Combination therapy of hydroxycarbamide with anagrelide in patients with essential thrombocythemia in the evaluation of Xagrid® efficacy and long-term safety study

by Luigi Gugliotta, Carlos Besses, Martin Griesshammer, Claire Harrison, Jean-Jacques Kiladjian, Ruth Coll, Jonathan Smith, Brihad Abhyankar, and Gunnar Birgegård

Haematologica 2013 [Epub ahead of print]

doi:10.3324/haematol.2012.083097

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors’ final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Combination therapy of hydroxycarbamide with anagrelide in patients with essential thrombocythemia in the evaluation of Xagrid® efficacy and long-term safety study

Luigi Gugliotta,1 Carlos Besses,2 Martin Griesshammer,3 Claire Harrison,4 Jean-Jacques Kiladjian,5 Ruth Coll,6 Jonathan Smith,6 Brihad Abhyankar,7 and Gunnar Birgegård8

1Department of Hematology “L. e A. Seragnoli”, St Orsola-Malpighi Hospital, Bologna, Italy
2Department of Hematology, Hospital del Mar-IMIM, Barcelona, Spain
3Hematology and Oncology, Johannes Wesling Klinikum Minden, Minden, Germany
4Department of Hematology, Guy’s and St Thomas’ NHS Foundation Trust, London, United Kingdom
5AP-HP, Hôpital Saint-Louis, Centre d’Investigations Cliniques, Paris, France
6Shire Pharmaceuticals Ltd, Basingstoke, United Kingdom
7Former employee of Shire Pharmaceuticals Ltd, Basingstoke, United Kingdom, and
8Department of Hematology, Uppsala University, Uppsala, Sweden

Running head: Hydroxycarbamide + anagrelide in the EXELS study
Correspondence: Luigi Gugliotta, Department of Hematology, “L. e A. Seragnoli”,
St Orsola-Malpighi Hospital, 40138 Bologna, Italy.
E-mail: luigi.gugliotta@unibo.it.

Trial registration: clinicaltrials.gov identifier: NCT00567502; Protocol No.: SPD422-401
Acknowledgments

The study was designed by the international EXELS steering committee (J-J Kiladjian, C Besses, M Griesshammer, L Gugliotta, C Harrison), chaired by G Birgegård. Under the direction of the authors, Kerry Acheson and Sasha Mitchell, employees of iMed Comms, provided writing assistance for this publication. Editorial assistance in formatting, proofreading, copy editing, and fact checking was also provided by iMed Comms. iMed Comms was funded by Shire for support in writing and editing this manuscript. Although the sponsor was involved in the design, analysis, interpretation, and fact checking of information, the content of this manuscript, the ultimate interpretation, and the decision to submit it for publication in Haematologica was made by the authors independently. The authors acknowledge the contribution of all investigators who participated in this study (Appendix).

Funding

The study was supported by Shire Development LLC, the sponsor, and was agreed with the European agency as a Post Approval Commitment and overseen by the international EXELS steering committee (J-J Kiladjian, C Besses, M Griesshammer, L Gugliotta, C Harrison), chaired by G Birgegård. Editorial assistance in writing, formatting, proofreading, copy editing, and fact checking was provided by iMed Comms and funded by Shire.
Abstract

Limited information is available regarding the use of cytoreductive combination therapy in high-risk patients with essential thrombocythemia. This analysis aims to evaluate the clinical relevance and patterns of cytoreductive combination treatment in European high-risk patients with essential thrombocythemia in the Evaluation of Xagrid® Efficacy and Long-term Safety study. From 3643 patients, 347 (9.5%) received combination therapy. Data were recorded at each 6-month update. Of 347 patients who received combination therapy, 304 (87.6%) received hydroxy-carbamide + anagrelide. Monotherapies received before this combination were hydroxy-carbamide (n=167; 54.9%) and anagrelide (n=123; 40.5%). Median weekly doses of hydroxy-carbamide and anagrelide were: 7000 and 10.5 mg when used as prior monotherapy; 3500 and 7.0 mg when used as add-on treatment. Overall, median platelet counts were 581x10⁹/L and 411x10⁹/L before and after starting hydroxy-carbamide + anagrelide, respectively. In patients with paired data (n=153), the number of patients with platelet counts < 400x10⁹/L increased from 33 (21.6%) to 74 (48.4%, P<0.0001), and with platelet counts < 600x10⁹/L, from 82 (53.6%) to 132 (86.3%, P<0.0001). Hydroxy-carbamide + anagrelide was discontinued in 158 patients: 76 (48.1%) stopped hydroxy-carbamide, 59 (37.3%) stopped anagrelide, 19 (12.0%) stopped both and 4 (2.5%) had another therapy added. The most frequent reasons for discontinuation were intolerance/side effects, lack of efficacy, and therapeutic strategy. Combination therapy, usually hydroxy-carbamide + anagrelide, is used in approximately 10% of all high-risk patients with essential thrombocythemia and may be a useful approach in treating patients for whom monotherapy is unsatisfactory. This trial is registered on clinicaltrials.gov (NCT00567502).

Key words: Essential thrombocythemia, anagrelide, hydroxy-carbamide, combination therapy, EXELS study.
Introduction

Essential thrombocythemia (ET) is a chronic myeloproliferative neoplasm characterized by proliferation of megakaryocytes and an increased risk of developing thrombohemorrhagic complications. Current therapy for ET is not curative, and is therefore guided by the need to minimize the incidence of thrombohemorrhagic events, to control disease-related symptoms, and to reduce primary and iatrogenic disease progression, where possible.

