Effect of P2Y₁₂ inhibitors on inflammation and immunity

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Summary
Platelet P2Y₁₂ inhibitors form a major part of the treatment strategy for patients with acute coronary syndromes (ACS) due to the importance of the platelet P2Y₁₂ receptor in mediating the pathophysiology of arterial thrombosis. It has been increasingly recognised that platelets also have a critical role in inflammation and immune responses. P2Y₁₂ inhibitors reduce platelet release of pro-inflammatory α-granule contents and the formation of pro-inflammatory platelet-leukocyte aggregates. These are important mediators of inflammation in a variety of different contexts. Clinical evidence shows that P2Y₁₂ inhibition by clopidogrel is associated with a reduction in platelet-related mediators of inflammation, such as soluble P-selectin and CD40L, following atherothrombosis. Clopidogrel in addition to aspirin, compared to aspirin alone, also reduces markers of systemic inflammation such as tumour necrosis factor (TNF) α and C-reactive protein (CRP) following ACS. The more potent thienopyridine P2Y₁₂ inhibitor, prasugrel, has been shown to decrease platelet P-selectin expression and platelet-leukocyte aggregate formation compared to clopidogrel. The PLATO study suggested that the novel P2Y₁₂ inhibitor ticagrelor might improve clinical outcomes from pulmonary infections and sepsis compared to clopidogrel in patients with ACS. Ticagrelor is a more potent P2Y₁₂ inhibitor than clopidogrel and also inhibits cellular adenosine uptake via equilibrative nucleoside transporter (ENT) 1, whereas clopidogrel does not. Further examination of the involvement of these mechanisms in inflammation and immunity is therefore warranted.

Keywords
Platelets, P2Y₁₂ inhibitors, antiplatelet medications, inflammation, infection

Introduction
Whilst it has long been recognised that platelets have a central role in thrombosis, there has been increasing recognition of their major role in inflammation and immune responses. Highlighting this important role, the PLATElet inhibition and patient Outcomes (PLATO) study suggested that the novel platelet P2Y₁₂ inhibitor ticagrelor might differ in its effects on immune responses compared to clopidogrel (1). In PLATO, ticagrelor was associated with significantly fewer pulmonary infections and deaths related to infection than clopidogrel (1–3). Ticagrelor is a more potent and consistent P2Y₁₂ inhibitor than clopidogrel (4), but in addition, ticagrelor inhibits cellular uptake of adenosine by inhibition of ENT1 (5). There are therefore many mechanisms by which ticagrelor and clopidogrel could differ in their effects on inflammation and immune responses. This review article summarises the current evidence for the effects of P2Y₁₂ inhibitors on inflammation. Of the currently used platelet P2Y₁₂ inhibitors, clopidogrel has been available for the longest. Therefore the majority of the currently available evidence regarding the effects of P2Y₁₂ inhibition on inflammation derives from studies of clopidogrel.
also therefore possible that P2Y₁₂ inhibitors may also affect inflammation by direct effects on these cell types, which will be covered at a later stage in this review.

**Clopidogrel**

Clopidogrel is a second-generation thienopyridine, the active metabolite of which covalently binds to the platelet P2Y₁₂ receptor. Clopidogrel, in addition to aspirin, has been shown to reduce the incidence of adverse cardiovascular events compared to placebo in patients with non-ST elevation acute coronary syndrome (NSTE-ACS) (12), ST-elevation myocardial infarction (MI) (STEMI) (13) and following percutaneous coronary intervention (PCI) (14). In patients with atherosclerotic disease there is considerable variation in response to clopidogrel, in part due to polymorphisms of CYP2C19, which encodes for the main enzyme responsible for its metabolism (15), and in part due to drug-drug interactions (16), age, weight and co-morbidities such as diabetes mellitus (17). Interestingly, it has also been shown that cytochrome p450s are downregulated by inflammation, which may reduce the formation of the active metabolite of clopidogrel (18).

**Effect of clopidogrel on inflammation related to atherothrombosis**

Inflammation is, at all stages, an important component of the pathophysiology of coronary artery disease and atherothrombosis, including the initial development of a coronary plaque and during ACS (19, 20). Antiplatelet medications may influence inflammation relatively directly, by modulating leukocyte responses for example, but are also likely to indirectly influence inflammation by reducing myocardial necrosis, due to their antithrombotic effect.

