Summary

NOVEL STRATEGIES OF ANTITHROMBOTIC PROPHYLAXIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: COMPARISON OF DIFFERENT DOSING REGIMENS OF ADMINISTRATION OF LOW-DOSE ACETYLSALICYLIC ACID. Aspirin Regimens in ESsential thrombocythemia (ARES)

Test drug: acetylsalycilic acid, CardioAspirin® 100 mg per os

Brief description of the study including design, comparison, duration, dose and patient population. Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPN) characterized by primary thrombocytosis and high thrombotic risk, with a reported event rate from 1.3 to 6.6%/yr.(1) Once daily (od), low-dose aspirin (75-100 mg) is currently recommended as antithrombotic prophylaxis in ET based on: i) evidence from non-ET, high-risk patients; ii) observational studies in ET; iii) extrapolation of data from a trial on a different MPN where aspirin 100 mg od reduced major vascular events vs. placebo.(2) The benefit/risk profile of aspirin in ET has never been assessed in randomized trials, and recommending the same od aspirin range for ET as for non-ET patients implies assuming similar pharmacodynamics (PD). We have shown that the antiplatelet effect of aspirin 100 mg od is shortened in ET,(3) reflecting accelerated renewal of the drug target, i.e. platelet cyclooxygenase (COX)-1-dependent thromboxane (TX) A_2 synthesis. The reduced inhibition of platelet COX-1 during the 24-hour dosing interval can be largely, but not entirely, overcome by twice-daily 100 mg aspirin.(3) However, a shorter dosing interval might inhibit endothelial prostacyclin (PGI_2) production,(4) thus lowering the net antithrombotic effect of aspirin.

ARES is a multi-centric, phase II, dose-finding, randomized trial, designed into two sequential parts, named 'part A' (placebo controlled) and 'part B' (open label) that enrolled ET patients diagnosed according to the WHO 2008 criteria, between 18 and 75 years, already on aspirin 100 mg daily since at least 1 month, according to the judgement of the referring Hematologist.

Part A was a parallel-arm, placebo-controlled, double-blind, randomized study comparing aspirin 100 mg once, twice (bid), or three- (tid) times daily. Placebo was used so that all ET patients took a study medication (active or placebo) 3 times daily. The calculated sample size was 100 patients per arm. After 2 weeks, serum TXB_2 and urinary PGI_2 metabolite were measured to identify the best multiple-dosing regimen giving the highest serum TXB_2 inhibition with the lowest inhibitory effect on PGI_2.

Part B had a randomized, open-label design and enrolled patients largely participating already to Part A, who were randomized to aspirin 100 mg twice daily (bid) which was the best multiple regimen identified in Part) vs. standard aspirin 100 mg od, for 20 months, with a planned sample size of 112 patients per arm. The main objective was the persistency of the superior biochemical efficacy of the experimental vs. standard dosing regimen, as assessed by repeated serum TXB_2 measurements over 11 study visits.

Primary Objectives. Part A assessed whether a bid or a tid 100 mg aspirin daily dosing was superior to standard 100 mg once-daily (od) aspirin in inhibiting platelet TXA_2, without affectingvascular PGI_2. Part B evaluated the long-term persistence of superior biochemical efficacy of a multiple vs. od dosing aspirin regimen.

EudraCT: 2016-002885-30

Phase: II, dose-finding study

Date of contract: 23/02/2017

Date of Ethic Committee approval: at the Coordinating Center 21/07/2016

Period covered: from FPFV of part A to LPLV of part B: 12/12/2017-24/10/2020

Date of early termination: NA, the study was completed as scheduled

Report Author(s): Valerio De Stefano, Bianca Rocca, Alberto Tosetto, Carlo Patrono

Principal Investigator: Prof. Valerio De Stefano, Director of the Hematology Laboratory Service, Fondazione Policlinico Universitario A. Gemelli IRCCS- Rome.

The ARES trial was performed in accordance with the Good Clinical Practices (GCP).

1.1 Study Administration and Investigators

Principal Investigator: Prof. Valerio De Stefano, Director of the Hematology Laboratory Service, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome

Other PIs at each participating Unit. Unit 2: Prof. Bianca Rocca, Istituto di Farmacologia, Università Cattolica del Sacro Cuore, Roma; Unit 3: Dr. Marco Ruggeri, Unità Operativa Complessa di Ematologia, Ospedale San Bortolo, Vicenza; Unit 4: Prof. Francesco Rodeghiero, Fondazione Progetto Ematologia, Vicenza; Unit 6: Dr. Francesca Palandri, Unità Operativa di Ematologia, Azienda Ospedaliero-Universitaria di Bologna Policlinico Sant'Orsola-Malpighi, Bologna; Unit 7 Prof. Alessandro M. Vannucchi, Dipartimento di Medicina Sperimentale e Clinica, CRIMM (Centro di Ricerca e Innovazione per le Malattie Mieloproliferative), Azienda Ospedaliero Universitaria Careggi, Firenze; Unit 8: Dr. Elena Maria Elli, Clinica Ematologica e Unità Trapianto di Midollo Osseo, Ospedale San Gerardo, ASST Monza, Università Milano-Bicocca, Monza; Unit 9: Prof. Mauro Di Ianni, Dipartimento Oncologico Ematologico, Centro Diagnosi e Terapia dell'Emofilia e delle Malattie Rare del Sangue, Ospedale di Pescara; Unit 10: Dr. Alessandra Iurlo. U.O.C. Oncoematologia, Fondazione IRCCS Ca'Granda, Ospedale Maggiore Policlinico, Milano; Unit 11: Prof. Maria Luigia Randi, Clinica Medica 1, Dipartimento di Medicina DIMED, Azienda Università- Ospedale Padova; Unit 12: Prof. Giorgina Specchia, U.O. Ematologia con Trapianto, Azienda Ospedaliero-Universitaria Policlinico Consorziale di Bari; Unit 13: Dr. Eloise Beggiato, Ematologia UAOU Città della Salute e della Scienza, Ospedale Molinette, Torino.

Name and affiliation of laboratories used in the study. Laboratorio Istituto di Farmacologia, Università Cattolica del Sacro Cuore, Roma; Personnel: Prof. Bianca Rocca, Dr. Giovanna Petrucci. External Laboratory for PGIM centralized measurements: Istituto Cardiologico Monzino IRCCS, Milano, Referent personnel: Dr. Viviana Cavalca.

Name and affiliations of all members of any committees involved with the study e.g.-steering committee independent review committees for specific parameters:

Steering Committee: Prof. C. Patrono (Study Chairman, Dipartimento di Bioetica e Sicurezza, Sezione di Farmacologia, Università Cattolica, Rome, Italy), Prof. V. De Stefano (Study Coordinator, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy), Prof. F. Rodeghiero (Hematology Project Foundation, Vicenza, Italy), Prof. B. Rocca (Dipartimento di Bioetica e Sicurezza, Sezione di Farmacologia, Università Cattolica, Rome, Italy), Dr. A. Tosetto (Hematology Department, Ospedale San Bortolo, Vicenza).
Data Safety Monitoring Board (DSMB): An independent DSMB was nominated to further protect the safety of the patients by monitoring the progress and results. DSMB included: Prof.
V. Di Lazzaro (Neurologist, Campus Biomedico University, Rome, Italy), Dr. I. Martinelli (Hematologist-Angiologist, Ospedale Maggiore Policlinico, Milan, Italy), Dr. M. Testa (Cardiologist, Ospedal S. Andrea, 2nd School of Medicine, Sapienza University, Rome, Italy) who were not Investigators in the study nor otherwise associated with it.

Name and affiliation of contract research organisations:

Unità Ematologia, Ospedale S. Bortolo di Vicenza (Dr. Alberto Tosetto) for data management, randomization, study monitoring, drug management and Clinical Trial Center.

Fondazione Policlinico Universitario A. Gemelli IRCCS (Dr. Betty Polikar, Dr. Elena Carafelli) for the operational oversight and Pharmacovigilance.

Name and address of relevant Sponsor study personnel:

Names and affiliations of all Investigators and Sponsor personnel have been reported in the Appendix 1 of this FSR.

1.3 Table of Contents

1.4 List of Abbreviations and Definition of Terms

AE: adverse event

bid: twice daily

COX: cyclooxygenase

CRNMB: clinically relevant non-major bleeding

EMA: European Medicine Agency

ET: Essential Thrombocythemia

FSR: final study report

MPN: myeloproliferative neoplasms

od: once daily

PD: pharmacodynamics

PG: prostaglandin

PGI_2: prostacyclin

PGIM: urinary prostacyclin metabolite

PV: Polycythemia Vera

SAE: serious adverse event

tid: three times daily

TX: thromboxane

TXM: urinary thromboxane metabolite

2 Synopsis

A brief synopsis (max 3 pages) that summarises the study, including numerical data and relevant statistical information e.g. p-values, in the results sections, should be included.

Name of finished product: acetylsalycilic acid, CardioAspirin® 100 mg enterico coated formulation

Name of active ingredient(s): acetylsalycilic acid

Study title: NOVEL STRATEGIES OF ANTITHROMBOTIC PROPHYLAXIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: COMPARISON OF DIFFERENT DOSING REGIMENS OF ADMINISTRATION OF LOW-DOSE ACETYLSALICYLIC ACID

Principal investigator: Prof. Valerio De Stefano, Director of the Hematology Laboratory Service, Fondazione Policlinico Universitario A. Gemelli IRCCS- Rome.

Study centre locations: the trial included 11 clinical Centers, one Core Lab, and one Foundation (Vicenza) for randomization and database collection

Study period: from FPFV of part A to LPLV of part B: 12/12/2017-24/10/2020

Phase: II, dose finding

Objectives of study: Part A assessed whether a multiple (bid or tid) 100 mg aspirin daily dosing was superior to standard 100 mg od aspirin in inhibiting platelet TXA_2, without affectingvascular PGI_2. Part B evaluates the long-term persistence of superior biochemical efficacy of a multiple vs. od dosing aspirin regimen.

Methodology (including brief description of study design, study population, visits and assessments): ET patients already prescribed aspirin 100 mg od by their Physician were enrolled in this trial comprising 2 sequential parts, and sample sizes were calculated as follows:

Part A was a parallel-arm, controlled, double-blind, randomized study comparing aspirin 100 mg once, twice, or three-times daily. Each arm was designed to enroll 100 patients. Placebo was used so that all patients took study medication 3 times daily. After 2 weeks, serum TXB_2 and urinary PGI_2 metabolite were measured to identify the best multiple-dosing regimen giving the highest TXB_2 inhibition with the lowest inhibitory effect on PGI_2.

Part B: 224 patients, largely the same ET patients participating to Part A, were randomized in an open-label fashion to aspirin 100 mg bid (identified as the best multiple regimen in Part A) vs. standard aspirin 100 mg od, for 20 months. Persistency of the superior biochemical efficacy of the experimental vs. standard dosing regimen was checked by repeated serum TXB_2 measurements across 10 study visits post randomization.

Number of subjects: Part A: patients enrolled 251; Part B patients enrolled 242.

Criteria for enrollment: Inclusion Criteria were all of the following: ET diagnosis according to WHO 2008 criteria; age between 18 and 75 years; ongoing aspirin 100 mg od since ≥1 month, according to the referring Hematologist; patient understanding and voluntarily signing the informed consent.

Exclusion Criterion was at least one of the following: platelet count >1,000,000/μL on 3 occasions over the 2 months before enrolment; creatinine level >1.5x upper limit of normal;liver disease defined as AST and/or ALT values >3x upper limit of normal; BMI >35 kg/m^2; history of major bleeding that in the referring Hematologist's judgement could expose the patient to increased risk of bleeding recurrence; active cancer or cancer in complete remission from less than one year, except for treated early-stage squamous or basal cell skin carcinomas; pregnancy or lactation; use of non-steroidal anti-inflammatory drugs (NSAIDs) >3 times/week; use of antiplatelet agents other than aspirin 100 mg od; use of oral anticoagulants including vitamin K antagonists, anti-Xa or -IIa agents; use of heparins or fondaparinux; chronic use of steroids (prednisone >5 mg/die or equivalent).

Inclusion and exclusion criteria were never modified during the entire study.

Experimental drug, dose, route of administration and batch numbers: Acetylsalycilic acid, CardioAspirin®, 100 mg twice- or three –times daily; 410,000 tablets, product name BAY e4465/19256 IIR, batch 13196002, EXP DATE 02/2021. Placebo, 4,800 tablets, product name: BAY e4465/19256 IIR, batch number 13196001, EXP DATE 08/2021

Oral route of administration for both placebo and acetylsalycilic acid.

Duration of treatment: part A: 14 days; part B: 20 months.

Reference therapy: acetylsalycilic acid, CardioAspirin®, 100 mg once-daily, per os

Criteria for evaluation of efficacy and safety: In both part A and B, serum TXB_2, a regulatory-validated biomarker of platelet COX-1 activity,(5) was measured at randomization and at each study visit as primary surrogate endpoints of efficacy.(5) Urinary PGIM excretion was measured in Part A as a primary surrogate endpoint of vascular safety.(6)

Statistical methodology. Part A: based on previous studies,(3, 7) we assumed that the mean ± standard deviation (SD) of serum TXB_2 in ET patients on aspirin 100 mg of 22±33 ng/ml. We tested two hypotheses: i) the 100 mg bid regimen as more effective than 100 mg od, yielding a ≥50% reduction in serum TXB_2, and ii) the 100 mg tid regimen as more effective than 100 mg bid, yielding a ≥50% reduction in serum TXB_2. Testing these hypotheses with an α-error of 0.05 and a β-error of 0.2 (80% power) required 70 patients per treatment arm. Anticipating a 30% dropout, we estimated 100 patients/arm to ensure adequate statistical power. For the co-primary endpoint of urinary PGIM, the study had 80% power to test the hypothesis that any experimental aspirin regimen would reduce urinary PGIM by >30% as compared with the od regimen. This PGIM threshold was selected based on the following considerations: urinary PGIM was minimally affected (20-40% variation) by aspirin 75-100 mg daily in healthy subjects; this threshold corresponded to the intra-subject coefficient of variation upon repeated measurements of PGIM excretion over time; traditional NSAIDs, including high-dose aspirin, reduced urinary PGIM excretion by 60-80%.(8) Part B: tested the long-term persistence of a superior biochemical efficacy of the optimized multiple-dosing treatment identified in part A according toboth the best TXB_2 associated with the lower degree of PGIM inhibition as compared to the standard once-daily regimen. One hundred and twelve patients per arm (standard of care vs. optimized dosing regimen) were calculated to be needed to assess with an alpha-error of 0.05and 80% power, a reduction ≥50% in serum TXB_2 with the optimized regimen 100 mg bid vs the

standard aspirin regimen (100 mg od), in at least 6 out of 10 determinations performed over the 20 months of part B study duration from Visit 2 to Visit 11 included.

Differences in mean serum TXB_2 values were evaluated by one-way analysis of variance, using Scheffe multiple-comparison test to allow comparisons of the 3 different treatments in Part A. Differences between qualitative and quantitative variables were tested with the chi-square and Wilcoxon signed-rank tests, respectively. A linear regression model was used to evaluate possible differences in serum TXB_2 response in effect of platelet count and cytoreductive therapy. The R statistical software version 3.6.1 was used for data analysis and plotting.(9) Differences between qualitative and quantitative variables were tested with the chi-square and Wilcoxon signed-rank tests, respectively. A linear regression model was used to evaluate possible differences in serum TXB_2 response in effect of platelet count and cytoreductive therapy. The R statistical software version 3.6.1 was used for data analysis and plotting.

Summary and conclusions. Results from part A of the ARES trial show that the currently recommended od low-dose aspirin regimen used for cardiovascular prophylaxis appears largely inadequate in reducing platelet activation in the majority of ET patients and it has a wide inter.individual variability. The antiplatelet response to low-dose aspirin was markedly improved by shortening the dosing interval to 12 hours (bid regimen), with no improvement by the tid regimen and with no effect on vascular PGIM. Moreover, Part B of the study showed that the bid regimen could steadily maintain an optimal platelet inhibition as compared to the standard od regimen in ET patients over 20 months of treatment. The bid experimental regimen was associated with a significant benefit in terms of subjective microvascular symptoms reported by the patients, without major issues in terms of safety, as indicated by the lack of significant differences of bleeding between the two randomized aspirin regimens. We observed a trend toward a lower incidence of major thrombosis in the bid arm.

