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ORIGINAL ARTICLE

# Platelet $P2Y_{12}$ inhibiting therapy in adjunct to vascular dose of rivaroxaban or aspirin: a pharmacodynamic study of dual pathway inhibition vs. dual antiplatelet therapy

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**Aims** Dual pathway inhibition (DPI) by adding a vascular dose of rivaroxaban to a single antiplatelet agent has emerged as a promising antithrombotic strategy. However, in most studies the antiplatelet agent of choice used in adjunct to a vascular dose of rivaroxaban was aspirin, and data on a $P2Y_{12}$ inhibitor and how this DPI regimen compares with standard dual antiplatelet therapy (DAPT) are limited.

**Methods and results** This investigation was a substudy analysis conducted in selected cohorts of patients with stable atherosclerotic disease enrolled from a larger prospective, open-label, parallel-group pharmacodynamic (PD) study. We analysed data from 40 patients treated with either clopidogrel- or ticagrelor-based DAPT first, and clopidogrel- or ticagrelor-based DPI thereafter. PD measures explored key pathways involved in thrombus formation and included markers of (1) $P2Y_{12}$ reactivity, (2) platelet-mediated global thrombogenicity, (3) cyclooxygenase-1 activity, (4) thrombin receptor-activating peptide (TRAP)-induced platelet aggregation, (5) tissue factor (TF)-induced platelet aggregation, and (6) thrombin generation. Compared with DAPT, on a background of the same $P2Y_{12}$ inhibitor (clopidogrel or ticagrelor), DPI was associated with reduced thrombin generation, increased markers of cyclooxygenase-1 activity and TRAP-induced platelet aggregation, and no differences in markers of $P2Y_{12}$ signalling, platelet-mediated global thrombogenicity, and TF-induced platelet aggregation. In an analysis according to $P2Y_{12}$ inhibitor type, ticagrelor reduced markers of platelet-mediated global thrombogenicity, $P2Y_{12}$ signalling, and rates of high platelet reactivity compared with clopidogrel.

**Conclusion** Compared with DAPT with aspirin and a $P2Y_{12}$ inhibitor, the use of a $P2Y_{12}$ inhibitor in adjunct to a vascular dose of rivaroxaban as part of a DPI strategy is associated with similar effects on platelet-mediated global thrombogenicity but reduced thrombin generation. A DPI strategy with ticagrelor is associated with enhanced antithrombotic efficacy, the clinical implications of which warrant larger scale investigations.

**Clinical trial registration** ClinicalTrials.gov identifier: NCT03718429.

**Keywords** Rivaroxaban • Dual pathway inhibition • Dual antiplatelet therapy • Atherosclerotic disease • Clopidogrel • Ticagrelor • Pharmacodynamic • $P2Y_{12}$ inhibitor

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# Introduction

Aspirin has represented the cornerstone of antiplatelet therapy since the late 1980s given its undisputed benefits in the secondary prevention of cardiovascular disease.[1] About a decade later, oral $P2Y_{12}$ inhibitors were tested in association with aspirin, a strategy called dual antiplatelet therapy (DAPT), showing consistent reduction of both local and systemic arterial thrombotic events compared with aspirin alone.[2,3] Nevertheless, such benefits occurred at the expense of increased bleeding, particularly with the use of more potent $P2Y_{12}$ inhibitors.[2–5] Pharmacodynamic (PD) investigations have shown that in the presence of potent $P2Y_{12}$ blockade aspirin provides limited antithrombotic effects, setting the foundations for clinical investigations evaluating the safety and efficacy of $P2Y_{12}$ inhibitor monotherapy.[6–9] A number of trials have consistently shown that after a brief period of DAPT (i.e. 1–3 months), a strategy of $P2Y_{12}$ inhibitor monotherapy among patients undergoing percutaneous coronary intervention (PCI) reduces bleeding without any trade-off in ischaemic events compared with a standard DAPT regimen (i.e. 12 months).[3,9,10] Moreover, in patients who maintained DAPT for 6–18 months after PCI, chronic treatment with $P2Y_{12}$ inhibitor monotherapy was superior to aspirin monotherapy in preventing future adverse clinical events.[11] Nevertheless, ischaemic recurrences continue to occur long term, particularly among high-risk patients, underscoring the need to identify alternative targets promoting atherothrombotic events.

Thrombin plays a key role in thrombotic as well as inflammatory processes.[12] A strategy of dual pathway inhibition (DPI), achieved by selective anti-Xa inhibition using rivaroxaban at a vascular dose regimen in adjunct to a single antiplatelet agent, reduces the risk of ischaemic recurrences in patients with atherosclerotic disease manifestations.[13,14] Nevertheless, of over 50 000 patients included in randomized controlled trials (RCTs) testing this strategy, only ~3000 have used a DPI strategy with a $P2Y_{12}$ inhibitor rather than aspirin as an antiplatelet agent.[15,16] Although the available trial data from phase II testing conducted in patients with acute coronary syndrome (ACS) showed DPI with a $P2Y_{12}$ inhibitor to have a safety profile similar to that of DAPT, this was underpowered to make any conclusions on efficacy and whether the use of different $P2Y_{12}$ inhibitors among DPI strategies translates into different outcomes remains unknown.[15] To this extent, PD investigations can provide important insights for the rationale of larger scale clinical testing. The aim of this investigation was to assess comparative PD profiles of DPI vs. DAPT regimens, on a background of a $P2Y_{12}$ inhibitor (clopidogrel or ticagrelor), among patients with atherosclerotic disease manifestations.

# Methods

## Study design and patient population

This subgroup analysis was conducted in a selected cohort of 40 patients from a larger scale prospective, open-label, parallel-group PD study enrolling 80 patients with stable atherosclerotic disease, including coronary artery disease (CAD) and peripheral artery disease (PAD) (NCT03718429). A description of the study design has been previously published.[17] In brief, included patients could have been on three different antiplatelet regimens—aspirin, aspirin plus clopidogrel (C-DAPT), or aspirin plus ticagrelor (T-DAPT)—as per standard of care at the discretion of their treating physician. Specific study inclusion and exclusion criteria are provided in the Supplementary material online. Each of these three cohorts was treated with adjunctive vascular dose rivaroxaban (2.5 mg/bid) for 7–10 days, after which aspirin therapy was suspended for 7–10 days. A flow diagram of the study design is illustrated in the Supplementary material online, *Figure S1*. PD assessments were conducted while on standard-of-care antiplatelet therapy (visit 1), 7–10 days after adjunctive treatment with vascular dose rivaroxaban (visit 2), and 7–10 days after dropping aspirin (visit 3). There was also a fourth cohort of patients with atrial fibrillation treated with rivaroxaban 20 mg.