The primary goal of cytoreductive therapy, recommended for patients with ET categorized as high-risk (> 60 years, or platelet count > 1500x10⁹/L, or a history of thrombohemorrhagic events), is to attain a complete clinicohematologic response (platelet count ≤ 400x10⁹/L, no disease-related symptoms, normal spleen size, white blood cell [WBC] count < 10x10⁹/L).

According to European LeukemiaNet (ELN) guidelines, hydroxycarbamide (HC) is currently recommended as first-line therapy in high-risk patients with ET, although its use should be carefully considered in younger patients (< 40 years old). Anagrelide is indicated as second-line therapy in Europe for high-risk patients with ET who are intolerant to their current first-line therapy. Other second-line therapies for management of ET include busulfan (licensed indication), interferon-α (IFN; unlicensed indication), and pipobroman (unlicensed indication).

Long-term treatment with cytoreductive agents can be accompanied by side effects often leading to dose reductions, which may in turn lead to reduced efficacy. As a means to overcome inadequate efficacy, or to avoid dose-limiting toxicities with monotherapy, combination therapy of two cytoreductive drugs, usually HC plus anagrelide, has been reported by the Anagrelide Study Group in around one-fifth of treated patients with ET. Moreover, a combination of anagrelide with either HC or IFN has been mentioned as a practical option for treatment of selected patients with ET and recent clinical data are now available. There are currently no guidelines in place to guide the use of combination therapy in ET, thus the decision to undertake this treatment is at the discretion of the treating physician.

Evaluation of Xagrid® Efficacy and Long-term Safety (EXELS) is a post-approval commitment observational study designed to monitor the safety and pregnancy outcomes of anagrelide and other cytoreductive therapies in a large European cohort of patients with ET. In the EXELS study, a cohort of patients was identified as being treated with anagrelide in combination with another cytoreductive drug. The aim of this subanalysis is to describe the use of combination therapy in patients with ET, with a focus on HC + anagrelide, and to discuss its role in clinical practice.
Methods

**Trial design**

EXELS is an ongoing phase IV, observational, multicenter, safety study in high-risk patients with ET being treated with cytoreductive therapy (NCT00567502).

The primary objective of the EXELS study is to monitor safety and pregnancy outcomes of anagrelide and other cytoreductive therapies in high-risk patients with ET. Secondary objectives include assessment of efficacy (platelet reduction and incidence of thrombohemorrhagic events) and drug utilization (drug type, drug dose, and duration of exposure).

The study is being conducted in 13 European countries: Denmark, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Norway, Portugal, Spain, Sweden, and the United Kingdom. All participating centers obtained ethical approval prior to enrolling patients and the study was conducted in accordance with the Declaration of Helsinki.

The first patient was registered in May 2005, and the last in April 2009; study completion is expected in June 2014. The analyses described here were performed on a planned data-cut in September 2011, 2.5 years after registration of the last patient.

**Participants**

Patients with a diagnosis of ET (according to Polycythemia Vera Study Group\textsuperscript{17} or World Health Organization\textsuperscript{1} criteria), with one or more high-risk features (> 60 years; history of thrombohemorrhagic events; initial platelet count of > 1000x10\textsuperscript{9}/L) and receiving cytoreductive therapy, were eligible for inclusion in the study. Exclusion criteria were limited to the contraindications listed in the product information for each cytoreductive therapy used. Written, informed consent was obtained from all patients prior to study participation.

**Treatment**

EXELS is a non-interventional study; the choice of cytoreductive therapy was determined prior to study registration and patients were managed according to local clinical practice at the discretion of the investigators.

Data related to thrombohemorrhagic events as collected per the predefined events in the protocol, suspected serious adverse events, platelet count, and any changes to medication were recorded at each 6-month update. Patients with multiple events of the same predefined
event category were only counted once for each treatment received. All safety evaluations and procedures were performed in accordance with routine clinical practice.

**Statistical methods**

Data were collected using an electronic data capture system and were summarized using descriptive statistics. Combination treatments were included in the current analyses if they: involved anagrelide in combination with another cytoreductive therapy; were taken for at least 30 days; and were the first combination received by the patient.

To eliminate data-input errors, a small number of data points were excluded using the following thresholds (considered to be outside of clinical practice): HC weekly doses reported as < 3500 mg or > 35,000 mg; anagrelide weekly doses of > 70 mg; platelet counts of < 10x10^9/L or > 10,000x10^9/L; WBC < 0.5x10^9 or > 150x10^9; hemoglobin (Hb) < 5 g/dL or > 22 g/dL; and hematocrit < 10% or > 70%..

**Results**

A total of 3643 high-risk patients with ET were enrolled in the EXELS study. Patient characteristics prior to starting combination therapy are shown in Table 1. At the time of the planned data-cut in September 2011, 347 patients (9.5%) had received anagrelide in combination with another cytoreductive drug (HC, IFN, pipobroman, or other) at some stage during the observation period (Table 1). HC + anagrelide was the most frequently prescribed combination therapy at registration or as first combination during the follow-up (n=304, 87.6%). The study investigators were not required to provide their reasons for starting patients on combination therapy. However, they did provide reasons for stopping monotherapy and therefore, by inference, the reasons for stopping the combination.

**HC + anagrelide combination therapy – patients**

In the 304 patients receiving HC + anagrelide, prior anagrelide monotherapy had been used in 123 patients (40.5%), HC monotherapy in 167 patients (54.9%), and 14 patients (4.6%) had previously received no cytoreductive therapy. The median duration of prior monotherapy was 54 weeks (range: 0.1-459) with anagrelide, and 139 weeks (range: 1-1431; P=0.0001) with HC.