Efficacy (including primary and secondary variables).

Part A: Evaluable patients assigned to the bid and tid regimens showed significantly reduced inter-individual variability and lower median values of serum TXB_2: 19.3[9.7-40], 4.0[2.1-6.7], and 2.5[1.4-5.65] ng/ml in the od (n=85), bid (n=79) and tid (n=79) arms, respectively. Urinary PGIM was comparable in the three arms. Urinary TXM was significantly reduced by 35% inboth experimental arms. Patients in the tid arm reported a higher abdominal discomfort score.

Part B: 242 patients were randomized to a bid vs. od low dose aspirin regimen for 20 months and underwent 11 visits. Serum TXB_2 was persistently and significantly higher in the od vs. bid arm: 24.2 [12.2-46.2] ng/ml versus 4.31 [2.53-6.61] ng/ml, respectively ($p<0.001$). Bleeding did not differ between the two randomized arms while there was a trend toward more major thromboses in the od vs. bid arms during study part B. Microvascular symptoms were significantly reduced by the bid regimen over the entire study period.

SAE and AE are reported in Appendix 4.

No deaths occurred over the study period

date of report: 29/Jul/2021

3 Ethics

3.1 Independent Ethics Committee (IEC)

The protocol, patient information sheet, diaries and consent form were approved by the Institutional Ethics Committee of the PI on 21/07/2016. Two substantial Amendments were made: #1 (13/07/2017) included one new trial site (Unit 13) and a prolongation of the study overall from 36 to 40 months to accommodate possible delays of the Centers in recruiting patients, importantly the time on treatment for the patients in part A and B was not changed and remained as in the protocol; #2 (2-7-2020) included the change of PIs in Units 8 and 9 as well as a minor modification of the time frame for 'breakfast' and 'after dinner' on the basis of part A experience to increase feasibility for the patients and ensure a 12 h interval intake for the bid regimen.

3.2 Ethical Conduct of the Study

ARES study was conducted in accordance with the Declaration of Helsinki. Specifically, the Investigators conducted the study in compliance with the 2004 revision of the 1964 declaration of Helsinki and in accordance with Good Clinical Practice requirements.

3.3 Subject / Patient Informed Consent

ET patients fulfilling all the eligibility criteria signed the written informed consent at Visit 1 during both part A and part B of the trial. After signing the informed consent, patients started the 'run in' phase in part A or started the randomized treatment in part B. No deviations from this procedure occurred in any ARES Center.

4 Investigational Plan

4.1 Introduction

ET is a MPN characterized by primary thrombocytosis and enhanced risk of arterial and venous thromboses.(1) Its prevalence is around 20 per 100,000 individuals, with estimates likely to increase due to the rise of occasional asymptomatic diagnoses. Up to 50% of ET patients experience a thrombotic event in their life, such as a myocardial infarction, ischemic stroke, transient ischemic attack or venous thromboembolism.(1) Annual thrombotic event rates range from 1.3 to 6.6% in patients on cytoreductive agents with or without co-administration of antiplatelet drugs.(1) Thrombosis is the main cause of mortality approximating 0.5%/year, which ranks higher than in the general population.(1) Several groups have reported evidence of platelet activation in ET.(1) In particular, we have previously described enhanced urinary excretion of TXA_2 metabolites in ET patients.(10) TXM excretion is a validated biomarker of in vivo platelet activation, that has been found consistently increased in different diseases characterized by enhanced cardiovascular risk or low antiplatelet drug response, and predictive of cardiovascular events in high risk patients.(11) Thus, data in ET suggest a pathogenetic link between persistent platelet activation and thrombotic complications, requiring anti-platelet therapy. On the basis of the thrombotic diathesis and persistent platelet activation, low-dose aspirin (75-100 mg once daily [od]) is currently recommended for both secondary and primary cardiovascular prevention in ET patients.(2) This recommendation is mainly based on retrospective, observational analyses and on extrapolation from an aspirin trial in a different MPN, i.e. polycythemia vera.(12)However, aside from cohort analyses, controlled trials formally assessing the efficacy and safety of low-dose aspirin in ET were never performed. Thus, the recommendation and off-label usage of the same aspirin dose range (75 to 100 mg) and a od dosing regimen for ET patients as for non-MPN patients, implies assuming similar antiplatelet PD, which we have recently reported to be altered in the majority of ET patients.(3)

The mechanism of action of low-dose aspirin in preventing atherothrombosis relies on the irreversible acetylation of platelet prostaglandin (PG)H-synthase 1, hampering the access of arachidonic acid to the catalytic site of cyclooxygenase (COX) activity and inhibiting the subsequent biosynthesis of TXA_2.(13) In spite of aspirin short half-life (20 min in the human circulation), blockade of platelet COX-1 activity lasts for the entire platelet life-span due to the limited platelet capacity for new protein synthesis, thus allowing once-daily dosing.(14) Moreover, aspirin acetylates a variable fraction of COX-isozymes in the bone marrow megakaryocytes and pro-platelets, as suggested by a 24-48 hour delay between aspirin withdrawal and reappearance of TXA_2 biosynthesis in peripheral platelets. Thus, under normal conditions of platelet formation, a 24-hour dosing interval of a short-lived drug is ensured by a favorable combination of the irreversible inhibition of a slowly renewable drug target (platelet COX-1) and an effect on progenitors, leading to a new platelet progeny with a largely non- functioning enzyme throughout the dosing interval.(15) Therefore, at steady state, low-dose aspirin, given once daily inhibits platelet TXA_2 biosynthesis by approximately 97 to 99% in healthy subjects, as assessed by a surrogate biomarker of efficacy, i.e. the measurement of serum TXB_2, endorsed by the European Medicines Agency.(5) Once-daily, low-dose aspirin reduces by approximately 25% the rate of major cardiovascular events, in high-risk, non-MPN patients.(16)

At variance with non-MPN subjects, od low-dose aspirin has been shown to be insufficient to fully inhibit platelet TXA_2 production in approximately 80% of ET patients.(3) While changes in

aspirin pharmacokinetics seem unlikely, faster renewal of the drug target in ET, due to enhanced platelet turnover is both biologically and pharmacologically plausible. Accelerated platelet turnover might generate unacetylated COX-1 and/or COX-2 during the 24-hour dosing interval, which would account for partial recovery of TX-dependent platelet function.(17) Inadequate suppression of platelet TXA_2 production during the 24-hour dosing interval can be largely (though not completely) overcome by a twice daily regimen of low-dose aspirin.(3) Thus, the abnormal megakaryopoiesis characterizing ET, might account for a shorter lasting antiplatelet effect of low-dose aspirin through faster renewal of platelet COX-1, which could be rescued by modulating the aspirin dosing interval rather than the dose.(3) However, multiple dosing of any drug can be associated with a lower compliance in the 'real world'. The COX-2 isozyme is expressed in vascular endothelial cells and accounts for PGI_2 biosynthesis under physiological shear conditions.(4) In humans, PGI_2 has vasodilator and platelet-inhibiting effects, counteracting pro-thrombotic signals, including platelet TXA_2.(4) Once-daily, plain or enteric- coated aspirin within the low-dose range seems to have limited inhibitory effects on in vivo PGI_2 biosynthesis, while it fully inhibits platelet TXA_2 generation in healthy subjects possibly because of differential rates of recovery of endothelial COX-2 vs. platelet COX-1 during the 24-hour dosing interval.(8) It is unknown whether shortening the aspirin dosing interval may affect endothelial PGI_2 production. The balance TXA_2-PGI_2 needed to be explored while testing different aspirin dosing regimens, within the low-dose range (<325 mg), as this balance may be relevant to the net anti-thrombotic effect of aspirin. Thus the unmet therapeutic needs in ET are: 1- lack of randomized clinical trials of antiplatelet prophylaxis; 2 - widely held assumption that astandard aspirin regimen is adequate for all ET patients, while in fact we and Others have shown that a standard 24-hour dosing regimen is inadequate to achieve persistent, high-grade inhibition of platelet TXA_2 production in the vast majority of ET patients; 3 - a consistent residual risk of major thrombosis in spite of aspirin treatment, as shown by several studies.

4.2 Study Objectives

This study had two primary objectives:

1- To investigate whether aspirin regimens based on twice or three times daily inhibits platelet-derived TXA_2, without significantly affecting in vivo PGI_2 biosynthesis, as compared to the standard, once daily 100 mg regimen. Serum TXB_2 was measured as a surrogate biomarker of aspirin efficacy, according to the EMA guideline.(5) A major urinary PGIM was used as a surrogate biomarker of vascular safety.(6) The comparison between aspirin 100 mg twice- or three-times daily vs. 100 mg od was designed to test a superiority hypothesis of serum TXB_2 levels associated with each experimental regimen vs. standard treatment. PGIM comparisons was designed as non-inferiority of any multiple daily dosing vs the standard od regimen.

2- To evaluate the long-term persistence of superior biochemical efficacy of an optimized, multiple daily dosing regimen of aspirin, as compared to the standard 100 mg od regimen. Biochemical efficacy was assessed by measurements of serum TXB_2 every 3 months over 20 months.

Objective 1 was addressed in part A of the study by a randomized, parallel-arm, double blind, placebo-controlled study of 2 weeks aimed at identifying the aspirin regimen to be further evaluated on a longer follow-up in the second part of the study.

Objective 2 was addressed in part B by a an open-label, randomized study comparing standard 100 mg od aspirin versus the best multiple daily dosing regimen identified in part A, with a follow-up of 20 months.

Secondary objectives were:

1. Safety. The safety of the multiple daily aspirin regimen was assessed in an exploratory fashion by recording major bleeding and clinically relevant non-major bleeding (CRNMB) (study protocol Appendix 1) as well as any upper gastrointestinal nonbleeding adverse events (NB-UGI AE) which was considered attributable to aspirin. Any thrombotic complication (major and minor) was also recorded. These objectives were explored in part B of the study, over 20-month treatment, with descriptive statistics.

2. Tolerability. The tolerability was assessed in an exploratory fashion by recording: a. the gastrointestinal symptoms (study protocol Appendix 4); b. the MPN symptom burden as scored by the MPN-SAF questionnaire modified to capture all microvascular symptoms and a pain numeric rating scale (NRS) for erythromelalgia. These questionnaires were administered to the patients both in part A and B of the trial.

3. Stability over time of in vivo platelet activation, as assessed by the urinary biomarker TXM, evaluated in both Part A and B of the study, and analyzed with descriptive statistics as exploratory, non-invasive substudy.

4. Stability over time of the pattern of plasma von Willebrand factor and analyzed with descriptive statistics in 8 clinical Units as exploratory substudy.

4.3 Study Design

Test drug: acetylsalycilic acid, CardioAspirin® 100 mg, enterico coated formulation, po

Part A was a parallel-arm, controlled, double-blind, randomized study comparing aspirin 100 mg once, twice, or three-times daily. Each arm was planned to enrol 100 ET patients, already on standard aspirin regimen (100 mg once-daily). Placebo was used so that all patients took study medication 3 times daily. After 2 weeks, serum TXB_2 and urinary PGI_2 metabolite were measured to identify the best multiple-dosing regimen giving the highest TXB_2 inhibition with the lowest inhibitory effect on PGI_2.

Part B: 224 ET patients were planned to be randomized with an open-label design to the best multiple regimen identified in part A vs. standard aspirin 100 mg od, for 20 months. Persistency of the superior biochemical efficacy of the experimental vs. standard dosing regimen was planned to be checked by repeated serum TXB_2 measurements over 10 study visits during part B.

The study included a Steering Committee that reviewed serum TXB_2 and PGIM measurements at the end of study part A and decided the best regimen to be randomized and compared in an open-label fashion to the standard regimen of aspirin during study part B. The study also included an independent Data Safety Monitoring Board committee created to further protect the safety of the patients by monitoring the progress and results. Data Safety Monitoring Board was blinded to study treatment in part A and B to monitor safety results, SAEs, and suspected/actual thrombotic

or bleeding events on a continual basis and could request an unplanned review of all safety data if a safety concern raised.

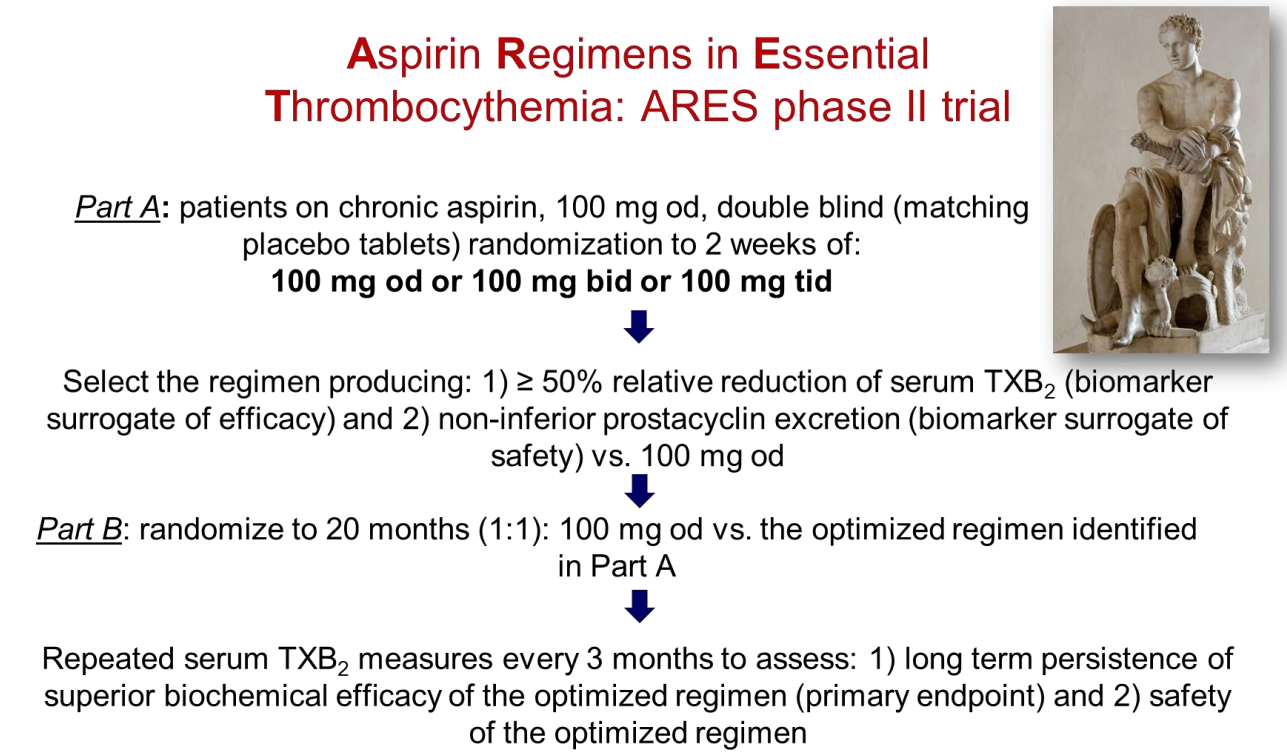
No interim analyses were planned.

Inclusion and exclusion criteria are detailed in section 4.4 of this FSR.

Prohibited concomitant medication at randomization and during the study were: NSAIDs >3 times/week; antiplatelet agents other than aspirin 100 mg od; oral anticoagulants includingvitamin K antagonists, anti-Xa or -IIa agents; heparins or fondaparinux; chronic use of steroids (prednisone >5 mg/die or equivalent).

Two substantial Amendments were approved: amendment #1 (13/07/2017) added a new trial site (Unit 13); amendment #2 (2/7/2020) included the change of the PIs in Units 8 and 9.

A diagram of the ARES trial is reported below:



4.4 Selection of Study Population

Inclusion Criteria were all of the following: age between 18 and 75 years; ET diagnosis according to WHO 2008 criteria; ongoing aspirin 100 mg daily since at least 1 month, according to the judgement of the referring Hematologist; understanding and voluntarily signing an informed consent.

