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Facilitation through Aggrastat or cangrelor Bolus and infusion Over prasugreL: a mUlticenter randomized open-label trial in patientS with ST-elevation myocardial inFarction referred for primAry percutaneouS inTERvention (FABOLUS FASTER) Trial: Design and Rationale

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Abstract

Antithrombotic therapy is a critical component of the management of ST-elevation myocardial infarction (STEMI) patients treated with primary percutaneous coronary intervention (PCI). Rapid and profound inhibition of platelet reactivity has been shown to mitigate the ischemic risks and improve myocardial salvage.

High residual platelet reactivity (HRPR) has been reported up to 4 or 6 hours after loading dose of prasugrel or ticagrelor, therefore, multiple alternative strategies, including crushed or chewed oral tables or intravenous agents, have been investigated to provide a more rapid and sustained inhibition of platelet function and bridge the initial treatment gap. The FABOLUS-FASTER is the first investigator-initiated, multicentre, open-label, prospective, randomized study to directly compare the pharmacodynamics effects of cangrelor, tirofiban, chewed or integer prasugrel. This study will add new insights in the management of antiplatelet therapy in patients with STEMI undergoing primary PCI and might be hypothesis-generating for future clinical trials in this field. The trial is registered on clinicaltrials.gov NCT02978040, and EudraCT 2017-001065-24.

Keywords: primary PCI, cangrelor, tirofiban, prasugrel, platelet aggregation

Abbreviations

AAR=area at risk

AUC=area under the curve

ACS=acute coronary syndrome

ACT=activated clotting time

ADP=adenosine diphosphate

ATP=adenosine triphosphate

cMRI=magnetic resonance imaging

ECG=electrocardiogram

HPRP=high residual platelet reactivity

IPA=inhibition of platelet activity

LTA=light transmittance aggregometry

MS=myocardial salvage

MSI=myocardial salvage index

MVO=microvascular obstruction

PAM=prasugrel active metabolite

PCI=percutaneous coronary intervention

PD=pharmacodynamic

PK=pharmacokinetic

STEMI=ST-elevation myocardial infarction

UFH=unfractionated heparin

BACKGROUND

Antithrombotic therapy is crucial in the management of ST-elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (PCI)[1-4]. Multiple pathways are involved in platelet activation, adhesion, aggregation and fibrin synthesis; hence, several anti-thrombotics including oral and/or parenteral anti-platelet and anticoagulation agents are to be administered in the acute phase of STEMI[5,6]. The Facilitation through Aggrastat or cangrelor Bolus and infusion Over prasugreL: a mUlticenter randomized open-label trial in patientS with ST-elevation myocardial inFarction referred for primAry percutaneouS inTERvention (FABOLUS FASTER) aims at investigating the acute pharmacodynamic effects of cangrelor, tirofiban or prasugrel, administered at standard 60 mg loading dose as integer or chewed tablets, in STEMI patients undergoing primary PCI.

Oral P2Y12 inhibitors

The combination of aspirin and oral inhibitors of ADP-activated platelet P2Y12 receptor such as prasugrel or ticagrelor has been shown to reduce ischemic recurrences as compared to aspirin and clopidogrel and represents the current standard of care[1-4]. However, high residual platelet reactivity (HRPR) has been consistently reported up to 4 or 6 hours after prasugrel or ticagrelor loading dose, with a large inter-individual variability[7-9]. The delayed onset and attenuated antiplatelet effects have been attributed to impaired drug absorption resulting in reduced drug bioavailability. Therefore, strategies to increase bioavailability of orally administered P2Y12 inhibitors have been further investigated in STEMI patients, including higher loading regimens, which overall proved ineffective, or crushed/chewed tablets[10-16]. Two small-scale independent studies suggested that crushed standard loading dose of ticagrelor or prasugrel can achieve more pronounced platelet inhibition compared with standard whole tablets soon after drug administration[13,14]. The MOJITO (Mashed Or Just Integral pill of TicagrelOr) trial found that in 82 patients a crushed standard loading dose of ticagrelor determined a more prominent platelet

inhibition (assessed with VerifyNow system) 1 hour after drug administration compared with integer tablets[13]. Similarly, the CRUSH trial enrolled 52 STEMI patients undergoing primary PCI and found that crushed prasugrel accelerated drug absorption, and thus, provided faster and greater antiplatelet effects (demonstrated by VerifyNow and whole blood vasodilator-stimulated phosphoprotein [VASP] assays) compared with integer tablets[14]. A pharmacokinetic analysis supported the pharmacodynamic findings by showing that crushed as compared to integer prasugrel was associated with faster drug absorption and higher plasma concentrations of prasugrel active metabolite (PAM) at 30 min and 1 h but not at 2 h[14]. In the IPAAD-Tica (The inhibition of platelet-aggregation after administration of three different ticagrelor formulations) 91 patients with stable angina were randomly assigned to integer, crushed or chewed ticagrelor tablets and platelet reactivity was assessed by VerifyNow 20 and 60 min after loading dose. Chewed ticagrelor was associated with significantly reduced PRU values compared with crushed or integer tablets at both 20 and 60min, and crushed ticagrelor loading dose determined significantly lower PRU values compared to integer tablets at 20 min but not at 60 min[16].

