THROMBOPROPHYLAXIS OF PATIENTS WITH JAK2-POSITIVE MYELOPROLIFERATIVE NEOPLASMS

MIRIAM KIMPTON

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Preface

Research Approval: The Research Ethics Board of the Ottawa Hospital Research Institute, where indicated, approved the research presented here.

Miriam Kimpton is the principal investigator for all work presented here. She led the conceptual work and design of the studies, sought Research Ethics Board approval (where appropriate), supervised research staff, acquired the data, performed the analysis and interpretation of the data, and wrote the manuscripts.

Marc Carrier is the M.Sc. thesis supervisor. He provided supervision and feedback on all steps of the research presented within this thesis. He approves the submission of this thesis and attests to the candidate's contributions.

Aurélien Delluc is a member of the M.Sc. thesis advisory committee. He provided guidance on study conception, data analysis, and manuscript writing.

Dean Fergusson is a member of the M.Sc. thesis advisory committee. He provided guidance on study conception, data analysis, and manuscript writing.

Tobias Tritschler participated in the systematic review as the second reviewer.

Abstract

Patients with JAK2-positive myeloproliferative neoplasms (JAK2MPN) form a rare patient population, at increased risk of arterial and venous thrombosis. Limited research is available to guide the thromboprophylaxis of these patients. Nonetheless, guidelines and expert opinions recommend the use of low-dose aspirin to decrease the risk of thrombotic complications. In order to build a research program on the thromboprophylaxis of JAK2MPN patients and assess the feasibility of conducting a randomized controlled trial comparing low-dose apixaban to low-dose aspirin for thromboprophylaxis in this patient population, we completed a systematic review and meta-analysis to provide reliable rates of thrombosis and bleeding on aspirin, we performed a survey of practice and a modified Delphi process to assess for heterogeneity in clinical practice and determine future research needs, and we developed a multi-centre, pilot randomized controlled trial on the feasibility of enrolling and retaining this patient population.

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Chapter 1: Introduction and Thesis Outline

Background

Myeloproliferative neoplasms are a group of chronic hematological malignancies, first distinguished by the presence of the BCR-ABL1 mutation. The presence of the BCR-ABL1 mutation is the defining feature of chronic myeloid leukemia. BCR-ALB1 negative myeloproliferative neoplasms (MPN) classically refer to polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These diseases are characterized by the uncontrolled production of hematological cells. In the case of PV, the primarily affected blood cells are the erythrocytes and nearly all cases of PV are defined by the presence of the JAK2(-V617F) driver mutation[1]. ET is categorised by the excessive production of megakaryocytes. About sixty percent of ET patients harbour the JAK2 driver mutation[2]. As for PMF, its predominant features are the unruly growth of atypical megakaryocytes and granulocytes, along with reticulin fibrosis. PMF may be further classified as pre-fibrotic, where patients are found to have a more indolent clinical course similar to that of ET, or overtly fibrotic, where specific therapies to counter the devastating effects of severe cytopenias must be initiated[3, 4]. As for ET, around 50-60% of PMF patients will have the JAK2 mutation[5].

When defined by the presence of the JAK2-mutation, patients with PV, ET, or PMF may be referred to as having JAK2-positive MPN (JAK2MPN), with some experts describing a biological continuum of disease based on the occurrence of the JAK2-mutation[6]. The JAK2 mutation leads to a pro-coagulant environment through the higher expression of P-selectin, which

heightens the endothelium's pro-adhesive quality and increases the presence of tissue factor, both on the surface of monocytes and at the site of thrombus formation[7-9]. An association has been found between high JAK2(-V617F) mutation allele burden and increased thrombotic risk[10].

MPN are rare diseases. Recent data from the Swedish Cancer Register and Swedish Blood Cancer Register show that the age-standardized incidence of PV is 1.48 (95% confidence interval [CI], 1.42–1.54) per 100 000 person-years, while it is 1.60 (95% CI, 1.53–1.66) per 100 000 person-years in ET, and 0.52 (95% CI, 0.48–0.56) per 100 000 person-years in PMF[11]. Given the chronicity of these diseases, though, patients with PV, ET, and pre-fibrotic PMF may live decades following the diagnosis of their disease[12, 13].

Thrombotic complications are a major source of morbidity and mortality in this patient population. In a large, international cohort of PV patients with a median follow up of nearly 7 years, arterial and venous thrombotic complications affected 12% and 9% of patients, respectively[14]. In unselected ET patients, an international cohort study with a median follow-up of 6.2 years revealed a 12% cumulative incidence of arterial or venous thrombosis. Importantly, the presence of the JAK2 mutation in multivariable analysis demonstrated a hazard ratio of 2.6 (p value 0.009)[15]. Similar thrombotic rates have been reported in JAK2-positive early PMF patients and JAK2-positive ET patients[3]. MPN patients are at particularly elevated risk of thrombosis near the time of diagnosis[16]. A recent meta-analysis revealed a pooled prevalence of thrombosis at time of diagnosis of 28.6% (95% CI, 22.0–36.3%; I^2 95%) in PV patients, 20.7% (95% CI, 16.6–25.5%; I^2 93%) in ET patients irrespective of their mutational

status, and 9.5% (95% CI, 5.0–17.4%; I^2 94%) in PMF patients, also not accounting for the presence of the JAK2 mutation[17].

Current guidelines recommend the use of low-dose aspirin for the thromboprophylaxis of JAK2MPN patients. In PV, all current guidelines, including the European Leukemia Network[18] and the National Comprehensive Cancer Network[19], are in agreement regarding the recommended use of low-dose aspirin thromboprophylaxis, where no contraindication to this intervention is found. In ET, where the mutational status imparts important information on the thrombotic risk, guidelines also recommend the use of low-dose aspirin thromboprophylaxis in JAK2-positive ET patients[18, 19]. As for PMF, where a dearth of evidence prohibits informed guideline recommendations on thromboprophylaxis, experts do consistently recommend the use of low-dose aspirin thromboprophylaxis in patients with risk factors for thrombosis, including the presence of the JAK2 mutation[4, 20, 21].

Despite the above clinical guideline and expert recommendations, there is little evidence available to support the use of low-dose aspirin thromboprophylaxis in JAK2MPN patients. The ECLAP trial represents the largest randomized controlled trial to date for the use of aspirin thromboprophylaxis in PV patients. Unfortunately, after accounting for the number of analyses for the two primary composite endpoints, this trial did not reach statistical significance regarding the efficacy of low-dose aspirin compared to placebo for the reduction of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular, with a relative risk (RR) of 0.41 (95% CI, 0.15 to 1.15; P=0.09); or nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (when adjusting for the multiple statistical comparisons), RR of 0.40 (95% CI, 0.18 to 0.91; P=0.03)[22].

Furthermore, in another large, international cohort of PV patients, where 84% were receiving low-dose aspirin and 73% cytoreductive therapies, the rate of thrombotic complications remained significantly elevated at 2.62% (95% CI, 2.34-2.94) per patient-year[23]. Additionally, a Cochrane meta-analysis did not show a significant reduction in the risk of fatal thrombotic events and all-cause mortality, when aspirin thromboprophylaxis was compared to no thromboprophylaxis in PV patients[24]. Of note, the recommendations for aspirin thromboprophylaxis in JAK2-positive ET patients and JAK2-positive PMF patients are mostly based on the extrapolation of the evidence from PV patients presented above, since no randomized controlled trials for this intervention have been completed in this patient population to date. A systematic review on the use of anti-platelet therapy for the thromboprophylaxis of ET patients revealed that the risk-benefit ratio of this intervention is highly uncertain, due to the imprecision and inconsistency of the available data[25]. Therefore, the efficacy and safety of thromboprophylactic interventions in JAK2MPN patients remains an area of unmet research needs, which has recently been identified and emphasized by a group of international MPN experts[26].

Challenges of studying patients with myeloproliferative neoplasms

The study of MPN patients, and specifically the research on thromboprophylaxis in this patient population, has been fraught with difficulties. Many of these obstacles are borne from the fact that each sub-type of MPN (PV, ET, and PMF) is considered to be a rare disease. Research into rare diseases faces challenges based on the small number of potential participants and even

smaller event rates (which impact potential statistical inferences from the available data), the lack of knowledge on the natural evolution of the disease (this is likely even truer for chronic, rare diseases), and the scarcity of evidence for the currently adopted therapeutic approaches (leaving in question the nature of an adequate comparator for newer therapies)[27, 28]. Such challenges must be met with careful planning of subsequent research and, at times, use of innovative research methods[28].

For JAK2MPN patients, one of the particular difficulties comes from the lack of centralized care. Since these patients suffer from a chronic disease, it is not uncommon for them to be followed in community clinics (by hematologists, oncologists, or internists), instead of tertiary centres, where most patients with hematological malignancies normally receive care. Even at a tertiary centre such as the Ottawa Hospital, JAK2MPN patients are dispersed amongst various clinical settings, including benign hematology clinics, thrombosis clinics, and malignant hematology clinics. Because of the different locations where these patients may be found, the proper identification and enrollment of JAK2MPN patients for the purpose of participating in research present logistical problems not seen in other patient populations.

Such recruitment challenges have been clearly illustrated by previous studies in MPN patients. Although it is the largest randomized controlled trial to date, the above described ECLAP trial did not meet its target sample size, with only 516 patients recruited from a target of 940 patients [22]. Similarly, the CYTO-PV study, which compared a strategy of intensive hematocrit control through phlebotomy (for a target hematocrit of less than 45%) to a less-intensive hematocrit control (target hematocrit of 45-50%) in PV patients, was forced to abandon its target sample size after 4 years of recruitment and only about one third of patient enrolled[29].

As explored in this thesis, one possible strategy to overcome some of the past difficulties faced by researchers and improve recruitment of MPN patients in clinical trials would be to combine different sub-types of MPN patients based on the presence of the JAK2 mutation, given that this mutation confers a shared pathophysiology and clinical course, when it comes to thrombotic complications experienced by these patients.

Research Questions for the Thesis:

1. What are the rates of thrombotic and bleeding complications in patients with JAK2MPN receiving aspirin thromboprophylaxis?

2. What is the minimal clinically important difference (MCID) in the cumulative rate of thrombosis over 20 years, which would lead physicians to adopt a new anti-thrombotic agent for the thromboprophylaxis of JAK2MPN patients?

3. Does the lack of evidence for primary and secondary thromboprophylaxis lead to heterogeneity of practice in the management of JAK2MPN patients?

4. Is the recruitment of JAK2MPN patients in a randomized controlled trial evaluating the efficacy of low-dose apixaban compared to low-dose aspirin feasible?

Thesis Objectives and Outline

The following objectives will be addressed within this thesis:

Objective 1. Conduct a systematic review and meta-analysis.

a. Calculate the rate of thrombotic complications (both arterial and venous), expressed as events per 100 patients-years (with its associated 95% CI) in JAK2MPN patients receiving low-dose aspirin thromboprophylaxis.

b. Calculate the rate of major bleeding expressed as events per 100 patients-years (with its associated 95% confidence interval) in JAK2MPN patients receiving low-dose aspirin thromboprophylaxis.

Objective 2. Perform a modified Delphi process and a survey of practice of Canadian physicians.

a. Determine the MCID in the rate of cumulative thrombotic complications over 20 years, which would prompt a change in practice regarding the thromboprophylaxis of JAK2MPN patients.

b. Describe the current clinical practice of Canadian physicians in the primary and secondary thromboprophylaxis of JAK2MPN patients.

c. Assess the role of direct oral anticoagulants in the thromboprophylaxis of JAK2MPN patients.

d. Identify areas of unmet research needs surrounding the thromboprophylaxis of JAK2MPN patients.

Objective 3. Write a protocol for a multi-centre, pilot randomized controlled trial on the feasibility of comparing low-dose apixaban to low-dose aspirin for the thromboprophylaxis of JAK2MPN patients.

a. Review the existent literature on the use of low-dose aspirin for the thromboprophylaxis of JAK2MPN patients.

b. Review the literature of the use of low-dose direct oral anticoagulants in other patient populations.

c. Formulate a hypothesis on the feasibility of recruiting JAK2MPN patients in a full-size randomized trial on the efficacy of low-dose apixaban.

d. Develop a detailed protocol to determine the eligibility criteria, the identification of participants and enrollment process, the randomization of participants, the assignment of treatment arms, the distribution of study medication, the necessary follow-up, the retainment of participants, the evaluation of clinical outcomes, and the analysis of feasibility endpoints.

In order to meet the above objectives, three manuscripts have been prepared for publication in peer-reviewed medical journals:

Manuscript 1: Risk of thrombosis and major bleeding in JAK2-positive myeloproliferative neoplasm patients receiving low-dose aspirin: a systematic review and meta-analysis.

Manuscript 2: Delphi Process and Practice Survey of Canadian Hematologists and Thrombosis Experts on the Prevention and Management of Thrombotic Complications in JAK2-Positive Myeloproliferative Neoplasm Patients.

Manuscript 3: Apixaban for the thromboprophylaxis of JAK2-postive myeloproliferative neoplasms: rationale and design of the AIRPORT-MPN pilot trial.

Chapter 2: Systematic Review and Meta-Analysis

Risk of thrombosis and major bleeding in JAK2-positive myeloproliferative neoplasm patients receiving low-dose aspirin: a systematic review and meta-analysis.

Miriam Kimpton[1,2], Tobias Tritschler[3], Aurélien Delluc[1,2], Dean Fergusson[4], Marc Carrier[1,2,4]

[1]Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada

[2]Clinical Epidemiology Program, The Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada

[3]Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[4]School of Epidemiology and Public Health, University of Ottawa, Ottawa, ON, Canada

Corresponding Author:
Miriam Kimpton
501 Smyth Road, PO Box 201A, Ottawa, ON, K1H 8L6
mkimpton@toh.ca

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ABSTRACT

Background: Patients with JAK2-positive myeloproliferative neoplasms (MPN) are at heightened risk of arterial and venous thrombosis. Low-dose aspirin is recommended in this patient population to decrease the thrombotic risk but comes at the cost of increased bleeding.

Purpose: To determine reliable rates of thrombotic complications (both arterial and venous) and major bleeding in JAK2-positive MPN patients, receiving thromboprophylaxis with low-dose aspirin.

Data Sources: Electronic databases (EMBASE, MEDLINE, and the Cochrane Central Registry of Controlled Trials [CENTRAL]) were searched from inception to February 20, 2020.

Study Selection: Retrospective cohort studies, prospective cohort studies, and randomized controlled trials reporting on thrombotic or bleeding complications in JAK2-positive MPN patients receiving low-dose aspirin were identified. There were no restrictions on time period or language.

Data Extraction: Data extraction and risk of bias assessment was performed independently by two reviewers.

Data Synthesis: Six studies were included, involving a total of 659 patients with JAK2-positive MPN receiving low-dose aspirin. The pooled rate of thrombotic events (both arterial and venous) was 1.8 (95% confidence interval [CI] 1.4-2.2, I^2=0%) per 100 patient-years. The pooled major bleeding rate was 1.0 (95% CI 0.6-1.6, I^2 =54.2%) per 100 patient-years. The included

studies were deemed to be at moderate-to-high risk of bias. Certainty of evidence for the thrombotic and major bleeding rates was judged to be low.

Limitations: Inconsistency due to the heterogeneity of thrombotic and major bleeding definitions used by the included studies, indirectness in estimating thrombotic and major bleeding rates in a sub-population, inability to extract the necessary data for our patient population from most studies at the full-text review.

Conclusion: Patients with JAK2-positive MPN remain at risk of thrombosis, despite the use of low-dose aspirin. Additional studies to evaluate new thromboprophylactic options are needed.

This review's protocol was deposited in the in the uO Research website, managed by the University of Ottawa.

Keywords: myeloproliferative disorder, aspirin, thrombosis, hemorrhage

BACKGROUND

Patients with Philadelphia-negative, JAK2(V617F)-positive myeloproliferative neoplasms (MPN) are at elevated risk of both arterial and venous thrombotic complications[1-4]. This particular mutation is present in >95% of patients with polycythemia vera (PV), and 50-60% of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF)[5-7]. The presence of the JAK2 mutation in the vascular endothelium has been associated with thrombogenicity via the expression of P-selectin, which leads to a pro-adhesive phenotype[8]. Current guidelines and expert opinions recommend the use of thromboprophylaxis with low-dose aspirin to prevent thrombosis in patients with JAK2-positve MPN[9-11]. The evidence for aspirin thromboprophylaxis, however, remains limited to one randomized controlled trial in the PV population[12]. Additionally, since these are rare diseases, reliable rates of thrombotic and bleeding complications in JAK2-positive MPN patients receiving standard of care therapy are lacking. A recent systematic review aimed to better quantify the thrombotic risk in ET patients [13]. Because of the inclusion of a very heterogeneous patient population (such as patients with driver mutations other than JAK2), the low quality of the selected studies, and the imprecision of the reported results, a meta-analysis could not be done.

We sought to determine reliable rates of arterial and venous thrombotic complications in JAK2-positive MPN patients receiving low-dose aspirin thromboprophylaxis. Secondary objectives included providing reliable rates of major bleeding and clinically relevant non-major bleeding (CRNMB) on low-dose aspirin, and thrombosis-associated mortality in our patient population.

METHODS

This systematic review's protocol was written in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) Statement[14]. The protocol was deposited in the uO Research website (available at http://hdl.handle.net/10393/40206), managed by the University of Ottawa.

Eligibility Criteria

Types of Participants:

Adult patients with a diagnosis of PV, JAK2-positive ET (JAK2ET) or JAK2-positive PMF (pre-fibrotic or overt) on low-dose aspirin thromboprophylaxis were included. All patients with a diagnosis of PV were assumed to have the JAK2 mutation. Only ET and PMF (pre-fibrotic or overt) patients in whom the presence of the JAK2 mutation was confirmed were included.

Intervention and Comparator:

The studied intervention was the use of low-dose aspirin. Since the goal of the systematic review was to calculate reliable rates of thrombotic complications on aspirin, having a comparator arm was not a necessary criterion for study inclusion.

Outcome Measures:

The primary outcome was the rate of arterial and venous thrombotic complications in JAK2-positive MPN patients, receiving low-dose aspirin thromboprophylaxis. A common definition of arterial and venous thromboembolic events across all studies could not be used. Therefore, the definition for an arterial or venous thrombotic outcome adopted by each included study was

applied. The secondary outcomes of interest were: the thrombotic complications rates on low-dose aspirin therapy, stratified by JAK2-positive MPN subtypes (PV; JAK2ET, and JAK2-positive PMF [pre-fibrotic or overt]); the rates of bleeding complications on aspirin (for the population as a whole and stratified by MPN sub-type), defined as major bleeding[15] and CRNMB[16] per the International Society on Thrombosis and Haemostasis (ISTH); the rates of thrombosis-associated mortality; and the rates of major bleeding-associated mortality.

Study Designs:

Randomized controlled trials, prospective cohort studies, and retrospective cohort studies reporting on one of the outcomes of interest in JAK2-positive MPN patients were included.

Cross-sectional, case-control, case series with fewer than 10 patients, and case reports were excluded.

Search Strategy, Study Selection, and Data Extraction

A comprehensive search strategy was created with the help of an information specialist using medical subject heading terms and key words (see Appendix for full strategy). Electronic databases (EMBASE, MEDLINE, and the Cochrane Central Registry of Controlled Trials [CENTRAL]) were searched from inception to February 20, 2020, without any restriction on time period or language. Additional references were obtained through hand searching. Grey literature was not searched.

With the help of the systematic review software Covidence[17], screening of titles and abstracts, followed by full-text review was done in duplicate, by two independent reviewers

(MK and TT). Discrepancies were resolved by consensus. Reasons for study exclusion at the full-text review stage were noted and reported in the PRISMA flow diagram.

A standardized form was created to facilitate data extraction, which was also completed by two independent reviewers (MK and TT). Presence of discrepancies in the collected data led to discussions, with resolution attained via consensus.

Risk of Bias Assessment

The quality of the non-randomized studies was assessed using the Risk Of Bias In Non-randomised Studies of Interventions (ROBINS-I) tool[18]. The randomized controlled trials were evaluated using the revised Cochrane risk-of-bias tool for randomized trials (RoB2) tool[19]. The risk of bias assessment was done in duplicate, also by two independent reviewers (MK, TT) with all conflicts resolved through discussion.

Data synthesis

The rate of thrombotic events was calculated and expressed as number of events per 100 person-years, with their associated 95% confidence intervals (CI). If the number of person-years was not provided by the study, it was estimated by multiplying the median follow-up duration with the number of participants. Where a total follow-up period was provided instead of a median follow-up duration, losses to follow up and deaths were assumed to occur consistently throughout the follow-up period (with participants lost to follow up or who had died contributing to half of the total time period in person-years). In studies reporting event rates (events per 100 person-years) along with incidence rate ratios for our study's population and outcome of interest, an event rate for thrombotic complications on low-dose aspirin in JAK2-

positive MPN patients was calculated by multiplying the provided overall event rate with the incidence rate ratios for low-dose aspirin and the presence of the JAK2 mutation. Where necessary, the rate was abstracted from graphical representations of the data.

The Cochrane's Q (statistically significant at $p<0.10$) and the I^2 statistic (>75% considered to represent high heterogeneity) were used to assess for statistical heterogeneity. After assessing the heterogeneity, data was analysed through a random effect model to better account for the inter-study heterogeneity. Sensitivity analysis based on the exclusion of studies deemed to be at high risk of bias was not done, given the result of the risk of bias assessment. Data synthesis was done with the StatsDirect[20] software.

Meta-Bias Assessment

The presence of meta-bias was assessed by repeating the analyses for the primary and secondary outcomes using a fixed effect model (to assess for small sample bias). A funnel plot was also to be done, if more than 10 studies were included in the meta-analysis, to assess for publication bias.

Confidence in Cumulative Evidence

The quality of evidence for the rate of thrombotic and major bleeding complications was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group methodology by focusing on inconsistency, indirectness, risk of bias across studies, publication bias, and random error[21].

RESULTS

Search results

The database search resulted in 1,500 records being identified. Eight records were added following review of the references and input from content experts (Figure 1). After duplicate removal, 1,500 studies were screened by abstract and 1,403 were found to be irrelevant. A total of 97 full-text articles were reviewed for eligibility (Table A1). From those, 6 studies were included and provided the necessary data for extraction.

Characteristics of the included studies

Three retrospective cohort studies, one prospective cohort study, and two randomized controlled trials were included, involving a total of 659 patients with JAK2-positive MPN. Three studies involved patients with PV and three studies involved patients with JAK2ET. There was heterogeneity in the definitions of arterial and venous thrombotic outcomes used by the included studies. Heterogeneity was also present in the definition of major bleeding adopted by each study. None of the included studies used the ISTH definition for CRNMB. Recorded bleeding events not meeting criteria for major bleeding were therefore captured as non-major bleeding events. The characteristics of the included studies are summarised in Table 1. The included studies were judged to be at moderate-to-high risk of bias per the ROBINS-I and RoB2 tools (Tables 2 and 3).

Thrombotic events during low-dose aspirin thromboprophylaxis

The pooled rate of thrombotic events (both arterial and venous) in JAK2-positive MPN patients was 1.8 (95% CI, 1.4-2.2, I^2=0%) per 100 patient-years (Figure 2). From the available data, it was not possible to calculate a case-fatality rate of thrombotic events in JAK2-positive MPN patients exposed to aspirin thromboprophylaxis.

Major bleeding events during low-dose aspirin thromboprophylaxis

The pooled rate of major bleeding events in this patient population during low-dose aspirin thromboprophylaxis was 1.0 (95% CI, 0.6-1.6, I^2 =54.2%) per 100 patient-years (Figure 3). The case-fatality of major bleeding for our specific patient population could not be calculated.

Subgroup Analyses

In PV patients receiving low-dose aspirin thromboprophylaxis, the pooled rate of thrombotic complications was 1.9 (95% CI, 1.1-2.9, I^2 =0%) per 100 patient-years (Figure A1). For major bleeding events, the pooled rate was 0.5 (95% CI, 0.1-1.0, I^2 =0%) per 100 patient-years (Figure A2). The pooled rate of non-major bleeding events was 2.7 (95% CI, 1.7-3.9, I^2 =0%) per 100 patient-years (Figure A3).

In JAK2ET patients treated with low-dose aspirin as thromboprophylaxis, the pooled rate of thrombotic events was 1.7 (95% CI, 1.4-2.2, I^2 =28.5%) per 100 patient-years (Figure A4), while the pooled rate of major bleeding was 1.3 (95% CI, 0.8-2.0, I^2 =59.1%) per 100 patient-years (Figure A5). A pooled non-major bleeding rate could not be calculated in JAK2ET patients.

The pooled rate for arterial complications in patients receiving low-dose aspirin thromboprophylaxis was 1.0 (95% CI, 0.6-1.5, I^2 =16.9%) per 100 patient-years (Figure A6), and

was 0.7 (95% CI, 0.3-1.3, I^2 =48.4%) per 100 patient-years for venous thrombotic events (Figure A7).

Meta-bias assessment

Analyses were repeated using a fixed effect model to account for small sample bias. The rates of thrombotic events and major bleeding events remained consistent with this analytic strategy at 1.8 (95% CI, 1.4-2.2) per 100 patient-years and 1.1 (95% CI, 0.8-1.4) per 100 patient-years, respectively. Since fewer than 10 studies were included, a funnel plot analysis was not done for potential publication bias.

Confidence in Cumulative Evidence

Based on the quality of the evidence, confidence in the estimates for overall thrombotic and major bleeding rates was deemed to be low, meaning that the true rates may be significantly different than the ones presented here. This assessment is due to the inconsistency in the different thrombotic definitions (both arterial and venous) and bleeding definitions adopted by the included studies, the use of observational data, the inability to assess for publication bias, and the indirectness of calculating rates for a sub-population of patients using median follow up duration for the entire cohort and incidence rate ratios.

DISCUSSION

Patients with MPN are at elevated risk of arterial and venous thrombosis, but this risk has been poorly characterized in the past, primarily due to the rarity of the disease and heterogeneity of

the patient population. With a greater molecular understanding of these diseases, it is now possible to define a group of MPN patients at higher risk for thrombotic complications through a common pathophysiological pathway determined by the presence of the JAK2 driver mutation. Reliable rates of thrombotic complications in this particular cohort of patients are needed to assess their baseline risk with the current standard of care. This meta-analysis suggests that the pooled rate of arterial and venous thrombotic events in JAK2-positive MPN patients receiving thromboprophylaxis with low-dose aspirin is 1.8 (95% CI, 1.4-2.2) per 100 patient-years. This rate is similar in PV and JAK2ET patients receiving low-dose aspirin thromboprophylaxis. To put this rate into perspective, in a large study assessing the natural history of PV patients over 20 years, the reported thrombosis rate was 3.4 per 100 patient-years[22]. In unselected ET patients not receiving anti-platelet therapy (where, according to current guidelines, only a proportion of them ought to receive low-dose aspirin thromboprophylaxis), the median baseline thrombotic risk was found to be 2 per 100 patient-years. In high-risk ET patients, the baseline thrombotic risk was 2.5 to 11 per 100-patient years[13]. Caution must be taken, though, when comparing historical data with more current data, because improvements in the management of MPN patients other than the use of low-dose aspirin thromboprophylaxis (such as the use of cytoreductive therapy strict hematocrit control in PV for example) were shown to also impact the rate of thrombosis[2].

The prospective studies included here recruited prevalent cases of patients with a JAK2-positive MPN diagnosis. Also, in two of the three retrospective cohort studies of ET patients included here, low-risk ET patients (i.e. younger patients without a prior history of thrombosis) were analyzed, meaning that patients who had experienced a thrombotic complication around the

time of MPN diagnosis were not included in these studies. As such, the calculated pooled thrombosis rate presented here likely represents the rate of thrombotic complications over a more indolent phase of the disease. This is an important distinction because JAK2-positive MPN are chronic diseases, with some patients surviving their diagnosis for decades[1, 23-25]. The aim of the management of MPN patients is therefore to reduce their risk of cumulative complications, including thrombotic events, over several years. Although the rate in itself may appear low, it must be interpreted through the lens of a chronic disease, where such a rate could result in a cumulative incidence of thrombotic complications of greater than 30% over a 20-year period, especially as patients accumulate other risk factors associated with thrombotic complications.

It is also important to note that the rate of major bleeding complications on low-dose aspirin thromboprophylaxis is significant, especially when compared to the rate of thrombotic complications. In JAK2ET patients, the major bleeding risk is almost as substantial as the thrombotic risk. Current anti-thrombotic therapies are associated with a decreased risk of thrombosis, at the cost of an increased risk of bleeding. When evaluating an anti-thrombotic medication in clinical practice, it is thus imperative to consider the clinical net benefit derived from this medication, by taking into account both the decrease in thrombotic disease burden and the increase in bleeding events, along with their associated morbidity and mortality. In ET patients receiving any kind of anti-platelet therapy, however, a recent systematic review was unable to calculate the pooled relative risk of thrombosis and major bleeding, due to the presence of high risk of bias, as well as inconsistency and imprecision of the included studies' results. A median relative risk for thrombosis with anti-platelet therapy, compared to the

baseline risk, was reported to be 0.74 (95% CI, 0.29-1.87), while the median relative risk for major bleeding was 1.30 (95% CI, 0.66-2.59). The authors concluded that it is very uncertain whether anti-platelet therapy reduces the risk of thrombosis or increases the risk of major bleeding in ET patients [13]. In JAK2ET patient receiving low-dose aspirin thromboprophylaxis, just as in ET patients treated with any type of anti-platelet therapy, it remains to be seen whether the benefit of decreased cumulative incidence of thrombotic complications with this intervention outweighs its associated risk of major bleeding. More research in this area is eagerly awaited. It is possible that other anti-thrombotic agents, with different mechanisms of action, will be able to provide a better net clinical benefit by further decreasing the risk of thrombotic events, without a significant associated increased risk of major bleeding.