Exclusion Criteria were at least one of the following: - platelet count >1,000,000/μL on three occasions over the 2 months before enrolment; - creatinine level >1.5x upper limit of normal; -

liver disease defined as AST and/or ALT values >3x upper limit of normal; - BMI >35 kg/m^2; - history of major bleeding that in the referring Hematologist's judgement may expose the patient to increased risk of bleeding recurrence; - active cancer or cancer in complete remission from less than one year, except for treated early-stage squamous or basal cell skin carcinomas; - pregnancy or lactation; - use of NSAIDs >3 times/week; - use of antiplatelet agents other than aspirin 100 mg od; - use of oral anticoagulants including vitamin K antagonists, anti-Xa or -IIa agents; - use of heparins or fondaparinux; - chronic use of steroids (prednisone >5 mg/die or equivalent).

Inclusion and exclusion criteria were never modified during the entire study.

Reasons for Withdrawal/Replacement of Study Subjects. Patients were free to withdraw from the study at any time. During part A, patients requiring to start cytoreduction or to modify cytoreductive dose had to exit the study according to the study protocol. This criterion did not apply to study part B.

Patients exited the study at any time (part A or B) in case of at least one of the following: major bleeding (defined in study protocol Appendix 1); NB-UGI AE judged as attributable to aspirin, i.e. documented symptomatic ulcer or perforation only if > grade 1; any thrombosis requiring treatment modification (i.e. starting a different ormultiple antithrombotic drugs; any other reason requiring permanent discontinuation of aspirin.

Patients with CRNMB events could either exit or remain in the study according to the judgement of the referring Hematologist: in the case of patients with CRNMB continued the study, a temporary discontinuation of aspirin could not exceed 10 days.

In case of temporary discontinuation of study drug (e.g. major trauma, major surgery), thepatient resumed randomized aspirin dosing as soon as possible according to the local practice, and had top have his/her next study visit between 4 and no later than 6 weeks after resuming treatment. Primary reason for discontinuation was recorded in the eCRF. Temporarydiscontinuation of aspirin was not allowed in study part A and applied only to study part B.

The Investigator could withdraw patients from the study for any of the following reasons: intercurrent major illness, pregnancy, disease progression to myelofibrosis or acute leukemia, or any other reason that in his/her judgment required study drug discontinuation in the interest of patient's safety. The primary reason for patient's withdrawal was recorded in the source documents.

Patients who withdrew between part A and B (between the end of part A and the beginning of part B) were replaced, whenever possible.

Withdrawals that occurred during part A or during part B (after the randomization visit) were not replaced.

4.5 Study Materials

Study drug: acetylsalycilic acid, CardioAspirin® 100 mg; matching placebo, p.o.

Formulation: enteric coated

Dose: Part A - 100 mg once, twice- or three-times daily for 14 days; Part B - 100 mg once- or twice-daily for 20 months

Placebo: placebo was used only in Part A to match for each patient the intake of 3 tablets/day, independently of the randomized treatment.

Route of administration: per os

Storage requirements: 15-25°C

No changes of drug dose, formulation or posology were made during the trial.

Laboratory disposable materials were supplied to each Center to be used for collecting, processing and storing biological samples (urine, plasma and serum) during both Part A and B.In particular the following material was supplied: standard urine beakers with screw cap (MV Medical, ref: 70.25.18ECSI-R); VACUETTE® serum 6 ml tubes, without anticoagulant (Z- serum Clot Activator; Greiner Bio-One; ref: 456089); Cryogenic vials 2 ml with screw cap (MV Medical, ref: BC607001); Safe-Lock microcentrifuge 2 ml tubes (Eppendorf , ref: 022363344); Centrifuge 15 ml tubes with screw cap (MV Medical, ref: 601052); Cryopen (Thermo Scientific, ref: 339993); Pasteur pipettes PE plastic (VWR International, ref: 612-3751); Cryoboxes and 9x9dividers (VWR International, ref: 479-1407).

4.6 Methods for Assigning Subjects to Treatment Groups

The specific methods used for assigning subjects to treatment groups should be described. Where randomisation codes have been used the following, information should be given: method for generation of randomisation codes; holders of the randomisation codes.

Part A. Patients were allocated to the 3 different aspirin regimens in a 1:1:1 ratio using a double blind procedure, and randomized to receive a drug package containing three bottles, as follows:

	Bottle 1	Bottle 2	Bottle 3
Treatment A	Aspirin 100 mg	Placebo	Placebo
Treatment B	Aspirin 100 mg	Placebo	Aspirin 100 mg
Treatment C	Aspirin 100 mg	Aspirin 100 mg	Aspirin 100 mg

Each drug package was coded by a unique, four digit UPIN number. The UPIN number with the corresponding treatment arm was kept at the Study Randomization and Drug Dispensation Team (Unit 4). Participating Centers received drug packages at the beginning of the study from Unit 4.

Patients were trained to take one pill from bottle 1 at breakfast (7-9 am), one from bottle 2 at lunch (1-2 pm) and one from bottle 3 after dinner (8-9 pm). Randomization was centrally stratified by Center and sex, using a REDCap software. After secure login, the Field Investigator enrolled into the database and randomized the patient. Based on the randomization table, REDCap returned the researcher the UPIN of the drug package for the enrolled patient.

Part B. After selection of the most appropriate dosing regimen by the Steering Committee upon revision of serum TXB_2 and PGIM measurements of part A, patients were randomized in an

open-label fashion to the standard vs. a bid dosing regimen of aspirin, stratified by Center and sex. After secure login, the Field Investigator enrolled the patient into the part B database, and randomized the patient based on a randomization table; the randomization code was maintained at the Study Randomization and Drug Dispensation Team, but not disclosed to the data analysis team until the end of data analysis.

A copy of the complete randomisation list with randomisation numbers, subjects identification and treatment assigned, is included in the Appendix 8 of this FRS.

Procedures for breaking the randomization code. The Investigator had to notify a request for code breaking in study part A, if needed, to alberto.tosetto@aulss8.veneto.it. No code breaking occurred during the part A of the study.

4.7 Dosage Regimen

Part A: Patients were trained to take one pill from bottle 1 at breakfast (7-9 am), one from bottle 2 at lunch (1-2 pm) and one from bottle 3 after dinner (8-9 pm). The dosage regimens are indicated in the Table below:

	Bottle 1	Bottle 2	Bottle 3
Treatment A	Aspirin 100 mg	Placebo	Placebo
Treatment B	Aspirin 100 mg	Placebo	Aspirin 100 mg
Treatment C	Aspirin 100 mg	Aspirin 100 mg	Aspirin 100 mg

Part B: patients were randomized in an open-label fashion to aspirin 100 mg once daily, at breakfast, or 100 mg twice daily, at breakfast and dinner.

4.8 Study Blinding

During Part A, patients, physicians and laboratory investigators were blinded as to randomized treatment. Statistical analysis and reporting was made only after complete data lock of the data entered by the centralized laboratory.

At variance with part A, part B was open-label. However, laboratory Investigators were not aware of the assigned randomized treatment.

Statistical analysis and reporting was made only after complete data lock of the data entered by the centralized laboratory.

4.9 Drug Accountability

Study medication accountability was performed by pill counting of the returned bottles at each visit.

4.10 Treatment Compliance

Part A: Compliance was assessed by pill counting in the study bottles returned by the patients at the end of the 14-day randomized treatment and by reviewing the patient's daily

diary, where he/she recorded daily timing of tablet intake, any drug other than their usual therapy and any symptom or comment that they deemed relevant.

Part B: Compliance was assessed by pill counting in the study bottles returned by the patients at each study visit (10 visits in total) over the 20-month randomized treatment and by reviewing the patient's daily diary, where he/she recorded daily timing of tablet intake, any drug other than their usual therapy and any symptom or comment that they deemed relevant.

4.11 Prior and Concomitant Medication

There were no restrictions for medications taken by the patient prior to entering ARES trial. During the trial the permitted concomitant medications were: all cytoreductive drugs (i.e. hydroxyurea, pipobroman, busulphan, interferon, anagrelide), proton pump inhibitors according to current regulatory indications approved in Italy. In case of occasional need of anti-inflammatory/antipyretic drugs, patients were allowed to take paracetamol (up to 2 gr daily) and avoid ibuprofen or naproxen, due to the known PD interaction with aspirin.(6) Prohibited concomitant medications were: NSAIDs >3 times/week; antiplatelet agents other than aspirin 100 mg od; oral anticoagulants including vitamin K antagonists, anti-Xa or -IIa agents; heparins or fondaparinux; chronic use of steroids (prednisone >5 mg/die or equivalent).

Occasional NSAID intake was specifically inquired at each study visit, both in part A and B, and recorded in a specific study form (Scheda Raccolta Campioni per Misurazione TXB_2 sierico - versione 2.0-31/01/2018). In addition, patients were asked to report in their daily diary any drug intake other than the usual prescribed therapy, including NSAIDs.

5 Study Assessments (including efficacy and safety variables)

Measurement of efficacy and safety variables. Efficacy variables: for serum TXB_2 measurements, peripheral venous blood was collected without anticoagulant, incubated within 5 minutes (18) for 1 hour at 37° C, centrifuged 10 minutes at 1,200g, and the supernatant serum was stored at -40°C until assayed,(19) and measured by a previously described, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-validated immunoassay.(18, 20, 21) The major urinary TXM, 11-dehydro-TXB_2, was measured in 1-ml urine samples by a GC/MS- validated immunoassay.(22, 23) Urinary prostanoid values were expressed as pg/mg of urinary creatinine, measured by a commercial kit (Creatinine Colorimetric Detection Kit; Enzo Life Sciences, Farmingdale, NY).

Safety variable: the major urinary PGIM, 2,3-dinor-6-keto-$PGF_{1\alpha}$ (24) was measured by LC-MS/MS method, as previously described.(25)

Personnel responsible for measurements: prof. Bianca Rocca and dr. Giovanna Petrucci at Unit 2 (serum TXB_2 and TXM), and dr. Viviana Cavalca at the external Laboratory of the Istituto Cardiologico Monzino in Milan (PGIM). The personnel responsible for measurements had a well-established track in the field.

Methods used to standardise or compare results between centres. Considering that the primary study endpoint of ARES was serum TXB_2, i.e. a surrogate biomarker of aspirin efficacy, a preliminary exercise of feasibility, reproducibility and validation of this biomarker across all participating Centers was performed. Three out of 11 Centers made pre-analytical errors, which were identified and corrected. The results of this preliminary exercise feasibility/reproducibility program confirmed the importance of controlling reproducibility of biomarkers in multi-center trials and the results have been published.

Briefly, the reference range of serum TXB_2 values was calculated as mean±1 standard deviation of 101 serum samples from healthy volunteers (43% females, median age 33 [30-49, interquartile range] years) from previous studies (15, 26, 27) and the database of the Institute of Pharmacology of the Catholic University in Rome, which were measured in the same centralised Lab, using the same described pre- and analytical procedures (3). We considered the inter-assay coefficient of variation, calculated as *standard deviation/mean*100* of the same sample measured in different assays. Thus given a mean serum TXB_2 value of 295±121 ng/ml, and 6% inter-assay variability, we considered as lower limit of the normal range a concentration of 184 ng/ml. Each Center collected samples from 5 healthy subjects. We considered the Centers as compliant with the procedure if they had at least 4 out of 5 samples measuring ≥184 ng/ml. Centers with ≥2 samples out of range were inquired about the procedure and asked to repeat blood sampling and the pre-analytical procedure a second time after correcting the possible errors. Fifty-five healthy volunteers (60% females, median age 34 [29-48] yrs) were recruited in the ARES Centers. The logged time interval between blood sampling and 37°C incubation was 1 [1-3.5] min (n=55) and the time between the end of incubation of the samples and serum freezingwas 31 [13-75] min (n=55) with no significant differences between Centers. The serum TXB_2

values of the first series of measurements in 3 out of 11 Centers had ≥2 values ≤184 ng/ml. These Centers were further inquired on their procedures and instrumentation to assess theconditions of 37°C incubation of the blood samples. One Center used a dry heating instrument (cell incubator) rather than a water bath, to incubate whole blood, one Center had a water bath not reaching the correct temperature in spite of the displayed value, one Center used to wrap up the tubes with rubber before placing them in the water bath. These conditions likely caused an actual incubation temperature of the samples <37°C or a delay in reaching the correct temperature in the sample. These Centers then modified their incubation conditions and repeated the procedure. As a control for method reproducibiliy, 3 Centers with appropriate serum TXB_2 values repeated the procedure as well. In the second series of measurements, all 6 Centers had values within the expected range.

Laboratory personnel who processed and measured the blood samples were blind as regarding to the assigned treatment in the entire ARES trial.

Methods and definition of adverse events and serious adverse events.

Adverse Event (AE) was defined as any untoward medical occurrence in a subject or clinical- trial subject administered a medicinal product and which does not necessarily had to have a causal relationship with this treatment. An AE could therefore be any unfavourable and unintended sign (e.g. an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Serious Adverse Event (SAE) was any adverse event/reaction which resulted in death, was life-threatening, required in-patient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, was a congenital anomaly/birth defector was considered an important medical event.

Death*:* in case death was the only information available at the time of the SAE notification, it had to be reported as such. Nevertheless, the cause of death had to be further investigated since death was considered as an outcome and not as an event. Deaths had to be always SAEs. For fatal cases, the Principal Investigator of the Center (and/or the designees) had to fill-in an "AE" eCRF form, an "SAE" form and immediately notify them to the relevant Ethics Committee.

Life-threatening event was an event/reaction which posed the subject at risk of death at the time of the event/reaction; it did not refer to an event/reaction that hypothetically might have caused death if more severe.

Hospitalization was ab events that requiring hospitalization for one of the reasons reported below and were not considered to be SAEs:

- hospitalization planned before entry to the clinical study which is part of the normal treatment or monitoring of the studied indication and not associated with any deterioration in condition;

- hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition;

- hospitalization for treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen.

Important Medical Event was any event that might not be immediately life threatening or result in death or hospitalization but that jeopardized the subject or required intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Reaction: was an adverse reaction, the nature or severity of which was not consistent with the applicable Product Information (e. g. Investigator's Brochure for an unauthorized investigational product or the Summary of Product Characteristics for an authorized product).

Criteria used for assessing AE and personnel responsible for these assessments. AE, SAE, adverse reactions and related degree of severity were defined in agreement with current rules (European Directive 2003/94/CE) (see previous paragraph). This study used a marketed aspirin formulation (Cardioaspirin®, Bayer S.p.A). Each severe AE or unexpected reaction had to be reported by the local Investigator to the Pharmacovigilance of the Clinical Trial Center at the Fondazione Policlinico Gemelli (Rome) and to the Coordinator's Ethics Committee. Patients hadto exit the study in case of pregnancy, any major bleeding, symptomatic ulcer endoscopically documented, ulcer perforation (>grade 1), thrombosis requiring treatment modification (i.e.starting one or more different antithrombotic drugs). The Investigator was responsible for the managing of the events meeting the definition and criteria of a AE or SAE, as provided in the protocol. All AEs occurring between between the date of informed consent signature and the date of study completion had to be recorded in the eCRF (and in the SAE form, if applicable). Each subject had to be monitored regularly by the Investigator and study personnel for AE occurring throughout the study.

5.1 Primary Variables

The primary measurements and end-points used to determine efficacy or safety should be listed together with the rationale for their selection.

Part A primary measurements: to investigate whether bid or tid aspirin regimens modified platelet-derived TXA_2, without significantly affecting *in vivo* PGI_2 biosynthesis, as compared to the standard, once daily 100 mg regimen, serum TXB_2 was measured as a surrogate biomarker of aspirin efficacy, according to the EMA guideline.(5) PGIM was measured as a surrogate biomarker of vascular safety.(8) The comparison between aspirin 100 mg twice- or three-times daily vs. 100 mg od was performed to test a superiority hypothesis in terms of serum TXB_2 levels associated with each new regimen vs. standard treatment. PGIM comparisons was based tothe non-inferiority of any multiple daily dosing vs standard od regimen.

