minimum of ten patients were recruited into both the *JAK2*-positive and *JAK2*-negative groups. Efficacy was analyzed in all patients who received at least one dose of anagrelide and had at least one post-baseline platelet count and known *JAK2* mutation status (full analysis set). Safety was analyzed in all enrolled patients who received at least one dose of anagrelide (safety set).

For the primary endpoint and other treatment response endpoints, the odds ratio (OR) and 95% confidence interval (CI) were calculated for the difference in the proportion of patients achieving a response between the *JAK2*-positive and *JAK2*-negative groups. Patients without an available platelet sample within the specified time window were excluded from the analysis. The efficacy results from the last observation carried forward (LOCF) sensitivity analysis are also reported here as this approach considers the imbalance in patients with suitable platelet samples between the *JAK2*-positive and *JAK2*-negative groups. For *JAK2*-positive patients, platelet counts were cross-tabulated with the allele burden at 6 and 12 months; separate summaries were produced for the allele burden in granulocyte DNA and platelet RNA. ADRs were recorded using the Medical Dictionary for Regulatory Activities (MedDRA), version 15.1 or newer.

## Role of the funding source

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## Results

### Patients

Forty-seven patients were enrolled and received at least one dose of anagrelide. Of these, 23 (48.9%) were *JAK2*-positive and 24 (51.1%) were *JAK2*-negative. Forty-six patients had at least one post-baseline platelet count and known *JAK2* status (22 [47.8%] *JAK2*-positive and 24 [52.2%] *JAK2*-negative) and were included in the analysis of efficacy. Fifteen patients in the *JAK2*-positive group and 21 patients in the *JAK2*-negative group completed the study. The most common reason for withdrawal was an adverse event (AE) not related to anagrelide ( $n=6$ , 12.8%; *JAK2*-positive,  $n=4$

[including three due to transformation to myelofibrosis]; *JAK2*-negative,  $n=2$ ). Three patients (6.4%; *JAK2*-positive,  $n=2$ ; *JAK2*-negative,  $n=1$ ) withdrew from the study due to an ADR.

Patient demographics and baseline characteristics were generally well balanced between the *JAK2*-positive and *JAK2*-negative groups (Table 1), except for median (range) time since ET diagnosis (8.2 years [0–22] versus 5 years [0–16]) and median (range) platelet count ( $584.0 \times 10^9/L$  [310–1,158] versus  $752.5 \times 10^9/L$  [323–2,126]), respectively. Mean (standard deviation) age was 58.0 (14.87) years, 59.6% of patients were female, and most were Caucasian (97.9%).

At baseline, most *JAK2*-positive patients had a low ( $<50\%$ ) granulocyte DNA and platelet RNA allele burden (each  $n=18$  [78.3%]). The distribution of allele burden differed between granulocyte DNA and platelet RNA. Approximately half of *JAK2*-positive patients ( $n=11$  [47.8%]) had a granulocyte DNA allele burden of 25% to  $<50\%$ , with less than a third ( $n=7$  [30.4%]) having a granulocyte DNA allele burden  $<25\%$ . In contrast, the majority of *JAK2*-positive patients ( $n=17$  [73.9%]) had a platelet RNA allele burden of 25% to  $<50\%$ , and only one (4.3%) *JAK2*-positive patient had a platelet RNA allele burden  $<25\%$ .

## Treatment response

At 12 months in the safety analysis set, the median (range) platelet count was  $416.6 \times 10^9/L$  (235–854) in the *JAK2*-positive group and  $425.0 \times 10^9/L$  (165–1,279) in the *JAK2*-negative group. At 12 months, 35 patients (14 patients in the *JAK2*-positive group and 21 patients in the *JAK2*-negative group) had an available platelet sample for evaluation. Of these 35 patients, 26 (74.3%) achieved at least a partial platelet response ( $\leq 600 \times 10^9/L$ ) (95% CI 57.9, 85.8). The proportion of patients who achieved at least a partial response was higher in the *JAK2*-positive group ( $n=12$  [85.7%]; 95% CI 60.1, 96.0) than in the *JAK2*-negative group ( $n=14$  [66.7%]; 95% CI 45.4, 82.8). The odds of patients in the *JAK2*-positive group achieving at least a partial platelet response were 3.00 times higher than in the *JAK2*-negative group, although this difference was not statistically significant (95% CI 0.44, 33.97) (Figure 1A).

