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ORIGINAL ARTICLE

Long-term follow-up of essential thrombocythemia patients treated with anagrelide: subgroup analysis according to *JAK2/CALR/MPL* mutational status

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Abstract

Background: Anagrelide represents a treatment option for essential thrombocythemia, although its place in therapy remains controversial. *Aim:* To assess the impact of mutational status in response rates and development of adverse events during long-term use of anagrelide. *Methods:* We retrospectively evaluated 67 patients with essential thrombocythemia treated with anagrelide during 68 (4–176) months. *Results:* Mutational frequencies were 46.3%, 28.3%, and 1.5% for *JAK2*V617F, *CALR* and *MPL* mutations. Anagrelide yielded a high rate of hematologic responses, which were complete in 49.25% and partial in 46.25%, without differences among molecular subsets. The rate of thrombosis during treatment was one per 100 patient-years, without excess bleeding. Anemia was the major adverse event, 30.3% at 5-yr follow-up, being more frequent in $CALR^+$ ($P < 0.05$). Myelofibrotic transformation developed in 14.9% (12.9%, 21%, and 12.5% in $JAK2V617F^+$, $CALR^+$, and triple-negative patients, respectively, P = NS) and those treated >60 months were at higher risk, OR (95% CI) 9.32 (1.1–78.5), $P < 0.01$, indicating the need for bone marrow monitoring during prolonged treatment. *Conclusion:* Although $CALR^+$ patients were at higher risk of developing anemia, anagrelide proved effective among all molecular subsets, indicating that mutational status does not seem to represent a major determinant of choice of cytoreductive treatment among essential thrombocythemia therapies.

Key words Essential thrombocythemia; anagrelide; calreticulin; janus kinase 2

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Anagrelide is a platelet-lowering agent which acts by blocking megakaryocyte maturation, polyploidization and, as shown recently, proplatelet formation (1–3). Its clinical effectiveness in essential thrombocythemia (ET) was established by several centers (4–10). Consistent with its lack of effect on myeloid progenitors, anagrelide does not reduce leukocyte counts, although anemia has emerged as a frequent adverse effect of anagrelide therapy (6, 7, 10). In the PT1 clinical trial, patients randomized to receive hydroxyurea experienced lower frequency of arterial vascular events but higher incidence of venous thrombosis compared to those allocated to anagrelide, whereas the latter experienced excess bleeding and higher frequency of myelofibrotic transformation (11). However, these differences were not seen in the ANAHYDRET study (12). Concerns about the potential for this drug to promote bone marrow fibrosis have prompted the recommendation of performing periodic follow-up bone marrow biopsies during treatment (13).

Whereas hydroxyurea remains the first-line therapy for patients with essential thrombocythemia (ET) (14), anagrelide was, according to the EXELS study, the second most commonly used cytoreductive drug for high-risk patients across european countries (15). It is most commonly used in patients refractory or intolerant to hydroxyurea or in younger patients in whom concerns about the leukemogenic potential of hydroxyurea may be an issue.

Clinical and laboratory phenotype of patients with ET is strongly influenced by the presence of *JAK2*V617F, *CALR,* or *MPL* mutations (16–19). Furthermore, it has been shown that *JAK2*V617F-positive patients show increased sensitivity to hydroxyurea and achieve better control of platelet counts, while requiring lower doses (16), indicating that molecular subsets may show different response to therapy.

In this study, we report the long-term follow-up of patients with ET treated with anagrelide at a single institution and analyze whether mutation status influences the response rate and development of adverse events in this cohort.