Our study has a number of limitations. Overall, the quality of the evidence contained in our meta-analysis was found to be low. The included studies were determined to be at moderate to high risk of bias. Additionally, they had small sample sizes. Use of a fixed effect model, however, did not reveal a significant small sample size bias. Furthermore, although the rates of thrombosis reported among the included studies were consistent, we were unable to extract data from many of the studies reviewed at the full-text stage, because the vast majority of articles did not report outcomes specific to our target patient population. As such, it is possible that the actual rate of thrombosis differs from the one presented here and that future studies specifically involving MPN patients with the JAK2 driver mutation will lead to a different appreciation of the thrombosis rate with low-dose aspirin thromboprophylaxis. The three Alvarez-Larran studies defined the arterial and venous thrombotic endpoints consistently, but there was heterogeneity among the definitions used by the other included studies. Of note,

none of the studies explicitly defined deep vein thrombosis as either exclusively proximal or proximal and distal, which may impact the severity of the reported venous thrombotic outcomes since the clinical significance of a distal deep vein thrombosis remains unclear [26]. There was also heterogeneity in the major bleeding definitions adopted by the included studies, which leads to a certain amount of reservation regarding the pooled major bleeding rate. Of note, the included studies took place over nearly two decades, and two of the included studies predate the chosen ISTH major bleeding definition, which may explain the lack of consensus in bleeding definitions used. Moreover, no data could be extracted on JAK2-positive PMF patients, which remains an area of unmet research needs. Also, with the available data, we were not able to account for the impact of other interventions known to decrease the risk of thrombosis, such as the use of cytoreductive therapies or maintaining a hematocrit of less than 45% (in PV patients). Granular, patient-level data would be required for further analysis on this topic. A recent population-based study showed that MPN patients are at higher risk of thrombotic complications during the first year following MPN diagnosis[27]. Unfortunately, it was not possible from the available data to calculate the rate of thrombotic complications from the time of MPN diagnosis. Finally, indirectness in the presented results was introduced by including studies reporting incidence rate ratios for our outcomes of interest, based on the presence of the JAK2 mutation and the use of low-dose aspirin thromboprophylaxis.

CONCLUSION

Patients with JAK2-positive MPN remain at risk of thrombosis despite the use of low-dose aspirin thromboprophylaxis. When considered from the perspective of a chronic disease, the rate of thrombosis on low-dose aspirin presented here leads to a significant cumulative thrombotic risk over a patient's lifetime. More research is needed to identify better therapeutic options with the aim of further decreasing this thrombotic risk.

Author	Year	Design	MPN Type	MPN Definition	Arterial Thrombotic Definition	Venous Thrombotic Definition	Major Bleeding Definition	Number of patients*	Patient-years	Thrombotic Events#	Major Bleeding	Non-Major Bleeding‡
Alvarez-Larran	2010	Retrospective	ET (low-risk)	WHO 2008	Stroke, TIA, RAO, CAD, PAD	CVST, DVT, PE, BCS, SPVT	Modified ISTH^	82	1000	19.1	17.6	N/A
Alvarez-Larran	2013	Retrospective	ET (high-risk)	WHO 2008	Stroke, TIA, RAO, CAD, PAD	CVST, DVT, PE, BCS, SPVT	Modified ISTH^	89	1000	20.2	14.4	N/A
Alvarez-Larran	2016	Retrospective	ET (low-risk)	WHO 2008	Stroke, TIA, RAO, CAD, PAD	CVST, DVT, PE, BCS, SPVT	Modified ISTH^	162	1000	11.6	7.1	N/A
De Gaetano	1997	RCT	PV	PVSG	Stroke, TIA, MI, AAT (not otherwise defined)	VTE (not otherwise defined)	N/A	60	80	3	0	1
Landolfi	2004	RCT	PV	Current clinical practice	Stroke, TIA, MI, death from cardiovascular causes	PE, DVT	Other~	253	739	12	3	20
Robier	2014	Prospective	PV	WHO 2001	Not defined	Not defined	N/A	13	6.5	0	0	0

Table 1. Characteristics of the included studies.

AAT: acute arterial thrombosis; BCS: Budd-Chiari syndrome; CAD: coronary artery disease; CVST: cerebral venous sinus thrombosis; DVT: deep vein thrombosis; ET: Essential Thrombocythemia; ISTH: International Society on Thrombosis and Haemostasis; MI: myocardial infarction; MPN: myeloproliferative neoplasm; N/A: Not available; PAD: peripheral artery disease; PE: pulmonary embolism; PV: Polycythemia Vera; PVSG: Polycythemia Vera Study Group; RAO: retinal artery occlusion RCT: randomized controlled trial; SPVT: splenoportal vein thrombosis; TIA: transient ischemic attack; VTE: venous thromboembolism; WHO: World Health Organization.

*Number of patients included in the study with the JAK2 mutation and receiving low-dose aspirin thromboprophylaxis.

#Includes arterial and venous thrombotic events.

^ Symptomatic bleeding in a critical organ or an overt hemorrhage requiring transfusion or associated with a decrease in hemoglobin of ≥20 g/L without transfusion.

~ Non-fatal major bleeding (defined as any bleeding requiring transfusion and/or hospitalization) or fatal.

‡ None of the studies adopted the ISTH definition for clinically relevant non-major bleeding, but rather collected all non-major bleeding events within this category.

Observational study	Confounding Bias	Selection Bias	Classification Bias	Deviation from intervention	Missing Data Bias	Measurement of outcomes	Selected Reporting
Alvarez-Larran 2010	Serious (presence of microvascular symptoms, not used in propensity score)	Moderate (aspirin treated as time-varying variable)	Moderate	Moderate (CV co-interventions not captured, use of propensity score)	No information	Serious	Moderate
Alvarez-Larran 2013	Moderate (propensity score used)	Moderate (aspirin treated as time-varying variable)	Moderate	Moderate (CV co-interventions not captured, use of propensity score)	No information	Serious	Moderate
Alvarez-Larran 2016	Moderate (propensity score used)	Moderate (aspirin treated as time-varying variable)	Moderate	Moderate (CV co-interventions not captured, use of propensity score)	No information	Serious	Moderate
Robier	Serious	Low	Low	Serious (CV co-interventions not captured)	Low	Serious	Low

Table 2. Risk of bias assessment for observational studies, using the ROBINS-I tool.
CV: cardiovascular.

Randomized controlled trial	Randomization	Intended Interventions	Missing outcome data	Measurement of outcome bias	Selection of reported results	Overall
De Gaetano	Low	Low	High	Uncertain	Low	Some concerns
Landolfi	Low	Unclear	Unclear	Low	Low	Some concerns

Table 3. Risk of bias assessment for randomized controlled trial, using the RoB2 tool.

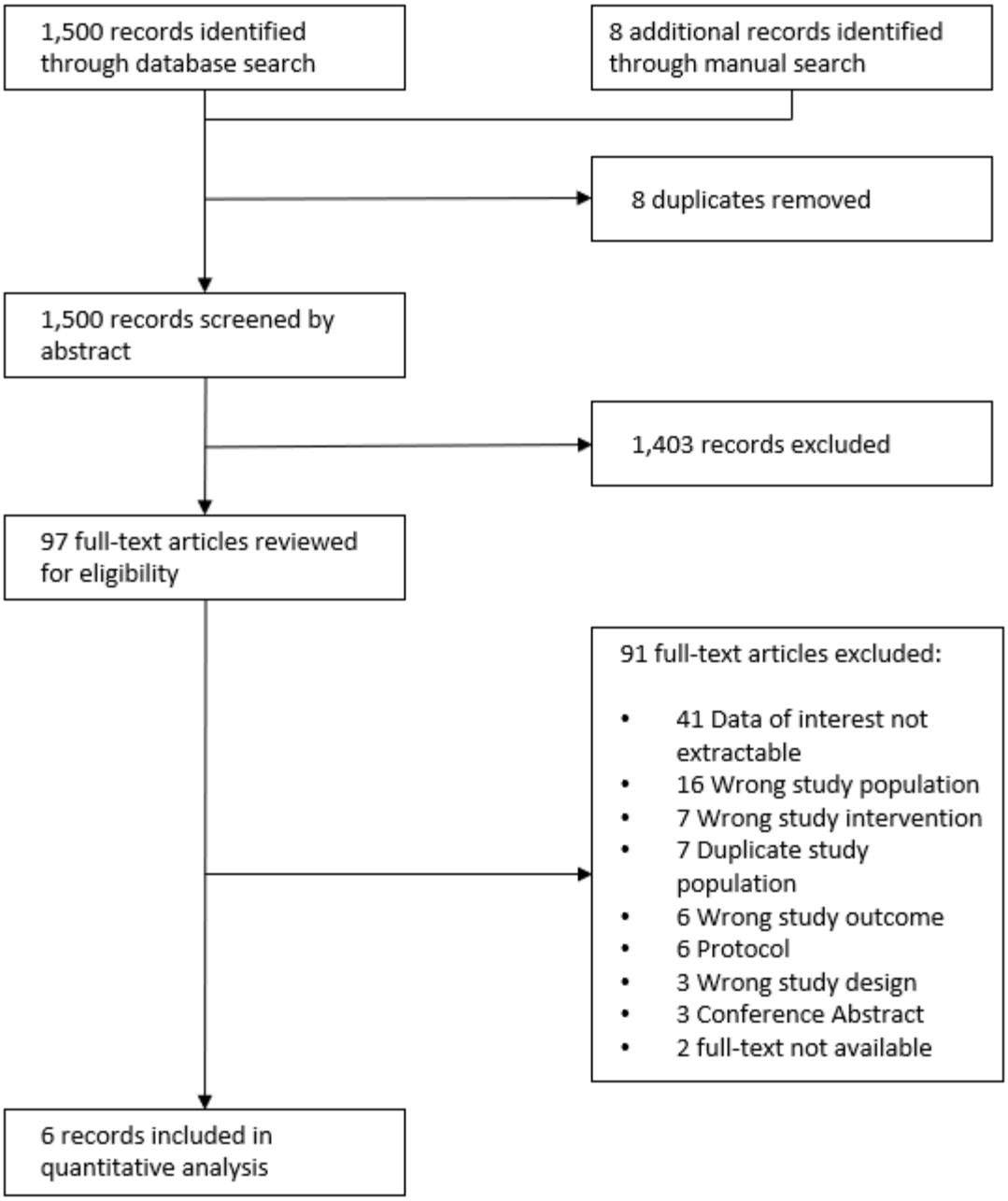


Figure 1. Prisma Flow Diagram.

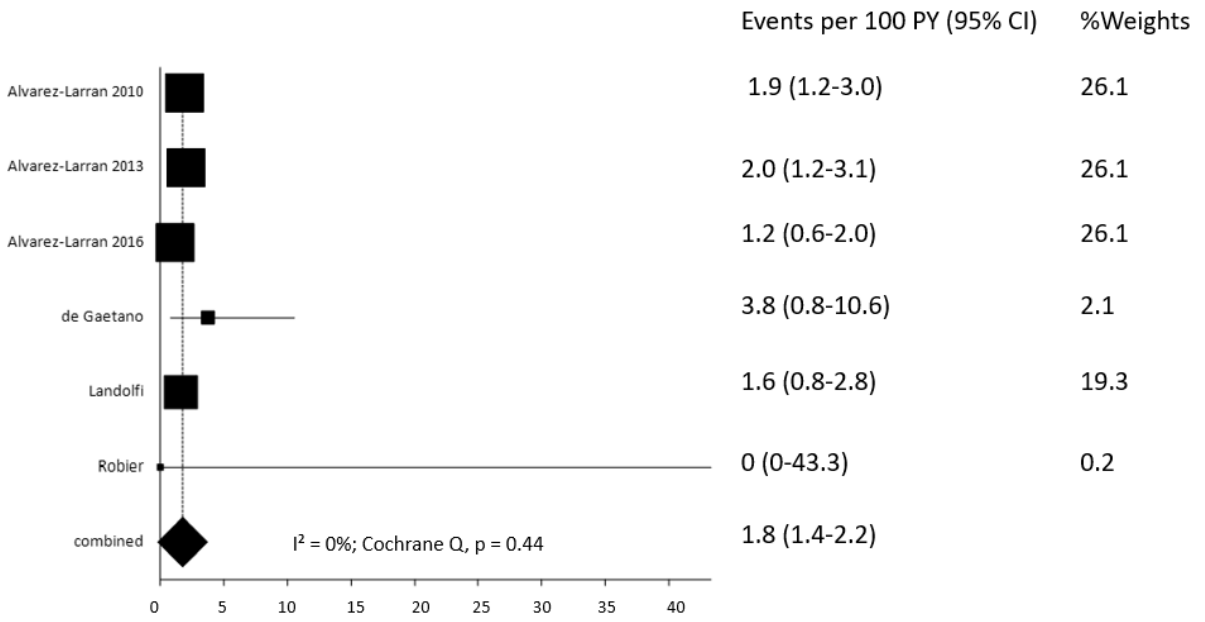


Figure 2. Pooled rate of thrombosis on low-dose aspirin thromboprophylaxis in patients with JAK2-positive myeloproliferative neoplasms.

CI: confidence interval, PY: patient-years

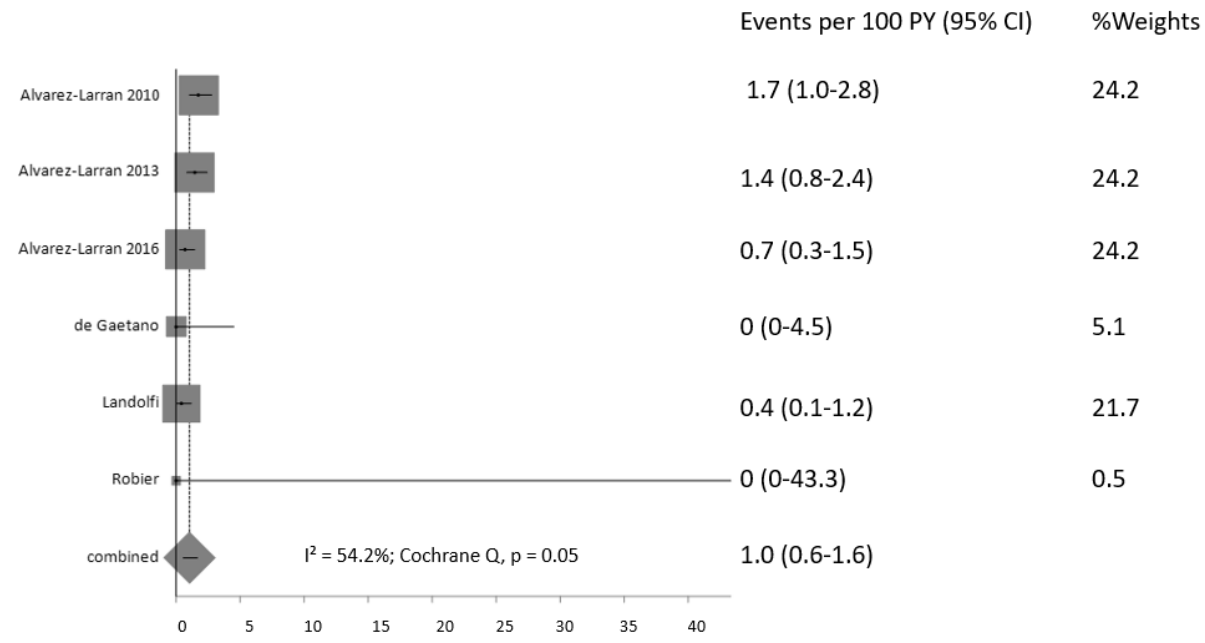


Figure 3. Pooled rate of major bleeding on low-dose aspirin thromboprophylaxis in patients with JAK2-positive myeloproliferative neaoplasms.

CI: confidence interval, PY: patient-years

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ANNEX

Table A1. Studies excluded at full text screening.

First Author	Year of Publication	Title	Reason for Exclusion
Alvarez-Larran	2009	Post-polycythaemic myelofibrosis: Frequency and risk factors for this complication in 116 patients.	Data of interest not extractable
Anger	1990	Idiopathic myelofibrosis: A retrospective study of 103 patients.	Wrong study population
Bai	2008	Risk factors of long-term incidences of thrombosis, myelofibrosis and evolution into malignance in polycythemia vera: A single center experience from China.	Data of interest not extractable
Barbui	2010	Thrombosis in primary myelofibrosis: Incidence and risk factors.	Data of interest not extractable
Barbui	2017	No correlation of intensity of phlebotomy regimen with risk of thrombosis in polycythemia vera: evidence from European Collaboration on Low-Dose Aspirin in Polycythemia Vera and Cytoreductive Therapy in Polycythemia Vera clinical trials.	Wrong study population
Barbui	2012	Disease characteristics and clinical outcome in young adults with essential thrombocythemia versus early/prefibrotic primary myelofibrosis.	Data of interest not extractable
Barbui	2015	Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia.	Data of interest not extractable

Barbui	2015	Patterns of presentation and thrombosis outcome in patients with polycythemia vera strictly defined by WHO-criteria and stratified by calendar period of diagnosis.	Data of interest not extractable
Bertozzi	2017	Thrombotic and hemorrhagic complications in idiopathic erythrocytosis	Data of interest not extractable
Billot	2011	Neurological disorders in essential thrombocythemia.	Wrong study population
Bjorn	2016	Ruxolitinib is manageable in patients with myelofibrosis and severe thrombocytopenia: A report on 12 Danish patients.	Wrong study design
Buxhofer-Ausch	2012	Leukocytosis as an important risk factor for arterial thrombosis in WHO-defined early/prefibrotic myelofibrosis: An international study of 264 patients.	Data of interest not extractable
Cacciola	2017	Optimal timing of aspirin intake in essential thrombocythemia.	Conference abstract
Campbell	2012	Correlation of blood counts with vascular complications in essential thrombocythemia: Analysis of the prospective PT1 cohort.	Data of interest not extractable
Campbell	2005	Definition of subtypes of essential thrombocythaemia andrelation to polycythaemia vera based on JAK2V617Fmutation status: a prospective study.	Data of interest not extractable
Campo	2009	Long-term outcome after percutaneous coronary intervention in patients with essential thrombocythemia.	Wrong study population
Carobbio	2010	Hydroxyurea in essential thrombocythemia: Rate and clinical relevance of responses by European LeukemiaNet criteria.	Data of interest not extractable
Carobbio	2007	Leukocytosis is a risk factor for thrombosis in essential thrombocythemia: Interaction with treatment, standard risk factors, and Jak2 mutation status.	Duplicate study population
Carobbio	2011	Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study on 891 patients.	Data of interest not extractable
Chistolini	1990	Essential thrombocythemia: A retrospective study on the clinical course of 100 patients.	Wrong study population
Cho	2009	Comparison of clinicopathologic findings according to JAK2 V617F mutation in patients with essential thrombocythemia.	Data of interest not extractable
Chornenki	2018	The real-world outcomes of treating Polycythemia Vera: Physician adherence to treatment guidelines.	Data of interest not extractable
Cortelazzo	1995	Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis.	Wrong study population
Dan	2006	Clinical features of polycythemia vera and essential thrombocythemia in Japan: Retrospective analysis of a nationwide survey by the Japanese Elderly Leukemia and Lymphoma Study Group.	Wrong study design
De Stefano	2018	Benefit-risk profile of cytoreductive drugs along with antiplatelet and antithrombotic therapy after transient ischemic attack or ischemic stroke in myeloproliferative neoplasms.	Data of interest not extractable
De Stefano	2008	The Aspirin Regimens in Essential Thrombocythemia (ARES) phase II randomized trial design: implementation of the serum thromboxane B2 assay as an evaluation tool of different aspirin dosing regimens in the clinical setting	Protocol

De Stefano	2018	Hydroxyurea prevents arterial and late venous thrombotic recurrences in patients with myeloproliferative neoplasms but fails in the splanchnic venous district. Pooled analysis of 1500 cases.	Data of interest not extractable
De Stefano	2016	High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with Vitamin K antagonists.	Data of interest not extractable
Desterro	2019	Essential thrombocythaemia treated with recombinant interferon: 'real world' United Kingdom referral centre experience.	Data of interest not extractable
Dillinger	2012	Twice daily aspirin to improve biological aspirin efficacy in patients with essential thrombocythemia.	Wrong study outcome
Dombi	2017	Anagrelide reduces thrombotic risk in essential thrombocythaemia vs. hydroxyurea plus aspirin,	Data of interest not extractable
Dombi	2017	[An audit of the Hungarian Philadelphia negative chronic myeloproliferative neoplasia registry. Evaluation of the essential thrombocythaemic patients]	Duplicate study population
Egli	1998	Polycythemia vera: Clinical findings and course in 86 patients.	Full-text not available
FAST	2015	Hydroxyurea Versus Aspirin and Hydroxyurea in Essential Thrombocythemia	Protocol
Finazzi	2004	A prospective analysis of thrombotic events in the European collaboration study on low-dose aspirin in polycythemia (ECLAP).	Data of interest not extractable
Finazzi	2005	Acute leukemia in polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study.	Wrong study outcome
Finazzi	2012	Incidence and risk factors for bleeding in 1104 patients with essential thrombocythemia or prefibrotic myelofibrosis diagnosed according to the 2008 WHO criteria.	Data of interest not extractable
Frohli	1983	[Prevention of vascular complications in polycythemia vera and primary thrombocythemia treated with low doses of acetylsalicylic acid]	Full-text not available
Gangat	2009	Predictors of pregnancy outcome in essential thrombocythemia: a single institution study of 63 pregnancies.	Wrong study population
Giraudet	2011	[Pregnancy and essential thrombocytemia].	Wrong study population
GISP	1997	Gruppo Italiano Studio Policitemia, Vera Low-dose aspirin in polycythaemia vera: a pilot study.	Duplicate study population
Gisslinger	2013	Anagrelide compared with hydroxyurea in WHO-classified essential thrombocythemia: The ANAHYDRET Study, a randomized controlled trial.	Data of interest not extractable
Godfrey	2017	Hydroxycarbamide plus aspirin vs aspirin alone in intermediate risk essential thrombocythemia: results of the PT-1 international, prospective, randomized clinical trial.	Data of interest not extractable
Godfrey	2018	Hydroxycarbamide plus aspirin versus aspirin alone in patients with essential thrombocythemia age 40 to 59 years without high-risk features.	Data of interest not extractable
Gotlib	2019	A randomized, open-label, multicenter, phase 2 study to evaluate the efficacy, safety, and pharmacokinetics of KRT-232 compared with ruxolitinib in patients with phlebotomy-dependent polycythemia vera.	Conference abstract
Griesshammer	2016	Outcomes of 121 pregnancies in patients with polycythemia vera (PV) Haematologica.	Conference abstract

Haider	2016	Validation of the revised International Prognostic Score of Thrombosis for Essential Thrombocythemia (IPSET-thrombosis) in 585 Mayo Clinic patients.	Wrong study intervention
Hansen	1979	The effect of acetyl salicylic acid and dipyridamole on thromboembolic complications in splenectomized patients with myelofibrosis.	Wrong study population
Harrison	2005	Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia.	Wrong study population
Hintermair	2018	Evaluation of vascular events in patients with myeloproliferative syndromes and mutations of either the januskinase-2 or calreticulin gene at the university hospital Krems from 2008 to 2015.	Data of interest not extractable
Hoekstra	2011	Long-term follow-up of patients with portal vein thrombosis and myeloproliferative neoplasms.	Data of interest not extractable
Ianotto	2017	Administration of direct oral anticoagulants in patients with myeloproliferative neoplasms.	Data of interest not extractable
ISCLAP	2007	International Study using Clopidogrel and Aspirin for the treatment of Polycythemia Vera.	Protocol
Jakovic	2018	The WHO diagnostic criteria for polycythemia vera-role of red cell mass versus hemoglobin/hematocrit level and morphology.	Wrong study outcome
Kander	2015	Bleeding complications in BCR-ABL negative myeloproliferative neoplasms: prevalence, type, and risk factors in a single-center cohort.	Data of interest not extractable
Kees	2005	Elevated plasma osteoprotegerin levels are associated with venous thrombosis and bleeding in patients with polycythemia vera.	Duplicate study population
Komrokji	2015	Results of a phase 2 study of pacritinib (SB1518), a JAK2/JAK2(V617F) inhibitor, in patients with myelofibrosis.	Wrong study intervention
Landolfi	2007	Leukocytosis as a major thrombotic risk factor in patients with polycythemia vera.	Duplicate study population
Landolfi	2012	Polycythemia vera: gender-related phenotypic differences.	Duplicate study population
Lekovic	2014	The importance of cardiovascular risk factors for thrombosis prediction in patients with essential thrombocythemia.	Data of interest not extractable
Lekovic	2015	Predictors of survival and cause of death in patients with essential thrombocythemia.	Duplicate study population
Lim	2015	Prediction of thrombotic and hemorrhagic events during polycythemia vera or essential thrombocythemia based on leukocyte burden.	Data of interest not extractable
Linardi	2008	Diagnosis and treatment of polycythemia vera: Brazilian experience from a single institution.	Data of interest not extractable
Low-PV	2016	The Benefit/Risk Profile of AOP2014 in Low-risk Patients With PV	Protocol
Marchioli	2005	Vascular and neoplastic risk in a large cohort of patients with polycythemia vera.	Data of interest not extractable
Marchioli	2013	CYTO-PV Cardiovascular Events and Intensity of Treatment in Polycythemia Vera.	Data of interest not extractable
Melillo	2009	Outcome of 122 pregnancies in essential thrombocythemia patients: A report from the Italian registry.	Wrong study population
Najean	1997	Treatment of polycythemia vera: use of 32P alone or in combination with maintenance therapy using hydroxyurea in 461 patients greater than 65 years of age.	Wrong study intervention

Nand	1996	Leukemogenic risk of hydroxyurea therapy in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis.	Wrong study population
Nussenzveig	2009	Imatinib mesylate therapy for polycythemia vera: final result of a phase II study initiated in 2001.	Wrong study intervention
Palandri	2009	Long-term follow-up of 386 consecutive patients with essential thrombocythemia: Safety of cytoreductive therapy.	Wrong study population
Parasuraman	2018	Cytoreductive treatment patterns among US veterans with polycythemia vera.	Wrong study intervention
Paysin	2014	JAK2 V617F mutation status of 232 patients diagnosed with chronic myeloproliferative neoplasms.	Data of interest not extractable
Peterson	2011	Published guidelines versus real-life practice in the diagnosis and treatment of essential thrombocythemia.	Data of interest not extractable
Posfai	2014	Stroke in essential thrombocythemia.	Wrong study design
PT1	2004	A randomised trial to compare ASPIRIN versus HYDROXYUREA/ASPIRIN in 'intermediate risk' primary thrombocythaemia and ASPIRIN only with observation in 'Low risk' primary thrombocythaemia.	Protocol
Rener	2012	Characteristics and treatment of patients with essential thrombocythemia during the period of 2008-2011	Data of interest not extractable
Ricksten	2008	Rapid decline of JAK2V617F levels during hydroxyurea treatment in patients with polycythemia vera and essential thrombocythemia.	Wrong study outcome
Rossi	1998	Acute coronary disease in essential thrombocythemia and polycythemia vera.	Data of interest not extractable
Ruggeri	1993	Is ticlopidine a safe alternative to aspirin for management of myeloproliferative disorders?	Wrong study population
Ruggeri	2010	Treatment outcome in a cohort of young patients with polycythemia vera.	Data of interest not extractable
Ruxo-BEAT	2015	The Ruxo-BEAT Trial in Patients With High-risk Polycythemia Vera or High-risk Essential Thrombocythemia	Protocol
Schafer	1982	Deficiency of platelet lipoxygenase activity in myeloproliferative disorders.	Wrong study outcome
Schwarz	2016	Thrombosis in thrombocythemic Ph- myeloproliferations is associated with higher platelet count prior to the event: results of analyses of prothrombotic risk factors from a registry of patients treated with anagrelide.	Data of interest not extractable
Sever	2012	The characteristics and treatment of patients with primary myelofibrosis at the university medical centre Ljubljana in the period 2008-2012.	Wrong study population
Shaikh	2016	Clinico-Pathological Profile And Outcomes Of Patients With Polycythaemia Vera, Essential Thrombocythaemia And Idiopathic Myelofibrosis: A Tertiary Care Center Experience From Southern Pakistan.	Wrong study outcome
Steurer	2004	Anagrelide for thrombocytosis in myeloproliferative disorders: a prospective study to assess efficacy and adverse event profile.	Wrong study intervention
Vannucchi	2007	Prospective identification of high-risk polycythemia vera patients based on JAK2V617F allele burden.	Data of interest not extractable
Varghese	2013	Essential thrombocythaemia: a single institution experience of 16 years.	Wrong study population
Verstovsek	2016	Ruxolitinib Versus Best Available Therapy in Patients With Polycythemia Vera: 80-week Follow-Up From the RESPONSE Trial.	Data of interest not extractable

Yacoub	2019	Pegylated interferon alfa-2a for polycythemia vera or essential thrombocythemia resistant or intolerant to hydroxyurea	Wrong study intervention

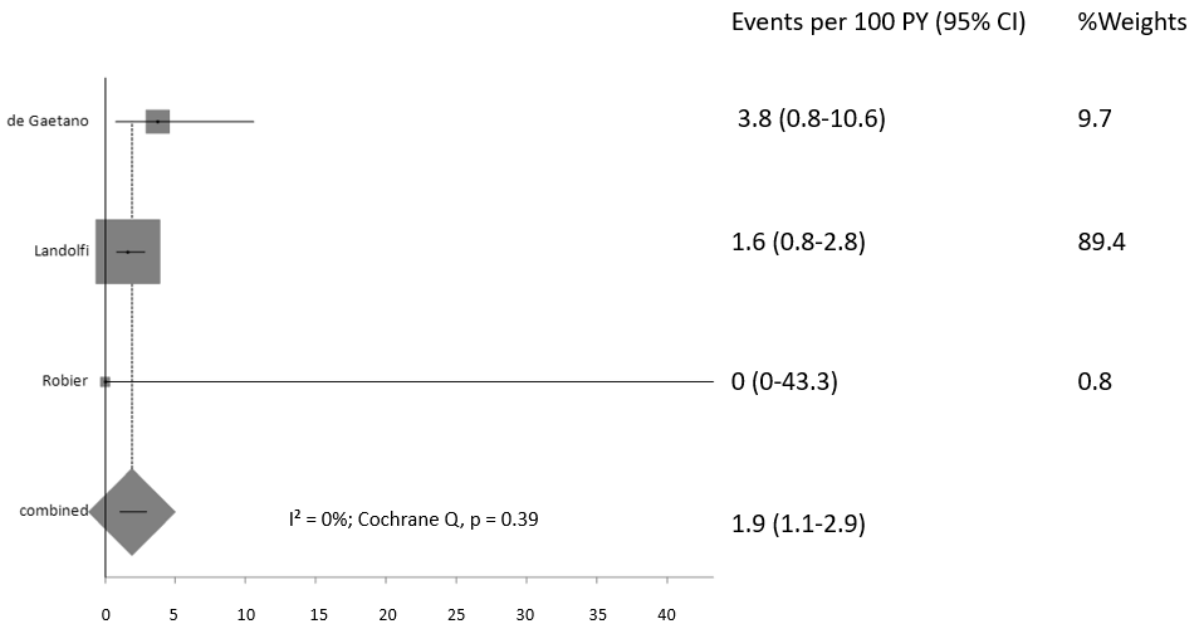


Figure A1. Pooled rate of thrombosis in polycythemia vera patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