Tirofiban

Tirofiban is a small molecule, nonpeptide tyrosine derivative which belongs to the class of glycoprotein (GP) IIb/IIIa inhibitors (GPI) [6]. By inhibiting the binding of fibrinogen and von Willebrand factor to the GP IIb/IIIa receptor on the surface of the platelet, GPIs are currently considered the most potent inhibitors of platelet aggregation.

A dedicated meta-analysis focusing on the benefits and risks of this drug as compared to placebo and abciximab has been published [17]. It included 31 studies and 20,006 patients (12,874 comparing tirofiban versus heparin plus placebo or bivalirudin alone, and 7,132 versus abciximab). At 30-day, tirofiban significantly decreased mortality (OR=0.68 [0.54-0.86]; p=0.001), and death or myocardial infarction (MI) (OR=0.69 [0.58-0.81]; p<0.001) when compared with placebo. This benefit persisted at follow-up but was counterbalanced by a higher risk of minor bleedings or

thrombocytopenia. There was no difference between tirofiban and abciximab in 30-day mortality (OR=0.90 [0.53, 1.54]; p=0.70), while in the overall group tirofiban trended to increase the composite of death or MI (OR=1.18 [0.96, 1.45]; p=0.11), although this trend was not present at medium-term follow-up or when assessing studies using the 25μ/kg bolus of tirofiban.

Current guidelines recommend the use of GPI, including tirofiban in selected situations[1-3].

In the FABOLUS PRO (Facilitation through Aggrastat By drOpping or shortening Infusion Line in patients with ST-segment elevation myocardial infarction compared to or on top of PRasugrel given at loading dOse) trial, 100 STEMI patients randomly received prasugrel 60 mg versus 25 μg/kg tirofiban bolus with or without post-bolus 2-h infusion of tirofiban, with or without concomitant prasugrel. Inhibition of platelet activity (IPA) at light transmittance aggregometry was performed throughout 24 h. Compared with prasugrel-treated patients, those in the tirofiban group had a significantly higher IPA to 20 μmol/l ADP stimulation as (87±31 vs. 36±35, p<0.0001) at 30 min. Similarly, the degree of platelet inhibition remained suboptimal for at least 2h in prasugrel-treated patients. When concomitant clopidogrel was given, the post-bolus tirofiban infusion was necessary to maintain a high level of IPA beyond 1h. Conversely, the bolus-only tirofiban and concomitant prasugrel were associated with the higher and more consistent IPA levels after both ADP and thrombin receptor-activating peptide stimuli compared with either therapy alone. There is currently no head to head comparison between tirofiban and cangrelor on the degree of platelet inhibition.

Cangrelor

Cangrelor, is an intravenous reversible inhibitor of ADP-induced platelet aggregation. In most recent guidelines it is to be considered in P2Y12 inhibitor–naive patients undergoing PCI, including primary PCI, with the same class of recommendation attributed to GPI [2]. Cangrelor is an ATP analogue and it is meant to achieve almost complete and immediate inhibition of ADP-induced platelet aggregation when administered as a bolus plus infusion. The plasma half-life is approximately 3 to 5 minutes and platelet function is entirely restored within 1 hour after cessation