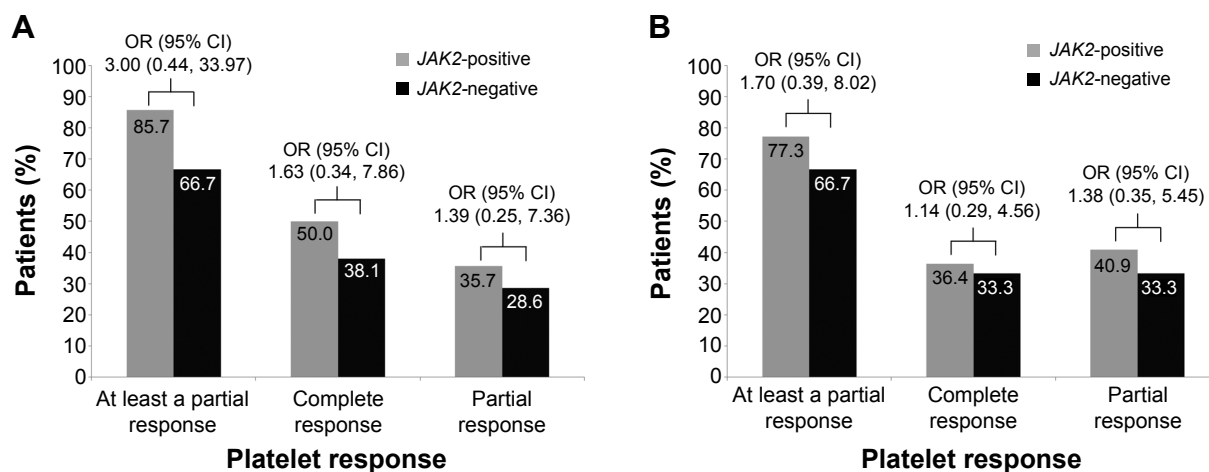
By using the LOCF approach in the sensitivity analysis, 33 (71.7%) patients achieved at least a partial response (95% CI 57.5, 82.7). The response rate in the *JAK2*-positive group was more similar to the *JAK2*-negative group than in the main analysis. The proportion of patients with at least a partial response was higher in the *JAK2*-positive group ( $n=17$

**Table 1** Patient demographics and baseline characteristics (safety set)

Characteristic	JAK2-positive (N=23)	JAK2-negative (N=24)	Total (N=47)
Age, years			
Mean (SD)	57.4 (14.00)	58.6 (15.94)	58.0 (14.87)
Median (range)	59.0 (36–85)	60.5 (27–89)	60.0 (27–89)
Sex, n (%)			
Male	9 (39.1)	10 (41.7)	19 (40.4)
Female	14 (60.9)	14 (58.3)	28 (59.6)
JAK2 mutation status, n (%)			
Positive	23 (100.0)	0	23 (48.9)
Negative	0	24 (100.0)	24 (51.1)
Allele burden: granulocyte DNA, % <sup>a</sup>	N=22		
Mean (SD)	35.5 (16.17)	n/a	n/a
Median	30.5	n/a	n/a
Q1, Q3	21.0, 44.0	n/a	n/a
Range	17–72	n/a	n/a
Allele burden: platelet RNA, % <sup>a</sup>	N=22		
Mean (SD)	39.0 (11.91)	n/a	n/a
Median	35.0	n/a	n/a
Q1, Q3	30.0, 47.0	n/a	n/a
Range	24–68	n/a	n/a
Hematological parameters, median (range)			
Platelet count, ×10 <sup>9</sup> /L	584.0 (310–1,158)	752.5 (323–2,126)	646.0 (310–2,126)
Hematocrit, fraction of l	0.419 (0.30–0.50)	0.370 (0.29–0.46)	0.387 (0.29–0.50)
Hemoglobin, g/L	137.0 (97–157)	122.0 (84–158)	130.0 (84–158)
White blood cells, ×10 <sup>9</sup> /L	7.70 (4.1–18.1)	6.81 (3.6–15.2)	7.03 (3.6–18.1)
Prior cytoreductive therapy, n (%) <sup>b</sup>	22 (95.7)	19 (79.2)	41 (87.2)
Busulfan	1 (4.3)	0	1 (2.1)
Hydroxycarbamide	21 (91.3)	18 (75.0)	39 (83.0)
Interferon <sup>c</sup>	3 (13.07)	4 (16.7)	7 (14.9)
Pipobroman	0	1 (4.2)	1 (2.1)

**Notes:** <sup>a</sup>Baseline is the first reported allele burden value available in the study. <sup>b</sup>Percentages are based on all patients in the safety set for each JAK2 status group. Medications were coded using the World Health Organization's Drug Dictionary version 2013SEP01. Patients were counted once per category per treatment group. Prior medications include all medications received by the patient in the 30 days prior to the commencement of anagrelide. <sup>c</sup>Includes interferon, interferon alfa, interferon alfa-N1, and peginterferon alfa-2A.