**Effect of clopidogrel on mediators that are contained within platelet α granules**

In patients with ACS, it has been shown that soluble P-selectin and CD40L levels decrease after a loading dose of clopidogrel (8). Whilst a higher clopidogrel loading dose (600 or 900 mg) further decreased platelet aggregation responses, it did not appear to further lower levels of sCD40L, PAI-1 or von Willebrand factor (vWF) compared to a 300 mg loading dose in the ALBION study (21). Clopidogrel treatment prior to PCI decreases platelet P-selectin and CD40L expression after PCI (22). Heitzer et al. also showed that clopidogrel, in addition to aspirin, reduced levels of sCD40L and RANTES in stable coronary artery disease (CAD) patients (23). However, clopidogrel monotherapy did not appear to change levels of soluble P-selectin, sCD40L, transforming growth factor (TGF)-β or MCP-1 compared to aspirin monotherapy in stable coronary artery disease patients in the ASCET study (24).

**Effect of clopidogrel on pro-inflammatory cytokines and C-reactive protein (CRP)**

Ruptured plaques contain activated leukocytes and, in particular, monocytes/macrophages have been implicated in mediating systemic inflammation related to ACS (20, 25). In addition, platelet-

### Table 1: Effect of clopidogrel on inflammatory markers.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Treatment</th>
<th>Timing of measurements</th>
<th>Difference between treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. (30) 115 NSTE-ACS patients</td>
<td>Clopidogrel + aspirin vs aspirin alone</td>
<td>Baseline, 1 week and 1 month</td>
<td>CRP significantly reduced: 2.40 vs 3.49 at 30 days (p&lt;0.05) TnFα significantly reduced: 44 vs 63 at 30 days (p&lt;0.05)</td>
</tr>
<tr>
<td>DOUBLE study (32) 54 STEMI patients</td>
<td>Clopidogrel maintenance dose: 150 mg vs 75 mg</td>
<td>Baseline, 1 week and 1 month</td>
<td>Trend for lower CRP: 1.7 vs 3.1 at 1 week (p=0.06)</td>
</tr>
<tr>
<td>ALBION study (21) 103 NSTE-ACS patients</td>
<td>Clopidogrel loading dose: 900 mg vs 600 mg vs 300 mg</td>
<td>Baseline, 6 hours and 24 hours post PCI</td>
<td>NS</td>
</tr>
<tr>
<td>CADET study (33) 184 ACS patients</td>
<td>Clopidogrel vs aspirin</td>
<td>Baseline, 1 month, 3 months and 6 months</td>
<td>NS</td>
</tr>
<tr>
<td>CLEAR-PLATELETS study (31) 60 elective PCI patients</td>
<td>Clopidogrel loading dose: 600 mg vs 300 mg</td>
<td>Baseline and 18–24 hours post PCI</td>
<td>No significant difference in CRP TnFα significantly reduced: approx. 35 % decrease from baseline vs 30 % increase from baseline (p&lt;0.001)</td>
</tr>
<tr>
<td>Vivekananthan et al. (22) 833 PCI patients</td>
<td>Clopidogrel pretreatment vs no clopidogrel pretreatment</td>
<td>Baseline, immediately after PCI and 18–24 hours after PCI</td>
<td>CRP significantly reduced: change from baseline of 0.15 vs 0.43 (p=0.03)</td>
</tr>
<tr>
<td>ASCET study (37) 206 SCAD patients</td>
<td>Clopidogrel vs aspirin</td>
<td>Baseline, 1 month and 1 year</td>
<td>NS</td>
</tr>
<tr>
<td>Heitzer et al. (23) 103 SCAD patients</td>
<td>Clopidogrel + aspirin vs aspirin alone</td>
<td>Baseline and 5 weeks</td>
<td>CRP decreased in patients treated with clopidogrel but did not in those treated with aspirin alone (p&lt;0.01)</td>
</tr>
<tr>
<td>Azar et al. (82) 73 SCAD patients</td>
<td>Clopidogrel + aspirin vs aspirin alone</td>
<td>Baseline and 8 weeks</td>
<td>NS</td>
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</tbody>
</table>