In the patients receiving HC + anagrelide, 177 (58.2%) were female and the mean age at registration was 60.5 years (mean age 55.4 and 68.7 years in those receiving anagrelide monotherapy or HC monotherapy, respectively). Patients receiving HC + anagrelide were
younger than the overall EXELS population who received monotherapy (60.5 vs. 65.3 years, respectively; \( P=0.0002 \)).

Across the countries included in the study, there was a considerable variation in the percentage of patients receiving HC + anagrelide therapy: Spain had the lowest (15/341; 4.4%) and Greece the highest (53/235; 22.6%) use of combination therapy (Figure 1).

**HC + anagrelide combination therapy – treatment**

The median weekly doses of HC and anagrelide during prior monotherapy were 7000 mg and 10.5 mg, respectively. These initial median doses were not adjusted when a second agent was added. However, the median weekly dose of the added agent was lower during combination treatment than during monotherapy (HC 3500 mg and anagrelide 7 mg).

The median duration of treatment for all patients who had received/continued to receive HC + anagrelide was 91.1 weeks (range: 4.3-723.0). At the time of the data-cut, the median treatment duration for patients who were continuing to take HC + anagrelide was 152.9 weeks (range: 16.0-723.0); median treatment duration for patients who discontinued HC + anagrelide before the data-cut was 30.5 weeks (range: 4.3-491.1).

In total, 196/304 patients (64.5%) received anti-aggregatory therapy (e.g. low-dose aspirin, clopidogrel, or ticlopidine/dipyridamole) prior to receiving HC + anagrelide, and 70.7% (n=215/304) received concurrent anti-aggregatory therapy during combination therapy. Of the patients who stopped combination therapy, 58.9% (n=93/158) received concurrent anti-aggregatory therapy within 1 month.

**HC + anagrelide combination therapy – blood cell counts**

In patients receiving HC + anagrelide, the median platelet count recorded at diagnosis was 1001x10^9/L (range: 432-2401). The last median platelet count recorded up to 6 months prior to starting combination therapy was 581x10^9/L; this was reduced to a median platelet count of 411 and 434x10^9/L at first and last evaluation, respectively, during combination therapy (Table 2). In patients who had received prior HC, median platelet count decreased from 566x10^9/L to 411x10^9/L with the initiation of combination therapy. Similarly, platelet count decreased from 612x10^9/L to 417x10^9/L in patients who had received prior anagrelide. There was no significant difference between the prior treatment groups at any time point (Table 2).

Paired data on platelet counts at 6 months prior to initiation of combination therapy and during the first 6 months of combination therapy were available in 153 cases. Significant increases in the proportion of patients with a platelet count \( \leq 400x10^9/L \) (from 33, 21.6% to
Similarly, when comparing platelet counts at 6 months prior to initiation of combination therapy and the last 6 months of combination therapy (paired data available in 98 cases), significant increases in the proportion of patients with a platelet count $\leq 400 \times 10^9$/L (from 21, 21.4% to 39, 39.8%; $P=0.0044$) and $\leq 600 \times 10^9$/L (from 46, 46.9% to 79, 80.6%; $P<0.0001$) were observed.

Triplicate data on platelet counts at 6 months prior to initiation of combination therapy, during the first 6 months of combination therapy, and during the last 6 months of combination therapy were available for 93 patients receiving HC + anagrelide. Proportions of patients with a platelet count $\leq 400 \times 10^9$/L at 6 months prior to initiation of combination therapy, and during the first 6 months and the last 6 months of combination therapy, were 21 (22.6%), 45 (48.4%), and 38 (40.9%) and proportions with a platelet count $\leq 600 \times 10^9$/L were 45 (48.4%), 80 (86.0%), and 75 (80.6%), respectively. These data were consistent with the paired data tested above.

Overall, similar trends in the proportions of patients reaching platelet counts $\leq 600 \times 10^9$/L were observed when all available data were considered; 52.9%, 79.1%, and 77.0% for before combination, and during the first and final 6 months of combination therapy, respectively (Figure 2). Three cases of thrombocytopenia were reported, of which two led to discontinuation; anagrelide was discontinued in one patient, and HC was discontinued in the other.

The median Hb level of patients receiving HC + anagrelide < 6 months prior to combination start was 11.9 g/dL (range: 8-16). At the time of last testing during combination therapy, median hemoglobin levels were 14.1 g/dL (range: 9-15) in patients with no prior monotherapy (n=6), compared with 10.8 g/dL (range: 7-16) in patients with prior HC monotherapy (n=55) and 12.2 g/dL (8-16) in patients with prior anagrelide monotherapy (n=33). At the time of last testing during combination therapy, there was a greater proportion of patients with Hb levels < 10 g/dL among patients who had received prior monotherapy with HC (n=18/87; 20.7%) than with anagrelide (n=5/55; 9.1%). The median minimum Hb levels were similar between patients who achieved a platelet response versus those who did not achieve a platelet response both during the first 6 months and the last 6 months of combination therapy (all between 10.3 and 10.9 g/dL).

The median WBC count in patients receiving HC + anagrelide at 6 months prior to initiation of combination therapy was $7.0 \times 10^9$/L (range: 2-44) and was similar to that observed during the first 6 months of combination therapy, $7.8 \times 10^9$/L (range: 2-61) and the last 6 months of combination therapy, $7.1 \times 10^9$/L (range: 1-35). At the time of last testing during combination therapy...
therapy, 29/121 (24.0%) patients had a WBC count ≥ 10x10⁹/L. There was a greater proportion of patients with a WBC count ≥ 10x10⁹/L among those who had received prior monotherapy with anagrelide (n=14/45; 31.1%) than with HC (n=13/68; 19.1%).

