



## New Antithrombotic Drugs

### Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines

*Jeffrey I. Weitz, MD, FCCP; John W. Eikelboom, MBBS; and Meyer Michel Samama, MD*

**This article focuses on new antithrombotic drugs that are in or are entering phase 3 clinical testing. Development of these new agents was prompted by the limitations of existing antiplatelet, anticoagulant, or fibrinolytic drugs. Addressing these unmet needs, this article (1) outlines the rationale for development of new antithrombotic agents; (2) describes the new antiplatelet, anticoagulant, and fibrinolytic drugs; and (3) provides clinical perspectives on the opportunities and challenges faced by these novel agents.**

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**Abbreviations:** ACS = acute coronary syndrome; ADP = adenosine diphosphate; aPTT = activated partial thromboplastin time; CYP = cytochrome P450; HR = hazard ratio; LMWH = low-molecular-weight heparin; MI = myocardial infarction; NAPc2 = nematode anticoagulant peptide c2; PAD = peripheral arterial disease; PAI-1 = type 1 plasminogen activator inhibitor; PAR = protease-activated receptor; PCI = percutaneous coronary intervention; PE = pulmonary embolism; PF4 = platelet factor 4; PLATO = Study of Platelet Inhibition and Patient Outcomes; RR = relative risk; TAFI = thrombin activatable fibrinolysis inhibitor; TIMI = thrombolysis in myocardial infarction; TRAP = thrombin receptor agonist peptide; t-PA = tissue plasminogen activator; u-PA = urokinase plasminogen activator

Arterial and venous thrombosis is a major cause of morbidity and mortality. Arterial thrombosis is a common cause of myocardial infarction (MI), ischemic stroke, and limb gangrene; venous thrombosis includes DVT, which can be complicated by the post-thrombotic syndrome, and pulmonary embolism (PE), which can be fatal or can lead to chronic thromboembolic pulmonary hypertension.

Arterial thrombi, which form under high shear conditions, consist of platelet aggregates held together by small amounts of fibrin.<sup>1</sup> Because of the predominance of platelets, strategies to inhibit arterial thrombogenesis focus mainly on drugs that block platelet function but include anticoagulants for prevention of cardioembolic events in patients with atrial fibrillation or mechanical heart valves. Fibrinolytic drugs are used for rapid restoration of antegrade blood flow in patients with acute MI who do not undergo a primary percutaneous coronary intervention (PCI) and for treatment of acute ischemic stroke.

Venous thrombi, which form under low shear, are composed mainly of fibrin and trapped RBCs and contain relatively few platelets.<sup>1</sup> With the predominance of fibrin in venous thrombi, anticoagulants are

the mainstay for the prevention and treatment of VTE. Systemic or catheter-directed fibrinolytic therapy is used for treatment of massive PE and for management of selected patients with submassive PE, whereas catheter-directed fibrinolytic therapy is used in some patients with extensive iliofemoral DVT.

Limitations of existing antithrombotic drugs have prompted a search for novel agents. Focusing on new drugs for the prevention and treatment of arterial and venous thrombosis, this chapter (1) outlines the rationale for development of new antithrombotic drugs; (2) describes the new antithrombotic drugs, focusing primarily on those in phase 2 or 3 clinical testing; and (3) provides perspective on the unmet needs in antithrombotic therapy.

#### 1.0 RATIONALE FOR DEVELOPMENT OF NEW ANTITHROMBOTIC DRUGS

New antithrombotic drugs have been developed to overcome the limitations of existing agents. Most of the advances have been in the area of antiplatelet drugs and anticoagulants. The development of new fibrinolytic agents has lagged.

Although IV glycoprotein IIb/IIIa antagonists have a role in patients undergoing PCI, the need for these agents has declined because of the development of more potent oral antiplatelet drugs. Currently available oral antiplatelet drugs include aspirin, clopidogrel, prasugrel, and dipyridamole. The efficacy of aspirin and clopidogrel has clearly established cyclooxygenase-1, a key enzyme in thromboxane A<sub>2</sub> synthesis, and P2Y<sub>12</sub>, the major adenosine diphosphate (ADP) receptor on platelets, as important targets for antiplatelet drugs. Although the benefits of aspirin for secondary prevention of atherothrombotic cardiovascular events clearly outweigh the risk of bleeding, aspirin is of limited usefulness for primary prevention, including primary prevention in patients with type 2 diabetes mellitus.<sup>2</sup> In addition, recent meta-analyses question the usefulness of aspirin for prevention of cardiovascular events in patients with peripheral arterial disease (PAD).<sup>3</sup> Building on this latter information, an expert panel of the US Food and Drug Administration found insufficient evidence to support over-the-counter use of aspirin for prevention of cardiovascular events in such patients.<sup>4</sup> These issues highlight the limitations of aspirin.

On its own, clopidogrel has been shown to be only marginally more effective than aspirin.<sup>5</sup> The combination of aspirin plus clopidogrel is superior to aspirin alone in patients at high risk for cardiovascular events,<sup>6-9</sup> but combination therapy is associated with a significant risk of bleeding and has yielded disappointing results in patients with stable cardiovascular disease.<sup>10,11</sup> Although the combination of aspirin plus dipyridamole is superior to aspirin alone for secondary prevention in patients with cerebrovascular

disease,<sup>12</sup> the efficacy of this combination is similar to that of clopidogrel.<sup>13</sup>

The limitations of existing antiplatelet drugs reflect, at least in part, their capacity to attenuate only a single pathway of platelet activation. Because platelets can be activated via multiple pathways, the potential for bypassing the inhibitory effects of these drugs remains high when there is a potent stimulus for platelet activation. Consequently, it is not surprising that breakthrough cardiovascular events occur, and these should not necessarily be labeled as simple treatment failures.

Another factor that may contribute to breakthrough cardiovascular events is individual variability in the response to antiplatelet drugs. Such variability may reflect poor compliance, pharmacogenetic factors, increased platelet turnover, drug interactions, baseline and residual platelet hyperreactivity, and other factors.<sup>14,15</sup> Decreased responsiveness to aspirin and/or clopidogrel is common in patients with acute coronary syndromes, particularly in those with diabetes.<sup>16</sup> In patients undergoing PCI, a reduced biologic response to aspirin plus clopidogrel has been associated with a poorer outcome. The decreased response may reflect, at least in part, reduced metabolic activation of clopidogrel.<sup>15</sup> The cytochrome P450 (CYP) 2C19 enzyme plays a critical role in this process, and clopidogrel-treated patients with reduced function variants of the *CYP2C19* gene have lower levels of clopidogrel metabolites and diminished platelet inhibition.<sup>17</sup> It is estimated that 26% of the white population carries one loss-of-function variant of this gene, and about 2% carry two such alleles. These percentages are slightly higher in blacks and substantially higher in Asians. In patients undergoing PCI, those with one or two *CYP2C19* loss-of-function alleles are at increased risk of subsequent cardiovascular events compared with noncarriers.<sup>17</sup> These findings have prompted some experts to recommend tailored antiplatelet therapy based on periprocedural platelet function or genetic testing.<sup>18,19</sup> However, the value of this approach has not been established. Instead, the limitations of existing antiplatelet agents has prompted the development of newer and more potent drugs, some directed against proven targets involved in platelet activation and others against new targets. These agents include novel inhibitors of the thromboxane A<sub>2</sub> receptor, new P2Y<sub>12</sub> antagonists, and inhibitors of protease activated receptor-1 (PAR-1), the major thrombin receptor on platelets.

On the anticoagulant front, most of the recent attention has focused on the development of new oral agents to replace vitamin K antagonists.<sup>20</sup> Rivaroxaban, a direct factor Xa inhibitor, and dabigatran etexilate, a direct thrombin inhibitor, have been licensed in many countries for short-term thromboprophylaxis

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**Affiliations:** From the Thrombosis and Atherosclerosis Research Institute and Department of Medicine (Drs Weitz and Eikelboom) and the Department of Biochemistry and Biomedical Sciences (Dr Weitz), McMaster University, Hamilton, ON, Canada; and the Hôtel-Dieu University Hospital (Dr Samama), Paris, France.

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**Correspondence to:** Jeffrey I. Weitz, MD, FCCP, Thrombosis and Atherosclerosis Research Institute, 237 Barton St E, Hamilton, ON, L8L 2X2, Canada; e-mail: [weitzj@taari.ca](mailto:weitzj@taari.ca)

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after elective hip or knee arthroplasty, and dabigatran etexilate has recently been licensed in the United States and Canada for stroke prevention in patients with atrial fibrillation. Several other direct factor Xa inhibitors, including apixaban and edoxaban, are in advanced stages of development for these and other indications (Table 1).

There have been few advances in fibrinolytic therapy over the past 5 years reflecting, at least in part, the shift from systemic fibrinolytic therapy to catheter-directed interventions for patients with acute MI and the challenge of improving on the efficacy of tissue plasminogen activator (t-PA) and the convenience of its longer-acting derivatives, such as reteplase and tenecteplase.<sup>21</sup> Nonetheless, there still is a need for safer fibrinolytic drugs that can extend the window for treatment of patients with acute ischemic stroke and for agents that produce more rapid and localized clot lysis when used for catheter-based procedures. Focusing on these indications, desmoteplase, a recombinant analog of the full-length plasminogen activator found in the saliva of the vampire bat, is undergoing phase 3 evaluation for treatment of acute ischemic stroke,<sup>22</sup> whereas human plasmin is being explored for catheter-based treatment of ischemic stroke or acute peripheral artery occlusion.<sup>23</sup>

## 2.0 NEW ANTIPLATELET AGENTS

New antiplatelet agents in advanced stages of development target the thromboxane A<sub>2</sub>, ADP, or thrombin receptors on platelets (Fig 1). Like clopidogrel, the novel ADP receptor antagonists target P2Y<sub>12</sub>, whereas the thrombin receptor antagonists target PAR-1.

### 2.1 Thromboxane A<sub>2</sub> Receptor Antagonists

The thromboxane A<sub>2</sub> receptor is a G-protein-coupled receptor on platelets that is activated not only by thromboxane A<sub>2</sub> but also by its cyclic endoperoxide precursors. Thromboxane A<sub>2</sub> receptor antagonists were developed, at least in part, to overcome the variable response to aspirin.<sup>24</sup> Although aspirin blocks thromboxane A<sub>2</sub> synthesis in most individuals, elevated urinary levels of its stable metabolite, thromboxane B<sub>2</sub>, have been associated with an increased risk of cardiovascular events.<sup>25,26</sup> Elevated levels of urinary thromboxane B<sub>2</sub> may reflect incomplete blockade of cyclooxygenase-1 in platelets and/or the shuttling of endoperoxide intermediates to platelets from other cells. The thromboxane A<sub>2</sub> receptor antagonists include terutroban, which is specific for the thromboxane A<sub>2</sub> receptor, and picotamide, which not only blocks the thromboxane A<sub>2</sub> receptor but also inhibits thromboxane A<sub>2</sub> synthetase.

**Table 1—[Section 1.0] Comparison of the Pharmacologic Properties of the New Oral Anticoagulants That Are Approved or in the Most Advanced Stages of Clinical Development**

Property	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Target	Thrombin	Factor Xa	Factor Xa	Factor Xa
Molecular weight	628	436	460	548
Bioavailability, %	6	80	50	50
Dose frequency	od/bid	od/bid	bid	od
Tmax, h	2	3	3	1-2
Half-life, h	12-17	7-11	9-14	9-11
Protein binding, %	35	95	87	54
CYP metabolism, %	None	32	15	< 4
P-gp transport	Yes	Yes	Yes	Yes
Renal excretion, %	80	66	25	35
Extrarenal excretion, %	20	34	75	65

CYP = cytochrome P450; od = once daily; P-gp = p-glycoprotein efflux transporter; Tmax = time to maximum concentration.

**2.1.1 Terutroban:** A selective inhibitor of the thromboxane A<sub>2</sub> receptor on platelets, terutroban (previously known as S18886) is orally active.<sup>27</sup> Peak plasma concentrations are achieved within 1 to 2 h of oral administration, and the drug has a half-life of 6 to 10 h. Terutroban inhibits thromboxane A<sub>2</sub>-induced platelet aggregation in a dose-dependent fashion with maximum inhibition obtained with drug levels > 10 ng/mL; a concentration that can be achieved with daily doses of 10 to 30 mg.<sup>28</sup>

Single-dose administration of 10 mg of terutroban to 12 patients with coronary artery disease who were receiving aspirin (100 mg/d) improved forearm blood flow after acetylcholine infusion compared with the results in eight aspirin-treated patients who received placebo.<sup>29</sup> In patients with PAD randomized to terutroban, aspirin, or placebo, terutroban produced dose-dependent inhibition of platelet aggregation in response to thromboxane, ADP, or collagen and was at least as potent as aspirin.<sup>30</sup> These phase 2 findings prompted the phase 3, double-blind Prevention of Cerebrovascular and Cardiovascular Events of Ischemic Origin with Terutroban in Patients with a History of Ischemic Stroke or Transient Ischemic

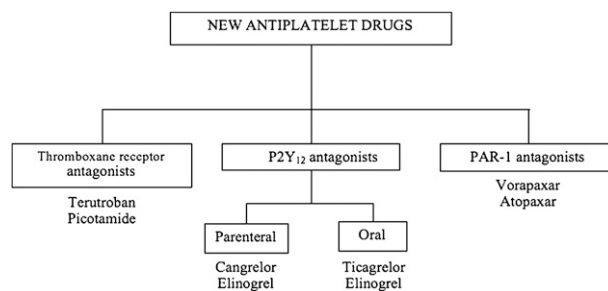


FIGURE 1. New antiplatelet drugs. PAR = protease-activated receptor.

Attack (PERFORM) trial, which compared terutroban (30 mg/d) with aspirin (100 mg/d) for secondary prevention in 19,119 patients with a recent history of ischemic stroke or transient ischemic attacks.<sup>31,32</sup> The primary efficacy end point, a composite of fatal or nonfatal ischemic stroke or MI or vascular death, occurred in 11% of patients receiving either terutroban or aspirin (hazard ratio [HR], 1.02; 95% CI, 0.94-1.12). There was a small increase in minor bleeding with terutroban compared with aspirin (12% and 11%, respectively; HR, 1.11; 95% CI, 1.02-1.12), but no difference in other safety end points.<sup>33</sup>

**2.1.2 Picotamide:** A derivative of methoxy-isophthalic acid, picotamide not only inhibits the thromboxane A<sub>2</sub> receptor but also inhibits thromboxane synthetase at equivalent concentrations.<sup>34</sup> In contrast to aspirin, picotamide does not interfere with prostacyclin production. In a double-blind, placebo-controlled study in 2,304 patients with PAD, treatment with picotamide (300 mg bid) or placebo was administered for 18 months.<sup>35</sup> End points of the study included major events (cardiovascular death, MI, stroke, or amputation) and minor events (unstable angina, transient ischemic attacks, hypertension, renal failure, or worsening of PAD symptoms). Although the intention-to-treat analysis revealed an 18.9% reduction in major plus minor events with picotamide, this difference was not statistically significant. However, the on-treatment analysis showed a 22.8% reduction in the same end points. Bleeding side effects were similar in the two groups. A post hoc subgroup analysis of the data from the 438 patients with diabetes included in the study revealed a 45.2% reduction in major and minor end points with picotamide compared with placebo.<sup>36</sup> These findings prompted a randomized trial comparing picotamide (600 mg bid) with aspirin (320 mg/d) in 1,209 patients with diabetes with

PAD.<sup>37</sup> The primary efficacy end point was overall mortality, whereas the secondary end point was the composite of death and cardiovascular events. At 2 years, the overall mortality rates with picotamide and aspirin were 3.0% and 5.5%, respectively (relative risk [RR], 0.55; 95% CI, 0.31-0.98). Cardiovascular events occurred in 7.1% of patients given picotamide and 8.7% of those treated with aspirin. The difference in the combined end point of mortality plus cardiovascular events between the two groups did not reach statistical significance. Bleeding events were infrequent with both picotamide and aspirin (1.3% and 2.0%, respectively). Although these results are promising, additional studies are needed to establish the role of picotamide in patients with diabetes with PAD.

## 2.2 ADP Receptor Antagonists

Three reversible P2Y<sub>12</sub> inhibitors are in phase 3 development: cangrelor, ticagrelor, and elinogrel (Table 2). Although these agents are chemically distinct and have different pharmacologic profiles, they share certain properties. In contrast to the thienopyridines, the reversible P2Y<sub>12</sub> inhibitors do not require metabolic activation and they bind directly and reversibly to the P2Y<sub>12</sub> receptor. Because of the reversible binding, their inhibitory effects decrease as drug concentrations fall.

**2.2.1 Cangrelor:** An adenosine triphosphate analog, cangrelor is a direct competitive inhibitor of P2Y<sub>12</sub>.<sup>37</sup> In contrast to clopidogrel or prasugrel, cangrelor does not require hepatic conversion to an active metabolite. The drug is only active when administered IV and it produces almost immediate and dose-proportional inhibition of ADP-induced platelet aggregation. Cangrelor is rapidly inactivated by dephosphorylation and has a half-life of 3 to 5 min. Upon cessation of

**Table 2—[Section 2.2] Pharmacologic Characteristics of Direct-Acting Reversible P2Y<sub>12</sub> Inhibitors**

Characteristic	Cangrelor	Ticagrelor	Elinogrel
Molecular weight	776	523	562
Route of administration	IV	Oral	IV or oral
Site of action	ADP binding site	Site distinct from ADP binding site	ADP binding site
Type of inhibition	Competitive	Noncompetitive	Competitive
Time to peak activity	30 min	2 h	20 min and 12 h for IV and oral formulation, respectively
Frequency of oral administration	Inactive	bid	bid
Half-life	3-5 min	6-12 h	Not reported
Metabolism	Dephosphorylation	O-deethylation and oxidation	None
Elimination	27% Renal; 58% feces	30% Renal; 70% feces	40% Renal; 60% feces
Time to recovery of platelet function	60 min	3-5 d	Not reported
Stage of development	Phase 3	Completed phase 3; licensed in United States, Europe, and Canada	Phase 2

ADP = adenosine diphosphate.

therapy, therefore, there is recovery of platelet function within 60 min.<sup>37,38</sup>

The interaction of cangrelor with P2Y<sub>12</sub> prevents the binding of the active metabolites of clopidogrel or prasugrel. This phenomenon complicates the transitioning of patients from cangrelor to clopidogrel or other thienopyridines, which can only exert their inhibitory effects once cangrelor dissociates from P2Y<sub>12</sub>.

Cangrelor has been evaluated in a two-part phase 2 trial in patients undergoing PCI.<sup>39</sup> For part one, 200 patients were randomized to an 18- to 24-h infusion of cangrelor (in doses of 1, 2, or 4 µg/kg/min) or placebo in addition to aspirin plus heparin. In the second part, an additional 199 patients were randomized to IV cangrelor (at a dose of 4 µg/kg/min) or abciximab prior to PCI. In the first part of the study, the primary end point, the combination of major and minor bleeding up to 7 days, occurred in 13% of patients given cangrelor and in 8% of those given placebo, a difference that was not statistically significant.<sup>39</sup> In the second part, major plus minor bleeding occurred in 7% and 10% of those randomized to cangrelor or abciximab, respectively. The 30-day composite of adverse cardiac events (death, MI, or unplanned repeat coronary intervention) was not significantly different in patients randomized to cangrelor or abciximab (7.6% and 5.3%, respectively).

Building on these phase 2 data, cangrelor was investigated in two double-blind phase 3 trials. In the Cangrelor vs Standard Therapy to Achieve Optimal Management of Platelet Inhibition (CHAMPION)-PCI trial, 8,877 patients scheduled for PCI were randomized to receive IV cangrelor (30 µg/kg bolus followed by an infusion of 4 µg/kg/min) or placebo starting 30 min prior to PCI and continuing for at least 2 h after completion of the procedure.<sup>40</sup> Patients received 600 mg of clopidogrel or placebo at the time of the infusion. At the end of the infusion, the transition from IV cangrelor to clopidogrel was facilitated by administration of 600 mg of clopidogrel to those in the cangrelor group and administration of placebo to those in the clopidogrel group. All patients received aspirin. The primary efficacy end point, a composite of all-cause mortality, MI, or ischemia-driven revascularization within 48 h of PCI, occurred in 7.5% of those randomized to cangrelor and 7.1% of those given placebo (HR, 1.05; 95% CI, 0.88-1.24;  $P = .59$ ). Rates of major bleeding were similar with cangrelor or clopidogrel (3.6% and 2.9%, respectively;  $P = .06$ ), but rates of minor bleeding were higher with cangrelor (17.6% and 15.2%, respectively;  $P = .003$ ).

In the CHAMPION PLATFORM trial, 5,362 patients with non-ST-elevation MI or unstable angina with at least one coronary lesion amenable to PCI were randomized to receive cangrelor (in the same

regimen that was used in the CHAMPION-PCI trial) or placebo at the time of PCI.<sup>41</sup> Patients in the cangrelor group received 600 mg of clopidogrel after the cangrelor infusion stopped, whereas those in the placebo group received 600 mg of clopidogrel after the procedure. The primary efficacy end point, which was the same as that used in CHAMPION-PCI, occurred in 7.0% of those given cangrelor and in 8.0% of patients randomized to placebo (HR, 0.87; 95% CI, 0.71-1.07;  $P = .17$ ). Major bleeding was more frequent with cangrelor than with placebo (5.5% and 3.5%, respectively;  $P < .001$ ). Based on the negative results of these two trials, more work is needed to determine the role of cangrelor in patients with acute coronary syndrome (ACS) undergoing PCI.

**2.2.2 Ticagrelor:** An orally active agent belonging to the cyclopentyl-triazolopyrimidine class, ticagrelor acts as a direct inhibitor of P2Y<sub>12</sub>.<sup>42</sup> Ticagrelor binds to the receptor at a location distinct from the ADP binding site and blocks ADP-mediated receptor activation in a noncompetitive fashion, likely through an allosteric mechanism. Like cangrelor, ticagrelor does not require hepatic conversion to an active metabolite. Consequently, the drug has a rapid onset of action and within 30 min achieves a level of inhibition of ADP-induced platelet exceeding that obtained with a 300- or 600-mg loading dose of clopidogrel.<sup>43</sup> The peak inhibitory effect of ticagrelor is seen about 2 h after a loading dose of ticagrelor of 180 mg or a maintenance dose of 90 mg bid.