Part B primary measurements: to evaluate the long-term persistence of a superior biochemical efficacy of an optimized, multiple daily dosing regimen of aspirin, as compared to the standard 100 mg od regimen, biochemical efficacy was assessed by repeated measurements of serum TXB_2, every 3 months over 20 months to check whether multiple (bid) daily dosing was superior to once-daily dosing throughout the dosing interval.

5.2 Secondary Variables

Secondary measurements and end-points of efficacy or safety.

Safety. The safety of the bid daily aspirin regimen during part B was assessed in an exploratory fashion by recording major bleeding and clinically relevant non-major bleeding (CRNMB) (defined in the Appendix 1 of study protocol version 3.0) and any upper gastrointestinal nonbleeding adverse events (NB-UGI AE) considered attributable to aspirin. Any thrombotic complication (major and minor) was also recorded. These objectives were explored in part B of the study, over 20-month treatment, with descriptive statistics.

Tolerability. The tolerability was assessed in an exploratory fashion by recording: 1) the gastrointestinal symptoms (study protocol Appendix 4); 2) the MPN symptom burden as scored by the MPN-SAF questionnaire modified to capture all microvascular symptoms and a pain numeric rating scale (NRS) for erythromelalgia.

Stability over time of *in vivo* platelet activation, as assessed by the urinary TXM, and its correlation with the extent of serum TXB_2 inhibition, was evaluated in Part A and B of the study, and analyzed with descriptive statistics.

Stability over time of the pattern of plasma von Willebrand factor levels was evaluated in a pre-defined sub-study during part B in 8 selected ARES Centers, and analyzed with descriptive statistics.

5.3 Measurements/Assessments

Details of measurements or assessments carried out during the study.

Routine hematochemical analyses and the mutational profile of the patients were performed in routine laboratories of each participating Institution. Clinical and laboratory characteristics of the patients were collected through Research Electronic Data Capture (REDCap).(28)

The thrombotic risk was assessed according to the International Prognostic Score of Thrombosis in Essential Thrombocythemia (IPSET-thrombosis) system, a validated prognostic score that includes age, previous thrombosis, cardiovascular risk factors, and the JAK2 V617F mutation.(29)

Compliance was assessed at each study visit by pill counting and reviewing the patient's daily diary, where patients recorded daily timing of tablet intake, any drug other than their usual therapy and any symptom or comment that they deemed relevant.

Gastrointestinal symptoms and MPN symptom burden, as scored by the MPN-SAF questionnaire modified to capture all microvascular symptoms and a pain numeric rating scale (NRS) for erythromelalgia were assessed at each study visit by ad-hoc questionnaires, as detailed in section 5.2.

For serum TXB_2 measurements, peripheral venous blood was collected without anticoagulant, incubated within 5 minutes(18) for 1 hour at 37° C, centrifuged 10 minutes at 1,200g, and the supernatant serum was stored at -40°C until assayed.(19) Serum TXB_2 was measured by a previously described, liquid chromatography-tandem mass spectrometry (LC-MS/MS)-validated immunoassay.(18, 20, 21)

The major urinary PGIM (24) was measured by LC-MS/MS method, as previously described.(25) The major urinary TXM was measured in 1-ml urine samples by a GC/MS- validated immunoassay.(22, 23) Urinary prostanoid values were expressed as pg/mg of urinary creatinine.

6.0 Data Quality Assurance

Quality control and quality assurance procedures used to assure the quality of the data. For SOPs and monitoring, a contract was made with the Unità Ematologia, Ospedale S. Bortolo, Vicenza (Dr. Alberto Tosetto, Dr. Laura Lissandrini, Dr. Andrea Timillero) which included data management, randomization, study monitoring and drug management.

Primary and secondary monitoring procedures. Source data verification was performed by the Unità Ematologia, Ospedale S. Bortolo, Vicenza (Dr. Alberto Tosetto, Dr. Laura Lissandrini, Dr. Andrea Timillero) regarding data management, randomization, study monitoring, drug management. The training of the investigators/co-workers/monitors was performed at two Investigators' Meetings on 28/11/2016 and 12/11/2018.

Inter-centre standardisation. Inter-center standardization was implemented with an ad-hoc feasibility and reproducibility study performed before starting patient's recruitment. This study has been published and results are reported in section 5.0 of this FSR.

Auditing at the Investigator's sites were not performed.

All ARES Investigators conducted the study in compliance with the 2004 revision of the 1964 declaration of Helsinki and in accordance with Good Clinical Practice requirements described in the current ICH guidelines. Prior to undergoing any study-specific procedure, all subjects had to consent in writing to participate. The process of obtaining the informed consent was in compliance with the Italian regulations. The ICF incorporated privacy working that complies with relevant data protection and privacy legislation in Italy.

7. Data Management Procedures

The computer hardware/software used were: REDCap version 10, running on a secure HTTPS server used to manage study data, analyses were performed on a Windows 64 bit based PC using R software (version 3.6 and above), and R studio (version 1.1 and above). Raw data and R scripts are stored in the Center for Open Science OSF repository (osf.io) and available upon request to dr. Alberto Tosetto.

8 Statistical Considerations

8.1 Planned Statistical Methods

Statistical hypothesis and sample size for Part A. Based on previous findings (3, 7) we assumed a mean±SD serum TXB_2 in ET patients on aspirin 100 mg od and 100 mg bid of 22±33 and 5.0±6.0 ng/ml respectively. We planned to test with α -error of 0.05 and a β -error of 0.2 (power 80%) the following hypothesis:

Hypothesis	Required sample size
100 mg bid superior to 100 mg od, with ≥50% reduction in serum TXB_2	70 patients /arm
100 mg tid superior to 100 mg bid, with ≥50% reduction in serum TXB_2	70 patients /arm

Anticipating a 30% dropout over the entire study duration (i.e. between part A and part B, and during part B), 100 patients were planned to be enrolled in each study arm to ensure adequate statistical power.

For urinary PGIM, we assumed a mean±SD PGIM value in ET patients on aspirin 100 mg od of 195±119 pg/mg creatinine.(30) Using the above sample size (n=70 patients per arm), the study had 80% power to test the hypothesis that any experimental treatment could reduce urinary PGIM by >30%. This threshold of PGIM inhibition vs. the standard 100 mg od dosing was considered reasonably safe based on the following considerations: PGIM is minimally affected by low-dose aspirin in healthy subjects,(8, 30) in ET subjects a 100 mg bid dose did not significantly modify PGIM as compared to 100 mg od,(30) this threshold corresponds to the intra-subject coefficient of variation of repeated measurements of PGIM excretion over time.

Statistical hypothesis and sample size for Part B. The same ET patients were planned to be randomized in part B of the study to test the long-term persistence of superior biochemical efficacy of the optimized vs standard dosing regimen. To check the hypothesis of a superiority ofthe best multiple-dosing treatment identified in part A according to both the best TXB_2 associated with the lower degree of PGIM inhibition as compared to the standard once-daily regimen, 112 patients per arm (standard of care vs. optimized dosing regimen) were calculated tobe needed to assess with an α-error of 0.05 and 80% power, a reduction ≥50% in serum TXB_2 with the optimized regimen (100 mg bid) vs the standard aspirin regimen (100 mg od), in at least 6 out of 10 determinations (60%) performed over the 20 months of part B duration from Visit 2to Visit 11 included.

Differences in mean serum TXB_2 values were evaluated by one-way analysis of variance, using Scheffe multiple-comparison test to allow comparisons of the 3 different treatments in Part A. Analysis of covariance using multiple regression with dummies for the different treatments were used if, at single univariate analysis, differences with $p<0.05$ in the distribution of gender, age, platelet count, JAK2 mutational status, spleen size, AST, ALT or creatinine, type of cytoreductive drug (if any) were present between the treatment subgroups. Analyses were carried out per treatment and per protocol. Data were analysed using the Stata and R Foundationsoftware. The Sweave software package was used for the final report. No planned interim or

exploratory analyses were carried out, other than the analysis of the primary endpoints data after study part A in order to identify the optimal experimental treatment that was then used in part B.

8.2 Determination of Sample Size

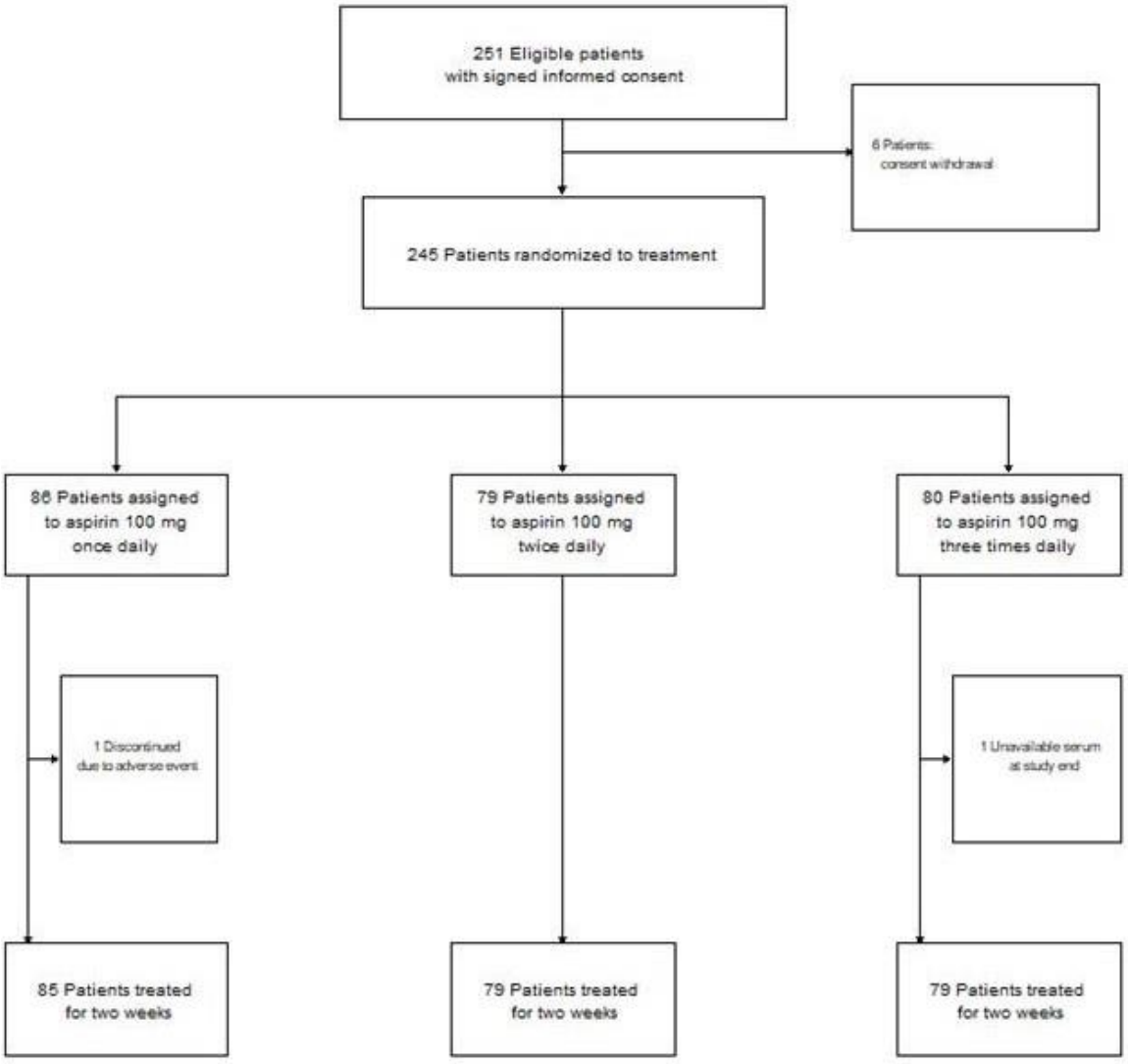
Sample size calculation is reported in the previous section 8.1.

9 Changes in the Conduct of the Study or Planned Analysis

All analyses were conducted as planned in the original protocol with no changes.

10 Results

I. Part A. The results of ARES part A have been published. The main findings are summarized as follows.
The **CONSORT diagram** of part A is reported below.



Two-hundred and 51 eligible, aspirin-treated, consenting ET patients were enrolled and started the

run-in phase. Six patients withdrew their consent during this phase for personal reasons, thus 245 patients underwent randomization at Visit 2. The demographic, clinical, and laboratory characteristics of these patients are detailed in **Table 1**. There were no statistically significant differences among the three treatment arms. One patient assigned to aspirin 100 mg od exited the study before Visit 3 for abdominal pain, and one patient had no serum sample available at Visit 3. Thus, 243 patients were evaluable at the end of the study and were included in the analyses. Compliance at Visit 3 is reported in **Table 2**: 218 out of 243 patients (90%) took all nine pills in the three days preceding visit 3 and were considered fully compliant. None of the patients reported NSAID intake in the three days preceding visit 3.

Co-primary endpoints: serum TXB_2 level at visit 2 averaged 19 [3.4-140.4] ng/ml (median and interquartile range; n=245) and was similar across the three treatment arms (**Table 3**). Serum TXB_2 at visit 2 displayed a substantial interindividual variability, spanning two to three orders of magnitude, with the vast majority of ET patients showing evidence of incomplete platelet COX-1 inactivation. After two weeks of randomized aspirin treatment, serum TXB_2 values of patients assigned to either the 100 mg bid or tid regimen were reduced by 80 to 90% versus their baseline values and were significantly lower than serum TXB_2 values of patients assigned to 100 mg od (**Table 3**). In the latter group, serum TXB_2 values showed remarkably similar inter-individual variability before and after 2-week treatment, indicating the stability of the poor aspirin responsiveness phenotype in ET. Patients assigned to the bid and tid regimens showed substantially and significantly reduced inter-individual variability in addition to lower median values of serum TXB_2 (**Table 3**). Data were also analyzed as the individual ratio of serum TXB_2 values at visit 3 vs. visit 2, considering that all patients at visit 2 were on aspirin 100 mg od. This analysis was performed to minimize the effect of variables such as the platelet count, turnover rate, and body weight known to influence aspirin responsiveness.(3, 31) In fact, we found that there was a slight but statistically significant effect of the platelet count on the response to bid and tid dosing (β coefficient: -0.02, for every $100x10^9$/L platelet increase, p=0.049). We found no effect of cytoreduction (β coefficient: -0.06, p=0.23). Patients randomized to the od regimen had a serum TXB_2 visit 3:visit 2 ratio averaging 1.03 (**Table 3**), indicating no appreciable short- term change in platelet COX-1 inhibition. The visit 3:visit 2 ratio of the bid and tid regimens averaged 0.14 and 0.13, respectively **(Table 3),** consistent with comparable, profound suppression of residual platelet TXA_2 production by both experimental aspirin regimens. The improved PD response was independent of previous thrombosis. Urinary PGIM excretion was similar across the treatment groups at Visit 2 (**Table 3**) and was not affected by either experimental regimen as compared to the respective baseline excretion rate, to any statistically significant extent (**Table 3**).

Secondary endpoints: baseline urinary TXM excretion averaged 428 [158.8-1063.7] pg/mg creatinine (n=245), without significant differences among the three treatment arms (**Table 3**). Urinary TXM at visit 2 displayed substantial interindividual variability, spanning one to two orders of magnitude, as would be expected from patients with variably and incompletely reducedTXA_2 biosynthesis.(20) After two weeks of randomized aspirin treatment, urinary TXM excretion rates of patients assigned to either the 100 mg bid or tid regimen were similarlyreduced by 30 to 40% versus their baseline values, with reduced interindividual variability, and were significantly lower than TXM excretion of patients assigned to 100 mg od (**Table 3**). In the od arm, TXM values were remarkably superimposable between visit 2 and 3, confirming the stability of the rate of platelet activation *in vivo*. Moreover, there was a positive, significant association between individual serum TXB_2 ratios at visit 3:visit 2 and the corresponding urinary TXM visit 3/visit 2 ratios (correlation coefficient, $r^2=0.12$ $p<0.0001$). Therefore, optimization of aspirin PD in ET patients effectively reduces *in vivo* platelet activation. Two-hundred and thirty- nine patients (98%) completed the SODA (32), MPN Symptom Assessment Form (MPN-SAF) (33), and PNRS questionnaire at Visits 2 and 3. Patients in the aspirin 100 mg tid arm showed a significantly higher score of GI disturbances as compared to the other arms, although none of thepatients had GI

adverse events requiring medical intervention. No major differences were observed in the microvascular disturbance scores, except for one query related to sleepingdifficulties that were apparently reduced in the bid arm. There were no major bleeding nor adverse cardiovascular events during the 2-week randomized treatment, as well as during the following 2 weeks of observation after Visit 3.