NS: non-significant difference. SCAD: stable coronary artery disease.
monocyte aggregates form after ACS (26) and upregulate monocyte pro-inflammatory functions, such as the release of pro-inflammatory cytokines including tumour necrosis factor (TNF)α, interleukin (IL)-1β, IL-8 and monocyte chemotactic protein (MCP)-1 (27, 28). Pro-inflammatory cytokines such as these induce CRP production by the liver, leading to increased levels (29). Clopidogrel has been shown to affect reduce markers of systemic inflammation, including TNFα and CRP, in patients with ACS (Tables 1–3). Levels of TNFα are significantly higher in patients with ACS than in healthy volunteers (30). Clopidogrel, in addition to aspirin, significantly decreases levels of TNFα and CRP compared to aspirin alone in patients with ACS (30). An increased maintenance dose of 150 mg of clopidogrel, compared to 75 mg, in addition to aspirin, led to approximately 50% lower levels of CRP at one week in STEMI patients, although this was not statistically significant (p=0.06) (32). Neither the CLEAR-PLATELETs (31) nor the ALBION study showed an effect of increasing the loading dose of clopidogrel on subsequent levels of CRP (21), perhaps suggesting that higher levels of P2Y12 inhibition must be sustained to achieve a reduction in CRP. The CADET study showed that clopidogrel monotherapy does not appear to significantly differ in its effects on CRP compared to aspirin monotherapy in patients with ACS (33). It is apparent from these studies that changes in levels of CRP at the time of ACS are more subtle than might be anticipated.

To show an effect of clopidogrel on these small changes, it appears it was necessary to use clopidogrel in conjunction with aspirin, whereas replacing aspirin with clopidogrel does not have a significant effect. Studies may also need a large sample size to demonstrate significant differences in these small changes in CRP. It is possible that more upstream mediators of inflammation, such as TNFα, may be more able to demonstrate an effect of P2Y12 inhibition.

Pretreatment with clopidogrel before PCI is associated with significantly lower subsequent levels of IL-1α, IL-2, IL-6, IL-13 and TNFα than no pretreatment (34). Whilst this study only showed a non-significant trend towards lower levels of CRP, another study has shown that pre-treatment with clopidogrel prior to PCI was associated with lower levels of CRP (22). In addition, in the CLEAR-PLATELETs study, an increased loading dose of clopidogrel significantly reduced subsequent levels of TNFα in PCI patients (31). Of course, it is unknown whether these changes reflect a direct effect of clopidogrel on inflammation or an indirect effect through reduction in periprocedural MI.

Compared to ACS and PCI, the inflammation related to chronic atherosclerosis involves distinct molecular and cellular pathways (35), with a less well recognised role of platelet P2Y12. Despite this, in patients with stable coronary artery disease, and hence no significant myocardial necrosis, high levels of P2Y12 reactivity during treatment with clopidogrel correlates with increased levels of CRP and WBC (36). Heitzer et al. also showed that levels of CRP, sCD40L and RANTES reduced after the initiation of clopidogrel, in addition to aspirin, in patients with symptomatic stable CAD (23). The ASCET study did not show any difference between clopidogrel monotherapy and aspirin monotherapy on levels of CRP, TNFα, IL-6, IL-10 and MCP-1 in patients with stable CAD (37).

### Effect of clopidogrel on other types of inflammation

Bacterial lipopolysaccharide (LPS), also known as endotoxin, is a potent activator of the innate immune system. During LPS-induced inflammation in mice and rats, clopidogrel appears to have a number of beneficial effects, including a reduction in levels of IL-6 and TNFα and a reduction in liver and lung injury (38, 39). However, in a pig model, clopidogrel did not significantly affect LPS-induced increases in levels of IL-6 or TNFα (40). A study of pigs whose coronary arteries were injured by angioplasty or irradiation showed that three months of clopidogrel treatment was associated with development of significantly fewer proliferative cells and inflammatory cells compared to one month of treatment (41).