When compared with clopidogrel in 200 patients with atherosclerosis treated with aspirin, ticagrelor, in doses of 100 or 200 mg bid or 400 mg once daily, produced more rapid and more potent inhibition of ADP-induced platelet aggregation.<sup>44</sup> The Dose Confirmation Study Assessing Antiplatelet Effects of ticagrelor vs Clopidogrel in non-ST-elevation MI (DISPERSE 2) compared ticagrelor plus aspirin with clopidogrel plus aspirin in 990 patients with non-ST-segment elevation ACS.<sup>45</sup> Patients were randomized to receive ticagrelor (90 or 180 mg bid) or clopidogrel (75 mg once daily). One-half of the patients randomized to ticagrelor were given a loading dose of 270 mg, whereas the other one-half only received the maintenance dose. The primary end point, a combination of major and minor bleeding, occurred in 10.2% of patients given either dose of ticagrelor and in 9.2% of those treated with clopidogrel.

Building on the promising phase 2 data, ticagrelor (180 mg loading dose followed by 90 mg bid thereafter) was compared with clopidogrel (300 to 600 mg loading dose followed by 75 mg daily thereafter) for prevention of cardiovascular events in 18,624 patients with acute coronary syndrome in the Study of

Platelet Inhibition and Patient Outcomes (PLATO) trial.<sup>46</sup> At 12 months, the primary efficacy end point—a composite of cardiovascular death, MI, or stroke—occurred in 9.8% of patients treated with ticagrelor and in 11.7% of those given clopidogrel (HR, 0.84; 95% CI, 0.77-0.92;  $P < .001$ ). The rate of MI was lower with ticagrelor than with clopidogrel (5.8% and 6.9%, respectively;  $P = .005$ ), as were the rates of cardiovascular mortality (4.0% and 5.1%, respectively;  $P = .001$ ) and all-cause mortality (4.5% and 5.9%, respectively;  $P < .001$ ). In contrast, the rate of stroke was similar with ticagrelor and clopidogrel (1.5% and 1.3%, respectively;  $P = .22$ ). Although there was no significant difference in the rates of major bleeding with ticagrelor and clopidogrel (11.6% and 11.2%, respectively;  $P = .43$ ), ticagrelor was associated with a higher rate of major bleeding not related to coronary artery bypass graft surgery (4.5% and 3.8%, respectively;  $P = .03$ ), including more fatal intracranial bleeds (0.1% and 0.01%, respectively;  $P = .02$ ).

Of the 18,624 patients entered in the PLATO trial, an invasive strategy was planned for 72%. In this subset, the primary composite end point occurred in 9.0% of patients randomized to ticagrelor and in 10.7% of those given clopidogrel (HR, 0.84; 95% CI, 0.75-0.94;  $P = .0025$ ). Rates of major bleeding were similar with ticagrelor and clopidogrel (11.6% and 16.5%, respectively;  $P = .88$ ).

PLATO-STEMI focused on the 7,544 patients with ST-elevation MI included in the PLATO trial who were scheduled to undergo primary PCI.<sup>47</sup> Of these patients, 75% received a stent, the majority of which were bare metal stents. In this subset, the primary efficacy end point occurred in 9.4% of patients randomized to ticagrelor and in 10.8% of those given clopidogrel (HR, 0.87; 95% CI, 0.75-1.01;  $P = .07$ ). The rate of MI was lower with ticagrelor than with clopidogrel (4.7% and 5.8%, respectively;  $P = .03$ ), as was all-cause mortality (5.0% and 6.1%, respectively;  $P = .05$ ). Definite stent thrombosis occurred in 1.6% of patients taking ticagrelor and in 2.4% of those given clopidogrel (HR, 0.60; 95% CI, 0.45-0.95;  $P = .03$ ). There was no increase in the rate of major bleeding with ticagrelor compared with clopidogrel (9.0% and 9.2%, respectively;  $P = .76$ ).

Outcomes in the 1,899 patients enrolled in the PLATO trial who underwent coronary artery bypass graft surgery postrandomization were reported in PLATO-CABG.<sup>48</sup> The protocol recommended withholding ticagrelor (or placebo) for 1 to 3 days and clopidogrel (or placebo) for 5 days prior to surgery, but among the 1,261 patients who underwent coronary artery bypass graft surgery within 7 days of stopping study drug, only 30.1% stopped within 2 days, 43.8% stopped within 3 to 5 days, and 26.1% stopped  $> 5$  days prior to surgery. The corresponding

numbers for clopidogrel were 27.7%, 37.9%, and 34.5%. In this subset, the primary efficacy end point occurred in 10.6% of patients randomized to ticagrelor and 13.1% of those who received clopidogrel (HR, 0.84; 95% CI, 0.60-1.16;  $P = .29$ ). The rate of cardiovascular death was lower with ticagrelor than with clopidogrel (4.1% and 7.9%, respectively;  $P < .01$ ), as was all-cause mortality (4.7% and 9.7%, respectively;  $P < .01$ ) but there was no difference in MI or stroke and no reduction in CABG-related bleeding.

Side effects of ticagrelor include dyspnea, which is usually mild and dose related,<sup>49</sup> asymptomatic bradycardia with ventricular pauses,<sup>46</sup> and a modest increase in the levels of uric acid. The mechanisms responsible for these side effects are unclear. One possible explanation relates to the capacity of ticagrelor to inhibit adenosine reuptake by erythrocytes, thereby increasing circulating levels of adenosine. In addition to explaining the dyspnea and the bradycardia, the resultant adenosine-induced vasodilatation and increased myocardial perfusion could also endow ticagrelor with beneficial effects that are independent of P2Y<sub>12</sub> blockade.

**2.2.3 Elinogrel:** A reversible P2Y<sub>12</sub> inhibitor available in both IV and oral formulation, elinogrel is a competitive inhibitor of P2Y<sub>12</sub> that blocks ADP binding to the receptor.<sup>50</sup> Consequently, the drug inhibits platelet aggregation in response to low concentrations of ADP, but its inhibitory effects can be overcome with higher ADP concentrations. This phenomenon could endow elinogrel with a favorable risk-benefit profile if ADP concentrations are higher with hemostatic plug formation than with intravascular thrombosis.

The Early Rapid Reversal of Platelet Thrombosis with Intravenous Elinogrel before PCI to Optimize Acute MI (ERASE MI) dose-escalation pilot study evaluated the safety and tolerability of a single IV bolus of elinogrel, in doses of 10, 20, 40, or 60 mg, compared with placebo given before the start of the diagnostic angiogram preceding primary PCI in 70 patients with ST-elevation MI.<sup>51</sup> All patients received a 600-mg loading dose of clopidogrel followed by a second 300-mg loading dose of clopidogrel 4 h after the procedure. The primary outcome was major bleeding, which was infrequent and occurred at a rate with all doses of elinogrel that was similar to the rate with placebo. However, the trial was stopped early for administrative reasons and subsequent studies used oral elinogrel in place of clopidogrel after the initial IV elinogrel bolus.

In the phase 2 Intravenous and Oral Administration of Elinogrel vs Clopidogrel to Evaluate Tolerability and Efficacy in Nonurgent PCI Patients

(INNOVATE-PCI) study, 652 patients scheduled for nonurgent PCI were randomized to clopidogrel (300 to 600 mg load followed by 75 mg daily thereafter) or to elinogrel (80 mg IV bolus followed by oral dosing of 50, 100, or 150 mg bid thereafter).<sup>52</sup> The Data Safety Monitoring Board recommended discontinuation of the 50-mg oral dose and suggested increasing the IV dose to 120 mg. The study was not powered for efficacy, but patients given elinogrel exhibited greater platelet inhibition than those treated with clopidogrel.

Dyspnea occurred more frequently with elinogrel than with clopidogrel. Abnormal liver function tests also were more common with elinogrel, but the abnormalities appeared to resolve over time, even if the drug was continued.<sup>51,52</sup>

Elinogrel will soon undergo phase 3 evaluation in aspirin-treated patients with a history of MI within the past 6 months to 5 years. Such patients will be randomized to elinogrel (in one of two doses) or to placebo for approximately 29 months. The primary efficacy outcome will be the composite of cardiovascular mortality, MI, or stroke. A phase 3 trial evaluating IV and oral elinogrel in patients with ACS is likely to follow.

### 2.3 PAR-1 Antagonists

PAR-1 belongs to a family of G-protein-coupled receptors that are activated by proteolytic cleavage.<sup>53</sup> Human platelets express PAR-1 and PAR-4, both of which can be activated by thrombin to induce platelet secretion and aggregation. Although activation of either receptor can cause platelet aggregation independently of the other, PAR-1 and PAR-4 act synergistically to induce platelet activation. However, the affinity of PAR-1 for thrombin is 40-fold higher than that of PAR-4. Consequently, PAR-1 is activated by relatively low concentrations of thrombin, whereas PAR-4 activation requires higher thrombin concentrations. Therefore, PAR-1 is considered to be the major thrombin receptor on human platelets.<sup>53</sup>

PAR-1 also is found on endothelial cells, smooth muscle cells, fibroblasts, and cardiac myocytes.<sup>53</sup> Thrombin-mediated activation of PAR-1 on these cells may contribute to the proliferative and proinflammatory effects of thrombin. Therefore, it is possible that PAR-1 antagonism will not only attenuate arterial thrombosis but may also modulate other thrombin-mediated processes, including restenosis. Two orally active PAR-1 antagonists are under investigation; vorapaxar and atopaxar (Table 3).

**2.3.1 Vorapaxar:** A synthetic tricyclic 3-phenylpyridine analog of himbacine, an alkaloid isolated from the bark of Australian magnolia trees that is used in several natural products, vorapaxar is a potent and

specific competitive inhibitor of PAR-1.<sup>54</sup> The drug has excellent oral bioavailability and produces dose-dependent inhibition of thrombin- or thrombin receptor agonist peptide (TRAP)-induced platelet aggregation. Vorapaxar does not affect platelet aggregation in response to other agonists nor does it affect thrombin-mediated conversion of fibrinogen to fibrin. Vorapaxar has a long half-life of 126 to 269 h and inhibits TRAP-induced platelet aggregation for up to 4 weeks. Although the binding of vorapaxar to PAR-1 is reversible, dissociation of the drug from the receptor is slow, which may explain the long half-life. Vorapaxar is metabolized by CYP3A4.<sup>53</sup>

In phase 1 studies, vorapaxar did not appear to prolong the bleeding time when administered to healthy volunteers.<sup>55</sup> A phase 2 study in 1,031 patients scheduled for coronary angiography and possible PCI randomized patients to vorapaxar (at loading doses of 10, 20, or 40 mg) or placebo in a 3:1 ratio.<sup>56</sup> A total of 573 patients underwent PCI, all of whom received aspirin, clopidogrel, and an anticoagulant (either heparin or bivalirudin). Those randomized to vorapaxar received maintenance therapy at doses of 0.5, 1.0, or 2.5 mg once daily for 2 months. The primary outcome, a combination of thrombolysis in myocardial infarction (TIMI) major and minor bleeding, occurred in 3.3% of the 151 patients randomized to placebo and in 2.8% of the 422 patients given vorapaxar. Major bleeding occurred in 1.3% and 0.7%, respectively. The efficacy end point of death, major adverse coronary events, or stroke occurred in 8.6% of patients randomized to placebo and in 6.2% of those given vorapaxar.

The safety of vorapaxar observed in the Thrombin Receptor Antagonist (TRA)-PCI study was confirmed in two small randomized trials conducted in Japanese patients. In the first, a total of 117 patients undergoing PCI for a non-ST elevation ACS were randomized to vorapaxar for 60 days (either 20- or 40-mg loading dose, followed by 1- or 2.5-mg daily maintenance dose) or placebo in addition to standard-of-care antithrombotic therapy.<sup>57</sup> No differences were observed in the rate of the primary safety end point

**Table 3—[Section 2.3] PAR-1 Antagonists: Comparison of the Features of Vorapaxar and Atopaxar**

Feature	Vorapaxar	Atopaxar
Molecular weight	591	608
Onset of action, h	2	3.5
Half-life, h	250	23
Route of elimination	Feces	Feces
Metabolism	CYP3A4	CYP3A4
Stage of development	Phase 3	Phase 2

PAR-1 = protease-activated receptor 1. See Table 1 legend for expansion of other abbreviation.

(TIMI major and minor bleeding), but compared with placebo, vorapaxar reduced the rate of nonfatal MI (42.9% and 16.9%, respectively;  $P = .013$ ). In the second study, which evaluated the safety of vorapaxar in patients with a history of ischemic stroke,<sup>58</sup> vorapaxar (1 or 2.5 mg daily) or placebo was administered to 90 such patients for 60 days. All patients received aspirin. Event rates were low, and vorapaxar appeared to be safe in this setting.

Building on these phase 2 results, vorapaxar underwent phase 3 evaluation in two large clinical trials: Thrombin Receptor Antagonists for Clinical Event Reduction (TRA-CER)<sup>59</sup> and Thrombin Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events (TRA2P-TIMI 50).<sup>60</sup> TRA-CER was a randomized, double-blind trial that compared vorapaxar with placebo on top of standard-of-care treatment with aspirin and/or clopidogrel in 13,000 patients with non-ST-elevation ACS. Follow-up in the trial was terminated early because of safety concerns. After a median follow-up of 502 days, the rates of the primary efficacy outcome (a composite of cardiovascular death, MI, stroke, hospitalization for recurrent ischemia, or urgent coronary revascularization) in the vorapaxar and placebo groups were 18.5% and 19.9%, respectively (HR, 0.29; 95% CI, 0.85-1.01;  $P = .07$ ). The composite of cardiovascular death, MI, or stroke was lower with vorapaxar than with placebo (14.7% and 16.4%, respectively; HR, 0.89; 95% CI, 0.81-0.98;  $P = .02$ ). Rates of moderate and severe bleeding were higher with vorapaxar than with placebo (7.2% and 5.2%, respectively) and there was more intracranial bleeding with vorapaxar (1.1% and 0.2%). Thus, addition of vorapaxar to standard therapy did not reduce the composite end point, but significantly increased the risk of bleeding.

TRA2P-TIMI 50 is a randomized double-blind trial compared vorapaxar (2.5 mg daily) with placebo on top of standard antiplatelet therapy (with aspirin and/or a thienopyridine) in about 26,500 patients with a history of MI, stroke, or PAD. After safety review, vorapaxar was discontinued in patients with a stroke prior to entry or during the course of the trial because of excess bleeding.

**2.3.2 Atopaxar:** A reversible PAR-1 antagonist, atopaxar binds PAR-1 with high affinity and blocks thrombin and TRAP-induced platelet aggregation.<sup>53</sup> Like vorapaxar, atopaxar is a small molecule chemically identified as 1-(3-*tert*-butyl-4-methoxy-5-morpholinophenyl)-2-(5,6-diethoxy-7-fluoro-1-imino-1,3-dihydro-2H-isindolyl-2) ethanone hydrobromide. Because atopaxar inhibits TRAP binding to PAR-1, it is likely that the drug interacts with PAR-1 at or near the tethered ligand binding site. Atopaxar exhibits good oral bioavailability and is rapidly absorbed.

Maximal platelet inhibition is achieved within 3 to 5 h of dosing and the half-life is about 23 h. Like vorapaxar, atopaxar does not appear to prolong the bleeding time when administered to healthy volunteers.<sup>53</sup>

In the first of the phase 2 Lessons from Antagonizing the Cellular Effect of Thrombin in Japanese Patients (J-LANCELOT) trials, 241 patients with ACS (unstable angina or non-ST-elevation MI) were given atopaxar (loading dose of 400 mg followed by either placebo or atopaxar 50, 100, or 200 mg daily thereafter) for 12 weeks.<sup>61</sup> More than 95% of the patients were also taking aspirin and clopidogrel. In the second trial, 263 patients with coronary artery disease were randomized to placebo or the same doses of atopaxar for 24 weeks.<sup>54</sup> All patients were taking aspirin, and about 40% were also receiving clopidogrel. The primary end point in these studies was bleeding events; the secondary end points were major adverse cardiovascular events, including cardiovascular death, MI, stroke, or recurrent ischemia, and inhibition of platelet aggregation. There was a nonsignificant trend toward an increase in bleeding with increasing doses of atopaxar. The combination of major plus minor bleeding and minimal bleeding requiring medical attention was similar in patients receiving placebo and those given atopaxar (6.6% and 5.0%, respectively, in the first study and 1.5% in both groups in the second study). The studies were underpowered for efficacy, but at trough levels, atopaxar produced a mean of > 90% inhibition of platelet aggregation with the 100- or 200-mg doses and 20% to 60% inhibition with the 50-mg dose.<sup>54</sup>

The clinical development plan for atopaxar is uncertain. Unexplained increased transaminase levels were noted in up to 15% of patients receiving atopaxar.<sup>61</sup> In addition, prolongation of the QTc interval was seen with higher doses of atopaxar in the phase 2 studies.<sup>61</sup> Additional studies are needed to determine the clinical significance of these findings.

### 3.0 NEW ANTICOAGULANTS

Anticoagulants can inhibit the initiation or propagation of coagulation, or, by targeting thrombin, they can attenuate fibrin formation. Drugs that target the tissue factor/factor VIIa complex block the initiation of coagulation, whereas those that inhibit factor IXa or factor Xa, or their cofactors, factor VIIIa and factor Va, block the propagation of coagulation. Finally, anticoagulants that target thrombin attenuate fibrin generation (Fig 2). New anticoagulants can be further subclassified as direct or indirect inhibitors (Fig 3). Direct inhibitors bind directly to the target enzyme and block substrate interactions. In contrast, indirect inhibitors exert their anticoagulant effects by binding



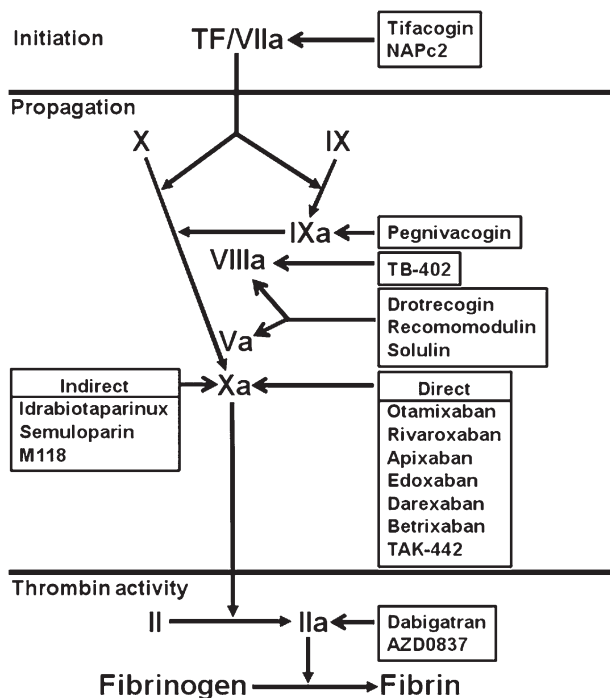


FIGURE 2. Sites of action of new anticoagulants in more advanced stages of development. NAPc2 = nematode anticoagulant peptide c2.

to naturally occurring plasma cofactors, such as antithrombin or heparin cofactor II, thereby accelerating their interaction with clotting enzymes.

### 3.1 Inhibitors of Initiation of Coagulation

Drugs that target the factor VIIa/tissue factor complex inhibit the initiation of coagulation. Only

parenteral agents in this category have reached phase 2 or 3 clinical testing (Table 4). These include tifa-cogin, which is a recombinant form of tissue factory pathway inhibitor, recombinant nematode anticoagulant peptide (NAPc2), and active site inhibited factor VIIa (factor VIIai).

**3.1.1 Tifacogin:** A recombinant form of tissue factory pathway inhibitor, tifa-cogin has been evaluated in patients with sepsis. The drug has a half-life of minutes, which necessitates IV infusion, and is cleared by the liver. In a phase 2 trial,<sup>62</sup> 210 patients with sepsis were randomized to receive one of two doses of tifa-cogin (25 or 50 mg/kg/h) by continuous infusion or placebo for 4 days. Compared with placebo, tifa-cogin produced a 20% relative reduction in 28-day mortality. Major bleeding occurred in 9% of patients treated with tifa-cogin and in 6% of those given placebo, a nonsignificant difference. Building on these results, a phase 3 trial compared tifa-cogin with placebo in 1,754 patients with severe sepsis.<sup>63</sup> The primary end point, 28-day mortality, was similar with tifa-cogin and placebo (34.2% and 33.9%, respectively), whereas the rate of bleeding was significantly higher with tifa-cogin (6.5% and 4.8%, respectively). A post hoc subgroup analysis suggested a benefit of tifa-cogin in the 780 patients with severe community-acquired pneumonia; in this subset, 28-day mortality was 27.9% with tifa-cogin and 32.7% with placebo.<sup>64</sup> Differences in mortality were greater in patients with more severe disease who did not receive adjunctive heparin. Based on this analysis, a phase 3 double-blind trial compared two doses of tifa-cogin with placebo in about 2,100 patients with severe community-acquired

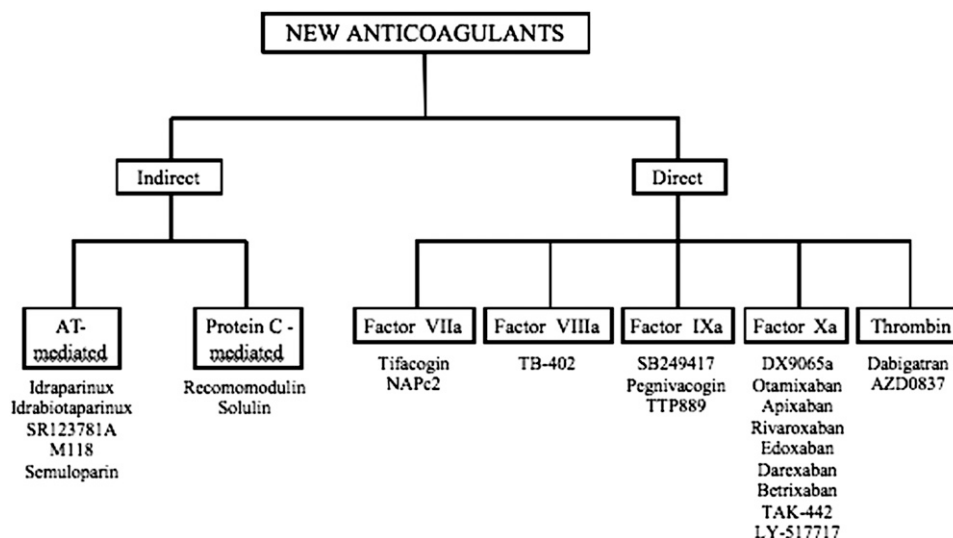


FIGURE 3. Classification of new anticoagulants. Indirect anticoagulants act in an AT-dependent fashion or exert their effect via the protein C pathway. Direct anticoagulants do not require a plasma cofactor. Instead, these agents directly target a specific coagulation enzyme. AT = antithrombin. See Figure 2 legend for expansion of other abbreviation.