For the purpose of this analysis, comparative PD assessments were conducted in patients on DAPT with either clopidogrel (C-DAPT) or ticagrelor (T-DAPT) and DPI with either clopidogrel (C-DPI) or ticagrelor (T-DPI) (Supplementary material online, *Figure S1*). The study was performed at the University of Florida Health Science Center (Jacksonville, FL, USA). Laboratory personnel performing PD testing were blinded to treatment assignment. Patients were screened and recruited at the outpatient clinics of our institution. The study complied with the Declaration of Helsinki, was approved by the Western Institutional Review Board, and all patients gave their written informed consent.

## Blood sampling and laboratory assessments

Peripheral venous blood samples were drawn through a short venous catheter inserted into a forearm vein and collected in citrate, ethylenediamine tetraacetic acid (EDTA), and serum tubes. To avoid spontaneous platelet activation, the first 2–4 mL of blood was discarded. In order to provide a comprehensive evaluation of different pathways leading to thrombus formation, we used a large number of assays, including vasodilator-stimulated phosphoprotein (VASP), VerifyNow $P2Y_{12}$ reaction unit (PRU), light transmittance aggregometry (LTA), and a thrombin generation assay. A detailed description of the assays is provided in the Supplementary material online, Methods S2, and has been previously reported.[17–20]

PD assessments were performed to provide insights on the following key pathways involved in thrombus formation: (1) $P2Y_{12}$ signalling, by using VerifyNow PRU, VASP, and LTA following stimuli with adenosine diphosphate (ADP) (20 $\mu$mol/L); (2) platelet-mediated global thrombogenicity, by using LTA following stimuli with a combination of 2 $\mu$g/mL collagen-related peptide + 5 $\mu$mol/L ADP + tissue factor (TF)-$CaCl_2$ (CATF), which leads to activation of multiple platelet signalling pathways; (3) cyclooxygenase-1 activity, by using LTA following collagen (3 $\mu$g/mL) and arachidonic acid (AA, 1 mmol/L) stimuli; (4) TF-induced platelet aggregation, by using LTA following TF-$CaCl_2$ stimuli; (5) thrombin-receptor-mediated platelet aggregation, by using LTA following thrombin receptor-activating peptide (TRAP, 15 $\mu$mol/L) stimuli; and (6) thrombin generation. VASP results are reported as platelet reactivity index (PRI, %), Verify Now results as PRUs, LTA results as maximum platelet aggregation (MPA, %), and thrombin generation as lag reaction time (min), peak thrombin ($\mu$M), peak time (min), velocity index, and area under the curve (AUC).

## Study endpoints and sample size calculation

Since there are limited reports exploring the PD effects of vascular dose rivaroxaban in addition to $P2Y_{12}$ inhibiting therapy, we provided a comprehensive assessment of markers of thrombosis by multiple PD assays embracing different key pathways of thrombus formation. The endpoints were the comparisons between DPI vs. DAPT (intragroup) and C-DPI vs. T-DPI (intergroup) of VerifyNow PRU, VASP, LTA following ADP (20 $\mu$mol/L), CATF, TF (TF-$CaCl_2$), AA (1 mmol/L),

**Table 1 Baseline characteristics**

| | Clopidogrel-based therapy ($n = 20$) | Ticagrelor-based therapy ($n = 20$) | *P*-value |
|---|---|---|---|
| Age | 64.4 ± 9.2 | 59.4 ± 8.9 | 0.092 |
| Male | 14 (70%) | 16 (80%) | 0.758 |
| Race | | | 0.476 |
| White | 11 (55%) | 8 (40%) | — |
| Black | 8 (40%) | 12 (60%) | — |
| Other | 1 (5%) | 0 | — |
| BMI | 31.3 ± 6.2 | 29.8 ± 5.5 | 0.443 |
| Current smoker | 16 (80%) | 16 (80%) | 0.985 |
| Hypertension | 20 (100%) | 19 (95%) | 0.562 |
| Diabetes | 12 (60%) | 10 (50%) | 0.848 |
| Dyslipidaemia | 20 (100%) | 18 (90%) | 0.240 |
| Family history of CAD | 6 (30%) | 1 (5%) | 0.044 |
| Peripheral arterial disease | 12 (60%) | 1 (5%) | <0.001 |
| Previous stroke or TIA | 2 (10%) | 2 (10%) | 1 |
| Hepatic dysfunction | 0 | 0 | — |
| Prior myocardial infarction | 9 (45%) | 16 (80%) | 0.015 |
| Prior PCI | 13 (65%) | 19 (95%) | 0.010 |
| Prior CABG | 3 (15%) | 6 (30%) | 0.461 |
| Ejection fraction | 52.4 ± 10.9 | 44.8 ± 11.8 | 0.078 |
| Medications | | | |
| Statins | 20 (100%) | 20 (100%) | 20 (100%) |
| Beta-blockers | 16 (80%) | 20 (100%) | 0.031 |
| ACE/ARB | 18 (90%) | 13 (65%) | 0.067 |
| Nitrates | 5 (25%) | 6 (30%) | 0.969 |
| Calcium channel blockers | 12 (60%) | 8 (40%) | 0.362 |
| Creatinine (mg/dL) | 1.10 ± 0.26 | 1.08 ± 0.36 | 0.853 |
| Platelet count ($\times 10^9$/L) | 233 ± 61 | 327 ± 87 | 0.303 |
| Haematocrit (%) | 40.7 ± 3.9 | 40.4 ± 4.4 | 0.575 |
| Haemoglobin (g/dL) | 13.2 ± 1.3 | 13.5 ± 1.6 | 0.819 |

BMI, body mass index; CAD, coronary artery disease; TIA, transient ischaemic attack; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; ACE, angiotensin-converting enzyme; and ARB, angiotensin II receptor blocker.