Patients and methods

Patients

Consecutive unselected patients with a diagnosis of ET followed at the Instituto de Investigaciones Médicas Alfredo Lanari, who were started on anagrelide therapy between march 1993 and June 2010 and received at least one-month treatment were included in this retrospective study. Patients were eligible for anagrelide treatment if they fulfilled one of the following criteria: age >60 yrs, history of thrombosis and/or major hemorrhage and/or platelet counts higher than 1000×10^9/L. Diagnosis was established according to the criteria used at the time of diagnosis (20). In an attempt to correct for changes in diagnostic criteria over time, bone marrow biopsy, which had been performed in all patients at diagnosis, was revaluated when available. For this study, only patients with absent or mild reticulin fibrosis or classified as grade 0–1 on a 0–3 scale (21) and the absence of two of the minor criteria required for a diagnosis of primary myelofibrosis (PMF) according to the WHO criteria (22), comprising anemia, leukoerythroblastic peripheral blood picture, splenomegaly, increased LDH and constitutional symptoms, were included. Follow-up bone marrow biopsies were performed in patients in whom transformation to myelofibrosis was presumed because of suggestive clinical or laboratory features. Post-ET myelofibrosis (MF) was diagnosed according to the WHO criteria based on the presence of increased bone marrow fibrosis respect to baseline and fibrosis grades 2–3 on a three-graded scale and at least two of the following criteria: anemia, leukoerythroblastic peripheral blood picture, splenomegaly, increased LDH and constitutional symptoms (23). The study was approved by the Institutional Ethics Committee and is in accordance with the Helsinki Declaration.

Molecular studies

DNA was isolated by standard procedures from peripheral blood leukocytes. Screening for *JAK2* V617F and *MPL* W515L, W515K and S505N mutations was performed by allele-specific PCR, as described (17, 24). For analysis of *CALR* exon 9 mutations, a fragment of the gene was amplified (25) and the selected PCR product was purified from the agarose gel to sequence the entire exon 9. Bidirectional Sanger sequencing was done using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) and run in a 3500 Genetic Analyzer (Applied Biosystems).

Anagrelide treatment

Anagrelide starting dose was 2 mg daily administered in four divided doses of 0.5 mg each for the first 7 d and then titrated for each patient individually to achieve and maintain optimal platelet response. Dose was increased by no more than 0.5 mg/d each week to achieve optimal control of platelet counts. During maintenance therapy, anagrelide was administered in divided 0.5–1 mg doses, two to four times daily, according to total daily dose and never exceeded 4 mg/d or 1 mg/dose. Platelet counts were monitored at weekly intervals during the first month. After achievement of stable platelet counts, platelets were assessed every two or three months. No other cytoreductive drugs were administered concomitantly to anagrelide treatment. Aspirin 100 mg daily was indicated in patients with $<1000 \times 10^9$/L platelets who had a history of previous arterial thrombosis, the presence of cardiovascular risk factors or microvascular disturbances persisting after normalization of platelet counts or at the discretion of the physician. Oral anticoagulants were prescribed in patients with venous thrombosis.

Evaluation of clinical and laboratory features

Clinical events, laboratory parameters and adverse events were assessed retrospectively by chart review. Platelet counts were evaluated by phase contrast microscopy. European LeukemiaNet (ELN) clinicohematologic response criteria were applied (26). Complete response was considered as platelet counts below 400×10^9/L, white blood cell counts below 10×10^9/L, no disease-related symptoms, and normal spleen size. Partial response in patients not fulfilling criteria for complete response but platelets lower than 600×10^9/L or platelet decrease greater than 50% from baseline and no response, any response that did not satisfy criteria for partial response. Thrombotic episodes other than superficial thrombophlebitis were considered major. Microvascular disturbances included erythromelalgia and/or photopsia. All bleeding events except for brief epistaxis, nonextensive ecchymoses, and gingival hemorrhage were recorded. Major

haemorrhage was defined as that associated with a decrease in hemoglobin ≥20 g/L or overt hemorrhage requiring blood transfusion. Adverse events were classified according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Those reported in the first month of anagrelide therapy were considered to be early adverse events, whereas those that continued to be reported after the first month of treatment constituted late side effects.

Statistical analysis

Continuous variables between groups were analyzed by either *t*-test or Mann–Whitney *U*-test (for comparison of two groups) or by one-way ANOVA followed by Tukey's multiple comparisons test or Kruskal–Wallis test followed by Dunn's multiple comparisons test (for comparison of three groups), while the chi-square or Fisher's exact test were used to analyze categorical variables. Two-sided *P*-values <0.05 were considered significant. Differences in the rate of thrombosis and bleeding were calculated by using the log–rank test. Myelofibrosis-free survival curve was prepared by the Kaplan–Meier method. Analysis was performed using the GraphPad Prism 5 (La Jolla, CA, USA) and Stata 11 (College Station, TX, USA) softwares.

