CASE HISTORY

The discovery and development of rivaroxaban, an oral, direct factor Xa inhibitor

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Abstract | The activated serine protease factor Xa is a promising target for new anticoagulants. After studies on naturally occurring factor Xa inhibitors indicated that such agents could be effective and safe, research focused on small-molecule direct inhibitors of factor Xa that might address the major clinical need for improved oral anticoagulants. In 2008, rivaroxaban (Xarelto; Bayer HealthCare) became the first such compound to be approved for clinical use. This article presents the history of rivaroxaban's development, from the structure–activity relationship studies that led to its discovery to the preclinical and clinical studies, and also provides a brief overview of other oral anticoagulants in advanced clinical development.

Thromboembolic disorders
A group of conditions characterized by an increased incidence of thrombi in the vasculature, such as deep-vein thrombosis, pulmonary embolism, systemic embolism or coronary and cerebral ischaemia.

Unfractionated heparin
(UFH). An anticoagulant administered intravenously or subcutaneously. It binds to antithrombin, greatly increasing its activity and resulting in the inhibition of factors Xa, IXa, XIa, XIIa and thrombin (factor IIa).

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Anticoagulants are used for the prevention and treatment of venous and arterial thromboembolic disorders. Many approaches have been explored in the development of antithrombotic drugs that inhibit enzymes in the coagulation pathways. However, most currently approved drugs for the prevention and treatment of thromboembolic disorders have been on the market for a long time, in some cases for decades. Unfractionated heparin (UFH), which was discovered in 1916 (REF. 1) targets multiple factors in the coagulation cascade[2], but has a number of limitations, including a parenteral route of administration, frequent laboratory monitoring of coagulation activity and the risk for patients of developing potentially life-threatening heparin-induced thrombocytopaenia[2,3]. Low-molecular-weight heparins (LMWHs), which were developed in the 1980s, promote the inactivation of both thrombin (factor IIa) and, to a greater extent, factor Xa[2].

LMWHs have largely replaced UFH owing to their lower risk of causing bleeding, lower levels of binding to plasma proteins and endothelium, good bioavailability, longer half-life and superior pharmacokinetic properties compared with UFH[2]. However, their use remains limited because of the need for parenteral administration[2], which can be inconvenient, especially in an outpatient setting, with patients needing to be trained to self-inject after discharge, and nurse visits required for those unable to do so[4]. Both UFH and LMWHs are indirect inhibitors of coagulation, and their activity is mediated by plasma cofactors, principally antithrombin and, to a lesser extent for UFH, heparin cofactor II (REF. 2).

Warfarin, the prototype vitamin K antagonist (VKA), was originally discovered in 1941 (REF. 5). Until recently, the VKAs were the only available oral anticoagulants, as well as the most frequently prescribed. However, VKAs have numerous well-documented drawbacks, including unpredictable pharmacokinetics and pharmacodynamics, a slow onset and offset of action, a narrow therapeutic window, multiple food–drug and drug–drug interactions[6], and considerable inter-individual and intra-individual variability in dose response. In addition, regular coagulation monitoring and dose adjustment are required to keep patients within the target international normalized ratio (INR) range, usually 2.0–3.0, which can be costly[6]. Furthermore, establishing the optimal dose of warfarin is complicated by variations in warfarin sensitivity due to common genetic polymorphisms, particularly in *CYP2C9* and *VKORC1* (REF. 7). Attempts have been made to use pharmacogenetics to estimate dose, although the clinical value of such assessments is debatable[8]. Nonetheless, LMWHs and VKAs are still the basis of contemporary thromboprophylaxis and treatment. However, the difficulties and shortcomings that surround the practicalities and clinical management of these established anticoagulants — particularly parenteral administration, the need for monitoring and the lack of predictable response — has spurred the development of new agents that are less burdensome for the patient and health-care system, and address both patients' and physicians' unmet needs. TABLE 1 contrasts the characteristics of the VKAs with those of a