collagen (3 $\mu$g/mL), (TRAP, 15 $\mu$mol/L) stimuli, and thrombin generation parameters (lag reaction time, peak thrombin, peak time, velocity index, and AUC). Moreover, given the variability in response to $P2Y_{12}$ inhibitors, particularly clopidogrel, rates of high platelet reactivity (HPR), defined as PRU > 208 assessed by VerifyNow, were assessed according to the drug being used and its impact on other PD markers investigated.[21] In line with recommendations for pilot investigations, and because there were no preliminary data exploring the PD effects of vascular dose rivaroxaban in addition to antiplatelet therapy, we arbitrarily chose a sample size of 20 patients per treatment group.[22] During study treatment, ischaemic cardiac events (including death, myocardial infarction, stroke, and urgent revascularization) and serious adverse events (bleeding and other adverse events) were collected. Bleeding was defined by the Bleeding Academic Research Consortium (BARC) definition.[23]

## Statistical methods

Categorical variables are expressed as frequencies and percentages. Comparisons between categorical variables were performed using two-tailed Fisher's exact test or Pearson's $\chi^2$ test. Continuous variables are presented as mean ± SD. Student's *t* test and the Mann–Whitney *U* test were used to compare continuous variables according to normal distribution tested by the Kolmogorov–Smirnov test. A two-tailed $P < 0.05$ was considered to indicate a statistically significant difference for all the analyses performed. Statistical analysis was performed by our group using SPSS version 25.0 software (SPSS Inc., Chicago, IL, USA).

# Results

## Patient population

A total of 40 patients were included in this analysis: 20 patients treated with clopidogrel and 20 patients treated with ticagrelor. Both groups dropped aspirin and then started vascular dose rivaroxaban (Supplementary material online, *Figure S1*). *Table 1* shows the baseline characteristics. Rates of PAD and family history of coronary artery disease were more common in the clopidogrel group, while previous myocardial infarction and previous percutaneous coronary intervention were more common in the ticagrelor group. There were no clinical ischaemic or BARC 2– 5 bleeding clinical events during the conduct of the present study.

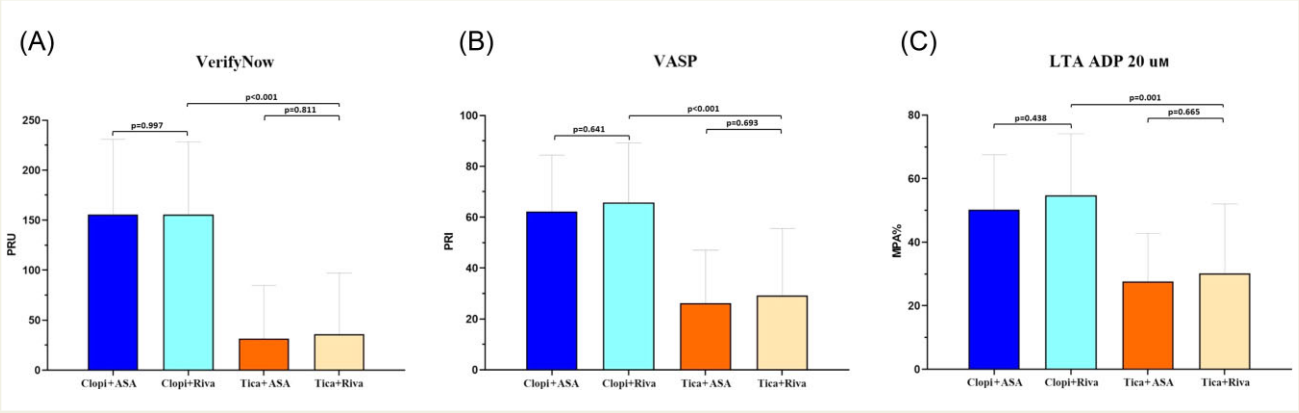


**Figure 1** Markers of $P2Y_{12}$ signalling. (*A*) VerifyNow; (*B*) vasodilator-stimulated phosphoprotein; (*C*) adenosine diphosphate-induced platelet aggregation measured by light transmittance aggregometry. Data are presented as mean; error bars indicate standard deviation. *P*-values refer to intra- and intergroup comparisons. LTA, light transmittance aggregometry; MPA, maximum platelet aggregation; PRI, platelet reactivity index; PRU, $P2Y_{12}$ reaction units; and VASP, vasodilator-stimulated phosphoprotein.

