Overview of pleiotropic effects of platelet P2Y12 receptor inhibitors

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Summary

Dual antiplatelet therapy consisting of one of the P2Y12 receptor inhibitors in conjunction with aspirin is the mainstay of treatment for patients with acute coronary syndromes (ACS) and those undergoing percutaneous coronary interventions (PCI). In recent years, multiple extra-platelet features of P2Y12 receptor antagonists have been reported in numerous clinical trials. The aim of this review is to summarise reported pleiotropic effects of clopidogrel, prasugrel, ticagrelor and other P2Y12 receptor blockers. We included observations made both in human and in animal models, together with proposed mechanisms of action for described features. If confirmed in randomised studies and properly applied to everyday practice, the observed extra-platelet actions could enable us to improve efficacy of ACS and post-PCI treatment, as well as to confine mortality and occurrence rate of cardiovascular events.

Keywords

Clopidogrel, off-target effect, P2Y12 receptor, prasugrel, ticagrelor

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Introduction

Dual antiplatelet therapy, consisting of P2Y12 receptor inhibitor and aspirin, represents the cornerstone of treatment for patients with acute coronary syndromes (ACS) and those undergoing percutaneous coronary interventions (PCI). The P2Y12 receptor is a Gi-coupled adenosine diphosphate (ADP) receptor (►Figure 1), which plays a pivotal role in platelet activation and aggregation. Stimulation of platelet P2Y12 receptor by ADP leads to a complex cascade of actions, which can result in arterial atherothrombosis, an important cause of adverse cardiovascular events. That is why the P2Y12 receptor has become an important molecular target in the development of antithrombotic drugs (1).

Since 1991, when ticlopidine became the first P2Y12 antagonist ever approved by the Food and Drug Administration (FDA), several subsequent P2Y12 blockers have been introduced to clinical use. P2Y12 receptor inhibitors can be classified based on their chemical structure. Thienopyridine group includes ticlopidine, clopidogrel and prasugrel, all of which are orally administered prodrugs leading to irreversible P2Y12 receptor inhibition. The second group - non-thienopyridine derivatives (ticagrelor, cangrelor and elinogrel) - provides reversible blockade of the P2Y12 receptor and, in contrast to the former, does not require hepatic bioactivation (2-10) (►Table 1). There is substantial evidence that these compounds also exert extra-platelet effects. The potential explanation is that apart from platelets, the P2Y12 receptor can also be found in a wide variety of tissues including certain subregions of the brain (11), vascular smooth muscle cells (12, 13), leukocytes (14), macrophages (15), microglial (16) and dendritic cells (17), thus increasing the amount of potential effectors for P2Y12 blockers (1) (►Figure 2). Furthermore, the pleiotropic effects of P2Y12 receptor inhibitors may also result from mechanisms different than their interaction with the P2Y12 receptor.

Inflammatory processes, endothelial function, vascular tone, adenosine plasma levels and cardioprotection were reported to be affected by P2Y12 blockers (12, 18-36). These off-target actions can be beneficial, but might also be associated with adverse side effects. The antiplatelet activity of clopidogrel, prasugrel, ticagrelor and other P2Y12 receptor antagonists, is well known and indisputable, however the pleiotropic effects of these substances, although observed in numerous studies, still warrant further extensive investigation to determine their clinical significance and detailed underlying mechanisms.

The aim of this review is to summarise available knowledge regarding the pleiotropic effects of P2Y12 receptor inhibitors. We included observations made both in human and in animal models, together with proposed mechanisms of action for described features.

A search covering the period from January 1991 through January 2014 was conducted by two independent investigators using MEDLINE, CENTRAL and Google Scholar databases. Proceedings from the Scientific Sessions of the American College of Car-

diology (http://www.acc.org), American Heart Association (http://www.heart.org), European Society of Cardiology (http://www.escardio.org), Transcatheter Cardiovascular Therapeutics (http://www.tctmd.com) and EuroPCR (http://www.europcr.com) were also considered. The following keywords were applied: 'antiplatelet therapy', 'cangrelor', 'clopidogrel', 'elinogrel', 'off-target', 'prasugrel', 'pleiotropic', and 'ticagrelor'. References of retrieved studies were searched manually for additional studies and reviews. No language restrictions were applied.

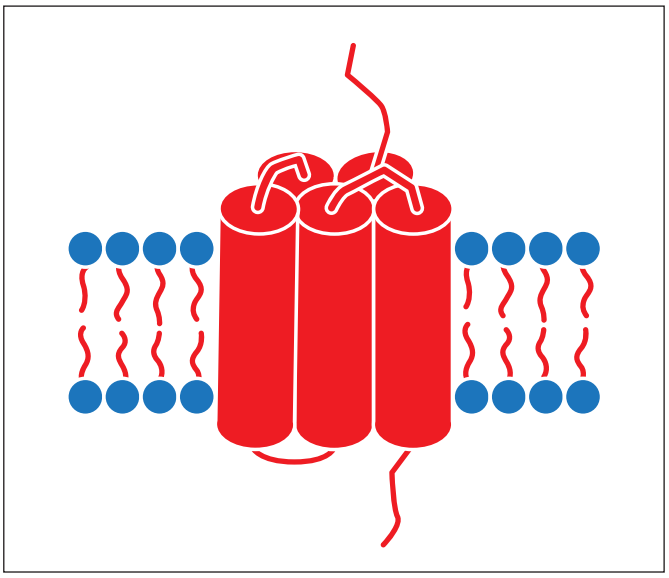

Figure 1: The P2Y12 receptor.

Ticagrelor and adenosine

A significant reduction in a composite of death from vascular causes, myocardial infarction or stroke, without concomitant increase in overall major bleeding complications was observed in the ticagrelor arm of the PLATO trial, a multicentre, randomised study comparing ticagrelor with clopidogrel in a broad spectrum of ACS patients (3). The unprecedented disproportion between robust mortality benefits observed in the PLATO trial and only a moderate decrease in the occurrence of myocardial infarction implied possible existence of clinically relevant pleiotropic effects of ticagrelor (▶Table 2) (37, 38). Moreover, the subsequent analysis of causes of deaths in the PLATO trial revealed that significantly fewer deaths attributed to sepsis and pulmonary adverse events occurred in the ticagrelor group compared with the clopidogrel group (39).

On the other hand, dyspnoea and ventricular pauses together with transient elevations in uric acid and creatinine concentrations are known adverse effects of ticagrelor (▶Table 3). Dyspnoea related to ticagrelor is not caused by impaired cardiac or pulmonary function, is usually transient, of mild or moderate severity and tends to occur early in the course of treatment (40). Although premature discontinuation of therapy due to dyspnoea was infrequent in the PLATO trial, it occurred significantly more commonly in patients treated with ticagrelor than in those receiving clopidogrel (41). In the same study, ventricular pauses occurred more often in ticagrelor-treated patients. However, they were predominantly asymptomatic, nocturnal and deprived of clinical consequences (42).

The aforenamed side effects are also typical for adenosine. Hence, it has been hypothesised that dyspnoea and ventricular pauses observed in ticagrelor-treated patients, could be caused by increased adenosine plasma levels. Van Giezen et al. demonstrated in a canine model that ticagrelor inhibits adenosine reuptake in erythrocytes, leading to a rise of its blood concentration and to a significant dose-dependent increase in adenosine-mediated coronary blood flow (28). A similar response to adenosine in the presence of ticagrelor was later confirmed in human subjects. The maintenance dose of ticagrelor was reported to augment adenosine-induced coronary blood flow velocity in non–ST-elevation ACS patients undergoing PCI (29). Moreover, Bonello et al. have demonstrated that ticagrelor significantly increases adenosine plasma concentration compared with clopidogrel in ACS patients

Table 1: Characteristics of P2Y12 receptor antagonists.