Results

Patients

Seventy patients were analyzed. After thorough review, three patients were excluded because of grade 2 or 3 bone marrow fibrosis associated with clinical or laboratory features consistent with primary myelofibrosis (PMF) and sixty-seven patients were available for further analysis. Patient features are listed in Table 1. Fifty-four (80.6%) patients were women. Age at diagnosis and at the start of anagrelide treatment was 37 (9–80) and 38.3 (10–80) years old, respectively. The young age of this cohort reflects the fact that young patients were referred from other centers for anagrelide treatment. Thirty-one (46.3%) were *JAK2*V617F-positive, while among *JAK2*V617F-negative patients, only one (1.5%), whose clinical course has been previously described (25), harboured the *MPL*W515L mutation and 19 (28.3%) were found to have exon 9 *CALR* mutations. Among these, 11 (57.9%) patients displayed the type 1 *CALR* variant, which consists of a 52-bp deletion (p.L367 fs*46), and 7 (36.8%) had the type 2 *CALR* variant, 5-bp TTGTC insertion (p.K385 fs*47), while one patient carried a 46-bp deletion resulting in Q365 fs*50. Triple-negative patients, for example those negative for *JAK2*V617F, *MPL,* and *CALR* mutations, comprised 23.9% of the total population. Compared to *JAK2*V617F, *CALR* mutations were associated with lower hemoglobin values ($P = 0.0002$), lower leukocyte counts ($P = 0.02$), and less frequent thrombotic events ($P = 0.003$). Triple-negative patients were younger ($P = 0.002$) and displayed higher platelet counts ($P = 0.004$) than *JAK2*V617F$^+$ patients, while differing significantly from *CALR*$^+$ patients regarding hemoglobin levels, which were higher in the former ($P = 0.04$).

Bone marrow fibrosis was revaluated in 27 patients in whom initial bone marrow biopsy was available for revision and classified as grades 0 or 1 on a 0–3 scale, while in 40 patients in whom bone marrow biopsy was not available for revision, reticulin fibrosis had been reported to be absent or mildly increased. Overall, fibrosis was grade 0 or absent in 50 (74.6%) patients, while 17 (25.4%) patients had grade 1 or mild reticulin fibrosis. Forty-one patients had been previously treated with hydroxyurea [28] or alpha interferon (5), while eight patients had received more than one sequential treatment, including hydroxyurea, alpha interferon, radioactive phosphorus (1), and lomustine (1). Overall, follow-up since diagnosis was 110 (12–281) months.

Table 1 Clinical and laboratory features of 67 patients with essential thrombocythemia

	Total	*JAK2*V617F$^+$	*CALR*$^+$	Triple-negative	*MPL*$^+$	*P* value
Number of patients, *n* (%)	67	31	19	16	1	–
Female, *n* (%)	54 (80.6)	25 (80.6)	17 (89.5)	11 (68.7)	1	0.3
Age at diagnosis (yrs)	37 (9–80)	45 (15–80)	30 (17–68)	30 (9–60)	40	**0.01**
Platelet count at diagnosis ($\times 10^9$/L)	1250 (608–3742)	1016 (608–3742)	1330 (870–2150)	1524 (900–2240)	780	**0.01**
Hemoglobin at diagnosis (g/L)	133.5 (101–172)	138.0 (116–172)	121.0 (101–145)	130 (110–150)	133	**0.0004**
Leukocyte count at diagnosis ($\times 10^9$/L)	8.80 (5.5–17.4)	9.4 (6.4–17.4)	8.4 (5.8–12.8)	9.2 (5.5–16.8)	6.5	0.09
Prior thrombosis, *n* (%)	14 (20.9)	11 (35.5)	0	2 (12.5)	1	**0.006**
Microvascular disturbances, *n* (%)	24 (35.8)	11 (35.5)	8 (42.1)	4 (25)	1	0.5
Prior haemorrhage, *n* (%)	9 (13.4)	4 12.9)	2 (10.5)	2 (12.5)	1	1.0
Prior cytoreductive treatment, *n* (%)	41 (61.2)	20 (64.5)	10 (52.6)	10 (62.5)	1	0.7
Patients receiving aspirin, *n* (%)	40 (59.7)	20 (64.5)	10 (52.6)	9 (56.2)	1	0.7
Follow-up (months)	110 (12–281)	105 (15–219)	115 (29–264)	98 (12–281)	120	0.6

Values are reported as median (range).