Table 1. Characteristics of 245 randomized ET patients overall and according to the assigned treatment.

	All N=245	100 mg od N=86	100 mg bid N=79	100 mg tid N=80
Sex:				
Male, n (%)	112 (45.7)	40 (46.5)	36 (45.6)	36 (45.0)
Female, n (%)	133 (54.3)	46 (53.5)	43 (54.4)	44 (55.0)
Age at diagnosis (years)	53.0 [42.0-63.0]	52.0 [41.2-62.8]	59.0 [43.5-65.5]	48.5 [39.8-58.0]
Age at enrollment (years)	60.0 [51.0-67.0]	59.0 [50.2-66.0]	62.0 [53.0-69.0]	58.0 [49.8-66.0]
BMI (kg/m^2)	24.9 [22.7-27.3]	24.9 [22.7-26.9]	24.5 [22.5-26.0]	25.2 [23.0-28.7]
Leukocytes ($x10^9/L$)	7.00 [5.6-8.5]	7.26 [5.6-8.3]	6.90 [5.4-8.8]	7.08 [5.8-8.4]
Platelet count ($x10^9/L$)	521 [422-641]	512 [418-629]	521 [404-622]	532 [424,660]
Hematocrit (%)	41.7 [39.1-44.3]	41.4 [38.3-44.4]	42.2 [39.5-44.3]	41.4 [39.6-43.8]
JAK2 genotype:				
Wild type, n (%)	99 (40.4)	38 (44.2)	31 (39.2)	30 (37.5)
Mutated, n (%)	145 (59.2)	48 (55.8)	48 (60.8)	49 (61.3)
Not available, n (%)	1 (0.4)	0 (0.0)	0 (0.0)	1 (1.3)
CALR mutation:				
Type 1, n (%)	19 (7.8)	7 (8.1)	6 (7.7)	6 (7.5)
Type 2, n (%)	16 (6.5)	5 (5.8)	6 (7.7)	5 (6.3)
Other, n (%)	95 (38.9)	31 (36.0)	29 (37.2)	35 (43.8)
Not available, n (%)	115 (46.7)	43 (50.0)	38 (47.4)	34 (42.5)
Microvascular symptoms, n (%)	25 (10.2)	10 (11.6)	9 (11.4)	6 (7.5)
Previous thrombosis:				
MPN-related, n (%)*	10 (4.1)	3 (3.5)	2 (2.5)	5 (6.2)
Any thrombosis, n (%)	28 (11.4)	10 (11.6)	8 (10.1)	10 (12.5)
Cytoreductive therapy:				
No, n (%)	98 (40.0)	41 (47.7)	28 (35.4)	29 (36.2)
Yes, n (%)	147 (60.0)	45 (52.3)	51 (64.6)	51 (63.7)
TXB_2 before randomization ng/ml	19 [9.3-43.2]	17.1 [8.3-32.8]	20.0 [11.6-56.4]	23.5 [9.8-47.8]

Quantitative values are reported as medians and [interquartile range], unless indicated. Abbreviations: BMI: body mass index; TX: thromboxane. *defined as any major thrombosis within 2 years before diagnosis and at any time afterwards.

There were no significant differences between the randomized groups, according to Kruskal-Wallis test or chi squared for continuous or discrete variables, respectively.

Table 2. Compliance with aspirin treatment according to pill counting in 243 evaluable ET patients.

	Fully compliant N=218	**Partially compliant N=21**	**Non compliant N=4**	**P global**
Definition	All 9 pills in the three days before V3	6-8 pills in the three days before V3	No pill in the three days before V3	
Sex:				0.23
Male, n (%)	104 (47.7)	6 (28.6)	2 (50.0)	
Female, n (%)	114 (52.3)	15 (71.4)	2 (50.0)	
Median age at enrollment (years)	60 [51.3-67.0]	54 [45.0-66.0]	54 [43.3-64.0]	0.21
Median TXB_2 at Visit 2 (ng/ml)	18.6 [8.9-42.9]	22.8 [13.6-37.0]	46.5 [24.0-107.8]	0.40
Treatment				0.60
100 od, n (%)	73 (33.5)	10 (47.6)	2 (50.0)	
100 bid, n (%)	71 (32.6)	7 (33.3)	1 (25.0)	
100 tid, n (%)	74 (33.9)	4 (19.0)	1 (25.0)	

Quantitative values are reported as medians and [interquartile range], unless otherwise indicated. Abbreviations: TX: thromboxane; P value according to the Kruskal-Wallis test or chi squared for continuous or discrete variables, respectively.

Table 3. Median values of serum TXB_2, urinary PGIM and urinary TXM before (Visit 2) and after (Visit 3) the randomized aspirin regimen in 243 evaluable ET patients.

	100 mg od N=85	100 mg bid N=79	100 mg tid N=79	P global	P bid vs.tid
$sTXB_2$ at V2 (ng/ml)	17.0 [8.2-33.0]	20.0 [11.6-56.4]	23.3 [9.6-46.4]	0.098	0.41
$sTXB_2$ at V3 (ng/ml)	19.3 [9.7-40.0]	4.0 [2.1-6.7]	2.5 [1.4-5.7]	<0.001	0.04
$sTXB_2$ V3/V2 ratio	1.0 [0.77-1.5]	0.1 [0.08-0.3]	0.1 [0.08-0.2]	<0.001	0.24
PGIM at V2 (pg/mg creatinine)	84 [50-123]	76 [47-132]	83 [53-123]	0.96	0.74
PGIM at V3 (pg/mg creatinine)	89 [54-127]	87 [46-121]	80.0 [47-131]	0.70	0.90
PGIM V3/V2 ratio	1.1 [0.7-1.5]	0.9 [0.7-1.4]	0.9 [0.6-1.6]	0.48	0.88
TXM at V2 (pg/mg creatinine)	485 [336-693]	641 [437-864]	515 [379-738]	0.02	0.09
TXM at V3 (pg/mg creatinine)	457 [313-674]	367 [237-541]	344 [229-487]	0.001	0.37
TXM V3/V2 ratio	0.9 [0.7-1.3]	0.7 [0.5-0.8]	0.7 [0.5-0.8]	<0.001	0.71

Data are medians and [interquartile range]. Abbreviations: TX: thromboxane; PGIM: urinary prostacyclin metabolite; TXM: urinary thromboxane metabolite; V: visit. P values refer to Spearman test (P global) and to Wilcoxon test for the bid vs tid comparison.

II. Part B. Two-hundred and 42 patients were enrolled and 2.670 biological samples werecollected across 10 Centers. 242 patients were randomized to aspirin 100 mg once vs. twice daily, the regimen that had been selected on the basis of Part A. During part A, 185 blood samples with serum TXB_2 below the lower threshold (0.50 ng/ml) were excluded from the analysis likely due to pre-analytic handling errors.

The **CONSORT diagram of part B** is depicted below

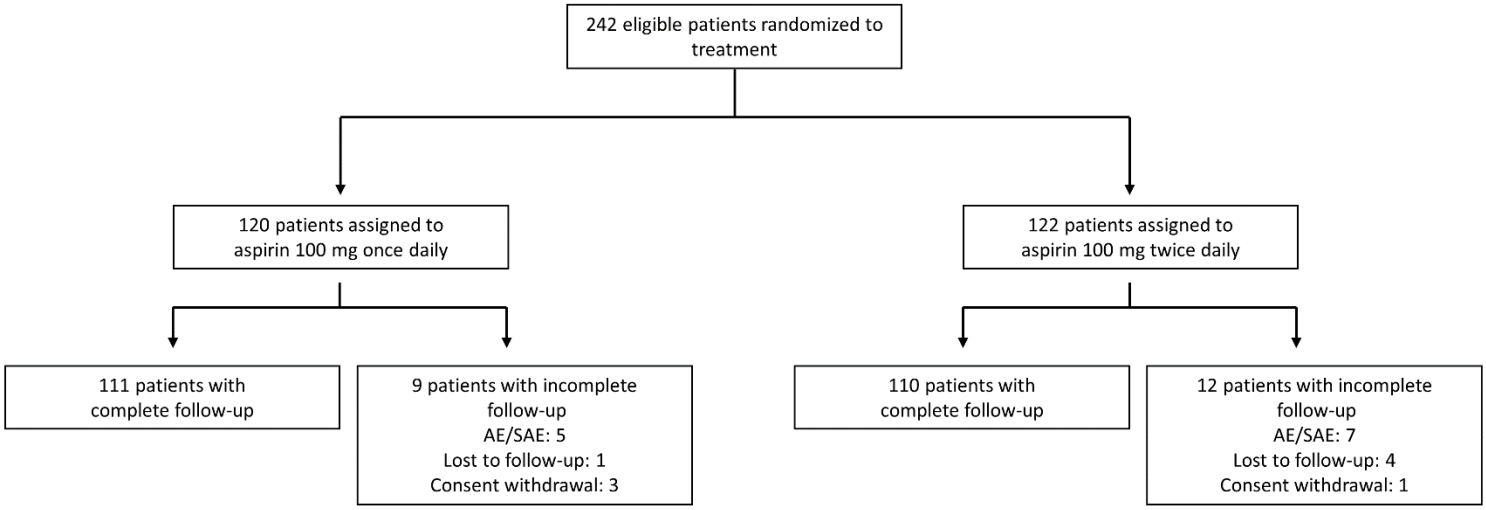


Table 4. Main clinical and haematological characteristics of patients in Part B

	[ALL] *N=242*	100 od *N=120*	100 bid *N=122*	p.overall
Sex:				0.992
F n (%)	130 (53.7%)	65 (54.2%)	65 (53.3%)	
M n (%)	112 (46.3%)	55 (45.8%)	57 (46.7%)	
Age (yrs)	59.2 (11.4)	58.8 (11.5)	59.6 (11.3)	0.590
AGE AT DIAGNOSIS	52.1 (12.8)	52.0 (12.5)	52.1 (13.2)	0.957
BMI	26.9 (21.9)	25.7 (4.03)	28.0 (30.5)	0.406
HCT (%)	42.1 (5.06)	41.9 (5.67)	42.3 (4.38)	0.558
WBC (10^9/L)	7.16 (2.14)	7.29 (2.06)	7.03 (2.22)	0.352
PLTS (10^9/L)	568 (184)	574 (193)	563 (176)	0.628
JAK-PCR:				0.210
WT	93 (38.4%)	41 (34.2%)	52 (42.6%)	
Mutated	148 (61.2%)	78 (65.0%)	70 (57.4%)	
Not done	1 (0.41%)	1 (0.83%)	0 (0.00%)	
CALR:				0.035

	[ALL]	100 od	100 bid	p.overall
	N=242	*N=120*	*N=122*	
Type1	20 (8.33%)	14 (11.8%)	6 (4.96%)	
Type2	15 (6.25%)	3 (2.52%)	12 (9.92%)	
Other	90 (37.5%)	45 (37.8%)	45 (37.2%)	
Not done	115 (47.9%)	57 (47.9%)	58 (47.9%)	
MPL:				0.817
WT	109 (45.4%)	51 (43.2%)	58 (47.5%)	
Mutated	4 (1.67%)	2 (1.69%)	2 (1.64%)	
Not done	127 (52.9%)	65 (55.1%)	62 (50.8%)	
cytoreduction:				0.529
No n (%)	93 (38.4%)	49 (40.8%)	44 (36.1%)	
Yes n (%)	149 (61.6%)	71 (59.2%)	78 (63.9%)	
Hydroxyurea:				0.899
No n (%)	119 (49.2%)	60 (50.0%)	59 (48.4%)	
Yes n (%)	123 (50.8%)	60 (50.0%)	63 (51.6%)	
anagrelide:				0.845
No n (%)	222 (91.7%)	111 (92.5%)	111 (91.0%)	
Yes n (%)	20 (8.26%)	9 (7.50%)	11 (9.02%)	

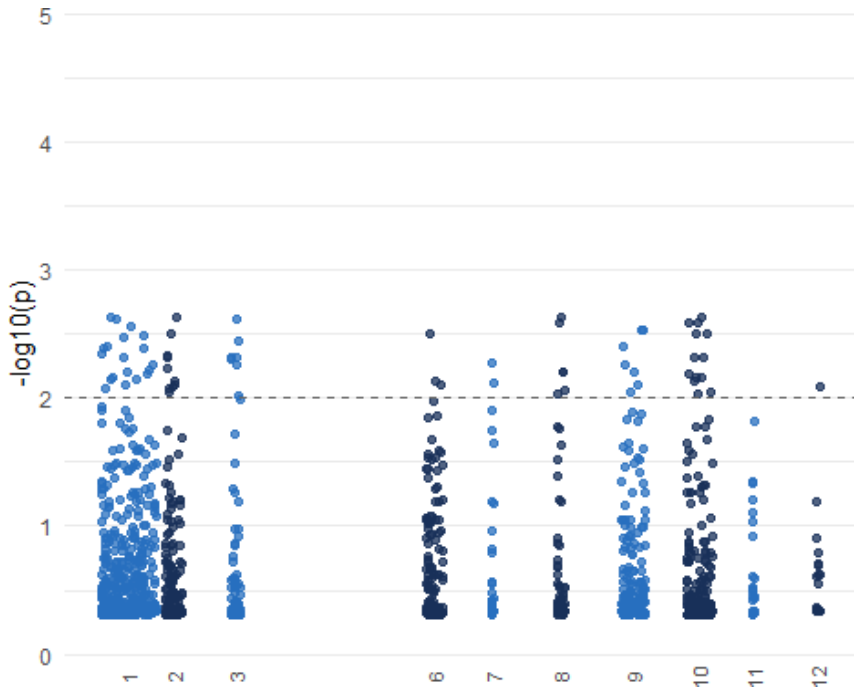
The main results of Part B are reported in **Table 5** and in two Manhattan plots, including the median number of visits per patient, mean and median value of serum TXB_2 during the randomized interval (visit 2 to 11), CV indicates the ration SD/mean intra-patient, the "outlier" indicates % of visits whose values were >3 folds the median value of serum TXB_2. The AUC was calculated across the entire length of time in study.

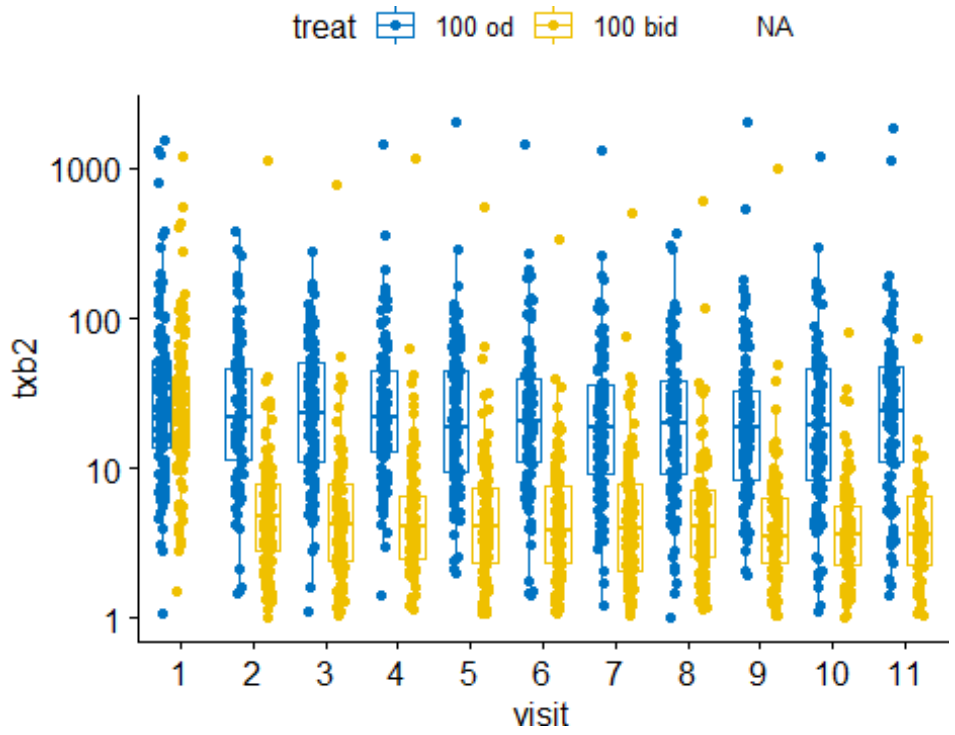
Compliance was assessed as number of tablet intake in the 3 days preceding the study visit/expected number of tables, similarly to part A.