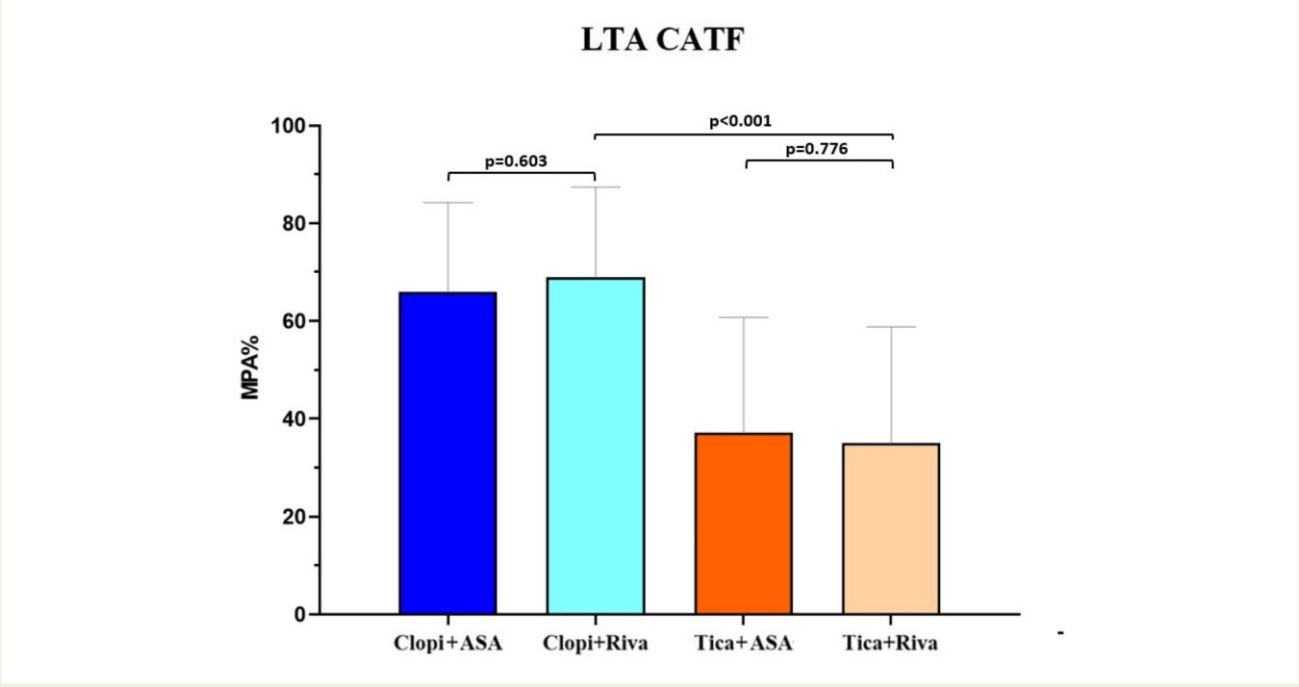


**Figure 2** Platelet-mediated global thrombogenicity as assessed by CATF-induced platelet aggregation. Data are presented as mean; error bars indicate standard deviation. *P*-values refer to intra- and intergroup comparisons. CATF, collagen-related peptide + ADP + TF-CaCl; LTA, light transmittance aggregometry; and MPA, maximum platelet aggregation.

## Pharmacodynamic findings

### Markers of $P2Y_{12}$ signalling and platelet-mediated global thrombogenicity

As compared with DAPT, DPI with either clopidogrel or ticagrelor led to similar levels of PRU ($P = 0.997$ and $P = 0.811$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively), PRI ($P = 0.641$ and $P = 0.693$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively), and ADP-induced MPA ($P = 0.438$ and $P = 0.665$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively). Ticagrelor was associated with lower levels of $P2Y_{12}$ signalling compared with clopidogrel in the setting of both DAPT and DPI (*Figure 1* and Supplementary material online, *Table S1*). There were no differences between DPI and DAPT on platelet-mediated global thrombogenicity as measured by CATF-induced MPA irrespective of $P2Y_{12}$ inhibiting therapy: clopidogrel ($P = 0.603$ for C-DPI vs. C-DAPT) or ticagrelor ($P = 0.776$ for T-DPI vs. T-DAPT) (*Figure 2* and Supplementary material online, *Table S1*).

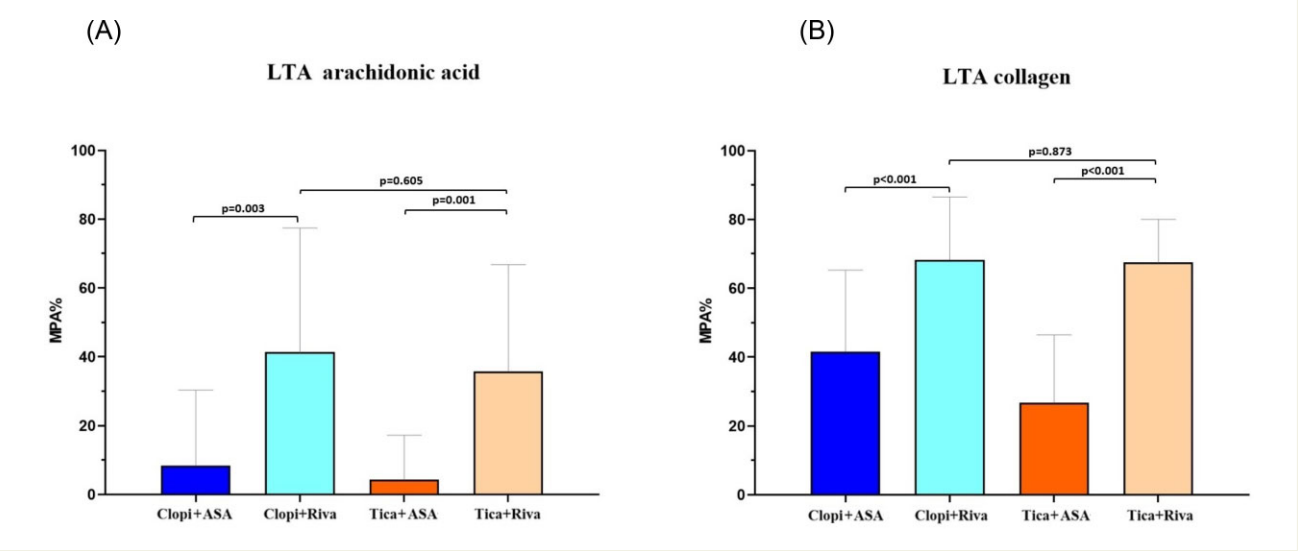


**Figure 3** Markers of cyclooxygenase-1 activity. (*A*) Arachidonic acid-induced aggregation measured by light transmittance aggregometry. (*B*) Collagen-induced aggregation measured by light transmittance aggregometry. Data are presented as mean; error bars indicate standard deviation. *P*-values refer to intra- and intergroup comparisons. LTA, light transmittance aggregometry; and MPA, maximum platelet aggregation.

### Markers of cyclooxygenase-1 activity, tissue factor-induced platelet aggregation and thrombin receptor-activating peptide-induced platelet aggregation

AA-induced MPA and collagen-induced MPA were increased with DPI compared with DAPT ($P = 0.003$ and $P < 0.001$ for C-DPI vs. C-DAPT; $P = 0.001$ and $P < 0.001$ for T-DPI vs. T-DAPT, respectively). There were no differences in these two markers of cyclooxygenase-1 activity between clopidogrel- and ticagrelor-based DPI ($P = 0.605$and $P = 0.873$, respectively) (*Figure 3* and Supplementary material online. *Table S1*).

TF-$CaCl_2$-induced MPA did not differ with either DAPT vs. DPI strategies ($P = 0.568$ and $P = 0.421$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively) or between clopidogrel- and ticagrelor-based DPI ($P = 0.961$) (*Figure 4* and Supplementary material online, *Table S1*).