P values show comparison between *JAK2*V617F$^+$, *CALR*$^+$, and triple-negative patients. Bold values indicate $P<0.05$. The small number of *MPL*$^+$ patients did not justify inclusion in statistical analysis.

Treatment response to anagrelide

Time from diagnosis to the beginning of anagrelide was 14 (1–124) months, while overall duration of anagrelide treatment was 68 (4–176) months. Overall response rate was 95.5% whereas complete response, according to ELN criteria, was achieved in 49.25% in this cohort, 46.25% had a partial response and 4.5% had no response to anagrelide. The proportion of patients achieving complete vs. partial vs. no response did not differ among *JAK2*V617F$^+$, *CALR*$^+$, and triple-negative patients (Fig. 1). Time to response was 14.9 ± 10.7 d and tended to be longer for *CALR*$^+$ compared to *JAK2*V617F$^+$ and triple-negative patients, 20.7 ± 13.2, 13.4 ± 9.5, and 12.8 ± 8.6 d, respectively, P = NS. Responses were maintained during follow-up. Administered maintenance dose did not vary significantly over time, 1.71 ± 0.54, 1.68 ± 0.72, and 1.56 ± 0.51 mg daily at 1, 3, and 5 yrs, respectively, P = NS, without differences among molecular subgroups (data not shown), indicating a sustained effect of anagrelide. The mean maximum and minimum daily dose was 3.6 and 0.5 mg, respectively. White blood cell counts did not vary significantly over time and were 8.8 (3.4–18), 8.7 (4.5–23) and 9.56 (3.3–34) × 10^9/L before anagrelide and at 2- and 5-yr follow-up, respectively, P = NS, consistent with the fact that anagrelide has no effect on myeloid progenitors.

Thrombohemorrhagic events during anagrelide treatment

Before anagrelide was started, 14 (21%) patients had experienced major thrombotic events, which were more frequent in *JAK2*V617F$^+$ than *JAK2*V617F$^-$ patients, 35.5% vs. 8.3%, P = 0.01, OR 6.05 (95% CI 1.5–24.35), as previously described for this cohort (17). These episodes occurred at the time or shortly after diagnosis, 3 yrs after diagnosis and 1 yr before diagnosis in 11, 1, 1, and 1 patient, respectively. During treatment with anagrelide, 4 (6%) patients had major vascular events, Table 2. Two of these four patients had had previous thrombosis, before anagrelide was started, whereas in the two others the first thrombotic event occurred after the onset of treatment, although one of them had transiently discontinued anagrelide and had high platelet counts at the time of the thrombotic event. During the observation time following diagnosis to the start of anagrelide, the rate of thrombosis was 2.15 per 100 patient-years, whereas the rate of thrombosis during treatment was one per 100 patient-years, P = NS. The frequency of bleeding episodes did not increase during treatment with anagrelide. Before the start of anagrelide, 9 (13.4%) patients had experienced bleeding episodes, whereas 6 (8.9%) patients had bleeding during anagrelide treatment. The rate of mayor bleeding was 1.8 vs. 0.75 per 100 patients-year, respectively, P = NS. Interestingly, all patients who bled during anagrelide were concomitantly treated with aspirin or oral anticoagulants. Microvascular disturbances, including acroparesthesia and photopsia, persisted during treatment in a significant proportion (28%) of patients, occurring at normal or mildly elevated platelet counts.