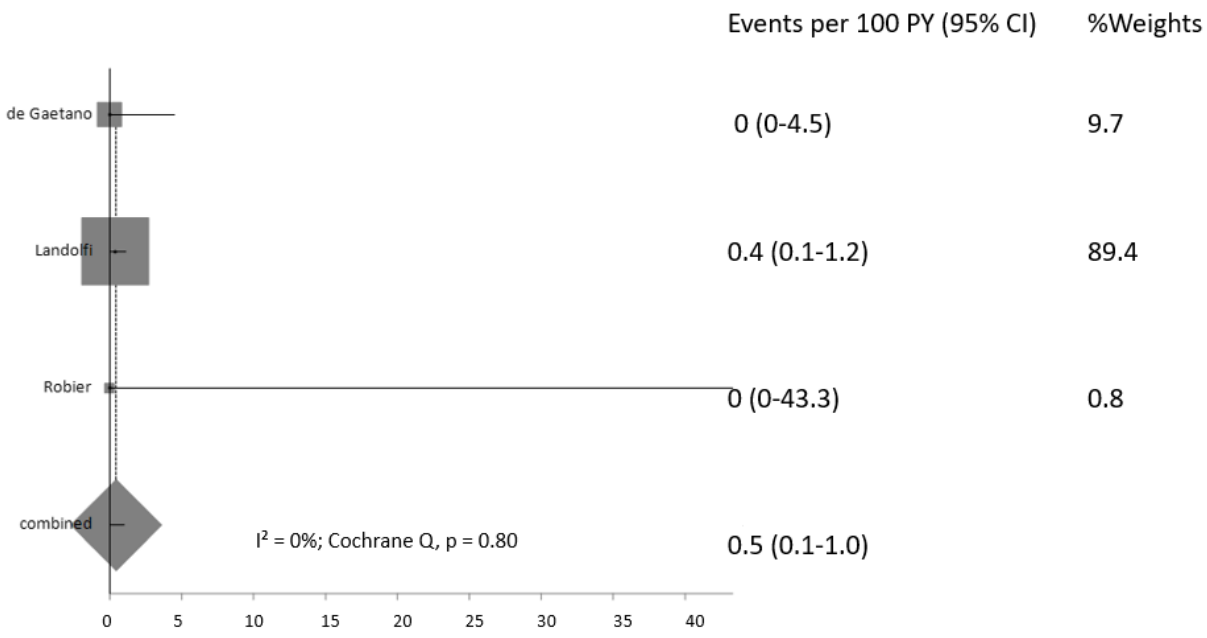


Figure A2. Pooled rate of major bleeding in polycythemia vera patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

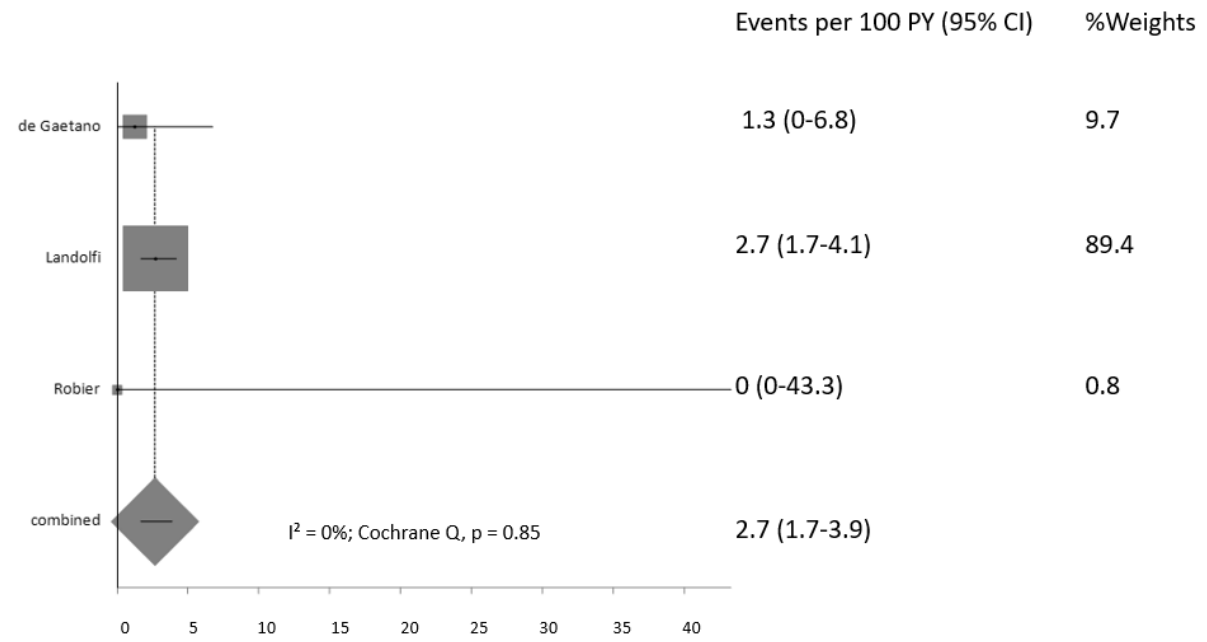


Figure A3. Pooled rate of non-major bleeding in polycythemia vera patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

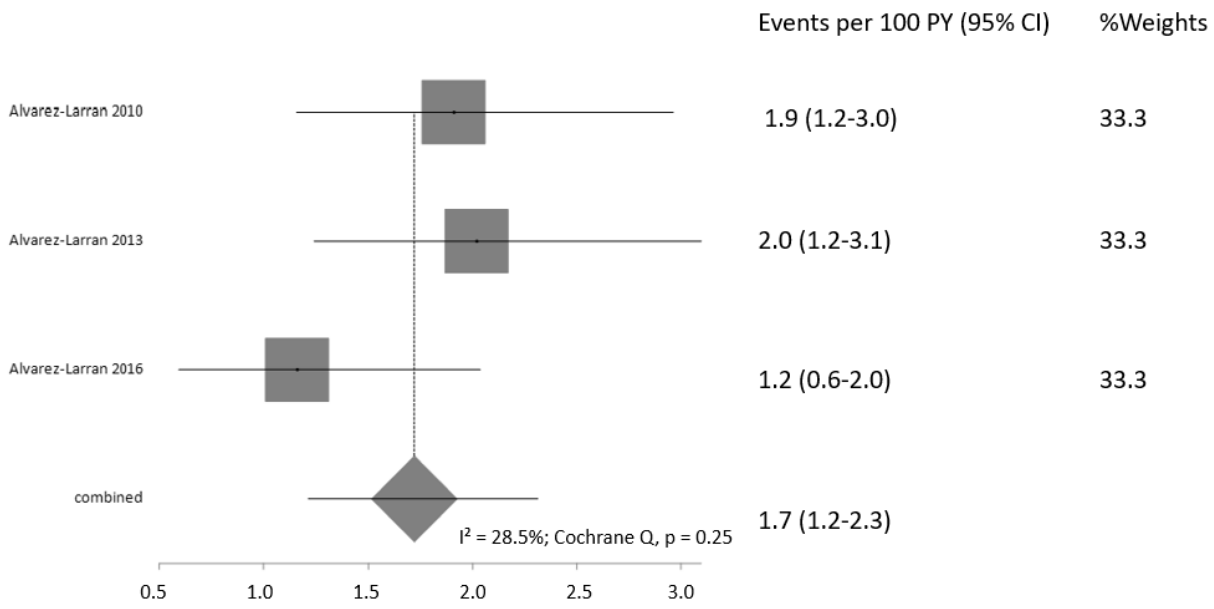


Figure A4. Pooled rate of thrombosis in JAK2-positive essential thrombocythemia patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

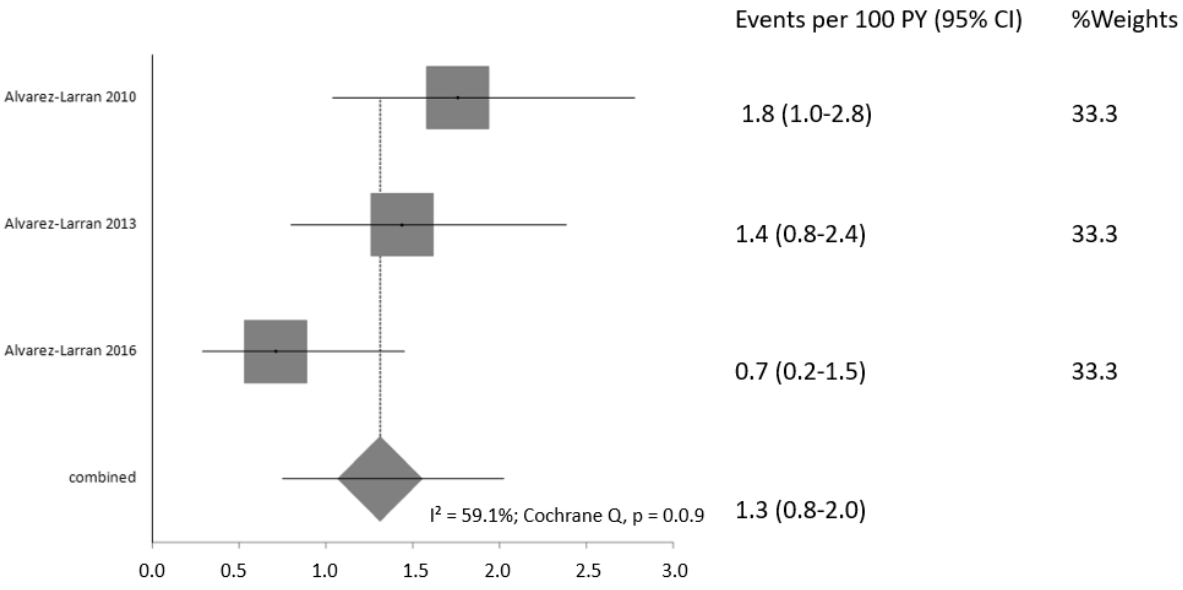


Figure A5. Pooled rate of major bleeding in JAK2-positive essential thrombocythemia patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

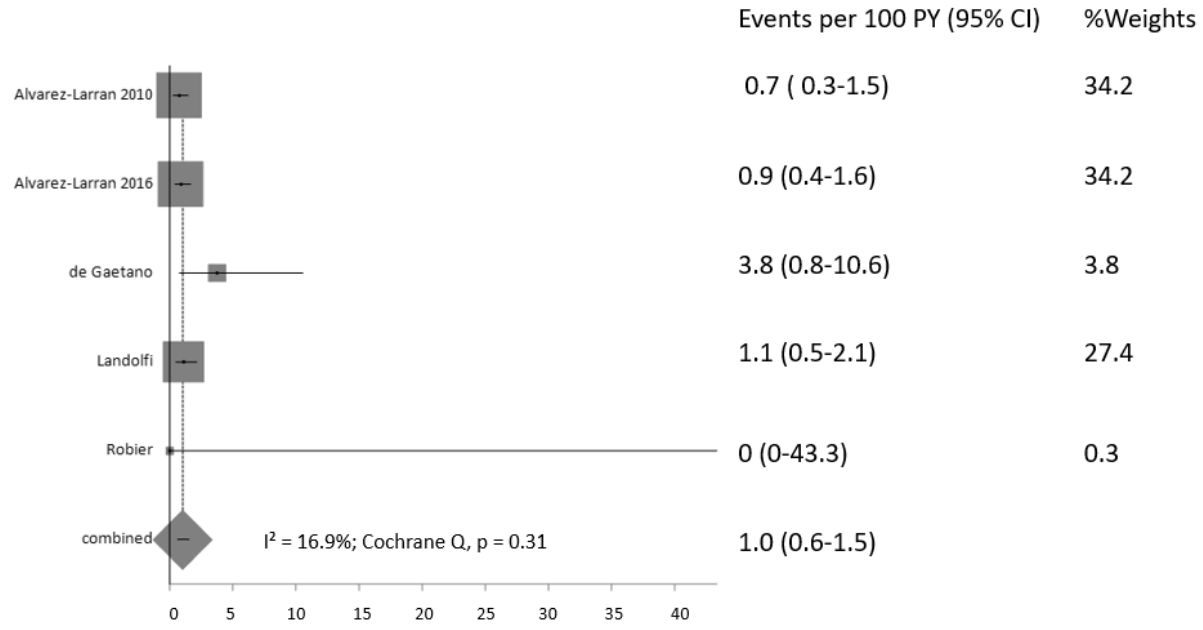


Figure A6. Pooled rate of arterial thrombosis in JAK2-positive myeloproliferative neoplasm patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

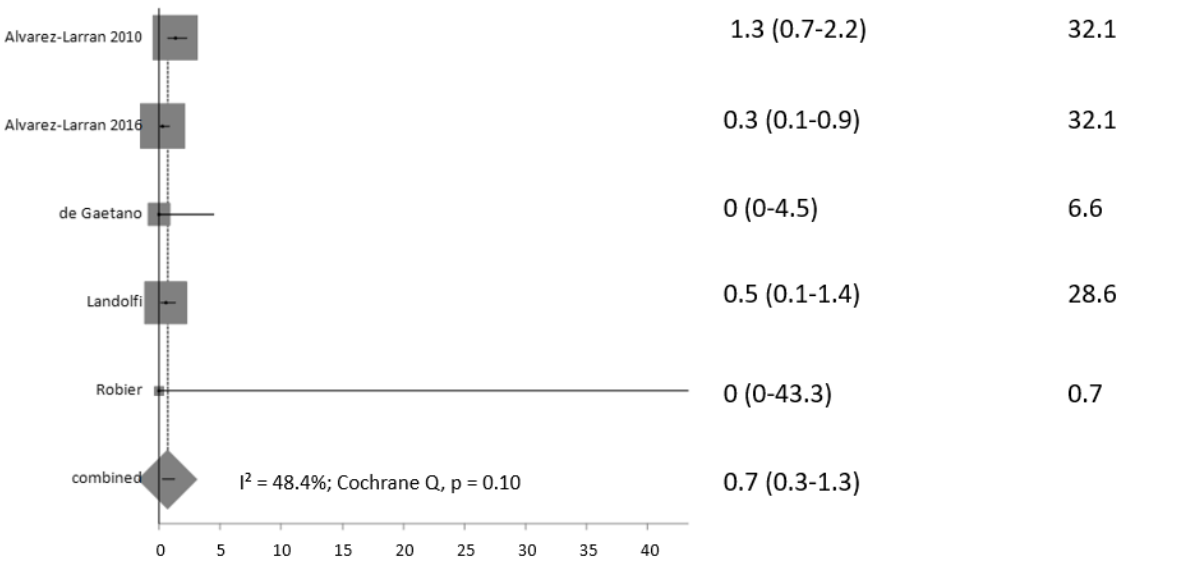


Figure A7. Pooled rate of venous thrombosis in JAK2-positive myeloproliferative neoplasm patients receiving low-dose aspirin thromboprophylaxis.

CI: confidence interval, PY: patient-years

SEARCH STRATEGY

Database: Embase Classic+Embase <1947 to 2020 February 19>, Ovid MEDLINE(R) ALL <1946 to February 20, 2020>, EBM Reviews - Cochrane Central Register of Controlled Trials <January 2020>

Search Strategy:

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1 exp Myeloproliferative Disorders/ (494830)
2 myeloproliferative.tw,kw. (30693)
3 Thrombocyth?em*.tw,kw. (12123)
4 Myelofibros*.tw,kw. (18436)
5 Bone Marrow Fibros*.tw,kw. (1845)
6 Myeloid metaplasia*.tw,kw. (2839)
7 Myeloscleros*.tw,kw. (523)
8 Primary Polycyth?em*.tw,kw. (353)
9 (Polycyth?em* adj2 Vera).tw. (15642)
10 Polycyth?em* Vera.kw. (2677)
11 Osler-Vaquez Disease.tw,kw. (5)
12 or/1-11 (504949)
13 exp Aspirin/ or aspirin*.tw,kw. (303220)
14 (acetylsalicylic acid* or acetyl salicylic acid).tw,kw. (28310)
15 or/13-14 (308724)
16 12 and 15 (5191)
17 exp animals/ not humans/ (17785787)
18 16 not 17 (3219)
19 18 use medall (726) Medline
20 18 use cctr (65) Cochrane
21 myeloproliferative disorder/ (16554)
22 myeloproliferative neoplasm/ (6037)
23 myeloproliferative.tw. (29982)
24 thrombocytosis/ (13126)

25 thrombocythemia/ (10722)
26 Thrombocyth?em*.tw. (11891)
27 myeloid metaplasia/ (12155)
28 Myelofibros*.tw. (18053)
29 Bone Marrow Fibros*.tw. (1818)
30 myelofibrosis/ (17064)
31 Myeloid metaplasia*.tw. (2638)
32 Myeloscleros*.tw. (517)
33 polycythemia vera/ (18338)
34 Primary Polycyth?em*.tw. (350)
35 (Polycyth?em* adj2 Vera).tw. (15642)
36 Osler-Vaquez Disease*.tw. (5)
37 or/21-36 (77651)
38 *acetylsalicylic acid/ (84473)
39 (Aspirin or acetylsalicylic acid or acetyl salicylic acid).tw. (196640)
40 38 or 39 (215464)
41 37 and 40 (2444)
42 (conference review or conference abstract).pt. (3741089)
43 exp animals/ not exp humans/ (10046681)
44 41 not (42 or 43) (1888)
45 44 use emczd (1268) Embase
46 19 or 20 or 45 (2059)
47 remove duplicates from 46 (1500)
48 47 use medall (718)
49 47 use emczd (743)
50 47 use cctr (39)

Chapter 3: Modified Delphi Process and Survey of Canadian Physicians

Delphi Process and Practice Survey of Canadian Hematologists and Thrombosis Experts on the Prevention and Management of Thrombotic Complications in JAK2-Positive Myeloproliferative Neoplasm Patients

Miriam Kimpton[1,2], Marc Carrier[1,2], Aurélien Delluc1,2

[1]Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada

[2]Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

Corresponding Author:

Miriam Kimpton

501 Smyth Road, PO Box 201A, Ottawa, ON, K1H 8L6

mkimpton@toh.ca

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Figure: 5

References: 25

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Abstract

The thromboprophylaxis of patients with JAK2-positive myeloproliferative neoplasms (JAK2MPN) is particularly challenging, given their risk of both thrombotic and hemorrhagic complications. Additionally, high quality data is limited to guide practice for both primary and secondary thromboprophylaxis in this patient population. Recently, the introduction of low-dose direct oral anticoagulants for the thromboprophylaxis of other patient populations offers other possible options for the prevention of arterial and venous thrombosis in JAK2MPN patients.

We conducted a modified Delphi process and practice survey of 20 Canadian thrombosis or hematology experts to define the minimal clinically important difference (MCID), which would lead to a change in their practice regarding the thromboprophylaxis of JAK2MPN patients, and to document practice heterogeneity in the primary and secondary thromboprophylaxis of this patient population.

Although half of the surveyed physicians believed that an absolute risk reduction of 10% in both arterial and venous thrombotic events combined over a 20-year follow up period would be significant enough to lead to a change in practice, consensus was not achieved (coefficient of variation of 33%, pre-determined threshold for consensus of ≤ 30%). Practice heterogeneity was noted when choosing a management strategy for the secondary thromboprophylaxis of these patients.

Our survey highlights some unmet research needs on the topic of thromboprophylaxis of JAK2MPN patients. Subsequent iterations of the modified Delphi process will be necessary to

define an MCID, which will help guide the design of future randomized controlled trials on the efficacy of new anti-thrombotics for the thromboprophylaxis of JAK2MPN patients.

Introduction

Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) are chronic, clonal diseases of the bone marrow, which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Patients with these diseases appear to be at increased risk of both thrombotic and hemorrhagic complications[1]. The presence and allele burden of the JAK2 mutation further increases the risk of thrombotic complications[2-7]. In PV patients and high-risk ET patients not receiving low-dose aspirin thromboprophylaxis on the basis of their hematological disease, the rate of thrombotic complications was shown to be 3.4 per 100 patient-years and 2.5 to 11 per 100-patient years, respectively[8, 9]. Current guidelines therefore recommend the use of low-dose aspirin thromboprophylaxis in all PV patients and ET patients at increased risk of thrombotic complications (including those with the JAK2 driver mutation)[10, 11]. Our own systematic review and meta-analysis of JAK2-positive MPN (JAK2MPN) patients receiving thromboprophylaxis with low-dose aspirin, however, showed a residual thrombotic rate (both arterial and venous) of 1.8 (95% CI, 1.4-2.2) per 100 patient-years, with a rate of major bleeding of 1.0 (95% CI, 0.6-1.6, I^2 =54.2%) per 100 patient-years. The current management of JAK2MPN patients therefore presents particular challenges for clinicians relating to the prevention of thrombotic complications (both arterial and venous), while minimizing the risk of bleeding. These difficulties are due to the fact that JAK2MPN are rare diseases[12], and, consequently, there is a limited body of high-quality data currently published on this topic[8, 13]. A prior international survey of hematologists found an important variability in practice for the treatment of arterial and venous thrombotic complications in MPN patients, which was not influenced by the physician's overall expertise with MPN[14]. It

remains to be seen whether similar diversity in clinical practice exists regarding the choice of primary thromboprophylaxis in this patient population.

Documenting such variability in clinical practice is an important first step in recognizing meaningful research questions by identifying clinical equipoise. This exercise may also help determine which patients are more likely to be recruited in a full-size trial comparing different primary and secondary thromboprophylactic strategies by detecting physicians' current treatment patterns and assessing the potential role of new anti-thrombotic therapies within their practice. Recently, randomized clinical trials have evaluated the use of a very-low-dose direct anticoagulant (rivaroxaban 2.5mg twice daily) plus aspirin in patients with stable cardiovascular disease or peripheral vascular disease [15], or after a revascularization procedure in patients with peripheral vascular disease[16]. In those patients, the addition of a very-low-dose direct oral anticoagulant to aspirin led to additional reduction of the thrombotic risk, when compared to aspirin alone, but at the cost of increased bleeding. Furthermore, two randomized clinical trials have assessed the use of low-dose direct oral anticoagulants (apixaban 2.5mg twice daily[17] or rivaroxaban 10mg daily[18]), compared to placebo, in ambulatory cancer patients receiving chemotherapy and found that this intervention led to a reduction in venous thromboembolic events. Of note, MPN patients were excluded from these trials. It is currently unknown whether clinicians have adopted direct oral anticoagulants (specifically apixaban or rivaroxaban) for the thromboprophylaxis of JAK2MPN patients by extrapolating from the available evidence in other patient populations.

Additionally, physician surveys offer the opportunity to determine the minimal clinically significant difference (MCID) on a specified topic, which would prompt a change in practice. The

MCID may be obtained by achieving consensus on such a value through a process called a modified Delphi process[19]. This process involves bringing together a group of subject-matter experts in order to take part in an anonymous and iterative process, where the previous rounds inform the subsequent ones, with the aim of achieving a consensus (as it is done for the MCID) or probe held beliefs on topics with limited evidence[20]. Identifying the MCID is critical when planning a randomized controlled trial, given that it informs the sample size calculation by providing the effect size needed for the results of the clinical trial to be of value to clinicians. Since JAK2MPN are chronic diseases, even small changes in the yearly rate of thrombotic complications can lead to an important cumulative incidence in thrombotic issues, and thereby produce an improvement in quality of life for a substantial number of patients living with these diseases. Achieving consensus on the target improvement in thrombotic incidence and identifying the willingness of physicians to conciliate a variety of factors which play a part in the risk-benefit balance of thromboprophylactic therapies (such as bleeding risk or efficacy of a particular medication at decreasing certain types of thrombotic complications over others), will help guide the design of a full-size randomized controlled trial evaluating the superiority of apixaban, a direct oral anticoagulant, compared to low-dose aspirin, the current standard of care, in preventing thrombotic outcomes in JAK2MPN patients.

The primary aim of the study was to identify the MCID in the absolute rate of thrombotic complications, which would be practice changing regarding the choice of primary thromboprophylaxis in JAK2MPN patients. The secondary aims were to describe practice variability associated with the primary thromboprophylaxis and secondary thromboprophylaxis of JAK2MPN patients and the willingness of physicians to incorporate direct oral anticoagulants

into the care regimen of JAK2MPN patients, by extrapolating what is known regarding the efficacy of these agents in other patient populations.

Methods

Physician members of the Canadian Venous Thromboembolism Clinical Trials and Outcomes Research (CanVECTOR) network and the Canadian Hematology Society (CHS) were asked to participate in our survey. These organizations are voluntary associations of clinicians, trainees, researchers, and other health professionals with an interest in hematology or thrombosis. The physician members of these organizations were chosen in order to provide a broad view of clinical practice, given that they were expected to have varying degrees of expertise in MPN and thrombosis management, and to work in the diverse clinical settings where JAK2MPN patients may find care, such as academic and community practices.

Survey respondents gained access to the survey via a hyperlink included in the invitation letter (Annex A), which was distributed through the regular manner in which these organizations communicate with their members. For the organizations using email to communicate with their own members, a reminder email to encourage survey participation was sent 7 days following the initial invitation. Participation in the survey was completely voluntary. Informed consent was assumed to have been granted by the physicians who choose to complete the survey. The survey was self-administered through the online software Survey Monkey, using the University of Ottawa's Enterprise license. All data amassed through this survey was anonymous. This questionnaire included four questions to capture demographic data, four questions as part of

the modified Delphi process, 10 clinical vignettes to determine practice variation in primary and secondary thromboprophylaxis of this rare patient population, and three questions to explore the preferred anti-thrombotic therapy to be assessed through future research (Annex B). If consensus was not reached following the first round of the modified Delphi process, then subsequent iterations of the modified Delphi process were to take place. For these ensuing rounds of the modified Delphi process, participants were to be provided with a summary of the results of the prior iteration, with the goal to guide consensus where possible. Ottawa Hospital Research Institute Research Ethics Board approval was obtained for the dissemination of the survey. The first iteration of the survey took place between October 2020 and January 2021.

Descriptive statistics were compiled to report the baseline characteristics of the survey participants. Consensus for the modified Delphi process was defined as having achieved a coefficient of variation of ≤ 30%[21, 22]. If it appeared that participants would not be able to reach a consensus (based on a lack of change in participants responses between survey iterations), then the modified Delphi process would be terminated.

Results

For the first iteration of the survey, there were 20 respondents out of a possible 126 physician members from the CanVECTOR organization (16%). The survey was also sent out in paper from through the Canadian Hematology Society's Newsletter, but none of the responses included in this interim analysis is believed to have been from CHS members. Of the respondents who provided demographic data, all were practicing in academic centres and most (63.2%) identified

the field of thrombosis as their main area of practice (Table 1). The majority of participants had more than 10 years in practice. Fifty-eight percent of respondents reported having a practice where research occupied about 50% or more of their time.

Type of Practice	
Academic	19 (100%)
Community	0
Years in Practice	
< 5	6 (31.6%)
5-10	5 (26.3%)
> 10	8 (42.1%)
Main Areas of Practice	
Thrombosis and MPN	5 (26.3%)
Thrombosis	12 (63.2%)
MPN	1 (5.3%)
Neither Thrombosis nor MPN	1 (5.3%)
Time dedicated to Research	
> 70%	5 (26.3%)
About 50%	6 (31.6%)
About 25%	8 (42.1%)

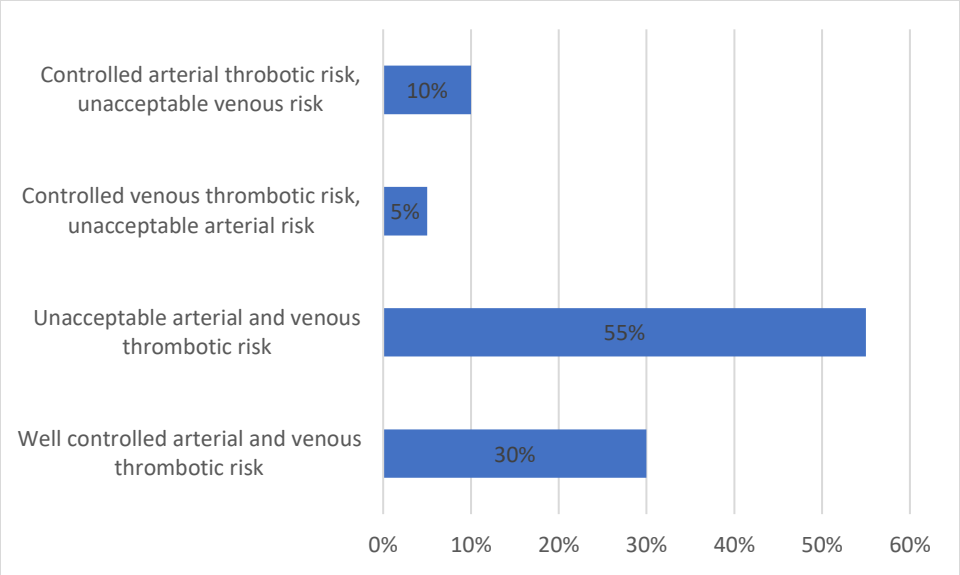
No significant research time	0 (0%)

Table 1. Baseline characteristics of survey participants.
Based on 19 respondents for this portion of the survey.
Totals may not come to 100% due to rounding.
MPN: myeloproliferative neoplasms.