TRAP-induced MPA was reduced with DAPT compared with DPI ($P = 0.002$ and $P = 0.046$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively). There was no difference in this marker between clopidogrel- and ticagrelor-based DPI ($P = 0.861$) (*Figure 4* and Supplementary material online, *Table S1*).

### Markers of thrombin generation

Compared with DAPT, DPI with either clopidogrel or ticagrelor reduced peak thrombin ($P < 0.001$ for both C-DPI vs. C-DAPT and T-DPI vs. T-DAPT), the thrombin velocity index ($P < 0.001$ for both C-DPI vs. C-DAPT and T-DPI vs. T-DAPT), and the thrombin AUC ($P < 0.001$ and $P = 0.003$, $P < 0.001$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively) (*Figure 5* and Supplementary material online, *Table S1*). Moreover, DPI was associated with increased thrombin lag phase time ($P = 0.007$ and $P = 0.015$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively) and thrombin peak time ($P = 0.024$ and $P = 0.050$ for C-DPI vs. C-DAPT and T-DPI vs. T-DAPT, respectively). Among DPI strategies, there were no differences in markers of thrombin generation between clopidogrel- and ticagrelor-based DPI ($P = 0.068$ for peak thrombin, $P = 0.322$ for thrombin velocity index, $P = 0.077$ for thrombin lag phase time, and $P = 0.122$ for thrombin peak time) except for a reduced thrombin AUC with C-DPI compared with T-DPI ($P = 0.043$) (*Figure 5* and Supplementary material online, *Table S1*).

### High platelet reactivity

HPR rates were significantly higher among clopidogrel- and ticagrelor-based regimens ($P = 0.025$) but did not differ between DPI and DAPT strategies when they were compared on the background of the same $P2Y_{12}$ inhibitor (i.e. C-DPI vs. C-DAPT and T-DPI vs. T-DAPT). Specifically, 12 patients with HPR were found in the overall population and were distributed as follows: C-DAPT = 5, C-DPI = 5, T-DAPT = 1, and T-DPI = 1. The presence of HPR was associated with significantly increased levels of other markers of $P2Y_{12}$ signalling, including ADP-induced MPA and PRI ($P < 0.001$ for both). Moreover, patients with HPR status also had increased CATF- ($P < 0.001$), collagen- ($P = 0.002$), TRAP- ($P = 0.025$), and TF- ($P = 0.028$) induced MPA. HPR status affected neither the AA-induced MPA nor the markers of thrombin generation (Supplementary material online, *Table S2*).

## Discussion

In this PD study comparing DPI vs. DAPT with either clopidogrel or ticagrelor as $P2Y_{12}$ inhibiting therapy, we found that on the background of the same $P2Y_{12}$ inhibitor (clopidogrel or ticagrelor), a DPI strategy using a vascular dose of rivaroxaban (1) was as effective as DAPT in reducing platelet-mediated global thrombogenicity; (2) significantly reduced thrombin generation; (3) did not affect markers

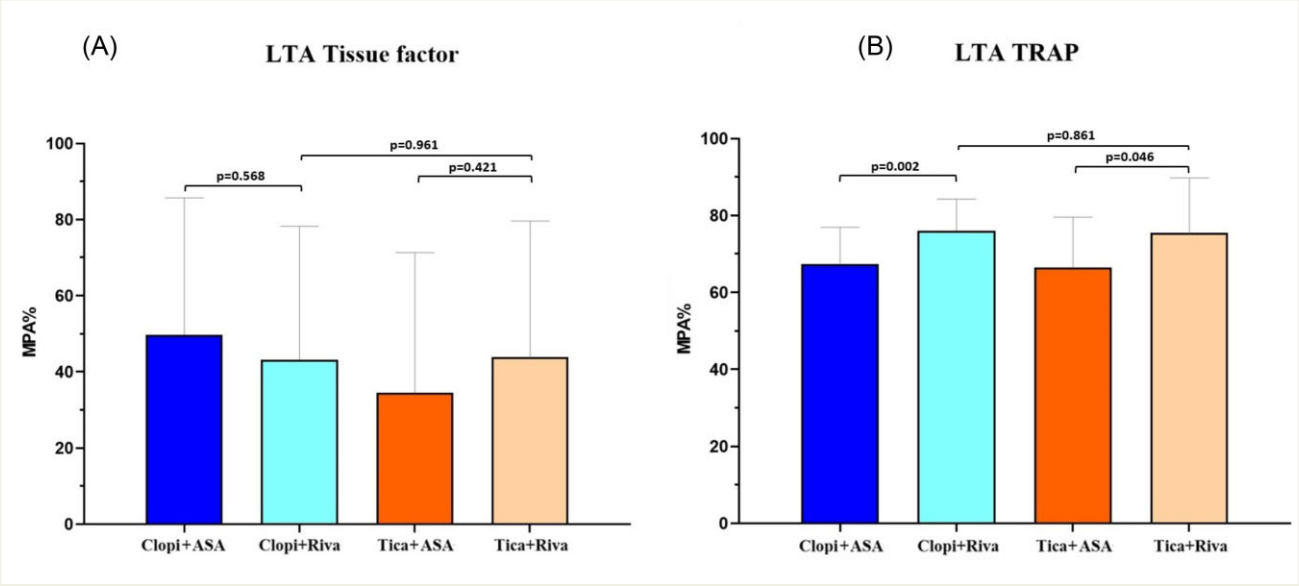


**Figure 4** Tissue factor and thrombin receptor-activating peptide-induced platelet aggregation. (*A*) Tissue factor-induced platelet aggregation measured by light transmittance aggregometry. (*B*) Thrombin receptor-activating peptide-induced maximum platelet aggregation measured by light transmittance aggregometry. Data are presented as mean; error bars indicate standard deviation. *P*-values refer to intra- and intergroup comparisons. LTA, light transmittance aggregometry; MPA, maximum platelet aggregation; and TRAP, thrombin receptor-activating peptide.

of $P2Y_{12}$ signalling and TF-induced platelet aggregation; and (4) was associated with increased markers of cyclooxygenase-1 activity and TRAP-induced platelet aggregation. Moreover, among both DAPT and DPI regimens, a ticagrelor-based strategy reduced markers of $P2Y_{12}$ reactivity, HPR rates, and platelet-mediated global thrombogenicity, compared with clopidogrel-based strategies. *Figure 6* provides a summary of these key PD findings.