**Safety and tolerability**

Predefined events were reported in 39 patients (12.8%) receiving HC + anagrelide as first combination, compared with 765 patients (21.0%) across the entire EXELS study population (n=3643; Table 3). The predefined events of cardiovascular symptoms (palpitations, tachycardia, hypotension, light headedness, dizziness, syncope, dyspnea on exertion, or peripheral edema) were the most frequently reported, and occurred in 15 patients (4.9%) receiving HC + anagrelide as first combination and 152 patients (4.2%) in the total EXELS population. Transformations (including acute myeloid leukemia, myelofibrosis, and polycythemia vera) were the next most common, occurring in 7 patients (2.3%) receiving HC + anagrelide compared with 129 patients (3.5%) across the entire EXELS study population. Death not attributed to a predefined event occurred in 4 patients (1.3%) in the HC + anagrelide group compared with 131 (3.6%) patients in the overall EXELS population. All other predefined events had an incidence of < 2.0% in both the HC + anagrelide group and the total EXELS population (Table 3). The major thrombotic event rate in the HC + anagrelide group was 1.43% per patient year, and was higher in patients who stopped combination therapy compared with patients who continued combination therapy (2.89% vs. 0.82% per patient year). The major thrombotic rate was similar between patients who received prior anagrelide or HC therapy (0.41% and 0.31% per patient year, respectively).

A total of 158 (52.0%) patients discontinued HC + anagrelide combination therapy (Table 4); of these, HC was discontinued in 76 patients (48.1%), anagrelide was discontinued in 59 patients (37.3%), and both therapies were discontinued in 19 patients (12.0%). In a further 4 patients an additional therapy was added to their existing combination. Of the 95 patients who had received prior HC, 41 (43%) discontinued HC, 37 (39%) discontinued anagrelide, 13 (14%) discontinued both agents and 4 (4%) had an additional therapy added. Of the 58 patients who had received prior anagrelide, 32 (55%) discontinued HC, 21 (36%) discontinued anagrelide and 5 (9%) discontinued both agents. The most common reasons reported by the investigators as causes for discontinuation were intolerance or side effects (n=75/158, 50.0%), lack of efficacy (n=35/158, 22.2%), and therapeutic strategy (including change of treatment) (n=34/158, 21.5%). In patients who had received prior HC, the frequency of discontinuation of either anagrelide or HC because of intolerance or side effects was similar (n=18/37, 49% and n=19/41, 46%, respectively). Intolerance or side effects was
also the most frequent reason for discontinuing anagrelide or HC in patients who had received prior anagrelide (n=12/21, 57% and n=16/32, 50%, respectively).

At the time of the data-cut, a general trend was observed of increasing proportions of patients receiving HC + anagrelide over time, from registration (3.4%) to 5 years after registration (5.5%).

Discussion

The EXELS study provides valuable evidence of the cytoreductive therapies employed by physicians to treat high-risk patients with ET in a real-world setting across 13 European countries. Data reported elsewhere suggest that European physicians adhere to ELN guidelines and generally prescribe HC as first-line and anagrelide as second-line therapy. However, data from this subanalysis indicate that clinical practice extends beyond the scope of current guidelines to include combination cytoreductive therapy, with or without concurrent anti-aggregatory therapy, in patients who do not respond adequately to monotherapy. The investigators were not required to provide their reasons for starting patients on combination therapy because of the observational nature of the study (just the reasons for stopping a monotherapy and by inference the reason for stopping the combination). However, combination therapy may have been commenced with the aim of improving platelet response without increasing the dose of the monotherapy drug, while attempting to minimize toxicity. In addition, patients who received anagrelide as prior monotherapy may have been started on combination therapy rather than switched to HC because of the leukemogenic risk associated with HC, especially in patients ≤ 60 years. In these cases, adding a low dose of HC may have been considered useful in improving the response without increasing the risk of significant toxicity.

It is notable that irrespective of patients’ initial monotherapy, discontinuations were most frequently attributed to intolerance. Of the patients who had received prior HC, neither HC nor anagrelide was discontinued more frequently. However, in those who had received prior anagrelide, 36% discontinued anagrelide while 55% discontinued HC; this suggests that some patients were being transitioned slowly from HC to anagrelide. Results from a recent study demonstrated that anagrelide was not inferior to HC in preventing thrombotic complications in patients with ET, supporting the rationale for this transition. However, for some patients it is possible that the treatment strategy changed during the course of switching the patients from one therapy to another if the combination was found to be effective.
In the current study, it was noted that patients receiving HC + anagrelide were younger than the overall EXELS population of patients who received monotherapy (60.5 vs. 65.3 years, respectively; \( P = 0.0002 \)). It appears that some physicians may have considered platelet counts of \(< 600 \times 10^9/L\) indicative of insufficient efficacy of monotherapy (median prior to combination therapy was \(581.0 \times 10^9/L\)) and elected to initiate combination therapy. Data suggest additional efficacy was achieved since median platelet counts reduced to \(411.0 \times 10^9/L\) after initiation of combination therapy. It is not known why physicians elected to add a second therapy rather than further increasing the dose of the initial therapy. However, it is reasonable to hypothesize that they had concerns about the tolerability of increasing the dose of the initial therapy and chose to add an agent with different pharmacologic characteristics at a low dose.

Patients had received HC monotherapy for a significantly longer time period than anagrelide monotherapy \((P < 0.0001)\) before beginning combination therapy. It is possible that patients who started on anagrelide were switched to combination therapy more rapidly because they did not obtain a satisfactory response in a relatively short time and/or experienced more intolerable AEs.