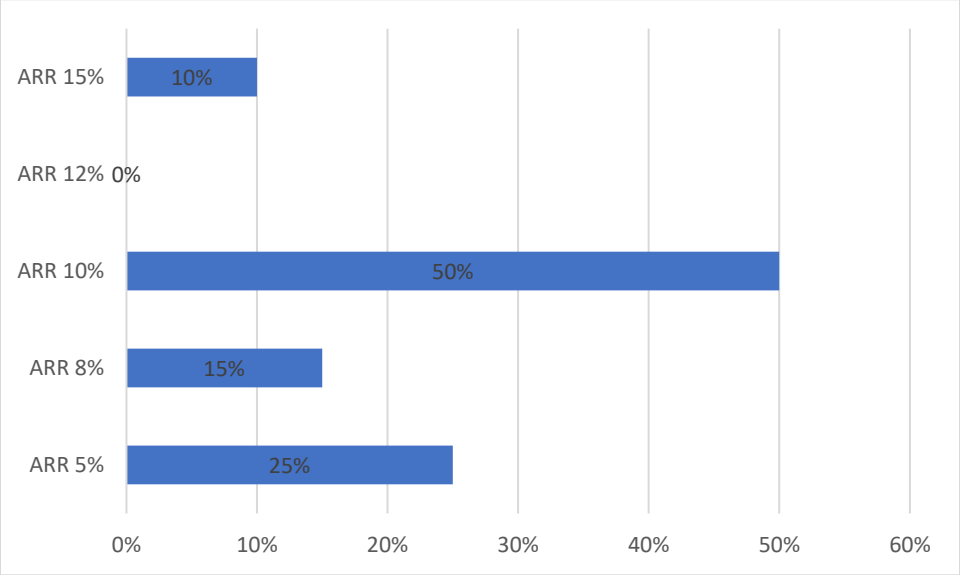
Most respondents (70%) believed that the thrombotic risk (either arterial, venous, or both) remained unacceptably high, despite the current management recommendations. Consensus was not achieved during the first iteration of the modified Delphi process (Figure 1). Half of the surveyed physicians believed that an absolute risk reduction of 10% in both arterial and venous thrombotic events combined over a 20-year follow up period would be significant enough to lead to a change in practice (coefficient of variation of 33%). Most participants were willing to accept a more efficacious alternative anti-thrombotic therapy when compared to aspirin (with one third relative reduction in thrombotic events) only if the absolute risk difference in major bleeding over 20 years did not increase or increased only by 3% (40% of respondents for each answer). Sixty-five percent of participants were willing to accept an alternative anti-thrombotic therapy with superior efficacy at reducing venous thrombotic events, while having no impact on the risk of arterial thrombotic complications.

Figure 1. First iteration of the modified Delphi process

a) What is your perception of the thrombotic risk of JAK2MPN patients with the current regimen of low-dose aspirin, optimization of cardiovascular risk factors, and cytoreductive therapies (where appropriate)?



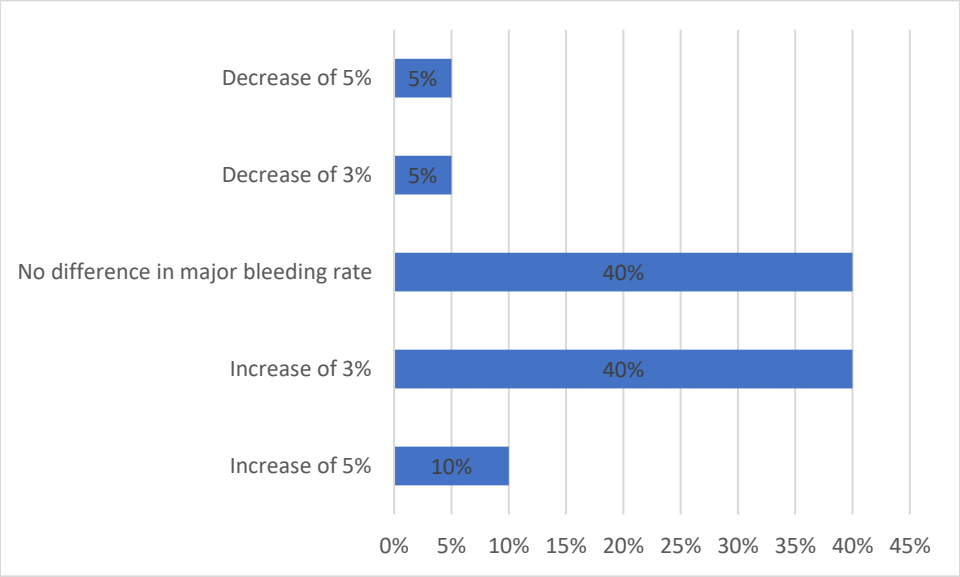
b) What absolute risk reduction in thrombotic events (arterial and venous) over a 20-year follow up period would you consider significant enough to lead to a change in your practice?



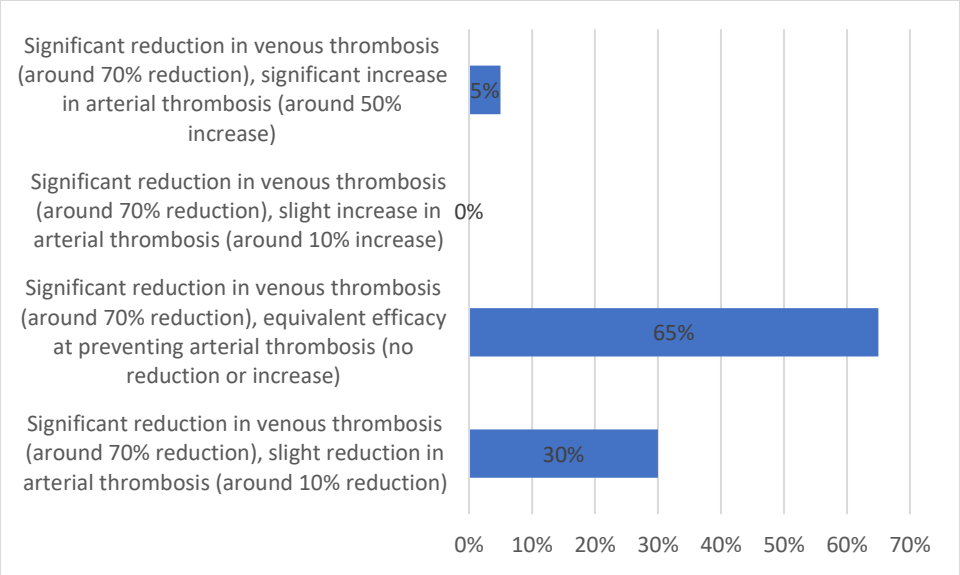
ARR: Absolute risk reduction

c) If a new anti-thrombotic medication were superior to aspirin at preventing thrombotic complications (with a one third relative reduction in thrombotic events), how would your

perception of the tolerated bleeding risk change? What absolute risk difference in major bleeding (compared to aspirin) over 20 years with this new agent would seem acceptable to you?



d) JAK2MPN patients experience both arterial and thrombotic complications. If a new anti-thrombotic agent, when compared to aspirin, reduced the cumulative thrombotic incidence but had different efficacy for the reduction of arterial and venous events, what is the least advantageous efficacy combination whereby you would still recommend this new medication (assuming major bleeding risk similar between the two therapies)?

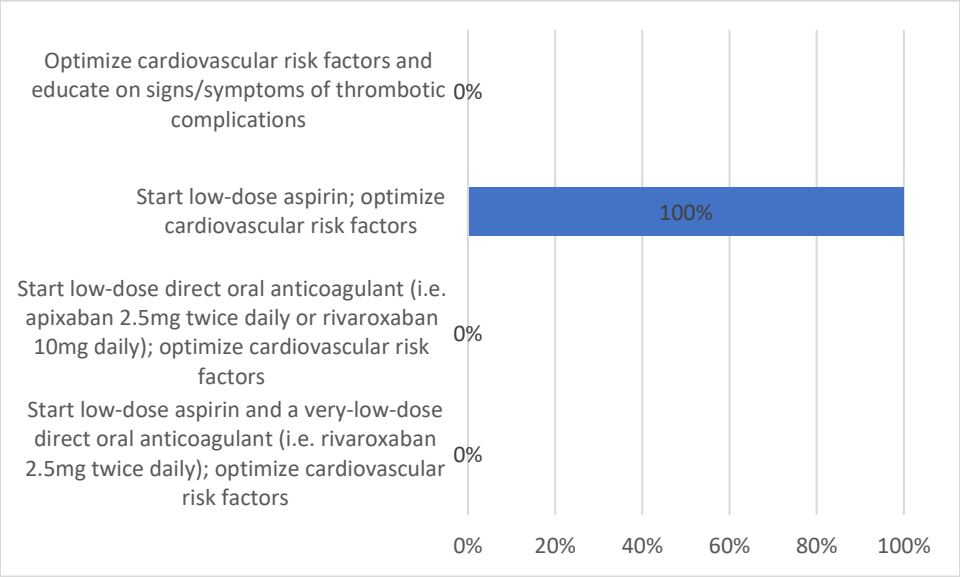


Nineteen physicians responded to the survey of practice questions. Responses regarding the primary thromboprophylaxis of well-controlled JAK2MPN patients were homogeneous (Figure 2). All respondents reported managing well-controlled PV and JAK2-positive ET patients with low-dose aspirin for primary thromboprophylaxis, as recommended by current guidelines. Ninety percent of participants reported the same strategy for well-controlled JAK2-positive PMF patients, in accordance with expert opinion.

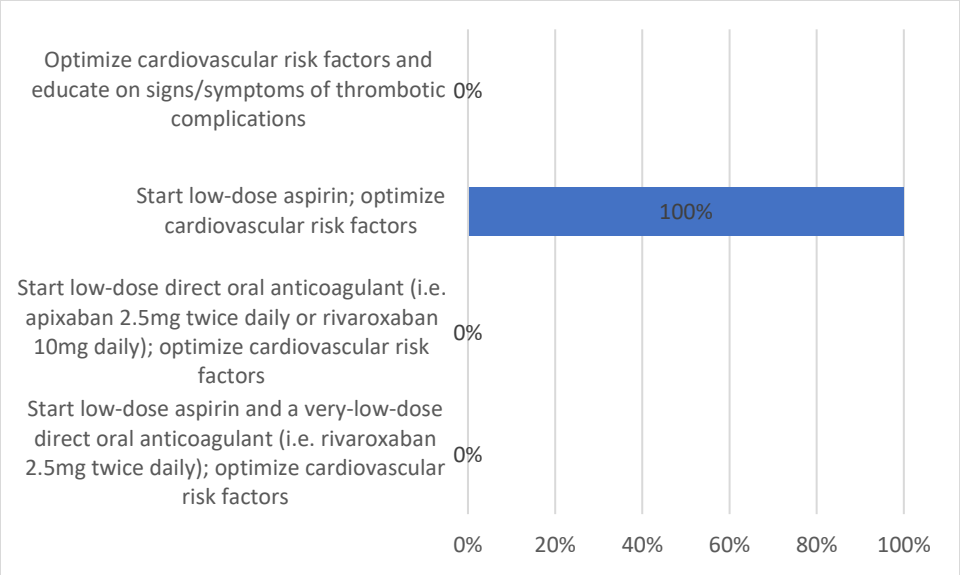
Figure 2. Responses for the primary thromboprophylaxis of MPN patients

a) 70F with JAK2-positive polycythemia vera (PV), no prior thrombotic events and no cardiovascular risk factors, apart from dyslipidemia. No bleeding risk factor. Hydroxyurea has been initiated and phlebotomies have been arranged to achieve a target hematocrit of less than 45%. Based on your current clinical practice, how would you manage this patient's

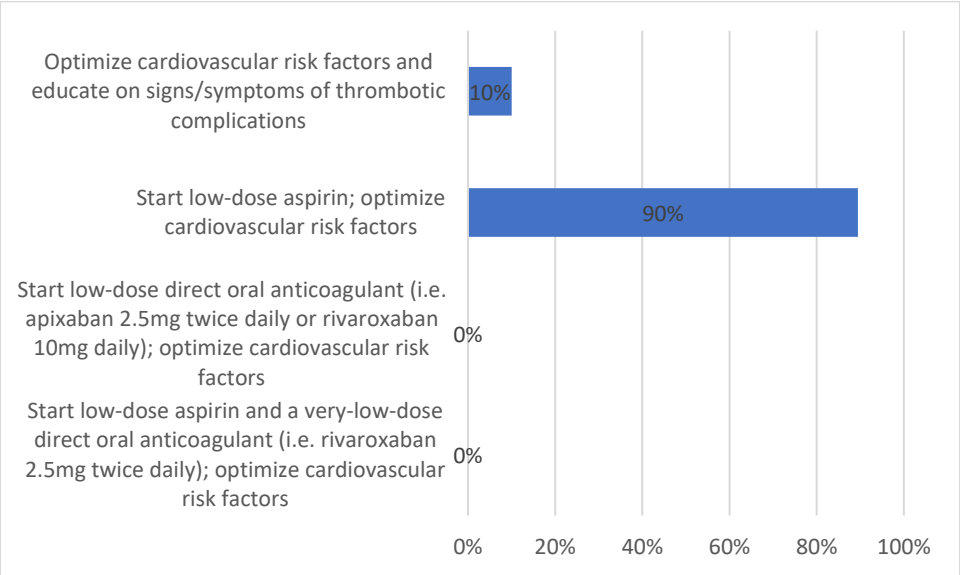
thrombotic risk (do not take into consideration governmental coverage or the patient's financial situation)?



b) 70F with JAK2-positive essential thrombocythemia, no prior thrombotic events and no cardiovascular risk factors, apart from dyslipidemia. No bleeding risk factor. Platelets are 700. Hydroxyurea has been initiated. Based on your current clinical practice, how would you manage this patient's thrombotic risk (do not take into consideration governmental coverage or the patient's financial situation)?



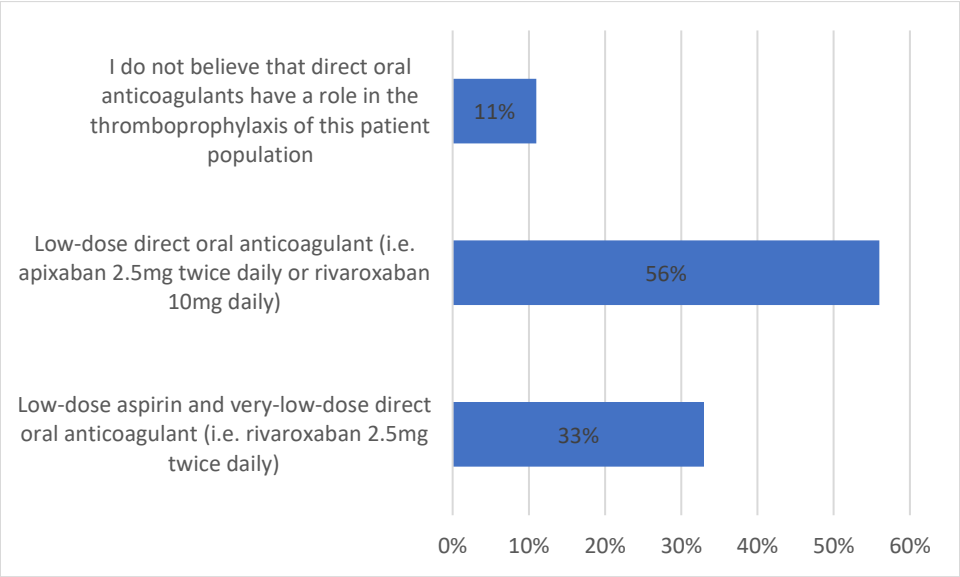
c) 70F with JAK2-positive pre-fibrotic primary myelofibrosis, no prior thrombotic events and no cardiovascular risk factors, apart from dyslipidemia. No bleeding risk factor. Platelets are 700. Hydroxyurea has been initiated. Based on your current clinical practice, how would you manage this patient's thrombotic risk (do not take into consideration governmental coverage or the patient's financial situation)?



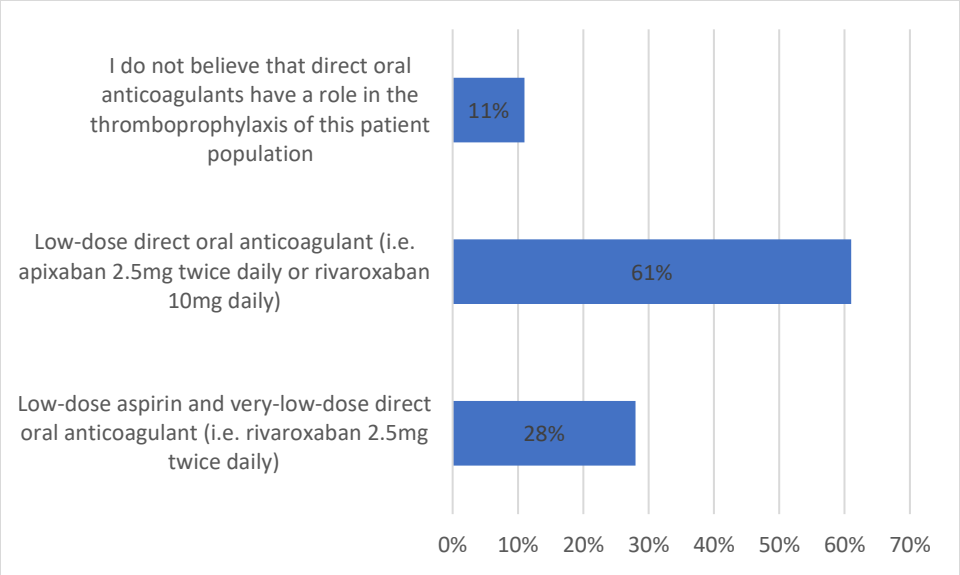
When asked which anti-thrombotic strategy for the primary thromboprophylaxis of JAK2MPN patients would warrant additional research efforts, the greatest proportion of participants identified low-dose anticoagulants (such as rivaroxaban 10mg daily or apixaban 2.5mg twice daily) as the best option (Figure 3).

Figure 3. Anti-thrombotic strategy warranting additional research endeavors for the primary thromboprophylaxis of myeloproliferative patients.

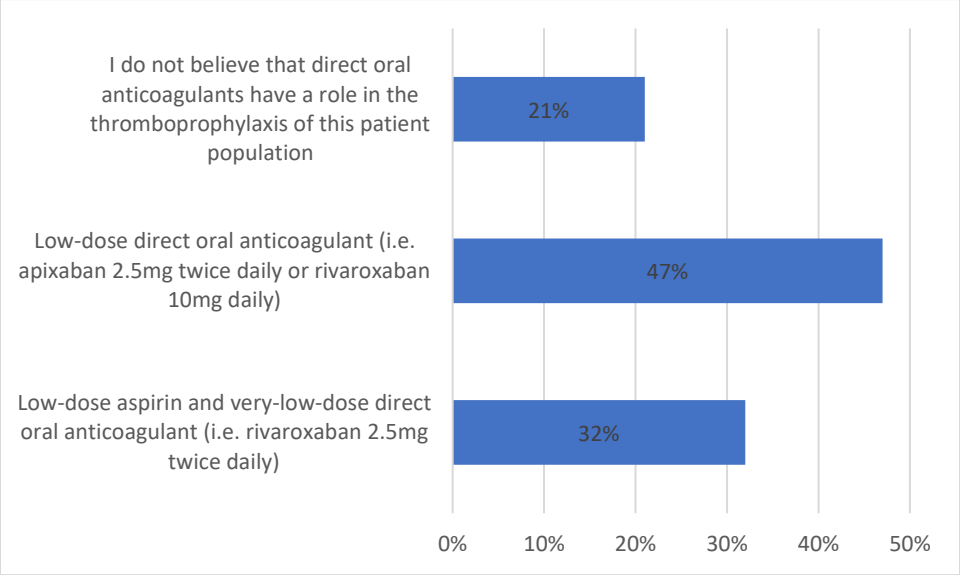
a) For the primary thromboprophylaxis of well-controlled polycythemia very patients: If you do not use direct oral anticoagulants in patients such as the one described above, which strategy do you believe warrants additional research?



b) For the primary thromboprophylaxis of well-controlled JAK2-positive essential thrombocythemia patients: If you do not use direct oral anticoagulants in patients such as the one described above, which strategy do you believe warrants additional research?



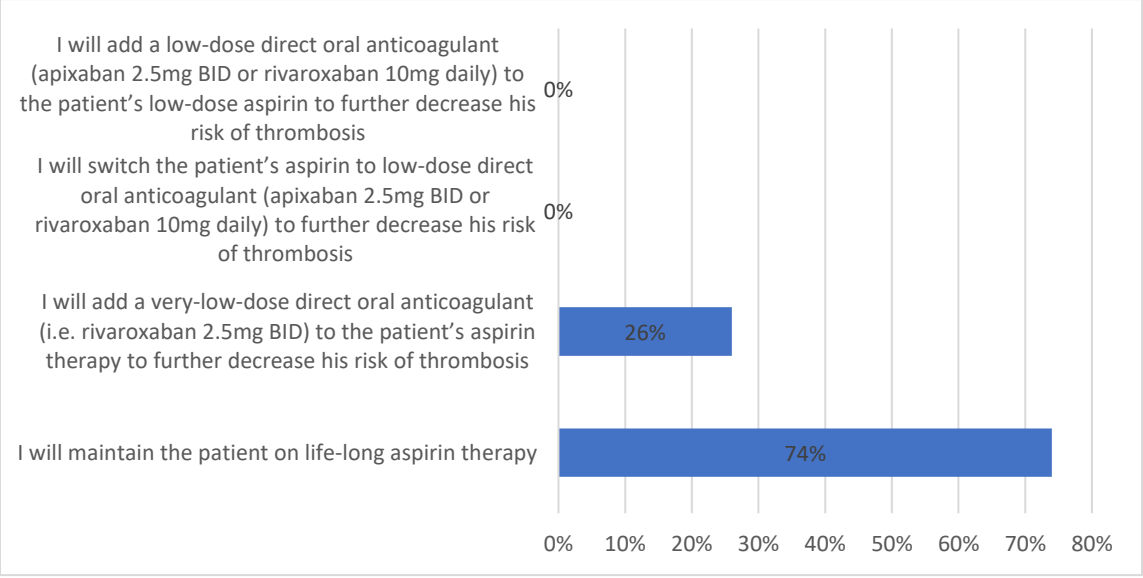
c) For the primary thromboprophylaxis of well-controlled JAK2-positive pre-fibrotic primary myelofibrosis patients: If you do not use direct oral anticoagulants in patients such as the one described above, which strategy do you believe warrants additional research?



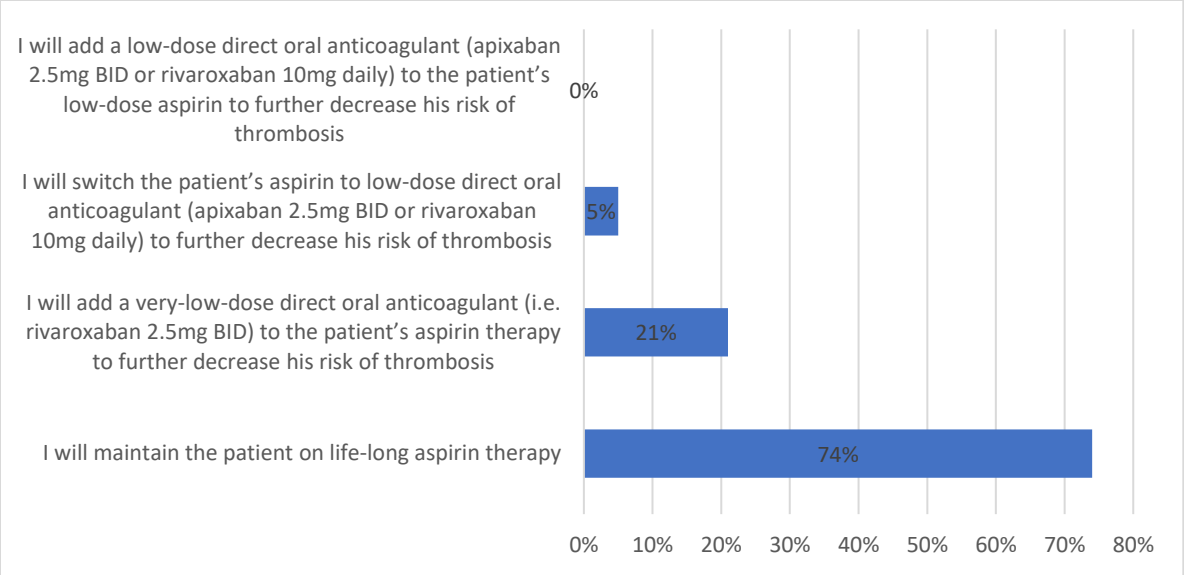
In PV patients with a prior arterial thrombotic complication, the majority of participants reported choosing to maintain therapy with low-dose aspirin for this indication (Figure 4).

Figure 4. Responses for the secondary prophylaxis of polycythemia patients with a prior arterial thrombotic complication.

a) 68M with PV, on hydroxyurea and aspirin. His hematocrit is currently 43%. The patient has a history of coronary artery disease, with a bare metal stent inserted 7 years ago for a non-ST-elevation myocardial infarction that he sustained around the time of PV diagnosis. He has not experienced any other thrombotic events since then. He is felt to be at low risk of bleeding. How would you manage this patient's thrombotic risk?



b) 68M with PV, on hydroxyurea and aspirin. His hematocrit is currently 43%. The patient has a history of a transient ischemic attack, which he sustained 7 years ago around the time of PV diagnosis. He has not experienced any other thrombotic events since then. He is felt to be at low risk of bleeding. How would you manage this patient's thrombotic risk?

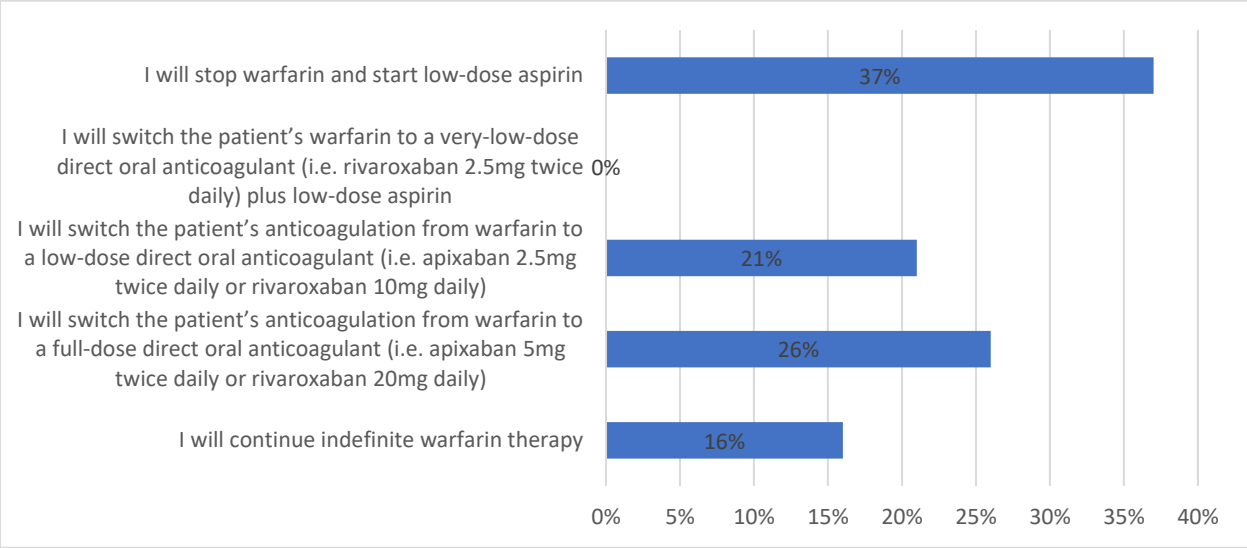


Some heterogeneity was noted in the secondary thromboprophylaxis of PV patients with a history of VTE (Figure 5). The greatest proportion of respondents preferred discontinuing anticoagulation (and re-starting low-dose aspirin) in a well-controlled PV patient with a provoked proximal DVT, following a minimum of 3 months of treatment. In a patient with a prior DVT without provoking factors (apart for the presence of PV), the majority of physicians felt comfortable managing these patients with a direct oral anticoagulant (following 6 months of treatment with warfarin), regardless of the lability of the INR (a measure of the quality of the anticoagulation therapy achieved with warfarin).

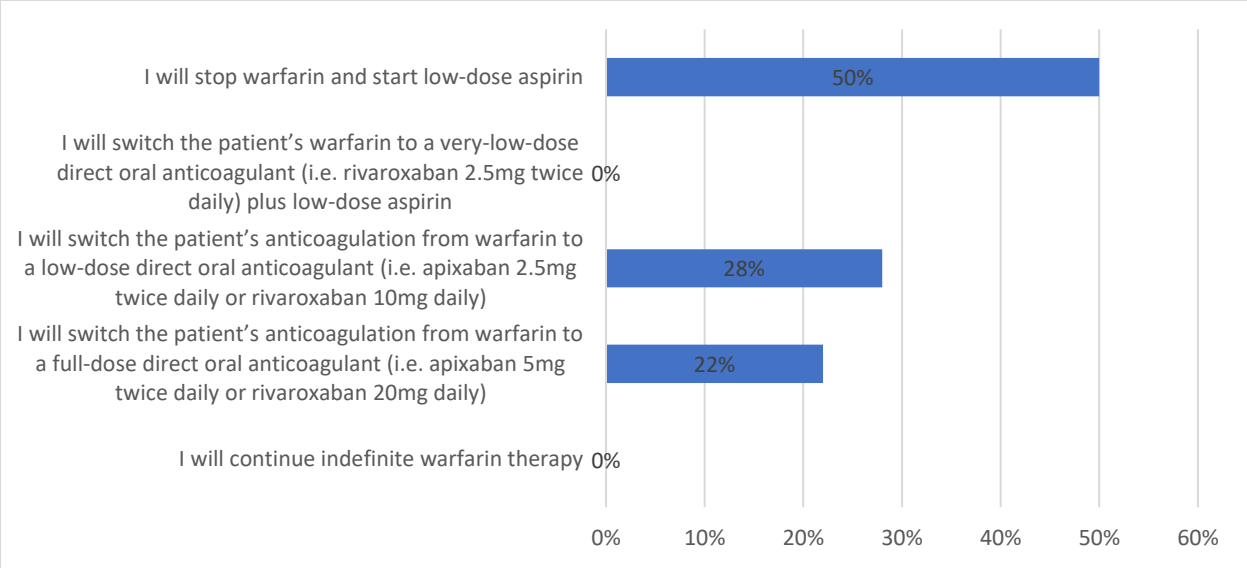
Figure 5. Responses for the secondary prophylaxis of polycythemia patients with a prior venous thrombotic complication.

a) 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a provoked proximal DVT (provoked by surgery), which he sustained 2 years ago

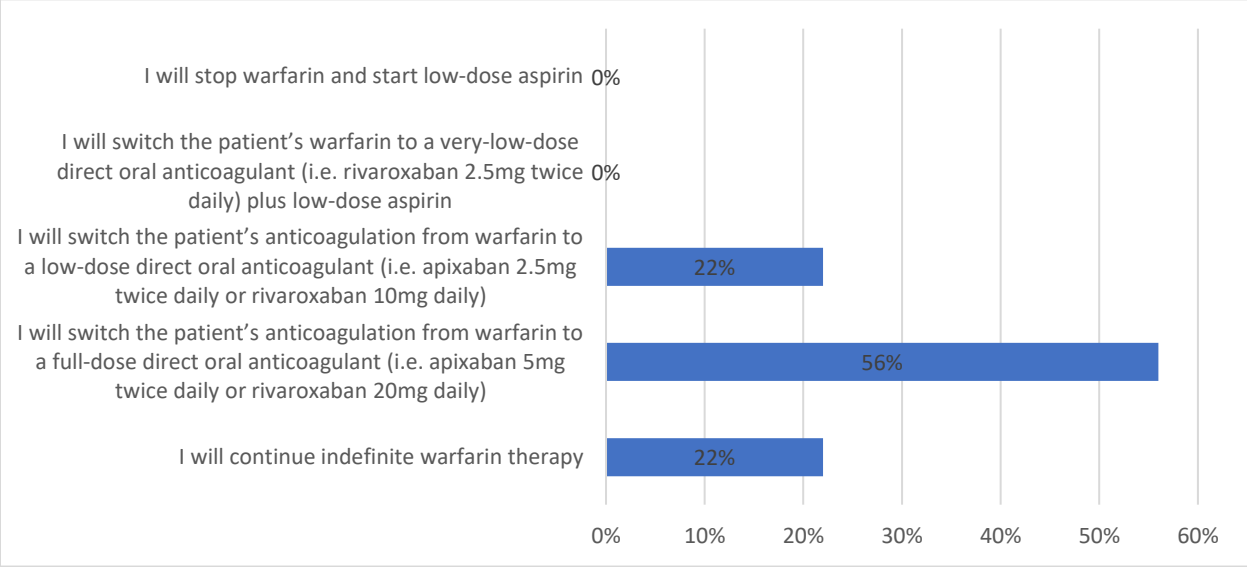
around the time of PV diagnosis. He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?