of infusion[6,18-20]. After animal studies, this agent was tested in humans. In a study of 39 patients with unstable angina or non-Q wave myocardial infarction, who were receiving aspirin and heparin, cangrelor (initially identified as AR-C69931MX) was administered intravenously with stepped dose increments over 3 h to a plateau at either 2 μg/kg/min for 21 h (Part 1; n=12) or up to 69 h (Part 2; n=13) or 4 μg/kg/min for up to 69 h (Part 3: n=14)[21]. This study assessed safety parameters, platelet aggregation (PA) induced by ADP 3 μmol/L (impedance aggregometry), bleeding time (BT) and cangrelor plasma concentrations. In patients completing the study (n=33) cangrelor was well tolerated. At 30-day no deaths or serious adverse events occurred, but trivial bleeding (56%) was frequent. Mean IPA was 36.2±39.2, 20.7±25.9 and 40.7±36.7% at 1 h post-infusion,, and 96.0±8.6, 94.9±14.4 and 98.7±2.1% at 24 h, for Parts 1, 2 and 3 respectively. Platelet aggregation test was based on the whole blood impedance aggregometry (Chronolog), and the stimulation was done with ADP 3 μmol/L. In a subsequent study, clopidogrel moderately inhibited platelet P2Y12 receptor activation while cangrelor induced a substantially greater P2Y12 receptor inhibition[20]. In this study, however, the 13 patients with acute coronary syndromes (ACS) receiving cangrelor (Group 1; stepped dose increments of cangrelor achieving a plateau after 3 h of 2 or 4 μg/kg per min in 8 and 5 patients, respectively), and 8 patients undergoing coronary stenting receiving clopidogrel (Group 2, 300 mg loading dose followed from 75 mg daily) were studied using a whole blood single-platelet counting aggregation assay that was collected at 30 min and 21h from infusion (and 69h in some patients) onset for cangrelor and 4-7 days for clopidogrel. An initial experience of cangrelor in patients undergoing PCI was obtained in a 2-part randomized trial comparing different dosages of cangrelor (1 or 2 or 4 μg/kg/min for 18-24h) with placebo in 200 patients and then comparing cangrelor (4 μg/kg/min for 18-24h) vs abciximab (0.25 mg/kg bolus followed by 0.125 μg/kg per minute up to 10 μg per minute for up to 12h) in 199 patients[22]. Cangrelor was associated with an acceptable risk of bleeding and adverse cardiac events, and less prolongation of bleeding time compared with abciximab, but this study did not include any pharmacodynamic

analysis. The CHAMPION-PLATFORM and CHAMPION-PCI trials compared cangrelor with placebo in clopidogrel treated patients with ACS or stable CAD undergoing PCI[23,24]. In both trials, cangrelor was administered with an intravenous bolus of 30 μg/kg followed by infusion of 4 μg/kg/min for at least 2h or until the conclusion of the index procedure, whichever was longer (at the clinician's discretion, the infusion could be continued for 4h). In these trials, the enrolment was stopped when an interim analysis concluded that the trials would be unlikely to show superiority for the primary endpoint. The platelet function substudy of these 2 trials tested the pharmacodynamic effects of cangrelor vs clopidogrel and was the largest pharmacodynamic experience with cangrelor up to that time[19]. This study demonstrated the potent P2Y12 receptor inhibitory effects of cangrelor, as well as its rapid onset/offset of action based on VerifyNow analysis in 167 patients (84 treated with cangrelor + clopidogrel and 83 treated with placebo + clopidogrel), mainly from CHAMPION-PCI trial. Moreover, this study included LTA assessment in 44 patients showing significantly greater inhibition in cangrelor-treated patients (LTA ADP 20 μM median 20.0 % vs 69.5 %, $p<0.001$; and ADP 5 μM median: 2.5 % vs 49.0 %, $p<0.001$), but these values referred to the final (after 6 min) platelet aggregation not the max aggregation. An additional study tested the PK and PD effects of cangrelor bolus and infusion at the regimen currently used[18]. Twenty-two healthy volunteers were randomly assigned to 15-μg/kg bolus followed by a 2-μg/kg/min infusion (n=12) or 30-μg/kg bolus followed by a 4-μg/kg/min infusion (n=10) and the infusion was continued for 60 minutes. The higher dose provided more consistent and pronounced inhibition on ADP–induced P-selectin expression, but no significant differences were observed between the 2 regimens in terms of platelet aggregation or time to recovery of platelet function. It is important to note that, however, this study was conducted in healthy volunteers, and again platelet aggregation was assessed by a whole blood test but not LTA.

To date, there are limited data on the effects of cangrelor used in combination with oral P2Y12 inhibitors in patients undergoing primary PCI and questions have emerged on the potential for drug-drug interactions during the transition from cangrelor to oral P2Y12 inhibitors. Indeed, it is

generally recommended that clopidogrel and prasugrel should be administered at the end of the cangrelor infusion, while for ticagrelor the simultaneous administration could not be an issue, however, evidence on these interactions are still limited[25]. In the recent CANTIC study, 50 STEMI patients undergoing primary PCI were randomly assigned to cangrelor or placebo (bolus followed by 2h infusion)[26]. All patients received crushed tablets of ticagrelor 180-mg loading dose at the time of cangrelor/placebo bolus administration. Pharmacodynamic analyses were performed at 8 time points (expressed as P2Y12 reaction units by VerifyNow and platelet reactivity index by vasodilator-stimulated phosphoprotein). This study supported the efficacy of cangrelor in bridging the gap of platelet inhibition associated with the use of oral P2Y12 inhibition induced by ticagrelor, without any apparent drug-drug interaction[26].