**Table 4—[Section 3.4] Inhibitors of the Factor VIIa-Tissue Factor Complex**

Drug	Route of Administration	Mechanism of Action	Stage of Development
Tifacogin	IV	Inhibits factor VIIa in a factor Xa-dependent fashion	Phase 3
NAPc2	Subcutaneous	Inhibits factor VIIa in a factor X- or Xa-dependent fashion	Phase 2
Factor VIIai	IV	Competes with factor VIIa for tissue factor	Halted

NAPc2 = nematode anticoagulant peptide c2.

pneumonia.<sup>65</sup> Although enrollment was completed in 2008, the results of this trial have not been reported.

**3.1.2 NAPc2:** An 85-amino acid polypeptide originally isolated from the canine hookworm, *Ancylostoma caninum*,<sup>66</sup> recombinant NAPc2 is expressed in yeast. NAPc2 binds to a noncatalytic site on factor X or factor Xa.<sup>67</sup> Once bound to factor Xa, the NAPc2/factor Xa complex inhibits tissue factor-bound factor VIIa. Because it binds factor X with high affinity, NAPc2 has a half-life of approximately 50 h after subcutaneous injection.<sup>68</sup> Consequently, the drug can be given on alternate days.

Initial clinical trials with NAPc2 focused on venous thromboprophylaxis. In a phase 2 dose-finding study,<sup>69</sup> 293 patients undergoing elective knee arthroplasty were given subcutaneous NAPc2 on the day of surgery and every second day thereafter to a maximum of 4 doses. The best results were observed with a NAPc2 dose of 3.0 µg/kg administered 1 h after surgery. With this dose, the rate of venographically-detected DVT in the operated leg was 12%, whereas the rate of proximal DVT was 1%. Major bleeding occurred in 2% of patients.

In a series of phase 2 studies, NAPc2 was evaluated in patients with unstable angina or non-ST-elevation ACS and in those undergoing PCI. Addition of NAPc2 to usual antithrombotic therapy in 203 patients with non-ST-elevation ACS reduced levels of prothrombin fragment 1.2 in a dose-dependent fashion without increasing the risk of bleeding.<sup>70</sup> In a second study, adjunctive NAPc2 (in doses ranging from 3.5-10 µg/kg) suppressed levels of prothrombin fragment 1.2 in patients undergoing elective PCI.<sup>71</sup> Despite these promising initial results, the disease focus for NAPc2 shifted from thrombosis to cancer to capitalize on emerging evidence that tissue factor plays a role in tumor progression. However, a phase 2 study examining the feasibility of administering escalating doses of twice-weekly subcutaneous NAPc2 as an adjunct to chemotherapy for metastatic colon cancer was suspended.<sup>72</sup> The data from this study

have not been reported and development of NAPc2 appears to have halted, at least temporarily.

**3.1.3 Factor VIIai:** Recombinant factor VIIa that has its active site irreversibly blocked competes with factor VIIa for tissue factor binding, thereby attenuating the initiation of coagulation by the factor VIIa/tissue factor complex. Based on promising results in animal models of thrombosis,<sup>73,74</sup> factor VIIai, given in doses ranging from 50 to 400 mg/kg with or without adjunctive heparin was compared with heparin alone in 491 patients undergoing elective PCI.<sup>75</sup> Factor VIIai, with or without adjunctive heparin, produced no significant reduction in the primary end point, a composite of death, MI, need for urgent revascularization, abrupt vessel closure, or bailout use of glycoprotein IIb/IIIa antagonists or heparin at day 7 or at hospital discharge. Rates of major bleeding were similar with factor VIIai and heparin. Because of these disappointing results, factor VIIai has not been developed further for treatment of arterial thrombosis.

### 3.2 Inhibitors of Propagation of Coagulation

Propagation of coagulation can be inhibited by drugs that target factors IXa or Xa or by agents that inactivate their respective cofactors, factors VIIIa and Va, respectively.

**3.2.1 Factor IXa Inhibitors:** Both parenteral and oral factor IXa inhibitors have been developed (Table 5). The parenteral agents include factor IX-directed monoclonal antibodies and pegnivacogin, a factor IXa-directed aptamer. Development of TTP889, the only oral factor IXa inhibitor, has been halted.

**3.2.1.1 Factor IX-Directed Antibodies—**Several monoclonal antibodies directed against various factor IX epitopes have been developed. Of these, SB 249417, a humanized mouse monoclonal antibody directed against the Gla-domain of factor IX, has undergone the most extensive investigation. The

**Table 5—[Section 3.2.1] Factor IXa Inhibitors**

Drug	Route of Administration	Mechanism of Action	Stage of Development
SB249417	IV	Partial inhibitor of factor IXa	Halted
Pegnivacogin	IV	Factor IXa-directed inhibitory RNA aptamer	Phase 2
TTP889	Oral	Inhibits factor IXa incorporation into intrinsic tenase	Halted

antibody exhibited antithrombotic activity comparable to that of enoxaparin in a rat arterial thrombosis model, but produced less prolongation of the activated partial thromboplastin time (aPTT).<sup>76</sup> In a rat model of thromboembolic stroke, the antibody reduced infarct volumes and neurologic deficits to a greater extent than t-PA.<sup>77</sup>

SB249417 has undergone limited evaluation in humans. In a phase 1 study in 26 human volunteers, a 50-min infusion of antibody prolonged the aPTT in a dose-dependent fashion.<sup>78</sup> Development of the antibody has been stopped.

**3.2.1.2 Pegnivacogin**—A specific factor IXa-directed RNA aptamer, pegnivacogin, which was previously known as RB006, was isolated from a library of 104 nucleic acid species.<sup>79</sup> Cholesterol was conjugated to the 5' end of pegnivacogin to extend its circulating half-life to about 12 h. Pegnivacogin binds to both factor IX and factor IXa with high affinity and not only inhibits factor IXa activity, but also blocks the activation of factor IX by the factor VIIa-tissue factor complex, but not by factor XIa. As such, pegnivacogin prolongs the aPTT in a dose-dependent fashion but has no effect on the prothrombin time. A unique feature of pegnivacogin is that its anticoagulant activity can be rapidly reversed with a complementary aptamer, anivamersen (previously known as RB007). Anivamersen exerts its inhibitory effect by binding to pegnivacogin and releasing it from factor IX or factor IXa.

In a phase 1 study, pegnivacogin and anivamersen were evaluated in 85 healthy volunteers.<sup>80</sup> These individuals received increasing IV bolus doses of pegnivacogin or placebo followed 3 h later by IV bolus doses of anivamersen or placebo. At pegnivacogin doses of 30 to 60 mg, there was a dose-dependent prolongation of the aPTT and activated clotting time that was rapidly restored to baseline values with anivamersen administration.

Two early phase 2 studies evaluated the safety and tolerability of pegnivacogin and anivamersen in patients with coronary artery disease.<sup>81,82</sup> In the first of these studies, 50 patients with stable coronary artery disease taking aspirin and/or clopidogrel received an IV bolus of pegnivacogin at doses of 15, 30, 50, or 75 mg. Pegnivacogin prolonged the aPTT in a concentration-dependent fashion. Subsequent administration of anivamersen (at doses of 30, 60, 100, or 150 mg) restored the aPTT to baseline levels within a median of 1 min (25th and 75th percentiles, 1 and 2 min, respectively) with no rebound increase through 7 days.<sup>81</sup> In the second study, 24 patients undergoing nonurgent PCI were randomized in a 5:1 ratio to receive pegnivacogin or unfractionated heparin with immediate or delayed reversal of pegnivacogin after the procedure.<sup>82</sup> All procedures were

successful and both reversal strategies enabled successful sheath removal.

In the phase 2 Randomized, Active-Controlled, Dose-Ranging Study Assessing the Safety, Efficacy and Pharmacodynamics of the REG1 Anticoagulation System (RADAR) study, 640 patients with ACS scheduled for cardiac catheterization were randomly allocated to receive open-label pegnivacogin (1 mg/kg) or unfractionated heparin in a 4:1 ratio prior to PCI. After the procedure, patients given pegnivacogin then were given anivamersen at four different doses designed to produce 25%, 50%, 75%, or 100% reversal.

Recruitment into the lowest-dose anivamersen arm was stopped early because of a higher rate of major bleeding compared with heparin (20.0% and 10.1%, respectively). Rates of major bleeding with doses of anivamersen that produced 50%, 75%, or 100% reversal were 10.6%, 8.4%, and 7.3%, respectively. There was a trend for fewer ischemic events with pegnivacogin than with heparin (3.0% and 5.7%, respectively), but the total number of events was small. Sheaths were removed at a mean of 24 min after the procedure in patients given pegnivacogin and after 3 h in those given heparin. Three allergic reactions were reported with pegnivacogin. Although two were mild, one patient required hemodynamic support. The mechanism responsible for these reactions has not been elucidated.

**3.2.1.3 TTP889**—An orally active partial inhibitor of factor IXa inhibitor, TTP889 exhibited antithrombotic activity in rat and porcine arteriovenous shunt models. A small phase 1 study was reported as showing a predictable pharmacokinetic profile after single or multiple dose administration and a half-life of about 20 h. In a phase 2 proof-of-concept study, 261 patients undergoing surgery for hip fracture were given conventional anticoagulant prophylaxis for 1 week and were then randomized to receive once daily oral TTP889 (at a dose of 300 mg) or placebo for 3 weeks, at which point bilateral venography was performed.<sup>83</sup> The primary efficacy outcome was VTE, which occurred in 32.1% of patients randomized to TTP889 and in 28.2% of those given placebo ( $P = .58$ ). There were no major bleeding events and only two clinically relevant nonmajor bleeding events with TTP889. Because of the negative results, development of TTP889 was halted.

**3.2.2 Factor Xa Inhibitors:** New factor Xa inhibitors include agents that block factor Xa indirectly or directly. Indirect inhibitors are given parenterally and act by catalyzing factor Xa inhibition by antithrombin. In contrast, direct factor Xa inhibitors bind directly to the active site of factor Xa, thereby blocking its interaction with its substrates. New

direct factor Xa inhibitors include otamixaban, which is given IV, and a number of orally active drugs. Unlike the heparin/antithrombin complex, which has limited capacity to inhibit factor Xa incorporated into the prothrombinase complex,<sup>84,85</sup> direct factor Xa inhibitors inhibit both free and platelet-bound factor Xa.<sup>86,87</sup> This property may endow these agents with an advantage over indirect factor Xa inhibitors.

**3.2.2.1 Indirect Factor Xa Inhibitors**—The prototype for most of the new indirect factor Xa inhibitors is fondaparinux, a first-generation synthetic analog of the antithrombin-binding pentasaccharide found in heparin or low-molecular-weight heparin (LMWH). Based on the results of well-designed randomized clinical trials, fondaparinux is licensed for prevention of VTE in patients undergoing high-risk orthopedic surgery and, in some countries, for VTE prevention in general surgical or medical patients. Fondaparinux also is approved as a substitute for heparin or LMWH for initial treatment of VTE. In addition, fondaparinux has been licensed in Europe and Canada, but not in the United States, as an alternative to heparin or LMWH for the treatment of ACS. Three of the newer indirect factor Xa inhibitors, idraparinux, idrabiotaparinux, and SR123781A, are second- and third-generation variants of fondaparinux, whereas M118 and semuloparin are LMWH derivatives (Table 6).

**Idraparinux:** A hypermethylated derivative of fondaparinux, idraparinux binds antithrombin with such high affinity that its plasma half-life of 80 h is similar to that of antithrombin.<sup>88</sup> Because of its long half-life, idraparinux can be given subcutaneously on a once-weekly basis. In a phase 2 dose-finding trial, idraparinux was compared with warfarin in 659 patients with proximal DVT.<sup>89</sup> After a 5- to 7-day course of enoxaparin, patients were randomized to receive once-weekly subcutaneous idraparinux (2.5, 5.0, 7.5, or 10 mg) or warfarin (dose-adjusted to achieve an international normalized ratio [INR] of 2-3) for 12 weeks. The primary end point, thrombus burden, as assessed by measuring changes in compression ultrasound and perfusion lung scan findings,

was similar in all idraparinux groups and did not differ from that in the warfarin group. There was a clear dose-response for major bleeding in patients given idraparinux, with an unacceptably high frequency in those given the 10-mg dose. Patients given the lowest dose of idraparinux had less bleeding than those randomized to warfarin ( $P = .029$ ). Based on these results, a once-weekly 2.5 mg dose of idraparinux was chosen for further trials.

The phase 3 Van Gogh DVT and PE trials<sup>90</sup> randomized 2,904 patients with acute symptomatic DVT and 2,215 patients with PE to either a 3- to 6-month course of once-weekly subcutaneous idraparinux (at a dose of 2.5 mg) or to conventional therapy with LMWH or heparin followed by a vitamin K antagonist with the dose adjusted to achieve an INR between 2 and 3. In the patients with DVT, the rate of recurrent VTE at 3 months was similar in the idraparinux and conventionally treated groups (2.9% and 3.0%, respectively). Clinically relevant bleeding events were less common with idraparinux than with conventional treatment (4.5% and 7.0%, respectively;  $P = .004$ ). In the patients with PE, idraparinux was less effective than conventional therapy at 3 months. Thus, recurrent VTE occurred in 3.4% of patients given idraparinux and in 1.6% of those receiving conventional therapy. Clinically relevant bleeding occurred in 5.8% of those given idraparinux and in 8.2% of those treated with heparin or LMWH followed by a vitamin K antagonist. The discordant results in the DVT and PE trials highlight the importance of adequate levels of anticoagulation for initial PE treatment because the majority of the recurrences occurred early. These findings suggest that patients with PE may require higher initial doses of idraparinux than patients with DVT.

The efficacy of long-term idraparinux was evaluated in the Van Gogh extension study.<sup>91</sup> In this trial, 1,215 patients who had completed 6 months of initial treatment of DVT or PE with either idraparinux or a vitamin K antagonist were randomized to an additional 6 months of treatment with either once-weekly subcutaneous idraparinux or with placebo. Compared with placebo, idraparinux produced a 72.9% relative reduction in the risk of recurrent VTE ( $P = .002$ ),

**Table 6—[Section 3.2.2.1] Antithrombin-Dependent Indirect Factor Xa Inhibitors**

Drug	Route of Administration	Mechanism of Action	Stage of Development
Idraparinux	Subcutaneous	Only inhibits factor Xa	Halted
Idrabiotaparinux	Subcutaneous	Biotinylated form of idraparinux	Phase 3
SR123781A	Subcutaneous	Synthetic hexadecasaccharide that inhibits factor Xa and thrombin equally well	Halted
M118	IV or subcutaneous	Novel LMWH that inhibits factor Xa to a greater extent than thrombin	Phase 2
Semuloparin	Subcutaneous	Ultra-LMWH that mainly inhibits factor Xa	Phase 3

LMWH = low-molecular-weight heparin.

reducing recurrent events from 3.7% to 1%. Major bleeding occurred in 3.7% of those given idraparinux and included three fatal intracranial bleeding events. In contrast, there were no major bleeding events in the placebo group.

In the phase 3 AMADEUS trial, subcutaneous idraparinux (2.5 mg once weekly) was compared with warfarin (dose-adjusted to achieve a target INR of 2 to 3) for stroke prevention in patients with atrial fibrillation.<sup>92</sup> Although the plan was to randomize 5,940 such patients to treatment of 18 months, the trial was stopped after randomization of only 4,576 patients with a mean follow-up of 10.7 months because of an excess of clinically relevant bleeding with idraparinux (19.7 and 11.3 per 100 patient-years, respectively;  $P < .0001$ ), including an excess of intracranial bleeding with idraparinux compared with warfarin (1.1 and 0.4 per 100 patient-years, respectively;  $P = .014$ ). There were 18 cases of thromboembolism with idraparinux and 27 cases with warfarin (0.9 and 1.3 per 100 patient-years, respectively; HR, 0.71; 95% CI, 0.39-1.30), a result that met the pre-specified noninferiority criterion (noninferiority  $P = .007$ ).

The results of the Van Gogh extension and the AMADEUS studies suggest that although idraparinux is a highly effective anticoagulant, it causes excessive bleeding. Based on this information, development of idraparinux was halted, and attention was shifted to idrabiotaparinux.

**Idrabiotaparinux:** A biotinylated form of idraparinux previously known as SSR126517E, idrabiotaparinux exhibits the same pharmacokinetic and pharmacodynamic profile as idraparinux. Like idraparinux, idrabiotaparinux is given subcutaneously on a once-weekly basis. The only difference between the drugs is that the anticoagulant activity of idrabiotaparinux can be rapidly neutralized by IV administration of avidin. A large tetrameric protein derived from egg white, avidin binds the biotin moiety of idrabiotaparinux with high affinity, thereby forming a 1:1 stoichiometric complex that is rapidly cleared by the kidneys.

In the phase 3 Bioequipotency Study of SSR126517E and Idraparinux in Patients with Deep Venous Thrombosis of the Lower Limbs (EQUINOX) trial, 757 patients with DVT were randomized to receive equimolar doses of once-weekly subcutaneous idrabiotaparinux or idraparinux (3 mg and 2.5 mg, respectively) for 6 months.<sup>93</sup> Recurrent VTE occurred in 2.3% of the patients randomized to idrabiotaparinux and in 3.2% of those given idraparinux. The rates of clinically relevant bleeding in the idrabiotaparinux and idraparinux groups were 5.2% and 7.3%, respectively ( $P = .29$ ). Both agents inhibited factor Xa to a similar extent.

Reversibility of idraparinux was demonstrated in two studies.<sup>94</sup> First, in a double-blind phase 1 study, 41 healthy males received idrabiotaparinux prior to being randomized to a 30-min infusion of either 100 mg of avidin or placebo. In eight of these subjects, idrabiotaparinux plus avidin was readministered 10 to 14 months later. Avidin infusion rapidly reduced anti-factor Xa activity by 66.1% to 90.3%. Similar results were obtained with readministration of idrabiotaparinux and avidin. The second study involved a subset of 55 patients who received idrabiotaparinux in the EQUINOX trial. These patients were randomized to receive either 100 mg avidin ( $n = 33$ ) or placebo ( $n = 22$ ). Avidin was well tolerated and reduced the anti-Xa activity by 67% to 97%.

The ongoing phase 3 Clinical Study Assessing SSR126517E Injections Once-weekly in Pulmonary Embolism Therapeutic Approach (CASSIOPEA) trial is comparing 3 to 6 months of subcutaneous idrabiotaparinux (3 mg once weekly) with conventional anticoagulant therapy for prevention of recurrent VTE in 3,200 patients with acute PE. The phase 3 Evaluation of Once-weekly Biotinylated Idraparinux vs Oral Adjusted-dose Warfarin to Prevent Stroke and Systemic Thromboembolic Events in patients with Atrial Fibrillation (BOREALIS-AF) trial, which compared the same idrabiotaparinux regimen with warfarin for prevention of stroke or systemic embolism in patients with atrial fibrillation, was stopped early, but the results are not yet available. With only the EQUINOX and CASSIOPEA trial results to support its use, it is likely that the role of idrabiotaparinux will be limited.

**SR123781A:** A synthetic hexadecasaccharide, SR123781A is composed of the antithrombin-binding synthetic pentasaccharide plus a thrombin-binding sulfated tetrasaccharide joined together by a central nonsulfated heptasaccharide. SR123781A binds antithrombin with high affinity.<sup>95</sup> In addition to catalyzing factor Xa inhibition by antithrombin, SR123781A is long enough to bridge antithrombin to thrombin, thereby enhancing thrombin inhibition. Like heparin, therefore, SR123781A catalyzes the inhibition of both factor Xa and thrombin.<sup>95</sup> Unlike heparin, however, SR123781A does not bind platelet factor 4 (PF4) or fibrin. Because it does not bind PF4, heparin-induced thrombocytopenia is unlikely to occur with SR123781A. Without affinity for fibrin, SR123781A does not promote the formation of the ternary heparin/thrombin/fibrin complex that protects fibrin-bound thrombin from inhibition by the antithrombin/heparin complex.<sup>96</sup> In contrast to heparin, therefore, SR123781A appears capable of inhibiting fibrin-bound thrombin.<sup>97</sup>

SR123781A is administered subcutaneously. It exhibits almost complete bioavailability after

subcutaneous administration and produces a dose-proportional increase in anti-factor Xa activity and the aPTT. The drug is primarily cleared by the kidneys, where it is excreted intact.

Subcutaneous SR123781A, at doses ranging from 0.25 to 4 mg once daily, was compared with enoxaparin (40 mg once daily) in 1,023 patients undergoing elective hip arthroplasty.<sup>98</sup> SR123781A reduced the rate of VTE in a dose-dependent fashion and the rates of VTE with the 2.0- and 4.0-mg doses (7.0% and 4.4%, respectively) were similar to that with enoxaparin (8.7%). Major bleeding occurred in 0.6% and 5.8% of patients given the 2.0- and 4.0-mg doses of SR123781A, respectively, and in 0.6% of those given enoxaparin. A phase 2 study comparing two different doses of SR123781A with heparin plus a glycoprotein IIb/IIIa antagonist in 180 patients with non-ST-elevation ACS undergoing PCI has been completed, but the results have not been reported.