Ticlopidine, the first thienopyridine P2Y12 inhibitor to be used in clinical practice, was associated with neutropaenia, which limited its use. Clopidogrel, a second generation thienopyridine, can also rarely cause neutropaenia, which, according to a case report, resolves after switching to ticagrelor (42). This suggests that

### Table 2: Effect of prasugrel on inflammatory markers.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Treatment</th>
<th>Timing of measurements</th>
<th>Difference between treatment groups</th>
</tr>
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<tbody>
<tr>
<td>JUMBO study (50) 9 PCI patients</td>
<td>Prasugrel + aspirin vs clopidogrel + aspirin</td>
<td>Baseline, 4 and 24 hours after PCI</td>
<td>Platelet expressed sCD40L and P-selectin significantly lower at 4 and 24 hours</td>
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</table>

### Table 3: Effect of ticagrelor on inflammatory markers.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Treatment group comparison</th>
<th>Timing of measurements</th>
<th>Difference between treatment groups</th>
</tr>
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<tbody>
<tr>
<td>PLATO study (1) 18,421 ACS patients</td>
<td>Ticagrelor + aspirin vs clopidogrel + aspirin</td>
<td>Baseline, discharge, 1 month and 6 months</td>
<td>CRP significantly higher at discharge: mean 28 vs 26 (p&lt;0.001) IL-6 significantly higher at discharge: mean 5.4 vs 4.9 (p&lt;0.001)</td>
</tr>
<tr>
<td>DISPERSE study (72) 990 NSTEMI-ACS patients</td>
<td>Ticagrelor + aspirin vs clopidogrel + aspirin</td>
<td>Baseline, discharge and 1 month</td>
<td>No significant difference in CRP, IL-6, MPO or sCD40L Median CRP at discharge: 14 vs 11 Median IL-6 at discharge: 5.8 vs 5.0</td>
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</tbody>
</table>
the neutropenia may not be mediated by platelet P2Y\textsubscript{12} receptors. In the PLATO study, neutrophil counts appeared to increase slightly one month after discontinuing clopidogrel (1). This was not seen in ticagrelor-treated patients and suggests a subtle suppression of neutrophil counts by clopidogrel that is likely to be independent of its effects on platelet P2Y\textsubscript{12} receptors.

Interestingly, there are also at least nine case reports of clopidogrel inducing a characteristic form of arthritis associated with a rash (43). Similarly, a rat model has also previously shown that clopidogrel may potentiate peptidoglycan polysaccharide-induced arthritis (44). If clopidogrel does rarely cause arthritis, it would appear that prasugrel may not have the same effect (43). This suggests that this is not due to P2Y\textsubscript{12} inhibition or the active metabolite of clopidogrel, which is structurally very similar to the active metabolite of prasugrel.

**Summary of the effect of clopidogrel on inflammation**

In patients with ACS and stable coronary artery disease, clopidogrel reduces levels of CRP when it is used in addition to aspirin compared to aspirin alone (23, 30). Increasing the maintenance dose of clopidogrel in patients with STEMI has also previously shown a trend towards lower levels of CRP (32). However, the balance of evidence does not suggest additional anti-inflammatory effects of clopidogrel monotherapy compared to aspirin monotherapy. There is also limited evidence that clopidogrel may have off-target effects on inflammation that are not mediated by P2Y\textsubscript{12} events compared to clopidogrel in patients with ACS who are planned to undergo PCI (46).

**Effect of prasugrel on markers of platelet-leukocyte interactions**

Prasugrel active metabolite potently inhibits ADP-induced platelet P-selectin expression and platelet-leukocyte aggregate formation in vitro (47–49) (Table 4). Inhibition of platelet-leukocyte aggregate formation by prasugrel active metabolite has been shown to decrease neutrophil activation, as demonstrated by lower expression of Mac-1 (49). Prasugrel has a greater inhibitory effect on platelet CD40L and P-selectin expression than clopidogrel in patients undergoing PCI (50). Prasugrel, in addition to aspirin, reduces ADP-induced platelet P-selectin and platelet-monocyte aggregate formation compared to clopidogrel in addition to aspirin in patients with stable coronary artery disease (51).