Almost 80% of patients achieved platelet levels \(\leq 600 \times 10^9/L\) within 6 months of starting HC + anagrelide, which was a marked increase from 53% prior to the combination. Thus, many patients with an unsatisfactory platelet response on monotherapy achieved an additional platelet-lowering effect with combination therapy. Furthermore, no significant difference in platelet levels was observed at any time point between patients who had received prior HC monotherapy and those who had received prior anagrelide monotherapy, suggesting that the platelet-lowering effects of HC + anagrelide are independent of prior monotherapy.

The ELN guidelines define a complete platelet response as \(\leq 400 \times 10^9/L\) and a partial platelet response as \(\leq 600 \times 10^9/L\).\(^2\) During the period of this study, physicians treating patients in EXELS were probably aiming to achieve complete platelet responses and the responses observed in our study were comparable to those observed in previous prospective studies.\(^{19-21}\) These prospective studies indicated that a platelet response is important in reducing the thrombosis rate. However, recently an expert panel concluded that the ELN response criteria is insufficient as a measure of benefit for patients with ET.\(^22\) This criticism was based on evidence from two retrospective studies that suggested complete clinicohematological responses and platelet count responses do not translate to reduced thrombosis risk.\(^{23,24}\) However, the low thrombosis rate observed in these retrospective studies was similar to that observed in the prospective studies,\(^{19-21}\) which is perhaps not surprising considering the majority of patients in both retrospective studies achieved a complete or partial response.
Therefore, this criticism of the response criteria needs to be supported by prospective studies.

As expected, patients receiving long-term cytoreductive therapy tended to develop some degree of anemia. At the latest time point available, more patients who had received prior HC had Hb levels of 8-10 g/dL than those who had received prior anagrelide; treatment-naïve patients were generally not anemic. These findings suggest that Hb levels are not fully recovered when anagrelide is added to prior HC monotherapy, possibly because the dose of HC is not reduced. Additionally, the lower dose of HC added to anagrelide monotherapy is not sufficient to cause marked anemia. Furthermore, when anagrelide and HC were started at the same time, where it is likely that both agents were given at slightly lower doses than in monotherapy, the patients also appeared to have a reduced risk of HC-induced anemia.

Although the most frequent reason for stopping HC + anagrelide was intolerance/side effects, the majority of patients who discontinued the combination stopped HC and continued with anagrelide monotherapy, similar to findings documented in the Registro Italiano Trombocitemia (RIT) report. This may simply reflect physicians loosely following the recommended treatment algorithm (namely first-line HC, second-line anagrelide). In the current study, the number of patients who received anti-aggregatory therapy remained relatively constant before, during, and following discontinuation of HC + anagrelide therapy. This is perhaps a little surprising, as many of the patients continued with anagrelide monotherapy and the use of anti-aggregatory therapy with anagrelide is often discouraged.25

The overall incidence of predefined events was, unexpectedly, lower in the population receiving the combination than in the overall EXELS population. This may be because patients who received the combination tended to be younger and therefore have a reduced probability of developing concurrent conditions. In this study, the rate of major thrombotic events in the HC + anagrelide group was 1.43% per patient year, which is comparable to the thrombotic event rate reported in previous HC monotherapy studies (1.66%23 and 2.4%24 per patient year).

The findings of this analysis support those of previously published reports of combination cytoreductive therapy. The efficacy and safety of HC + anagrelide combination therapy have been reported in three small studies of patients with ET (Pugliese et al, 2012, n=8; Christoforidou et al, 2008, n=8; D’Adda et al, 2008, n=4). From the limited data available from these studies, the authors concluded that using HC and anagrelide in combination in lower doses than usually prescribed as monotherapy is being used by some physicians in selected patients with ET. Furthermore, in these studies it was found that the
frequency and severity of AEs (in particular, the hematological toxicity of HC) were reduced with combination therapy.

**Summary**

The EXELS study is the largest observational cohort of high-risk patients with ET reported to date. The study provides valuable data for the analysis of current cytoreductive therapies preferred by physicians. It has become evident in this analysis that combination therapy, particularly that of HC + anagrelide, is being employed by physicians in around 10% of patients. Also it was noted that patients received HC and anagrelide in combination, with or without concurrent anti-aggregatory therapy.

These real-world data have highlighted that a switch from monotherapy to HC + anagrelide combination therapy in a subgroup of high-risk patients with ET, while not recommended by current guidelines, is being utilized in clinical practice. Although platelet levels of \( \leq 600 \times 10^9/L \) were achieved in almost 80% of patients receiving HC + anagrelide therapy, combination therapy was discontinued in approximately 50% of patients, most frequently for tolerability issues.

Further studies are warranted to define those patients in whom combination therapy may be an appropriate treatment option.
Appendix

The authors would like to thank all the investigators who participated in the study:

**Denmark:** Ole Weis Bjerrum, Hans Hasselbalch, Carsten Helleberg, Herdis Larsen, Torben Mourits-Andersen, Dorte Ronnov-Jess, Hanne Vestergaard; **Finland:** Eeva Juvonen, Marita Nurmi, Karri Penttila; **France:** Jean-Francois Abgrall, Sylvia Bellucci, Dominique Bordessoule, Jean-Yves Cahn, Natalie Cambier, Nicole Casadevall, Driss Chaoui, Sylvain Choquet, Brigitte Dupriez, Mustapha Kamel Ghomari, Jean-Jacques Kiladjian, Laurence Legros, Michel Leporrier, Gerard Sebahoun, Michel Tulliez, Jean Francois Viallard, Eric Wattel; **Germany:** Annette Bittrich, Martin Griesshammer, Bernhard Heinrich, Erhard Hiller, Georg Jacobs, Hendrik Kroening, Axel Matzdorff, Andreas Mohr, Friedrich Overkamp, Yolanda Rodemer, Burkardt Schmidt, Stephen Schmitz, Frank Stegelmann, Hans Tesch, Wolfgang Weber, Juergen Wehmeyer, Johann Weiss, Manfred Welslau, Wolfgang Zeller; **Greece:** Evangelos Biasoulis, Vasileia Garypidou, Anna Kioumi, Despoina Kyriakou, Eudokia Mandala, Panayiotis Panayiotidis, Helen Papadakis, Basil Seitandidis, Argris Symeonidis, Elina Verversou, Michalis Vougarelis, Panayiotis Zikos; **Ireland:** Gerard Crotty; **Italy:** Alessandro Andriani, Marino Brunori, Emma Cacciola, Silvana Capalbo, Vincenzo Capparella, Luigi Cavanna, Mario Cazzola, Riccardo Centurioni, Felicetto Ferrara, Gianluca Gaidano, Giovanni Garozzo, Riccardo Ghio, Marco Gobbi, Luigi Gugliotta, Eraldo Lanzi, Anna Marina Liberati, Marcellina Mangoni, Guglielmo Mariani, Massimo Martelli, Vincenzo Martinelli, Maria Gabriella Mazzucconi, Vincenzo Mettivier, Pellegrino Musto, Ubaldo Occhini, Alessandro Polacco, Giovanni Quarta, Maria Luigia Randi, Umberto Recine, Giuseppe Rossi, Stefano Sacchi, Giuseppe Saglio, Potito Rosario Scalzulli, Giorgina Specchia, Valerio de Stefano, Alessia Tieghi, Alessandro M Vannucchi, Giuseppe Visani, Alfonso Zaccaria; **Norway:** Waleed Ghanima, Marit Rinde, Tove Skjelbakken; **Portugal:** Pureza Pinto; **Spain:** Alberto Álvarez-Larrán, José Luis Bello, Carlos Besses, Juan Carlos Hernandez-Boluda, Felix Carbonell, Jesus Cesar, Cristalina Fernandez, Jose Julio Hernandez, Luis Hernandez-Nieto, Esperanza Lavilla, Javier Loscertales, Francisca Ferrer Marin, Jose R Mayans, Jesus M Hernandez Rivas, Francisco J de la Serna, Ana Villegas, Blanca Xicoy; **Sweden:** Jesper Aagesen, Tomas Ahlgren, Gunnar Birgegard, Honar Dylman, Peter Johansson, Olle Linder, Eva Lofvenberg, Jan Samuelsson, Kristina Wallman; **The Netherlands:** S Zweegman; **UK:** Sara Ali, Nigel O Connor, Roger Evely, Savio Fernandes, Claire Harrison, Mary F McMullen, Don Milligan, Beverley Paul, Shalal Sadullah, Charles Singer, Chris Tiplady, Peter Williamson.
Authorship and disclosures
LG takes primary responsibility of this paper. LG, J-JK, GB, MG, CH, CB, BA, and RC made substantial contributions to the conception and design of the study. LG, J-JK, GB, MG, CH, and CB contributed to the acquisition of data. JS participated in the statistical analysis and all authors contributed to analysis and interpretation of data, and revising the article critically for important intellectual content. Although the sponsor was involved in the design, collection, analysis, interpretation, and fact checking of information, the content of this manuscript, the ultimate interpretation, and the decision to submit it for publication in Haematologica was made by the authors independently.

LG and J-JK received funding from Shire for contributing to the EXELS study steering committee meetings. LG, J-JK, and GB also received funding for travel costs to the steering committee meetings. The Institution of Medical Sciences, Uppsala University (GB), received an unrestricted grant for a 7-year follow-up study of anagrelide and the present study. CH received money from Shire for medical and educational support, and consulting/honorarium.

Furthermore, Guy’s and St Thomas’ NHS Foundation Trust (CH) received a grant from Novartis and CH received honoraria from Novartis, as well as payment from PeerVoice for the development of educational presentations. LG received honoraria from Shire for hospital meetings. GB received honoraria from Shire for speaking at satellite symposia and educational sessions at hematology meetings, as well as for participating in an advisory board. CB received honoraria for participating in advisory boards for Novartis and Shire. RC is a Shire employee and holds Shire stock/stock options, BA is a former Shire employee, and JS is a statistical contractor employed on a 12-month contract with Shire. MG reported no potential conflicts of interest. J-JK has received honoraria for advisory boards, speaking at independent lectures and research grants from Shire and Novartis. He has also received research grants from Novartis and Celgene.
References


### Tables

#### Table 1. Summary of patient characteristics prior to starting combination therapy.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>First combination (≥ 10 patients)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>HC + anagrelide</strong> (n=304)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>127 (42)</td>
</tr>
<tr>
<td>Male</td>
<td>127 (42)</td>
</tr>
<tr>
<td>Female</td>
<td>177 (58)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61 (18-95)</td>
</tr>
<tr>
<td>Hematological parameters</td>
<td>580 (148-3056)</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>7 (2-44)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>38 (28-50)</td>
</tr>
<tr>
<td>WBC count (x10^9/L)</td>
<td>12 (8-16)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>12 (8-16)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>38 (28-50)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>38 (28-50)</td>
</tr>
<tr>
<td>History of thrombohemorrhagic events at diagnosis, n (%)</td>
<td>81 (27)</td>
</tr>
<tr>
<td>Time from diagnosis to combination start (months)</td>
<td>53 (0.392)</td>
</tr>
<tr>
<td>Prior monotherapy, n (%)†</td>
<td>123 (41)</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>123 (41)</td>
</tr>
<tr>
<td>HC</td>
<td>167 (55)</td>
</tr>
<tr>
<td>IFN</td>
<td>-</td>
</tr>
<tr>
<td>Pipobroman</td>
<td>-</td>
</tr>
<tr>
<td>None</td>
<td>14 (5)</td>
</tr>
<tr>
<td>Anti-aggregatory therapy within 1 month prior to combination start, n (%)</td>
<td>196 (65)</td>
</tr>
<tr>
<td>Any anti-aggregatory therapy</td>
<td>196 (65)</td>
</tr>
<tr>
<td>Low dose aspirin</td>
<td>180 (59)</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>15 (5)</td>
</tr>
<tr>
<td>Ticlopidine/dipyridamole</td>
<td>9 (3)</td>
</tr>
</tbody>
</table>