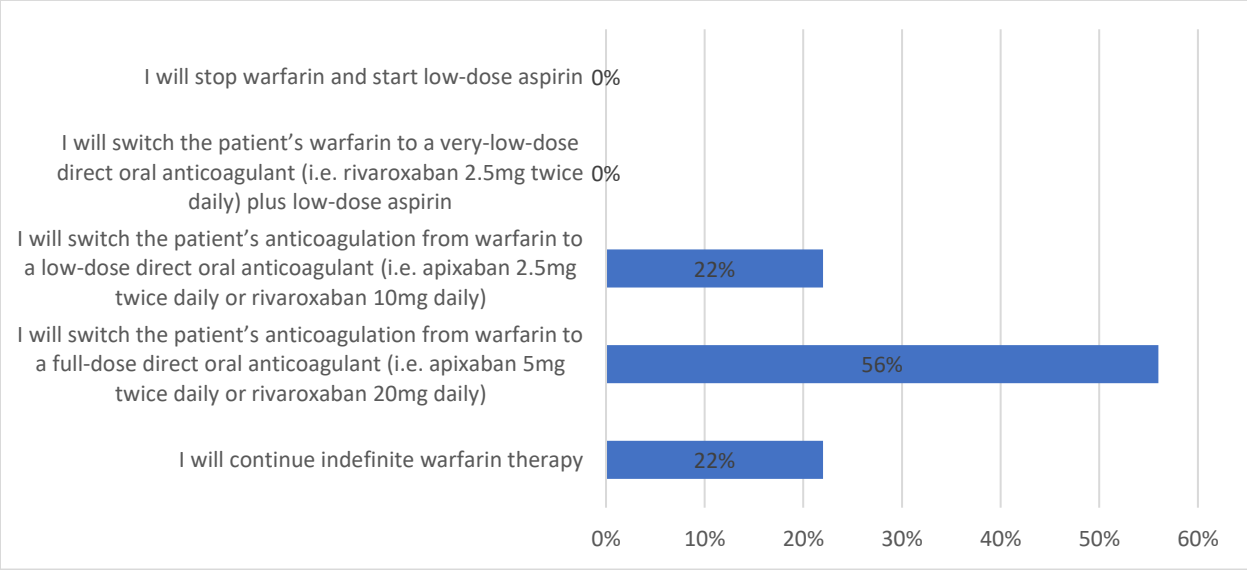
b) 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a provoked proximal DVT (provoked by surgery), which he sustained 3 months ago around the time of PV diagnosis. He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?



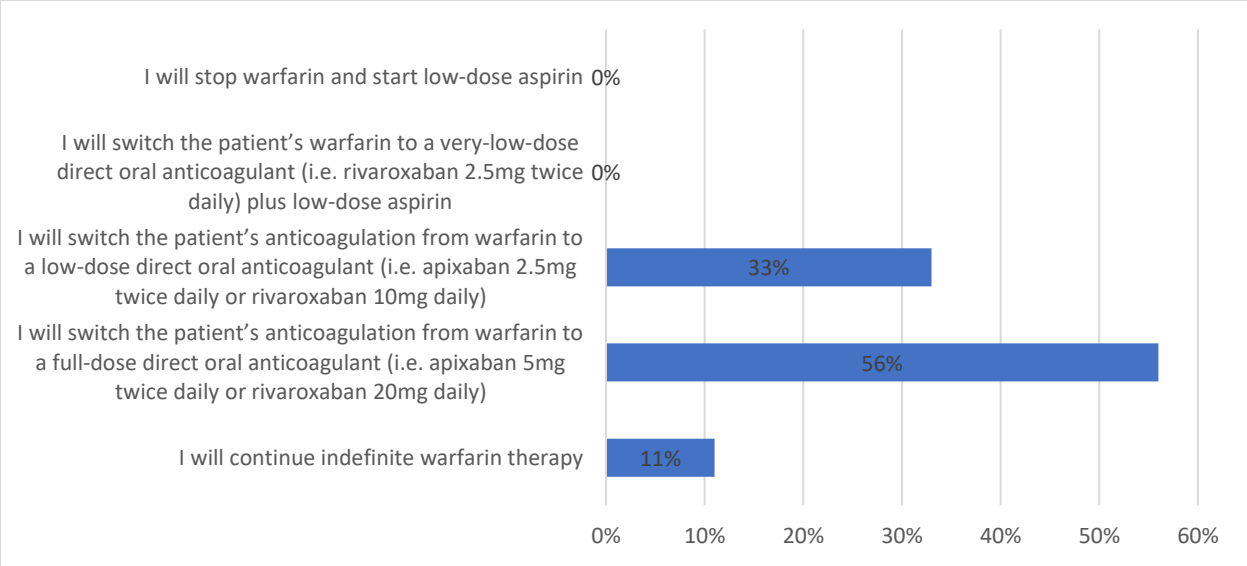
c) 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a proximal DVT which he sustained 2 years ago around the time of PV diagnosis (no other provoking factors). He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?



d) 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a proximal DVT which he sustained 6 months ago around the time of PV diagnosis (no other provoking factors). He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?



e) 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a proximal DVT which he sustained 2 years ago around the time of PV diagnosis (no other provoking factors). He has not experienced any other thrombotic events since then. His INR measurements have been labile. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?



Discussion

The results of this survey are informative regarding the risk-benefit balance to achieve, when proposing a novel primary thromboprophylactic agent in JAK2MPN patients. Although consensus on MCID was not reached during this first iteration of the modified Delphi process, it is encouraging to note that half of the surveyed Canadian hematologists and thrombosis experts selected a 10% reduction in absolute thrombotic risk over a 20-year follow up period, as being a meaningful difference (coefficient of variation 33%; pre-determined threshold for consensus ≤ 30%). Based on the previously published data in other patient populations[15, 23], such an MCID is expected to be attainable with the introduction of low-dose direct oral anticoagulants (such as rivaroxaban 10mg daily or apixaban 2.5mg twice daily) for the primary thromboprophylaxis of JAK2MPN patients. Subsequent iterations of the modified Delphi process, however, are needed in order to draw formal conclusions on this topic. Additionally, the use of low-dose direct oral anticoagulants was identified by the largest proportion of survey

participants as the therapeutic option, which would warrant additional research endeavors. This information will be invaluable when designing a full-scale randomized controlled trial on the efficacy of different anti-thrombotic strategies in this patient population.

The current practice of the surveyed physicians centred on the use of low-dose aspirin for the primary thromboprophylaxis of PV and JAK2-positive ET patients, in accordance with the current guidelines. Slightly more heterogeneity was noted regarding the use of low-dose aspirin for the primary thromboprophylaxis of JAK2-positive PMF patients, which reflects the dearth of evidence in this sub-population of patients, leading to a lack of strong guideline recommendations on the topic. Heterogeneity was observed in the management of PV patients requiring secondary venous thromboprophylaxis. It is interesting to note that about a quarter of physicians reported having already adopted the use of low-dose direct oral anticoagulants for the secondary venous thromboprophylaxis of JAK2MPN patients, despite the lack of evidence for this practice within the cancer population. Secondary venous thromboprophylaxis is a research topic previously identified as being a priority for future research by international MPN experts[24]. Our survey further bolsters the urgent need for additional research in this area.

As for the topic of secondary arterial thromboprophylaxis, the majority of respondents reported prescribing low-dose aspirin for this indication. Only about a quarter of participants stated having adopted the COMPASS trial regimen (low-dose aspirin plus very-low-dose rivaroxaban)[15] in PV patients with stable CAD. Although PV patients were not specifically sought for recruitment during the COMPASS trial, their hematological condition did not preclude them from participating, leaving open the possibility that a small number of the

COMPASS trial participants may have had or been diagnosed with PV during the trial period. Of note, 1084 patients developed cancer during the trial, including 594 identified with non-gastrointestinal or genitourinary cancers[25]. It remains unclear whether the physicians' reticence to adopt the COMPASS trial regimen within our survey is due to a general lack of confidence in the benefit of this regimen, when considering the accompanying increased bleeding risk, or whether physicians are particularly worried regarding the consequence of increased bleeding risk in PV patients.

Our study has several limitations. There was a poor response rate of 16% amongst the hematologists and thrombosis experts of the CanVECTOR network, leading to possible selection bias. This observation likely reflects the discomfort many physicians experience when managing patients with rare diseases. This poor initial response rate has led to a small number of answers being available for our analysis. It is possible that the results presented here will change, based on the compilation of subsequent survey data. Furthermore, few of the current survey participants identified themselves as MPN experts and all were from academic institutions, prohibiting any conclusions on the management of JAK2MPN patients within the community. Expanding the survey to other organizations, such as the CHS and the Canadian MPN Research Foundation will likely improve the number of respondents and the variety of participants.

The first iteration of our survey suggests areas of unmet research needs and an appetite from practicing physicians to adopt new anti-thrombotic strategies, such as low-dose direct oral anticoagulants, for the thromboprophylaxis of JAK2MPN patients. It is expected that subsequent iterations of the modified Delphi process will lead to a consensus regarding the MCID in efficacy, which would warrant a change in the current thromboprophylactic practice.

This information will be crucial for the design of randomized controlled trials on the efficacy of anti-thrombotic strategies in this patient population, which are eagerly awaited.

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Annex A

Dear CHS (or CanVECTOR network, CMPNRF, ASH, EHA, ISTH) physician member,

We invite you to participate in a practice survey regarding the primary and secondary thromboprophylaxis of patients with Philadelphia chromosome-negative myeloproliferative neoplasms, which include polycythemia vera, essential thrombocythemia, and primary myelofibrosis. These diseases are rare, chronic hematological malignancies. The presence and allele burden of the JAK2 mutation are associated with an increased thrombotic risk. As such, we would very much appreciate your help in understanding your current practice surrounding the management of JAK2-positive myeloproliferative neoplasm patients and in determining factors that would prompt you to adopt a new thromboprophylaxis strategy in these patients. We expect the survey to take 10 minutes to complete. All answers will be anonymous.

This study has been approved by The Ottawa Hospital Research Institute Research Ethics Board.

Please click on the link below to participate in our short survey.

Sincerely,

Miriam Kimpton

Annex B

Background:

JAK2-positive myeloproliferative neoplasms (JAK2MPN) include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These are chronic diseases and patients can live for decades after a diagnosis is made. There is limited evidence to guide the care of MPN patients, partly because MPN are rare diseases. The presence of the JAK2 mutation is associated with an increased risk for thrombosis. Based on two, small randomized controlled trials in PV patients, guidelines recommend that PV patients receive low-dose aspirin (such as aspirin 100mg daily) to prevent a first episode of venous or arterial thrombosis. Guidelines have also extrapolated this recommendation to JAK2-positive ET patients. There is very little data to help decide on the best management of PMF patients, but experts

recommend the use of low-dose aspirin in patients with factors that increase their risk for thrombosis, including the presence of the JAK2-mutation, in part because patients with (pre-fibrotic) PMF have a very similar clinical course to patients with ET. During the long-term follow up of JAK2MPN patients receiving low-dose aspirin thromboprophylaxis, the yearly rate of venous and arterial thrombotic complications is around 2 per 100 patients. Recently, randomized clinical trials have evaluated the use of a very-low-dose direct anticoagulant (rivaroxaban 2.5mg twice daily) plus aspirin in patients with stable cardiovascular disease or peripheral vascular disease, or after a revascularization procedure in patients with peripheral vascular disease. In those patients, the addition of a very-low-dose direct oral anticoagulant to aspirin has led to additional reduction of the thrombotic risk, when compared to aspirin alone. Furthermore, two randomized clinical trials have assessed the use of low-dose direct oral anticoagulants (apixaban 2.5mg twice daily or rivaroxaban 10mg daily) in ambulatory cancer patients receiving chemotherapy compared to placebo and found that this intervention led to a reduction in venous thromboembolic events. Of note, MPN patients were excluded from these trials. Acknowledging that there is limited evidence to guide clinical practice when it comes to the management of MPN patients and that the recent available data presented above was gathered from patient populations other than MPN patients, we would like to find out how you manage certain common scenarios faced by JAK2MPN patients.

<u>Modified Delphi Process:</u>

1. What is your perception of the thrombotic risk of JAK2MPN patients with the current regimen of low-dose aspirin, optimization of cardiovascular risk factors, and cytoreductive therapies (where appropriate)?
    a. The thrombotic risk of JAK2MPN patients (both arterial and venous) is well-controlled with the current regimen
    b. The thrombotic risk of JAK2MPN patients (both arterial and venous) remains unacceptably elevated
    c. The venous thrombotic risk of JAK2MPN patients is well-controlled but the arterial thrombotic risk remains unacceptably elevated
    d. The arterial thrombotic risk of JAK2MPN patients is well-controlled but the venous thrombotic risk remains unacceptably elevated

2. What absolute risk reduction in thrombotic events (arterial and venous) over a 20-year follow up period would you consider significant enough to lead to a change in your practice?
    a. 15%
    b. 12%
    c. 10%
    d. 8%
    e. 5%

3. If a new anti-thrombotic medication were superior to aspirin at preventing thrombotic complications (with a one third relative reduction in thrombotic events), how would your perception of the tolerated risk of bleeding change? What absolute risk difference in major bleeding (compared to aspirin) over 20 years with this new agent would seem acceptable to you?
    a. Increase of 5%
    b. Increase of 3%
    c. No difference in major bleeding rate
    d. Decrease of 3%
    e. Decrease of 5%

4. JAK2MPN patients experience both arterial and thrombotic complications. If a new anti-thrombotic agent, when compared to aspirin, reduced the cumulative thrombotic incidence but had different efficacy for the reduction of arterial and venous events, what is the **least** advantageous efficacy combination whereby you would still recommend this new medication (assuming major bleeding risk similar between the two therapies):
    a. Significant reduction in venous thrombosis (around 70% reduction), slight reduction in arterial thrombosis (around 10% reduction)
    b. Significant reduction in venous thrombosis (around 70% reduction), equivalent efficacy at preventing arterial thrombosis (no reduction or increase)

c. Significant reduction in venous thrombosis (around 70% reduction), slight increase in arterial thrombosis (around 10% increase)
d. Significant reduction in venous thrombosis (around 70% reduction), significant increase in arterial thrombosis (around 50% increase)

Survey of Practice:

5. 70F with JAK2-positive **polycythemia vera** (PV), no prior thrombotic events and no cardiovascular risk factors, apart from dyslipidemia. No bleeding risk factor. Hydroxyurea has been initiated and phlebotomies have been arranged to achieve a target hematocrit of less than 45%.

   Based on your current clinical practice, how would you manage this patient's thrombotic risk (do not take into consideration governmental coverage or the patient's financial situation)?

   a. Start low-dose aspirin and a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily); optimize cardiovascular risk factors
   b. Start low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily); optimize cardiovascular risk factors
   c. Start low-dose aspirin; optimize cardiovascular risk factors
   d. Optimize cardiovascular risk factors and educate on signs/symptoms of thrombotic complications

6. If you do not use direct oral anticoagulants in patients such as this one, which strategy do you believe warrants additional research?

a. Low-dose aspirin and very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily)
b. Low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)
c. I do not believe that direct oral anticoagulants have a role in the thromboprophylaxis of this patient population

7. 70F with JAK2-positive **essential thrombocythemia**, no prior thrombotic events and no cardiovascular risk factors, apart from dyslipidemia. No bleeding risk factor. Platelets are 700. Hydroxyurea has been initiated.

Based on your current clinical practice, how would you manage this patient's thrombotic risk (do not take into consideration governmental coverage or the patient's financial situation)?

a. Start low-dose aspirin and a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily); optimize cardiovascular risk factors

b. Start low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily); optimize cardiovascular risk factors

c. Start low-dose aspirin; optimize cardiovascular risk factors

d. Optimize cardiovascular risk factors and educate on signs/symptoms of thrombotic complications

8. If you do not use direct oral anticoagulants in patients such as this one, which strategy do you believe warrants additional research?

a. Low-dose aspirin and very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily)

b. Low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

c. I do not believe that direct oral anticoagulants have a role in the thromboprophylaxis of this patient population

9. 70F with JAK2-positive **pre-fibrotic primary myelofibrosis**, no prior thrombotic events and no cardiovascular risk factors, apart from dyslipidemia. No bleeding risk factor. Platelets are 700. Hydroxyurea has been initiated.

Based on your current clinical practice, how would you manage this patient's thrombotic risk (do not take into consideration governmental coverage or the patient's financial situation)?

a. Start low-dose aspirin and a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily); optimize cardiovascular risk factors

b. Start low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily); optimize cardiovascular risk factors

c. Start low-dose aspirin; optimize cardiovascular risk factors

d. Optimize cardiovascular risk factors and educate on signs/symptoms of thrombotic complications

10. If you do not use direct oral anticoagulants in patients such as this one, which strategy do you believe warrants additional research?

a. Low-dose aspirin and very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily)

b. Low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

c. I do not believe that direct oral anticoagulants have a role in the thromboprophylaxis of this patient population

11. 68M with PV, on hydroxyurea and aspirin. His hematocrit is currently 43%. The patient has a history of coronary artery disease, with a bare-metal stent inserted 7 years ago for a **non-ST-elevation myocardial infarction** that he sustained around the time of PV diagnosis. He has not experienced any other thrombotic events since then. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will maintain the patient on life-long aspirin therapy

b. I will add a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg BID) to the patient's aspirin therapy to further decrease his risk of thrombosis

c. I will switch the patient's aspirin to low-dose direct oral anticoagulant (apixaban 2.5mg BID or rivaroxaban 10mg daily) to further decrease his risk of thrombosis

d. I will add a low-dose direct oral anticoagulant (apixaban 2.5mg BID or rivaroxaban 10mg daily) to the patient's low-dose aspirin to further decrease his risk of thrombosis

12. 68M with PV, on hydroxyurea and aspirin. His hematocrit is currently 43%. The patient has a history of a **transient ischemic attack,** which he sustained 7 years ago around the time of PV diagnosis. He has not experienced any other thrombotic events since then. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will maintain the patient on life-long aspirin therapy

b. I will add a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg BID) to the patient's aspirin therapy to further decrease his risk of thrombosis

c. I will switch the patient's aspirin to low-dose direct oral anticoagulant (apixaban 2.5mg BID or rivaroxaban 10mg daily) to further decrease his risk of thrombosis

d. I will add a low-dose direct oral anticoagulant (apixaban 2.5mg BID or rivaroxaban 10mg daily) to the patient's low-dose aspirin to further decrease his risk of thrombosis

13. 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a **provoked** proximal DVT (provoked by surgery), which he sustained **2 years ago** around the time of PV diagnosis. He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will continue indefinite warfarin therapy

b. I will switch the patient's anticoagulation from warfarin to a full-dose direct oral anticoagulant (i.e. apixaban 5mg twice daily or rivaroxaban 20mg daily)

c. I will switch the patient's anticoagulation from warfarin to a low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

d. I will switch the patient's warfarin to a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily) plus low-dose aspirin

e. I will stop warfarin and start low-dose aspirin

14. 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a **provoked** proximal DVT (provoked by surgery), which he sustained **3 months ago** around the time of PV diagnosis. He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will continue indefinite warfarin therapy

b. I will switch the patient's anticoagulation from warfarin to a full-dose direct oral anticoagulant (i.e. apixaban 5mg twice daily or rivaroxaban 20mg daily)

c. I will switch the patient's anticoagulation from warfarin to a low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

d. I will switch the patient's warfarin to a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily) plus low-dose aspirin

e. I will stop warfarin and start low-dose aspirin

15. 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a proximal DVT which he sustained **2 years ago** around the time of PV diagnosis **(no other provoking factors)**. He has not experienced any other

thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will continue indefinite warfarin therapy

b. I will switch the patient's anticoagulation from warfarin to a full-dose direct oral anticoagulant (i.e. apixaban 5mg twice daily or rivaroxaban 20mg daily)

c. I will switch the patient's anticoagulation from warfarin to a low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

d. I will switch the patient's warfarin to a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily) plus low-dose aspirin

e. I will stop warfarin and start low-dose aspirin

16. 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a proximal DVT which he sustained **6 months ago** around the time of PV diagnosis **(no other provoking factors)**. He has not experienced any other thrombotic events since then. His INR measurements are mostly at target. He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will continue indefinite warfarin therapy

b. I will switch the patient's anticoagulation from warfarin to a full-dose direct oral anticoagulant (i.e. apixaban 5mg twice daily or rivaroxaban 20mg daily)

c. I will switch the patient's anticoagulation from warfarin to a low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

d. I will switch the patient's warfarin to a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily) plus low-dose aspirin

e. I will stop warfarin and start low-dose aspirin

17. 68M with PV, on hydroxyurea and warfarin. His hematocrit is currently 43%. The patient has a history of a proximal DVT which he sustained **2 years ago** around the time of PV diagnosis **(no other provoking factors)**. He has not experienced any other thrombotic events since then. **His INR measurements have been labile.** He is felt to be at low-risk of bleeding. How would you manage this patient's thrombotic risk?

a. I will continue indefinite warfarin therapy and attempt to address the issues related to the labile INR

b. I will switch the patient's anticoagulation from warfarin to a full-dose direct oral anticoagulant (i.e. apixaban 5mg twice daily or rivaroxaban 20mg daily)

c. I will switch the patient's anticoagulation from warfarin to a low-dose direct oral anticoagulant (i.e. apixaban 2.5mg twice daily or rivaroxaban 10mg daily)

d. I will switch the patient's warfarin to a very-low-dose direct oral anticoagulant (i.e. rivaroxaban 2.5mg twice daily) plus low-dose aspirin

e. I will stop warfarin and start low-dose aspirin

Demographic data:

18. Where do you practice?

    a. Academic centre

    b. Community

19. How many years have you been in practice?

    a. < 5 year

    b. 5-10 years

    c. > 10 years

20. What are main areas of your practice?

    a. Thrombosis and myeloproliferative neoplasms (MPN) are main areas of my practice

    b. Thrombosis is a main area of my practice

    c. MPN are main areas of my practice

    d. Neither thrombosis or MPN are main areas of my practice

21. What importance do research activities have in your practice?

    a. More than 70% of my time is spent doing research

b. About 50% of my time is spent doing research

c. About 25% of my time is spent doing research

d. Research is not a main area of focus for me

Chapter 4: Pilot Randomized Controlled Trial Protocol

Apixaban for the thromboprophylaxis of JAK2-postive myeloproliferative neoplasms: rationale and design of the AIRPORT-MPN pilot trial.

Miriam Kimpton[1,2], Marc Carrier[1,2], Aurélien Delluc[1,2]

[1]Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada

[2]Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

Corresponding Author:

Miriam Kimpton

501 Smyth Road, PO Box 201A, Ottawa, ON, K1H 8L6

mkimpton@toh.ca

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Abstract

Patients with JAK2-positive myeloproliferative neoplasms (JAK2MPN), such as polycythemia vera, essential thrombocythemia, and primary myelofibrosis, are at elevated risk of both arterial and venous thrombotic complications, owing to the pathophysiology of their malignancy. Low-dose aspirin is therefore recommended in these patients to prevent thrombotic complications. Evidence for the use of aspirin in JAK2MPN patients is limited, in part because of challenges faced when studying patients with a rare disease. Low-dose apixaban has been shown to be effective and safe for the thromboprophylaxis of other patient populations. We propose to do a pilot, randomized controlled trial assessing the feasibility of evaluating low-dose apixaban compared to low-dose aspirin for the thromboprophylaxis of JAK2MPN patients. The primary outcomes of the pilot study will include 1) the average monthly subject recruitment rate of all study sites during a 6-month recruitment period; 2) the recruitment of 44 JAK2MPN patients in 6 months (39 prevalent cases and 5 incident cases). Secondary outcomes will further relate to both feasibility and clinical endpoints on efficacy and safety.

Introduction

Myeloproliferative neoplasms (MPN) are a group of clonal hematopoietic disorders characterized by the unregulated production of various cells lineages. These clonal disorders are first divided by the presence of the BCR-ABL1 mutation, which is the defining feature of chronic myeloid leukaemia. Within the BCR-ABL1-negative MPN, further sub-divisions are made, based on the clinical presentation and presence of specific somatic mutations. Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are three BCR-ABL1-negative MPN with common pathophysiological characteristics and a propensity for thrombus formation.

The role of the JAK2 mutation in thrombus formation

The somatic JAK2 V617F mutation (and to a lesser extent other JAK2 mutation derivatives) occupies a particularly central role in the development of many BCR-ABL1-negative MPN. High JAK2 V617F allele burden has been associated with an increased risk of thrombotic complications[1]. The higher exposure of P-selectin by endothelial cells due to Weibel-Palade body degranulation, leading to a pro-adhesive phenotype of the endothelium, has been proposed as a mechanistic explanation for the presence of this associated heightened thrombotic risk[2, 3]. Furthermore, P-selectin has been found to help up-regulate tissue factor expression on monocytes and tissue factor concentration at developing thrombus sites. As a result, the P-selectin-enhanced thrombus formation pathway leads to a clot that is not only

platelet rich, but also tissue factor and fibrin rich[4]. These observations suggest possible diverse targets for anti-thrombotic therapies in this patient population.

Thrombosis and the use of low-dose aspirin in polycythemia vera, essential thrombocythemia, and primary myelofibrosis

PV is defined by an elevation in red blood cell production, the presence of the gain-of-function JAK2 mutation, and tri-lineage hyperplasia of the bone marrow[5]. PV patients are at an elevated risk for both arterial and venous thromboembolic events, which occur in similar proportions and represent a major cause of death in this patient population[6]. In an international study of 1,545 PV patients, 12% of patients suffered an arterial thrombosis, while 9% of patients were diagnosed with a venous thrombotic complication, during the mean follow-up period of 6.9 years[7]. For this reason, current guidelines, including the European Leukemia Network[8] and the National Comprehensive Cancer Network[9], recommend low-dose aspirin therapy to mitigate the risk of arterial and venous thrombosis in all PV patients, in the absence of contraindication for thromboprophylaxis. Despite the use of aspirin, however, PV patients remain at an elevated risk for thrombotic complications. In the above-mentioned international study, the rate of thrombotic complications remained significant at 2.62% (95% confidence interval (CI) 2.34-2.94) per patient-year despite 84% and 73% of the patients receiving aspirin and cytoreductive therapies, respectively[10]. In another study where 58.3% of the patients received anti-platelet therapy and 6.7% anticoagulation therapy, the cumulative rate of cardiovascular death and non-fatal thrombotic complications was even more substantial at 5.5

per 100 person-years, with thrombotic cerebrovascular events accounting for 1.4 events per 100 person-years[6]. Furthermore, in the largest randomized controlled trial comparing aspirin to placebo in this patient population, aspirin did not significantly reduce the risk of nonfatal myocardial infarction (MI), nonfatal stroke, or death from cardiovascular causes (relative risk (RR)=0.41; 95% CI 0.15-1.15; P=0.09) or the risk of nonfatal MI, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (RR=0.40; 95% CI 0.18-0.91; P=0.03), after accounting for the number of analyses involved in the assessment of these two primary endpoints[11]. Finally, a Cochrane meta-analysis comparing low-dose aspirin to no treatment in PV patients demonstrated no reduction in the risk of fatal thrombotic events and all-cause mortality in patients receiving low-dose aspirin[12].

ET is characterized by the proliferation of the megakaryocytic lineage. Three distinct mutations have been associated with ET: CALR, MLP, and JAK2. The JAK2 mutation accounts for 60% of ET patients[13]. Guideline recommendations are more heterogenous regarding the role of thromboprophylaxis in patients with ET, with some recommending aspirin for all patients[9], while orders identifying a group of patients less likely to benefit from this intervention, such as CALR-mutated patients[8]. However, all guidelines recommend the use of thromboprophylaxis with low dose aspirin in JAK2-positive ET (JAK2ET) patients. The difference in recommendations may be explained by the fact that there are fewer studies, with lower methodological quality, which have assessed thromboprophylaxis in ET patients. A recent systematic review of anti-thrombotic therapies in ET patients (the vast majority having received low-dose aspirin) found only low-quality data regarding anti-thrombotic therapies in this patient population and a modest relative risk reduction of 26%, suggesting that the risk of thrombosis remains elevated,

despite anti-thrombotic therapy[14]. Furthermore, a study by Rumi et al. found that JAK2ET patients have a very similar risk of developing thrombosis when compared to PV patients[15]. Some experts have also described PV and JAK2ET as a biological continuum of the same disease[16]. Finally, a retrospective study from Alvarez-Larran et al. identified a group of low-risk CALR-mutated ET patients who may not benefit from aspirin thromboprophylaxis, since the use of aspirin in these patients was associated with an increase in the risk of bleeding, without an improvement in the risk of thrombosis[17]. Therefore, based on the available data regarding the clinical progression of JAK2ET patients and our understanding of the molecular basis of this disease, offering thromboprophylaxis to this patient population appears to be beneficial.