STUDY RATIONALE

The use of an intravenous antiplatelet agent including a parenteral P2Y12 inhibitor such as cangrelor or a GPI, such as tirofiban, on top of oral therapy with aspirin and a P2Y12 inhibitor confers the benefit of a more rapid and stronger antagonism of platelet reactivity, thus bridging the initial gap in platelet inhibition associated with the use of oral P2Y12 inhibitors in STEMI patients. The association of parenteral and oral antiplatelet agents might therefore mitigate the increased risk of early thrombotic complications and improve myocardial salvage conferred by mechanical revascularisation. However, the association of multiple potent antithrombotics increases the bleeding risk by a magnitude that is depending on patient characteristics and procedural features. Cangrelor was shown in a propensity-matched study to be associated to less bleeding risk and comparable ischemic protection as compared to GPI administered at standard prolonged post-bolus infusion [27]. Whether this postulated differential safety profile of these two agents comes from a different degree of ADP-induced inhibition of platelet aggregation, the inherent mechanism of action (i.e. ADP as opposed to GP antagonism) or overall duration of treatment (i.e. 2h versus $\geq$ 12h infusion) remains speculative.

The comparative effectiveness of cangrelor and tirofiban on-label regimens in inhibiting platelet reactivity remains unknown. Similarly, it remains unclear if the standard loading dose of prasugrel, when chewed, might mitigate the absorption delay observed with all oral P2Y12 inhibitors in STEMI patients and therefore potentially reduce the need for concomitant parenteral antiplatelet agents.

METHODS

Study design and patient population

The FABOLUS FASTER is an investigator-initiated, multicentre, open-label, randomized study. The study is registered on clinicaltrials.gov NCT02978040, and EudraCT 2017-001065-24. The purpose of this study is to compare the efficacy with respect to the inhibition of platelet aggregation of cangrelor, tirofiban, or prasugrel, administered as integer or chewed tablets in STEMI patients undergoing primary PCI. The study design and flow-chart are summarised in **Figures 1 and 2**.

Patients undergoing primary PCI due to suspected STEMI are screened at admission and, after adequate explanation, those consenting are enrolled in the study by dedicated staff previously identified at each participating centre. No screening log is mandated by study and only randomized patients with written consent are to be reported. Eligibility criteria are reported in **Table 1**. Briefly, adult patients presenting with STEMI and referred for primary PCI within 12 hours (or 24h if ongoing ischemia is demonstrated) will be enrolled. Key exclusion criteria are: unconsciousness or other conditions that make the patient incapable of providing informed consent and receiving oral loading dose of prasugrel, bleeding diathesis, recent administration of fibrinolytics or GPI or P2Y12 inhibitors or cangrelor, recent major surgery or bleeding, chronic dialysis, previous intracranial haemorrhage, previous stroke or recent TIA, known hypersensitivity to study drugs, chronic oral anticoagulant therapy, pregnancy or breastfeeding, limited life expectancy.

Patients are being randomly assigned in a 1:1:1 fashion to tirofiban (≥40 patients, bolus + 2 hour infusion, followed by prasugrel at 60 mg loading dose at the time of infusion discontinuation), cangrelor (≥40 patients, bolus + 2 hour infusion, followed by prasugrel at 60 mg loading dose at the time of infusion discontinuation) or prasugrel 60 mg (≥40 patients, at the beginning of the PCI, administered as either integer or chewed tablets). Patients assigned to the prasugrel arm undergo an immediate 1:1 sub-randomization to either chewed or integer oral loading dose of prasugrel. Randomization is concealed and stratified according to centre and to time from onset of symptoms to PCI (<3 hours, 3-6 hours, >6 hours) with blocks of 3 or 6 alternating randomly for the first randomization, and stratified according to centre with blocks of 2 or 4 alternating randomly for the second randomization, within the electronic data capture system ICE provided by Advice Pharma Group (https://advicepharma.com/en/technologies/ice/).

Medications and procedures

All patients have to receive aspirin before primary PCI (150-300 mg orally or 80-150 mg i.v., then 81-325 mg daily). Patients allocated to Cangrelor receive the on-label regimen in terms of bolus of 30 µg/Kg followed by infusion at 4 µg/Kg/min for 2 h (or to the end of PCI). Tirofiban will be administrated according to the single high dose bolus (SHDB) of 25 µg/Kg followed by infusion at 0.15 µg/Kg/min for 2 h (or to the end of PCI) (or an infusion rate of 0.075 µg/Kg/min for patients with known creatinine clearance <30 ml/min). At the end of infusion, oral prasugrel at a loading dose of 60 mg is to be administrated as integer tablets, followed by 10 mg daily (or 5 mg daily if body weight < 60 kg or age > 75 years old) in accordance to the instructions for use and international guidelines. In the prasugrel arm, no intravenous anti-platelet drug is to be administered. Patients randomized to prasugrel receive either integer or chewed prasugrel at an identical loading dose of 60 mg, then 10 mg daily (or 5 mg daily if body weight < 60 kg or age > 75 years old). In the chewed prasugrel arm, patients are instructed to chew pills for at least 10–15 s followed by oral administration of 150 mL of water as previously described [16].