**Abbreviations:** n/a, not applicable; Q, quartile; SD, standard deviation.



**Figure 1** Treatment response comparisons at 12 months for the JAK2-positive and JAK2-negative groups.

**Notes:** (A) Full analysis set; (B) LOCF sensitivity analyses.

**Abbreviations:** CI, confidence interval; LOCF, last observation carried forward; OR, odds ratio.

[77.3%]; 95% CI 56.6, 89.9) than in the *JAK2*-negative group (n=16 [66.7%]; 95% CI 46.7, 82.0). The odds of patients in the *JAK2*-positive group achieving at least a partial response in the LOCF sensitivity analysis were 1.7 times higher than in the *JAK2*-negative group (Figure 1B). However, this difference was not statistically significant (95% CI 0.39, 8.02).

At 12 months, 15 patients (42.9%) had achieved a complete platelet response ( $\leq 400 \times 10^9/L$ ) (95% CI 28.0, 59.1). A higher proportion of *JAK2*-positive patients (n=7 [50.0%]; 95% CI 26.8, 73.2) achieved a complete platelet response than *JAK2*-negative patients (n=8 [38.1%]; 95% CI 20.8, 59.1). The odds of patients in the *JAK2*-positive group achieving a complete platelet response were 1.63 times higher than in the *JAK2*-negative group, although this difference was not statistically significant (95% CI 0.34, 7.86) (Figure 1A).

In the sensitivity analysis using the LOCF approach, 16 (34.8%) patients achieved a complete platelet response at 12 months (95% CI 0.29, 4.56). The proportion of patients who achieved a complete response was higher in the *JAK2*-positive group (n=8 [36.4%]; 95% CI 19.7, 57.0) than in the *JAK2*-negative group (n=8 [33.3%]; 95% CI 18.0, 53.3). The odds of patients in the *JAK2*-positive group achieving a complete platelet response in the LOCF sensitivity analysis were 1.14 times higher than in the *JAK2*-negative group, although this difference was not statistically significant (95% CI 0.29, 4.56) (Figure 1B).

Platelet responses to anagrelide treatment were also observed at 6 months, although the difference in platelet responses between the *JAK2*-positive and *JAK2*-negative

groups at this time was not as pronounced as that observed at 12 months (Figure 2).

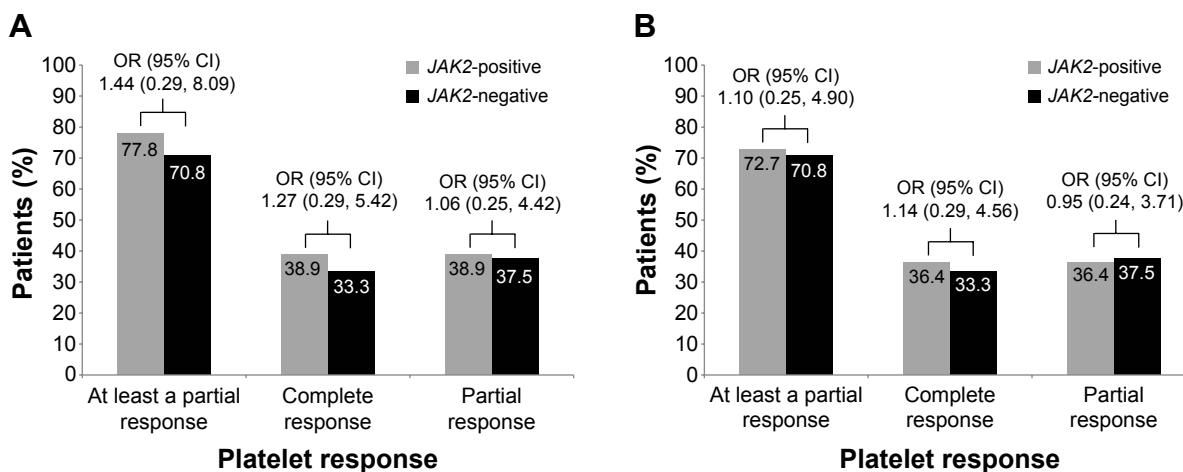
## Allele burden

In *JAK2*-positive patients, no clear relationship was identified between platelet response and baseline allele burden. The median allele burden for the 12 patients who achieved at least a partial response at 12 months and had a suitable platelet sample was 41.0% (range: 17–47) for granulocyte DNA and 49.5% (range: 31–58) for platelet RNA. There was no clinically relevant change in allele burden from baseline over the 12 months of treatment with anagrelide (data not shown).

## Exposure and safety

The mean (standard deviation) daily dose of anagrelide was 1.40 (0.482) mg/day overall, 1.32 (0.378) mg/day in the *JAK2*-positive group, and 1.47 (0.563) mg/day in the *JAK2*-negative group. Total exposure was similar in *JAK2*-positive (19.6 person-years) and *JAK2*-negative patients (22.8 person-years).