The need for less aggressive antithrombotic drug regimens to prevent local ischaemic events with newer generation stent platforms, together with the increased understanding of the prognostic relevance of bleeding events, has sparked the interest for antithrombotic regimens associated with reduced bleeding risk but still able to preserve efficacy by minimizing the risk of thrombotic complications.[24,25] DAPT with aspirin and a $P2Y_{12}$ inhibitor has represented the standard of care in patients undergoing PCI and after completion of DAPT, patients most commonly stop the $P2Y_{12}$ inhibitor and maintain aspirin indefinitely.[26] A $P2Y_{12}$ inhibitor monotherapy has recently emerged as a promising strategy among patients with atherosclerotic disease manifestations.[12] The rationale for investigating $P2Y_{12}$ inhibitor monotherapy stems from the fact that the platelet $P2Y_{12}$ receptor signalling pathway plays a key role in platelet activation and amplification processes, and *in vitro* and *ex vivo* PD investigations suggest that aspirin provides limited antithrombotic effects in addition to potent $P2Y_{12}$ blockade.[6–9] Moreover, aspirin carries a well-established gastrointestinal toxicity leading to increased gastrointestinal bleeding.[8,9] These PD findings have been recently endorsed by a number of clinical studies showing that among patients undergoing PCI, irrespective of clinical presentation (ACS and chronic coronary syndromes), an early withdrawal of aspirin, after either 1 or 3 months, reduced bleeding without any trade-off in ischaemic events.[9,10] Most recently, the HOST-EXAM trial showed that in patients 6–18 months post-PCI, compared with aspirin, clopidogrel significantly reduced the primary composite endpoint of all-cause death, non-fatal myocardial infarction, stroke, readmission due to ACS, and BARC bleeding type 3 or greater during a 24-month follow-up.[11] These findings are in line with the CAPRIE trial reported over 20 years ago showing that long-term administration of clopidogrel to patients with atherosclerotic vascular disease was more effective than aspirin in reducing the combined risk of ischaemic stroke, myocardial infarction, and vascular death and with better gastrointestinal tolerance .[27] However, a major limitation of using clopidogrel monotherapy is the broad range of interpatient responses, leading to HPR, a marker of thrombotic risk, in a considerable number of patients, prompting precision medicine approaches for the selection of antiplatelet therapy.[21,28]

The high rate of ischaemic recurrences among high-risk patients supports the rationale for targeting adjunctive antithrombotic pathways.[12] To this extent, inhibition of thrombin-mediated signalling processes using a vascular dose of rivaroxaban has been proposed in adjunct to antiplatelet therapy, aiming at providing a wider blockade of both thrombotic and inflammatory pathways.[12] Indeed, thrombin not only is an important mediator of the coagulation cascade, but also acts as a potent inducer of platelet activation and is involved in the complex interplay between coagulation and inflammatory pathways, playing a key role in plaque progression and destabilization.[29,30] Importantly, the production of thrombin is considerably elevated in patients who experienced an ACS, and may remain elevated; notably these patients are at risk of recurrent cardiovascular events.[31,32] These findings underscore the potential for investigating strategies modulating thrombin generation in high-risk patients with atherosclerotic disease.[12]

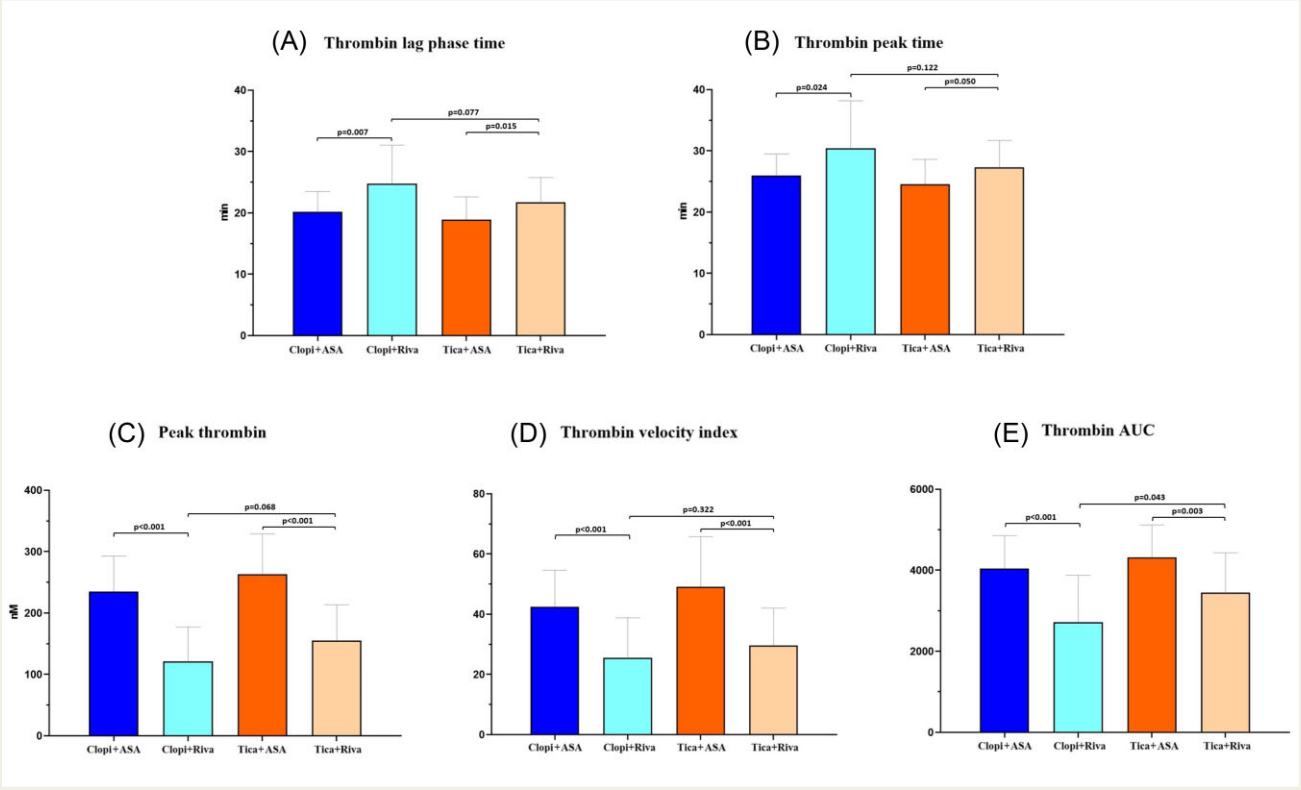


**Figure 5** Markers of thrombin generation. (*A*) Lag phase time; (*B*) peak time; (*C*) peak; (*D*) velocity index; and (*E*) area under the curve. Data are presented as mean; error bars indicate standard deviation. *P*-values refer to intra- and intergroup comparisons. AUC, area under the curve.