Anticoagulation is to be achieved with concomitant unfractionated heparin (UFH) or enoxaparin whereas bivalirudin is not allowed to avoid potential confounding effects. UFH is to be administrated as bolus of 50-70 UI/Kg in the two parenteral antiplatelet arms or at 70-100 UI/Kg bolus in the prasugrel arm, then adjusted with additional adjunctive boluses in order to achieve an activated clotting time (ACT) of at least 250s during the procedure. UFH administration after the end of the procedure is discouraged unless clinically indicated.

Blood samples are obtained at randomization and before the administration of study drug (baseline) and then, counting from drug bolus termination (tirofiban and cangrelor arms) or oral loading dose of prasugrel, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and between 4 to 6 hours thereafter. ECG is recorded before and immediately after PCI and 90 minutes thereafter, as well as at discharge. Patients providing consent to participate to the imaging sub-study, will undergo cardiac magnetic resonance imaging (cMRI) at 3±1 days and 5±1 months. Clinical data collection (vital signs, medications, clinical events and serious adverse events) is collected throughout 30 days.

Pharmacodynamic analysis

LTA analysis remains the gold standard of platelet function evaluation[28], and is being performed as previously described[9]. Briefly, at each participating centre, blood samples anticoagulated with 0.129 mol/l sodium citrate will be collected. Platelet-rich plasma, obtained by centrifuging whole blood for 10 min at 70g, will be stimulated with 5 and 20 μmol/l ADP and 5 and 15 μmol/l thrombin receptor agonist peptide (TRAP), and aggregation will be assessed using a light transmission aggregometer. The 100% line will be set using platelet-poor plasma and the 0 baseline established with platelet-rich plasma (adjusted from $18x10^9$/l up to $30x10^9$/l). Platelet aggregation is being evaluated considering the maximal percentage of platelet aggregation in response to stimulus (Agg_{max}). The percentage of late platelet aggregation (Agg_{late}) is also being collected for further analyses.

Multiple electrode aggregometry (Multiplate, Roche Diagnostics, Switzerland) is being performed as previously described[29] by using hirudin-anticoagulated whole blood samples. Blood will be diluted with saline and then stimulated with ADP and TRAP reagents. After 1:2 dilution of whole blood with 0.9% NaCl solution and stirring for 3 min in the test cuvettes at 37°C, ADP, and TRAP stimulation will be added and platelet aggregation will be continuously recorded for 6 minutes. This measurement simulates platelet adhesion, activation, and aggregation on a metal surface during continuous stirring in an ex vivo setting, mimicking the development of stent thrombosis upon platelet activation. Changes in impedance are plotted over time resulting in an aggregation curve, similarly to LTA, and the efficacy of platelet inhibition is expressed with the area-under-the aggregation-curve (AUC) value.

Pharmacokinetic analysis

A pharmacokinetic analysis will be performed within the Prasugrel arm in order to compare integer versus chewed Prasugrel administration. Prasugrel Active Metabolites (PAM) will be measured at each time point under the secondary hypothesis that chewed as compared to integer prasugrel will lead to higher PAM bioavailability at 30 minutes (and other time points: 15min to 4-6h hours). Blood samples for the determination of PAM plasma concentration will be collected into pre-cooled EDTA tubes at the established time points (baseline, 15 min, 30 min, 1h, 2h, 3h and 4-6h) and will be treated with 25µl of 500 mM 3′-methoxyphenacyl bromide in acetonitrile within 30 s of collection to derivatize and stabilize the PAM as previously described[14,30]. Centrifugation of the sample will be performed within 30 minutes (2800 rpm for 15 min at 4°C), and plasma samples prepared from these blood samples will be stored at -20/-80°C in polypropylene tubes until they will be shipped to the central laboratory at University Hospital of Verona (LURM) for analysis. Plasma concentrations of PAM will be determined using validated liquid chromatography methods and tandem mass spectrometric detection.

Endpoints

The primary endpoint is the percentage of inhibition of platelet activity (IPA) assessed with light transmission aggregometry (LTA) in platelet-rich plasma after the addition of ADP 20 µmol/l at 30 minutes from drug administration (bolus or oral loading dose). Percentage IPA is defined as 100%*(baseline platelet aggregregation minus at time t platelet aggregation) / baseline platelet aggregration[9]. Baseline is at time 0 minutes just before drug administration, follow-up measurements are at time 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and 4 to 6 hours since drug administration.

Secondary endpoints include LTA using TRAP at 5 and 15 µmol/l, as well as ADP at 5 µmol/l at all different time points and up to 6 hours and ADP at 20 µmol/l at time points before and later than 30 minutes (15 minutes, 1h, 2h, 3h, 4-6h).