Table 5. Main results of study part B

	[ALL] *N=242*	100 od *N=120*	100 bid *N=122*	p.overall
n.visits	11.0 [11.0;11.0]	11.0 [11.0;11.0]	11.0 [11.0;11.0]	0.663
Serum TXB_2 mean	9.75 [4.19;25.9]	24.2 [12.2;46.2]	4.31 [2.53;6.61]	<0.001
Serum TXB_2 median	7.94 [3.67;22.0]	19.2 [10.2;37.7]	3.86 [2.31;6.03]	<0.001
CV	0.60 [0.42;0.80]	0.60 [0.37;0.80]	0.59 [0.44;0.77]	0.644
Outliers, %	10.0 [0.00;20.0]	10.0 [0.00;20.0]	10.0 [0.00;20.0]	0.392
Serum TXB_2 <10 ng/ml, %	70.0 [10.0;100]	10.6 [0.00;50.0]	100 [80.0;100]	<0.001
AUC mean (days*ng/mL)	484 [223;1259]	1224 [645;2282]	232 [125;399]	<0.001
Compliance	100 [95.0;100]	100 [100;100]	98.3 [90.0;100]	<0.001

The Manhattan plot below reports serum TXB_2 values according to the recruiting Center. No significant differences were observed.





The Manhattan plot depicted above represents the individual serum TXB_2 values in each study visit and randomized arm. At visit 1 (pre-randomization) all patients were on standard aspirin 100 mg od.

Bleeding and thrombosis complications according to the randomized treatment are reported in the **Table 6 of section 10.3,** without statistically-significant differences among the two arms.

The results of the Brief Fatigue Inventory and MPN-SAF according to the randomized arm are reported in the **Table 7** below, showing a significantly lower score (and therefore a better outcome) for the subjectively reported fatigue, and hands burn in the bid vs. od arm

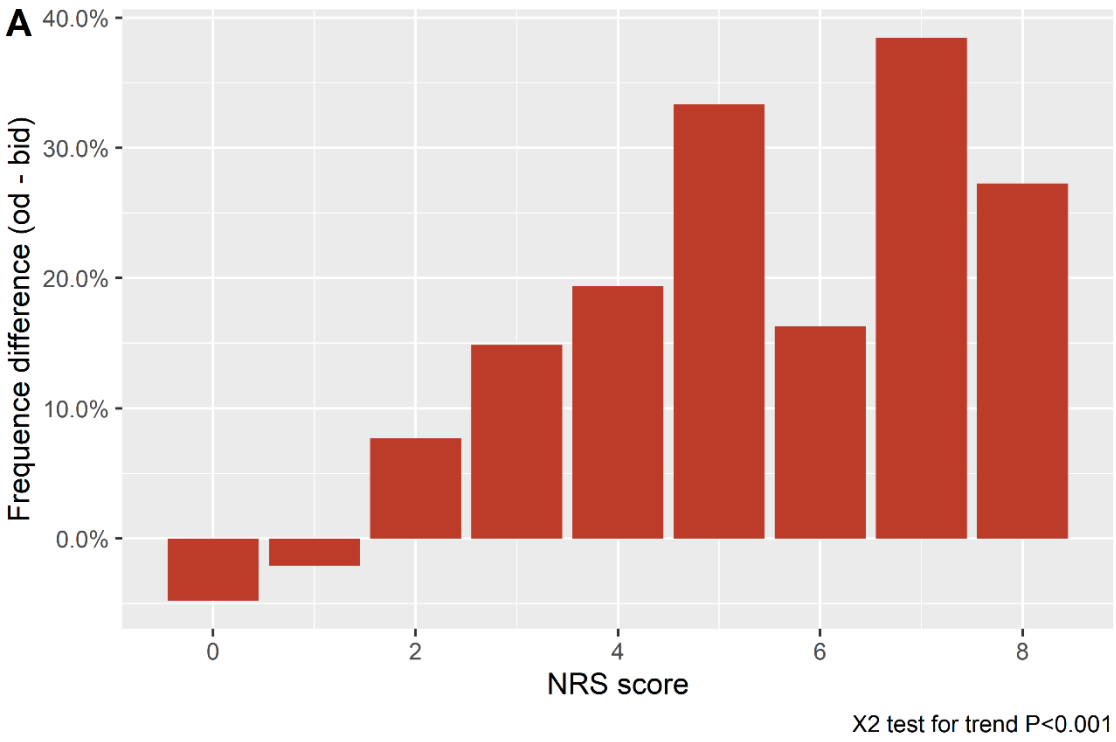
Table 7. Brief Fatigue Inventory and MPN-SAF scores in the overall and randomized populations

	[ALL]	**100 od**	**100 bid**	**p.overall**
	N=240*	***N=119***	***N=121***	
fatigue	*1.76 (1.92)*	*2.03 (2.10)*	*1.50 (1.69)*	*0.033*
repletion	1.19 (1.83)	1.24 (1.78)	1.14 (1.88)	0.676
abd_pain	0.65 (1.19)	0.66 (1.12)	0.64 (1.26)	0.907
abd_disc	1.01 (1.58)	0.99 (1.57)	1.03 (1.60)	0.839
inactive	1.15 (1.85)	1.35 (2.09)	0.95 (1.55)	0.092
headache	1.22 (1.87)	1.28 (1.79)	1.16 (1.96)	0.619
concentr	1.53 (2.12)	1.74 (2.24)	1.33 (1.98)	0.143
dizziness	1.15 (1.86)	1.32 (2.10)	0.99 (1.58)	0.163
tingle	1.55 (1.99)	1.63 (2.02)	1.47 (1.97)	0.527

	[ALL]	100 od	100 bid	p.overall
	N=240*	***N=119***	***N=121***	
insomnia	1.64 (2.16)	1.75 (2.16)	1.53 (2.17)	0.425
sadness	*1.37 (1.92)*	*1.64 (2.05)*	*1.11 (1.76)*	*0.031*
sexual	1.57 (2.57)	1.81 (2.68)	1.34 (2.45)	0.159
cough	0.55 (1.20)	0.53 (1.16)	0.57 (1.24)	0.793
night.swe	1.29 (1.93)	1.34 (2.02)	1.24 (1.84)	0.687
itching	1.15 (1.97)	1.17 (1.85)	1.12 (2.08)	0.837
bone.pain	1.04 (1.99)	1.14 (2.01)	0.95 (1.97)	0.464
fever	0.05 (0.36)	0.09 (0.50)	0.01 (0.10)	0.092
weight.los	0.17 (0.78)	0.21 (0.75)	0.12 (0.81)	0.394
qol	2.16 (2.19)	2.13 (2.21)	2.19 (2.17)	0.833
sight.dist	1.19 (1.91)	1.34 (1.96)	1.05 (1.86)	0.253
hear.dist	1.23 (2.03)	1.30 (2.01)	1.17 (2.05)	0.613
hands.burn	*0.88 (1.62)*	*1.10 (1.78)*	*0.66 (1.43)*	*0.036*
feet.burn	0.35 (1.17)	0.46 (1.42)	0.23 (0.86)	0.129

* Two out of 242 patients had no available questionnaires and then were lost at follow-up

The microvascular symptoms were significantly ameliorated by the bid vs. od treatment, as shown by the lower grades reported by the patients in scoring their hand and foot pain, reported in the Figure below which represents the difference in the frequence of each point of the score (from 0 to 10) between the ob and bid arms. The microvascular symptom questionnaires were available in 240 out of 242 patients.



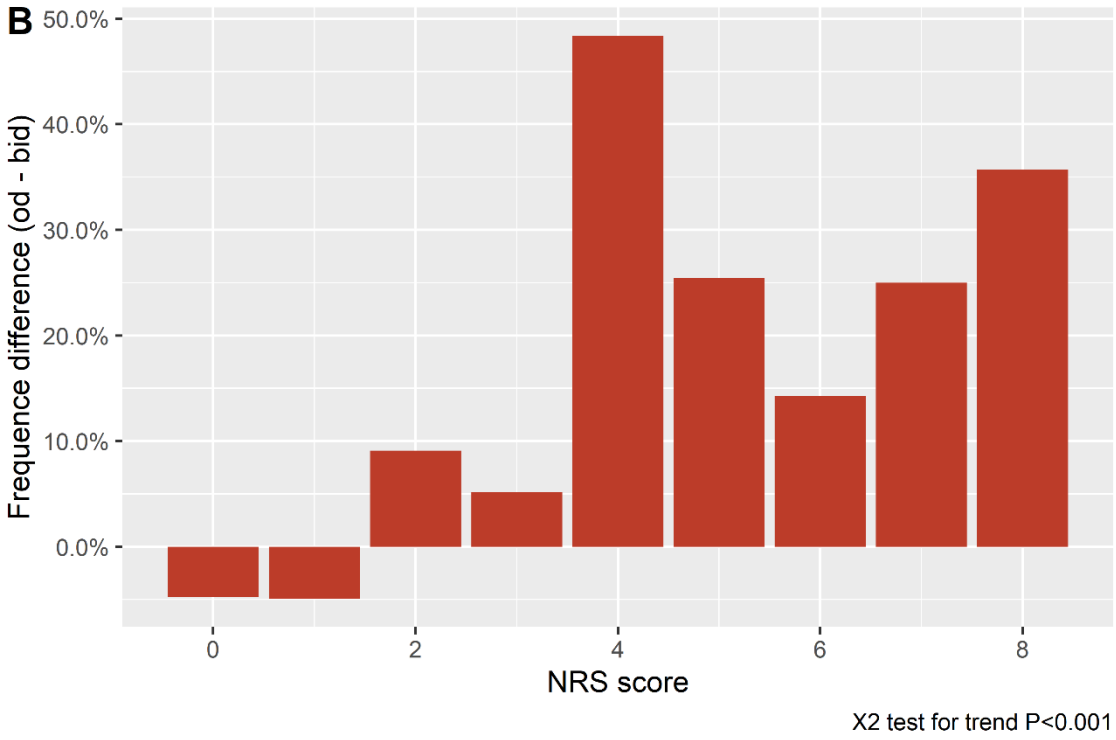


Table 8. SODA questions according to the randomized arm.

SODA Question 1

Score class	100 od	100 bid
[0,10]	1,093	1,047
(10,20]	54	47
(20,30]	35	38
(30,40]	14	26
(40,50]	6	12
(50,60]	3	10
(60,70]	0	7
(70,80]	3	6
(80,90]	1	2
(90,100]	3	4

Pearson chi2 = 19.37 Pr = 0.022

SODA Question 2

score	100 od	100 bid
0	612	586
1	277	312
2	179	139
3	95	89
4	49	47
5	25	29
6	18	24
7	3	14
8	6	5
9	7	13

Pearson chi2 = 18.00 Pr = 0.035

With both SODA questionnaires, we observed a small, but statistically significant, increase of the frequency of higher scores (associated with more severe GI symptoms) in the bid treatment arm.
SODA questionnaires were available in 240 out of 242 patients

10.1 Study Subjects/Patients

a) Disposition of Subjects

Part A: 251 eligible, aspirin-treated, consenting ET patients were enrolled and started the run-in phase in 10 Centers, one Center did not recruit any patient (Bergamo). Six patie4ts voluntary withdrew their consent during this phase for personal reasons, thus 245 patients underwent randomization at Visit 2. See CONSORT diagram in section 10.0.
Thirty two patients withdrew between part A and B for disease progression, cancer, withdrawal of informed consent or were lost at f.u.
Part B. Two-hundred and 42 patients were enrolled across 10 Centers. One Center did not recruit patients also in part B (Bergamo). Out of 242 patients, 4 voluntary withdrew the informed consent; 12 patients withdrew duringpart B for SAE/AE, and 5 were lost at f.u. The analysis was performed as ITT.
The CONSORT diagram of part B is reported in section 10.0.
Basic demographics of the patients recruited in part A and B of the study are reported in **Tables 1** and **4** of **Section 10**, respectively.

Aside from voluntary withdrawal of the informed consent, the reasons for patient exiting the study are summarised in Appendix 4 to this FSR, which includes also a detailed listing, subdivided by treatment group.

Blinding was never broken during part A, while part B had an open label design. Primary response variables were assessed prior to withdrawal whenever possible.

b) Protocol Deviations

We did not record any protocol deviation during both part A and B (no subjects who entered without fulfilling the inclusion/exclusion criteria; no subjects developed withdrawal criteriaduring, the study; no subjects received incorrect study treatment or doses; no subjects received prohibited concomitant medication). We had 34 missing visit because of COVID pandemics

10.2 Efficacy Evaluation

a) Data Sets Analysed

Part A: 251 eligible, aspirin-treated (the vast majority for primary prevention), consenting ET patients were enrolled, 6 patients voluntary withdrew their consent during before randomization for personal reasons, thus 245 patients were randomized and included in the ITT analysis.
Part B: 242 patients were enrolled and evaluated on an ITT basis, 2,670 biological samples were collected across 10 Centers. Out of 242 patients, 185 blood samples with serum $TXB_2 < 0.50$ ng/ml were excluded from the analysis likely due to pre-analytic handling errors. 18 patients dropped out during part B for SAE, diseaseprogression, lost at f.u. or voluntary withdrew of the informed consent, as indicated in **Appendix4.**

b) Demographic and Other Baseline Characteristics

Basic demographics and characteristics of the patients enrolled in part A and B of the study are reported in **Tables 1** and **4** of section 10.0, respectively, overall and according to the assigned randomized treatment.

c) Treatment Compliance

Compliance was assessed in the entire trial by pill counting of the study drug returned at each visit. It was expressed as tablets taken/expected. Compliance is reported in **Tables 2** and **5** of section 10.0, for part A and B, respectively.

d) Efficacy Results

The results of all analyses related to the efficacy variables, both primary and secondary of part A are presented by treatment group in **Table 3** of section 10.0, including the size of each groups. The results of all analyses related to the efficacy variable of part B are presented by treatment group on the **Table 5** of section 10.0, including the size of each groups.

e) Statistical Issues

Differences between qualitative and quantitative variables were tested with the chi-square and Wilcoxon signed-rank tests, respectively. A linear regression model was used to evaluate possible differences in serum TXB_2 response in effect of platelet count and cytoreductive therapy. The R statistical software version 3.6.1 was used for data analysis and plotting.(9)

Moreover, since the number of dropouts has been limited in each part of the study (see CONSORT diagrams in section 10.0), their impact on the ITT analysis of the trial was negligible.

ARES has used a block randomization. The p values were tested only for the primary hypotheses. Regarding covariates, serum TXB_2 (the primary endpoint) was adjusted for platelet count only in a pre-defined analysis.
procedure for dealing with missing data: multiple imputation (MAR) modelling is used for TXB_2 data in case of multivariate analysis
The primary endpoint data have been always analyzed overall and stratified by Center, without any significant difference observed both for part A and B
Adjustment made for inter-centre variability: this analysis is ongoing for part B and was made for part A
subset analyses: exploratory subset analysis performed by gender, age, platelet count, presence of JAK2 mutation, and cytoreduction based on potential clinical relevance and biologic plausibility

f) Drug Dose, Drug Concentration and Relationship to Response

The different dose regimes of aspirin used in the study part A and B and the corresponding values of serum TXB_2 at each dose regimen are indicated in **Tables 3 and 5** of section 10.0, respectively. Drug concentration was not planned to be measured according to the protocol.

g) Drug-drug and drug-disease interactions

We did not foresee nor identify any apparent relationship between aspirin response and concomitant therapy or past concomitant diseases in terms of DDI. The previously-known DDI between aspirin and NSAIDs (6) was prevented during the trial by specific exclusion criteria, patient's information and direct questions to the patient at each study visit.

h) Efficacy Conclusions (conclusions relating to both the primary and secondary variables should be summarised with reference to any relevant statistical information).