M118: Obtained by depolymerization of unfractionated heparin, M118 is a novel LMWH that has a mean molecular weight of 6,500 and an anti-factor Xa to anti-factor IIa ratio of 1.4.<sup>99</sup> The drug has a subcutaneous bioavailability of about 70% and a half-life of about 1 h after IV administration and 2 to 3 h after subcutaneous delivery. The anticoagulant activity is reversed with protamine sulfate and can be monitored using the aPTT.

In the phase 2 EMINENCE study, 503 patients undergoing PCI were randomized to receive either IV M118 (in doses of 50, 75, or 110 units/kg) or 70 units/kg of unfractionated heparin.<sup>100</sup> The primary end point (a composite of death, MI, stroke, thrombocytopenia, catheter thrombus, bailout use of a glycoprotein IIb/IIIa antagonist, or any bleeding up to 20 days) occurred in 31.1% of patients given heparin and in 22.7%, 28.3%, and 30.1% of those randomized to the 50, 75, or 110 units/kg dose of M118, respectively. The rates of adverse events were similar across all study groups.

Semuloparin: An ultra-LMWH, semuloparin is synthesized from unfractionated heparin by selective and controlled depolymerization. With a mean molecular weight of 2,000 to 3,000, most of the semuloparin molecules are too short to bridge anti-thrombin to thrombin. Consequently, semuloparin has high anti-factor Xa activity and only minimal activity against thrombin. The drug is administered subcutaneously and exhibits 98% bioavailability. Peak plasma levels are achieved 3 h after subcutaneous injection and the half-life is 16 to 20 h, which enables once-daily administration. Excretion of the drug is primarily via the kidneys.

In phase 3 trials, semuloparin has been evaluated for postoperative thromboprophylaxis after hip or knee arthroplasty or after surgery for hip fracture,

and for VTE prevention after major abdominal surgery. Enoxaparin was the comparator in all of these studies. Semuloparin also has been compared with placebo for VTE prevention in patients receiving chemotherapy for treatment of cancer. A trial comparing semuloparin with enoxaparin for thromboprophylaxis in hospitalized, medically ill patients was stopped.<sup>101</sup>

In the Evaluation of AVE5026 as Compared to Enoxaparin in Patients Undergoing Total Hip Replacement Surgery (SAVE-HIP1) trial, semuloparin (20 mg once daily) was compared with enoxaparin (40 mg once daily starting 12 h prior to surgery) in 2,326 patients undergoing elective hip arthroplasty.<sup>102</sup> Both treatments were given for 7 to 10 days. In the 1,849 patients with evaluable venograms, the primary efficacy end point, a composite of VTE or all-cause mortality, occurred in 6.3% of those randomized to semuloparin and in 11.1% of those given enoxaparin (OR, 0.54; 95% CI, 0.38-0.76;  $P < .001$ ). Rates of major bleeding were 0.3% and 1.2% with semuloparin and enoxaparin, respectively (OR, 0.28; 95% CI, 0.08-0.83) and rates of clinically relevant nonmajor bleeding with semuloparin and enoxaparin were 0.7% and 1.0%, respectively (OR, 0.73; 95% CI, 0.28-1.83).

The same dose regimens were compared in 1,003 patients undergoing surgery for hip fracture in the SAVE-HIP2 trial.<sup>103</sup> In the 753 patients with evaluable venograms, the primary efficacy end point, a composite of VTE or all-cause mortality, occurred in 17.7% of those randomized to semuloparin and in 22.0% of those given enoxaparin (OR, 0.77; 95% CI, 0.53-1.12). The rates of major bleeding with semuloparin and enoxaparin were 1.0% and 0.6%, respectively, whereas the rates of clinically relevant nonmajor bleeding were 1.0% and 0.2%, respectively.

Semuloparin was compared with enoxaparin (30 mg bid starting 12 to 24 h after surgery) in 1,150 patients undergoing elective knee arthroplasty in the SAVE-KNEE trial.<sup>104</sup> In the 855 patients with evaluable venograms, the primary efficacy end point, a composite of VTE or all-cause mortality, occurred in 24.5% of those randomized to semuloparin and in 28.1% of those given enoxaparin (OR, 0.83; 95% CI, 0.60-1.14). The rates of major bleeding with semuloparin and enoxaparin were 0.5% and 0.7%, respectively, whereas the rates of clinically relevant nonmajor bleeding were 2.1% and 1.1%, respectively.

A meta-analysis of the results of the SAVE-HIP1 and 2 and the SAVE-KNEE trials, which included 4,479 patients undergoing major orthopedic surgery, demonstrated that compared with a 7- to 10-day course of enoxaparin (at a dose of 40 mg once daily for hip fracture or 30 mg bid for knee arthroplasty), semuloparin reduced any VTE or all-cause mortality

by almost one-third (OR, 0.70; 95% CI, 0.58-0.85;  $P = .0003$ ) with similar rates of major or clinically relevant nonmajor bleeding (1.7% and 1.8%, respectively; OR, 0.95; 95% CI, 0.60-1.50).<sup>105</sup> In the SAVE-HIP3 trial, 509 patients undergoing surgery for hip fracture all received semuloparin for 7 to 10 days, and 469 patients were then randomized in a 2:1 fashion to either continued semuloparin or to placebo for an additional 19 to 23 days.<sup>106</sup> In the 332 patients with evaluable venograms, the primary efficacy end point, a composite of VTE or all-cause mortality, occurred in 3.9% of those randomized to extended semuloparin and in 18.6% of those given placebo (OR, 0.18; 95% CI, 0.07-0.45;  $P < .001$ ). The rates of major bleeding and clinically relevant nonmajor bleeding with semuloparin were both 0.3%; there were no bleeding events with placebo.

In the SAVE-ABDO trial, semuloparin (20 mg once daily started 8 h after surgery) was compared with enoxaparin (40 mg once daily started 12 to 24 h after surgery) in 4,413 patients undergoing major abdominal surgery who were  $> 60$  years of age or, if younger, had risk factors for VTE.<sup>107</sup> Both treatments were administered for 7 to 10 days. Originally conceived as a superiority trial, there was revision to a noninferiority design after an interim analysis. In the 3,030 patients with evaluable venograms, the primary efficacy end point, a composite of VTE or all-cause mortality, occurred in 6.3% of those randomized to semuloparin and in 5.5% of those given enoxaparin (OR, 1.16; 95% CI, 0.84-1.59), a difference that failed to meet the prespecified noninferiority margin of 1.25. The rates of major VTE (proximal DVT or PE) or all-cause mortality were 2.2% and 2.3% with semuloparin and enoxaparin, respectively. Major bleeding occurred in 2.9% of patients given semuloparin and in 4.5% of those given enoxaparin, whereas the rates of clinically relevant nonmajor bleeding were 1.2% in both groups.

The SAVE-ONCO trial compared semuloparin (20 mg once daily) with placebo in  $> 3,000$  patients with cancer who were receiving chemotherapy.<sup>108</sup> Although the trial has been completed, the results have not been reported.

Overall, the results with semuloparin have been mixed. The need for a new injectable anticoagulant in this patient population is limited, given the availability of new oral anticoagulants. The disappointing results of the SAVE-ABDO trial suggest that the 20 mg once daily dose of semuloparin is inappropriate for all thromboprophylactic indications.

**3.2.2.2 Direct Factor Xa Inhibitors**—Direct factor Xa inhibitors include parenteral agents, such as DX9065a and otamixaban, as well as several orally active drugs. All of the direct factor Xa inhibitors are small molecules that reversibly block the active

site of factor Xa (Table 7). The large number of oral factor Xa inhibitors highlights the continued focus on development of oral anticoagulants that can replace vitamin K antagonists, such as warfarin.

**DX-9065a:** A synthetic nonpeptidic direct factor Xa inhibitor, DX9065a is administered parentally, has a dose-dependent half-life that ranges from 40 min to 5 h, and is cleared by the kidneys.<sup>109-111</sup> DX9065a was evaluated in patients with non-ST-elevation ACS and in patients undergoing PCI. In the ACS trial, 402 patients were randomized to weight-adjusted heparin or to low- or high-dose DX-9065a.<sup>112</sup> The primary efficacy end point, a composite of death, MI, urgent revascularization, or ischemia, occurred in 33.6%, 34.3%, and 31.3% of patients, respectively. Major bleeding occurred in 3.3% of those randomized to heparin and in  $< 1\%$  of those who received DX-9065a. In the PCI trial, 175 patients were randomized to open-label DX-9065a or to heparin in one of four sequential phases.<sup>113</sup> Although thrombotic events were rare in all phases of the study, enrollment in the phase evaluating the lowest dose of DX-9065 was stopped because of catheter thrombosis. Major bleeding events were uncommon, and there was no apparent dose response. Although promising, DX9065a has not undergone further clinical evaluation.

**Otamixaban:** A parenteral direct factor Xa inhibitor, otamixaban has a rapid onset of action, produces a predictable anticoagulant effect, has a short half-life, and  $< 25\%$  of the drug is cleared by the kidneys.<sup>114,115</sup> These features render otamixaban as a potential candidate to replace heparin in patients with ACS and those undergoing PCI. This possibility was evaluated in two phase 2 dose-finding trials.

The Study of Otamixaban vs Unfractionated Heparin (UFH) and Eptifibatid in Percutaneous Coronary Intervention (SEPIA-PCI) trial compared five different doses of otamixaban with unfractionated heparin in 947 patients undergoing nonurgent PCI.<sup>116</sup> The primary outcomes were change in blood levels of prothrombin fragment 1.2 (F1.2) and anti-factor Xa activity. The highest dose of otamixaban produced greater suppression of F1.2 than unfractionated

**Table 7—[Section 3.2.2.2] Direct Factor Xa Inhibitors**

Drug	Route of Administration	Stage of Development
DX-9065a	IV	Stopped at phase 2
Otamixaban	IV	Phase 3
Apixaban	Oral	Phase 3
Rivaroxaban	Oral	Phase 3; licensed for some indications
Edoxaban	Oral	Phase 3
Darexaban	Oral	Halted
Betrixaban	Oral	Phase 2
TAK-442	Oral	Phase 2
LY-517717	Oral	Halted

heparin, and increasing doses of otamixaban were associated with increasing anti-Xa levels. Otamixaban also had a dose-dependent effect on rates of any bleeding, which were significantly higher with the two highest otamixaban doses than with unfractionated heparin. Rates of ischemic events were similar across treatment groups.

The Study of Otamixaban vs Unfractionated Heparin and Eptifibatide in Non-ST Elevation Acute Coronary Syndrome (SEPIA-ACS)-1-TIMI 42 trial compared five different doses of otamixaban with the combination of heparin plus eptifibatide for the prevention of major cardiovascular events in 3,241 patients with non-ST-elevation ACS.<sup>117</sup> All patients received aspirin and clopidogrel and 62.7% underwent PCI. Although the rates of the primary efficacy outcome, a composite of death, MI, urgent revascularization, or bailout glycoprotein IIb/IIIa use at 7 days, were not significantly different across the five doses of otamixaban or compared with heparin plus eptifibatide, rates were numerically lowest with the two intermediate doses of otamixaban. The lowest-dose otamixaban arm was stopped early because of excess thrombotic complications, and the rates of thrombotic complications were numerically higher with all doses of otamixaban than with heparin plus eptifibatide. Otamixaban was associated with a significant dose-dependent increase in the primary safety outcome, non-coronary artery bypass graft surgery-related TIMI major or minor bleeding, but bleeding rates with intermediate doses of otamixaban were similar to those with heparin plus eptifibatide. On the basis of these results, a phase 3 trial of the same design has been initiated; this trial will evaluate two different doses of otamixaban.<sup>118</sup>

Rivaroxaban: A direct factor Xa inhibitor, rivaroxaban is an active compound with about 80% oral bioavailability. Plasma levels of rivaroxaban peak 2 to 3 h after administration, and the terminal half-life is 7 to 11 h.<sup>119,120</sup> Rivaroxaban is eliminated by the kidneys and in the feces. One-third of the administered drug is cleared as unchanged active drug by the kidneys, one-third is metabolized by the liver via CYP3A4-dependent and CYP3A4-independent pathways and then excreted in feces, and one-third is metabolized to inactive metabolites, which are then excreted by the kidneys. The pharmacokinetic and pharmacodynamic profile of rivaroxaban is predictable and dose-dependent and is not influenced by age, gender, or body weight. Potent inhibitors of both CYP3A4 and P-glycoprotein, such as ketoconazole or ritonavir, are contraindicated because they increase plasma drug concentrations.

As outlined in Ageno et al,<sup>121</sup> rivaroxaban has been investigated for the prevention and treatment of VTE, for stroke prevention in atrial fibrillation, and

for prevention of recurrent ischemia in patients with ACS. The drug is licensed in the United States, Canada, and Europe for VTE prevention after hip or knee arthroplasty and is under consideration for approval for VTE treatment and for stroke prevention in atrial fibrillation.

Apixaban: An oral direct factor Xa inhibitor, apixaban is an active drug with an oral bioavailability of > 45%. Like rivaroxaban, apixaban inhibits both free and clot-associated factor Xa activity.<sup>122</sup> In healthy men, levels of apixaban in plasma peak about 3 h after oral administration, and the drug is cleared with a terminal plasma half-life of 8 to 14 h.<sup>123</sup> Apixaban is eliminated via multiple pathways, including oxidative metabolism and renal and intestinal routes. Potent inhibitors of CYP3A4, such as ketoconazole or ritonavir, are contraindicated because they increase plasma drug concentrations.

Prevention of VTE: Apixaban has been evaluated for VTE prevention in three phase 3 randomized controlled trials: two in patients undergoing knee arthroplasty (n = 6,252) and one in patients undergoing hip arthroplasty (n = 5,407). All three trials used the same apixaban dose regimen of 2.5 mg bid starting 12 to 24 h after surgery, which was administered for 10 to 14 days in patients undergoing knee arthroplasty and for 35 days in those undergoing hip arthroplasty. In the ADVANCE-1 trial, apixaban was similarly effective to enoxaparin 30 mg bid (starting 12-24 h after surgery) for the prevention of total VTE or all-cause mortality (9.0% and 8.8%, respectively; RR, 1.02; 95% CI, 0.78-1.32) in patients undergoing knee arthroplasty, although the prespecified statistical criteria for noninferiority were not met.<sup>124</sup> In the ADVANCE-2 trial, apixaban was superior to enoxaparin 40 mg once daily (starting 12 h preoperatively) for the prevention of total VTE or all-cause mortality (1.51% and 24.4%, respectively; RR, 0.62; 95% CI, 0.51-0.78; *P* < .0001) in patients undergoing knee arthroplasty.<sup>125</sup> In the ADVANCE-3 trial, patients undergoing hip arthroplasty were randomized to apixaban or enoxaparin (40 mg once daily starting 12 h preoperatively) for 35 days.<sup>126</sup> Once again, apixaban was superior to enoxaparin (1.4% and 3.9%, respectively; RR, 0.36; 95% CI, 0.22-0.54; *P* < .001). Rates of major or clinically relevant nonmajor bleeding were numerically lower with apixaban than with enoxaparin in all three trials, but the differences only reached statistical significance in the ADVANCE-1 trial. Based on the results of the ADVANCE-2 and ADVANCE-3 trials, apixaban was approved by the European Commission for prevention of VTE in patients undergoing elective hip or knee replacement surgery.

Apixaban also was evaluated for thromboprophylaxis in medical patients. In the phase 3, multicenter,



double-blind, randomized ADOPT trial, a 30-day regimen of apixaban (2.5 mg bid) was compared with enoxaparin (40 mg once daily, given for 6 to 14 days) in acutely ill medical patients.<sup>127</sup> At 30 days, the primary efficacy outcome (a composite of symptomatic VTE, asymptomatic proximal DVT detected by routine ultrasonography or VTE-related mortality) occurred in 2.71% of patients given apixaban and in 3.06% of those treated with enoxaparin (RR, 0.87; 95% CI, 0.62-1.23). By day 30, rates of major bleeding in the apixaban and enoxaparin groups were 0.47% and 0.19%, respectively (RR, 2.58; 95% CI, 1.02-7.24;  $p = .04$ ). Therefore, extended thromboprophylaxis with apixaban was not superior to a shorter course with enoxaparin, and there was more bleeding with apixaban.

Treatment of VTE: The phase 3 multicenter, double-blind, randomized AMPLIFY trial is comparing apixaban (10 mg bid for 1 week followed by 5 mg bid thereafter) with conventional anticoagulant therapy (heparin or LMWH followed by dose-adjusted warfarin) for treatment of patients with acute VTE.<sup>128</sup> The phase 3 multicenter, double-blind, randomized AMPLIFY-EXT trial is comparing apixaban (at doses of either 2.5 or 5 mg bid) with placebo for prevention of recurrent VTE in patients who have completed a minimum of a 6-month course of anticoagulation therapy for a first episode of VTE.<sup>129</sup>

Stroke prevention in atrial fibrillation: The multicenter, double-blind, randomized phase 3 Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial compared apixaban (5 mg bid) with warfarin (dose-adjusted to achieve an INR of 2-3) in 18,201 patients with atrial fibrillation with at least one additional risk factor for stroke.<sup>130</sup> The rate of primary efficacy outcome, a composite of stroke (ischemic or hemorrhagic) and systemic embolism, was 1.27% per year in the apixaban group and 1.60% per year in the warfarin group (HR, 0.79; 95% CI, 0.66-0.95;  $P < .001$  for noninferiority and  $P < .01$  for superiority). Annual rates of major bleeding with apixaban and warfarin were 2.13% and 3.09%, respectively (HR, 0.69; 95% CI, 0.60-0.80;  $P < .001$ ) and rates of hemorrhagic stroke were 0.24% and 0.47%, respectively (HR, 0.51; 95% CI, 0.35-0.75;  $P < .001$ ). The rates of ischemic stroke were similar with apixaban and warfarin (0.97% and 1.05%, respectively). All-cause mortality was lower with apixaban than with warfarin (3.52% and 3.94%, respectively;  $P = .047$ ). Therefore, apixaban was superior to warfarin in preventing stroke and systemic embolism and produced less bleeding. Enrollment has been completed and patients are now undergoing follow-up. In the multicenter, double-blind, phase 3 AVERROES trial, the same apixaban regimen was compared with aspirin in

5,600 patients with atrial fibrillation who were ineligible for vitamin K antagonist treatment or could not tolerate such therapy.<sup>131</sup> Compared with aspirin, treatment with apixaban reduced the rate of stroke or systemic embolism from 3.6% to 1.6% (RR, 0.46; 95% CI, 0.33-0.64). Rates of major bleeding were similar with apixaban and aspirin (1.4% and 1.2%, respectively).

Acute coronary syndromes: In the phase 2 Apixaban for Prevention of Acute Ischemic and Safety Events (APPRAISE) study, varying doses of apixaban (2.5 mg bid, 10 mg once daily, 10 mg bid, or 20 mg once daily) were compared with placebo in 1,715 patients with recent ST-elevation or non-ST-elevation ACS.<sup>132</sup> Nearly all patients received aspirin, and 76% also received clopidogrel. The primary outcome was bleeding. Recruitment into the two higher dose arms of apixaban was stopped early because of excess bleeding. Compared with placebo, apixaban (2.5 mg bid and 10 mg once daily) increased bleeding in a dose-dependent fashion. There was a trend for fewer ischemic events in patients given apixaban, a phenomenon that was attenuated in those taking aspirin plus clopidogrel compared with those receiving aspirin alone. Building on these phase 2 results, the multicenter, double-blind, randomized phase 3 APPRAISE-2 trial compared apixaban (5 mg bid) with placebo as adjuncts to antiplatelet therapy with aspirin with or without a thienopyridine for prevention of recurrent ischemic events in 7,392 patients with ACS.<sup>133</sup> The trial was stopped early because of excess bleeding, including intracranial bleeding when apixaban was given in conjunction with dual antiplatelet therapy, and no evidence of efficacy.

Edoxaban: An active drug with an oral bioavailability of at least 50%, edoxaban is rapidly absorbed from the gastrointestinal tract such that plasma concentrations peak 1 to 2 h after dosing. Elimination follows a biphasic pattern, and the terminal elimination half-life is approximately 8 to 10 h.<sup>134</sup> There is a dual mechanism of elimination; approximately 35% of the total administered oral dose is excreted via the kidneys, and the remainder is eliminated in the feces.<sup>134</sup>

Prevention of VTE: In a phase 2 dose-ranging study conducted in Japan, oral edoxaban (5, 15, 30, or 60 mg once daily) was compared with placebo in 593 patients undergoing knee arthroplasty.<sup>135</sup> Treatment was given for 11 to 14 days. Compared with placebo, edoxaban produced a significant and dose-related reduction in the primary efficacy end point, total VTE, which was reduced from 48.3% to 29.5%, 26.2%, 12.5%, and 9.1% with the 5-, 15-, 30-, and 60-mg dose of edoxaban, respectively. The rates of major plus clinically relevant nonmajor bleeding

were similar across all treatment groups, and there was no significant difference in the rates with edoxaban and that with placebo.