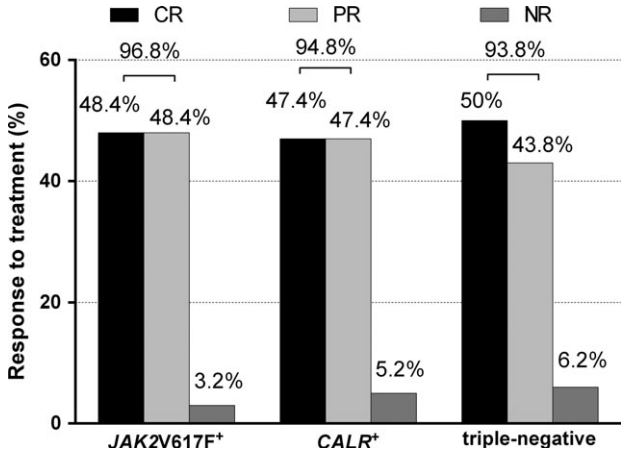

Figure 1 Response to anagrelide treatment in *JAK2*V617F$^+$, *CALR*$^+$, and triple-negative patients according to the European LeukemiaNet criteria (26). Bars represent proportion of patients achieving complete (CR) and partial response (PR) and those who had no response (NR) to anagrelide among molecular subgroups, P = NS, Fisher's exact test.

Hemoglobin decline during treatment with anagrelide

Hemoglobin levels decreased progressively during treatment with anagrelide, hemoglobin values at diagnosis, at 2- and 5-yr follow-up were 134.4 ± 14, 119.8 ± 13, and 117.5 ± 14 g/L, respectively, $P < 0.0001$. The decline in hemoglobin tended to be steeper in *JAK2*V617F$^+$ than in *CALR*$^+$ and triple-negative patients (Fig. 2). The difference between pretreatment hemoglobin and values at 2-yr follow-up was 16.1 ± 2, 13.5 ± 2, and 11.3 ± 2 g/L for

Table 2 Major vacular events occurring before and during treatment with anagrelide

	Before anagrelide	During anagrelide
Arterial thrombosis	10	4
Stroke	5	2
TIA	3	1
AMI	3	0
PAD	0	1
Venous thromboembolism	5	0
SVT	3	0
PTE/DVT	2	0

TIA means transient ischemic attack; AMI, acute myocardial infarction; PAD, peripheral arterial disease; SVT, splanchnic venous thrombosis; PTE, pulmonary embolism; DVT, deep venous thrombosis.

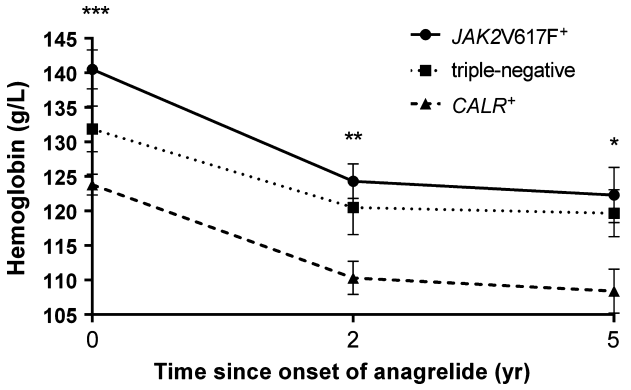

Figure 2 Decline in hemoglobin during the course of anagrelide treatment in *JAK2*V617F$^+$ and *CALR*$^+$ positive and triple-negative patients. Statistical analysis reveals significant differences in hemoglobin values among molecular subsets at all time points, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, one-way ANOVA.

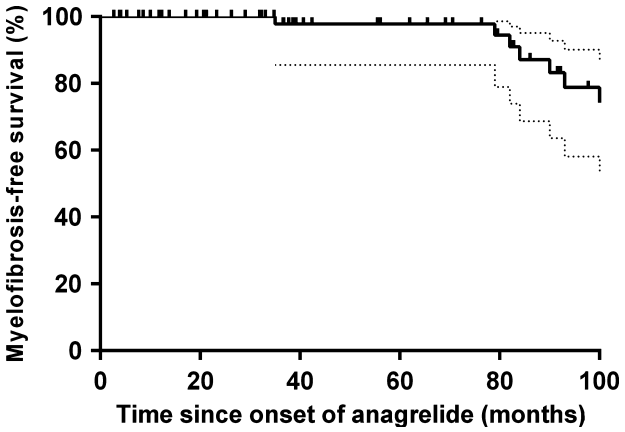

Figure 3 Myelofibrosis-free survival as the onset of anagrelide in patients with essential thrombocythemia. Dashed lines indicate 95% confidence interval.