Based on results from part A of the ARES trial, the currently recommended od low-dose aspirin regimen used for cardiovascular prophylaxis in ET appears largely inadequate to fully inhibit the COX-1-derived TXA_2 from platelets in the vast majority of ET patients and it is characterized by a wide inter-individual variability. The inhibition of platelet-COX-1 by low-dose aspirin was significantly improved by shortening the dosing interval to 12 hours, with no improvement by further reducing the dosing interval to three-times daily. The bid regimen did not have any significant effect on vascular PGIM. Moreover, the wide inter-individual variability in the platelet inhibition observed in patients on the standard regimen (spanning across 3 Logs), was largely restrained by the multiple-dosing regimens. Since no major differences in terms of further platelet-derived TXA_2 between the bid and tid regimen, and considering also that the tid regimen was associated to a significant increase in subjective GI disturbances base on the SODA questionnaires, the dose which was selected to be randomized in the part B of the trial was the bid regimen.

ARES part B showed that the bid regimen could steadily maintain an optimal platelet inhibition as compared to the standard od regimen in ET patients over 20 months of treatment. The

experimental regimen was associated with a significant and consistent benefit in terms of subjective microvascular symptoms reported by the patients across the 20-month treatment. No major issues were observed in terms of safety, as indicated by the lack of significant differences of bleeding between the two randomized aspirin regimens. We observed a trend toward a lower incidence of major thrombosis (MI, TIA, coronary revascularization) in the bid arm.

10.3 Safety Evaluation

All subjects entered into the study who received at least one dose of study medication were included in the safety analysis

a) Extent of Exposure (The extent of exposure to the study drugs should be described according, to the number of subjects exposed, duration of exposure and dose of the study drugs)

Part A: for 14 days, 79 patients were exposed to aspirin 100 mg bid (= 200 mg over 24 hours) and one placebo pill, 79 patients were exposed to aspirin 100 mg tid (=300 mg over 24 hours) and 85 patients were exposed to one tablet of aspirin 100 mg and two tablets of placebo daily.

Part B: 120 patients were exposed to aspirin 100 mg bid (=200 mg over 24 hours) for 20 months and 122 patients were exposed to aspirin 100 mg daily (reference treatment).

b) Adverse Events (Describe the overall incidence of treatment emergent adverse events (i.e. those which started during the active treatment phase or which were present at baseline and became worse during the study)

All AE are summarised in tabular form per patient in the Appendix 4 by treatment group, preferred term (AE/SAE), subdivided by body system, possibility of a causal relationship and severity. Headache was the most common adverse event (i.e. occurring in > 1% of the study population) as reported in Appendix 4, but it was judged as unrelated to the IMP, since it is either occasional or associated with the ET itself.

Events more specifically relevant to the study drug in terms of efficacy, i.e. adjudicated thrombotic and safety, i.e. bleeding complications, are reported in the Table below with the related statistics. Although not statistically significant, there were more major thromboses in the reference as compared to the experimental treatment, while there were slightly more CRNMB in the bid group, even though no major bleeding occurred in each group.

Table 9. Thrombotic and bleeding events in the 242 patients of ARES part B

	[ALL]	**100 od**	**100 bid**	**p.overall**
	N=242	***N=120***	***N=122***	
Major arterial thrombosis: Yes	4 (1.65%)	3 (2.50%)	1 (0.82%)	0.368
Minor thrombosis: Yes	2 (0.83%)	1 (0.83%)	1 (0.82%)	1.000
CRNMB: Yes	10 (4.13%)	2 (1.67%)	8 (6.56%)	0.102
Minor bleeding: Yes	9 (3.72%)	5 (4.17%)	4 (3.28%)	0.748

The median SODA scores thorough all visits was comparable between the two treatment arms (SODA question 1: 2.6 vs 5.2 for 100 od vs 100 bid, p=0.055; SODA question 2: 1.94 vs 2.14 for 100 od vs 100 bid, p=0.279).

According to the NPR questionnaires filled by the patients, microvascular symptoms occurred significantly more frequently in the reference (od) vs. experimental (bid) study arm (p<0.001).

No unexpected adverse events occurred during the study.

c) Deaths

No deaths occurred over the entire study

d) Other Serious Adverse Events

All SAE are summarised in tabular form by treatment group, preferred term and body system in the Appendix 4 of this FSR. Whether reported SAE have been considered as related or unrelated to the study drug is also reported in the Appendix 4.

e) Adverse Events Leading to Withdrawal

Whether AE led to withdrawal from the study or not is summarised in tabular form in the Appendix 4. We did not observe un-expected events, moreover bleeding complications did not differ in the two groups.

Other Significant Adverse Events

We did not observe any other significant and unexpected AE of specific relevance to the drug class or indication

f) Narratives: N/A

g) Clinical Laboratory Evaluations

No relevant changes in routine laboratory parameters were observed. No patients had abnormal values of hepatic or kidney laboratory indexes. One patient randomized to the standard od arm had acute pancreatitis, reported as a SAE, which was considered unrelated to aspirin (seeAppendix 4) and the patients continued the randomized treatment and did not exit the study.

h)Vital Signs, Physical Findings and Other Observations Related to Safety

We did not observe any relevant variation of vital signs, physical findings or any other observations related to safety, beyond the ones already listed in the Appendix 4 and discussed in the previous sections.

i) Safety Conclusions

The overall safety evaluation of the study should be reviewed and compared with any control groups. Particular reference should be made to the incidence of serious adverse events, deaths and adverse events which led to withdrawal and any significant differences between treatment groups. The implication for the use of the study drug i.e. necessary dosage changes, interactions, etc. should be summarised.

Part A. PGIM (the vascular safety biomarker) was not significantly different between the two experimental (bid and tid) regimens as compared to the standard od regimen of low-dose aspirin. Subjective gastric disturbances, as assessed by the SODA questionnaire, were slightly higher in the bid arm, however no major GI events occurred during part A. No other safety issues arisen

during this part of the study.

Part B. No major issues of safety occurred during part B of the study.

11 Discussion and Overall Conclusions

The way in which low-dose aspirin prevents atherothrombosis is through permanent inactivation of platelet COX-1, resulting in virtually complete (i.e. >97%) suppression of TXA_2 production throughout the 24-hour dosing interval.(14) There is consistency in the saturability of the acetylation of platelet COX-1,(34) suppression of TXA_2 formation(35) and reduction in atherothrombotic events at daily doses of aspirin in the range of 75 to 100 mg.(14) Although the clinical efficacy of low-dose aspirin has been evaluated in subjects at variable risk of vascular occlusion, spanning the whole spectrum from asymptomatic, healthy subjects(36) to patients with acute ischemic syndromes,(37) its use in MPNs has been largely based on extrapolation from non-MPN trials and from a single trial in PV.(12) In the absence of any aspirin trial in ET patients, justification for its use based on extrapolation from other clinical settings would require demonstrating comparable PD response (i.e., platelet TXA_2 suppression) in ET and non-ET subjects.

We designed the ARES study with two main objectives: i) to demonstrate improved antiplatelet efficacy and preserved endothelial safety of an optimized aspirin dosing regimen; ii) to assess long-term compliance with and tolerability of the selected regimen.(19)

We found high absolute values (about 10-fold higher than in non-ET subjects) and marked inter-individual variability in serum TXB_2, a validated biomarker of low-dose aspirin efficacy,(5, 21) with the vast majority of ET patients having biochemical evidence of inadequate platelet inhibition when treated with a standard low-dose aspirin regimen (**Figure 1 section 12**). It should be emphasized that most traditional NSAIDs (with the possible exception of high-dose naproxen),(38) inhibit platelet TXA_2 production by <95%, which would correspond to a residual serum TXB_2 >15-30 ng/ml (depending on platelet count), a level comparable to the average basal value measured in the recruited ET patients. Incomplete platelet COX-1 inhibition by NSAIDs has been shown to be insufficient to exert a cardioprotective effect, and to protect against COX- 2-dependent cardiotoxicity.(39)

We demonstrated with high statistical confidence that a bid regimen of aspirin reduced inter-individual variability in serum TXB_2 and substantially lowered (i.e., by ≈90%) the residual serum TXB_2 level (**Figure 1 and 2, section 12**). However, no further improvement was achievedby a tid regimen, suggesting that a ceiling effect was reached in matching accelerated renewal of the drug target with a shortened dosing interval (**Figure 1 and 2 section 12**). Both experimental regimens similarly reduced *in vivo* TXA_2-dependent platelet activation, as reflected by urinary TXM excretion (**Figure 3 section 12**), consistent with saturability of platelet COX-1 inactivationwith a 12-hour dosing interval of aspirin administration in ET. The apparent endothelial safety ofa bid regimen in sparing PGI_2 biosynthesis confirms the preliminary findings in a small sample of ET patients,(25) apparently at odds with earlier findings in healthy subjects (**Figure 4 section 12**).(8)

Based on the Part A results, we have chosen aspirin 100 mg bid as the experimental antiplatelet therapy regimen to be compared with the standard 100 mg od regimen for maintenance of superior antiplatelet efficacy, compliance and tolerability in the long-term part B of the ARES study, in which the same ET patients were re-randomized to one of the two aspirin regimens. ARES part B unequivocally confirmed that the superiority of the bid regimen as compared to the od regimen

was maintained over a long-term exposure (20 months) with no major safety issue,

and with a clear benefit on microvascular symptoms, which are very frequent in ET patients, undermine their daily life activities and quality of life and have been shown to be of platelet origin.(1) Serious vascular events, predictably, were too few to be analyzed with an adequate statistical power, however numerically more events occurred in the od vs. bid arm according to the ITT analysis.

We conclude that: i) the currently recommended aspirin regimen of 75-100 od for primary or secondary cardiovascular prophylaxis is largely inadequate in reducing platelet activation in the vast majority of ET patients; ii) the antiplatelet response to low-dose aspirin can be dramatically improved by a bid regimen, with no further improvement by a tid administration; iii) the bid regimen showed a long-term superiority, similar compliance and tolerability as compared to the standard od low-dose aspirin treatment.

12. <u>Tables, Figures and Graphs</u>

Demographic, efficacy and safety data are presented as summary tables, figures or graphs within the text of the report.

Additional Figures for the primary efficacy and safety parameters are reported below.

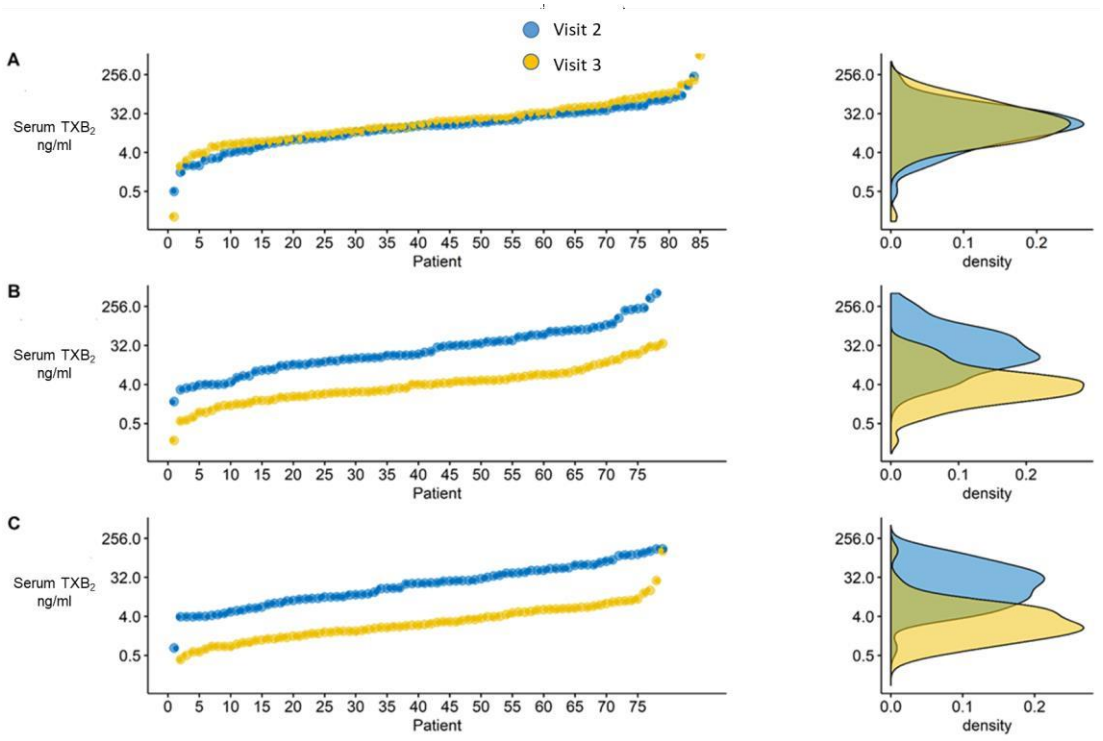


Figure 1. **Individual serum thromboxane (TX)B_2 values according to the randomized treatment**. Each panel depicts on the left side the individual values of serum TXB_2 at Visits 2 (randomization) and 3 (end of treatment); the right side of each panel shows the corresponding distribution of the data. Panel A: once-daily arm; Panel B: twice-daily arm; Panel C: three times daily arm.

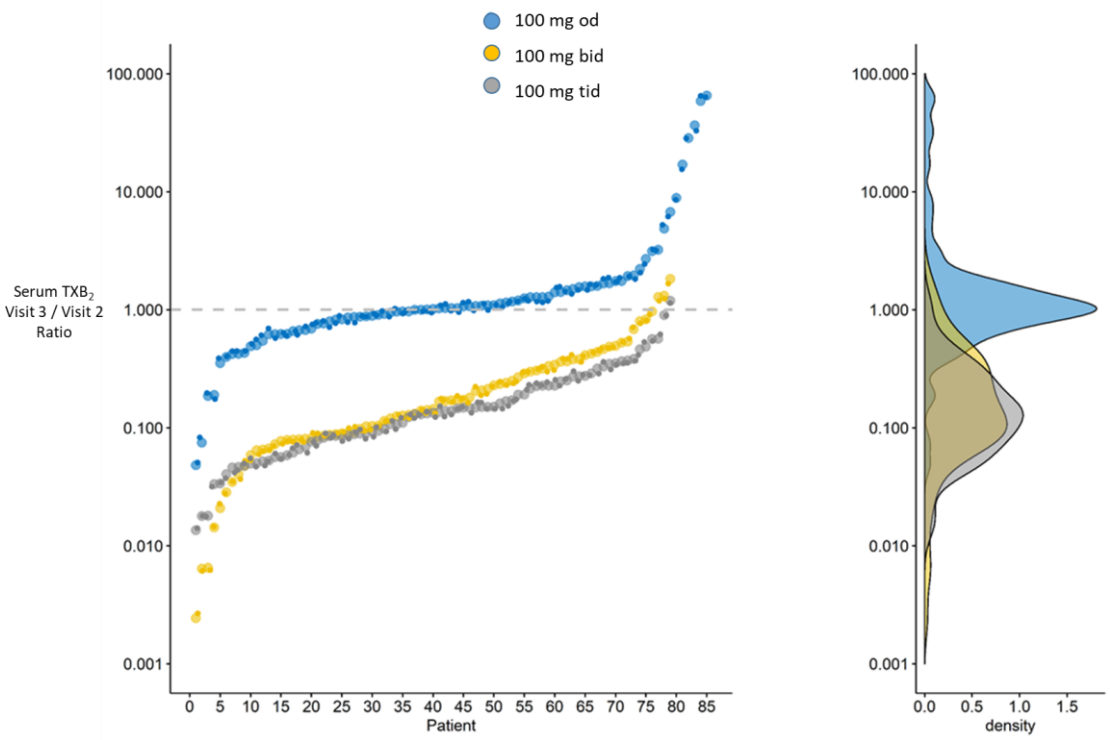


Figure 2. Intra-subject ratios of serum thromboxane (TX)B_2 values. Individual, intra-subject ratios of serum TXB_2 values measured at Visit 3 versus Visit 2 are represented for each treatment arm on the left side; the corresponding data distribution are represented on the right side of the figure.