A second phase 2 study compared oral edoxaban (in doses of 15, 30, 60, or 90 mg once daily) with subcutaneous dalteparin (at an initial dose of 2,500 units, followed by 5,000 units once daily thereafter) in 903 patients undergoing hip arthroplasty. Both treatments were given for 7 to 10 days.<sup>136</sup> The rates of the primary efficacy end point, total VTE, with edoxaban were 28.2%, 21.2%, 15.2%, and 10.6% in the 15-, 30-, 60-, and 90-mg dose groups, respectively, compared with a rate of 43.8% with dalteparin ( $P < .005$ ). The incidences of clinically relevant nonmajor bleeding were low and similar across all treatment groups.<sup>136</sup>

Building on these phase 2 results, oral edoxaban (30 mg once daily) was compared with subcutaneous enoxaparin in three double-blind phase 3 trials conducted in Japan. In the first trial, edoxaban was compared with enoxaparin (20 mg bid, the licensed dose in Japan) in 716 patients undergoing knee arthroplasty. Edoxaban was started 6 to 24 h after surgery, whereas enoxaparin treatment was initiated 24 to 36 h after surgery, which is the standard-of-care in Japan; both treatments were given for 11 to 14 days. The rate of the primary efficacy end point, total VTE, was lower with edoxaban than with enoxaparin (7.4% and 13.9%, respectively;  $P = .01$ ). Rates of the primary safety end point, the composite of major and clinically relevant nonmajor bleeding, were similar in both groups.<sup>135</sup> In the second trial, the same regimens of edoxaban and enoxaparin were compared in 610 patients undergoing knee arthroplasty in Japan. The primary efficacy outcome, total VTE, in the edoxaban and enoxaparin groups was 2.4% and 6.9%, respectively (absolute risk difference,  $-4.5%$ ; 95% CI,  $-8.6%$  to  $-0.9%$ ;  $P < .001$  for noninferiority;  $P = .016$  for superiority). Rates of major plus clinically relevant nonmajor bleeding with edoxaban and enoxaparin were 2.6% and 3.7%, respectively, a difference that was not statistically significant.<sup>137</sup> The third trial compared the same regimens of edoxaban and enoxaparin in patients undergoing surgery for hip fracture. Although the trial has been completed, the data have not yet been presented. Based on the results of these three studies, regulatory filing for prophylactic edoxaban in patients undergoing major orthopedic surgery has been submitted in Japan.

Treatment of VTE: The phase 3 double-blind HOKUSAI trial is comparing edoxaban 60 mg once daily (reduced to 30 mg once daily in selected patients) with warfarin (dose adjusted to achieve a target INR of 2-3) for treatment of VTE. Patients are randomized to edoxaban or warfarin after they have completed an initial course of therapy with heparin or LMWH for at least 5 days. Study drug is administered for 3, 6, or

12 months depending on whether the VTE is provoked or unprovoked.<sup>138</sup>

Stroke prevention in atrial fibrillation: In a phase 2 trial, edoxaban (at doses of 30 or 60 once daily or 30 or 60 mg bid) was compared with warfarin in 1,146 patients with atrial fibrillation. The rates of the primary end point, the composite of major plus clinically relevant nonmajor bleeding, were significantly higher in the groups randomized to 30 or 60 mg of edoxaban bid than the rate in patients given warfarin (7.8%, 10.6%, and 3.2%, respectively). In contrast, the rates with 30 or 60 mg of edoxaban once daily were similar to that with warfarin (3.0% and 3.8%, respectively).<sup>139</sup> Based on these data, the phase 3 double-blind ENGAGE-AF-TIMI 48 trial is comparing two doses of edoxaban (30 or 60 mg once daily with dose adjustments for drug clearance) with warfarin (dose adjusted to achieve a target INR of 2-3) for stroke prevention in 21,500 patients with atrial fibrillation.<sup>140</sup> Enrollment into the trial has been completed and patients are now being followed in this event-driven trial.

Darexaban: An oral inhibitor that inhibits factor Xa with a  $K_i$  of 31 nM, darexaban (formerly known as YM150) has high oral bioavailability, and drug levels peak about 2 h after oral drug administration. The half-life of darexaban is 14 to 18 h. Darexaban has a dual mechanism of elimination; one-half is eliminated via the kidneys and the remainder in the feces.

Prevention of VTE: Darexaban was first evaluated in 174 patients undergoing elective hip arthroplasty.<sup>141</sup> At once-daily doses of 3, 10, 30, or 60 mg, darexaban produced a statistically significant dose response for efficacy. No major bleeding events were reported and there was no dose-response trend for clinically relevant nonmajor bleeding. In a second phase 2 dose-finding study, 1,017 patients undergoing elective hip arthroplasty were randomized to once-daily oral darexaban (at doses of 5, 10, 30, 60, or 120 mg) or to subcutaneous enoxaparin (40 mg once daily starting 12 h prior to surgery) for 5 weeks. The primary efficacy end point, a composite of VTE or all-cause mortality, occurred in 18.9% of patients given enoxaparin. Darexaban reduced the rate of VTE in a dose-dependent fashion and the rates in the 30-, 60-, and 90-mg dose groups were 19.3%, 13.3%, and 14.5%, respectively. There was one major bleeding event with the 60-mg dose of darexaban and one with enoxaparin. Ongoing dose-finding studies were comparing once-daily and bid regimens of darexaban with enoxaparin for VTE prevention after hip arthroplasty<sup>142</sup> and with warfarin in patients undergoing knee arthroplasty.<sup>143</sup> Although there was an initial filing in Japan for darexaban use for VTE prevention in the orthopedic setting, the application was withdrawn because the regulatory agency requested additional clinical trials.

Stroke prevention in atrial fibrillation: In a phase 2 dose-finding study, once-daily darexaban (at doses of 30, 60, 120, or 240 mg) was compared with warfarin in 448 patients with atrial fibrillation. The warfarin dose was adjusted to achieve an INR of 2.0 to 3.0 for those up to age 69 years and an INR of 1.6 to 2.6 for those  $\geq 70$  years. Recruitment into the 240-mg darexaban dose arm was stopped early because of excess bleeding. Rates of major or clinically relevant nonmajor bleeding with the 30-, 60-, and 120-mg doses of darexaban were 2.2%, 2.2%, and 3.2%, respectively, whereas the rate was 2.1% with warfarin. A larger phase 2 dose-finding study is underway.<sup>144</sup>

Acute coronary syndrome: A phase 2 dose-finding study compared darexaban with placebo for prevention of recurrent ischemic events in 1,279 stabilized patients with acute coronary syndrome.<sup>145</sup> Three doses of darexaban were tested (10, 30, or 60 mg), with each dose given either four times or bid. The primary end point was a composite of major and clinically relevant nonmajor bleeding. All darexaban regimens produced more bleeding than placebo. With the 30-mg twice-daily dose, the rate of bleeding was 11.3% compared with 3.1% with placebo (HR, 3.8;  $P = .002$ ). There was no difference in efficacy between darexaban and placebo. Because of these results and the lack of a clear advantage of darexaban over other oral factor Xa inhibitors, development of darexaban has been halted.

Betrixaban: With oral bioavailability of 47% and a half-life of 19 h, betrixaban inhibits factor Xa with a Ki of 0.12 nM. The drug has minimal renal excretion. Betrixaban had antithrombotic activity in animal models and was well tolerated in humans in a phase 1 trial that included 64 subjects.<sup>146</sup>

Prevention of VTE: In the phase 2 EXPERT trial, oral betrixaban, at doses of 15 or 40 mg bid, was compared with subcutaneous enoxaparin (30 mg bid) for postoperative thromboprophylaxis in 215 patients undergoing elective knee arthroplasty.<sup>147</sup> Randomization was done in a 2:2:1 fashion and treatment was given for 10 to 14 days. VTE occurred in 20% and 15% of patients given 15 or 40 mg of betrixaban, respectively, and in 10% of those given enoxaparin. There were no major bleeding events in the 171 patients given betrixaban, and there was one major bleeding event in the 43 patients given enoxaparin.

Stroke prevention in atrial fibrillation: In the phase 2, EXPLORE-Xa study, oral betrixaban (at doses of 40, 60, or 80 mg once daily) was compared with warfarin (dose-adjusted to achieve and INR of 2-3) in 506 patients with atrial fibrillation. Major or clinically relevant nonmajor bleeding occurred in 1, 5, and 4 patients in the groups of patients given 40, 60, or 80 mg of betrixaban, respectively, and in four of

those given warfarin. It is unclear whether betrixaban will be taken to phase 3 for this indication.

TAK-442: With good oral bioavailability, TAK-442 is specific for factor Xa and inhibits the enzyme with a Ki of 1.8 nM. In healthy volunteers, TAK-442 produces a predictable and dose-proportional level of anticoagulation with a time to peak plasma concentration of 1 to 2 h and an elimination half-life of 9 to 13 h.<sup>146</sup> In a phase 2 dose-finding study, oral TAK-442 (in doses of 40 or 80 mg once daily or 10, 20, 40, or 80 mg bid) was compared with subcutaneous enoxaparin (30 mg bid) in 1,038 patients undergoing knee arthroplasty.<sup>148</sup> Both treatments were given for 10 to 14 days. The primary efficacy end point was composite of total VTE and all-cause mortality, whereas the primary safety end point was major bleeding. Recruitment into the TAK-442 10- and 20-mg bid arms of the study was stopped early because the rates of the primary efficacy end point were higher than that with enoxaparin (39.0%, 38.4%, and 22.0%, respectively). The primary efficacy end point occurred in 23.5%, 21.4%, 26.8%, and 14.3% of those receiving TAK-442 40 mg once daily, 40 mg bid, 80 mg once daily, or 80 mg bid, respectively. Rates of major bleeding were low and the rates of major plus clinically relevant nonmajor bleeding were similar across all treatment groups.<sup>147</sup>

A phase 2 dose-finding study compared varying doses and regimens of TAK-442 with placebo in patients with ACS, most of whom were also taking aspirin and clopidogrel.<sup>149</sup> Although the study has been completed, the results have not been reported.

LY-517717: With oral bioavailability of 25% to 82%, LY-517717 inhibits factor Xa with a Ki of 5 to 7 nM. LY-517717 has a half-life of about 25 h and is given once daily.<sup>146</sup> LY-517717 was evaluated in a phase 2 noninferiority study that randomized 511 patients undergoing hip or knee arthroplasty to one of six doses of LY-517717 (25, 50, 75, 100, 125, or 150 mg started 6 to 8 h after wound closure) or to once-daily subcutaneous enoxaparin (40 mg started the evening before surgery).<sup>150</sup> Both treatments were administered for a total of 6 to 10 doses. Randomization to the three lower doses of LY-517717 was stopped early due to lack of efficacy. The three higher doses of LY-517717 had efficacy similar to that of enoxaparin (17.1% to 24.0%, and 22.2%, respectively). Adjudicated major bleeding events were uncommon in all study arms. Further development of LY-517717 has been halted.

### 3.3 Factor Va and VIIIa Inhibitors

Factor Va is the major target of activated protein C. Activated protein C acts as an anticoagulant by proteolytically degrading and inactivating factor Va,

a key cofactor in thrombin generation. Factor Va is directly inhibited by drotrecogin alfa (activated), a recombinant form of activated protein C. Recomodulin, previously known as ART-123, and solulin are recombinant analogs of the extracellular domain of thrombomodulin that binds thrombin and enhances its capacity to activate protein C. Solulin differs from Recomodulin in that targeted amino acid substitutions render it relatively resistant to oxidation or protease degradation. TB-402 is a factor VIIIa-directed monoclonal antibody (Table 8).

**3.3.1 Drotrecogin Alfa (Activated):** A recombinant form of activated protein C, drotrecogin is licensed for treatment of patients with severe sepsis. Approval for this indication was based on a trial comparing drotrecogin with placebo in 1,690 patients with severe sepsis.<sup>151</sup> When given as an infusion of 24 µg/kg/h over 96 h, drotrecogin produced a 19% reduction in mortality at 28 days (from 30.8% to 24.7%;  $P = .005$ ). The rate of major bleeding was higher with drotrecogin than with placebo (3.5% and 2%, respectively;  $P = .06$ ). Since approval, two additional clinical trials, one in adults with sepsis and a low risk of death and the other in children with sepsis, were stopped prematurely due to lack of efficacy and the potential to cause harm because of bleeding.<sup>152,153</sup> Because of these results, drotrecogin has recently been withdrawn from the market.

**3.3.2 Recomodulin:** A recombinant analog of the extracellular domain of thrombomodulin,<sup>154</sup> Recomodulin binds thrombin and converts it from a procoagulant enzyme into a potent activator of protein C. Recomodulin has nearly 100% bioavailability after subcutaneous administration and a half-life of 2 to 3 days. In a phase 2a dose-ranging study in patients undergoing elective hip arthroplasty, the primary end point (a composite of venographically-detected DVT and symptomatic PE) occurred in

4.3% of the 94 patients given lower dose Recomodulin and in none of the 99 patients receiving the higher dose.<sup>155</sup> Major bleeding occurred in 1.6% and 5.7% of patients receiving low- or high-dose Recomodulin, respectively. In a double-blind study, Recomodulin was compared with placebo in 750 patients with sepsis associated with disseminated intravascular coagulation. Although the study has been completed, the results are not available.

**3.3.3 Solulin:** Another recombinant analog of thrombomodulin, low-dose solulin is being explored as an adjunctive treatment of patient with hemophilia. Solulin appears to stabilize thrombi in animal models of hemophilia, likely by promoting the activation of thrombin activatable fibrinolysis inhibitor (TAFI). Once activated, TAFIa releases lysine residues from the COOH-termini of the polypeptide chains of degrading fibrin. This phenomenon attenuates fibrin breakdown because these lysine residues serve as binding sites for plasmin. Studies in humans with hemophilia have not yet been initiated.

**3.3.4 TB-402:** A human IgG<sub>4</sub> monoclonal antibody that partially inhibits factor VIIIa, TB-402 was generated by introducing a point mutation into the gene encoding MAB-LE2E9, a factor VIII-directed monoclonal antibody.<sup>156,157</sup> TB-402 does not affect the interaction of factor VIII with von Willebrand factor, but it inhibits factor VIII activity by about 40%.<sup>158</sup> The antibody has a half-life of about 3 weeks, which may enable prolonged VTE prophylaxis with a single dose.

In a phase 1 study in healthy volunteers, increasing doses of TB-402 decreased factor VIII activity to plateau levels that were one-third to two-thirds lower than baseline.<sup>158</sup> The aPTT was correspondingly prolonged and remained so for at least 4 weeks, consistent with the long half-life. In a phase 2 study, a single dose of IV TB-402 (0.3, 0.6, or 1.2 mg/kg) was compared with enoxaparin in 316 patients undergoing knee arthroplasty.<sup>159</sup> All patients received a 40-mg subcutaneous dose of enoxaparin 12 h prior to surgery; they were then randomized to receive either TB-402 or enoxaparin 18 to 24 h after the procedure. The primary outcome, major or clinically relevant nonmajor bleeding, occurred in 3.8% of patients given enoxaparin and in 4.0%, 5.4%, and 8.0% of those given TB-402 at the 0.3-, 0.6-, and 1.2-mg/kg dose, respectively. The primary efficacy outcome, total VTE, occurred in 39.0% of the patients given enoxaparin and in 16.7%, 23.9%, and 24.1% of those receiving TB-402 at doses of 0.3, 0.6, and 1.2 mg/kg, respectively. Thus, there was no dose-response trend for efficacy with TB-402, but there was a dose-dependent increase in bleeding. At a dose of 0.3 mg/kg, TB-402 appeared to have efficacy and safety similar to that of

**Table 8—[Section 3.3] Inhibitors of Factor Va and/or VIIIa**

Drug	Route of Administration	Mechanism of Action	Stage of Development
Drotrecogin	IV	Proteolytically degrades and inactivates factors Va and VIIIa	Withdrawn from market
Recomodulin	Subcutaneous	Binds thrombin and promotes its activation of protein C	Phase 2
Solulin	Subcutaneous	Binds thrombin and promotes activation of protein C	Phase 2
TB-402	IV	Partially inhibits factor VIIIa	Phase 2

enoxaparin. Additional studies are needed to confirm these findings.

### 3.4 Inhibitors of Fibrin Formation

Thrombin, the enzyme that converts fibrinogen to fibrin, can be inhibited indirectly or directly. Indirect inhibitors that are specific for thrombin act by catalyzing heparin cofactor II. In contrast, direct thrombin inhibitors bind to the enzyme and block its interaction with substrates.

Direct thrombin inhibitors have properties that give them potential mechanistic advantages over indirect inhibitors.<sup>160,161</sup> First, because direct thrombin inhibitors do not bind to plasma proteins, they produce a more predictable anticoagulant response. Second, unlike heparin, direct thrombin inhibitors do not bind to PF4. Consequently, the anticoagulant activity of direct thrombin inhibitors is unaffected by the large quantities of PF4 released in the vicinity of platelet-rich thrombi. Finally, direct thrombin inhibitors inactivate fibrin-bound thrombin, as well as fluid-phase thrombin.

Four parenteral direct thrombin inhibitors (lepirudin, desirudin, argatroban, and bivalirudin) have been licensed in North America for limited indications. Lepirudin and argatroban are approved for treatment of patients with heparin-induced thrombocytopenia, whereas bivalirudin is licensed as an alternative to heparin in patients undergoing PCI with or without heparin-induced thrombocytopenia. Desirudin is approved for postoperative thromboprophylaxis in patients undergoing hip arthroplasty. Because these drugs are already licensed, they will not be discussed here. Flovagatran and pegmusirudin are parenteral direct thrombin inhibitors that have undergone phase 2 evaluation; neither has advanced to phase 3. There also are three new oral thrombin inhibitors: odiparcil, an indirect inhibitor whose development has been halted, and dabigatran etexilate and AZD0837, which are oral direct thrombin inhibitors (Table 9).

**3.4.1 Flovagatran:** A synthetic active site-directed small molecule formerly designated TGN 255, flovagatran reversibly inhibits thrombin. The drug exhibits predictable and dose-dependent pharmacokinetics after IV injection and has a short half-life. Because

flovagatran is mainly cleared via an extrarenal mechanism, its pharmacokinetic profile in patients with renal failure is reported to be similar to that in patients with normal renal function. Building on this property, flovagatran was investigated as an alternative to heparin during hemodialysis in patients with end-stage renal disease who had antibodies directed against the heparin/PF4 complex. A small phase 2 study in 38 such patients demonstrated that the drug produces a predictable anticoagulant effect that permits successful hemodialysis. Plans for future development of flovagatran are uncertain.

**3.4.2 Pegmusirudin:** A chemically modified hirudin derivative, pegmusirudin is manufactured by coupling two polyethylene glycol side chains to recombinant hirudin.<sup>162</sup> These side chains prolong the half-life of hirudin from 60 min after IV bolus injection to about 12 h in patients with normal renal function. Like hirudin, pegmusirudin is cleared by the kidneys, and its half-life is prolonged in patients with renal insufficiency. Capitalizing on this feature, the drug underwent phase 2 evaluation to prevent access graft occlusion in patients with end-stage renal disease who were undergoing routine hemodialysis. Given IV prior to each dialysis session, pegmusirudin not only provided anticoagulation during dialysis but also produced continued anticoagulation between dialysis sessions. Because of its prolonged anticoagulant effect, pegmusirudin produced excessive bleeding, and the study was stopped early. It is unlikely that pegmusirudin will undergo further development.

**3.4.3 Odiparcil:** An oral  $\beta$ -D-xyloside, odiparcil primes the synthesis of circulating dermatan sulfate-like glycosaminoglycans.<sup>163</sup> These glycosaminoglycans indirectly inhibit thrombin by catalyzing heparin cofactor II. Steady-state levels of glycosaminoglycans are achieved after 2 to 3 days of odiparcil administration. Like warfarin, therefore, odiparcil has a delayed onset of action. The anticoagulant activity of odiparcil can be partially reversed with protamine sulfate. In a phase 2 dose-finding trial, three different doses of oral odiparcil were compared with warfarin for thromboprophylaxis in patients undergoing knee arthroplasty.<sup>164</sup> Because of lack of efficacy, further development of odiparcil was halted.

**Table 9—[Section 3.4] Thrombin Inhibitors**

Drug	Route of Administration	Mechanism of Action	Stage of Development
Odiparcil	Oral	Primes the synthesis of dermatan sulfate-like glycosaminoglycans	Halted
Dabigatran etexilate	Oral	Prodrug of dabigatran, a reversible inhibitor of the active site of thrombin	Phase 3; licensed for some indications
AZD0837	Oral	Prodrug of AR-HO67637, a reversible inhibitor of the active site of thrombin	Phase 2

**3.4.4 Dabigatran Etxilate:** A prodrug of dabigatran, dabigatran etexilate has an oral bioavailability of about 6%. After absorption, dabigatran etexilate is rapidly and completely converted to dabigatran by esterases. Plasma levels of dabigatran peak 2 h after drug administration, and the half-life of dabigatran is 14 to 17 h. The elimination is mainly via the kidneys, with 80% of the drug excreted unchanged in the urine. Dabigatran exhibits predictable pharmacokinetics and pharmacodynamics with little effect of food. Drug-drug interactions are limited; potent P-glycoprotein inhibitors, such as quinidine, are contraindicated.

As detailed by Ageno et al,<sup>121</sup> dabigatran etexilate has been evaluated for the prevention and treatment of VTE and as an alternative to warfarin for stroke prevention in patients with atrial fibrillation. The drug is licensed in the United States, Canada, and Europe for stroke prevention in patients with atrial fibrillation and in Canada and in Europe for VTE prevention after hip or knee arthroplasty.

**3.4.5 AZD0837:** A follow-up to ximelagatran, AZD0837 is a prodrug, which undergoes rapid metabolism by CYP isoenzymes, including CYP2C9, CYP2C19, and CYP3A4, to AR-H69927, an intermediate that is then converted to AR-H067637, a selective and reversible direct inhibitor of thrombin.<sup>161</sup> The half-life of AR-H067637 is 9 to 14 h in healthy subjects and AZD0837 and its metabolites are excreted in the urine and the feces. The oral bioavailability of AZD0837 is in the range of 22% to 55%, and drug levels in plasma peak about 1.5 h after oral drug administration. Coadministration with food prolongs the time to peak by about 2 h. Both immediate-release and extended-release formulations of AZD0837 have been developed.<sup>161</sup> The extended-release form has the potential for once-daily administration. Both formulations of AZD0837 have been explored as alternatives to warfarin in patients with atrial fibrillation. In a phase 2 study, the immediate release formulation of AZD0837 (in doses of 150 or 350 mg bid) was compared with warfarin (dose-adjusted to achieve an INR of 2-3) in 250 such patients.<sup>165</sup> Total bleeding events were reported in six patients receiving the 150-mg dose of AZD0837, in 15 patients given the 350-mg dose, and in eight of those treated with warfarin. Elevations in alanine aminotransferase levels were infrequent, and the rates were similar with AZD0837 and warfarin.