A total of 15 (31.9%) patients experienced 20 ADRs (Table 2). Fewer patients in the *JAK2*-positive group had an ADR than in the *JAK2*-negative group (26.1% versus 37.5%). The most common ADR was headache (12.8%). All of the ADRs reported were of mild or moderate severity. None of the ADRs were fatal, serious, or severe and no deaths occurred during the study. Three patients were withdrawn due to an ADR (dermatitis and pruritus in the *JAK2*-positive group and headache in the *JAK2*-negative group).



**Figure 2** Treatment response comparisons at 6 months for the *JAK2*-positive and *JAK2*-negative groups.

**Notes:** (A) Full analysis set; (B) LOCF sensitivity analyses.

**Abbreviations:** CI, confidence interval; LOCF, last observation carried forward; OR, odds ratio.

**Table 2** Adverse drug reactions (safety set)

Category, n (%)	<i>JAK2</i> -positive (N=23)	<i>JAK2</i> -negative (N=24)	Total (N=47)
Any ADR	6 (26.1)	9 (37.5)	15 (31.9)
Severe ADRs	0	0	0
SADR	0	0	0
ADR causing withdrawal of anagrelide	2 (8.7) <sup>a</sup>	1 (4.2) <sup>b</sup>	3 (6.4)
Deaths	0	0	0
ADRs by preferred term			
Headache	2 (8.7)	4 (16.7)	6 (12.8)
Anemia	1 (4.3)	2 (8.3)	3 (6.4)
Palpitations	1 (4.3)	1 (4.2)	2 (4.3)
Tachycardia	1 (4.3)	1 (4.2)	2 (4.3)
Scotoma	0	1 (4.2)	1 (2.1)
Diarrhea	0	1 (4.2)	1 (2.1)
Hyperuricemia	0	1 (4.2)	1 (2.1)
Presyncope	1 (4.3)	0	1 (2.1)
Dermatitis	1 (4.3)	0	1 (2.1)
Pruritus	0	1 (4.2)	1 (2.1)

Notes: <sup>a</sup>Headache, n=1; dermatitis, n=1; <sup>b</sup>Pruritus.

Abbreviations: ADR, adverse drug reaction; SADR, serious adverse drug reaction.

There were no notable changes from baseline to 12 months in any hematological parameters other than platelets.

## Discussion

In this hypothesis-generating study, the overall platelet response rate to anagrelide in patients with ET was found to be high irrespective of *JAK2* mutation status. Our findings are in line with the efficacy of anagrelide observed in other studies in patients with ET.<sup>22,24–26</sup> Previous studies have shown that responses to anagrelide are unaffected by *JAK2* mutation status.<sup>27,29</sup> In the current study, we found the likelihood of having at least a partial response to anagrelide to be higher in *JAK2*-positive patients than in those who were *JAK2*-negative. However, the sensitivity analyses using LOCF showed more comparable treatment response findings between the *JAK2*-positive and *JAK2*-negative groups and lower ORs, indicating a similar sensitivity to anagrelide independent of *JAK2* mutation status.

No clear relationship or clinically relevant difference was identified between *JAK2* allele burden and platelet treatment response in this study, and there was no significant change in allele burden over the 12 months of anagrelide therapy. The majority of *JAK2*-positive patients had an allele burden of  $\geq 25\%$ – $50\%$ , regardless of whether this was measured using granulocyte DNA or platelet RNA methodology. *JAK2* allele burden appears to play a role in clinical phenotype and disease evolution in ET.<sup>9,13</sup>

Anagrelide was found to be well tolerated in this study, with a safety profile consistent with the SPC<sup>22</sup> and current

literature.<sup>26,30,31</sup> The frequency of ADRs was lower than that reported in the current literature, but there is no obvious explanation for this finding. The most commonly reported ADR was headache. All of the ADRs reported were of mild or moderate severity, no severe or serious ADRs occurred, and few patients were withdrawn due to an ADR. Three patients were withdrawn due to transformation to myelofibrosis, but these cases were not considered to be related to anagrelide because of the short time interval between anagrelide initiation and myelofibrosis diagnosis. No thrombotic or hemorrhagic complications were reported over the duration of this study. Furthermore, no differences in ADRs were evident between the *JAK2*-positive and *JAK2*-negative groups.

It should be noted that results from this study were not statistically significant (CIs were wide) and data should be interpreted with caution due to the small study size. In addition, the imbalance of patients who remained in the study at 12 months between groups could have added potential bias to the results. This is supported by the sensitivity analyses using LOCF, which showed more comparable treatment response findings between the two groups.

## Conclusion

In conclusion, the overall platelet response rate was high in both *JAK2*-positive and *JAK2*-negative patients with ET in this study. The odds of achieving at least a partial response were found to be slightly higher if patients had a *JAK2(V617F)* mutation than if they did not. However, no firm conclusions can be made and a larger well-controlled study would be needed to confirm the findings of this hypothesis-generating study.

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