	Clopidogrel	Prasugrel	Ticagrelor	Cangrelor
Chemical class	thienopyridine	thienopyridine	cyclopentyl-triazolo-pyrimidine	ATP analogue
Routes	oral	oral	oral	intravenous
Prodrug	yes (requires hepatic cytochrome P450 activation)	yes (requires hepatic cytochrome P450 activation)	no	no
Standard dosage	300 mg/600 mg loading dose	60 mg loading dose	180 mg loading dose	30 µg/kg bolus*
	75 mg qd	10 mg qd	90 mg bid	4 µg/kg/min*
Reversibility of binding	irreversible	irreversible	reversible	reversible
Excretion	50% renal, 46% biliary	68% renal, 27% feces	biliary	not dependent on hepatic or renal clearance mechanisms

ATP, adenosine triphosphate; bid, twice a day; qd, once a day. * doses used in the CHAMPION PHOENIX trial.

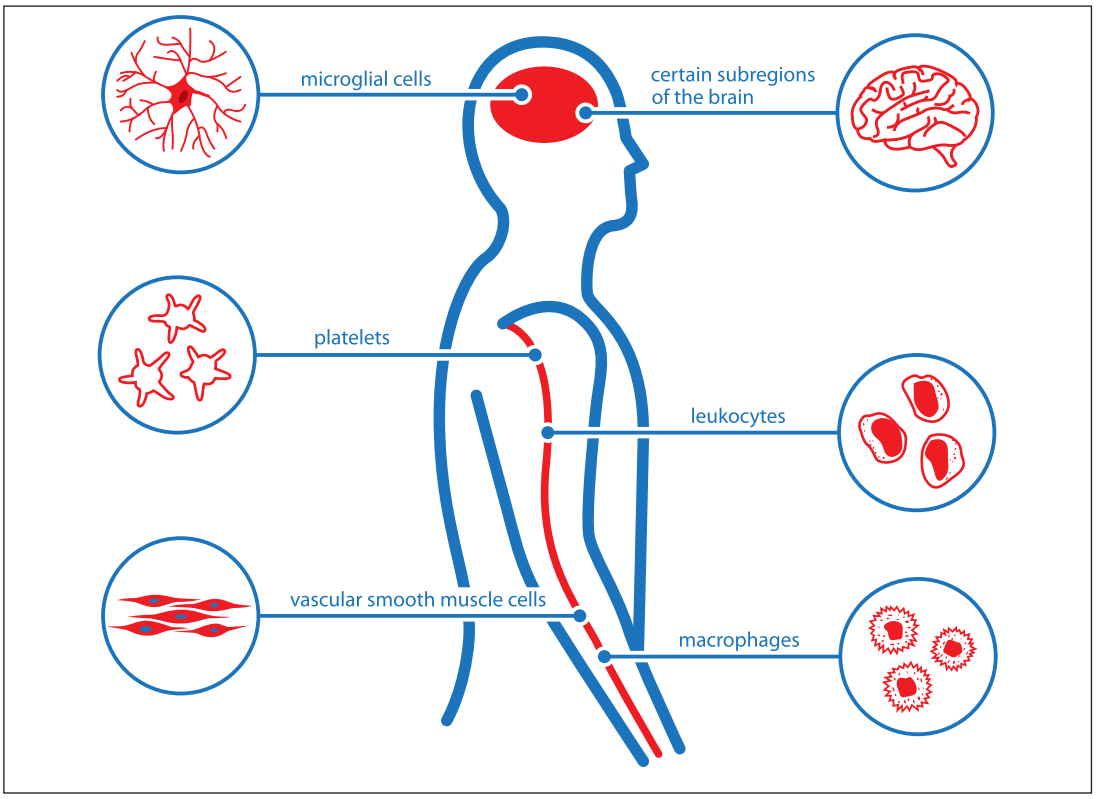

Figure 2: Tissue distribution of the P2Y12 receptor.

(27). Additionally, in the same study serum containing ticagrelor inhibited adenosine uptake by red blood cells compared with clopidogrel or controls. The authors suggested that this feature of ticagrelor is independent of P2Y12 receptor blockade. Similarly to the maintenance dose of ticagrelor, a single 180 mg loading dose of the drug also enhanced adenosine-induced coronary blood flow velocity in a double-blind, placebo-controlled, randomised study including 40 healthy male subjects (30). The fact that this potentially beneficial effect, as well as the increased sensation of adenosine-induced dyspnoea, can be reversed with theophylline, an adenosine receptor antagonist, indicates that these actions are mediated by adenosine receptors (30).

On the other hand, Cattaneo and Faioni hypothesised that the increased incidence of dyspnoea could be a result of inhibition of P2Y12 receptors on sensory neurons, especially when reversible P2Y12 inhibitors, like ticagrelor, cangrelor and elinogrel, are used. The blockade of neural P2Y12 receptors by reversible inhibitors is continuous, because the plasma drug concentration is maintained

Table 2: Incidence of major efficacy end points in the PLATO trial.

Endpoint	Ticagrelor, 90 mg bid	Clopidogrel, 75 mg qd	HR (95% CI)	P-value	NNT (95% CI)
	n=9333	n=9291			
	Patients with events, n (%)	Patients with events, n (%)			
Primary end point: death from vascular causes, myocardial infarction, or stroke	864 (9.8)	1014 (11.7)	0.84 (0.77–0.92)	<0.001	60.4 (39.7–126.3)
Death from vascular causes	353 (4.0)	442 (5.1)	0.79 (0.69–0.91)	0.001	102.6 (64.3–253.5)
Myocardial infarction	504 (5.8)	593 (6.9)	0.84 (0.75–0.95)	0.005	101.8 (60.3–326.6)
Stroke	125 (1.5)	106 (1.3)	1.17 (0.91–1.52)	0.22	na
Death from any cause	399 (4.5)	506 (5.9)	0.78 (0.69–0.89)	<0.001	85.4 (55.9–180.6)

bid, twice a day; CI, confidence interval; HR, hazard ratio; na, not applicable; NNT, number needed to treat; qd, once a day. NNT was calculated exclusively for differences that were statistically significant.

Table 3: The incidence of non-cardiac adverse events and premature discontinuation of the study drug in major randomised clinical trials on novel P2Y12 receptor inhibitors.

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
PLATO (3)	STEMI scheduled for primary PCI or moderate-to-high risk NSTE-ACS regardless of the treatment strategy	Ticagrelor: 180 mg loading dose, then 90 mg bid maintenance; additional 90 mg recommended pre-PCI performed >24 h post-randomisation	Clopidogrel: if pre-treated, no additional loading dose; if naive, standard 300 mg loading dose, then 75 mg qd maintenance; additional 300 mg allowed pre PCI	n=9333	n=9291	up to 12 months; median exposure to study drug 277 days	Dyspnoea	1270/9235 (13.8%)	721/9186 (7.8%)	<0.001	Overall premature discontinuation	2186/9333 (23.4%)	1999/9291 (21.5%)	0.002
							Bradycardia requiring pacemaker insertion	82/9235 (0.9%)	79/9186 (0.9%)	ns	Discontinuation of the study drug due to adverse events	690/9333 (7.4%)	556/9291 (6.0%)	<0.001
							Bradycardia leading to syncope	100/9235 (1.1%)	76/9186 (0.8%)	ns	Discontinuation of the study drug due to dyspnoea	79/9235 (0.9%)	13/9186 (0.1%)	<0.001
							Clinically relevant bradycardia	409/9235 (4.4%)	372/9186 (4.0%)	ns				
							Heart block	67/9235 (0.7%)	66/9186 (0.7%)	ns				
							Ventricular pauses ≥3 sec at 7-day Holter monitoring initiated at randomisation	84/1451 (5.8)	51/1415 (3.6)	0.01				
							Ventricular pauses ≥5 sec at 7-day Holter monitoring initiated at randomisation	29/1451 (2.0)	17/1415 (1.2)	ns				
							Ventricular pauses ≥3 sec at 7-day Holter monitoring initiated at 30 days	21/985 (2.1%)	17/1006 (1.7%)	ns				
							Ventricular pauses ≥5 sec at 7-day Holter monitoring initiated at 30 days	8/985 (0.8)	6/1006 (0.6)	ns				