The extended-release formulation has been evaluated in two phase 2 studies. The first compared the extended-release formulation of AZD0837 (in doses of 150, 300, and 450 mg once daily and 200 mg bid) with warfarin (dose-adjusted to achieve an INR between 2.0 and 3.0) in 955 patients with atrial fibril-

lation.<sup>166</sup> Total bleeding events were similar or lower with all doses of AZD0837 than with warfarin (5.3% to 14.7% with AZD0837 and 14.5% with warfarin). Adverse events were similar in frequency with AZD0837 and warfarin, but there were more gastrointestinal side effects, including diarrhea and nausea, with AZD0837. The frequency of elevations in the level of alanine aminotransferase was similar with AZD0837 and warfarin.

The second phase 2 study compared the extended-release formulation of AZD0837 (at doses of 150 or 300 mg once daily) with standard therapy (consisting of either nothing, aspirin, or clopidogrel), in 131 patients with atrial fibrillation who were unwilling or unable to take a vitamin K antagonist.<sup>167</sup> Treatment was given for a median duration of 6 weeks. Minor or clinically relevant nonmajor bleeding events occurred in none of the patients given the 150-mg dose of AZD0837, in five of those treated with the 300-mg dose, and in six of the patients receiving standard therapy. There were no major bleeding events. Side effects of AZD0837 include dyspepsia and a reversible elevation in the serum creatinine level that appears to be the result of decreased inhibition of the tubular secretion of creatinine.<sup>168</sup> Despite the promising clinical results with AZD0837, phase 3 trials have not yet been initiated.

#### 4.0 FIBRINOLYTIC THERAPY

Although traditional antithrombotic strategies have been aimed at inhibiting platelet function or blocking coagulation, a better understanding of fibrinolysis has identified potential methods to enhance endogenous fibrinolytic activity and has led to the development of new fibrinolytic agents. Strategies to enhance endogenous fibrinolysis include inhibitors of type 1 plasminogen activator (PAI-1), urokinase plasminogen activator (u-PA), TAFIa, or activated factor XIII (factor XIIIa). New fibrinolytic agents include alfimeprase, V10153, plasmin, and desmoteplase (Table 10).

**Table 10—[Section 4.0] New Fibrinolytic Agents**

Drug	Route of Administration	Mechanism of Action	Stage of Development
Alfimeprase	IV	Directly degrades fibrin and fibrinogen	Halted
BB10153	IV	Thrombin activatable plasminogen variant	Phase 2
Plasmin	IV	Directly degrades fibrin and fibrinogen	Phase 2
Desmoteplase	IV	A variant of t-PA with enhanced fibrin specificity	Phase 3

t-PA = tissue plasminogen activator.

## 4.1 Strategies to Enhance Endogenous Fibrinolysis

**4.1.1 PAI-1 Inhibitors:** As the major physiologic inhibitor of t-PA and u-PA, PAI-1 is an attractive target. PAI-1 activity can be reduced by (1) decreasing PAI-1 gene expression, or (2) reducing the activity of PAI-1. Lipid-lowering drugs, such as niacin and fibrates,<sup>169,170</sup> decrease PAI-1 synthesis in vitro. These agents are not specific for PAI-1, however, and also affect the synthesis of other proteins. More contemporary strategies focus on PAI-1 gene silencing.

Peptides have been identified that block PAI-1 activity either by preventing insertion of the reactive center loop into the body of the inhibitor after cleavage by the target protease<sup>171</sup> or by converting PAI-1 into its latent conformation.<sup>172</sup> However, the effectiveness of these agents has yet to be tested in vivo. More promising are small-molecule PAI-1 inhibitors, some of which exhibit antithrombotic activity in vivo.<sup>173</sup>

**4.1.2 u-PA Inhibitors:** Mesupron (WX-671) is an orally active prodrug of WX-UK1, a compound that inhibits u-PA and other serine proteases. Both Mesupron and WX-UK1 reduce tumor growth and metastasis in vitro and in various animal models.<sup>174,175</sup> A phase 1 study in 19 patients with advanced head and neck cancer revealed a dose-dependent increase in plasma levels of WX-671, and WX-671 was found in tissue samples collected after tumor resection,<sup>176</sup> suggesting that amounts of drug sufficient to inhibit u-PA are concentrated in the tumor.<sup>176</sup> In a phase 2 study, 95 patients with locally advanced, metastatic pancreatic cancer received either Mesupron (at doses corresponding to 200 or 400 mg of WX-UK1) or placebo in conjunction with weekly gemcitabine. Mesupron was well tolerated, and compared with placebo, overall survival increased from 10.2 months to 13.5 months. An ongoing study is evaluating Mesupron as an adjunct to capecitabine in women with HER2 receptor-negative breast cancer.

**4.1.3 TAFIa Inhibitors:** Studies in vitro indicate that TAFIa attenuates fibrinolysis by cleaving carboxy-terminal lysine residues from fibrin.<sup>177</sup> Removal of these lysine residues decreases plasminogen or plasmin binding to fibrin, thereby retarding the lytic process. Given this mechanism of action, inhibitors of TAFIa have the potential to enhance fibrinolytic activity, a concept supported by studies in dogs and rabbits demonstrating that a potato-derived TAFIa inhibitor increased plasminogen activator-induced thrombolysis.<sup>178-180</sup> These observations have prompted development of TAFIa-directed antibodies<sup>181</sup> and nanobodies,<sup>182</sup> as well as small molecule TAFIa inhibitors.<sup>183-185</sup> A potential limitation of some such

agents is paradoxical enhancement of TAFIa activity at low doses.<sup>186-188</sup> Presumably, this reflects allosteric modulation at the active site of the enzyme. If this phenomenon is common to all TAFIa inhibitors, optimal dosing of these agents will be problematic.

**4.1.4 Factor XIIIa Inhibitors:** A thrombin-activated transglutaminase, factor XIIIa cross-links the  $\alpha$ - and  $\gamma$ -chains of fibrinogen to form  $\alpha$ -polymers and  $\gamma$ -dimers, respectively. Cross-linking stabilizes the fibrin polymer and renders it more refractory to degradation by plasmin.<sup>189</sup> Inhibition of factor XIIIa, therefore, has the potential to increase the susceptibility of the thrombus to lysis.<sup>190</sup>

Tridegin, a peptide isolated from the giant Amazon leech, *Haementeria ghilianii*, is a specific inhibitor of factor XIIIa and enhances fibrinolysis in vitro when added before clotting of fibrinogen.<sup>191,192</sup> Destabilase, a leech enzyme that hydrolyzes cross-links, provides an alternative approach to reversing the consequences of factor XIIIa-mediated fibrin crosslinking.<sup>193,194</sup> Neither of these agents has been tested in humans.

## 4.2 New Fibrinolytic Agents

Existing fibrinolytic agents are plasminogen activators that act by converting plasminogen to plasmin. t-PA and u-PA, which are enzymes, do this directly by converting single-chain plasminogen into two-chain plasmin. In contrast, streptokinase accomplishes this indirectly. Streptokinase, which is not an enzyme, binds to plasminogen and the streptokinase/plasminogen complex then serves as the plasminogen activator. More recently licensed plasminogen activators are variants of t-PA. These include reteplase, a truncated t-PA variant with a longer half-life, and tenecteplase, a bioengineered t-PA variant that not only has a longer half-life than t-PA but also exhibits enhanced fibrin specificity and resistance to inhibition by PAI-1. Because of their longer half-lives, reteplase and tenecteplase can be given by bolus injection, thereby simplifying administration.

New fibrinolytic agents under development build on advances with t-PA derivatives. Direct-acting fibrinolytic drugs, such as alfineprase, VI0153, and plasmin, have been developed in an attempt to accelerate lysis, whereas desmoteplase has been developed because of its enhanced fibrin specificity (Table 7).

**4.2.1 Alfineprase:** A recombinant truncated form of fibrolase, alfineprase directly degrades fibrin and fibrinogen.<sup>195</sup> Fibrolase is a zinc metalloprotease originally isolated from the venom of the Southern copperhead snake, *Agkistrodon contortrix contortrix*. Like fibrolase, alfineprase directly degrades the  $\alpha$  chains

of fibrin and fibrinogen.<sup>195</sup> Because there is no need for plasmin generation, alteplase has the potential more, the action of alteplase is independent of the plasminogen content of the thrombus and alteplase is not inhibited by PAI-1. Finally, by degrading fibrinogen as well as fibrin, alteplase not only degrades preformed fibrin but also has the potential to inhibit fibrin generation.

In the circulation, alteplase is rapidly inhibited by  $\alpha_2$ -macroglobulin.<sup>196</sup> Neutralization by  $\alpha_2$ -macroglobulin limits the systemic effects of alteplase and may reduce its hemorrhagic potential. To bypass circulating  $\alpha_2$ -macroglobulin, alteplase must be administered directly into the thrombus. Therefore, clinical trials of alteplase have focused on catheter-directed lysis of peripheral arterial occlusions or on local delivery to restore flow in indwelling catheters blocked by thrombus.<sup>197</sup> Phase 3 studies with alteplase for these indications have been halted, at least temporarily, because key efficacy end points were not met. The full results of these trials have not yet been published.

**4.2.2 V10153:** A variant form of plasminogen, V10153 (previously known as BB10153) has its plasminogen activator cleavage site replaced with a thrombin cleavage site.<sup>198</sup> Like plasminogen, BB10153 binds to fibrin. Once bound to fibrin, BB10153 is converted to plasmin by fibrin-bound thrombin and not by plasminogen activators. After IV injection, BB10153 has a half-life of about 4.4 h in humans.<sup>199</sup> In a phase 2 dose-escalation study in 50 patients with acute MI, a single IV bolus of BB10153 produced a dose-dependent increase in drug levels, and, at doses in the 5- to 10-mg/kg range, 34% of patients achieved complete flow in the infarct-related artery.<sup>200</sup> Major bleeding occurred in three patients, whereas minor bleeding occurred in six. There were no intracranial bleeding events. Based on these data, V10153 is undergoing continued investigation for treatment of acute ischemic stroke.

**4.2.3 Plasmin:** Delivery of plasmin directly into a thrombus via a catheter has the potential to produce rapid lysis. Excess plasmin that fails to bind to fibrin will rapidly be inactivated by  $\alpha_2$ -antiplasmin, thereby preventing the generation of a systemic lytic state by unopposed plasmin.<sup>23</sup> Although there are plasma-derived, recombinant, and transgenic forms of human plasmin, a plasma-derived form is in the most advanced stages of development. Thus, an ongoing dose-escalation study is evaluating the safety of catheter-delivered plasma-derived plasmin (in doses of 20, 40, or 60 mg) in patients with acute ischemic stroke involving the middle cerebral artery who pre-

sent within 9 h of stroke symptom onset.<sup>201</sup> There are two ongoing studies in patients with acute peripheral arterial occlusion; the first is evaluating the safety of increasing doses of plasma-derived plasmin (ranging from 25 to 175 mg in 25-mg increments) injected directly into the thrombus,<sup>202</sup> and the second is comparing four different infusion strategies for catheter-directed delivery of 150 mg plasmin.<sup>203</sup>

**4.2.4 Desmoteplase:** A recombinant analog of the full-length plasminogen activator isolated from the saliva of the vampire bat, *Desmodus rotundus*, desmoteplase has >70% homology to t-PA. Like t-PA, desmoteplase binds to fibrin via its fibronectin finger-like domain, and its catalytic activity is enhanced in the presence of fibrin.<sup>204</sup> Once bound to fibrin, desmoteplase converts fibrin-bound plasminogen to plasmin and induces fibrin degradation.

In contrast to t-PA, desmoteplase lacks a second kringle domain. This endows desmoteplase with greater fibrin specificity than t-PA because it is the second kringle domain of t-PA that mediates its interaction with fibrin degradation products and promotes systemic plasmin generation and subsequent fibrinogen degradation.<sup>204</sup> Because it is more fibrin-specific than t-PA, desmoteplase may produce less bleeding.<sup>205</sup>

In the phase 2 Desmoteplase in Acute Ischemic Stroke (DIAS) study, 104 patients presenting within 3 to 9 h of the onset of symptoms of acute ischemic stroke and with evidence of perfusion/diffusion mismatch on MRI of the brain were randomized to IV desmoteplase (25, 37.5, or 50 mg) or placebo.<sup>206</sup> Because of an excessive rate of intracranial hemorrhage with fixed doses of desmoteplase in the first 47 patients enrolled in the study (26.7% compared with none with placebo), subsequent patients were given lower, weight-adjusted doses of desmoteplase (62, 90, or 120  $\mu$ g/kg). With these lower doses, the overall rate of intracranial hemorrhage with desmoteplase was 2.2%. Reperfusion rates up to 71.4% were observed with desmoteplase compared with 19.2% with placebo. These findings were confirmed in the Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS) study, which compared IV desmoteplase (90 or 125  $\mu$ g/kg) with placebo in 37 patients presenting within 3 to 9 h of onset of stroke symptoms.<sup>207</sup> There were no symptomatic intracranial hemorrhages, and reperfusion was achieved in 18.2% of patients given 90  $\mu$ g/kg desmoteplase, 53.3% of those treated with 125  $\mu$ g/kg desmoteplase, and in 37.5% of patients given placebo. Good clinical outcome at 90 days occurred in 28.6% and 60.0% of patients treated with 90 or 125  $\mu$ g/kg desmoteplase, respectively, compared with 25% of patients given placebo. Building on these findings, the DIAS-2 study followed the same design and randomized



193 patients to desmoteplase (90 or 120  $\mu\text{g}/\text{kg}$ ) or placebo 3 to 9 h after onset of symptoms of stroke.<sup>208</sup> Clinical response rates at day 90, the primary efficacy end point, were 47% and 36%, with the 90- or 120- $\mu\text{g}/\text{kg}$  doses of desmoteplase, respectively, compared with 46% with placebo. The high response in the placebo group may be explained by inclusion of patients with mild strokes, which may have reduced the potential to detect an effect with desmoteplase. An ongoing phase 2 trial in Japan is comparing desmoteplase (70 or 90  $\mu\text{g}/\text{kg}$ ) with placebo in patients with acute ischemic stroke.<sup>209</sup> The phase 3 DIAS-4 trial is comparing 90  $\mu\text{g}/\text{kg}$  of desmoteplase with placebo in patients presenting 3 to 9 h after onset of symptoms of stroke.<sup>210</sup>

## 5.0 CONCLUSIONS AND FUTURE DIRECTIONS

Aspirin and clopidogrel have an established role in the prevention and treatment of arterial thrombosis. Although effective, breakthrough thrombosis remains a problem, even when the drugs are used in combination. This has prompted the development of new antiplatelet drugs. The variable antiplatelet effects of fixed doses of clopidogrel have led to the development of new thienopyridines, such as prasugrel, which produces more potent and consistent inhibition of ADP-induced platelet aggregation. Direct-acting P2Y<sub>12</sub> inhibitors, such as ticagrelor, not only overcome the slow onset and offset of the thienopyridines but also offer more potent ADP receptor blockade. The challenge with these new agents is safety; when added to aspirin, these drugs produce more bleeding than clopidogrel. Therefore, finding the right dose, identifying the appropriate patients, and restricting the duration of therapy will be necessary to ensure that an optimal benefit-to-risk profile is obtained.

The greatest unmet need in anticoagulation therapy has been the replacement of warfarin with orally active agents that can be given in fixed doses without routine coagulation monitoring. Consequently, most of the recent attention has focused on new oral anticoagulants.

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## REFERENCES

- Freiman D. The structure of thrombi. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*. 2nd ed. Philadelphia, PA: JB Lippincott; 1987:1123-1135.
- Baigent C, Blackwell L, Collins R, et al; Antithrombotic Trialists' (ATT) Collaboration. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet*. 2009;373(9678):1849-1860.
- Berger JS, Krantz MJ, Kittelson JM, Hiatt WR. Aspirin for the prevention of cardiovascular events in patients with peripheral artery disease: a meta-analysis of randomized trials. *JAMA*. 2009;301(18):1909-1919.
- Food and Drug Administration. Internal analgesic, antipyretic, and antirheumatic drug products for over-the-counter human use; final rule for professional labeling of aspirin, buffered aspirin, and aspirin in combination with antacid drug products. *Fed Regist*. 1998;63(205):56802-56809.
- CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet*. 1996;348(9038):1329-1339.
- Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001;345(7):494-502.
- Chen ZM, Jiang LX, Chen YP, et al; COMMIT (CLOpidogrel and Metoprolol in Myocardial Infarction Trial) collaborative group. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet*. 2005;366(9497):1607-1621.
- Sabatine MS, Cannon CP, Gibson CM, et al; CLARITY-TIMI 28 Investigators. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med*. 2005;352(12):1179-1189.
- Mehta S, Tanguay J-F, Eikelboom JW, et al. Double-dose versus standard-dose clopidogrel and high-dose versus low-dose aspirin in individuals undergoing percutaneous

- coronary intervention for acute coronary syndromes (CURRENT-OASIS): a randomised factorial trial. *Lancet*. 2010;376(9748):1233-1243.
10. Bhatt DL, Fox KA, Hacke W, et al; CHARISMA Investigators. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med*. 2006;354(16):1706-1717.
  11. Diener H-C, Bogousslavsky J, Brass LM, et al; MATCH investigators. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet*. 2004;364(9431):331-337.
  12. Verro P, Gorelick P, Nguyen D. Aspirin plus dipyridamole versus aspirin for prevention of vascular events after stroke or TIA: a meta-analysis. *Stroke*. 2008;39(4):1358-1363.
  13. Sacco RL, Diener H-C, Yusuf S, et al; PROFESS Study Group. Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. *N Engl J Med*. 2008;359(12):1238-1251.
  14. Hankey GJ, Eikelboom JW. Aspirin resistance. *Lancet*. 2006;367(9510):606-617.
  15. Armero B, Ait Mokhtar O, Mancini J AP et al. Clopidogrel loading dose adjustment according to platelet reactivity monitoring in patients carrying the 2C19\*2 loss of function polymorphism. *J Am Coll Cardiol*. 2010;56(20):1630-1636.
  16. Braunwald E, Angiolillo DJ, Bates ER, et al. Assessing the current role of platelet function testing. *Clin Cardiol*. 2008;31(3 suppl 1):I10-16.
  17. Mega JL, Simon T, Collet JP, et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *JAMA*. 2010;304(16):1821-1830.
  18. Verstuyft C, Simon T, Kim RB. Personalized medicine and antiplatelet therapy: ready for prime time? *Eur Heart J*. 2009;30(16):1943-1963.
  19. Damani SB, Topol EJ. The case for routine genotyping in dual-antiplatelet therapy. *J Am Coll Cardiol*. 2010;56(2):109-111.
  20. Eikelboom JW, Weitz JI. New anticoagulants. *Circulation*. 2010;121(13):1523-1532.
  21. Kunadian V, Gibson CM. Thrombolytics and myocardial infarction [published online ahead of print November 11, 2010]. *Cardiovasc Ther*. doi:10.1111/j.1755-5922.2010.00239.x.
  22. Paciaroni M, Medeiros E, Bogousslavsky J. Desmoteplase. *Expert Opin Biol Ther*. 2009;9(6):773-778.
  23. Marder VJ, Novokhatny V. Direct fibrinolytic agents: biochemical attributes, preclinical foundation and clinical potential. *J Thromb Haemost*. 2010;8(3):433-444.
  24. Giannarelli C, Zafar MU, Badimon JJ. Prostanoid and TP-receptors in atherothrombosis: is there a role for their antagonism? *Thromb Haemost*. 2010;104(5):949-954.
  25. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation*. 2002;105(14):1650-1655.
  26. Eikelboom JW, Hankey GJ, Thom J, et al; Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) Investigators. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. *Circulation*. 2008;118(17):1705-1712.
  27. Kakkos SK, Nicolaidis AN. S-18886 Servier. *Curr Opin Investig Drugs*. 2002;3(9):1324-1327.
  28. Gaussem P, Reny J-L, Thalamas C, et al. The specific thromboxane receptor antagonist S18886: pharmacokinetic and pharmacodynamic studies. *J Thromb Haemost*. 2005;3(7):1437-1445.
  29. Belhassen L, Pelle G, Dubois-Rande JL, Adnot S. Improved endothelial function by the thromboxane A2 receptor antagonist S 18886 in patients with coronary artery disease treated with aspirin. *J Am Coll Cardiol*. 2003;41(7):1198-1204.
  30. Fiessinger JN, Bounameaux H, Cairols MA, et al; TAIPAD investigators. Thromboxane antagonism with terutroban in peripheral arterial disease. The TAIPAD study. *J Thromb Haemost*. 2010;8(11):2369-2376.
  31. Bousser M-G, Amarenco P, Charmorro A, et al. Rationale and design of a randomized, double-blind, parallel group study of terutroban 30 mg/day versus aspirin 100 mg/day in stroke patients: the prevention of cerebrovascular and cardiovascular events of ischemic origin with terutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study. *Cerebrovasc Dis*. 2009;27(5):509-518.
  32. Bousser M-G, Amarenco P, Chamorro A, et al; PERFORM Study Investigators. The Prevention of cerebrovascular and cardiovascular Events of ischemic origin with teRutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study: baseline characteristics of the population. *Cerebrovasc Dis*. 2009;27(6):608-613.
  33. Bousser M-G, Amarenco P, Chamorro A, et al; PERFORM Study Investigators. Terutroban versus aspirin in patients with cerebral ischaemic events (PERFORM): a randomised, double-blind, parallel-group trial. *Lancet*. 2011;377(9782):2013-2022.
  34. Modesti PA, Cecioni I, Colella A, Costoli A, Paniccia R, Neri Serneri GG. Binding kinetics and antiplatelet activities of picotamide, a thromboxane A2 receptor antagonist. *Br J Pharmacol*. 1994;112(1):81-86.
  35. Balsano F, Violi F; The ADEP Group. Effect of picotamide on the clinical progression of peripheral vascular disease. A double-blind placebo-controlled study. *Circulation*. 1993;87(5):1563-1569.
  36. Milani M, Longoni A, Maderna M. Effects of picotamide, an antiplatelet agent, on cardiovascular, events in 438 claudicant patients with diabetes: a retrospective analysis of the ADEP study. *Br J Clin Pharmacol*. 1996;42(6):782-785.
  37. Neri Serneri GG, Coccheri S, Marubini E, Violi F; Drug Evaluation in Atherosclerotic Vascular Disease in Diabetics (DAVID) Study Group. Picotamide, a combined inhibitor of thromboxane A2 synthase and receptor, reduces 2-year mortality in diabetics with peripheral arterial disease: the DAVID study. *Eur Heart J*. 2004;25(20):1845-1852.
  38. Fugate SE, Cudd LA. Cangrelor for treatment of coronary thrombosis. *Ann Pharmacother*. 2006;40(5):925-930.
  39. Greenbaum AB, Grines CL, Bittl JA et al. Initial experience with an intravenous P2Y12 platelet receptor antagonist in patients undergoing percutaneous coronary intervention: results from a 2-part, phase 2, multicenter, randomized, placebo- and active-controlled trial. *Am Heart J*. 2006;151(3):689.e1-689.e10.
  40. Harrington RA, Stone GW, McNulty S, et al. Platelet inhibition with cangrelor in patients undergoing PCI. *N Engl J Med*. 2009;361(24):2318-2329.
  41. Bhatt DL, Lincoff AM, Gibson CM, et al; CHAMPION PLATFORM Investigators. Intravenous platelet blockade with cangrelor during PCI. *N Engl J Med*. 2009;361(24):2330-2341.
  42. Tantry US, Bliden KP, Gurbel PA. AZD6140. *Expert Opin Investig Drugs*. 2007;16(2):225-229.
  43. Gurbel PA, Bliden KP, Butler KD, et al. Randomized double-blind assessment of the ONSET and OFFSET of the antiplatelet effects of ticagrelor versus clopidogrel