*JAK2*V617F$^+$, *CALR*$^+$, and triple-negative patients, respectively, whereas the decline at 5 yrs was 19.1 ± 2, 15.5 ± 4, and 13.7 ± 3 g/L, respectively, although differences were not significant at any time point. Higher (>135 g/L) initial hemoglobin levels at the start of anagrelide treatment was the only variable found to be significantly associated with greater probability of fall in hemoglobin (>20 g/L) at 2 and 5 yrs, OR 5.62 (95% CI: 1.40–22.5) and 5.25 (95% CI: 1.15–23.9), respectively, whereas no difference was found between patients who had mild or grade 1 compared to absent reticulin fibrosis (data not shown).

Anemia (hemoglobin values less than 110 g/L) developed in 20.8% and 30.3% of patients after 2 and 5 yrs of treatment, respectively, and was mild to moderate in most cases. Although a trend toward a greater reduction in hemoglobin levels was seen for *JAK2*V617F$^+$ patients, anemia was more frequent in those positive for *CALR* mutations, 46.15% vs. 11.43%, $P = 0.015$ and 60% vs. 11.43%, $P = 0.035$ at 2- and 5-yr follow-up, respectively, probably due to the fact that hemoglobin was lower at treatment onset for this latter subgroup. Anemia did not improve after anagrelide dose reduction but a rise in hemoglobin values was obtained after anagrelide discontinuation in some patients.

Transformation to postessential thrombocythemia myelofibrosis

Transformation to post-ET MF occurred in 10 (14.9%) patients, Fig. 3, and was 12.9%, 21%, and 12.5% for *JAK2*V617F$^+$, *CALR*$^+$, and triple-negative patients, respectively, P = NS. Initial bone marrow findings were revaluated in 7 of 10 patients who developed overt myelofibrosis during treatment with anagrelide and revealed grade 0 fibrosis in 4 and grade 1 fibrosis in 3, whereas fibrosis had been reported to be absent in the remaining three patients in whom initial bone marrow was not available for revision.

Median follow-up from diagnosis to myelofibrotic transformation was 102.5 (51–155) months, while overall follow-up for patients who did not develop myelofibrosis was 94 (12–281) months, P = NS. In the group with post-ET MF, duration of anagrelide treatment before transformation to myelofibrosis was 91.5 (35–138) months, while patients who did not develop myelofibrosis received anagrelide for 58 (4–153) months, $P = 0.02$. Patients who received anagrelide longer than 60 months were at higher risk of developing myelofibrosis, OR 9.32 (1.1–78.5), $P = 0.01$. Other than duration of anagrelide treatment, other factors, such as age, history of thrombosis, platelet and leukocyte counts at diagnosis, reticulin grade at diagnosis, anagrelide dose, and previous treatments, did not differ significantly between patients with or without myelofibrotic transformation (data not shown). After diagnosis of post-ET MF, anagrelide was discontinued in all cases and four patients were treated with hydroxyurea, three with alpha interferon whereas three did not receive cytoreductive treatment. Two patients were lost to follow up. Of eight evaluable patients, two patients experienced increase in hemoglobin values, normalization of LDH levels and peripheral blood smear abnormalities and decrease in spleen size after switching anagrelide for hydroxyurea, indicating improvement of myelofibrosis-associated features. Two other patients had mild increase in hemoglobin levels, while no clinical improvement was observed in the remaining patients. Bone marrow biopsy was not repeated after anagrelide discontinuation. During follow-up, two of the patients with myelofibrosis required treatment with erythropoietin because of anemia and one of them developed thrombocytopenia. All patients with post-ET MF remain alive after 58 (28–84) months following anagrelide discontinuation. There

were no leukemic transformations during the follow-up period in this cohort and 5 (5.9%) patients died whereas 6 (8.9%) patients were lost to follow up.

Toxicity

In this cohort of ET patients who tolerated anagrelide treatment for at least 1 month, its side-effect profile was similar to that reported in previous studies (1, 4–10). Early and late non-hematologic adverse events, including headache, diarrhea, palpitations, and edema, were mainly grades 1–2 and occurred in 79.1% and 32.8%, respectively, were well tolerated and very rarely led to anagrelide discontinuation. Only one patient had a severe cardiovascular adverse event, consisting in ventricular tachycardia. During long-term treatment, anemia was, as detailed above, the most frequent hematologic adverse event. No thrombocytopenia was observed during treatment. A high rate of anagrelide discontinuation was noted and was accounted for by several underlying reasons (Table 3).