Table 3: Continued

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
							Any neoplasm arising during treatment	132/9235 (1.4%)	155/9186 (1.7%)	ns				
							Malignant neoplasm arising during treatment	115/9235 (1.2%)	121/9186 (1.3%)	ns				
							Benign neoplasm arising during treatment	18/9235 (0.2%)	35/9186 (0.4%)	0.02				
							Increase in serum uric acid from baseline value at 1 month of treatment	14 ± 46%	7 ± 44%	<0.001				
							Increase in serum uric acid from baseline value at 12 months of treatment	15 ± 52%	7 ± 31%	<0.001				
							Increase in serum uric acid from baseline value at 1 month after end of treatment	7 ± 43%	8 ± 48%	ns				
							Increase in serum creatinine from baseline value at 1 month of treatment	10 ± 22%	8 ± 21%	<0.001				
							Increase in serum creatinine from baseline value at 12 months of treatment	11 ± 22%	9 ± 22%	<0.001				

Table 3: Continued

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
							Increase in serum creatinine from baseline value at 1 month after end of treatment	10 ± 22%	10 ± 22%	ns				
							Pulmonary adverse event* on-treatment^	275/9235 (3.0%)	331/9186 (3.6%)	0.019				
							Death after pulmonary adverse event on-treatment^	33/9235 (0.4%)	71/9186 (0.8%)	<0.001				
							Death after pulmonary adverse event with continuing study drug^	10/9235 (0.1%)	43/9186 (0.5%)	<0.001				
							Death attributed to sepsis by the investigator's assessment	7/9235 (0.1%)	23/9186 (0.3%)	0.003				
							Neutrophil count at randomisation	7.05 ± 3.10 x 10^9/L (n=3925)	7.05 ± 3.05 x 10^9/L (n=3919)	ns				
							Neutrophil count at 1 month of treatment with the study drug	5.01 ± 1.67 x 10^9/L (n=3563)	4.66 ± 1.63 x 10^9/L (n=3583)	<0.0001				
							Neutrophil count at 3 months of treatment with the study drug	4.85 ± 1.63 x 10^9/L (n=3155)	4.57 ± 1.50 x 10^9/L (n=3188)	<0.0001				

Table 3: Continued

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
							Neutrophil count at 6 months of treatment with the study drug	4.79 ± 1.57 x 10^9/L (n=2560)	4.53 ± 1.47 x 10^9/L (n=2525)	<0.0001				
							Neutrophil count at 1 month post discontinuation of the study drug	4.65 ± 1.63 x 10^9/L (n=2242)	4.74 ± 1.68 x 10^9/L (n=2252)	0.044				
TRITON-TIMI 38 (4)	ACS patients (moderate-to-high-risk NSTE-ACS or STEMI) with scheduled PCI	Prasugrel: 60 mg loading dose, then 10 mg qd maintenance	Clopidogrel: 300 mg loading dose, then 75 mg qd maintenance	n=6813	n=6795	up to 15 months; median exposure to study drug 14.5 months	Overall serious adverse events not related to haemorrhage	22.5%#	22.8%#	ns	Discontinuation of the study drug due to adverse events not related to haemorrhage	4.7%#	5.0%#	ns
							Severe thrombocytopenia	17/6813 (0.3%)	18/6795 (0.3%)	ns	Discontinuation of the study drug due to adverse events related to haemorrhage	2.5%#	1.4%#	<0.001
							Severe neutropenia	2/6813 (<0.1%)	10/6795 (0.2%)	0.02				
							All newly diagnosed cancers	88/6741 (1.3%)	67/6716 (1.0%)	0.094				
							Newly diagnosed cancers excluding colonic cancers	69/6741 (1.0%)	57/6716 (0.85%)	0.289				
							Newly diagnosed colonic cancer	13/6813 (0.2%)	4/6795 (0.1%)	0.03				

Table 3: Continued

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
TRILOGY ACS (5)	NSTE-ACS patients treated medically without revascularisation within 10 days after the index event	Prasugrel: 30 mg loading dose, then 10 mg qd maintenance in patients <75 years or 5 mg qd maintenance in patients ≥75 years	Clopidogrel: 300 mg loading dose, then 75 mg qd maintenance	n= 4663	n= 4663	up to 30 months; median exposure to study drug 14.8 months	Newly diagnosed nonbenign neoplasms	1.9%#	1.8%#	0.79				
CHAMPION PCI (6)	stable angina, NSTE-ACS, or STEMI with scheduled PCI	Cangrelor: 30 µg/kg IV bolus 30 min before PCI, followed by 4 µg/kg/min infusion for ≥2 h or the duration of PCI, whichever was longer, then clopidogrel: 600 mg loading dose at discontinuation of cangrelor infusion	Clopidogrel: 600 mg loading dose 30 min before PCI	n= 4367	n= 4341	Primary study end point reported at 48 h; secondary study end points reported at 48 h and at 30 days	Dyspnoea	1.0%#	0.4%#	0.001	Discontinuation of the study drug due to adverse events	0.5%#	0.5%#	na
CHAMPION PLATFORM (7)	NSTE-ACS P2Y12 inhibitor naive patients with scheduled PCI	Cangrelor: 30 µg/kg IV bolus followed by 4 µg/kg/min infusion for ≥ 2 h or the duration of the procedure, whichever was longer, then clopidogrel: 600 mg loading dose at discontinuation of cangrelor infusion	Clopidogrel: 600 mg loading dose immediately after PCI	n=2654	n=2645	Primary and secondary study end points reported at 48 h; adverse events reported at 48 h	Dyspnoea	37/2662 (1.4%)	14/2650 (0.5%)	0.002	Rates of discontinuation of the study drug were not reported by the study investigators			

Table 3: Continued

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
CHAMPION PHOENIX (8)	stable angina, NSTE-ACS and STEMI P2Y12 inhibitor naive patients who required PCI	Cangrelor: 30 µg/kg iv bolus followed by 4 µg/kg/min infusion for ≥ 2 h or the duration of the procedure, whichever was longer, then clopidogrel: 600 mg loading dose at discontinuation of cangrelor infusion, then 75 mg qd maintenance during first 48 h; thereafter, P2Y12 inhibitor per physician	Clopidogrel: 300 mg or 600 mg loading dose at the start or at the end of PCI; then 75 mg qd maintenance during first 48 h; thereafter, P2Y12 inhibitor per physician	n=5529	n=5527	Primary study end point and adverse events reported at 48 h; secondary study end points reported at 48 h and at 30 days	Dyspnoea	64/5529 (1.2%)	18/5527 (0.3%)	<0.001	Discontinuation of the study drug due to adverse events related to treatment	0.5%#	0.4%#	0.21
CHAMPION pooled safety population (9)				n=12565	n=12542		Dyspnoea	143/12565 (1.1%)	48/12542 (0.4%)	<0.0001	Discontinuation of the study drug due to adverse events	74/12565 (0.6%)	51/12542 (0.4%)	0.0402
											Discontinuation of the study drug due to dyspnoea	8/12565 (0.1%)	0/12542	0.0078