**Effect of prasugrel in other types of inflammation**

In mice, prasugrel attenuates LPS-induced increases in TNF\textalpha and thromboxane B\textsubscript{2} (49). In a human model of LPS administration, prasugrel inhibited platelet reactivity according to a number of measures; however there was a suggestion that vWF release after endotoxin administration might antagonise the inhibitory effect of prasugrel (52). There has also been a suggestion that prasugrel metabolites may directly affect neutrophil function, in a process that is not mediated by platelet P2Y\textsubscript{12} receptors (53).

**Summary of the effect of prasugrel on inflammation**

Prasugrel inhibits platelet-leukocyte interactions more potently than clopidogrel, as would be expected from its greater inhibition of platelet P2Y\textsubscript{12} receptors. It remains to be established whether this has an additional anti-inflammatory effect during ACS or other models of inflammation.
Possible mechanisms for effects of P2Y<sub>12</sub> inhibitors on inflammation

Platelet P2Y<sub>12</sub>-mediated mechanisms

As has been previously been discussed, the predominant mechanism of effect of P2Y<sub>12</sub> inhibitors on inflammation is generally presumed to be mediated by a reduction in platelet P2Y<sub>12</sub>-mediated platelet-leukocyte interactions. Inhibition of platelet P2Y<sub>12</sub> reduces the release of pro-inflammatory cytokines from platelet α granules and reduces the formation of platelet-leukocyte aggregates (6, 54) (Figure 1 and Table 4). α granules contain the cytokines soluble P-selectin, soluble CD40L, PF4, RANTES, IL-1β and MIP-1α amongst others, which generally upregulate a wide range of pro-inflammatory functions of leukocytes, particularly monocytes and macrophages. P-selectin-mediated platelet-leukocyte aggregate formation also upregulates leukocyte release of pro-inflammatory cytokines IL-1β, IL-8, TNFα and MCP-1 (27, 55) and expression of adhesion molecules (56).

Non-platelet P2Y<sub>12</sub>-mediated mechanisms

When P2Y<sub>12</sub> was originally cloned in humans in 2001, it was thought to be predominantly expressed on platelets, with a lesser expression in certain brain cells (57). It has since become apparent that P2Y<sub>12</sub> receptors are also expressed on murine dendritic cells, which are antigen-presenting cells that are related to monocytes (58). The function of dendritic cell P2Y<sub>12</sub> is not well defined, but may regulate dendritic cell endocytosis and IL-12 production (58–60). It has also been shown that lymphocytes may contain mRNA for P2Y<sub>12</sub> although the functional consequence of this is unknown and could be related to possible platelet contamination (61). So, whilst these studies show that dendritic cells and lymphocytes may express P2Y<sub>12</sub>, they do not appear to play as important a role as platelet P2Y<sub>12</sub>.

Vascular smooth muscle cells (VSMC) also express P2Y<sub>12</sub> and ADP stimulation causes vasoconstriction in rat tails, which can be inhibited by ticagrelor, but not clopidogrel or prasugrel treatment (62). The mechanism for this has not been established, but ticagrelor has a longer half-life than the active metabolites of clopidogrel and prasugrel (63) and may therefore have a greater effect on nucleated cells that can potentially recover from P2Y<sub>12</sub> inhibition. The active metabolite of prasugrel is able to inhibit ADP-induced MCP-1 expression by VSMCs in culture, which is also mediated by P2Y<sub>12</sub> (64). In addition, vascular P2Y<sub>12</sub>, as opposed to platelet P2Y<sub>12</sub>, has a role in atherogenesis and transplant arteriosclerosis in mouse models (65, 66). However, despite this clear role of vessel wall P2Y<sub>12</sub> in atherogenesis, administration of P2Y<sub>12</sub> inhibitors failed to inhibit atheroma formation.

There is also accumulating evidence that P2Y<sub>12</sub> receptors play a regulatory role in pulmonary inflammation induced by leukotriene (LT) E<sub>4</sub>, which is a potent pro-inflammatory lipid mediator involved in asthma (67). In particular, clopidogrel and platelet depletion reduce LTE<sub>4</sub> mediated expression of mRNA encoding IL-13 and MUC5AC and accumulation of airway eosinophils (68) in mice. In humans, however, prasugrel was associated with a slight reduction in airway reactivity in patients with asthma that was not statistically significant (69).