Other secondary endpoints will include the residual platelet reactivity assessed with Multiplate® technology (AUC at impedance aggregometry Multiplate® Electrode Aggregometry in whole blood) at all time frames and after ADP and TRAP as agonists.

For the pharmacokinetic analysis, the time for the maximum plasma concentration (Tmax), maximum observed plasma concentration (Cmax), and the area under the plasma concentration versus time curve from time 0 to the last measurable concentration (AUC_{0-t}) will be calculated. Moreover, to explore early exposure to PAM, AUC from time 0 to 2 h (AUC_{0-2}) will be also calculated.

Adverse clinical ischemic and bleeding events at 48 hours and 30 days will be also collected and include: death, cardiac death, non-fatal myocardial infarction, non-fatal stroke, transient ischemic attack, urgent target vessel revascularization, unplanned revascularization, definite/probable stent thrombosis, bleeding events, net adverse clinical event.

Other endpoints will be analysed for the pre-specified substudies.

Sample size and statistics

The Clinical Trial Unit of Bern provides statistical oversight and guidance. The total number of patients with primary endpoint assessment is 120, of whom at least 40 patients in the tirofiban group, at least 40 patients in the cangrelor group, and at least 40 patients in the prasugrel group (of which at least 20 integer and at least 20 chewed tablets).

Sample size is calculated to state the non-inferiority of cangrelor compared with tirofiban, superiority of both tirofiban and cangrelor compared with chewed prasugrel and to show the superiority of chewed prasugrel as compared to integer prasugrel.

The sample size was determined following these considerations, three primary non-inferiority tests each with alpha of 0.05/3 tests = 0.016 and one additional test also with alpha of 0.016 for consistency (chewed prasugrel vs integer prasugrel):

Assuming %IPA with 20 μmol/l ADP at 30 minutes from randomization of at least 94% with i.v. antiplatelet therapy, with a standard deviation of 8%, a non-inferiority margin of 9%, with an alpha of 0.016, 40 patients per group will give 99% of power to show non-inferiority of cangrelor compared to tirofiban. If non-inferiority of cangrelor will not be met, then the superiority of tirofiban over cangrelor will be tested (pre-specified secondary endpoint).

Assuming %IPA with 20 μmol/l ADP at 30 minutes of 55% with chewed prasugrel, with a standard deviation of 24% and an alpha of 0.016, this study will have 100% of power to show both superiority of Tirofiban (n=40) as compared to chewed Prasugrel (n=20) and superiority of Cangrelor (n=40) as compared to chewed Prasugrel (n=20).

Assuming %IPA with 20 μmol/l ADP at 30 minutes from randomization of 55% with chewed prasugrel, with a standard deviation of 24% and an alpha of 0.016, 20 patients per group will give 82% of power to show superiority of chewed Prasugrel compared with integer Prasugrel.

These assumptions were largely based on previous evidence[9].

The pairwise mean difference in platelet inhibition (difference in %IPA) will be estimated and reported with the 95% confidence intervals (Cangrelor vs Tirofiban, Cangrelor vs chewed Prasugrel, Tirofiban vs chewed Prasugrel; based on using mixed models - including data on all

time-points since PCI, random effects of site and patient as appropriate), non-inferiority will be tested using a z-test. Superiority tests will be conducted on platelet activity (% of IPA at LTA or AUC unit at Multiplate, as continuous variables, random effects of site and patient as appropriate) using mixed models (including data on all time-points since PCI) with Bonferroni post-hoc test. Mixed models will use the appropriate link function according to the type of primary or secondary endpoints measured (continuous, binary) and adjust for time-point (minutes to hours since PCI) and the interaction between randomized arm and time-point, random effects added of site and patient as appropriate. Rate of events will be compared with $\chi 2$ test and hazard ratios calculated with Cox-proportional-hazard model.

Pre-specified substudies

A pre-specified cMRI substudy will be performed in all patients providing consent to this additional study procedure. cMRI will be performed as previously described[31,32]. The relationship with all MRI parameters and platelet inhibition at each time point is to be explored. The primary cMRI endpoints are the Myocardial salvage Index (MSI) which is defined as Myocardial Salvage/Area at risk (MS/AAR) and the extent of intramyocardial haemorrhage. Many other secondary endpoints will be analysed, including microvascular obstruction (MVO), and infarct size. Among the pre-specified sub-analyses, we will explore the relationship between presence and extent of intramyocardial haemorrhage and allocated treatment, degree of platelet inhibition, and time to presentation.

An ECG/Angio substudy will investigate the impact of randomized treatment strategy on electrocardiographic and angiographic parameters. Anonymized ECGs and angiographies will be collected and analysed by a core-lab blinded to treatment strategy. Multiple parameters will be extracted and compared among the groups and associated with platelet reactivity. ECG analysis will be performed as previously described[33].