Table 1 | **Comparison of an ideal anticoagulant and a vitamin K antagonist**

Property	Ideal anticoagulant	Vitamin K antagonist
Administration	Oral	Oral
Onset/offset of action	Rapid (several hours)	Slow (several days)
Therapeutic window	Wide	Narrow
Variability in dose response	Little or no inter-individual or intra-individual variability	Considerable inter-individual and intra-individual variability
Interactions	Little or no interaction with food or other drugs	Multiple interactions with food and other drugs
PK/PD	Predictable	Unpredictable and variable
Coagulation monitoring	No routine monitoring required	Regular monitoring required
Dose adjustment	None required	Required
Efficacy	Highly effective in reducing thromboembolic events	Effective in reducing thromboembolic events when properly controlled
Safety profile	Good, especially with regard to bleeding	Difficulties in maintaining patients within the target therapeutic range (INR 2.0–3.0); contributes to an increased risk of bleeding

INR, international normalized ratio; PK/PD, pharmacokinetics/pharmacodynamics.

hypothetical 'ideal' anticoagulant, and FIG. 1 chronicles the historical development of anticoagulants.

Despite the accumulated knowledge on the coagulation system (FIG. 2), its complexity has presented numerous obstacles to the discovery and development of potent anticoagulants that are both effective and safe. In recent years, research has focused on new classes of anticoagulants that target a specific coagulation enzyme or step in the coagulation cascade[9,10], including inhibitors of the factor VIIa–tissue factor complex[10], inhibitors of factor IXa[11,12] and factor XIa[13,14], direct thrombin inhibitors[15,16], synthetic indirect and direct inhibitors of factor Xa (activated factor X)[17–20], and recombinant soluble thrombomodulin[21,22]. In addition, recombinant activated protein C (APC) mitigates the procoagulant state associated with sepsis[23,24].

In the search for new anticoagulant drugs, the activated serine protease factor Xa is a particularly promising target and has attracted great interest in recent years[18,25–27]. This article describes the discovery and development of the first oral, direct factor Xa inhibitor to be approved for clinical use — rivaroxaban (Xarelto; Bayer HealthCare). Rivaroxaban was approved by the European authorities in 2008 for the prevention of venous thromboembolism (VTE; comprising deep-vein thrombosis (DVT) and pulmonary embolism) after elective hip or knee replacement. The article then briefly considers the future clinical potential of rivaroxaban and other factor Xa inhibitors currently in advanced clinical development.

Factor Xa: function and biology

Factor X has long been known to have a key role in haemostasis[28] and factor Xa plays a central part in the blood coagulation pathway by catalysing the production of thrombin, which leads to clot formation and wound closure[20] (FIG. 2). Conversely, deficiency of factor Xa may disturb haemostasis. In the very rare factor X deficiency disorder (for which 1 in 500,000 is homozygous and 1 in 500 heterozygous), very low plasma and activity levels of factor Xa manifest as severe bleeding tendencies[29–31]. Studies of variants of factor X deficiency indicate that factor X plasma activity levels must be as low as 6–10% of the normal range (approximately 50–150% of the population average) to be considered a mild deficiency; cases with factor X activity levels below 1% are considered to be severe[29,32]. Thus it seems that factor X activity can be markedly suppressed without affecting haemostasis. An ideal anticoagulant would prevent thrombosis without inducing systemic hypocoagulation, and would thereby avoid unintended bleeding complications. Therefore, a factor Xa inhibitor could potentially have the properties of a desirable anticoagulant.

Validating factor Xa as a drug target

The first factor Xa inhibitors. Although factor Xa was identified as a promising target for the development of new anticoagulants in the early 1980s, the viability of factor Xa inhibition was not tested before the end of that decade. In 1987, the first factor Xa inhibitor, the naturally occurring compound antistasin, was isolated from the salivary glands of the Mexican leech *Haementeria officinalis*[33,34]. Antistasin is a 119 amino-acid polypeptide; kinetic studies revealed that it is a slow, tight-binding, potent factor Xa inhibitor (inhibition constant (K_i) of 0.3–0.6 nM) that also inhibits trypsin (half maximal inhibitory concentration (IC_{50}) is 5 nM in the presence of 1 nM trypsin)[35]. Another naturally occurring factor Xa inhibitor, the tick anticoagulant peptide (TAP), a single-chain, 60 amino-acid peptide, was isolated in 1990 from extracts of the soft tick *Ornithodoros moubata*[36]. Similarly to antistasin, TAP is a slow, tight-binding inhibitor of factor Xa (K_i of ~0.6 nM).