A strategy of DPI consisting of low-dose rivaroxaban plus antiplatelet therapy has been evaluated in several RCTs overall, including more than 50 000 patients with atherosclerotic disease manifestations, and has been shown to reduce the risk of ischaemic recurrences but at the expense of increased bleeding.[16] Nevertheless, of the 50 000 patients included in RCTs testing this strategy, only ~3000 have used a DPI strategy with a $P2Y_{12}$ inhibitor in lieu of aspirin.[15,16] The Study to Compare the Safety of Rivaroxaban Versus Acetylsalicylic Acid in Addition to Either Clopidogrel or Ticagrelor Therapy in Participants With Acute Coronary Syndrome 1 (GEMINI ACS 1) was a phase 2 trial published in 2017, randomizing 3037 patients with recent ACS to either aspirin or vascular dose rivaroxaban, on the background of a $P2Y_{12}$ inhibitor.[15] DPI combining a vascular dose of rivaroxaban with a $P2Y_{12}$ inhibitor was found to have a similar risk of clinically significant bleeding as DAPT with aspirin and a $P2Y_{12}$ inhibitor. However, although ischaemic event rates did not differ between treatment arms, the study was underpowered for efficacy.[15] Moreover, the study allowed the use of both clopidogrel and ticagrelor, preventing from assessing possible differences between DPI strategies using different $P2Y_{12}$ inhibitors. Following GEMINI-ACS-1, no other RCTs tested the use of DPI with a $P2Y_{12}$ inhibitor.

In this PD investigation, we provide mechanistic insights on the comparative use of DPI vs. DAPT on the background of the same $P2Y_{12}$ inhibitor. Moreover, we compared DPI strategies on a background of $P2Y_{12}$ inhibitors with different potencies. We found that, compared with DAPT, a $P2Y_{12}$ inhibitor-based DPI showed no differences in platelet-mediated global thrombogenicity but reduced thrombin generation, which is a marker of ischaemic event recurrences in patients with ACS.[31,32] Moreover, ticagrelor-based DPI was associated with lower HPR rates, translating into PD profiles indicative of enhanced antithrombotic efficacy, including reduced platelet-mediated global thrombogenicity, compared with clopidogrel-based DPI. These PD findings make a ticagrelor-based DPI strategy a promising antithrombotic regimen for patients with ACS as it allows for efficacious blockade of both cellular (i.e. platelets) and plasma (i.e. coagulation factors) components leading to thrombus formation, which are particularly amplified in this setting. Importantly, the PD effects specific to a DPI strategy using a $P2Y_{12}$ inhibitor instead of aspirin as well as the favourable safety and efficacy of aspirin-free approaches in large-scale clinical testing offset the concerns associated with the increase in markers of cyclooxygenase-1 activity and TRAP-induced platelet aggregation.[6–9] This is in line with a prior PD report showing that a DPI-based regimen using aspirin and vascular dose rivaroxaban did not result in any differences in platelet-mediated global thrombogenicity compared with DAPT using aspirin and clopidogrel with or without vascular dose rivaroxaban.[17] Collectively, these findings highlight the favourable PD profiles associated with a $P2Y_{12}$ inhibitor-based DPI regimen and provide important insights for the design of future clinical studies. The use of novel approaches to modulate coagulation processes by means of factor XI inhibition in combination with antiplatelet therapies is an ongoing area of large-scale clinical testing, including among patients with ACS.