Effect of combined P2Y<sub>12</sub> and ENT1 inhibition on inflammation

Clinical effects of ticagrelor

Ticagrelor is a novel class of antiplatelet medication that potently inhibits platelet P2Y<sub>12</sub> receptors and also inhibits cellular uptake of adenosine by inhibiting ENT1 (5). In the PLATelet inhibition and patient Outcomes (PLATO) study, ticagrelor reduced the incidence of adverse cardiovascular events in patients with ACS.
compared to clopidogrel (70). However, ticagrelor also unexpectedly reduced all-cause mortality to a greater degree than would be expected from previous trials of P2Y$_{12}$ inhibitors. This has raised the question as to whether or not ticagrelor has additional beneficial pleiotropic effects, such as modulation of host immunity (1).

In PLATO, ticagrelor was unexpectedly associated with fewer pulmonary infections and deaths related to infection than clopidogrel (1, 2, 71). Also, surprisingly, ticagrelor was associated with slightly higher levels of IL-6 and CRP at discharge than clopidogrel in PLATO, converse to what would be expected from a more potent P2Y$_{12}$ inhibitor (1) (▶Table 3). This demonstrates a differential effect of the medications on inflammatory responses, although the mechanisms have not yet been fully explained. It is likely that the smaller DISPERSE 2 study was underpowered to demonstrate these subtle effects and the differences in levels of CRP, IL-6, MPO and CD40L in ACS patients treated with ticagrelor or clopidogrel were not significant in this study (72).

**Potential adenosine-mediated effects of ticagrelor on inflammation**

The adenosine-mediated effects of ticagrelor are still relatively unexplored, since this mechanism has only been identified recently (5, 73). Ticagrelor has now also been shown to increase extracellular levels of adenosine in patients with ACS (74). This appears to have clinically relevant effects, since ticagrelor potentiates adenosine-induced coronary vasodilation and dyspnoea (75, 76).

Adenosine is a major modulator of inflammation and innate immune responses that acts on four different receptors (A$_1$, A$_{2A}$, A$_{2B}$ and A$_3$), which are differentially expressed in different cell types (77). Adenosine is a degradation product of ATP, ADP and AMP that is leaked into the extracellular space in pathological conditions that exert cellular stress, such as ischaemia and infection (78). Possible adenosine-mediated effects of ticagrelor are complex and may be technically difficult to characterise. At low
concentrations, adenosine predominantly acts on high affinity leukocyte A<sub>2a</sub> receptors (Figure 3) (79, 80). This has mostly pro-inflammatory effects, including potentiation of neutrophil chemotaxis and phagocytosis and macrophage phagocytosis (79, 80). At higher concentrations, adenosine predominantly acts on lower affinity leukocyte A<sub>2b</sub> and A<sub>3</sub> receptors (Figure 3). This has mostly anti-inflammatory effects, such as downregulation of the release of pro-inflammatory cytokines, including IL-6 and TNFα (79, 80). In keeping with this, dipyridamole, another inhibitor of cellular uptake of adenosine, inhibited the release of IL-6 and TNFα in a model of human sepsis (81). This may limit excessive innate immune activation during conditions of severe cellular stress, such as sepsis, which may prevent collateral damage from innate immune responses. In summary (Figure 3), possible adenosine-mediated effects of ticagrelor on inflammation are complex and may be pro-inflammatory or anti-inflammatory, depending on the context.

Summary of the effect of ticagrelor on inflammation

The possible effects of ticagrelor on inflammation are complex, due to its dual inhibition of platelet P2Y<sub>12</sub> receptors and ENT1. PLATO showed that ticagrelor was associated with slightly higher levels of IL-6 and CRP than clopidogrel at discharge and the mechanism underpinning this remains to be established. PLATO, however, suggests that the relative difference between ticagrelor and clopidogrel on inflammation might be associated with a beneficial effect on host immunity.

Conflicts of interest

R. F. Storey has received Institutional Research Grants from AstraZeneca, Accumetrics, and Merck; consultancy fees from AstraZeneca, Accumetrics, Aspen, and Thermo Fisher Scientific; as well as speaker fees from AStrazeneca, Accumetrics, and Medscape.

M. Thomas reports no conflicts of interest.

References