Discussion

Treatment options for ET remain limited. Whereas hydroxyurea represents the cytoreductive treatment of choice, there is no clear consensus regarding choice of second-line therapy (14). This represents an important issue considering that a significant proportion of patients are refractory or intolerant to hydroxyurea. Among second-line therapies, options include anagrelide and pegylated alpha interferon (27). In this study, we confirm that anagrelide is effective in reducing platelet counts, with a rapid onset of action in high-risk patients with essential thrombocythemia. A high rate of response was shown for all molecular subsets, with a similar proportion of patients achieving complete vs. partial response. The rate of thrombosis was similar to that of a recently reported cohort of patients treated with anagrelide (10), whereas the frequency of hemorrhagic events did not increase during treatment, in accordance with the aforementioned study (10).

A remarkable feature of this study was the progressive decline in hemoglobin seen during anagrelide treatment, which occurred early (within a few months) and worsened slowly but progressively thereafter. The only variable associated with greater decline in hemoglobin levels was higher hemoglobin values at the start of treatment, suggesting that greater expansion of the erythroid lineage, as seen in *JAK2*V617F-positive ET patients, may render erythroid progenitors more susceptible to the mechanisms underlying anagrelide-induced anemia, which at present remain largely unknown. Despite deeper hemoglobin decline in the *JAK2*V617F$^+$ group, development of anemia was a more frequent finding in *CALR*$^+$ patients, indicating that this subgroup might be at particularly higher risk of developing anemia. Anemia was mild to moderate and well tolerated in most patients.

Table 3 Reasons for anagrelide discontinuation

Anagrelide discontinuation for any cause, *n* (%)	26 (38.8)
Discontinuation due to myelofibrotic transformation, *n* (%)	10 (14.9)
Discontinuation due to anemia, *n* (%)	2 (3)
Discontinuation due to adverse events, *n* (%)	1 (1.5)
Discontinuation due to planned pregnancy, *n* (%)	7 (10.5)
Discontinuation due to minor or no response, *n* (%)	3 (4.5)
Others causes of discontinuation	3 (4.5)

The frequency of myelofibrotic transformation after 9-yr follow-up was 14.9% and was independent of mutation status. The reported frequency of post-ET MF is variable, ranging from 4 to 8% at 10 yrs and 9 to 15% at 15 yrs (28–30). The possible influence of anagrelide in the relatively higher frequency of myelofibrosis in this cohort cannot be ascertained because of lack of a control group. Whereas in the PT1 trial, in which diagnosis was based on the PVSG criteria, the frequency of post-ET MF was higher in patients receiving anagrelide vs. hydroxyurea (11), no differences were found in the more recent ANAHYDRET study, which included only patients with WHO-diagnosed 'true ET' (12). Current WHO criteria distinguish 'true ET' and early/prefibrotic PMF as two distinct clinicopathological entities (31), with a higher risk of overt myelofibrosis for the latter (32). Although none of the patients in this cohort fulfilled the WHO criteria for PMF at initial presentation (22), patients with prefibrotic/early PMF could have been included, which may have contributed to the higher frequency of overt myelofibrosis found. Considering that reproducibility of WHO histopathologic criteria in certain settings may be challenging (33, 34), availability of biomarkers for accurate differential diagnosis between 'true ET' and early/prefibrotic PMF would help address this issue. Careful follow-up of patients receiving anagrelide, particularly those treated for extended periods of time, searching for features suggestive of myelofibrotic transformation, may identify patients at risk and dictate early treatment discontinuation.

In conclusion, anagrelide yielded a high rate of durable platelet responses among all molecular subgroups. Anemia was the most frequent side effect of long-term therapy and *CALR*-mutated patients were found to be at particular risk. Longer duration of anagrelide treatment was identified as a risk factor for myelofibrotic transformation, indicating the need for bone marrow monitoring in patients who receive anagrelide over long periods of time. Mutational status does not seem to represent a major determinant of choice of cytoreductive treatment among essential thrombocythemia therapies.

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