A PK/PD substudy will be also performed to explore the role of prasugrel inactive metabolite R-95913 and its derivatized active metabolite (R-138727, PAM) and their relationship with PD profiles. According to the kinetics profile, the threshold exposure that is required to determine a meaningful pharmacodynamic response in platelets will be tentatively defined. To this aim, separate and cumulative analysis of kinetics data deriving from each time point and any single patients will be considered along with the maximum platelet response in ADP-induced platelet aggregation, irrespectively of timing of blood sample collection. Time to response, threshold response, and maximum response will be considered.

A cost-effectiveness analysis will be carried out by inputting direct and indirect costs in relation to outcomes assessed both in terms of clinical events, surrogate markers of outcomes, such as ST-segment elevation resolution or infarct size at MRI as well with respect to degree of platelet inhibition.

CLINICAL SIGNIFICANCE AND TRANSLATIONAL OUTLOOK

Ischaemic heart disease is the single most common cause of death worldwide and its frequency is increasing. STEMI remains frequent and is associated with substantial rates of mortality. In the last years, there was a fall in acute and long-term mortality following STEMI in parallel with greater use of reperfusion therapy, PCI, modern antithrombotic therapy, and secondary prevention. However, mortality in STEMI patients remains between 4-12% in-hospital and 10% at 1-year follow-up. Primary PCI is the standard of treatment of almost all STEMI patients and all patients receive DAPT, a combination of aspirin and an oral P2Y12 inhibitor. There is still limited evidence with respect to when the oral P2Y12 inhibitor should be initiated in STEMI patients and the only randomized trial testing pretreatment in these patients with ticagrelor did not show advantages of this strategy compared with administration at the time of PCI. Oral P2Y12 inhibitors are associated to delay in the onset of effect in STEMI patients undergoing primary PCI. Therefore, intravenous antiplatelet agents might be important in these patients to cover the initial gap in platelet inhibition

and help preventing the increased risk of early thrombotic complications. The FABOLUS FASTER study is the first to directly compare the new intravenous P2Y12 inhibitor cangrelor with the intravenous GPI tirofiban and the standard oral P2Y12 inhibitor prasugrel. Moreover, this study will provide additional pharmacokinetic and pharmacodynamic data on the oral assumption of prasugrel through chewed or standard intake of pills that could also be relevant to accelerate the absorption and effect of this drug. The study findings will add new relevant insights in the management of antiplatelet therapy in patients with STEMI undergoing primary PCI and might be also hypothesis-generating for future trials in this field.

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Compliance with Ethical Standards

Ethics Approval: The study was approved by the local Ethics Committees ("Kantonale Ethikkommission Bern (KEK)" for Bern, Switzerland; "Comitato Etico Università Federico II" for Naples and "Comitato Etico Area Vasta Emilia Centro - AVEC" for Ferrara, Italy) and the Italian Agency of the Drug (AIFA).

Human Subjects/Informed Consent Statement: All procedures followed are in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent is obtained from all patients previous to being included in the study.

Conflict of interest:

FG reports research grant support from the European Society of Cardiology (ESC Research grant).

SW reports research and educational grants from Abbott, Amgen, Bayer, BMS, Boston Scientific, Biotronik, CSL Behring, Edwards Lifesciences, Medtronic, Polares and Sinomed, outside the submitted work.

MV reports grants and personal fees from Abbott, Alvimedica, Amgen, Bayer, Bristol-Myers Squibb SA, Coreflow, Daiichi Sankyo, Vifor, Idorsia, Terumo, iVascular and AstraZeneca, outside the submitted work.

Other authors have nothing to disclose.