PMF is a clonal disease associated with the proliferation of atypical megakaryocytes and granulocytes. In the pre-fibrotic stage, the bone marrow is hypercellular, with minimal reticulin fibrosis. In the overt stage, there is significant reticulin or collagen fibrosis, with often associated osteosclerosis and extramedullary hematopoiesis[18]. PMF is also associated with a significant risk for thrombotic complications. In a competing risk analysis, the estimated rate of thrombosis for all PMF patients was 2.2 per 100 person-years. The same study found that the presence of the JAK2 mutation further increased the risk for thrombosis, with a hazard ratio (HR) of 1.92 (95% CI, 1.10-3.34). In a subset of PMF patients with the JAK2 mutation and leucocytosis (defined as a white blood cell count ≥15 x 10^9/L at diagnosis), the rate of thrombotic complications was 3.92 (95% CI, 1.87-8.22) per 100 person-years, with an HR of 3.13 (95% CI, 1.26-7.81)[19]. Another study comparing JAK2-positive ET patients to JAK2-positive early PMF patients found comparable thrombotic rates[20]. Of note, patients with overt PMF experience severe cytopenia (precluding the use of thromboprophylaxis) and follow a distinct

disease progression, when compared to the more indolent PV, ET, and pre-fibrotic PMF[21]. Therefore, although aspirin has not been directly studied in PMF patients, experts recommend thromboprophylaxis with low-dose aspirin in patients with risk factors for thrombosis, following an individualized assessment of the bleeding risk[21-23].

Direct oral anticoagulants thromboprophylaxis in non-MPN populations

Direct oral anticoagulants (DOAC) have been shown to be efficacious and safe for the primary and secondary thromboprophylaxis of various patient populations. In AVERROES, apixaban was compared to aspirin for the prevention of stroke and systemic embolism in patients with atrial fibrillation, leading to an HR of 0.45 (95% CI, 0.32-0.62)[24]. Additionally, there was no statistically significant increase in major bleeding or clinically relevant non-major bleeding (CRNMB) between the apixaban and aspirin groups. In ARISTOTLE, apixaban was also superior to warfarin in preventing stroke or systemic embolism, caused less bleeding, and resulted in lower mortality[25]. In AMPLIFY, two doses of apixaban (5mg BID and 2.5mg BID) were compared to placebo in patients eligible to receive extended anticoagulation for a venous thromboembolic event (VTE)[26]. In this study, neither dose of apixaban was associated with an increase in the relative risk of major bleeding, when compared to placebo. Major bleeding occurred in four patients (0.5%) who were receiving placebo, compared to two patients (0.2%) who were receiving apixaban 2.5mg BID. In the apixaban 2.5mg BID group, the RR for recurrent VTE or death from any cause, when compared to placebo, was 0.33 (95% CI, 0.22-0.48). The Einstein-Choice study compared another low dose DOAC, rivaroxaban, to low-dose aspirin for

the extended anticoagulation of patients with a diagnosis of VTE[27]. In patients receiving rivaroxaban 10mg daily, 1.2% suffered recurrent VTE, compared to 4.4% of patients assigned to the aspirin arm, for an HR of 0.26 (95% CI, 0.14–0.47). Importantly, there was no statistically significant increase in major bleeding, with an HR of 1.64 (95% CI, 0.39–6.84). More recently, the AVERT study compared the use of apixaban 2.5mg BID to placebo in ambulatory cancer patients initiating chemotherapy, who were at intermediate to high risk of VTE according to the Khorana score. MPN patients were excluded from this trial, given that they had not been included in the derivation and validation studies of the Khorana score. From the on-treatment analysis, apixaban was not associated with an increased risk of major bleeding when compared to placebo (HR 1.89; 95% CI, 0.39-9.24) and was superior at preventing VTE (HR 0.41; 95%CI, 0.26 to 0.65), in the modified intention-to-treat analysis.

Currently, there are no studies comparing apixaban to aspirin for the prevention of arterial thrombosis, but the COMPASS trial compared very low-dose rivaroxaban to aspirin and found a nearly statistically significant reduction in cardiovascular death, stroke or MI with an HR of 0.90 (95% CI, 0.79-1.03)[28]. Of note, in the COMPASS trial, very low-dose rivaroxaban was associated with a statistically significant increase in major bleeding when compared to aspirin, as defined by the International Society on Thrombosis and Haemostasis (ISTH), with a HR of 1.52 (95% CI, 1.20–1.92). Moreover, in the same trial, the addition of low-dose aspirin to very low-dose rivaroxaban was also associated with a statistically significant increase in major bleeding per ISTH criteria, when compared to aspirin monotherapy (HR 1.78; 95% CI, 1.41–2.23). The VOYAGER-PAD trial chose a similar therapeutic approach to the COMPASS trial (very-low dose rivaroxaban plus aspirin versus aspirin) but limited its target patient population to

patients with peripheral artery disease, post-revascularization surgery. In this population, the addition of very low-dose rivaroxaban to aspirin, compared to aspirin alone, was again seen to decrease the risk of thrombotic complications, at the expense of increased bleeding complications[29]. The addition of antiplatelet therapy to low or intermediate doses of rivaroxaban (based on renal function) compared to rivaroxaban monotherapy has also recently been studied in patients with atrial fibrillation and stable coronary artery disease during the AFIRE study[30]. In this large cohort of 2236 patients, monotherapy with rivaroxaban was found to be non-inferior to the combination of antiplatelet therapy (where low-dose aspirin was the chosen therapy in 70% of patients) and low/intermediate dose rivaroxaban in terms of efficacy to prevent stroke, systemic embolism, myocardial infarction, unstable angina requiring revascularization, or death from any cause in patients with atrial fibrillation and stable coronary disease. Also, monotherapy with rivaroxaban was superior to combination therapy for the primary safety outcome of major bleeding (HR 0.59; 95% CI, 0.39-0.89; P = 0.01 for superiority). Therefore, the results of COMPASS, VOYAGER-PAD, and AFIRE suggest that the addition of an antiplatelet agent to different doses of rivaroxaban is associated with an increase in major bleeding.

Rationale for the choice of apixaban thromboprophylaxis

Although aspirin is the current standard of care, strong evidence is lacking regarding its efficacy in reducing thrombotic events in this patient population. Furthermore, with aspirin, these patients remain at an unacceptably elevated risk for both arterial and venous thrombotic complications. Based on the evidence presented above, we hypothesize that monotherapy with

low-dose apixaban will be more effective at preventing thrombotic events in JAK2-positive MPN (JAK2MPN) patients, without increasing their bleeding risk, when compared to low-dose aspirin. Apixaban was chosen over rivaroxaban given that studies have consistently shown that apixaban is not associated with an increased bleeding risk, when compared to placebo or aspirin. Likewise, the addition of low-dose aspirin to apixaban was not chosen for the interventional arm, due to concerns over an increase in major bleeding, as described in the studies assessing the concomitant use of antiplatelet agents and different doses of rivaroxaban.

As such, we propose to do a multi-centre pilot randomized controlled trial evaluating the feasibility of a randomized controlled trial assessing the superiority of apixaban 2.5mg BID compared to low-dose aspirin as thromboprophylaxis of JAK2MPN patients.

The need for a pilot randomized controlled trial on feasibility

The study of BCR-ABL1-negative MPN patients poses certain notable challenges, due to the rarity of these diseases and their chronicity, with many of these patients surviving several years following diagnosis[31]. Therefore, PV, JAK2ET, and pre-fibrotic JAK2-positive PMF patients are followed in a broad range of clinical settings, from small community clinics (where participation in clinical research may be limited) to specialized clinics in tertiary centres. Such disease characteristics and logistics specificities may explain why prior interventional studies on the prevention of thrombotic complications in this patient population have not been successful at meeting their recruitment target[11, 32].

Since it is prudent to ensure the feasibility of a randomized controlled trial on efficacy prior to committing important financial and human resources, a pilot randomized controlled trial design is the optimal way to gather information on the possible recruitment and retention challenges that will need to be addressed before conducting a full-scale trial. During the pilot phase, experience is gathered on all aspects of the randomized controlled trial process, such as the identification of participants, the randomization and allocation procedures, the distribution of study medications, the follow-up period, and the documentation and adjudication of pre-defined clinical events. Furthermore, feasibility may be explicitly established *a priori* through the trial's primary endpoint, as it is proposed here. In our case, the number of participants recruited during a specific recruitment period at a pre-determined number of sites will help calculate the needed recruitment and follow up periods, in the case where a low primary endpoint event-rate for the full-scale trial is expected. By procuring a better understanding of the type of clinical centres which should be identified as future recruiting sites, and the expected number of sites needed, this pilot project will allow for a sounder design, as well as more rigorous planning and budgeting of the full-scale trial. Finally, the use of diverse feasibility endpoints as secondary outcomes (such as reasons for non-consent, adherence to therapy, and proportion of patients picking up the study medication during follow up) will allow for a deeper understanding of the acceptability of the intervention and the willingness of patients to participate in such a trial.

Methods

Study Design

This is a multicenter, prospective, open-label, blinded-endpoint (PROBE), randomized controlled trial. Four recruitment centres have been chosen based on their individual characteristics to include a variety of clinical centres where JAK2MPN patients receive care, from a community hospital setting to a specialized clinic within a Cancer Centre. Eligible study participants will be randomly assigned to continue receiving aspirin or to receive apixaban, on a 1:1 basis. We will compare aspirin 81mg once per day to low dose of apixaban, 2.5mg twice per day, as shown in Table 1.

Table 1: Study Treatment Arms

Arm 1 (Control Group)	Arm 2 (Interventional Group)
23 Participants	23 Participants
Aspirin 81mg once per day for 6 months Then treated & followed up as per standard of care	Apixaban 2.5mg twice per day for 6 months Then treated & followed up as per standard of care

The recruitment period for this feasibility pilot study will last for 6 months (Figure 1). Once recruited, patients will remain on the assigned study medication for 6 months, then be treated at the discretion of his/her treating physician. All patients will follow up in person at the 3-month and 6-month visits. A 1-month post-study conclusion visit (as mandated by Health Canada) will take place in person or via telephone. There will be no extra tests required while

taking part in the study. All laboratory, diagnostic, and/or subsequent treatment information will be collected based on its availability in the patient's medical files (per standard of care).

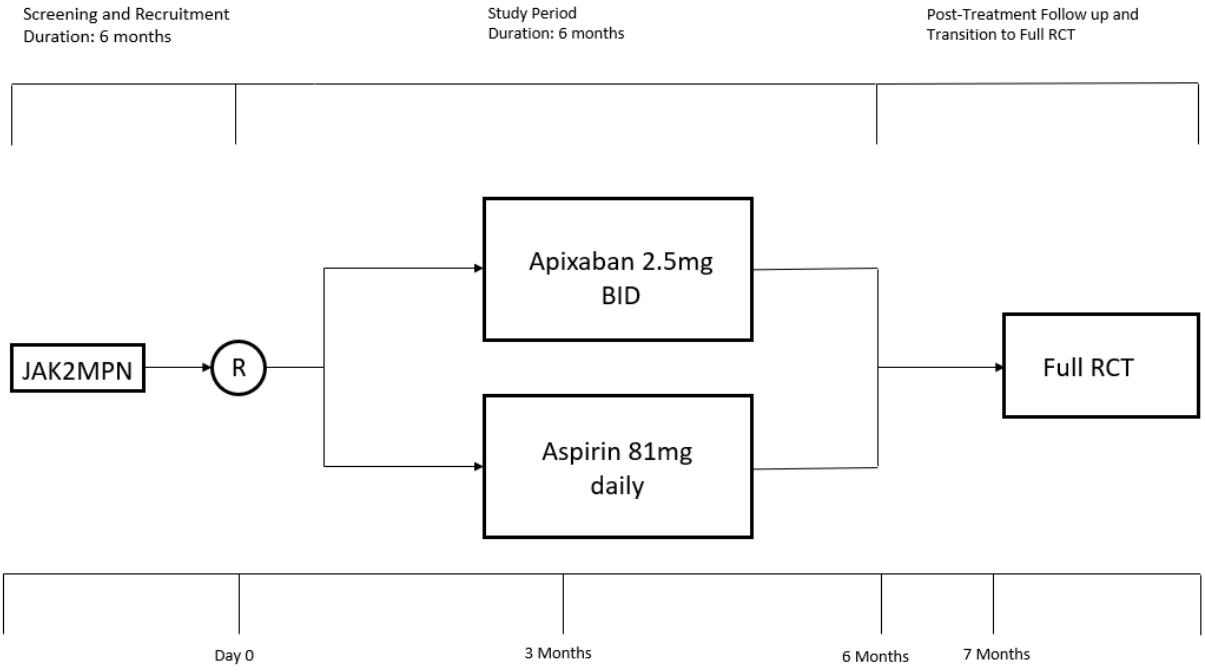


Figure 1. Study Design

JAK2MPN: JAK2-positive myeloproliferative neoplasm patient; R: Randomization; RCT: Randomized controlled trial

Sample Size of the Pilot Trial

A maximum enrollment total of 46 subjects is anticipated to ensure that at least 44 subjects will complete their study treatment (22 subjects per arm). This would represent a rate of 7.5 patients recruited per month, within a standard error of 0.08, if the target recruitment is completed within 6 months. As a time-to-event analysis is planned for the full-size trial, the sample size for the full-size trial will be informed by the recruitment rate calculated during the pilot phase, as well as the expected rate of thrombotic complications. The pilot project will also

allow us to gather data on the variance and precision of the rate of thrombotic event in this patient population.

Furthermore, the pilot project will provide us with the opportunity to collect important information on the incidence and prevalence of JAK2MPN. Incident cases will be defined as those diagnosed with a JAK2MPN within the previous year. Incident cases of MPN patients are known to be at higher risk for both arterial and venous thrombotic complications[33]. Once 39 prevalent cases have been recruited, the recruitment process will focus only on incident cases. The population included in the pilot project via the chosen four centres represent about 10% of the Canadian population. We estimate that about 1200 patients will be needed for the full-size trial, and that 400 of these patients will be recruited within Canada. Based on the limited published data on the incidence and prevalence of JAK2MPN[13, 34, 35], these 400 patients would represent 3.5% of the total Canadian JAK2MPN population. Therefore, we will aim to recruit 5-6 incident cases of JAK2MPN (representing 15% of all incident cases of JAK2MPN encompassed by our four centres) and 39 prevalent cases of JAK2MPN (representing 3.5% of all prevalent cases).

Patient Population

Adult patients with a confirmed diagnosis of PV, JAK2ET or JAK2-positive pre-fibrotic PMF (per local clinical definitions) and who are able to consent will be asked to take part in the study. Patients will be excluded if they have a known allergy to the study medications, have a need for specific antithrombotic therapy, have a contraindication to thromboprophylaxis, have severe

renal dysfunction (creatine clearance of less than 25 mL per minute) or liver disease, are taking a medication with a known interaction to apixaban, or are currently pregnant or breast-feeding. Patients with a previous history of VTE may be included in this trial for secondary thromboprophylaxis, provided that they have completed a minimum of 6 months of anticoagulation and that the treating physician would be agreeable to have the patient's treatment stepped down to thromboprophylaxis with aspirin at minimum.

Treatment Allocation

After acquiring informed consent from an eligible patient, a centralized, web-based randomization system will be used to allocate the patient to one of the treatment arms, using a 1:1 ratio. For the pilot trial, no stratification will be done, given the small sample size.

Trial Intervention

In the intervention arm, patients will receive apixaban 2.5mg. In the control arm, patients will receive low-dose aspirin (81mg daily). Patients will receive their assigned medications for the trial duration (6 months) or until a clinical endpoint occurs, which warrants a therapeutic change per the treating physician. Such clinical endpoints include venous and arterial thrombotic complications, and major bleeding (associated with the discontinuation of the study medication). At the end of the 6-month follow-up visit, it will be at the discretion of the

patient's treating physician to decide whether the patient will continue apixaban, as an off-label option.

Study Follow Up

Enrolled patients will be followed up for 7 months (6 months of treatment based on the allocated study arm and a 1-month post-treatment follow up). In person follow ups will take place at the 3-month and 6-month mark, while an in person or telephone follow up will be arranged for the 1-month post-treatment follow up. At each visit, review of the patient's most recent laboratory investigations (completed by the treating physician per standard of care) will be done. Compliance to the study medication will also be ensured using a medication diary. Clinical outcome and adverse event information will be systematically sought. The severity of adverse events will be graded using the Common Terminology Criteria for Adverse Events, Version 5.0. The SF-36 quality of life questionnaire will be administered at the recruitment, 3-month, and 6-month visits.

Study Outcomes

Primary outcomes of the pilot study include: 1) the average monthly subject recruitment rate of all study sites during a 6-month recruitment period; 2) the recruitment of 44 JAK2MPN patients in 6 months (39 prevalent cases and 5 incident cases).

Secondary outcomes of the pilot study relating to feasibility include: 1) screen-failure and consent rates; 2) study drug compliance as assessed by quantification of used and unused drug; 3) study visit compliance as assessed by the number of study visits (in person and/or phone call) completed; 4) study subject retention rate (i.e. subject withdrawal and lost-to-follow-up rates); 5) percentage of incident and prevalent cases included in the study.

Secondary clinical outcomes of the pilot study will be comprised of the rate of arterial and venous thrombotic events (defined as myocardial infarction, stroke, transient ischemic attack, peripheral arterial thrombosis, and VTE); the rates of major bleeding and clinically relevant non-major bleeding events (per the ISTH[36, 37]); thrombosis-associated mortality; bleeding-associated mortality; and all-cause mortality. Clinical outcomes will be blindly adjudicated by an expert committee.

Secondary quality of life outcomes will be measured using the SF-36 questionnaire for all patients receiving apixaban or aspirin.

Statistical Analysis of the Pilot Trial

An estimate of the mean monthly recruitment rate over a 6-month period at four centres with 95% CI will be provided as the primary outcome of the pilot trial.

If 42-46 patients are recruited during the 6 months of recruitment (representing a rate of 7.5 patients per month, with a standard error of 0.08), then the full-scale trial will be deemed feasible. The four chosen sites represent two larger academic sites, where the care of JAK2MPN

patients is concentrated within a few clinics, a smaller academic centre, and a community centre. The rate of each centre will also be calculated and analyzed to inform further decisions regarding the number and type of sites to be included in the full-size randomized controlled trial.

Descriptive statistics will be used to characterize subject characteristics at study entry, as well as the proportion of screened patients meeting eligibility criteria, of incident or prevalent cases, of participant retention, of adherence to study visit, and of adherence to medication.

An exploratory time-to-event analysis for secondary clinical outcomes will also be done during the analysis of the pilot results.

Statistical Analysis of the Full-Scale Trial

We hypothesize that apixaban at a dose of 2.5mg BID will be superior to the current intervention of low-dose aspirin in preventing arterial and VTE complications in this patient population. From the results presented above, it is expected that the use of apixaban 2.5mg BID will decrease the risk of venous events by 70% and will be at least as effective as aspirin in decreasing the risk of arterial events, for an overall risk reduction for both venous and arterial events of 35%.

Descriptive statistics (proportion and means with 95% confidence intervals) will be done to assess baseline patient characteristics and to identify failure of the randomization process.

Patients will be censored at the time of the last follow-up (if lost to follow up), at the time of death, or at the end of the study period. Only the first objectively confirmed component of the composite primary outcome will be captured in the analysis.

The statistical analysis for the primary efficacy endpoint will be performed using a modified intention-to-treat approach, which will include all patients who were randomized and have received at least one dose of the study medication. The analysis for the safety outcomes of major bleeding, clinically relevant non-major bleeding, and clinically relevant bleeding will be performed using an on-treatment approach. A time-to-event analysis will be done using Kaplan-Meier curves to estimate cumulative event rates for the primary and secondary outcome measures. Hazard ratios for the primary outcome will be estimated using Cox regression analyses, adjusting for the type of MPN, age, sex, and recruitment centre. For the primary outcome, a sensitivity analysis using death not attributed to a cardiovascular cause or bleeding as a competing risk will also be performed by estimating cumulative incidence functions, which will be compared using Gray's test.

Missing data will be assumed to occur at random and potentially lead to bias. A multiple imputation analysis will be performed with the aim to overcome this possible source of bias.

Discussion

Thrombotic complications are an important cause of morbidity and mortality in BRC-ABL1-negative MPN patients. Compared to age and sex-matched controls, the magnitude of risk is particularly increased during the first year since diagnosis, with a hazard ratio for arterial events

of 3.0, 2.0, and 1.5 and for venous events of 9.7, 4.3, and 3.2, at 3 months, 1 year, and 5 years from diagnosis, respectively[33]. The mutational profile, the presence of the JAK2 mutation, is one of the known risk factors, which further increases a patient's risk of thrombosis[1]. Despite these known risks, the thromboprophylaxis of BRC-ABL1-negative MPN patients remains a challenge, due to several disease-specific processes.

BRC-ABL1-negative MPN are rare diseases. A recent population-based study from Sweden estimates that the age-standardized incidence of BRC-ABL1-negative MPN (comprised of PV, ET, and PMF) is 4.45 (95% CI 4.34-4.56)/100 000 person-years, with the incidence higher in older persons compared to younger ones[38]. This low incidence rate partially explains why prior randomized controlled trials were not successful in meeting their sample size requirements[11, 32]. A pilot trial is therefore necessary to gather information on the logistical challenges associated with the identification and recruitment of both incident and prevalent cases of BRC-ABL1-negative MPN. This information will maximize the chances of success of a large, randomized controlled trial on the efficacy of novel anti-thrombotic interventions.

Even without the use of anti-thrombotic therapies, BRC-ABL1-negative MPN patients are at increased risk for bleeding because of disease-related factors. A prior observational study has suggested that the presence of the JAK2 V617F mutation may be associated with the development of acquired von Willebrand disease in patients with PV and ET, even with a platelet count of less than 1,000 x 10^9/L (the normally accepted threshold for disease suspicion)[39]. Advanced age, prior hemorrhage, leucocytosis, thrombocytosis (independent of the diagnosis of acquired von Willebrand disease), portal hypertension, and BRC-ABL1-negative MPN subtypes are other risk factors that have been associated with bleeding in these

patients[40]. Therefore, a careful evaluation of a patient's bleeding risk and thoughtful consideration of the recommended therapeutic regimen is of utmost importance in JAK2MPN patients. Although prior studies have mostly focused on the use of anti-platelet therapies in this patient population, there is a need to explore different anti-thrombotic strategies, which target different stipulated pathways of thrombosis formation, in order to find the optimal anti-thrombotic strategy.

Finally, every newly recommended therapy must also take into consideration patient preferences and values, which form the framework for the acceptability of the proposed intervention. BRC-ABL1-negative MPN are chronic diseases, meaning that BRC-ABL1-negative MPN patients often survive for several years following their BRC-ABL1-negative MPN diagnosis[31]. Assessing patients' willingness to participate in research and to adopt new therapeutic measures within their daily medication regimen, along with the impact of such changes on their quality of life, ought to be performed to inform the design of future trials. Reviewing the screening failure rate and measuring patients' quality of life during the pilot project will provide us with valuable information, with the aim to better understand the acceptability of our proposed intervention.

The pilot project described here on the feasibility of assessing the efficacy of apixaban thromboprophylaxis, compared to the current standard of care of low-dose aspirin, will provide the opportunity to gather additional knowledge on this rare patient population, and guide the development of a future randomized controlled trial on the efficacy of a direct-oral anticoagulant-based thromboprophylactic strategy.

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Chapter 5: Discussion

The work presented within this thesis forms the basis for a research program on the thromboprophylaxis of JAK2MPN patients. This research program is necessary in order to address significant knowledge gaps surrounding the thromboprophylaxis of JAK2MPN patients and identify areas are future research avenues. By defining our research population through the presence of the JAK2 mutation within the different MPN sub-types, we increase the pool of potential research participants and capture more high-risk patients, which are important aspects when doing research in rare diseases. Furthermore, this approach will allow us to take advantage of our growing knowledge of the pathophysiology of thrombosis due to this mutation.

Our systematic review and meta-analysis reenforces the uncertainty of the data regarding the use of aspirin thromboprophylaxis for this patient population. It also suggests that the rate of thrombosis, despite the use of low-dose aspirin, remains unacceptably elevated. Additional research is needed to better define the efficacy and safety of the current standard of care for the thromboprophylaxis of JAK2MPN patients. Furthermore, it illustrates that there is a potential role for other anti-thrombotic therapies, which target different mechanisms of thrombogenicity common to this patient population. Such alternative therapies may be able to further decrease the rate of thrombotic complications and obtain a better risk-benefit ratio between thrombotic complications and hemorrhagic ones in these patients.

Our survey of Canadian physicians demonstrated heterogeneity in multiple aspects of the management of JAK2MPN patients. It appears that physicians are interested in integrating

within their practice the use of novel anti-thrombotic therapies, and specifically direct oral anticoagulants, in order to better care for these patients. Although the first iteration of the modified Delphi process did not lead to agreement regarding the MCID, it is likely that subsequent iterations will yield the necessary information to better calculate the sample size needed to design a trial on efficacy, which will lead to clinically relevant results. The next step will be to open the survey internationally to capture a more diverse physician population and proceed to additional iterations of the modified Delphi process. Given the rarity of the disease, it will be essential to forge international partnerships to complete a full-size trial on the efficacy of a proposed, new thromboprophylactic intervention. Opening the survey to an internal group of physicians will help identify further heterogeneity of practice patterns and ensure that the MCID will reflect the opinions of a broad group of physicians operating in diverse clinical settings.

The pilot, multi-center, randomized controlled trial on the feasibility of comparing apixaban thromboprophylaxis to aspirin thromboprophylaxis, AIRPORT-MPN (ClinicalTrials.gov Identifier: NCT04243122), is set to open for enrollment in February 2021. Unfortunately, due to the COVID-19 pandemic, the opening AIRPORT-MPN trial had to be delayed from its anticipated date in mid-2020. This pilot study will allow for the determination of a monthly rate of recruitment, as well as identify logistical challenges for the recruitment of JAK2MPN patients. Specifically, we will be interested in elucidating how many incident and prevalent cases of the disease will be included, since the rate of thrombosis for incident and prevalent cases of JAK2MPN has been found to differ. Furthermore, although not powered on clinical outcomes, it

will offer additional information on the rate of thrombotic and bleeding events in these patients.

Together, the systematic review and meta-analysis, the modified Delphi process and survey of practice, and the pilot, randomized controlled trial on feasibility will provide the necessary information to decide on whether a full-size randomized controlled trial on low-dose apixaban compared to aspirin thromboprophylaxis in JAK2MPN patients is a worthwhile endeavor. The MCID and the monthly rate of recruitment will inform the sample size needed for such a trial, with a planned time-to-event analysis. Other feasibility outcomes such as the number of screen failures and consent rate, study drug compliance, study subject retention, and rate of recruitment per study site will guide the choice and number of needed study sites for the full-size trial. As a whole, this information will allow for more accurate planning and budgeting of the full-size trial. With the completion of these three complementary research projects, we will be in a good position to decide on the feasibility of a full-size randomized controlled trial on the efficacy of apixaban, compared to aspirin, in this specific patient population defined by a common driver mutation.

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Appendix

CLINICAL TRIAL PROTOCOL

Protocol Title: A Phase 2 Pilot Randomized Controlled Trial Assessing Feasibility of Thromboprophylaxis with Apixaban in JAK2-positive Myeloproliferative Neoplasm Patients (AIRPORT-MPN Study)

Study Sponsor:	The Ottawa Hospital Research Institute 501 Smyth Road, Ottawa ON, Canada K1H 8L6
Principal Investigators:	Dr. Miriam Kimpton Dr. Aurelien Delluc
Trial Coordinating Center:	Ottawa Blood Disease Centre The Ottawa Hospital, General Campus 501 Smyth Road, Box 180 Ottawa, ON, Canada K1H 8L6
Investigational Product:	Apixaban, sold under the brand name Eliquis ®
Study Protocol Number:	AIRPORT-MPN-001
ClinicalTrials.gov Identifier:	NCT04243122
Protocol Version:	Version 1.2 (Initial): January 28th, 2020

Confidentiality Statement:

This document contains confidential information. It is intended solely for the use of the principal investigators, co-investigators, staff, appropriate institutional review boards or ethical committees, and other required regulatory bodies.

Sponsor Signature Page

Protocol Title: A Phase 2 Pilot Randomized Controlled Trial Assessing Feasibility of Thromboprophylaxis with Apixaban in Polycythemia Vera and JAK2-positive Essential Thrombocythemia patients

Study Protocol Number: AIRPORT-MPN-001

Investigational Product: Apixaban, sold under the brand name Eliquis ®

Study Sponsor: The Ottawa Hospital Research Institute

Protocol Version: Version 1.2 (Initial): January 28th, 2020

Principal Investigators: Dr. Miriam Kimpton
Dr. Aurelien Delluc

_____ _____

Signature Date:

_____ _____

Signature Date:

Site Investigator Signature Page

Protocol Title: A Phase 2 Pilot Randomized Controlled Trial Assessing Feasibility of Thromboprophylaxis with Apixaban in Polycythemia Vera and JAK2-positive Essential Thrombocythemia patients

Study Protocol Number: OHRI-MPN-001

Investigational Product: Apixaban, sold under the brand name Eliquis ®

Study Sponsor: The Ottawa Hospital Research Institute

Protocol Version: Version 1.2 (Initial): January 28th, 2020

I have read and understand the protocol, and I agree that it contains all the ethical, legal and scientific information necessary to conduct this study. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated. I will work according to the principles of Good Clinical Practice (GCP) as described in the Food and Drug Act, Division 5 Health Canada., The Declaration of Helsinki (2004), and Good Clinical Practice (GCP) as described in the International Conference on Harmonization (ICH) document, "Guidance for Industry – E6 Good Clinical Practice: Consolidated Guidance" dated April 1996, or any subsequently revised version if applicable. Further, I will conduct the study in keeping with local and regulatory requirements.