TAP[37] and recombinant forms of antistasin[38] and TAP[38–40] were used to validate factor Xa as a viable drug target and to improve understanding of the role of factor Xa in thrombosis. The antithrombotic effects of these compounds were compared with those of direct thrombin inhibitors and of indirect thrombin and factor Xa inhibitors (that is, UFH) in animal models of thrombosis. These studies suggested that direct factor Xa inhibitors might be a more effective approach to anticoagulation[37,39], and might also offer a wider therapeutic window, particularly with regard to primary haemostasis[40,41].

In vitro *and* in vivo *studies.* Clot-bound factor Xa was shown to be enzymatically active *in vitro* and able to activate prothrombin to thrombin[42]. In addition, factor Xa was found to be an important contributor to clot-associated procoagulant activity *in vitro*[43]. Clot-bound factor Xa activity was resistant to inhibition by antithrombin[42], suggesting that the ability to directly inhibit clot-associated factor Xa, with no requirement for a cofactor, could provide an effective and highly localized approach to the prevention of thrombus growth. The clot-associated

Heparin-induced thrombocytopaenia
The process by which antibodies against the complex of heparin and platelet factor 4 activate platelets, resulting in a decrease in platelet numbers of more than 50%.

Low-molecular-weight heparins
(LMWHs). A class of anticoagulants derived from unfractionated heparin by chemical or enzymatic degradation. They induce a conformational change in antithrombin that greatly increases its anticoagulant activity.

Thrombin
Thrombin (also known as factor IIa) is the terminal enzyme of the coagulation cascade and converts fibrinogen into fibrin, which forms clot fibres. Thrombin also activates several other coagulation factors, as well as protein C.

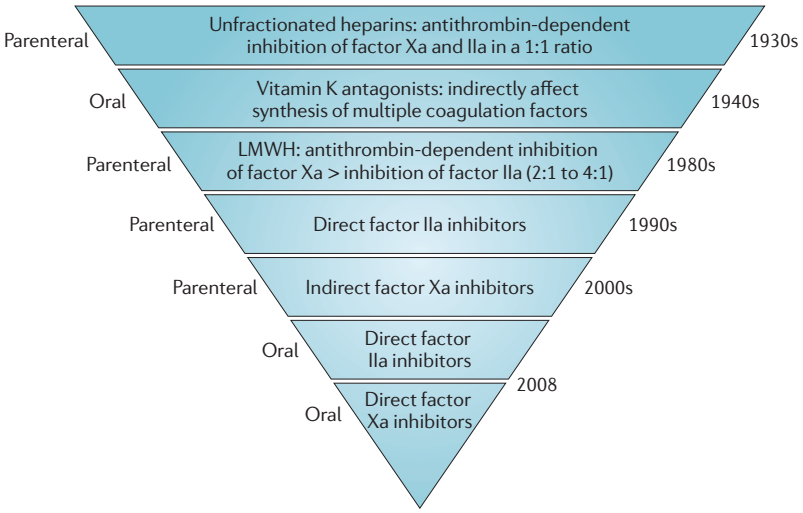


Figure 1 | **Development of anticoagulants over the past century.** The timeframe starts with the discovery of the heparins and the vitamin K antagonists, followed by the low-molecular-weight heparins. These were followed, in turn, by the discovery of the parenteral direct thrombin inhibitors, the development of the indirect factor Xa inhibitor, fondaparinux, and the work of the current decade that has resulted in oral direct inhibitors of both thrombin (factor IIa) and factor Xa. The inverted triangle reflects the narrowing of action and increasing specificity of the anticoagulants. LMWH, low-molecular-weight heparin.

generation of fibrinopeptide A (a byproduct of fibrinogen cleavage by thrombin) was inhibited to the same extent by both recombinant TAP and hirudin, a direct thrombin inhibitor. This observation suggested that procoagulant activity in the clot was due to *de novo* activation of prothrombin to thrombin, rather than to the activity of pre-existing thrombin[43]. It was subsequently shown that inhibition of factor Xa by recombinant TAP provided sustained *in vitro*[44] and *in vivo*[45] inhibition of clot-associated procoagulant activity, which may, in turn, protect against ongoing coagulation after cessation of anticoagulant treatment.