Table 3: Continued

Study name	Clinical setting	Study drug design		Number of participants		Follow-up time	Non-cardiac adverse events				Premature discontinuation of study drug			
		Experimental group	Control group	Experimental group	Control group		Type	Experimental group	Control group	p	Type	Rate in the experimental group	Rate in the control group	p
INNOVATE-PCI (10)	patients undergoing non-urgent PCI	Elinogrel: 80 or 120 mg loading dose IV, then 50, 100, or 150 mg PO bid maintenance	Clopidogrel: 300 mg or 600 mg loading dose, then 75 mg qd maintenance for up to 120 days	n=408 (elinogrel pooled)	n=208	up to 124 days; median exposure to study drug 117 days	Dyspnoea	50/408 (12.3%)	8/208 (3.8%)	0.0007	Overall premature discontinuation of the study drug	73/408 (17.9%)	30/208 (14.4%)	0.27
											Discontinuation of the study drug due to adverse events	30/408 (7.4%)	15/208 (7.2%)	0.95
											Discontinuation of the study drug due to dyspnoea	4/408 (0.1%)	nr	na

ACS, acute coronary syndrome; bid, twice a day; IV, intravenous; na, not applicable; nr, not reported; ns, not significant; NSTE-ACS, non-ST-elevation acute coronary syndrome; PCI, percutaneous coronary intervention; PO, per os; qd, once a day; STEMI, ST-elevation myocardial infarction. * Pulmonary adverse events include diagnoses of pneumonia, lower respiratory tract infection, lung infection, respiratory failure or any pleural disorder. ^ "on-treatment" means within 7 days of last dose; "continuing study drug" means dose given on third day after onset of adverse event; "after" means occurring after onset of the adverse event. # Crude numbers are not provided by the investigators.

by repeated dosing, whereas clopidogrel's interaction with the P2Y12 receptor on neurons may be temporary and transient, because these cells, unlike platelets, have a nucleus and are therefore able to rapidly replace the inhibited receptors with newly synthesised ones (43).

An alternative mechanism, possibly responsible for the potential adenosine-mediated off-target effects of ticagrelor, is adenosine triphosphate (ATP) release from red blood cells exposed to this substance. This phenomenon was observed by Öhman et al. in human erythrocytes examined *in vitro* (31). In circulation ATP is subsequently degraded to adenosine by enzymes present in endothelial cells, white blood cells and red blood cells themselves. Extracellular adenosine has a wide range of positive physiological effects including vasodilation, release of endothelial factors as well as cardioprotective and anti-inflammatory effects. These pleiotropic properties (▶Figure 3) may serve as a potential explanation for the mortality advantage of ticagrelor over clopidogrel observed in the PLATO trial (37, 44). Intriguingly, further analysis of the PLATO trial revealed that among patients undergoing coronary artery bypass surgery, the overall mortality was lower with ticagrelor, not only due to a reduction of cardiovascular deaths, but also on account of a decreased rate of bleeding and infection events (45). Moreover, even though adenosine adjunctive therapy exerts a positive influence on post-PCI and post-thrombolysis coronary flow, according to our meta-analysis it fails to improve clinical outcomes in ACS patients (46). Thus, the advantageous effects of ticagrelor are likely to involve other, complex and yet unknown mechanisms, possibly exceeding the explanation provided by the adenosine theory. The mortality advantage of ticagrelor over clopidogrel may also derive from the impact exerted by ticagrelor on the coronary atherosclerotic plaques. Lee et al. were first to report the presence of P2Y12 receptors in human coronary atherosclerotic plaques (47). Therefore, it is possible that P2Y12 antagonists apart from their antiplatelet actions may also have an additional anti-ischaemic effect by inducing plaque stabilisation. Ticagrelor, due to its pharmacokinetic superiority over clopidogrel, could probably be more powerful in this aspect.

Impact on endothelium and vascular tone

The influence of clopidogrel on endothelial function was a point of interest for Warnholtz et al. (18). The impact of 300 mg and 600 mg loading doses of clopidogrel on endothelium was assessed by measurement of flow-mediated dilation of the brachial artery in patients with stable coronary artery disease. The results were promising, showing a significant, dose-dependent improvement of endothelial function. However, further investigation of potential long-term advantages revealed, that the beneficial effect of clopidogrel on flow-mediated dilation of the brachial artery did not sustain after 28 days of treatment with a 75 mg maintenance dose (48). On the other hand, a substantial improvement in peripheral arterial function was observed in ticagrelor-treated patients with a history of ACS (32). No similar effect was observed with prasugrel or clopidogrel. Moreover, there were significantly fewer patients

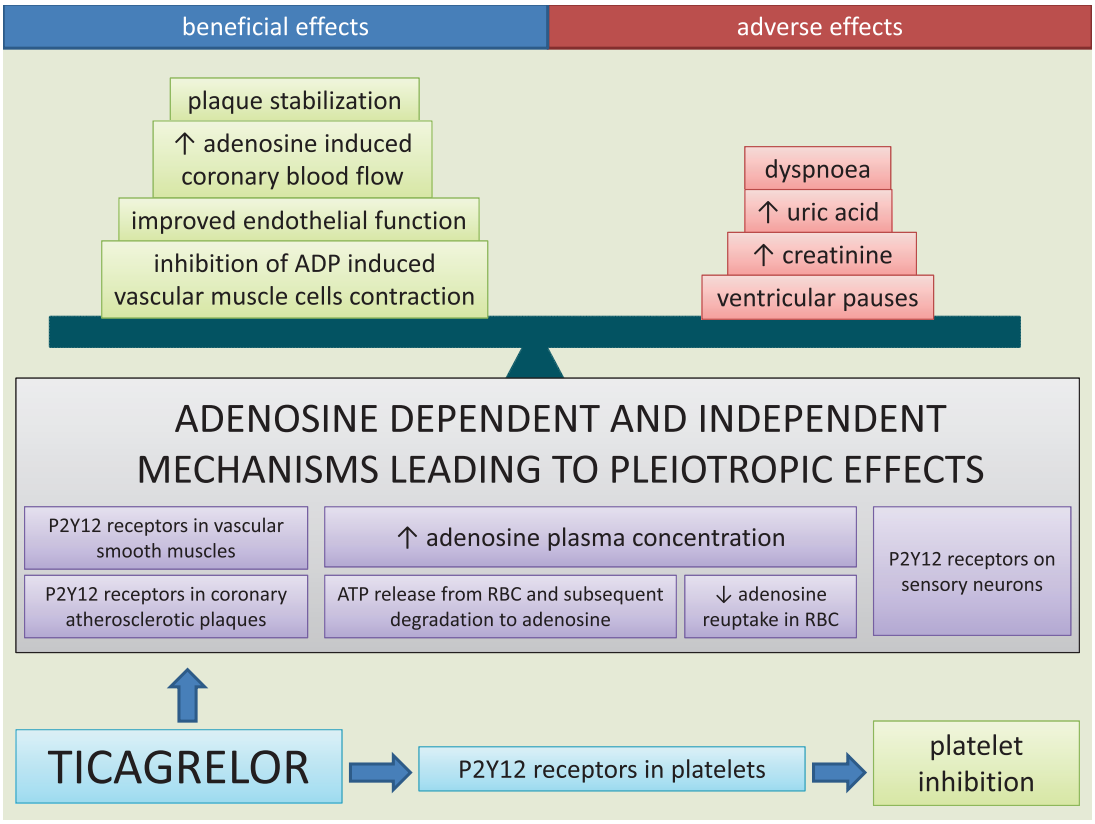

Figure 3: Potential underlying mechanisms of ticagrelor's off-target effects. ADP, adenosine diphosphate; ATP, adenosine triphosphate; RBC, red blood cells.

with endothelial dysfunction in the ticagrelor group compared with subjects treated with other antiplatelet agents (32).