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Figure Legend

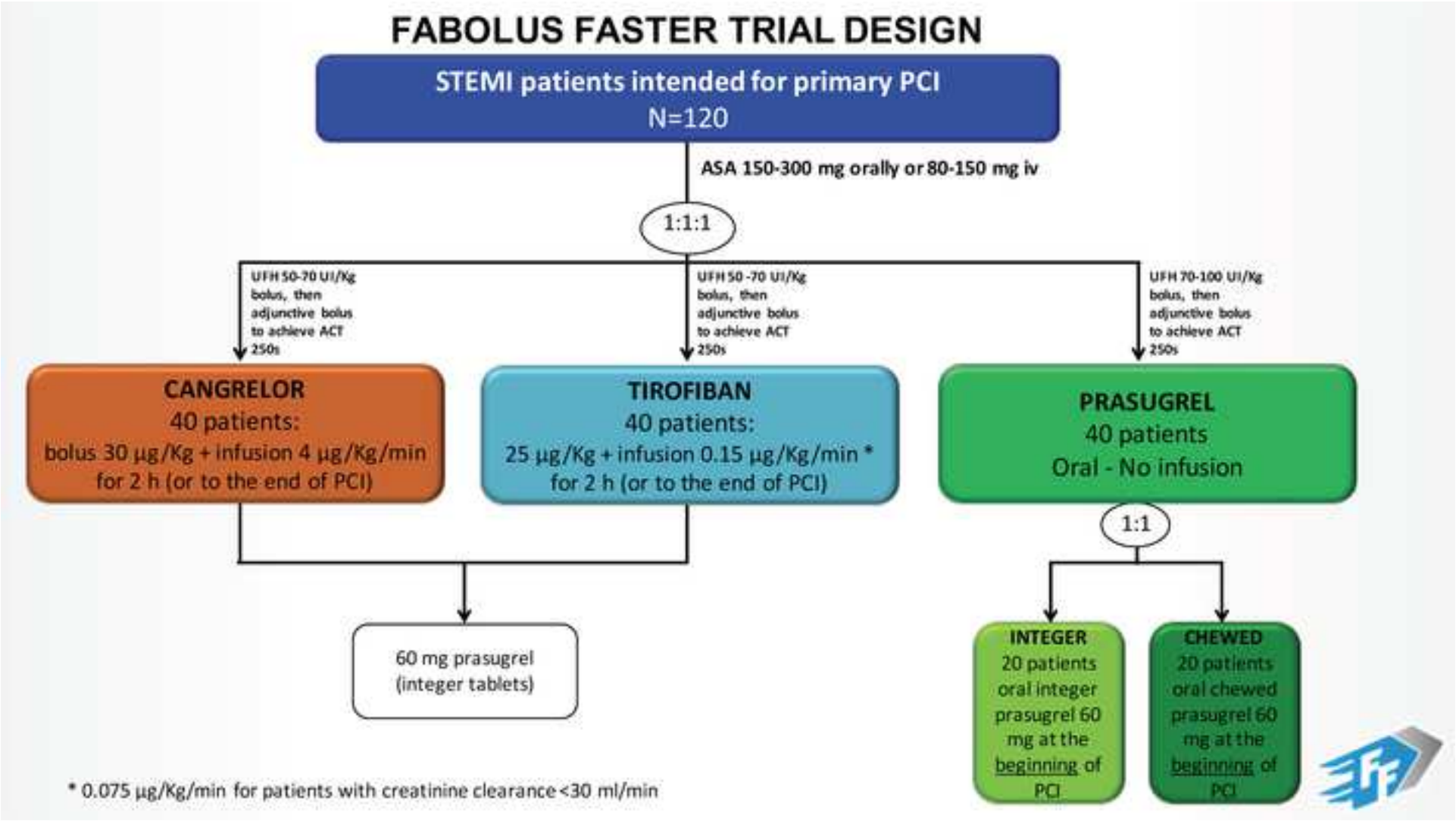
Figure 1. Design of the FABOLUS-FASTER study.

Figure 2. Procedures of the FABOLUS-FASTER study.

Table 1. Inclusion and exclusion criteria of the FABOLUS-FASTER study.

INCLUSION CRITERIA
• Signed Informed Consent
• Age greater than 18 years old
• Have symptoms of acute myocardial ischemia (i.e. new persistent anginal pain) that were lasting at least 20 min with an electrocardiographic ST-segment elevation > 1 mm in 2 or more contiguous ECG leads, or with a new (or presumably new) left bundle branch block or ST segment depression of ≥1 mm in ≥2 of leads V1-3 with a positive terminal T wave
• Referred for primary PCI either within 12 h of symptom onset or between 12 and 24 h after onset with evidence of continuing ischemia
EXCLUSION CRITERIA
• Unconsciousness
• Other conditions that make the patient incapable receiving integer loading dose of prasugrel
• Any contraindication and/or known hypersensitivity or allergy to aspirin, prasugrel, intravenous unfractionated heparin, cangrelor, tirofiban
• Any contraindication to primary PCI
• Administration of GPI or P2Y12-inhibitors or cangrelor < 7 days
• Chronic dialysis
• Recent (< 15 days) or current major bleeding
• Recent (< 15 days) major surgery
• Administration of fibrinolytics < 30 days
• Current use or indication to oral anticoagulant
• Previous stroke or TIA
• Inability to follow the procedures of the study (language problems, psychological disorders, dementia) or comorbidities associated with less than 6 months survival (active malignancies drug or alcohol abuse, etc.)
• Women who are pregnant or breast feeding or with potential to become pregnant during the course of the study (age < 55 years and last menstruation within the last 12 months) and did not undergo tubal ligation, ovariectomy or hysterectomy
• Participation in another study with investigational drug within the 30 days preceding and during the present study
• Enrolment of the investigator, his/her family members, employees and other dependent persons

Figure



Figure