- in patients with stable coronary artery disease: the ONSET/OFFSET study. *Circulation*. 2009;120(25):2577-2585.
44. Husted SE, Emanuelsson H, Heptinstall S, Sandset PM, Wickens M, Peters G. Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. *Eur Heart J*. 2006;27(9):1038-1047.
  45. Cannon CP, Husted SE, Harrington RA, et al; DISPERSE-2 Investigators. Safety, tolerability, and initial efficacy of AZD6140, the first reversible oral adenosine diphosphate receptor antagonist, compared with clopidogrel, in patients with non-ST-segment elevation acute coronary syndrome: primary results of the DISPERSE-2 trial. *J Am Coll Cardiol*. 2007;50(19):1844-1851.
  46. Wallentin L, Becker RC, Budaj A, et al; PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*. 2009;361(11):1045-1057.
  47. Steg PG, James S, Harrington RA, et al; PLATO Study Group. Ticagrelor versus clopidogrel in patients with ST-elevation acute coronary syndromes intended for reperfusion with primary percutaneous coronary intervention: A Platelet Inhibition and Patient Outcomes (PLATO) trial subgroup analysis. *Circulation*. 2010;122(21):2131-2141.
  48. Held C, Asenblad N, Bassand J-P, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes undergoing coronary artery bypass surgery: results from the PLATO (Platelet Inhibition and Patient Outcomes) trial. *J Am Coll Cardiol*. 2011;57(6):672-684.
  49. Storey RF, Bliden KP, Patil SB, et al; ONSET/OFFSET Investigators. Incidence of dyspnea and assessment of cardiac and pulmonary function in patients with stable coronary artery disease receiving ticagrelor, clopidogrel, or placebo in the ONSET/OFFSET study. *J Am Coll Cardiol*. 2010;56(3):185-193.
  50. Siller-Matula JM, Krumphuber J, Jilma B. Pharmacokinetic, pharmacodynamic and clinical profile of novel antiplatelet drugs targeting vascular diseases. *Br J Pharmacol*. 2010;159(3):502-517.
  51. Berger JS, Roe MT, Gibson CM, et al. Safety and feasibility of adjunctive antiplatelet therapy with intravenous elinogrel, a direct-acting and reversible P2Y12 ADP-receptor antagonist, before primary percutaneous intervention in patients with ST-elevation myocardial infarction: the Early Rapid ReversAl of platelet thromboSis with intravenous Elinogrel before PCI to optimize reperfusion in acute Myocardial Infarction (ERASE MI) pilot trial. *Am Heart J*. 2009;158(6):998-1004., e1.
  52. Leonardi S, Rao SV, Harrington RA, et al. Rationale and design of the randomized, double-blind trial testing INtraveNous and Oral administration of elinogrel, a selective and reversible P2Y(12)-receptor inhibitor, versus clopidogrel to eVAluate Tolerability and Efficacy in nonurgent Percutaneous Coronary Interventions patients (INNOVATE-PCI). *Am Heart J*. 2010;160(1):65-72.
  53. Smyth SS, Woulfe DS, Weitz JI, et al; 2008 Platelet Colloquium Participants. G-protein-coupled receptors as signaling targets for antiplatelet therapy. *Arterioscler Thromb Vasc Biol*. 2009;29(4):449-457.
  54. Tomasello SD, Angiolillo DJ, Goto S. Inhibiting PAR-1 in the prevention and treatment of atherothrombotic events. *Expert Opin Investig Drugs*. 2010;19(12):1557-1567.
  55. Chackalamannil S, Wang Y, Greenlee WJ, et al. Discovery of a novel, orally active himbacine-based thrombin receptor antagonist (SCH 530348) with potent antiplatelet activity. *J Med Chem*. 2008;51(11):3061-3064.
  56. Becker RC, Moliterno DJ, Jennings LK, et al; TRA-PCI Investigators. Safety and tolerability of SCH 530348 in patients undergoing non-urgent percutaneous coronary intervention: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet*. 2009;373(9667):919-928.
  57. Goto S, Yamaguchi T, Ikeda Y, Kato K, Yamaguchi H, Jensen P. Safety and exploratory efficacy of the novel thrombin receptor (PAR-1) antagonist SCH530348 for non-ST-segment elevation acute coronary syndrome. *J Atheroscler Thromb*. 2010;17(2):156-164.
  58. Shinohara Y, Goto S, Doi M, Jensen P. Safety of the novel protease-activated receptor-1 antagonist vorapaxar in Japanese patients with a history of ischemic stroke [published online ahead of print October 13, 2010]. *J Stroke Cerebrovasc Dis*.
  59. Tricoci P, Huang Z, Held C, et al. Thrombin-receptor antagonist vorapaxar in acute coronary syndromes [published ahead of print November 13, 2011]. *N Engl J Med*. doi:10.1056/NEJMoal109719.
  60. Morrow DA, Scirica BM, Fox KA, et al; TRA 2(o)P-TIMI 50 Investigators. Evaluation of a novel antiplatelet agent for secondary prevention in patients with a history of atherosclerotic disease: design and rationale for the Thrombin-Receptor Antagonist in Secondary Prevention of Atherothrombotic Ischemic Events (TRA 2 degrees P)-TIMI 50 trial. *Am Heart J*. 2009;158(3):335-341., e3.
  61. Goto S, Ogawa H, Takeuchi M et al. Double-blind, placebo-controlled Phase 2 studies of the protein-activated receptor 1 antagonist E555 (atopaxar) in Japanese patients with acute coronary syndrome or high-risk coronary artery disease. *Eur Heart J*. 2010;31(21):2601-2613.
  62. Abraham E, Reinhart K, Svoboda P, et al. Assessment of the safety of recombinant tissue factor pathway inhibitor in patients with severe sepsis: a multicenter, randomized, placebo-controlled, single-blind, dose escalation study. *Crit Care Med*. 2001;29(11):2081-2089.
  63. Abraham E, Reinhart K, Opal SM, et al; OPTIMIST Trial Study Group. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *JAMA*. 2003;290(2):238-247.
  64. Laterre P-F, Opal SM, Abraham E, et al. A clinical evaluation committee assessment of recombinant human tissue factor pathway inhibitor (tifacogin) in patients with severe community-acquired pneumonia. *Crit Care*. 2009;13(2):R36.
  65. National Institutes of Health Clinical Center. Tifacogin for the treatment of patients with severe community-acquired pneumonia. NCT00084071. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2004.
  66. Cappello M, Vlasuk GP, Bergum PW, Huang S, Hotez PJ. Ancylostoma caninum anticoagulant peptide: a hookworm-derived inhibitor of human coagulation factor Xa. *Proc Natl Acad Sci U S A*. 1995;92(13):6152-6156.
  67. Bergum PW, Cruikshank A, Maki SL, Kelly CR, Ruf W, Vlasuk GP. Role of zymogen and activated factor X as scaffolds for the inhibition of the blood coagulation factor VIIa-tissue factor complex by recombinant nematode anticoagulant protein c2. *J Biol Chem*. 2001;276(13):10063-10071.
  68. Vlasuk GP, Bradbury A, Lopez-Kininger L, et al. Pharmacokinetics and anticoagulant properties of the factor VIIa-tissue factor inhibitor recombinant Nematode Anticoagulant Protein c2 following subcutaneous administration in man. Dependence on the stoichiometric binding to circulating factor X. *Thromb Haemost*. 2003;90(5):803-812.
  69. Lee A, Agnelli G, Büller H, et al. Dose-response study of recombinant factor VIIa/tissue factor inhibitor recombinant nematode anticoagulant protein c2 in prevention of

- postoperative venous thromboembolism in patients undergoing total knee replacement. *Circulation*. 2001;104(1):74-78.
70. Giugliano RP, Wiviott SD, Stone PH, et al; ANTHEM-TIMI-32 Investigators. Recombinant nematode anticoagulant protein c2 in patients with non-ST-segment elevation acute coronary syndrome: the ANTHEM-TIMI-32 trial. *J Am Coll Cardiol*. 2007;49(25):2398-2407.
  71. Moons AHM, Peters RJ, Bijsterveld NR, et al. Recombinant nematode anticoagulant protein c2, an inhibitor of the tissue factor/factor VIIa complex, in patients undergoing elective coronary angioplasty. *J Am Coll Cardiol*. 2003;41(12):2147-2153.
  72. National Institutes of Health Clinical Center. Safety study of recombinant NAPc2 to prevent tumor progression and metastases in metastatic colon cancer. NCT00443573. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2007.
  73. Taylor FB Jr. Role of tissue factor and factor VIIa in the coagulant and inflammatory response to LD100 *Escherichia coli* in the baboon. *Haemostasis*. 1996;26(suppl 1):S3-91.
  74. Jang Y, Guzman LA, Lincoff AM, et al. Influence of blockade at specific levels of the coagulation cascade on restenosis in a rabbit atherosclerotic femoral artery injury model. *Circulation*. 1995;92(10):3041-3050.
  75. Lincoff AM. First clinical investigation of a tissue-factor inhibitor administered during percutaneous coronary revascularization: a randomized, double-blind, dose-escalation trial assessing safety and efficacy of FFR-FVIIa in percutaneous transluminal coronary angioplasty (ASIS) trial [abstract]. *JACC*. 2000;36:312.
  76. Toomey JR, Blackburn MN, Storer BL, Valocik RE, Koster PF, Feuerstein GZ. Comparing the antithrombotic efficacy of a humanized anti-factor IX(a) monoclonal antibody (SB 249417) to the low molecular weight heparin enoxaparin in a rat model of arterial thrombosis. *Thromb Res*. 2000;100(1):73-79.
  77. Toomey JR, Valocik RE, Koster PF, et al. Inhibition of factor IX(a) is protective in a rat model of thromboembolic stroke. *Stroke*. 2002;33(2):578-585.
  78. Chow FS, Benincosa LJ, Sheth SB, et al. Pharmacokinetic and pharmacodynamic modeling of humanized anti-factor IX antibody (SB 249417) in humans. *Clin Pharmacol Ther*. 2002;71(4):235-245.
  79. Rusconi CP, Scardino E, Layzer J, et al. RNA aptamers as reversible antagonists of coagulation factor IXa. *Nature*. 2002;419(6902):90-94.
  80. Dyke CK, Steinhubl SR, Kleiman NS, et al. First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase Ia pharmacodynamic evaluation of a drug-antidote pair for the controlled regulation of factor IXa activity. *Circulation*. 2006;114(23):2490-2497.
  81. Chan MY, Cohen MG, Dyke CK, et al. Phase Ib randomized study of antidote-controlled modulation of factor IXa activity in patients with stable coronary artery disease. *Circulation*. 2008;117(22):2865-2874.
  82. Cohen MG, Purdy DA, Rossi JS, et al. First clinical application of an actively reversible direct factor IXa inhibitor as an anticoagulation strategy in patients undergoing percutaneous coronary intervention. *Circulation*. 2010;122(6):614-622.
  83. Eriksson BI, Dahl OE, Lassen MR, et al; Fixit Study Group. Partial factor IXa inhibition with TTP889 for prevention of venous thromboembolism: an exploratory study. *J Thromb Haemost*. 2008;6(3):457-463.
  84. Rezaie AR. Prothrombin protects factor Xa in the prothrombinase complex from inhibition by the heparin-antithrombin complex. *Blood*. 2001;97(8):2308-2313.
  85. Brufatto N, Nesheim ME. The use of prothrombin(S525C) labeled with fluorescein to directly study the inhibition of prothrombinase by antithrombin during prothrombin activation. *J Biol Chem*. 2001;276(21):17663-17671.
  86. Krishnaswamy S, Vlasuk GP, Bergum PW. Assembly of the prothrombinase complex enhances the inhibition of bovine factor Xa by tick anticoagulant peptide. *Biochemistry*. 1994;33(25):7897-7907.
  87. Héroult J-P, Bernat A, Pflieger AM, Lormeau JC, Herbert JM. Comparative effects of two direct and indirect factor Xa inhibitors on free and clot-bound prothrombinase. *J Pharmacol Exp Ther*. 1997;283(1):16-22.
  88. Herbert J-M, Héroult J-P, Bernat A, et al. Biochemical and pharmacological properties of SANORG 34006, a potent and long-acting synthetic pentasaccharide. *Blood*. 1998;91(11):4197-4205.
  89. PERSIST investigators. A novel long-acting synthetic factor Xa inhibitor (SanOrg34006) to replace warfarin for secondary prevention in deep vein thrombosis. A Phase 2 evaluation. *J Thromb Haemost*. 2004;2(1):47-53.
  90. Buller HR, Cohen AT, Davidson B, et al; van Gogh Investigators. Idraparin versus standard therapy for venous thromboembolic disease. *N Engl J Med*. 2007;357(11):1094-1104.
  91. Buller HR, Cohen AT, Davidson B, et al; van Gogh Investigators. Extended prophylaxis of venous thromboembolism with idraparin. *N Engl J Med*. 2007;357(11):1105-1112.
  92. Bousser MG, Bouthier J, Büller HR, et al; Amadeus Investigators. Comparison of idraparin with vitamin K antagonists for prevention of thromboembolism in patients with atrial fibrillation: a randomised, open-label, non-inferiority trial. *Lancet*. 2008;371(9609):315-321.
  93. Equinox Investigators. Efficacy and safety of once weekly subcutaneous idrabiotaparin in the treatment of patients with symptomatic deep venous thrombosis. *J Thromb Haemost*. 2011;9(1):92-99.
  94. Paty I, Trellu M, Destors JM, Cortez P, Boëlle E, Sanderink G. Reversibility of the anti-FXa activity of idrabiotaparin (biotinylated idraparin) by intravenous avidin infusion. *J Thromb Haemost*. 2010;8(4):722-729.
  95. Herbert J-M, Héroult J-P, Bernat A, et al. SR123781A, a synthetic heparin mimetic. *Thromb Haemost*. 2001;85(5):852-860.
  96. Becker DL, Fredenburgh JC, Stafford AR, Weitz JI. Exosites 1 and 2 are essential for protection of fibrin-bound thrombin from heparin-catalyzed inhibition by antithrombin and heparin cofactor II. *J Biol Chem*. 1999;274(10):6226-6233.
  97. Héroult J-P, Cappelle M, Bernat A, et al. Effect of SanOrg123781A, a synthetic hexadecasaccharide, on clot-bound thrombin and factor Xa in vitro and in vivo. *J Thromb Haemost*. 2003;1(9):1959-1965.
  98. Lassen MR, Dahl OE, Mismetti P, Zielske D, Turpie AG. SR123781A: a new once daily synthetic oligosaccharide anticoagulant for thromboprophylaxis after total hip replacement surgery: the DRIVE (Dose Ranging Study in Elective Total Hip Replacement Surgery) study. *J Am Coll Cardiol*. 2008;51(15):1498-1504.
  99. Kishimoto TK, Qi YW, Long A, et al. M118—a rationally engineered low-molecular-weight heparin designed specifically for the treatment of acute coronary syndromes. *Thromb Haemost*. 2009;102(5):900-906.
  100. Rao SV, Melloni C, Myles-Dimauro S, et al; EMINENCE Investigators. Evaluation of a new heparin agent in percutaneous coronary intervention: results of the phase 2 evaluation of M118 IN pErcutaNeous Coronary intErvention (EMINENCE) Trial. *Circulation*. 2010;121(15):1713-1721.

101. National Institutes of Health Clinical Center. Evaluation of AVE5026 in the Prevention of Venous Thromboembolism in Acutely Ill Medical Patients with Restricted Mobility (SAVE-VEMED). NCT00714597. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2008.
102. Mouret P, Agnelli G, Fisher WD et al. The ultra-low-molecular-weight heparin (ULMWH) semuloparin for prevention of venous thromboembolism (VTE) after elective hip replacement surgery [abstract]. *Pathophysiol Haemost Thromb*. 2009;37(suppl 1):OC316.
103. Fisher WD, Agnelli G, George D et al. The ultra-low-molecular-weight heparin (ULMWH) semuloparin for prevention of venous thromboembolism (VTE) after hip fracture surgery [abstract]. *Pathophysiol Haemost Thromb*. 2009;37(suppl 1):P330.
104. Lassen MR, Agnelli G, Fisher WD et al. The ultra-low-molecular-weight heparin (ULMWH) semuloparin for prevention of venous thromboembolism (VTE) after elective knee replacement surgery [abstract]. *Pathophysiol Haemost Thromb*. 2009;37(suppl 1):OC331.
105. Turpie AG, Agnelli G, Fisher W et al. Benefit-to-risk profile of the ultra-low-molecular-weight heparin (ULMWH) semuloparin for prevention of venous thromboembolism (VTE): a meta-analysis of 3 major orthopedic surgery studies [abstract]. *Pathophysiol Haemost Thromb*. 2009;37(suppl 1):OC332.
106. Fisher W, Agnelli G, George D et al. Extended venous thromboembolism (VTE) prophylaxis after hip fracture surgery with the ultra-low-molecular-weight heparin (ULMWH) semuloparin [abstract]. *Pathophysiol Haemost Thromb*. 2009;37(suppl 1):OC681.
107. Kakkar AK, Agnelli G, Fisher WD, et al. The ultra-low-molecular-weight heparin semuloparin for prevention of venous thromboembolism in patients undergoing major abdominal surgery [abstract]. American Society of Hematology Annual Meeting; December 4-7, 2010; Orlando, FL.
108. National Institutes of Health Clinical Center. Evaluation of AVE5026 in the prevention of venous thromboembolism in cancer patients undergoing chemotherapy (SAVE-ONCO). NCT00694382. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2008.
109. Herbert J-M, Bernat A, Dol F, Hérault JP, Crépon B, Lormeau JC. DX 9065A a novel, synthetic, selective and orally active inhibitor of factor Xa: in vitro and in vivo studies. *J Pharmacol Exp Ther*. 1996;276(3):1030-1038.
110. Maruyama I, Tanaka M, Kunitada S, et al. Tolerability, pharmacokinetics and pharmacodynamics of DX-9065a, a new synthetic potent anticoagulant and specific factor Xa inhibitor, in healthy male volunteers. *Clin Pharmacol Ther*. 1996;66(3):258-264.
111. Becker RC, Alexander JH, Dyke CK, et al; XaNADU-1B Investigators. Effect of the novel direct factor Xa inhibitor DX-9065a on thrombin generation and inhibition among patients with stable atherosclerotic coronary artery disease. *Thromb Res*. 2006;117(4):439-446.
112. Alexander JH, Yang H, Becker RC, et al. First experience with direct, selective factor Xa inhibition in patients with non-ST-elevation acute coronary syndromes: results of the XaNADU-ACS trial. *J Thromb Haemost*. 2005;3(3):439-447.
113. Alexander JH, Dyke CK, Yang H, et al; XaNADU-PCI PILOT Investigators. Initial experience with factor-Xa inhibition in percutaneous coronary intervention: the XaNADU-PCI Pilot. *J Thromb Haemost*. 2004;2(2):234-241.
114. Paccaly A, Ozoux ML, Chu V, et al. Pharmacodynamic markers in the early clinical assessment of otamixaban, a direct factor Xa inhibitor. *Thromb Haemost*. 2005;94(6):1156-1163.
115. Hinder M, Frick A, Jordaan P, et al. Direct and rapid inhibition of factor Xa by otamixaban: a pharmacokinetic and pharmacodynamic investigation in patients with coronary artery disease. *Clin Pharmacol Ther*. 2006;80(6):691-702.
116. Cohen M, Bhatt DL, Alexander JH, et al; SEPIA-PCI Trial Investigators. Randomized, double-blind, dose-ranging study of otamixaban, a novel, parenteral, short-acting direct factor Xa inhibitor, in percutaneous coronary intervention: the SEPIA-PCI trial. *Circulation*. 2007;115(20):2642-2651.
117. Sabatine MS, Antman EM, Widimsky P, et al. Otamixaban for the treatment of patients with non-ST-elevation acute coronary syndromes (SEPIA-ACS1 TIMI 42): a randomised, double-blind, active-controlled, phase 2 trial. *Lancet*. 2009;374(9692):787-795.
118. National Institutes of Health Clinical Center. Effect of otamixaban versus unfractionated heparin + eptifibatide in patients with unstable angina/non ST elevation myocardial infarction undergoing early invasive strategy (TAO). NCT01076764. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2010.
119. Kubitzka D, Becka M, Wensing G, Voith B, Zuehlsdorf M. Safety, pharmacodynamics, and pharmacokinetics of BAY 59-7939—an oral, direct Factor Xa inhibitor—after multiple dosing in healthy male subjects. *Eur J Clin Pharmacol*. 2005;61(12):873-880.
120. Mueck W, Becka M, Kubitzka D, Voith B, Zuehlsdorf M. Population model of the pharmacokinetics and pharmacodynamics of rivaroxaban—an oral, direct factor xa inhibitor—in healthy subjects. *Int J Clin Pharmacol Ther*. 2007;45(6):335-344.
121. Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G. Oral anticoagulant therapy: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141(2)(suppl):e44S-e88S.
122. Jiang X, Crain EJ, Luetttgen JM, Schumacher WA, Wong PC. Apixaban, an oral direct factor Xa inhibitor, inhibits human clot-bound factor Xa activity in vitro. *Thromb Haemost*. 2009;101(4):780-782.
123. Raghavan N, Frost CE, Yu Z, et al. Apixaban metabolism and pharmacokinetics after oral administration to humans. *Drug Metab Dispos*. 2009;37(1):74-81.
124. Lassen MR, Raskob GE, Gallus AS, Pineo G, Chen D, Portman RJ. Apixaban or enoxaparin for thromboprophylaxis after knee replacement. *N Engl J Med*. 2009;361(6):594-604.
125. Lassen MR, Raskob GE, Gallus AS, Pineo G, Chen D, Hornick P; ADVANCE-2 investigators. Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. *Lancet*. 2010;375(9717):807-815.
126. Lassen MR, Gallus AS, Raskob GE, Pineo G, Chen D, Ramirez LM; ADVANCE-3 Investigators. Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. *N Engl J Med*. 2010;363(26):2487-2498.
127. Goldhaber SZ, Leizorovicz A, Kakkar AK, et al. Apixaban versus enoxaparin for thromboprophylaxis in medically ill patients [published ahead of print November 13, 2011]. *N Engl J Med*. doi:10.1056/NEJMoa1110899.
128. National Institutes of Health Clinical Center. Efficacy and safety study of apixaban for extended treatment of deep vein thrombosis or pulmonary embolism. NCT00633893. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2008.
129. National Institutes of Health Clinical Center. Efficacy and safety study of apixaban for the treatment of deep vein thrombosis or pulmonary embolism. NCT00643201. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2008.