I will provide copies of the protocol and access to all relevant information to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

_____ _____

Site Investigator's Signature Date

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GLOSSARY OF ABBREVIATIONS

AE	Adverse event
BID	bis in die (twice daily)
CBC	Complete blood count
CI	Confidence Interval
CIHR	Canadian Institute of Health Research
CIOMS	The Council for International Organizations of Medical Sciences
CRNMB	Clinically relevant non-major bleeding
CTCAE	Common Terminology Criteria for Adverse Events
DOAC	Direct oral anticoagulant
DSMB	Data and Safety Monitoring Board
ET	Essential thrombocythemia
GCP	Good Clinical Practice
HR	Hazard Ratio
ISTH	International Society on Thrombosis and Haemostasis
MB	Major bleeding
MF	Primary myelofibrosis
MI	Myocardial infarction
MPN	Myeloproliferative neoplasm
PAT	Peripheral arterial thrombosis
PV	Polycythemia Vera
RCT	Randomized controlled trial
RR	Relative risk
SAE	Serious adverse event
TIA	Transient ischemic attack
VTE	Venous thromboembolism

Introduction

Study Background

Polycythemia vera (PV) is a myeloproliferative neoplasm (MPN) defined by an elevation in red blood cell production, the presence of the gain-of-function JAK2 mutation, and tri-lineage hyperplasia of the bone marrow[1]. High JAK2 V617F allele burden has been associated with an increased risk of thrombotic complications[10]. The higher exposure of P-selectin by endothelial cells due to Weibel-Palade body degranulation, leading to a pro-adhesive phenotype of the endothelium, has been proposed as a mechanistic explanation for the presence of this associated heightened thrombotic risk[7]. PV patients are therefore at an elevated risk for both arterial and venous thromboembolic events, which occur in similar proportions and represent a major cause of death in this patient population[47]. In an international study of 1,545 PV patients, 12% of patients suffered an arterial thrombosis, while 9% of patients were diagnosed with a venous thrombotic complication, during the mean follow-up period of 6.9 years[14]. For this reason, current guidelines, including the European Leukemia Network[18] and the National Comprehensive Cancer Network[19], recommend low-dose aspirin therapy to mitigate the risk of arterial and venous thrombosis in all PV patients, in the absence contraindication for thromboprophylaxis. Despite the use of aspirin, however, PV patients remain at an elevated risk for thrombotic complications. In the above-mentioned international study, the rate of thrombotic complications remained significant at 2.62% (95% confidence interval (CI) 2.34-2.94) per patient-year despite 84% and 73% of the patients receiving aspirin and cytoreductive therapies, respectively[23]. In another study where 58.3% of the patients received anti-platelet therapy and 6.7% anticoagulation therapy, the cumulative rate of cardiovascular death and

non-fatal thrombotic complications was even more substantial at 5.5 per 100 person-years, with thrombotic cerebrovascular events accounting for 1.4 events per 100 person-years[47]. Furthermore, in the largest RCT comparing aspirin to placebo in this patient population, aspirin did not significantly reduce the risk of nonfatal myocardial infarction (MI), nonfatal stroke, or death from cardiovascular causes (relative risk (RR)=0.41; 95% CI 0.15-1.15; P=0.09) or the risk of nonfatal MI, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (RR=0.40; 95% CI 0.18-0.91; P=0.03), after accounting for the number of analyses involved in the assessment of these two primary endpoints[22]. Finally, a Cochrane meta-analysis comparing low-dose aspirin to no treatment in PV patients demonstrated no reduction in the risk of fatal thrombotic events and all-cause mortality in patients receiving low-dose aspirin[24].

Essential thrombocythemia (ET) is also an MPN, characterized by the proliferation of the megakaryocytic lineage. Three distinct mutations have been associated with ET: CALR, MLP, and JAK2. The JAK2 mutation accounts for 60% of ET patients[2]. Guideline recommendations are more heterogenous regarding the role of thromboprophylaxis in patients with ET, with some recommending aspirin for all patients[19], while orders identifying a group of patients less likely to benefit from this intervention, such as CALR-mutated patients[18]. However, all guidelines recommend the use of thromboprophylaxis with low dose aspirin in JAK2-positive ET (JAK2ET) patients. The difference in recommendations may be explained by the fact that there are fewer studies, with lower methodological quality, which have assessed thromboprophylaxis in ET patients. A recent systematic review of anti-thrombotic therapies in ET patients (the vast

majority having received low-dose aspirin) found only low-quality data regarding anti-thrombotic therapies in this patient population and a modest relative risk reduction of 26%, suggesting that the risk of thrombosis remains elevated, despite anti-thrombotic therapy[25]. Furthermore, a study by Rumi et al. found that JAK2ET patients have a very similar risk of developing thrombosis when compared to PV patients[32]. Some experts have also described PV and JAK2ET as a biological continuum of the same disease[6]. Finally, a retrospective study from Alvarez-Larran et al. identified a group of low-risk CALR-mutated ET patients who may not benefit from aspirin thromboprophylaxis, since the use of aspirin in these patients was associated with an increase in the risk of bleeding, without an improvement in the risk of thrombosis[33]. Therefore, based on the available data regarding the clinical progression of JAK2ET patients and our understanding of the molecular basis of this disease, offering thromboprophylaxis to this patient population appears to be beneficial.

Primary myelofibrosis (MF) is a clonal disease associated with the proliferation of atypical megakaryocytes and granulocytes. In the pre-fibrotic stage, the bone marrow is hypercellular, with minimal reticulin fibrosis. In the overt stage, there is significant reticulin or collagen fibrosis, with often associated osteosclerosis and extramedullary hematopoiesis[5]. MF is also associated with a significant risk for thrombotic complications. In a competing risk analysis, the estimated rate of thrombosis for all MF patients was 2.2 per 100 person-years. The same study found that the presence of the JAK2 mutation further increased the risk for thrombosis, with a hazard ratio (HR) of 1.92 (95% CI, 1.10-3.34). In a subset of MF patients with the JAK2 mutation and leucocytosis (defined as a white blood cell count ≥15 x 10^9/L at diagnosis), the rate of

thrombotic complications was 3.92 (95% CI, 1.87-8.22) per 100 person-years, with an HR of 3.13 (95% CI, 1.26-7.81)[34]. Another study comparing JAK2-positive ET patients to JAK2-positive early MF patients found comparable thrombotic rates[3]. Of note, patients with overt MF experience severe cytopenia (precluding the use of thromboprophylaxis) and follow a distinct disease progression, when compared to the more indolent PV, ET, and pre-fibrotic MF[4]. Therefore, although aspirin has not been directly studied in MF patients, experts recommend thromboprophylaxis with low-dose aspirin in patients with risk factors for thrombosis, following an individualized assessment of the bleeding risk[4, 20, 21].

Study Rationale and Current Treatments

Apixaban is a direct oral anticoagulant (DOAC) that specifically inhibits factor Xa. This drug has been shown to be efficacious and safe for the primary and secondary thromboprophylaxis of various patient populations. In AVERROES, apixaban was compared to aspirin for the prevention of stroke and systemic embolism in patients with atrial fibrillation, leading to an HR of 0.45 (95% CI, 0.32-0.62)[48]. Additionally, there was no statistically significant increase in major bleeding or clinically relevant non-major bleeding (CRNMB) between the apixaban and aspirin groups. In AMPLIFY, two doses of apixaban (5mg BID and 2.5mg BID) were compared to placebo in patients eligible to receive extended anticoagulation for a venous thromboembolic event[45]. In this study, neither dose of apixaban was associated with an increase in the relative risk of major bleeding, when compared to placebo. Major bleeding occurred in four patients

(0.5%) who were receiving placebo, compared to two patients (0.2%) who were receiving apixaban 2.5mg BID. In the apixaban 2.5mg BID group, the RR for recurrent venous thromboembolic event (VTE) or death from any cause, when compared to placebo, was 0.33 (95% CI, 0.22-0.48). The Einstein-Choice study compared another low dose direct oral Xa inhibitor, rivaroxaban, to low-dose aspirin for the extended anticoagulation of patients with a diagnosis of VTE[50]. In patients receiving rivaroxaban 10mg daily, 1.2% suffered recurrent VTE, compared to 4.4% of patients assigned to the aspirin arm, for an HR of 0.26 (95% CI, 0.14–0.47). Importantly, there was no statistically significant increase in major bleeding, with an HR of 1.64 (95% CI, 0.39–6.84). More recently, the AVERT study compared the use of apixaban 2.5mg BID to placebo in ambulatory cancer patients receiving chemotherapy. MPN patients were excluded from this trial, given that they had not been included in the derivation and validation studies of the Khorana score. From the on-treatment analysis, apixaban was not associated with an increased risk of major bleeding when compared to placebo (HR 1.89; 95% CI, 0.39-9.24) and was superior at preventing VTE (HR 0.41; 95%CI, 0.26 to 0.65), in the modified intention-to-treat analysis.

Currently, there are no studies comparing apixaban to aspirin for the prevention of arterial thrombosis, but the COMPASS trial compared very low-dose rivaroxaban to aspirin and found a nearly statistically significant reduction in cardiovascular death, stroke or MI with an HR of 0.90 (95% CI, 0.79-1.03)[37]. Of note, in the COMPASS trial, very low-dose rivaroxaban was associated with a statistically significant increase in major bleeding when compared to aspirin, as defined by the International Society on Thrombosis and Haemostasis (ISTH), with a HR of 1.52 (95% CI, 1.20–1.92). Moreover, in the same trial, the addition of low-dose aspirin to very

low-dose rivaroxaban was also associated with a statistically significant increase in major bleeding per ISTH criteria, when compared to aspirin monotherapy (HR 1.78; 95% CI, 1.41–2.23). The addition of antiplatelet therapy to low or intermediate doses of rivaroxaban (based on renal function) compared to rivaroxaban monotherapy has recently been studied in patients with atrial fibrillation and stable coronary artery disease during the AFIRE study[51]. In this large cohort of 2,236 patients, monotherapy with rivaroxaban was found to be non-inferior to the combination of antiplatelet therapy (where low-dose aspirin was the chosen therapy in 70% of patients) and low/intermediate dose rivaroxaban in terms of efficacy to prevent stroke, systemic embolism, myocardial infarction, unstable angina requiring revascularization, or death from any cause in patients with atrial fibrillation and stable coronary disease. Also, monotherapy with rivaroxaban was superior to combination therapy for the primary safety outcome of major bleeding (HR 0.59; 95% CI, 0.39-0.89; P = 0.01 for superiority). Therefore, both the results of the COMPASS trial and the AFIRE study demonstrate that the addition of an antiplatelet agent to different doses of rivaroxaban is associated with an increase in major bleeding.

Although aspirin is the current standard of care, strong evidence is lacking regarding its efficacy in reducing thrombotic events in this patient population. Furthermore, with aspirin, these patients remain at an unacceptably elevated risk for both arterial and venous thrombotic complications. Based on the evidence presented above, we hypothesize that monotherapy with low-dose apixaban will be more effective at preventing thrombotic events in JAK2-positive MPN (JAK2MPN) patients, without increasing their bleeding risk. Apixaban was chosen over rivaroxaban given that studies have consistently shown that it is not associated with an

increased bleeding risk, when compared to placebo or aspirin. Likewise, the addition of low-dose aspirin to apixaban was not chosen for the interventional arm, due to concerns over an increase in major bleeding, as described in the studies assessing the concomitant use of antiplatelets agents and different doses of rivaroxaban. Therefore, we propose to do a multi-centre pilot randomized controlled trial (RCT) evaluating the feasibility of a randomized controlled trial assessing the superiority of apixaban 2.5mg BID compared to low-dose aspirin as thromboprophylaxis of JAK2MPN patients.

Study Significance and Benefits

This pilot project is particularly important because MPN patients have special needs when it comes to the prevention of thrombosis, which means that they have been excluded in the past from the large trials that evaluated the use of medications to prevent thrombotic complications in cancer patients. Completing this pilot project will help to achieve equity in research by providing an opportunity for patients who normally do not get the chance to participate in clinical trials to be included in a research project. Through research endeavors such as this one, we hope to learn more about these conditions and offer better care to MPN patients.

Furthermore, since JAK2MPN are rare diseases, these patients are not always followed in highly specialized clinics, where they may be easily identified for the purpose of participating in a clinical trial. This pilot project is therefore a necessary step in order to gather invaluable information regarding the feasibility of a full-size RCT and to devise strategies to overcome

logistical challenges associated with the identification and recruitment of patients suffering from rare diseases.

Finally, the pilot study will form the basis for additional peer-review funding applications, such as Canadian Institute of Health Research (CIHR) grants. In preparation for these future grant applications, we have initiated negotiations with additional international and Canadian researchers who have expressed an interest in participating in the full-size RCT (once it is considered feasible).

Study Risks

All anticoagulant and antiplatelet therapies are associated with an increased risk of bleeding. In previous studies (as detailed in the previous section), apixaban has been shown to be as safe as aspirin, meaning that patients on apixaban did not have more major bleeding than patients on aspirin. Apixaban has also been shown to be better than aspirin at preventing venous blood clots, and as good as—or slightly better—than aspirin, the current standard-of-care, at preventing arterial clots. It is possible, however, that patients may find this change of treatment unacceptable, either because it is inconvenient or because participants are uncomfortable taking an anticoagulant.

Study Objectives and Endpoints

Primary Objective:

- To pilot test the feasibility and acceptability of designing a full-scale RCT to assess the superiority of apixaban compared to aspirin for the thromboprophylaxis of JAK2MPN patients.

Secondary Objectives:

- To identify additional feasibility measures, participation barriers and intervention preferences of JAK2MPN patients who choose not to participate in this pilot study
- To determine clinically meaningful outcomes of the subsequent full-scale RCT

Primary Endpoint of This <u>Pilot</u> Study:

- Average monthly subject recruitment rate of all study sites during a 6-month recruitment period
- Recruitment of 44 JAK2MPN patients in 6 months (39 prevalent cases and 5 incident cases)
- Feasibility will be measured through study accrual, attrition, and session completion
- Quality of life on apixaban and aspirin will be measured through the use of the SF-36 questionnaire

Secondary Endpoints of This Pilot Study:

- Screen-failure and consent rates
- Study drug compliance as assessed by quantification of used and unused drug
- Study visit compliance as assessed by the number of study visits (in person and/or phone call) completed
- Study subject retention rate (i.e. subject withdrawal and lost-to-follow-up rates)
- Percentage of incident and prevalent cases included in the study
- Rates of arterial and venous thrombotic events (MI, stroke, transient ischemic attack, peripheral arterial thrombosis, VTE) and all-cause mortality
- Rates of major bleeding and clinically relevant non-major bleeding events (per the International Society of Thrombosis and Hemostasis[54, 55])

Hypotheses of This Pilot Study:

- We expect that this pilot study will be considered feasible and acceptable.
- We expect that ≥90% of patients will pick up the study medication at the 3-month follow-up visit, and ≥80% of patients will pick up the study medication at the 6-month follow-up visit.

- We expect satisfactory adherence to both study intervention or standard-of-care aspirin therapy (defined as patients taking 80% or greater of the medication prescribed).

STUDY DESIGN

Study Overview

This is a multicenter, prospective, open-label, blinded-endpoint, phase II, randomized controlled trial. Eligible study participants will be randomly assigned to continue receiving aspirin or to receive apixaban, on a 1:1 basis. We would compare aspirin 81mg once per day to a low dose of apixaban, 2.5mg twice per day as shown in Table 1 below.

Table 1: Study Treatment Arms

Arm 1 (Control Group)	Arm 2 (Interventional Group)
23 Participants	23 Participants
Aspirin 81mg once per day for 6 months Then treated & followed up as per standard of care	Apixaban 2.5mg twice per day for 6 months Then treated & followed up as per standard of care

The recruitment period for this feasibility pilot study will last for 6 months. Once recruited, the patients will remain on the assigned study medication for at least 6 months, then be treated at the discretion of his/her treating physician. All patients will follow up in person at the 3-month and 6-month visits. A 1-month post-study conclusion visit (as mandated by Health Canada) will take place in person or via telephone.

There will be no extra tests required while taking part in the study. All laboratory, diagnostic, and/or subsequent treatment information will be collected based on its availability in the patient's medical files (per standard of care).

We anticipate obtaining both regulatory and ethics approvals for study sites by January 2020, enrolling the 1st study participant in March 2020, and completing patient recruitment by Summer 2020.

Sample Size

A maximum enrollment total of 46 subjects is anticipated to ensure that at least 44 subjects will complete their study treatment, 22 subjects per arm. This would represent a rate of 7.5 patients recruited per month, within a standard error of 0.08, if the target recruitment is completed within 6 months. As a time-to-event analysis is planned for the full-size trial, the sample size for the full-size trial will be informed by the recruitment rate calculated during the pilot phase, as well as the expected rate of thrombotic complications. The pilot project will also allow us to gather data on the variance and precision of the rate of thrombotic event in this patient population.

Furthermore, the pilot project will provide us with the opportunity to collect important information on the incidence and prevalence of JAK2MPN. Incident cases will be defined as those diagnosed with a JAK2MPN within the previous year. Incident cases of MPN patients are known to be at higher risk for both arterial and venous thrombotic complications[16]. Of note, once 39 prevalent cases have been recruited, the recruitment process will focus only on incident cases. The population included in the pilot project via the chosen four centres represent about 10% of the Canadian population. We estimate that about 1200 patients will be needed for the full-size trial, and that 400 of these patients will be recruited within Canada. Based on the limited published data on the incidence and prevalence of JAK2MPN[2, 52, 53], these 400 patients would represent 3.5% of the total Canadian JAK2MPN population. Therefore, we will aim to recruit 5-6 incident cases of JAK2MPN (representing 15% of all incident cases of JAK2MPN encompassed by our four centres) and 39 prevalent cases of JAK2MPN (representing 3.5% of all prevalent cases).

Patient Engagement Plan

Meaningful participant involvement plays an important role in helping produce and disseminate relevant evidence for decision making. This is especially true in clinical trials investigating patients with rare diseases, such as MPN, where more target approaches are necessary to both gather data and assist in delivering better patient-centered care. An independent and experienced patient-partner has been invited and will be involved in the planning and implementation of this pilot trial and potential full-size trial. Additionally, we will collect information regarding quality of life via the SF-36 questionnaire throughout the research

period. The involvement of the patient-partner and quality of life information will help guide recruitment and retention strategies.

Subject Selection

Subject Eligibility Criteria

This clinical trial can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom study participation is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Eligibility criteria are subject to review in the case of a Good Clinical Practice (GCP) or a regulatory authority audit. Any questions regarding a subject's eligibility should be discussed with the Study Coordinating Center (see protocol cover page) prior to enrollment.

Inclusion Criteria

Subjects must meet all of the following conditions to be eligible for enrollment into the study:

1. Male or female subjects aged 18 years or older,
2. Confirmed diagnosis of PV, JAK2ET or JAK2 pre-fibrotic MF, per local clinical definitions
3. Able and willing to comply with study procedures and follow-up examinations contained within the written consent form

4.3 Exclusion Criteria

The presence of any of the following conditions will exclude a subject from study enrollment:

1. Known allergy to apixaban or aspirin,
2. Another need for anticoagulation or specific anti-platelet therapy,
3. Contraindication to thromboprophylaxis (which would specifically include but not be limited to platelets less than $50x10^9$/L and acquired Von Willebrand disease),
4. Current pregnancy or breast-feeding,
5. Renal dysfunction (Creatine Clearance <25 mL/min),
6. Known liver disease
7. Currently on any medication with a known interaction to apixaban (refer to section 6.2.2)
8. Unwilling to use an effective means of contraception for women of childbearing potential
9. Overtly fibrotic myelofibrosis
10. Myelodysplastic/myeloproliferative neoplasms

4.4 Special Considerations for Enrollment Criteria

Patients with a previous history of VTE may be included in this trial for secondary thromboprophylaxis, provided that they have completed a minimum of 6 months of

anticoagulation and that the treating physician would be agreeable to have the patient's treatment stepped down to thromboprophylaxis with aspirin at minimum.

Additionally, as described in Section 3.2, we will aim to recruit a maximum of 39 prevalent cases of JAK2MPN. Therefore, once 39 prevalent cases have been recruited, we will include only incident cases (diagnosed within the previous year) for the remaining of the recruitment period.

Recruitment and Enrollment Procedures

Patient Enrollment and Randomization

Procedures that are to be performed as part of the practice of medicine and which would be done whether or not study entry was contemplated, such as for diagnosis or treatment of a disease or medical condition, may be performed and the results subsequently used for determining study eligibility without first obtaining consent.

Pre-screening to assess whether prospective patients are appropriate candidates for inclusion in this study and the process by which staff belonging to the primary circle of care of a patient refers the patient to the research team for formal consent and screening process are appropriate pre-entry/pre-screening activities. However, these pre-entry/pre-screening activities must be approved by the responsible research ethics board prior to its implementation at a site.

Upon obtaining a patient's informed consent, the qualified investigators or delegated research staff will then screen potential patients prior to enrollment based on information from the

medical history, available laboratory or biopsy results. The research staff should keep a record of patients who were screened as part of the study screening log. Ideally, every screened patient should be assigned a screening number.

Randomization or Treatment Allocation

Upon confirmation of a patient's eligibility by a delegated study investigator, the investigator or delegated research staff will use an Interactive Web Response System (IWRS) to assign the eligible patient a unique subject number (beginning with 2-digit site number + 2-digit patient number starting with 01). The subject number assigned at randomization will be different from the screening number. Randomization to either control or intervention will be performed in a 1:1 ratio. The subject number will be used for identification of the participant throughout the study.

Stratification

Eligible patients will be stratified based on the recruitment centre and use of cytoreductive therapies in the subsequent full-scale trial.

Due to the small sample size of this feasibility pilot study, the investigator's choice of cytoreductive therapies will only be documented at baseline & concomitant medication case report forms (CRFs), but not used for stratification during the randomization process.

Procedures for Handling Incorrectly Enrolled or Randomized Patients

Patients who fail to meet the eligibility criteria should not, under any circumstances, be enrolled or receive study medication. There can be no exceptions to this rule.

Where a patient does not meet all the eligibility criteria but is randomized in error, or incorrectly started on treatment, the following steps need to be taken:

1. The patient must be discontinued from the study, and the site must inform the trial coordinating center and the Sponsor-Investigator(s) immediately or as soon as the site becomes aware of the error, ensuring patient safety must always be the number one priority.
2. The treating physician may choose to continue with the randomized assigned therapy off-label, if he or she is satisfied that there are no concerns related to safety and disease management, but the patient must not continue in the study as the eligibility criteria are not discretionary. The rationale for continuing the assigned therapy off-label must be clearly documented.

Trial Blinding/Masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know which treatment is being administered.

All arterial or venous thromboembolic events, bleeding events, or deaths will be centrally reviewed and adjudicated by independent physicians who specialize in the adjudication process but who will be blind to subject treatment assignment to avoid bias.

Study INTERVENTION

Study Treatment

In the intervention arm, patients will receive apixaban 2.5mg. In the control arm, patients will receive low-dose aspirin (81mg daily). Patients will receive their assigned medications for at least 6 months.

At the end of the 6-month follow-up visit, it will be at the discretion of the patient's treating physician to decide whether the patient will continue apixaban as an off-label option, if the patient was initially assigned to apixaban. Whether the physician decides to continue the use of apixaban or to switch to aspirin, the decision and subsequent treatment plan will be documented along with its justification.

Concomitant Medications (Allowed & Prohibited)

In authorizing the use of any drug other than the study medication, the investigator should consider the clinical situation, the potential for masking symptoms of a more significant underlying event, and whether the use of the drug will compromise the outcome or integrity of the study. Medications specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication specifically prohibited during

the trial, discontinuation from the trial therapy may be required. The Site Investigator should discuss any questions regarding this issue with the Sponsor-Investigator. The final decision on any supportive therapy or medication rests with the Sponsor-Investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or schedule requires the mutual agreement of the investigator, the Sponsor and the subject.

i. Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. The use of over-the-counter or prescription NSAIDs will be recorded in the source documents and on the CRFs. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date will also be included on the CRF.

In this study, collection of concomitant medication information will focus on the initiation of cytoreductive therapies. Drugs of particular interest will include hydroxyurea, anagrelide, busulfan, ruxolitinib, interferon, pipobroman, and radioactive phosphorus. Finally, the use and frequency of phlebotomies will also be recorded.

All concomitant medications received within 14 days before the first dose of trial treatment through the Day 180 in-person Follow-up Visit should be recorded.

ii. Prohibited Concomitant Medications and/or Treatments

Subjects are prohibited from receiving the following medications or therapies upon study enrollment:

- Concomitant systemic treatment with strong inhibitors of both CYP 3A4 and P-glycopotein such as azole-antimycotics, e.g., ketoconazole, itraconazole, voriconazole, or posaconazole, and HIV protease inhibitors, e.g., ritonavir
- Concomitant systemic treatment with strong inducers of both CYP 3A4 and P-glycoprotein, such as rifampin, phenytoin, carbamazepine, phenobarbital or St. John's Wort
- Other anticoagulants, such as low-molecular-weight heparin, heparin, heparin derivatives, other DOACs, and warfarin
- Other antiplatelet agents, such as clopidogrel, ticagrelor, prasugrel, abciximab, eptifibatide or tirofiban
- The addition of one or more antiplatelet agent to apixaban

Although not contraindicated, the use of non steroidal anti-inflammatory drugs will be closely monitored (as described in Section 6.2.1).

Timing and Instruction on Dose Administration

All subjects should receive their assigned medications within 24 hours of randomization.

If a subject forgets to take his/her medication, the subject should take it when he/she remembers. However, the patient should not take extra medication (i.e. double dose) to compensate for a missed dosage. For a subject who inadvertently has a delay in administration of study drug of <6 hours, the planned dose should be taken. For a subject who inadvertently has a delay in administration of study drug of ≥6 hours, the dose should not be taken. Study drug administration may continue but the missed dose should not be made up and the planned timing of subsequent study drug dosing should not be altered.

All study medications (i.e. apixaban or aspirin) will be administered in outpatient setting.

Drug dispensing and return of medication

The clinic pharmacist or an alternative qualified person will be responsible for dispensing the study medication. The amount of drug necessary for a 3-month dosing period will be dispensed to the patient/care provider at the beginning of the study and again at the 3-month follow up, for a total on-treatment period of 6 months.

The patient will also be provided with a *Patient Medication Log* to document the exact date and time of the 1st dose of their study medication, last dose of their study medication (if applicable), as well as any missed doses.

Subjects should return all remaining tablets (any drug remaining in the opened bottles and the sealed bottles that were never opened) to the study site at the end of the treatment period. The *Subject Investigational Product Accountability Log* will serve as the source document for

drug supply to the subjects and will document the return of any unused drug for compliance assessments in the event of missing/lost *Patient Medication Log*.

Storage and Stability

Pre-labelled aspirin and apixaban in commercial format will be shipped to the site under appropriate conditions, and should be stored at room temperature (15° to 30°C) at the site and by the patient at home. During shipping and storage, excursions in temperature outside of the range of 5° to 40°C should be avoided.

Source of Study Medications

Both aspirin and apixaban will be supplied free of charge to the clinical site by the Sponsor for appropriate distribution to the subjects/caregivers. It is intended that the study drug will be provided in sufficient supply for the entire study.

Study Medication Accountability

Study personnel must ensure that all study medication supplies are kept in a secure locked area with access limited to authorized personnel. This study product must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study product to other investigators or clinics, or allow the supplies to be used other than as directed by this protocol.

The Site Investigator or his/her delegate must maintain accurate records of the receipt of all study medication shipped by OHRI Research Pharmacy or its designee, including, but not limited to, the date received, lot number, amount received, and the disposition of all study medication. Current dispensing records must also be maintained that include the date and amount of medication dispensed, relevant batch or bottle code numbers, and the subject's assigned study number.

At the time of dispensing the drug to the subject, the drug will be identified by the subject number. A drug-dispensing log will be maintained at each site. At each clinic visit by the subject, including the end of the study visit, all empty or partially used bottles in their original cartons and all unused medication must be returned to the site for accounting.

All used containers of study medication should be discarded according to standard institutional policy only after the study monitor (i.e. the Multi-Centre Trial Coordinator or delegate) has examined them. Depending upon the decision of the Sponsor, unused clinical supplies may be destroyed on site as dictated by the appropriate standard procedures at the participating institution or must be returned to the Sponsor or its designee after the study is completed. Records documenting the date of study medication destruction or shipping, relevant batch or bottle numbers, and amount destroyed or shipped should be kept.

Schedule of Events and study parameters

Schedule of Events

The proposed types and timing of data to be collected and recorded throughout the study are described in **Error! Reference source not found.** below. Please see Section 0 below for cross-r eferenced explanations of the study procedures described in the table.

Table 2: Schedule of Mandatory Events

	Screening[1] (Day -30 to Day 0)	Baseline Visit (Day 1)	Day 90 (±10) In-person Follow-up	Day 180 (±10) In-person Follow-up	Day 210 (±10) In-person/ phone call Follow-up
Eligibility	√				
Informed Consent	√				
Demography	√				
Medical History	√				
Physical Exam	√				
Review Laboratory Assessments[2]	√	√	√	√	
Concomitant Medication[3]	√	√	√	√	√
Randomization		√			
Study Drug Dispensing		√	√		
Patient Teaching		√			
SF-36 Quality of life questionnaire		√	√	√	
Treatment Compliance[4]			√	√	
Outcome Information			√	√	√
Adverse Events[5]			√	√	√

1. Screening visit and baseline visit can occur on the same date.
2. Results of laboratory/diagnostic tests will only be retrieved from existing medical records, if available; No research laboratory test is required.
3. All concomitant medications received within 14 days before the first dose of trial treatment through the Day 180 in-person Follow-up Visit should be recorded.
4. *Patient Medication Log* or *Subject Investigational Product Log* will be reviewed for compliance.
5. Adverse event reporting period starts upon initiation of study drug (apixaban only) and ends 30 days after the last dose of the study drug.

Explanation of Study Procedure

iii. Screening Visit Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

The screening visit procedures include:

- Obtaining patient's informed consent
- Review complete medical history
- Obtaining demographic information
    - Age, sex, race, ethnicity
- Review previous and concomitant medications
- Limited physical exam including measurement of the patient's height and weight
- Review subject eligibility criteria
- Review relevant laboratory, biopsy, diagnostic assessments
- Pregnancy test (if applicable)

iv. Baseline Visit Procedures

In this study, the screening and baseline visits can take place on the same date. In such cases, all screening and baseline visit procedures must also be performed on the same date.