*HC: hydroxycarbamide; IFN: interferon-α; WBC, white blood cell; Hb, hemoglobin; HCT, hematocrit.

†Two patients received Thromboreductin™ (anagrelide), 2 patients received HC + IFN, and 1 patient received busulfan as prior monotherapy. One patient who received busulfan + anagrelide received anagrelide as prior monotherapy.

*Six patients received anagrelide + other combination therapy.
Table 2. Summary of platelet count (x10^9/L) for patients on HC + anagrelide combination therapy, prior to and during therapy.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Total HC + anagrelide population</th>
<th>Prior HC</th>
<th>Prior anagrelide</th>
<th>No prior therapy</th>
<th>Prior HC vs. prior anagrelide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD) Median (range)</td>
<td>Mean (SD) Median (range)</td>
<td>Mean (SD) Median (range)</td>
<td>Mean (SD) Median (range)</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>n=170</td>
<td>n=95</td>
<td>n=72</td>
<td>n=3</td>
<td></td>
</tr>
<tr>
<td>Before combination*</td>
<td>674 (398) 581 (148-3056)</td>
<td>629 (320) 566 (148-1850)</td>
<td>677 (367) 612 (224-2401)</td>
<td>2036 (941) 1850 (1202-3056)</td>
<td>0.3708</td>
</tr>
<tr>
<td></td>
<td>n=279</td>
<td>n=153</td>
<td>n=113</td>
<td>n=13</td>
<td></td>
</tr>
<tr>
<td>First 6 months on combination</td>
<td>490 (383) 411 (58-5140)</td>
<td>509 (487) 411 (66-5140)</td>
<td>472 (193) 417 (79-1306)</td>
<td>426 (159) 408 (58-670)</td>
<td>0.4438</td>
</tr>
<tr>
<td></td>
<td>n=191</td>
<td>n=105</td>
<td>n=76</td>
<td>n=10</td>
<td></td>
</tr>
<tr>
<td>Last 6 months on combination</td>
<td>495 (230) 434 (79-1465)</td>
<td>507 (244) 449 (111-1465)</td>
<td>488 (222) 420 (79-1364)</td>
<td>426 (113) 408 (290-708)</td>
<td>0.5947</td>
</tr>
</tbody>
</table>

HC: hydroxycarbamide; SD: standard deviation.
*≤ 6 months before starting combination.
Table 3. Summary of predefined events experienced by at least 1% of the overall study population or patients receiving HC + anagrelide as first combination.

<table>
<thead>
<tr>
<th>Predefined event</th>
<th>Overall EXELS (n=3643)</th>
<th>HC + anagrelide (n=304)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Any predefined event</td>
<td>765 (21.0)</td>
<td>39 (12.8)</td>
</tr>
<tr>
<td>Major hemorrhagic events</td>
<td>71 (1.9)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Venous thrombotic events</td>
<td>57 (1.6)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>49 (1.3)</td>
<td>4 (1.3)</td>
</tr>
<tr>
<td>Congestive cardiac failure</td>
<td>42 (1.2)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Other cardiovascular symptoms</td>
<td>152 (4.2)</td>
<td>15 (4.9)</td>
</tr>
<tr>
<td>Stroke</td>
<td>56 (1.5)</td>
<td>4 (1.3)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>37 (1.0)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Transformation</td>
<td>129 (3.5)</td>
<td>7 (2.3)</td>
</tr>
<tr>
<td>Non-hematological malignancy</td>
<td>103 (2.8)</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Severe mucocutaneous disorders</td>
<td>47 (1.3)</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>Death not attributed to a predefined event</td>
<td>131 (3.6)</td>
<td>4 (1.3)</td>
</tr>
</tbody>
</table>

Table 4: Summary of patients discontinuing HC + anagrelide combination therapy (n=158).