130. Granger CB, Alexander JH, McMurray JJ, et al; ARISTOTLE Committees and Investigators. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2011;365(11):981-992.
131. Eikelboom JW, O'Donnell M, Yusuf S, et al. Rationale and design of AVERROES: apixaban versus acetylsalicylic acid to prevent stroke in atrial fibrillation patients who have failed or are unsuitable for vitamin K antagonist treatment. *Am Heart J*. 2010;159(3):348-353., e1.
132. Alexander JH, Becker RC, Bhatt DL, et al; APPRAISE Steering Committee and Investigators. Apixaban, an oral, direct, selective factor Xa inhibitor, in combination with antiplatelet therapy after acute coronary syndrome: results of the Apixaban for Prevention of Acute Ischemic and Safety Events (APPRAISE) trial. *Circulation*. 2009;119(22):2877-2885.
133. Alexander JH, Lopes RD, James S, et al; APPRAISE-2 Investigators. Apixaban with antiplatelet therapy after acute coronary syndrome. *N Engl J Med*. 2011;365(8):699-708.
134. Ogata K, Mendell-Harary J, Tachibana M, et al. Clinical safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel factor Xa inhibitor edoxaban in healthy volunteers. *J Clin Pharmacol*. 2010;50(7):743-753.
135. Fuji T, Fujita S, Tachibana S, Kawai Y. A dose-ranging study evaluating the oral factor Xa inhibitor edoxaban for the prevention of venous thromboembolism in patients undergoing total knee arthroplasty. *J Thromb Haemost*. 2010;8(11):2458-2468.
136. Raskob GE, Cohen AT, Eriksson BI, et al. Oral direct factor Xa inhibition with edoxaban for thromboprophylaxis after elective total hip replacement. A randomised double-blind dose-response study. *Thromb Haemost*. 2010;104(3):642-649.
137. Fuji T, Fujita S, Tachibana M et al. Efficacy and safety of edoxaban versus enoxaparin for the prevention of venous thromboembolism following total hip arthroplasty [abstract]. *Blood*. 2010;116(21):1360.
138. National Institutes of Health Clinical Center. Comparative investigation of low molecular weight (LMW) heparin/edoxaban tosylate (DU176b) versus (LMW) heparin/warfarin in the treatment of symptomatic deep-vein blood clots and/or lung blood clots (The Edoxaban Hokusai-VTE Study). NCT00986154. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2009.
139. Weitz JI, Connolly SJ, Patel I, et al. Randomised, parallel-group, multicentre, multinational phase 2 study comparing edoxaban, an oral factor Xa inhibitor, with warfarin for stroke prevention in patients with atrial fibrillation. *Thromb Haemost*. 2010;104(3):633-641.
140. Ruff CT, Giugliano RP, Antman EM, et al. Evaluation of the novel factor Xa inhibitor edoxaban compared with warfarin in patients with atrial fibrillation: design and rationale for the Effective aNticoagulation with factor xA next GEneration in Atrial Fibrillation-Thrombolysis In Myocardial Infarction study 48 (ENGAGE AF-TIMI 48). *Am Heart J*. 2010;160(4):635-641.
141. Eriksson BI, Turpie AG, Lassen MR, et al; ONYX study group. A dose escalation study of YM150, an oral direct factor Xa inhibitor, in the prevention of venous thromboembolism in elective primary hip replacement surgery. *J Thromb Haemost*. 2007;5(8):1660-1665.
142. National Institutes of Health Clinical Center. A study evaluating efficacy and safety of YM150 compared to enoxaparin in subjects undergoing hip replacement surgery (ONYX-3). NCT00902928. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2009.
143. National Institutes of Health Clinical Center. A study to evaluate the safety and efficacy of YM150 in patients with knee replacement surgery (PEARL). NCT00595426. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2008.
144. National Institutes of Health Clinical Center. A study evaluating safety and tolerability of YM150 compared to warfarin in subjects with atrial fibrillation (OPAL-2). NCT00938730. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2009.
145. Steg PG, Mehta SR, Jukema JW, et al. RUBY-1: a randomized, double-blind, placebo-controlled trial of the safety and tolerability of the novel oral factor Xa inhibitor darexaban (YM150) following acute coronary syndrome. *Eur Heart J*. 2011;32:2541-2554.
146. Eriksson BI, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor xa inhibitors in development. *Clin Pharmacokinet*. 2009;48(1):1-22.
147. Turpie AG, Bauer KA, Davidson BL, et al; EXPERT Study Group. A randomized evaluation of betrixaban, an oral factor Xa inhibitor, for prevention of thromboembolic events after total knee replacement (EXPERT). *Thromb Haemost*. 2009;101(1):68-76.
148. Weitz JI, Cao C, Eriksson BI, et al. A dose-finding study with TAK-442, an oral factor Xa inhibitor, in patients undergoing elective total knee replacement surgery. *Thromb Haemost*. 2010;104(6):1150-1157.
149. National Institutes of Health Clinical Center. Safety and efficacy of TAK-442 in subjects with acute coronary syndromes. NCT00677053. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2008.
150. Agnelli G, Haas S, Ginsberg JS, Krueger KA, Dmitrienko A, Brandt JT. A phase 2 study of the oral factor Xa inhibitor LY517717 for the prevention of venous thromboembolism after hip or knee replacement. *J Thromb Haemost*. 2007;5(4):746-753.
151. Bernard GR, Vincent JL, Laterre P-F, et al; Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study group. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med*. 2001;344(10):699-709.
152. Abraham E, Laterre P-F, Garg R, et al; Administration of Drotrecogin Alfa (Activated) in Early Stage Severe Sepsis (ADDRESS) Study Group. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. *N Engl J Med*. 2005;353(13):1332-1341.
153. Nadel S, Goldstein B, Williams MD, et al; REsearching severe Sepsis and Organ dysfunction in children: a gLobal perspective (RESOLVE) study group. Drotrecogin alfa (activated) in children with severe sepsis: a multicentre phase 3 randomised controlled trial. *Lancet*. 2007;369(9564):836-843.
154. Parkinson JF, Grinnell BW, Moore RE, Hoskins J, Vlahos CJ, Bang NU. Stable expression of a secretable deletion mutant of recombinant human thrombomodulin in mammalian cells. *J Biol Chem*. 1990;265(21):12602-12610.
155. Kearon C, Comp PC, Douketis JD, Royds R, Yamada K, Gent M. Dose-response study of recombinant human soluble thrombomodulin (ART-123) in the prevention of venous thromboembolism after total hip replacement. *J Thromb Haemost*. 2005;3(5):962-968.
156. Jacquemin M, Radcliffe CM, Lavend'homme R, et al. Variable region heavy chain glycosylation determines the anticoagulant activity of a factor VIII antibody. *J Thromb Haemost*. 2006;4(5):1047-1055.

157. Jacquemin M, Stassen J-M, Saint-Remy J-M, et al. A human monoclonal antibody inhibiting partially factor VIII activity reduces thrombus growth in baboons. *J Thromb Haemost.* 2009;7(3):429-437.
158. Verhamme P, Pakola S, Jensen TJ, et al. Tolerability and pharmacokinetics of TB-402 in healthy male volunteers. *Clin Ther.* 2010;32(6):1205-1220.
159. Verhamme P, Tangelder MJD, Verhaeghe R, et al; TB-402 Study Group. Single intravenous administration of TB-402 for the prophylaxis of venous thromboembolism after total knee replacement: a dose-escalating, randomized, controlled trial. *J Thromb Haemost.* 2011;9(4):664-671.
160. Weitz JI, Buller HR. Direct thrombin inhibitors in acute coronary syndromes: present and future. *Circulation.* 2002;105(8):1004-1011.
161. Weitz JI, Crowther M. Direct thrombin inhibitors. *Thromb Res.* 2002;106(3):V275-V284.
162. Avgerinos GC, Turner BG, Gorelick KJ, Papendieck A, Weydemann U, Gellissen G. Production and clinical development of a Hansenula polymorpha-derived PEGylated hirudin. *Semin Thromb Hemost.* 2001;27(4):357-372.
163. Toomey JR, Abboud MA, Valocik RE, et al. A comparison of the beta-D-xyloside, odiparil, to warfarin in a rat model of venous thrombosis. *J Thromb Haemost.* 2006;4(9):1989-1996.
164. Bates SM, Buller HR, Lassen MR et al. A phase 2 double-blind placebo-controlled parallel-group randomized study of extended prophylaxis with odiparil following total hip arthroplasty (THA) [abstract]. *J Thromb Haemost.* 2007; 5(suppl 2):653.
165. Olsson SB, Rasmussen LH, Tveit A, et al. Safety and tolerability of an immediate-release formulation of the oral direct thrombin inhibitor AZD0837 in the prevention of stroke and systemic embolism in patients with atrial fibrillation. *Thromb Haemost.* 2010;103(3):604-612.
166. Lip GY, Rasmussen LH, Olsson SB, et al; Steering Committee. Oral direct thrombin inhibitor AZD0837 for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation: a randomized dose-guiding, safety, and tolerability study of four doses of AZD0837 vs. vitamin K antagonists. *Eur Heart J.* 2009;30(23):2897-2907.
167. Lip GY, Rasmussen LH, Olsson SB, et al; Steering Committee. Oral direct thrombin inhibitor AZD0837 for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation: a phase 2 study of AZD0837 in patients who are appropriate for but unable or unwilling to take vitamin K antagonist therapy. *Thromb Res.* 2011;127(2):91-99.
168. Schützer KM, Svensson MK, Zetterstrand S, Eriksson UG, Wählander K. Reversible elevations of serum creatinine levels but no effect on glomerular filtration during treatment with the direct thrombin inhibitor AZD0837. *Eur J Clin Pharmacol.* 2010;66(9):903-910.
169. Fujii S, Sawa H, Sobel BE. Inhibition of endothelial cell expression of plasminogen activator inhibitor type-1 by gemfibrozil. *Thromb Haemost.* 1993;70(4):642-647.
170. Brown SL, Sobel BE, Fujii S. Attenuation of the synthesis of plasminogen activator inhibitor type 1 by niacin. A potential link between lipid lowering and fibrinolysis. *Circulation.* 1995;92(4):767-772.
171. Kvassman JO, Lawrence DA, Shore JD. The acid stabilization of plasminogen activator inhibitor-1 depends on protonation of a single group that affects loop insertion into beta-sheet A. *J Biol Chem.* 1995;270(46):27942-27947.
172. Eitzman DT, Fay WP, Lawrence DA, et al. Peptide-mediated inactivation of recombinant and platelet plasminogen activator inhibitor-1 in vitro. *J Clin Invest.* 1995;95(5):2416-2420.
173. Friederich PW, Levi M, Biemond BJ, et al. Novel low-molecular-weight inhibitor of PAI-1 (XR5118) promotes endogenous fibrinolysis and reduces postthrombolysis thrombus growth in rabbits. *Circulation.* 1997;96(3):916-921.
174. Ertongur S, Lang S, Mack B, Wosikowski K, Muehlenweg B, Gires O. Inhibition of the invasion capacity of carcinoma cells by WX-UK1, a novel synthetic inhibitor of the urokinase-type plasminogen activator system. *Int J Cancer.* 2004;110(6):815-824.
175. Setyono-Han B, Stürzebecher J, Schmalix WA, et al. Suppression of rat breast cancer metastasis and reduction of primary tumour growth by the small synthetic urokinase inhibitor WX-UK1. *Thromb Haemost.* 2005;93(4):779-786.
176. Meyer JE, Brocks C, Graefe H, et al. The oral serine protease inhibitor WX-671 - first experience in patients with advanced head and neck carcinoma. *Breast Care (Basel).* 2008;3(s2):20-24.
177. Sakharov DV, Plow EF, Rijken DC. On the mechanism of the antifibrinolytic activity of plasma carboxypeptidase B. *J Biol Chem.* 1997;272(22):14477-14482.
178. Redlitz A, Nicolini FA, Malycky JL, Topol EJ, Plow EF. Inducible carboxypeptidase activity. A role in clot lysis in vivo. *Circulation.* 1996;93(7):1328-1330.
179. Klement P, Liao P, Bajzar L. A novel approach to arterial thrombolysis. *Blood.* 1999;94(8):2735-2743.
180. Nagashima M, Werner M, Wang M, et al. An inhibitor of activated thrombin-activatable fibrinolysis inhibitor potentiates tissue-type plasminogen activator-induced thrombolysis in a rabbit jugular vein thrombolysis model. *Thromb Res.* 2000;98(4):333-342.
181. Develter J, Dewilde M, Gils A, Declerck PJ. Comparative study of inhibitory antibody derivatives towards thrombin activatable fibrinolysis inhibitor. *Thromb Haemost.* 2009;102(1):69-75.
182. Buelens K, Hassanzadeh-Ghassabeh G, Muyltermans S, Gils A, Declerck PJ. Generation and characterization of inhibitory nanobodies towards thrombin activatable fibrinolysis inhibitor. *J Thromb Haemost.* 2010;8(6):1302-1312.
183. Guimarães AHC, Barrett-Bergshoeff MM, Criscuoli M, Evangelista S, Rijken DC. Fibrinolytic efficacy of Amediplase, Tenecteplase and scu-PA in different external plasma clot lysis models: sensitivity to the inhibitory action of thrombin activatable fibrinolysis inhibitor (TAFI). *Thromb Haemost.* 2006;96(3):325-330.
184. Islam I, Bryant J, May K, et al. 3-Mercaptopropionic acids as efficacious inhibitors of activated thrombin activatable fibrinolysis inhibitor (TAFIa). *Bioorg Med Chem Lett.* 2007;17(5):1349-1354.
185. Owen DR, Bull DJ, Bunnage ME, Glossop MS, Maguire RJ, Strang RS. Oxygenated analogues of UK-396082 as inhibitors of activated thrombin activatable fibrinolysis inhibitor. *Bioorg Med Chem Lett.* 2010;20(1):92-96.
186. Schneider M, Nesheim ME. Reversible inhibitors of TAFIa can both promote and inhibit fibrinolysis. *J Thromb Haemost.* 2003;1(1):147-154.
187. Walker JB, Hughes B, James I, Haddock P, Kluff C, Bajzar L. Stabilization versus inhibition of TAFIa by competitive inhibitors in vitro. *J Biol Chem.* 2003;278(11):8913-8921.
188. Sanglas L, Arolas JL, Valnickova Z, Aviles FX, Enghild JJ, Gomis-Rüth FX. Insights into the molecular inactivation mechanism of human activated thrombin-activatable fibrinolysis inhibitor. *J Thromb Haemost.* 2010;8(5):1056-1065.

189. Mosesson MW. The roles of fibrinogen and fibrin in hemostasis and thrombosis. *Semin Hematol*. 1992;29(3):177-188.
190. Muszbek L, Adány R, Mikkola H. Novel aspects of blood coagulation factor XIII. I. Structure, distribution, activation, and function. *Crit Rev Clin Lab Sci*. 1996;33(5):357-421.
191. Seale L, Finney S, Sawyer RT, Wallis RB. Tridegin, a novel peptidic inhibitor of factor XIIIa from the leech, *Haementeria ghilianii*, enhances fibrinolysis in vitro. *Thromb Haemost*. 1997;77(5):959-963.
192. Finney S, Seale L, Sawyer RT, Wallis RB. Tridegin, a new peptidic inhibitor of factor XIIIa, from the blood-sucking leech *Haementeria ghilianii*. *Biochem J*. 1997;324(pt 3):797-805.
193. Baskova IP, Nikonov GI. Destabilase, the novel epsilon-(gamma-Glu)-Lys isopeptidase with thrombolytic activity. *Blood Coagul Fibrinolysis*. 1991;2(1):167-172.
194. Zavalova L, Lukyanov S, Baskova I, et al. Genes from the medicinal leech (*Hirudo medicinalis*) coding for unusual enzymes that specifically cleave endo-epsilon (gamma-Glu)-Lys isopeptide bonds and help to dissolve blood clots. *Mol Gen Genet*. 1996;253(1-2):20-25.
195. Deitcher SR, Funk WD, Buchanan J, Liu S, Levy MD, Toombs CF. Altimeprase: a novel recombinant direct-acting fibrinolytic. *Expert Opin Biol Ther*. 2006;6(12):1361-1369.
196. Toombs CF. Altimeprase: pharmacology of a novel fibrinolytic metalloproteinase for thrombolysis. *Haemostasis*. 2001;31(3-6):141-147.
197. Moll S, Kenyon P, Bertoli L, De Maio J, Homesley H, Deitcher SR. Phase 2 trial of altimeprase, a novel-acting fibrin degradation agent, for occluded central venous access devices. *J Clin Oncol*. 2006;24(19):3056-3060.
198. Comer MB, Cackett KS, Gladwell S, Wood LM, Dawson KM. Thrombolytic activity of BB-10153, a thrombin-activatable plasminogen. *J Thromb Haemost*. 2005;3(1):146-153.
199. Curtis LD, Brown A, Comer MB, Senior JM, Warrington S, Dawson KM. Pharmacokinetics and pharmacodynamics of BB-10153, a thrombin-activatable plasminogen, in healthy volunteers. *J Thromb Haemost*. 2005;3(6):1180-1186.
200. Gibson CM, Zorkun C, Molhoek P, et al. Dose escalation trial of the efficacy, safety, and pharmacokinetics of a novel fibrinolytic agent, BB-10153, in patients with ST elevation MI: results of the TIMI 31 trial. *J Thromb Thrombolysis*. 2006;22(1):13-21.
201. National Institutes of Health Clinical Center. A safety and dose finding study of plasmin (human) administered into the middle cerebral artery of stroke patients. NCT01014975. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2009.
202. National Institutes of Health Clinical Center. A dose escalation and safety study of plasmin (human) in acute lower extremity native artery or bypass graft occlusion (PRIORITY). NCT00418483. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2007.
203. National Institutes of Health Clinical Center. A study of intra-thrombus plasmin (human) in acute peripheral arterial occlusion. NCT01222117. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2010.
204. Stewart RJ, Fredenburgh JC, Weitz JI. Characterization of the interactions of plasminogen and tissue and vampire bat plasminogen activators with fibrinogen, fibrin, and the complex of D-dimer noncovalently linked to fragment E. *J Biol Chem*. 1998;273(29):18292-18299.
205. Mellott MJ, Ramjit DR, Stabilito II, et al. Vampire bat salivary plasminogen activator evokes minimal bleeding relative to tissue-type plasminogen activator as assessed by a rabbit cuticle bleeding time model. *Thromb Haemost*. 1995;73(3):478-483.
206. Hacke W, Albers GW, Al-Rawi Y, et al; DIAS Study Group. The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a phase 2 MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. *Stroke*. 2005;36(1):66-73.
207. Furlan AJ, Eyding D, Albers GW, et al; DEDAS Investigators. Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS): evidence of safety and efficacy 3 to 9 hours after stroke onset. *Stroke*. 2006;37(5):1227-1231.
208. Hacke W, Furlan AJ, Al-Rawi Y, et al. Intravenous desmoteplase in patients with acute ischaemic stroke selected by MRI perfusion-diffusion weighted imaging or perfusion CT (DIAS-2): a prospective, randomised, double-blind, placebo-controlled study. *Lancet Neurol*. 2009;8(2):141-150.
209. National Institutes of Health Clinical Center. Clinical study of desmoteplase in Japanese patients with acute ischemic stroke. NCT01104467. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2010.
210. National Institutes of Health Clinical Center. Efficacy and safety study of desmoteplase to treat acute ischemic stroke (DIAS-4). NCT00856661. ClinicalTrials.gov. Bethesda, MD: National Institutes of Health; 2009.