The baseline visit procedures include:

- Confirmation of subject's eligibility
- Review concomitant medications
- Randomization of study subject to either control or intervention arm
- Study drug dispensing
- SF-36 health survey
- Documentation of clinical measurements and/or laboratory assessments of special interests, where applicable/available:
    - Risk factors that have been reported to be predictive of higher rates of thrombosis or bleeding
        - History of thrombosis
        - Known thrombophilia
        - Age
        - Leucocytosis (white blood cell count above 11)
        - Bleeding diathesis
        - Thrombocytosis
        - Ristocetin cofactor screen for acquired von Willebrand disease

- Known cardiovascular risk factors
    - Hypertension
    - Smoking
    - Diabetes
    - Hypercholesterolemia
    - Family history of cardiovascular disease
- Dates and types of laboratory tests used for eligibility confirmation including the results:
    - CBC with differential
    - Iron studies (iron, ferritin TIBC)
    - BCR-ABL gene re-arrangement to exclude CML (if available)
    - Cytogenetic testing - JAK 2 (exon 12 and exon 14), CALR, MPL
    - EPO level
    - Hematocrit
    - C-reactive protein, ESR to exclude reactive causes
    - Bone marrow biopsy (if available)
    - Creatinine
    - Bilirubin and liver enzymes (if available)
    - Lipids studies (if available)

v. Day 90 and Day 180 Follow-up Visit Procedures (In-person)

Day 90 and Day 180 follow-up visit procedures include:

- Return of unused study medications, opened and/or sealed drug bottles
- Assessment of study drug compliance and accountability
- Evaluation of adverse events
    - Adverse events of special interests
        - Leukemic transformation, progression to overt fibrosis
- Evaluation of study outcome information (i.e. major bleeding, CRNMB, venous thromboembolism, arterial and venous thrombotic events, and death if applicable)
- Documentation of dates and types of laboratory tests done between baseline and this visit to monitor or manage the baseline MF, ET or PV, if known, including but not limited to:
    - CBC
    - Liver function tests
    - Renal function tests
- Subsequent treatment/follow-up plan & frequency
- Smoking history in the past 3 months
- Use of concomitant medications
- SF-36 health survey

Day 210 Follow-up Visit Procedures (Optional In-person or telephone)

For all enrolled participants, an assessment will occur at the 7-month month time point. If this assessment coincides with an in-person visit, as mandated by the patient's treating physician,

the assessment will be done in-person. If not, this final assessment will be done over the telephone.

Day 210 follow up assessment procedure:

- Evaluation of adverse events
    - Adverse events of special interests
        - Leukemic transformation, progression to overt fibrosis
- Evaluation of study outcome information (i.e. bleeding, venous thromboembolism, arterial and venous thrombotic events, and death if applicable)
- Documentation of dates and types of laboratory tests done between baseline and this visit to monitor or manage the baseline ET or PV, if known, including but not limited to:
    - CBC
    - Liver function tests
    - Renal function tests
- Smoking history in the past 3 months
- Use of concomitant medications

Adverse Event Assessments

Adverse Event Definitions

An adverse event is any untoward medical occurrence in a trial subject receiving a study drug; the event does not necessarily have a causal relationship with the study drug administration or device usage. For this protocol, untoward medical occurrences that should be reported as adverse events include the following:

- All adverse events that are suspected to be due to the study medication
- The worsening of a preexisting illness
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as 2 separate adverse events. The outcome of the accident (hip fracture secondary to the fall) should be recorded in source documents.
- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test)
- Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities not requiring clinical intervention or further investigation will be captured as part of overall routine laboratory monitoring assessment, and should not be reported as adverse events.
- A preexisting condition must be noted on the appropriate baseline case report form but should not be reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event reporting period. Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period

should be reported as the adverse event and the resulting appendectomy should be recorded in the source documents. If a surgical procedure was planned prior to entry into the trial, and the surgery is not performed because of a worsening of a baseline condition, this should not be reported as an adverse event.

The adverse event reporting period begins on the day of enrolment and ends 30 days after the last dose of the study drug for patients who are assigned to apixaban.

Patients who are in the non-experimental group (i.e. received aspirin) will not be subject to any AE reporting requirement because that's the standard-of-care for this patient population. However, if a patient in this group experiences any outcome event that meets the reporting requirements specified in section 11.3.1. or 11.3.2., the event will be captured and reported using the study outcome forms.

The following symptoms are commonly reported by patients with PV or ET, and thus, **not captured** as reportable adverse events.

- Headache or migraines
- Lightheadedness and dizziness
- Fatigue
- Tinnitus
- Diaphoresis and night sweats
- Facial plethora

- Gout arthritis and tophi
- Symptoms of splenomegaly, including pain and discomfort in the left upper quadrant, early satiety, dyspepsia, decreased appetite, and associated weight loss

Bleeding is a known side effect of all anticoagulation medications, including apixaban, and therefore, bleeding events will not qualify as Adverse Events (AEs). For this study, bleeding events meeting criteria for major bleeding and/or clinically relevant non-major bleeding will be recorded as secondary outcomes. However, if a bleeding event meets criteria for a Serious Adverse Event (SAE) it will be recorded and reported as an outcome and as a SAE.

Serious Adverse Event Definitions

In this protocol, an adverse event that results in 1 or more of the following outcomes is classified as serious:

- Death
- Life-threatening situation (i.e., with an immediate risk of death)
- Inpatient hospitalization or prolongation of existing hospitalization. The following are not considered hospitalizations:
    - Social admission (e.g., patient/subject has no place to sleep),
    - Administrative admission (e.g., for yearly physical exam),
    - Optional admission not associated with a precipitating medical AE (e.g., for elective surgery),

- Hospitalization for observation without an AE,
- Admission for treatment of a pre-existing condition not associated with the development of a new AE or with a worsening of the pre-existing condition (e.g., for work-up of persistent pre-treatment lab abnormality),
- Protocol-specified admission during a clinical study (e.g., for a procedure required by the study protocol.

- Persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Other important untoward medical occurrences that may not result in death, may not be life-threatening, and do not require hospitalization, but which may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent any of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment administration in an emergency room or at home.

When a fatal or other serious outcome is the primary efficacy endpoint in a clinical trial, the protocol should clearly indicate the serious event(s) that will be treated as disease-related and not subject to expedited reporting. In this study, the following events will not be subject to expedited reporting to Health Canada if they meet the definition of Serious Unexpected Adverse Drug Reactions.

- Major bleeding or clinically relevant non-major bleeding events

- Serious arterial and/or venous thrombotic events

Adverse Event Recording

All adverse events (both serious and nonserious) that occur in subjects during the adverse event reporting period defined in Section 8.1 must be recorded, whether or not the event is considered drug-related.

All adverse events are to be recorded in the source documents and on the case report forms using concise medical terminology, and, whenever possible, terms contained in MedDRA. In addition, the following information should be recorded:

- Time of last dose prior to onset of event
- Onset date and time
- Resolution date and time, or date and time of death
- Severity of the event
- Action taken
- Outcome of the event
- Relationship to study drug
- Indication of whether the event is serious or nonserious; if serious, the reason for this classification

The Adverse Event CRF should be printed for Investigator signature and date and as confirmation that the data provided has been reviewed and assessed for intensity, relationship to study drug (causality), and seriousness.

Grading of Adverse Event Severity

The severity of adverse events will be graded using the CTCAE, Version 5.0 (see the *Study Manual*). For each event, the highest severity grade attained should be reported. If a CTCAE criterion does not exist, the investigator should use the grades or adjectives defined in Table .

Table 3. Grading of Adverse Event Severity (Events not Graded in CTCAE)

Grade	Adjective	Description
Grade 1	Mild	Sign or symptom is present, but it is easily tolerated, is not expected to have a clinically significant effect on the subject's overall health and wellbeing, does not interfere with the subject's usual function, and is not likely to require medical attention
Grade 2	Moderate	Sign or symptom causes interference with usual activity or affects clinical status, and may require medical intervention
Grade 3	Severe	Sign or symptom is incapacitating or significantly affects clinical status and likely requires medical intervention and/or close follow-up
Grade 4	Life-threatening	Sign or symptom results in a potential threat to life

Note the distinction between the seriousness and the severity of an adverse event. Severity is a measure of intensity; thus, a severe reaction is not necessarily a serious reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed in Section 0 above.

Describing Relationship to Study Drug (Causality)

The following will be used to describe the causality of the AE: UNRELATED, POSSIBLY RELATED, or RELATED to the study drug. For the purpose of consistency, the causality grades are defined as follows:

Table 4. Description of Causality between Study Drug and AEs

Adjective	Description
Unrelated	There is not a reasonable possibility that the adverse event may have been caused by the study drug/intervention.
Possibly Related	The adverse event may have been caused by the study drug/intervention; however, there is insufficient information to determine the likelihood of this possibility.
Related	There is reasonable possibility that the adverse event may have been caused by the study drug/intervention.

Expectedness of the event

The following will be used to describe the grading of the AE: EXPECTED or UNEXPECTED. For the purpose of consistency grading will be defined in Table 5 as follows:

Table 5. Description of Expectedness of AEs

Adjective	Description

Expected	Identified in nature, severity, or frequency in the current protocol, informed consent, product monograph or within other current risk information.
Unexpected	Not identified in nature, severity, or frequency in the current protocol, informed consent or product monograph or within other current risk information.

Seriousness of the Event (Gravity)

Finally, each AE is to be classified by the Site Investigator as SERIOUS or NONSERIOUS. This classification of the gravity of the event determines the reporting procedures to be followed.

Follow-up of Unresolved Adverse Events

All adverse events should be followed until they are resolved, or the investigator assesses them as chronic or stable. The investigator should use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. A subject withdrawn from the study because of an adverse event must be followed by the investigator until the clinical outcome from the adverse event is determined.

Adverse Event Reporting Requirements

Site Investigator's SAE Reporting Requirements

If a SAE occurs, the Site Investigator is responsible for notifying the Trial Coordinating Centre in Ottawa within 24 hours of awareness of the event. In the rare event that the Site Investigator does not become aware of the occurrence of a SAE immediately (e.g., if an out-patient trial

participant initially seeks treatment elsewhere), the Site Investigator is to report the event within 24 hours after learning of it and document the time of his/her first awareness of the SAE. The submission should be accompanied by a notification email to the Sponsor-Investigator AND the Primary Contact for the Trial Coordinating Centre (i.e. the Multi-Center Trial Coordinator) to ensure that the sponsor is aware that an SAE has been reported.

The Multicentre Trial Coordinator can be contacted by telephone or email if the Site Personnel have questions about SAE reporting or recording processes.

Site Investigators are also responsible for reporting SAEs to their individual Research Ethics Board and/or Research and Development department according to local policies. Note that unexpected SAEs may be subject to expedited reporting to the responsible Research Ethics Board.

Timelines for Site Investigator's SAE reporting

It is important that the initial *Serious Adverse Event Reporting Form* be sent within 24 hours of awareness of the event, even if the event is ongoing and all sections of the form cannot be completed at that time. Subsequent follow-up reports may be sent as the pertinent information becomes available.

Table 6. REPORTING REQUIREMENTS FOR ADVERSE EVENTS		
Gravity	**Reporting Time**	**Type of Report**
SERIOUS	Within 24 hours	Initial report on SAE
	Within 15 days	Follow-up report on SAE
SERIOUS (life-threatening or death)	Within 24 hours	Initial report on SAE
	Within 7 days	Follow-up report on SAE
NON-SERIOUS	10 days after completion of Day 90 visit	Adverse Events CRF

Sponsor Investigator's SAE Reporting Requirements

The Sponsor-Investigator(s) is responsible for SAE reporting to regulatory authorities. Upon receipt of the *Serious Adverse Event Reporting Form* from the site, an email will be sent from the Study Coordinating Centre to the Site Investigator to confirm receipt.

The **Sponsor–Investigator** will:

- Review all SAEs and will comment to the Site Investigator via email on causality and grading in a timely manner,
- Notify *Health Canada* if the SAE is assessed to be related or possibly related, and unexpected by **either** the reporting health care professional or the sponsor. Only serious events that are **BOTH** related and unexpected are subject to expedited reporting to **Health Canada**. Serious, related and unexpected SAEs will be submitted to Health

Canada by the coordinating center via fax using the CIOMS form accompanied by the Adverse Drug Reactions (ADRs) for Clinical Trials, Expedited Reporting Summary Form. They will be reported according to the following criteria:

  - Where event is neither fatal or life threatening, within 15 calendar days after becoming aware of the information
  - Where it is fatal or life threatening, immediately where possible, and, in any event within 7 calendar days after becoming aware of the information.
  - Within 8 calendar days after having informed Health Canada, submit a complete report including assessment of the importance and implication of any findings, and relevant previous experience with the same or similar drugs.

- Serious but expected reactions as well as serious events that are considered unrelated to the study product by **both** the investigator and the sponsor will not be reported to Health Canada,
- For all SAEs, the **Study Coordinating Centre** will:
  - Submit the completed *Serious Adverse Event Case Report Form* to the **Data Safety Monitoring Board (DSMB)** for committee review.
  - Notify Site Investigators at *all participating centres* of the Serious Unexpected Adverse Drug Reactions so that each may report to their individual Research Ethics Board and / or Research and Development department according to local policies.

How to report:

When submitting an ADR report to Health Canada, a complete ADR Expedited Reporting Summary Form (Form 01-03) and the CIOMS Form should be attached and as applicable be mailed or faxed to:

Therapeutic Products Directorate

Pharmaceuticals

Fax: 613-941-2121

Treatment interruption AND PERMANENT DISCONTINUATION

In some circumstances, it may be necessary to temporarily interrupt treatment as a result of adverse experiences that may have an unclear relationship to the study drug. The study drug may be withheld by the Investigator at any time if there is concern about patient safety and for all aspects of the conduct of the protocol, since the safety of the individual patient is paramount. Treating Investigator may employ any means necessary to ensure patient safety, particularly in medical circumstances not anticipated by this protocol.

As such, the study medication (apixaban or aspirin) should be temporarily held in case of severe thrombocytopenia (platelet count < 50 x 10^9/L) or invasive procedures where the risk of major bleeding is elevated and therefore warrants discontinuation of thromboprophylaxis. In the case

of apixaban, the drug should also be interrupted if the creatinine clearance decreased below 25 ml/min.

Patients have the right to withdraw from the study at any time for any reason. The Investigator has the right to withdraw patients from the study due to medical reasons according to his/her discretion.

When patients discontinue study medication, the reason must be categorized in the case report form (CRF) as one of the following:

1. study completed;
2. adverse event(s);
3. disease progression;
4. protocol violation;
5. patient withdrew Informed Consent;
6. lost at follow-up (despite every effort made to contact the patient);
7. physician decision due to safety reasons;
8. sponsor decision;
9. death;
10. pregnancy.

If the patient discontinues for any reason (including discontinuation for pregnancy), with drug related adverse event ongoing at study end, he/she must be followed until resolution or

stabilization of the event or until it is reasonable to consider the event not drug related any more or until the start of a new treatment, whichever occurs first.

If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance every effort should be made to complete and report the observations as thoroughly as possible. All relevant information related to the reason for treatment discontinuation including contributory factors must be included on the CRF.

Withdrawal of subjects From Study Participation

Since this is a trial of relatively short-term- treatment, all subjects who receive study drug should remain in the study whenever possible. However:

- Any subject has the right to withdraw from the study at any time.
- Any subject who has been enrolled in the study but is later found to be ineligible must be discontinued from study participation.
- Any subject whose condition substantially changes after entering the study should be carefully evaluated by the investigator in consultation with the Sponsor-Investigator. Such subjects should be withdrawn from study treatment if continuing would place them at risk.
- Any subject who becomes significantly noncompliant with study drug administration, study procedures, or study requirements should be withdrawn from study treatment

when the circumstances increase risk to the subject or substantially compromise the interpretation of study results.

- The investigator, in consultation with the Sponsor-Investigator, may withdraw any subject from the study treatment, if, in the investigator's opinion, it is not in the subject's best interest to continue.

The date the subject is withdrawn from the study and the reason for discontinuation will be recorded in the source documents and on the case report forms.

When a subject is withdrawn from the study (regardless of the reason), the investigator should encourage that all of the evaluations required at the Day 90 study visit be performed and that any additional evaluations be completed that may be necessary to ensure that the subject is free of untoward effects. The subject should be encouraged to seek appropriate follow-up for any continuing health problems.

A patient who completes the initial portion of the study (through the Day 90 visit) and is then lost to follow-up during the following 3-month short-term follow-up period will not be considered to have prematurely withdrawn.

Statistics and data Management

Statistics Plan

Pilot project:

An estimate of the mean recruitment rate over a 6-month period at four centres with 95% CI will be provided as the primary outcome of the pilot trial.

If 42-46 patients are recruited during the 6 months of recruitment (representing a rate of 7.5 patient per month, with a standard error of 0.08), then the full-scale trial will be deemed feasible. The four chosen sites represent two larger academic sites, where the care of JAK2MPN patients is concentrated within a few clinics, a smaller academic centre, and a community centre. The rate of each centre will also be calculated and analyzed to inform further decisions regarding the number and type of sites to be included in the full-size RCT.

Descriptive statistics will be used to characterize subject characteristics at study entry, as well as the proportion of screened patients meeting eligibility criteria, of incident or prevalent cases, of participant retention, of adherence to study visit, and of adherence to medication. Frequencies of adverse events and laboratory abnormalities will be tabulated by MedDRA System Organ Class, Preferred Term, dose level, worst severity, timing, outcome of the event, relationship to study drug, and seriousness.

An exploratory time-to-event analysis for the primary and secondary outcomes of the full-scale trial (described below) will also be done during the analysis of the pilot results.

Full-scale trial:

We hypothesize that apixaban at a dose of 2.5mg BID will be superior to the current intervention of low-dose aspirin in preventing arterial and VTE complications in this patient population. From the results presented above, it is expected that the use of apixaban 2.5mg BID

will decrease the risk of venous events by 70% and will be at least as effective as aspirin in decreasing the risk of arterial events, for an overall risk reduction for both venous and arterial events of 35%.

Descriptive statistics (proportion and means with 95% confidence intervals) will be done to assess baseline patient characteristics and to identify failure of the randomization process.

Patients will be censored at the time of the last follow-up (if lost to follow up), at the time of death, or at the end of the study period. Only the first objectively confirmed component of the composite primary outcome will be captured in the analysis.

The statistical analysis for the primary efficacy endpoint will be performed using a modified intention-to-treat approach, which will include all patients who were randomized and have received at least one dose of the study medication. The analysis for the safety outcomes of major bleeding, clinically relevant non-major bleeding, and clinically relevant bleeding will be performed using an on-treatment approach. A time-to-event analysis will be done using Kaplan-Meier curves to estimate cumulative event rates for the primary and secondary outcome measures. Hazard ratios for the primary outcome will be estimated using Cox regression analyses, adjusting for the type of MPN, age, sex, and recruitment centre. For the primary outcome, a sensitivity analysis using death not attributed to a cardiovascular cause or bleeding as a competing risk will also be performed by estimating cumulative incidence functions, which will be compared using Gray's test.

Missing data will be assumed to occur at random and potentially lead to bias. A multiple imputation analysis will be performed with the aim to overcome this possible source of bias.

Data and Safety Monitoring Board

External oversight for this trial will be provided by an independent DSMB in accordance with the OHRI CRMC approved Study Monitoring Plan. The primary responsibility of the DSMB is to protect the safety and welfare of subjects participating in this clinical trial and to ensure the integrity of the clinical trial.

The DSMB will meet either in-person or remotely to discuss matters related to the safety of study participants (SAE/SUSAR), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness.

The DSMB will review the interim data from the study once a total of 20 subjects have been treated with either the study drug or standard-of-care for at least 3 months, and again when all subjects have completed their 6-month study treatment. The DSMB may review the safety data at other times as warranted by emerging results. Based on review of the safety data, the DSMB can recommend continuation of the study without modification(s), study interruption, study termination, or modification of the trial, where applicable.

Further information regarding the DSMB review process is provided in the Study DSMB Charter.

Study Outcome Adjudication Committee

At any time during the study follow-up period, if a patient experiences signs or symptoms suggestive of bleeding, arterial or venous thrombotic events, diagnostic tests will be performed at the discretion of the treating physician. All events, independent of their seriousness, will be

treated according to the standard of care at the local institution by the treating physician. Secondary outcomes (suspected major bleeding, CRNMB, suspected arterial and/or venous thrombotic events, all-cause mortality) will be sent to the blinded adjudication committee for review.

11.3.1 Methods for Assessing Secondary Outcome Measures: Arterial and Venous Thrombotic events & All-Cause Mortality

Venous thrombosis:

- Compressive ultrasonography showing a thrombosed venous segment measuring more than 5mm in diameter under compression in the deep vessels, or an inability to compress superficial or muscular vessel of the limbs or neck. A distal deep vein thrombosis of the lower extremities is located below the popliteal vessel, while of proximal deep vein thrombosis is located above the trifurcation.
- Computed tomography with contrast (of the head, neck, extremities, thorax, abdomen, or pelvis) showing an intra-luminal filling defect within the veins

Pulmonary embolism:

- V/Q scan interpreted as high probability for pulmonary embolism per PIOPED criteria and associated with an intermediate or high pre-test probability

- Computed tomography showing a filling defect in a subsegmental or larger pulmonary vessel
- Pulmonary angiography with a finding of intra-luminal filling defect in 2 or more views, or a sudden contrast cut-off in one or more vessels measuring greater than 2.5mm in diameter.

Peripheral arterial thrombosis:

- Doppler ultrasonography showing a lack of arterial flow or a thrombus in an arterial segment
- Computed tomography with contrast (of the head, neck, extremities, thorax, abdomen or pelvis) showing an occlusion of an arterial segment et/or a visceral infarction

Ischemic cerebral vascular ischemia:

- Focal and sudden neurological deficit, such as:
    - Unilateral paralysis
    - Paresthesia of an extremity
    - Aphasia
    - Mononuclear vision loss
    - Difficulty with balance or ambulation

and associated with cerebral imaging (magnetic resonance imaging or computed tomography) showing an occlusion of an arterial vessel and/or an ischemic plane, based on the imaging technique

- A transient ischemic attack, defined as a focal and sudden neurological deficit, ischemic in nature, and which completely resolves in less than 24hours (usually less than 30 minutes). The total duration must be greater than 10 seconds.

Ischemic heart disease:

- A non-ST elevation myocardial infarction is defined as an elevation in troponin in context of angina
    - Pressure-type chest pain radiating to the jaw or the left shoulder. The pain may be atypical
    - Acute ECG changes including: ST segment depression, horizontal or down-slopping (particularly ≥1mm in 2 or more contiguous leads); horizontal or convex ST elevation consistent with Prinzmetal angina, transient T wave inversion (if measuring >1mm). The ECG may also remain unchanged.
    - Post-acute ECG changes: deep and symmetrical T wave inversion in the anterior leads
    - Elevation in troponin detected 3-6 hours following the start of the ischemic event
- ST elevation myocardial infarction

    - Chest pain, usually retrosternal, constrictive and severe, radiating to the arms, jaws, back or shoulders, occurring at rest (or after exertion), lasting greater than 30 minutes and resistant to nitroglycerine therapy. The pain may be atypical.
    - ECG changes: ST elevation measuring more than 1mm in the limb and augmented limb leads (I, II, III, aVF, aVL, aVR) or 2mm in the precordial leads (V1-V6) in at least 2 contiguous leads, followed by T wave inversion (which may normalize afterwards). A Q wave may be noted.
    - Elevation in troponin detected 3-6 hours following the start of the ischemic event
- Morphologic proof of myocardial tissue death (by scintigraphy, coronary angiogram, stress test, or stress echocardiography)

Death related to a thrombotic event:

- Cardiovascular death: death attributed to MI or stroke by autopsy or radiological findings and/or without an alternative explanation
- Fatal PE: death attributed to PE by autopsy or radiological findings and/or without an alternative explanation

Malignancy-associated mortality:

- Death attributed to a hematological malignancy

11.3.2 Methods for Assessing Secondary Outcome Measure: Bleeding

Major bleeding and clinically relevant non-major bleeding events will be confirmed with investigational reports including clinic notes, hemoglobin results and imaging as available.

Major Bleeding will be defined as[54]:

- Fatal bleeding, and/or
- Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome, and/or
- Bleeding causing a fall in hemoglobin of ≥20 g/L, or leading to transfusion of ≥2 units of whole blood or red cells

Clinically relevant non-major bleeding will be defined as[55]:

- Any sign or symptom of hemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that does not fit the criteria for the ISTH definition of major bleeding but does meet at least one of the following criteria:
    - Requiring medical intervention by a healthcare professional

- Leading to hospitalization or increased level of care
- Prompting a face to face (i.e., not just a telephone or electronic communication) evaluation

Minor bleeding is any bleeding that does not meet the criteria for being clinically relevant but is abnormal for the patient compared to their baseline. This includes "nuisance bleeding", such as bleeding gums or increased bleeding while shaving, that does not require any evaluation or intervention as described above.

Obligations of the Investigator and the Sponsor

Compliance with Ethical and Regulatory Guidelines

The investigator is responsible for ensuring that the clinical study is performed in accordance with the Declaration of Helsinki **Error! Reference source not found.**), Health Canada Food and D rug Part C Division 5 regulations, and the International Conference on Harmonisation (ICH) GCP guidance documents.

Research Ethics Board

Prior to enrollment of subjects into the study, as required by the Health Canada, the protocol and informed consent document will be reviewed and approved by an appropriate REB. By signing the Qualified Investigator Undertaking Form (Health Canada Form 01-03), the investigator assures that approval of the trial protocol will be obtained from the REB and that

all aspects of the REB review will be conducted in accordance with current regulations. Amendments to the protocol will be subject to the same REB review requirements as the original protocol. Only changes necessary to eliminate apparent immediate hazards to the subjects may be initiated prior to REB approval. The investigator will also promptly notify the REB of any serious, unexpected drug reaction events, or any other information that may affect the safe use of the drug during the course of the trial.

A letter documenting the REB approval and a list of the names and titles of the REB members must be received by the Trial Coordinating Office prior to the initiation of the study. All correspondence with the REB should be retained in the investigator's study file.

As soon as possible after completion or termination of the study, the investigator will submit a final report to the REB and to the Coordinating Office. This report should include the dates of initiation and completion of the trial, a description of any changes in study procedures or amendments to the protocol, deviations from the protocol, the number and type of subjects evaluated, the number of subjects who discontinued (and the reasons for discontinuation), the number of subjects who completed the trial, and the results of the trial, including a description of any adverse events.

Informed Consent

The investigator assures that informed consent will be obtained from each subject prior to study entry and that the informed consent will be obtained in accordance with current regulations. The investigator or delegated staff will give each subject full and adequate verbal

and written information regarding the objective and procedures of the trial and the possible risks involved. An informed consent document will be provided to each subject in a language in which the subject is fluent (French or English). This information must be provided to the subject prior to undertaking any trial-related procedure. The written subject information must not be changed without prior approval by the Sponsor and the responsible REB. Each subject will be given a copy of the signed consent form. The original signed informed consent forms will be retained by the investigator with the study records.

Paper Case Report Forms

A paper case report form (CRF) is required and must be completed for each enrolled subject, with all required study data accurately recorded such that the information matches the data contained in medical records (e.g., physician's note, nurse notes, clinic charts and other study-specific source documents). These case report forms will be completed at each site by the investigator or investigator's designee (e.g., research coordinator, research nurses). The case report forms will be submitted to the Trial Coordinating Center via a secured web-based file transfer site. Any change of data will be made to the paper CRFs and then re-submitted to the file transfer site with an updated submission date to provide an audit trail.

It is the investigator's responsibility to ensure completion of data entry, and to review and approve all data entered uploaded to the file transfer site from the investigator's site. At all times, the investigator has final responsibility for the accuracy and authenticity of all clinical and laboratory data reported to Sponsor from the investigator's site.

The data contained therein are the sole property of the Sponsor and should not be made available in any form to third parties, except for authorized representatives of the Sponsor and of appropriate regulatory authorities, without written permission from the Sponsor.

Study Records

During the study, the investigator will maintain adequate records for the study, including medical records, source document records detailing the progress of the study for each subject, laboratory reports, printed and signed copies of the data summary documents of the data that has been captured in the completed paper CRFs for each subject, signed informed consent forms, study drug disposition records, correspondence with the REB, adverse event reports, and information regarding subject discontinuation and completion of the study. Current regulations require OHRI (or an authorized designee) to inspect all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the subjects enrolled in this trial. These regulations also allow the same records to be inspected by authorized representatives of Health Canada or other regulatory authorities.

Confidentiality

The names and identities of all research subjects will be kept in strict confidence and will not appear on paper case report forms or other records provided to or retained by the Sponsor (or its authorized designee). The names and identities of the subjects need not be divulged;

however, the records must nevertheless be inspected. This will be accomplished by blacking out the subject's name and replacing the name with the subject's study identification number on any record provided to or retained by the Sponsor. The informed consent form must include appropriate statements explaining these requirements.

Retention of Records

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link paper CRFs, and clinic records), all original signed informed consent forms, signed copies of the data summary documents of the data that has been submitted for each subject, and detailed records of study drug disposition. All records and documents pertaining to the study (including but not limited to those outlined in Section 0 above) will be maintained by the investigator until notification is received from the sponsor that the records no longer need to be retained. This will be for a period of at least 25 years.

The investigator must obtain the Sponsor's written permission before disposing of any records. In order to avoid any possible errors, the investigator will contact the Sponsor prior to the destruction of any study records. The investigator will promptly notify the Sponsor in the event of accidental loss or destruction of any study records. If the investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to the Sponsor.

Monitoring and Auditing

In accordance with Part C Division 5 of the Food and Drug Regulations, and/or relevant ICH guidelines, the Sponsor or a designee will periodically inspect all completed study case report forms (see Section 0), study documents, research facilities, and clinical laboratory facilities associated with this study at mutually convenient times during and after completion of the study. As specified in the Study Monitoring Plan, the monitoring visits provide the Sponsor with the opportunity to evaluate the progress of the study; verify the accuracy and completeness of data in the completed case report forms; ensure that all protocol requirements, applicable Health Canada and other relevant regulations, and investigator's obligations are being fulfilled; and resolve any inconsistencies in the study records. This includes inspection of all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the subjects in this trial. The names and identities of all research subjects will be kept in strict confidence and will not appear on electronic data entry forms or other records provided to or retained by the Sponsor. The investigator/institution guarantees direct access to source documents by the Sponsor and appropriate regulatory authorities.

The trial site may also be subject to review by the REB, to quality assurance audits performed by the Sponsor, and/or to inspection by Health Canada and/or other regulatory authorities.

Termination of the Study

The Sponsor reserves the right to discontinue the trial prior to inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons. The Site Investigator, after consultation with the Sponsor, reserves the right to discontinue the study for safety reasons at any time. After such a decision is taken, the Site Investigator must contact all subjects who are continuing their participation in the study and must do so within a time period set by the Sponsor. As directed by the Sponsor, all study materials must be collected and all case report forms completed to the greatest extent possible.

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