<table>
<thead>
<tr>
<th>Discontinued therapy, n (%)</th>
<th>Non-efficacious</th>
<th>Intolerance/ side effects</th>
<th>Therapeutic strategy</th>
<th>Economic</th>
<th>Missing/ unknown</th>
<th>Patient preference</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall population</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC (n=76)</td>
<td>17 (22)</td>
<td>36 (47)</td>
<td>22 (29)</td>
<td>-</td>
<td>2 (3)</td>
<td>3 (4)</td>
<td>-</td>
</tr>
<tr>
<td>Anagrelide (n=59)</td>
<td>16 (27)</td>
<td>31 (53)</td>
<td>8 (14)</td>
<td>-</td>
<td>2 (3)</td>
<td>5 (8)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>HC + anagrelide (n=19)</td>
<td>2 (11)</td>
<td>8 (42)</td>
<td>4 (21)</td>
<td>1 (5)</td>
<td>3 (16)</td>
<td>2 (11)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>Total† (n=158)</td>
<td>35 (22)</td>
<td>75 (47)</td>
<td>34 (22)</td>
<td>1 (1)</td>
<td>11 (7)</td>
<td>10 (6)</td>
<td>5 (3)</td>
</tr>
<tr>
<td><strong>Prior HC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC (n=41)</td>
<td>11 (27)</td>
<td>19 (46)</td>
<td>13 (32)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anagrelide (n=37)</td>
<td>13 (35)</td>
<td>18 (49)</td>
<td>3 (8)</td>
<td>-</td>
<td>2 (5)</td>
<td>2 (5)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>HC + anagrelide (n=13)</td>
<td>1 (8)</td>
<td>6 (46)</td>
<td>4 (31)</td>
<td>1 (8)</td>
<td>2 (15)</td>
<td>2 (15)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Total† (n=95)</td>
<td>25 (26)</td>
<td>43 (45)</td>
<td>20 (21)</td>
<td>1 (1)</td>
<td>8 (8)</td>
<td>4 (4)</td>
<td>2 (2)</td>
</tr>
<tr>
<td><strong>Prior anagrelide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC (n=32)</td>
<td>6 (19)</td>
<td>16 (50)</td>
<td>7 (22)</td>
<td>-</td>
<td>2 (6)</td>
<td>3 (9)</td>
<td>-</td>
</tr>
<tr>
<td>Anagrelide (n=21)</td>
<td>3 (14)</td>
<td>12 (57)</td>
<td>5 (24)</td>
<td>-</td>
<td>-</td>
<td>3 (14)</td>
<td>-</td>
</tr>
<tr>
<td>HC + anagrelide (n=5)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>-</td>
<td>-</td>
<td>1 (20)</td>
<td>-</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Total (n=58)</td>
<td>10 (17)</td>
<td>29 (50)</td>
<td>12 (21)</td>
<td>-</td>
<td>3 (5)</td>
<td>6 (10)</td>
<td>3 (5)</td>
</tr>
</tbody>
</table>

HC: hydroxycarbamide.
*Data includes patients who may have discontinued for more than one reason.
†Total includes 4 patients who did not stop either therapy; HC + anagrelide therapy ongoing, but an additional therapy started.
Figure legends

Figure 1. **Number of patients receiving HC + anagrelide by country enrolled.**

HC: hydroxycarbamide.
NB: All data values not reported are < 1%.

Figure 2. **Summary of platelet response rates prior to and during combination therapy for patients on HC + anagrelide.**

HC: hydroxycarbamide.
The bar chart shows the percentage of patients enrolled from various countries who have received HC + anagrelide therapy, categorized by prior monotherapy treatment.

- **Greece**: 22.6% of patients (53/235) had no prior monotherapy, 10.2% (5/48) had prior hydroxycarbamide monotherapy, and 9.8% (5/52) had prior anagrelide monotherapy.
- **Portugal**: 12.5% (1/8) had no prior monotherapy, 7.5% (2/27) had prior hydroxycarbamide monotherapy, and 3.7% (1/28) had prior anagrelide monotherapy.
- **Denmark**: 11.6% (31/267) had no prior monotherapy, 6.9% (21/304) had prior hydroxycarbamide monotherapy, and 2.7% (2/75) had prior anagrelide monotherapy.
- **Norway**: 10.8% (4/37) had no prior monotherapy, 5.1% (1/19) had prior hydroxycarbamide monotherapy, and 2.9% (1/35) had prior anagrelide monotherapy.
- **Germany**: 8.8% (38/432) had no prior monotherapy, 4.7% (20/427) had prior hydroxycarbamide monotherapy, and 2.9% (12/414) had prior anagrelide monotherapy.
- **France**: 8.0% (41/510) had no prior monotherapy, 4.8% (24/496) had prior hydroxycarbamide monotherapy, and 2.9% (15/511) had prior anagrelide monotherapy.
- **Finland**: 7.7% (8/104) had no prior monotherapy, 3.7% (3/81) had prior hydroxycarbamide monotherapy, and 3.1% (2/64) had prior anagrelide monotherapy.
- **Netherlands**: 7.7% (1/13) had no prior monotherapy, 2.9% (2/71) had prior hydroxycarbamide monotherapy, and 3.1% (1/32) had prior anagrelide monotherapy.
- **UK**: 7.0% (23/328) had no prior monotherapy, 3.7% (12/325) had prior hydroxycarbamide monotherapy, and 5.0% (16/324) had prior anagrelide monotherapy.
- **Italy**: 6.9% (75/1093) had no prior monotherapy, 3.7% (40/1058) had prior hydroxycarbamide monotherapy, and 3.8% (45/1166) had prior anagrelide monotherapy.
- **Ireland**: 6.7% (1/15) had no prior monotherapy, 2.9% (1/34) had prior hydroxycarbamide monotherapy, and 1.9% (1/53) had prior anagrelide monotherapy.
- **Sweden**: 6.7% (13/260) had no prior monotherapy, 3.1% (8/257) had prior hydroxycarbamide monotherapy, and 3.8% (10/261) had prior anagrelide monotherapy.
- **Spain**: 4.4% (15/341) had no prior monotherapy, 1.9% (6/315) had prior hydroxycarbamide monotherapy, and 3.8% (6/161) had prior anagrelide monotherapy.

*Note: (n=patients receiving HC + anagrelide/total patients enrolled)*
≤ 6 months before combination (n=170)

First 6 months on combination (n=279)

Last 6 months on combination (n=191)

Patients (%)

0 10 20 30 40 50 60 70 80 90 100

> 1000x10⁹/L
≥ 600-1000x10⁹/L
< 600x10⁹/L

Pa
tient s (%)

n=90
n=59
n=21

n=221

n=49

n=36
n=8

Time point (n=patients)