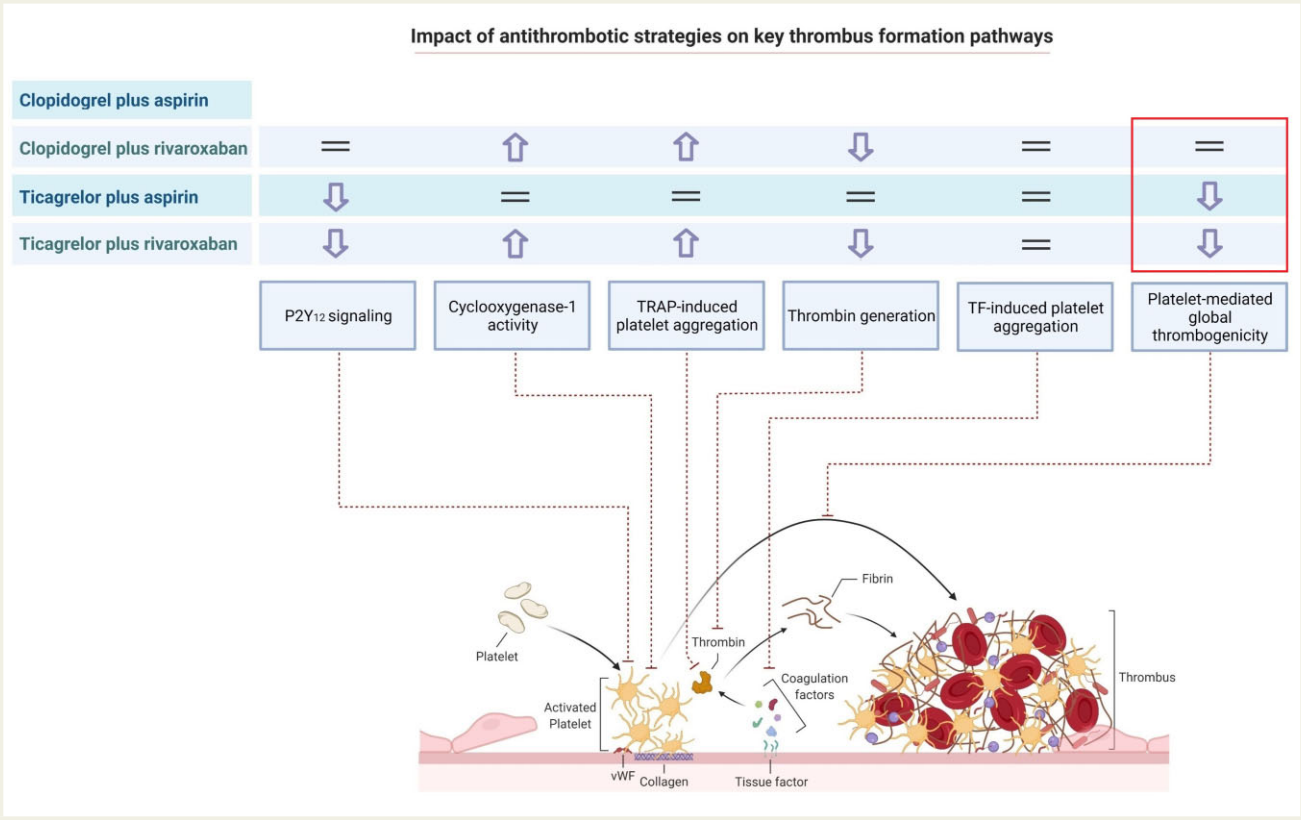


**Figure 6** Overall impact of tested antithrombotic strategies on key pathways of thrombus formation. Dual antiplatelet therapy with aspirin and clopidogrel was used as reference treatment. TF, tissue factor; vWF, von Willebrand factor; and TRAP, thrombin receptor-activating peptide.

*In vitro* and animal studies have suggested that rivaroxaban and ticagrelor may play an important role in reducing TF-induced platelet aggregation.[33] Indeed, TF is a potent initiator of the coagulation cascade and thrombin formation, and its inhibition has been proposed to support the potential clinical benefit of direct anti-X blockade vs. direct thrombin inhibitors (i.e. anti-II).[34] Nevertheless, in our study, neither the use of rivaroxaban vs. aspirin nor the use of ticagrelor vs. clopidogrel was associated with reduced TF-induced platelet aggregation. This may be attributed to the fact that we did not include monotherapy treatments (such as rivaroxaban or ticagrelor alone) and our findings on TF-induced platelet aggregation could have been blunted by the concomitant use of other antithrombotic agents. Moreover, plasma concentrations achieved by antithrombotic agents in our *in vivo* study conducted in human subjects may be lower compared with those used in prior experimental models.[33]

## Study limitations

This analysis has several limitations. First, the PD nature of this analysis does not allow us to draw any conclusions on clinical outcomes of the included antithrombotic regimens. However, this represents the only PD investigation that provides insights into how a DPI regimen using a $P2Y_{12}$ inhibitor compares with standard-of-care DAPT among patients with atherosclerotic disease manifestations. Second, although this was a prospective investigation, treatments were not randomized and differences in baseline characteristics could potentially impact PD measurements. Nevertheless, given the design of the study, the cohort of patients undergoing DPI was the same as those undergoing DAPT, eliminating the risk of such bias for intragroup comparisons. With regard to the intergroup comparisons, there were some differences in baseline characteristics that, however, reflect current recommendations for the use of clopidogrel and ticagrelor in patients with CAD or PAD. Third, it may be argued that the treatment duration with adjunctive rivaroxaban and aspirin withdrawal was limited; nevertheless, this was sufficient to achieve its full effects for the purpose of this PD investigation. Fourth, in this prospective PD investigation, we included patients with stable atherosclerotic disease manifestations and patients needed to be at least 3 months from their ACS if they had experienced one to be eligible for enrolment. Since the thrombotic milieu of a patient who experienced a recent ACS differs from that of a patient who has been stabilized, this could have led to an underestimation of the PD effects of the treatment regimens, including HPR rates, studied in our investigation.

# Conclusions

The use of a $P2Y_{12}$ inhibitor in a DPI strategy (i.e. in combination with a vascular dose of rivaroxaban) is associated with similar effects on platelet-mediated global thrombogenicity but reduced thrombin generation, as compared with the standard-of-care DAPT.

A ticagrelor-based DPI regimen is associated with lower HPR rates, translating into PD profiles indicative of enhanced antithrombotic efficacy, including reduced platelet-mediated global thrombogenicity, compared with a clopidogrel-based DPI. These PD findings provide important insights into the use of a $P2Y_{12}$ inhibitor-based DPI as compared with standard DAPT strategies, the clinical implications of which warrant investigation in larger scale studies.

# Supplementary material

Supplementary material is available at *European Heart Journal—Quality of Care and Clinical Outcomes* online.

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**Conflict of interest** F.F. declares that he has received payment as an individual for consulting fee or honorarium from AstraZeneca, Bayer, and Sanofi, and institutional payments for grants from PLx Pharma and The Scott R. MacKenzie Foundation. F.R. declares that he has received honoraria from Chiesi. T.G. declares that he has received research grants from Bristol Myers Squibb, Bayer, Daiichi Sankyo, and Ferrer/Chiesi. T.G. also declares that he has received consulting fees or honoraria from Amgen, AstraZeneca, Bristol Myers Squibb, Bayer, Ferrer/Chiesi, and Pfizer. L.K.J. declares that she has received consulting fees or honoraria from AstraZeneca, Bayer, Sanofi, Bristol Myers Squibb, Janssen, Portola, and PhaseBio, and her company has received grants from AstraZeneca, Bristol Myers Squibb, Bayer, Janssen, and Portola. D.J.A. declares that he has received consulting fees or honoraria from Abbott, Amgen, AstraZeneca, Bayer, Biosensors, Boehringer Ingelheim, Bristol Myers Squibb, Chiesi, Daiichi Sankyo, Eli Lilly, Haemonetics, Janssen, Merck, PhaseBio, PLx Pharma, Pfizer, and Sanofi, and his institution has received research grants from Amgen, AstraZeneca, Bayer, Biosensors, CeloNova, CSL Behring, Daiichi Sankyo, Eisai, Eli Lilly, Gilead, Janssen, Matsutani Chemical Industry Co., Merck, Novartis, Osprey Medical, Renal Guard Solutions, and The Scott R. MacKenzie Foundation. The remaining authors report no conflict of interest.

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