André et al. designed an animal research to establish whether bleeding induced by thienopyridines is entirely P2Y12-dependent (19). The results of their study suggested existence of an additional target, apart from the P2Y12 receptor, which contributed to increased volume of blood loss. Both prasugrel and clopidogrel augmented bleeding by inhibiting the constriction of mice mesenteric veins in response to mechanical injury (micropuncture). Furthermore, mesenteric veins from animals treated with maximal doses of clopidogrel and prasugrel responded only partially (in case of clopidogrel) or minimally (in case of prasugrel) to α,β-metATP. Use of elinogrel, a novel reversible P2Y12 antagonist, did not affect vasoconstriction stimulated by α,β-metATP and was associated with less bleeding (19). Similar results were obtained when the impact of clopidogrel on a rat caudal artery was researched (20). Even though, this pro-drug requires hepatic activation, thus having no ADP-antagonist activity *in vitro*, it caused relaxation of arterial tissues and had an impact on vascular smooth muscle cell proliferation. The observed influence on the vessel wall is believed to be endothelium-, β-adrenergic- and P2Y12 receptor-independent (30). It was previously reported that ticagrelor also interacts with vessel walls (12, 33). When administered orally, it prevents ADP-induced vascular smooth muscle cells contraction in a rat model (33). The vasorelaxant properties of ticagrelor are not abolished after removal of endothelium. On the contrary, in the same research, there was no evidence found, that clopidogrel or prasugrel diminish ADP-induced vascular smooth muscle cells contraction (33). Moreover, it was showed that high-dose, but not low-dose, aspirin impairs the vasorelaxant effect of ticagrelor on the ADP-induced vascular smooth muscle cells contraction (34). This observation provides a new insight into the knowledge regarding the possible interaction between ticagrelor and aspirin doses (North American Paradox).

Postconditioning-like activity of cangrelor

Cangrelor is a novel intravenous, reversible, predictable and potent P2Y12 antagonist with a rapid onset and offset of activity (49, 50). With its attributes it has a potential to become a substantial therapy improvement in patients undergoing PCI, particularly, as it has been recently reported to reduce the rate of ischaemic events including stent thrombosis, with no significant increase in severe bleeding compared with clopidogrel (51).

In a research by Yang et al. conducted in two different animal models aiming to determine whether cangrelor has any cardioprotective features, cangrelor administered 10 minutes (min) before reperfusion in rabbits subjected to 30-min cardiac ischaemia, led to an approximately 50% reduction of infarct size (35). It was postulated that the observed heart protection was rather cangrelor's postconditioning mimetic effect than its antiplatelet action. This assumption was based on the fact that cangrelor's protective effect was abrogated by antagonists of signal transduction specific

for ischaemic postconditioning and that at the same time these blockers did not affect cangrelor's ability to inhibit platelet aggregation. Furthermore, the combination of cangrelor and ischaemic postconditioning did not produce additional diminution in infarct size (35). A consistent cardioprotective influence of cangrelor was documented in a primate model (macaque monkeys), in which anatomical infarct size could be measured directly (36). In this study the existence of class effect of P2Y12 blockers was postulated. Both cangrelor and OM2, an investigational murine antibody against the primate collagen receptor glycoprotein VI, decreased infarct size significantly compared with the control group. The reduction of infarct size was comparable to that seen with ischaemic postconditioning. On the basis of their results, the authors suggested that postconditioning-mimetic interventions, shown to be efficacious in animal models, had only a mediocre effect in clinical trials, because patients treated with antiplatelet agents prior to revascularisation may have already been in a postconditioned state (36).

Anti-inflammatory effects

Inflammation is a vital pathogenetic factor of atherosclerosis and thrombosis, both of the latter being main underlying causes of cardiovascular disease. Platelets, apart from their role in haemostasis, participate in both - thrombus formation and inflammation following vascular injury. It has been suggested that inhibition of platelet function can affect certain inflammatory markers, especially those associated with activated platelets, such as CD40 ligand (CD40L), P-selectin, and C-reactive protein (CRP) (52). The modulation of inflammation by P2Y12 antagonists can be explained not only by inhibition of platelet activation, but also by reduction of platelet interactions with leukocytes, as well as by the direct interaction between antiplatelet drugs and white blood cells (WBC).

There is a growing body of evidence that therapy with clopidogrel leads to reduction in serum levels of CD40L, CRP, P-selectin and platelet-leukocyte aggregate formation (53). Interestingly, a recent sub-analysis of the PLATO trial indicated significantly lower leukocyte counts during treatment, but not at one-month post-discontinuation, in the clopidogrel group when compared with patients receiving ticagrelor (39). In contrast, with ticagrelor a higher increase in CRP concentration at discharge was noted, while interleukin (IL)-6 levels remained higher during the first month of treatment. However, it remains unclear whether these slight differences in inflammatory parameters affect all-cause, sepsis-related and pulmonary adverse events-related mortality risk in clopidogrel vs ticagrelor-treated ACS patients. Further research in this field should focus on the answer whether ticagrelor and clopidogrel have different effects on immune signalling (39).

The investigation of clopidogrel's influence on interactions between platelets and leukocytes showed that the drug impairs the formation of platelet-monocyte and platelet-neutrophil conjugates in ACS patients (21). Quinn et al. examined the effect of clopidogrel pretreatment on platelet inflammatory markers expression in patients undergoing PCI (22). They demonstrated that administration of clopidogrel before PCI is associated with decreased agonist-induced platelet P-selectin and CD40L expression after the procedure. Long-term clopidogrel therapy was proven to significantly diminish plasma concentrations of IL-1α, IL-2, IL-6, IL-13, tumour necrosis factor (TNF)-α and TNF-β as well as ADP-stimulated P-selectin expression in subjects who underwent PCI (23). Clopidogrel shows its ability to modulate inflammation not only in patients with coronary artery disease, but also in subjects suffering from peripheral artery disease. In a study by Donaldson et al. clopidogrel was among few substances, which attenuated local inflammatory response and lowered cytokine level in patients with superficial femoral artery stenosis (24). Moreover, Takeda et al. postulated that the expansion of stable plaque areas and reduction of unstable ones, observed with clopidogrel, might be due to anti-inflammatory actions of clopidogrel (25). However, this has been only observed in a murine model so far (25).

It was suggested in the literature, that patients presenting with high on-clopidogrel platelet reactivity could be deprived of the potential pleiotropic anti-inflammatory effects of this substance (54). An interesting observation was made, when Ge et al. cross-matched platelet aggregation six months after PCI with levels of plasma inflammatory markers in patients treated with clopidogrel (55). Higher concentrations of CRP, P-selectin, sCD40L and IL-6 were found in patients with impaired platelet response to the drug. This corresponds well with the findings of Bernlochner et al. who likewise observed a correlation between high on-clopidogrel platelet reactivity and elevated plasma levels of inflammatory markers (such as CRP, WBC count and fibrinogen) in patients after PCI (56). Another evidence for the anti-inflammatory effects of clopidogrel was provided by Angiolillo et al., who examined CRP serum level and expression of P-selectin in diabetic patients with coronary artery disease (CAD) treated with dual antiplatelet therapy (clopidogrel + aspirin) for 12 months after PCI and in whom clopidogrel was then discontinued (57). As expected, P2Y12 antagonist withdrawal resulted in an increase of inflammation biomarkers.

Prasugrel, a third-generation thienopyridine, has also been reported to modulate inflammation. It was observed that a mixture of prasugrel metabolites significantly decreased N-formyl-methionyl-leucyl-phenylalanin- and platelet-activating factor-induced neutrophil activation, suggesting existence of prasugrel's anti-inflammatory effects (58). Specific reversible antagonists of the P2Y12 and P2Y13 receptors were used to determine the role of these receptors in prasugrel-induced inhibition of neutrophils. Interestingly, the results of this study suggested that the interaction between prasugrel metabolites and neutrophils is P2Y12- and P2Y13-independent (58). Moreover, prasugrel was reported to diminish platelet-mediated inflammation by reducing platelet interactions with monocytes and leukocytes in a murine model, accompanied by decreased plasma concentrations of inflammatory markers. In the mentioned study, treatment with prasugrel of both healthy and endotoxin-treated mice, revealed a profound inhibition of platelet P-selectin expression, thromboxane A2 production and platelet- polymorphonuclear leukocytes adhesion. In ad-

dition, in endotoxin-treated mice *in vivo* prasugrel reduced thromboxane A2 and TNF-α synthesis and increased nitric oxide metabolites production (26).