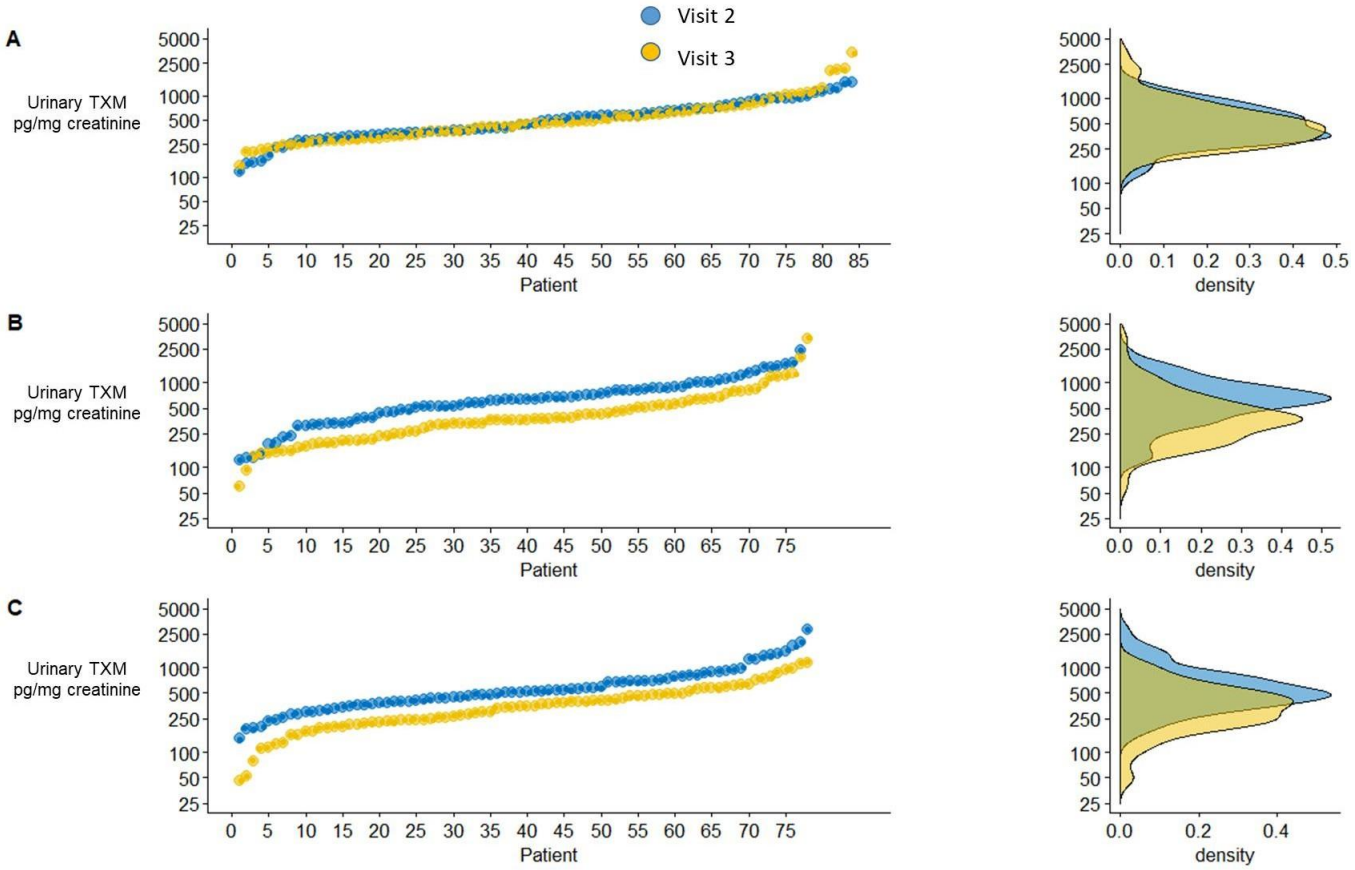


Figure 3. Individual urinary thromboxane metabolite (TXM) values according to the randomized treatment. Each panel depicts on the left side the individual values of urinary TXM excretion at Visits 2 (randomization) and 3 (end of treatment); the right side of each panel shows the corresponding distribution of the data. Panel A: once-daily arm; Panel B: twice-daily arm; Panel C: three times daily arm.

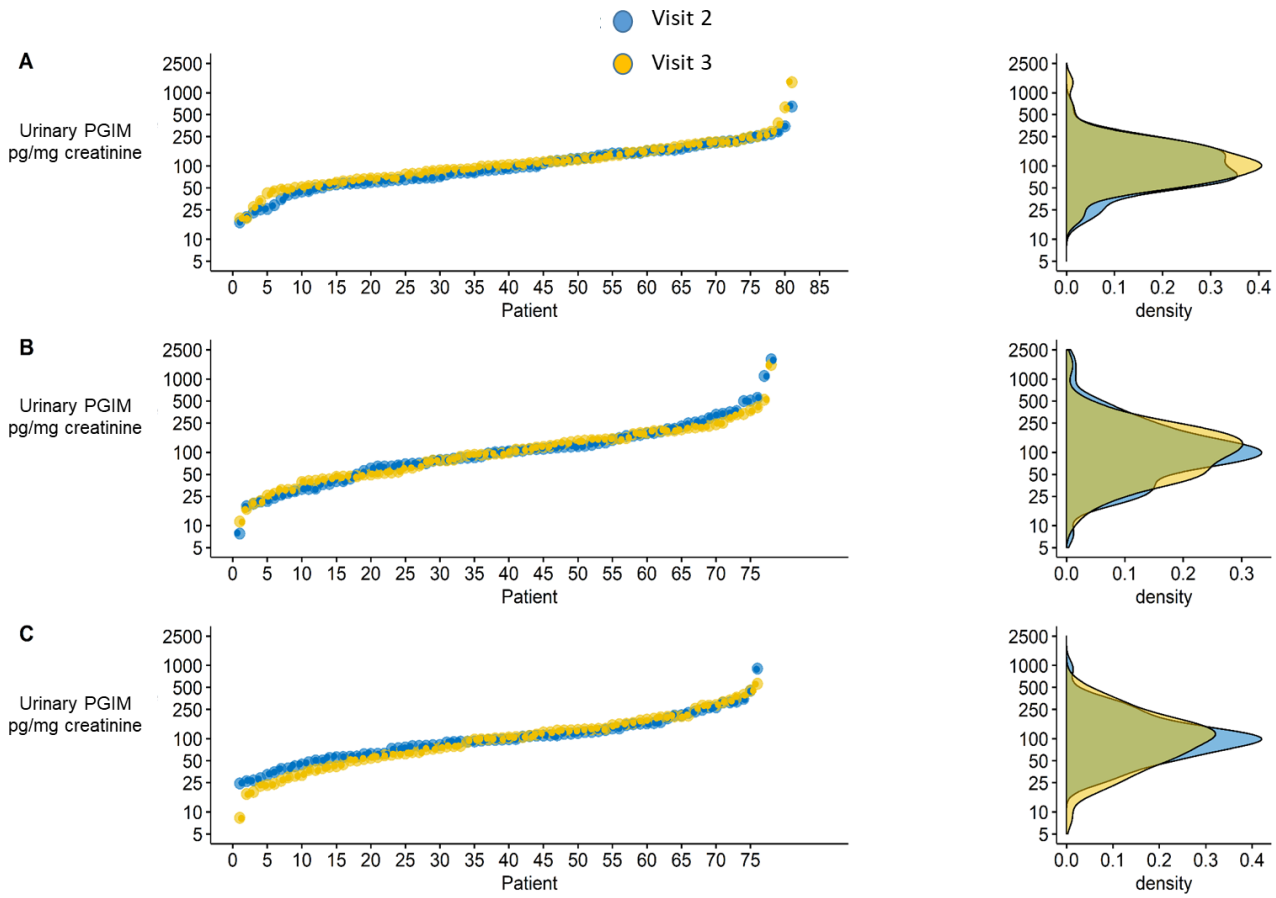


Figure 4. Individual urinary prostacyclin metabolite (PGIM) values according to the randomized treatment. Each panel depicts on the left side the individual values of urinary TXM excretion at Visits 2 (randomization) and 3 (end of treatment); the right side of each panel shows the corresponding distribution of the data. Panel A: once-daily arm; Panel B: twice-daily arm; Panel C: three times daily arm.

13.0 References

1. Patrono C, Rocca B, De Stefano V. Platelet activation and inhibition in polycythemia vera and essential thrombocythemia. Blood. 2013;121(10):1701-11.
2. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. Am J Hematol. 2020;95(12):1599-613.
3. Pascale S, Petrucci G, Dragani A, Habib A, Zaccardi F, Pagliaccia F, et al. Aspirin-insensitive thromboxane biosynthesis in essential thrombocythemia is explained by accelerated renewal of the drug target. Blood. 2012;119(15):3595-603.
4. Badimon L, Vilahur G, Rocca B, Patrono C. The Key Contribution Of Platelet And Vascular Arachidonic Acid Metabolism To The Pathophysiology Of Atherothrombosis. Cardiovasc Res. 2021.
5. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003340.pdf .
6. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci U S A. 1999;96(1):272-7.
7. Dragani A, Pascale S, Recchiuti A, Mattoscio D, Lattanzio S, Petrucci G, et al. The contribution of cyclooxygenase-1 and -2 to persistent thromboxane biosynthesis in aspirin-treated essential thrombocythemia: implications for antiplatelet therapy. Blood. 2009;115(5):1054-61.
8. FitzGerald GA, Oates JA, Hawiger J, Maas RL, Roberts LJ, 2nd, Lawson JA, et al. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man.J Clin Invest. 1983;71(3):676-88.
9. Team RDC. A language and environment for statistical computing (v 3.6.1). R Foundation for Statistical Computing, Vienna. 2019.
10. Rocca B, Ciabattoni G, Tartaglione R, Cortelazzo S, Barbui T, Patrono C, et al. Increased thromboxane biosynthesis in essential thrombocythemia. Thromb Haemost. 1995;74(5):1225-30.
11. Patrono C, Rocca B. Measurement of Thromboxane Biosynthesis in Health and Disease. Front Pharmacol. 2019;10:1244.
12. Landolfi R, Marchioli R, Kutti J, Gisslinger H, Tognoni G, Patrono C, et al. Efficacy and safety of low- dose aspirin in polycythemia vera. N Engl J Med. 2004;350(2):114-24.
13. Patrono C, Baigent C. Role of aspirin in primary prevention of cardiovascular disease. Nat Rev Cardiol. 2019;16(11):675-86.
14. Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med. 2005;353(22):2373-83.
15. Santilli F, Rocca B, De Cristofaro R, Lattanzio S, Pietrangelo L, Habib A, et al. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin "resistance". J Am Coll Cardiol. 2009;53(8):667-77.
16. Antithrombotic Trialists C, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373(9678):1849-60.
17. Giaretta A, Rocca B, Di Camillo B, Toffolo GM, Patrono C. In Silico Modeling of the Antiplatelet Pharmacodynamics of Low-dose Aspirin in Health and Disease. Clin Pharmacol Ther. 2017;102(5):823-31.
18. Petrucci G, Rizzi A, Cavalca V, Habib A, Pitocco D, Veglia F, et al. Patient-independent variablesaffecting the assessment of aspirin responsiveness by serum thromboxane measurement. Thromb Haemostasis. 2016;116(5):891-6.
19. De Stefano V, Rocca B, Tosetto A, Soldati D, Petrucci G, Beggiato E, et al. 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. Blood Cancer J. 2018;8(6):49.
20. Rocca B, Santilli F, Pitocco D, Mucci L, Petrucci G, Vitacolonna E, et al. The recovery of platelet cyclooxygenase activity explains interindividual variability in responsiveness to low-dose aspirin in patients with and without diabetes. J Thromb Haemost. 2012;10(7):1220-30.
21. Patrono C, Ciabattoni G, Pinca E, Pugliese F, Castrucci G, De Salvo A, et al. Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. Thromb Res. 1980;17(3-4):317-27.
22. Ciabattoni G, Maclouf J, Catella F, FitzGerald GA, Patrono C. Radioimmunoassay of 11-dehydrothromboxane B2 in human plasma and urine. Biochim Biophys Acta. 1987;918(3):293-7.
23. Pagliaccia F, Habib A, Pitocco D, Petrucci G, Zaccardi F, Di Stasio E, et al. Stability of urinary thromboxane A2 metabolites and adaptation of the extraction method to small urine volume. Clin Lab. 2014;60(1):105-11.
24. FitzGerald GA, Brash AR, Falardeau P, Oates JA. Estimated rate of prostacyclin secretion into the circulation of normal man. J Clin Invest. 1981;68(5):1272-6.

25. Cavalca V, Rocca B, Squellerio I, Dragani A, Veglia F, Pagliaccia F, et al. In vivo prostacyclin biosynthesis and effects of different aspirin regimens in patients with essential thrombocythaemia. Thromb Haemost. 2014;112(1):118-27.
26. Zaccardi F, Rizzi A, Petrucci G, Ciaffardini F, Tanese L, Pagliaccia F, et al. In vivo platelet activation and aspirin responsiveness in type 1 diabetes mellitus. Diabetes. 2016;503:6.
27. Petrucci G, Rizzi A, Cavalca V, Habib A, Pitocco D, Veglia F, et al. Patient-independent variablesaffecting the assessment of aspirin responsiveness by serum thromboxane measurement. Thromb Haemost. 2016;116(5):891-6.
28. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture(REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.
29. Barbui T, Finazzi G, Carobbio A, Thiele J, Passamonti F, Rumi E, et al. Development and validation of an International Prognostic Score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). Blood. 2012;120(26):5128-33; quiz 252.
30. Cavalca V, Rocca B, Squellerio I, Dragani A, Veglia F, Pagliaccia F, et al. In vivo prostacyclin biosynthesis and effects of different aspirin regimens in patients with essential thrombocythaemia. Thromb Haemostasis. 2014;112(1):118-27.
31. Petrucci G, Zaccardi F, Giaretta A, Cavalca V, Capristo E, Cardillo C, et al. Obesity is associated with impaired responsiveness to once-daily low-dose aspirin and in vivo platelet activation. J Thromb Haemost. 2019;17(6):885-95.
32. Rabeneck L, Wristers K, Goldstein JL, Eisen G, Dedhiya SD, Burke TA. Reliability, validity, and responsiveness of severity of dyspepsia assessment (SODA) in a randomized clinical trial of a COX-2-specific inhibitor and traditional NSAID therapy. Am J Gastroenterol. 2002;97(1):32-9.
33. Geyer HL, Kosiorek H, Dueck AC, Scherber R, Slot S, Zweegman S, et al. Associations between gender, disease features and symptom burden in patients with myeloproliferative neoplasms: an analysis by the MPN QOL International Working Group. Haematologica. 2017;102(1):85-93.
34. Patrignani P, Tacconelli S, Piazuelo E, Di Francesco L, Dovizio M, Sostres C, et al. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost. 2014;12(8):1320-30.
35. Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest. 1982;69(6):1366-72.
36. Patrono C, Baigent C. Role of aspirin in primary prevention of cardiovascular disease. Nat Rev Cardiol. 2019.
37. Patrono C, Morais J, Baigent C, Collet JP, Fitzgerald D, Halvorsen S, et al. Antiplatelet Agents for the Treatment and Prevention of Coronary Atherothrombosis. J Am Coll Cardiol. 2017;70(14):1760-76.
38. Capone ML, Tacconelli S, Sciulli MG, Grana M, Ricciotti E, Minuz P, et al. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. Circulation. 2004;109(12):1468-71.
39. Patrono C, Baigent C. Nonsteroidal anti-inflammatory drugs and the heart. Circulation. 2014;129(8):907- 16.

14. Appendices

The full list of the appendices relevant to and included in this FSR is summarized below.

Appendix 1 Names and affiliations of all Investigators and Sponsor personnel

Appendix 4 Adverse event/serious adverse event / unexpected adverse events/ withdrawals

Appendix 8 Randomisation list with randomisation numbers (Part A and B)