It has to be considered though, that the vast majority of patients suffering from CAD use P2Y12 receptor inhibitors concurrently with statins, whose anti-inflammatory effects have been confirmed in the past (59-61). This fact might have influenced the results of some of the studies exploring the anti-inflammatory activity of P2Y12 receptor blockers, as some of them might have included patients using statins. We have no knowledge regarding whether, and up to what extent, the anti-inflammatory effects of both statins and P2Y12 receptor antagonists may interact and what clinical outcomes it may produce.

Despite the considerable amount of literature describing the modulating effect of P2Y12 inhibitors on inflammatory markers, the clinical significance of these effects remains uncertain. Up to date, we are still lacking evidence that P2Y12 receptor antagonists actually provide considerable anti-inflammatory clinical benefit.

Effects on carcinogenicity

Another intriguing point are potentially distinct effects of platelet P2Y12 receptor inhibitors on carcinogenicity. However, major clinical trials were not designed to assess this outcome and their value to evaluate the incidence of cancers is limited by the insufficient duration of the follow-up. The TRITON-TIMI 38 investigators reported in the primary publication of the study on the slight, but statistically significant, excess of newly diagnosed colonic cancers in the prasugrel arm (►Table 3) (4). The overall incidence of cancers in the TRITON-TIMI 38 trial was higher in patients treated with prasugrel than in those receiving clopidogrel, but the difference did not reach statistical significance and the exclusion of colonic cancers from the analysis further attenuated the imbalance in the cancer incidence between the study arms. It was unclear whether the difference in the colonic cancer incidence between the prasugrel and clopidogrel groups was causally related, caused by detection bias or by chance. Numerous hypothetical mechanisms linking prasugrel with increased carcinogenicity have been proposed: direct hazard of prasugrel on cancer occurrence and/or progression, indirect modulation of tumour growth, or enhanced metastatic dissemination due to instability of platelet-tumour cell aggregates, and/or inability to contain the disease locally due to more potent long-term platelet inhibition (62). However, any solid research evidence supporting the causative role of these mechanisms is lacking. The TRITON-TIMI 38 investigators speculated that this observation may have resulted from the more potent antiplatelet effect of prasugrel bringing more events to medical attention (4). On the other hand, the FDA, when approving prasugrel, concluded that this discrepancy was likely due to chance, and the prasugrel advisory committee was in agreement (63). In support of this opinion, the TRILOGY ACS investigators observed comparable rates of newly diagnosed non-benign neoplasms in the prasugrel and clopidogrel groups (5). Similar findings were made among treated patients with no history of cancer or a history of previous cancer that had been cured before randomisation. These analyses were prespecified in the study protocol and all events were adjudicated by an independent oncology adjudication committee.

Interestingly, the FDA, when considering the prasugrel manufacturer's application for the drug approval, based on data from the CAPRIE and CHARISMA trials, found no evidence that clopidogrel increases cancer risk (64).

On the other hand, the results of the PLATO trial suggest that ticagrelor does not provide any harm in terms of carcinogenicity and may be even protective (3). Compared with clopidogrel, ticagrelor was associated with numerically lower rates of both any neoplasms and malignant neoplasms arising during treatment, while the difference between the study arms was statistically significant in favour of ticagrelor when considering benign neoplasms (►Table 3). Several mechanisms potentially contributing to the hypothetical anti-cancer effect of ticagrelor through stimulation of the adenosine receptors, such as inhibition of tumour growth, attenuation of blast transformation, and enhanced immunosuppression, were postulated (44, 65-68).

The effects of novel antiplatelet agents on the onset of cancer should be investigated in prospective phase IV mega-trials. Additionally, carefully designed preclinical and mechanistic studies addressing this issue are needed.

Pleiotropic effects of novel platelet P2Y12 receptor inhibitors as a potential explanation of differences in the results of landmark trials

Both novel P2Y12 antagonists, ticagrelor and prasugrel, offer us a chance to confine the reinfarction rate and stent thrombosis in interventionally treated ACS patients (3, 4, 69).

Despite these similarities, landmark clinical trials on ticagrelor and prasugrel indicated substantial differences in outcomes when these agents were compared with clopidogrel. Ticagrelor conferred clinical advantages in the PLATO trial in a broad spectrum of ACS patients, namely in subjects with ST-segment elevation myocardial infarction (STEMI) scheduled for primary PCI and in patients with moderate-to-high risk non-ST-ACS regardless of the treatment strategy. In the PLATO trial benefits of ticagrelor over clopidogrel in terms of the study primary end point were consistent among multiple subgroups, with the exception of substantial geographic variation in ticagrelor efficacy (3). Based on the results of two independent analyses, it was hypothesised that differences in the maintenance dose of aspirin were responsible for this regional difference (70). In the subgroup of patients treated with high-dose aspirin (at least 300 mg) in the PLATO trial, particularly in those enrolled in the United States, ticagrelor therapy was paradoxically associated with worse clinical outcomes than clopidogrel therapy (70). As a consequence of this observation, the FDA approved ticagrelor with a "Boxed Warning" indicating that aspirin daily maintenance doses of above 100 mg decrease its effectiveness (71).

Table 4: Overview of research data on pleiotropic effects of P2Y12 receptor antagonists.

Drug	Off-target effect	Type of research evidence supporting the presence of the off-target effect	Summary of the study findings on pleiotropic effects
Clopidogrel	Improvement of endothelial function	A double-blind, randomised study comparing the effects of 600 vs 300 mg loading doses of clopidogrel on ultrasonographically measured flow-mediated dilation of the right brachial artery in 58 patients with stable CAD (18).	Clopidogrel improves endothelial function in patients with stable CAD independently of changes in platelet oxidative stress and platelet-derived NO bioavailability thus favouring direct stimulating effects on the endothelium. A 600 mg loading dose of clopidogrel causes a significantly larger increase in flow-mediated dilation compared with a 300 mg loading dose of clopidogrel.
	Inhibition of vasoconstriction in response to mechanical injury or stimulation by α,β-metATP	An animal research in a murine model designed to investigate whether bleeding induced by clopidogrel, prasugrel and elinogrel is entirely P2Y12-dependent, and to compare therapeutic indexes of these drugs (19).	Both clopidogrel and prasugrel reduce constriction of murine mesenteric veins in response to mechanical injury (micropuncture) and in situ stimulation by α,β-metATP independently of the P2Y12 receptors. Compared with clopidogrel, prasugrel shows stronger anticontractile potency. The observed off-target effects of clopidogrel and prasugrel contribute to bleeding, are dose- and time-dependent and reversible#.
	Endothelium-independent vasorelaxation	An animal research in a murine model investigating whether ticlopidine and clopidogrel have vascular activity independent of hepatic bioactivation (20).	Clopidogrel and ticlopidine induce vasorelaxation of rat caudal arteries without hepatic bioactivation in a concentration-dependent manner. This action induced *in vitro* is independent of endothelium-derived NO, β-adrenergic receptors and P2 receptors.
	Reduction of plasma levels of inflammatory markers	A prospective, placebo-controlled, observational study assessing the effect of clopidogrel on platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation. The study and control groups comprised 23 patients with NSTE-ACS and 20 healthy individuals, respectively (21).	In patients with NSTE-ACS, clopidogrel reduces membrane P-selectin expression, as well as soluble P-selectin and CD40L plasma concentrations. In these patients clopidogrel also attenuates the agonist effects of ADP and TRAP on platelet aggregation, platelet secretion, formation of platelet-monocyte and platelet-neutrophil conjugates.
		A non-randomised, prospective study assessing the effect of clopidogrel pre-treatment on inflammatory marker expression in 79 patients undergoing PCI due to stable CAD or ACS (22).	Clopidogrel pretreatment reduces expression of platelet inflammatory markers (CD40L and CD62P) in patients undergoing PCI due to stable CAD or ACS.
		A non-randomised, prospective study assessing platelet reactivity and inflammatory markers in subjects undergoing non-emergent coronary stenting. The study population included 69 clopidogrel-naive patients and 41 patients receiving long-term clopidogrel treatment (23).	Patients receiving long-term clopidogrel treatment compared with clopidogrel-naive patients have lower expression of ADP-stimulated P-selectin and activated GP IIb/IIIa, lower ADP-induced platelet aggregation and lower levels of selected inflammatory markers (IL-α, IL-2, IL-6, IL-13, TNF-α, TNF-β).
		A non-randomised, prospective study assessing local production of cytokines and growth factors at culprit SFA plaques in 20 patients with peripheral artery disease (24).	Concentrations of sCD40L and TNF-β are increased at culprit sites of SFA stenosis. Statins lower sCD40L concentration at culprit SFA lesions, whereas both aspirin and clopidogrel tend to decrease TNF-β concentration at culprit SFA lesions.
	Atherosclerotic plaque stabilisation	An animal research in a murine model investigating the impact of antiplatelet therapy with cilostazol and clopidogrel on stability of atherosclerotic plaque. Atherosclerotic lesion volumes and components in the brachiocephalic artery were assessed by phase-contrast X-ray CT imaging and histochemistry (25).	Both cilostazol and clopidogrel reduce unstable plaque areas, increase stable ones and lead to regression of atherosclerotic plaque volume in the murine brachiocephalic artery model.

Table 4: Continued

Drug	Off-target effect	Type of research evidence supporting the presence of the off-target effect	Summary of the study findings on pleiotropic effects
Prasugrel	Inhibition of vasoconstriction in response to mechanical injury or stimulation by α,β-metATP	An animal research in a murine model designed to investigate whether bleeding induced by clopidogrel, prasugrel and elinogrel is entirely P2Y12-dependent, and to compare therapeutic indexes of these drugs (19).	The results of this study are described above and marked with #.
	Reduction of plasma levels of inflammatory markers	An animal research in a murine model designed to investigate the influence exerted by prasugrel on inflammatory processes (26).	In endotoxin-treated mice, prasugrel reduces *in vivo* thromboxane A2 and TNF-α synthesis, increases production of NO metabolites and inhibits platelet interactions with polymorphonuclear leukocytes and monocytes.
Ticagrelor	Increase in adenosine plasma concentration	A prospective, open-label, controlled, randomised study investigating the impact of ticagrelor on adenosine plasma concentration in patients with NSTE-ACS. ACS patients were assigned to therapy either with ticagrelor (n=30) or clopidogrel (n=30) (27). Twenty healthy subjects served as controls. Adenosine plasma concentration was measured by liquid chromatography. To assess the mechanism of variation in adenosine plasma concentration, adenosine deaminase concentration, adenosine uptake by RBCs and cAMP production by cells over-expressing adenosine receptors were assessed*.	Ticagrelor increases adenosine plasma concentration in patients with NSTE-ACS compared with clopidogrel by inhibiting adenosine uptake by RBCs, while serum adenosine deaminase activity is comparable in patients receiving ticagrelor and clopidogrel^.
	Inhibition of adenosine re-uptake in erythrocytes	A prospective, open-label, controlled, randomised study investigating the impact of ticagrelor on adenosine plasma concentration in patients with NSTE-ACS (27). Detailed design of the study is described above and marked with *.	The results of this study are described above and marked with ^.
		Research designed to examine ticagrelor's *in vitro* influence on adenosine re-uptake inhibition in different cell lines and to study the physiological consequences of adenosine re-uptake inhibition by ticagrelor in a canine model (28).	Ticagrelor inhibits adenosine re-uptake in erythrocytes in a canine model, leading to a significant dose-dependent increase in both adenosine-mediated and reactive hypoxia-induced coronary blood flow. Additionally, ticagrelor dose-dependently decreases *in vitro* adenosine uptake in human erythrocytes and in cell lines of rat, canine, or human origin&.
	Increase in adenosine-mediated coronary blood flow	A single-blind, crossover study, randomised study (29). NSTE-ACS patients undergoing PCI were assigned to receive either ticagrelor 90 mg bid (n=28) or prasugrel 10 mg qd (n=28) with a 15-day treatment period. At the end of each treatment period, CBFV by transthoracic Doppler echocardiography was assessed at baseline and under incremental doses of adenosine infusion.	Maximal CBFV area under the curve and maximal CBFV/baseline CBFV ratio were higher for ticagrelor-treated than for prasugrel-treated NSTE-ACS patients.
		A double-blind, placebo-controlled, crossover, randomised study investigating whether ticagrelor augments adenosine-induced coronary blood flow and the sensation of dyspnoea in 40 healthy subjects (30). CBFV was measured with transthoracic Doppler echocardiography at rest after multiple stepwise adenosine infusions given before and after study drug, and again after an infusion of theophylline.	Ticagrelor enhances adenosine-induced CBFV and the sensation of dyspnea in healthy male subjects via an adenosine-mediated mechanism.
		Research designed to examine ticagrelor's *in vitro* influence on adenosine re-uptake inhibition and to study the physiological consequences of adenosine re-uptake inhibition by ticagrelor in a canine model (28).	The results of this study are described above and marked with&.

Table 4: Continued

Drug	Off-target effect	Type of research evidence supporting the presence of the off-target effect	Summary of the study findings on pleiotropic effects
Ticagrelor	Increase in ATP release from red blood cells	An *in vitro* study investigating whether ticagrelor induces ATP release from human red blood cells (31). ATP release was studied in human blood collected from healthy volunteers using a luciferase-based bioluminescence assay.	Ticagrelor dose-dependently releases ATP from isolated human RBCs, but not from human WBCs or endothelial cells. The rapid effect indicates release through membrane channel. Ticagrelor affects the membrane potential of human RBCs by inducing dose-dependent depolarisation, which can be blocked by anion transport inhibitors.
	Improvement of endothelial function	A non-randomised, prospective study comparing endothelial function evaluated with peripheral arterial tonometry after forearm ischaemia induction in patients with previous ACS on maintenance dose of: clopidogrel 75 mg qd (n = 35), prasugrel 10 mg qd (n = 32), or ticagrelor 90 mg bid (n = 25) and without P2Y12 blocker (n = 35) (32).	Ticagrelor improves peripheral endothelial function in patients with previous ACS, compared with patients receiving clopidogrel, prasugrel or controls.
	Inhibition of ADP-induced vascular smooth muscle cells contraction	An *in vitro* study investigating whether ticagrelor inhibits ADP-mediated contraction in human large and small arteries, and whether ticagrelor exerts any effect on ADP-mediated contraction in murine thoracic aorta ring segments in the presence and absence of orally administered clopidogrel (12). Internal mammary artery segments and pericardial fat arteries used in this research were obtained from 9 patients undergoing coronary bypass surgery.	Ticagrelor significantly reduces contractile effects of ADP both in human internal mammary arteries and in pericardial fat arteries. The anticontractile effect of ticagrelor following ADP stimulation is also present in murine thoracic aorta ring segments independently of clopidogrel pretreatment.
		An animal study designed to assess the influence of clopidogrel, prasugrel and ticagrelor on vasoconstriction of rat caudal artery caused by stable ADP analogue (2-MeS-ADP), phenylephrine and arginine vasopressin (33).	Ticagrelor, unlike clopidogrel and prasugrel, inhibits 2-MeS-ADP-induced contraction of rat caudal artery in vessels with and without vascular endothelium. Vasorelaxant properties of ticagrelor in the presence of 2-MeS-ADP are not abolished when rat caudal arteries are perfused with phenylephrine and arginine vasopressin.
		An animal study designed to compare the anticontractile effects of low- and high-dose aspirin co-administered with ticagrelor on VSMC of rat tail arteries. Changes in perfusion pressure caused by stable ADP analogue (2-MeS-ADP) and phenylephrine-induced constriction of rat tail arteries were assessed (34).	Ticagrelor suppresses 2-MeS-ADP-induced VSMC contraction in endothelialised vessels pretreated only with low-dose aspirin. In rat tail arteries with preserved endothelium and pretreated with high-dose aspirin, no anticontractile effect of ticagrelor following 2-MeS-ADP stimulation is present.
Cangrelor	Postconditioning-mimetic effect	Animal studies in rabbit (35) and macaque monkey models investigating the potential direct cardioprotective effect of cangrelor (36).	Cangrelor decreases the infarct size when administered prior to reperfusion in ischaemic rabbit and macaque hearts. In rabbits, the cardioprotective effect of cangrelor is similar to the one seen with ischaemic postconditioning, and unlike cangrelor's anti-platelet activity, it is abrogated by signalling inhibitors of ischaemic postconditioning.

2-MeS-ADP, stable analogue of adenosine diphosphate; ACS, acute coronary syndrome; ADP, adenosine diphosphate; ATP, adenosine triphosphate; AUC, area under the curve; bid, twice a day; CAD, coronary artery disease; cAMP, cyclic adenosine monophosphate; CBFV, coronary blood flow velocity; CD40L, CD40 ligand; CT, computed tomography; IL-1α, interleukin-1α; IL-2, interleukin-2; IL-6, interleukin-6; IL-13, interleukin-13; NO, nitric oxide; NSTE-ACS: non-ST-elevation acute coronary syndrome; PCI, percutaneous coronary intervention; qd, once a day; RBC, red blood cell; sCD40L, soluble CD40 ligand; SFA, superficial femoral artery; TNF-α, tumour necrosis factor α; TRAP, thrombin receptor activating peptide; VSMC, vascular smooth muscle cells; α,β-metATP, α,β-methyleneadenosine 5-triphosphate.

Of major importance, ticagrelor therapy unexpectedly reduced all-cause mortality when compared with clopidogrel treatment in the PLATO trial, without any significant difference in the rates of major bleeding between the study arms (3).

Prasugrel was tested in the TRITION-TIMI 38 trial exclusively in ACS patients (moderate-to-high-risk non–ST-elevation ACS or STEMI) with scheduled PCI (4). Therefore, in contrast to the PLATO trial, its findings are not applicable to conservatively treated ACS patients. Furthermore, the TRILOGY ACS trial including non-ST-ACS patients treated conservatively failed to demonstrate any clinical benefits of prasugrel, as compared with clopidogrel (5). Interestingly, the TRILOGY ACS platelet function sub-

study indicated that even though prasugrel led to a substantially higher platelet inhibition, as compared with its less powerful counterpart - clopidogrel, it did not improve outcomes (72). Therapy with prasugrel in the TRITION-TIMI 38 trial was associated with an increased risk of major bleeding, including life-threatening bleeding and fatal bleeding, when compared with clopidogrel treatment (4). As a result of the discordance between the efficacy and safety results, the TRITON-TIMI 38 investigators decided to perform a series of post hoc exploratory analyses to identify the subgroups of patients who did not have a favourable net clinical benefit (defined as the rate of death from any cause, non-fatal myocardial infarction, non-fatal stroke, or non-fatal TIMI major bleeding unrelated to coronary artery bypass grafting). These analyses revealed net clinical harm of prasugrel therapy in subjects who had a previous stroke or transient ischaemic attack and the lack of net clinical benefit in patients 75 years of age or older and in patients with body weight of less than 60 kg (4). On the other hand, an indirect comparison of ticagrelor and prasugrel suggests a better protection from stent thrombosis with the latter (73).

All the above-discussed differences in the results of landmark trials on novel platelet P2Y12 receptor inhibitors may be potentially explained by pleiotropic effects of these agents, as the antiplatelet potencies of ticagrelor and prasugrel are comparable. The eagerly awaited results of the PEGASUS-TIMI 54 study, a randomised phase III trial assessing the efficacy of ticagrelor against placebo on a background of aspirin therapy in patients with a history of myocardial infarction, may hopefully provide a new insight into pleiotropic effects of ticagrelor. Unfortunately, adequately powered head-to-head trials with carefully designed sub-analyses comparing ticagrelor and prasugrel in the ACS setting, representing ideal tools to assess the mechanisms and clinical relevance of pleiotropic effects of novel platelet P2Y12 receptor inhibitors, are unlikely to be conducted in the nearest future.

Critical appraisal of the currently available data on pleiotropic effects of platelet P2Y12 receptor inhibitors

The issue of pleiotropic effects of platelet P2Y12 receptor inhibitors have received considerable attention of the cardiological community in recent years. Particularly, adenosine-related mechanisms, described in the ticagrelor-treated subjects, seem to be an interesting and promising explanation for the benefits observed in the PLATO trial. Nonetheless, currently we are still lacking an indisputable evidence directly associating the pleiotropic effects of prasugrel or ticagrelor with their improved clinical outcomes. Obviously, it cannot be excluded that the superiority of novel P2Y12 receptor blockers over clopidogrel presented in clinical trials, originates only from the more advantageous pharmacokinetics and more potent platelet inhibition, and may not be connected at all with the observed various extra-platelet activities of these compounds.

Distinguishing the clinical efficacy deriving directly from the antiplatelet activity and the effects deriving from the extra-platelet impact exerted by P2Y12 receptor blockers, is a hard task. Nevertheless, we believe that broadening the knowledge of P2Y12 receptor antagonists' off-target actions could potentially support more conscious clinical decisions and could help to optimise the ACS and post-ACS treatment as well as decrease the occurrence rate of adverse post-PCI cardiovascular events. Moreover, proper application of these pleiotropic features into everyday practice, in a long term could improve the clinical outcomes or even become the first step towards creating a tailored therapy for ACS and post-PCI patients. For that reason, it would be advisable to put more emphasis on evaluation of the pleiotropic actions of these compounds in future clinical trials, which is even more compelling when bearing in mind that a considerable amount of observations describing extra-platelet effects of diverse thienopyridines and non-thienopyridines have been made in a murine or other animal models and no confirmation of their presence in humans is available, yet.

On the other hand, it has to be considered, that the pleiotropic effects of P2Y12 receptor blockers may also exert a negative influence (►Table 3). Ticagrelor-induced dyspnoea for example, though usually transient and mild, still leads to premature discontinuation of treatment in small percentage of patients (40, 41), and shows that adverse off-target actions must not be underestimated and that physicians have to be aware of their potential occurrence.

In our opinion, it is crucial to pursue efforts to unveil and comprehend the full potential of the pleiotropic activity of P2Y12 receptor antagonists, particularly the newer, faster acting and more potent non-thienopyridines. Completing this goal could enable us to employ more effective therapy and confirm P2Y12 inhibitors' position of an important fixture in our armamentarium for the prevention of adverse cardiovascular events in ACS patients.

Conclusions

Undoubtedly, platelet P2Y12 receptor antagonists exhibit a wide range of actions beyond their primary antiaggregatory function, for which they are so commonly used. These agents have a potential to affect the pathogenesis of cardiovascular disease on many different levels. Modulation of inflammation, improvement of endothelial function, prevention of vasoconstriction, adenosine-like and postconditioning-like effects are only some of additional pathways potentially providing an extra gain for patients treated with P2Y12 blockers (►Table 4). However, even though many observations regarding this issue have been made, our knowledge about beneficial and adverse off-target effects displayed by P2Y12 receptor inhibitors and both, their clinical significance and detailed underlying mechanisms, remains unsatisfactory.

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Conflicts of interest

Dr. Marek Koziński and Dr. Grzegorz Grześk received honoraria for lectures from AstraZeneca. The other authors declare no conflict of interest.

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