Overall, these data suggested that direct factor Xa inhibitors might not be linked to the phenomenon of 'rebound' thrombosis that was associated with the direct and indirect thrombin inhibitors previously under investigation[46,47]. Rebound thrombosis can be thought of as a transient increase in thromboembolic events occurring shortly after the withdrawal of an antithrombotic medication[46,48]. Furthermore, low concentrations of a direct thrombin inhibitor may partly suppress the negative feedback on coagulation by APC, in contrast to factor Xa inhibition, which does not seem to measurably affect the thrombin–thrombomodulin–APC system[49]. However, whether these experimental observations have any clinical relevance remains to be determined.

The first synthetic factor Xa inhibitors

Although antistasin and TAP provided support for the concept of factor Xa inhibition, development of these compounds was discontinued. The reasons were never disclosed. Nonetheless, the encouraging results from studies using recombinant versions of the natural factor Xa inhibitors prompted several pharmaceutical companies to initiate chemistry programmes to develop selective, small-molecule, direct inhibitors of factor Xa, such as DX-9065a[50] and YM-60828 (REFS 51,52) (FIG. 3). Both these compounds contain a highly basic amidine residue, designed as mimics for the arginine of the natural substrate prothrombin.

DX-9065a, a widely studied non-peptidic small molecule, shows rapid, direct and reversible binding kinetics for factor Xa (K_i of 41 nM)[53]. At physiological pH it is a zwitterion with high water solubility and low lipophilicity[54]. However, human oral bioavailability was only 2–3%[54]. A small Phase II study was conducted in patients with non-ST-elevation acute coronary syndrome (ACS) who were randomized to low- or high-dose intravenous DX-9065a or UFH[55]. A non-significant trend was observed towards a reduction in ischaemic events and bleeding for DX-9065a compared with UFH[55].

Because of the success of indirect dual factor Xa and thrombin inhibitors, such as LMWHs, indirect inhibitors of factor Xa with greater selectivity, such as fondaparinux (Arixtra; GlaxoSmithKline)[56,57], were developed in parallel with direct factor Xa inhibitors.

Both UFH and LMWHs contain a unique pentasaccharide sequence that mediates binding to antithrombin. Binding induces a conformational change in antithrombin that potentiates its ability to inhibit coagulation factors. Inhibition of thrombin occurs through heparin chains of sufficient length to bridge antithrombin to thrombin in a ternary complex, after which antithrombin binds covalently to thrombin and the heparin chain dissociates[2]. However, such bridging is not necessary for antithrombin to be able to inhibit factor Xa. Most LMWH chains are too short to catalyse thrombin inhibition, but can nonetheless promote the inhibition of factor Xa. Thus, UFH has an anti-Xa to anti-IIa ratio of 1:1, LMWHs have anti-Xa to anti-IIa ratios from 2:1 to 4:1, depending on the molecular weight distribution of the preparation[2].

Fondaparinux is an analogue of the pentasaccharide sequence required to promote the binding of antithrombin to factor Xa[2]. The pentasaccharide structure is too short to enable bridging between antithrombin and thrombin. As a result, fondaparinux exclusively potentiates the anti-factor Xa activity of antithrombin and has no effect on thrombin[2]. The efficacy and safety of fondaparinux for the prevention of VTE after major orthopaedic surgery were investigated in four randomized, Phase III trials in patients undergoing surgery for hip fracture[58], hip replacement[59,60] and knee replacement[61]. A meta-analysis of these trials showed the superior efficacy of fondaparinux over the LMWH enoxaparin (Lovenox/Clexane; Sanofi–Aventis) in reducing the incidence of VTE. However, major bleeding occurred more frequently in the fondaparinux-treated group (P=0.008), although the incidence of clinically relevant bleeding (bleeding leading to death, reoperation or in a critical organ) did not differ between the treatment groups[62]. Fondaparinux provided the proof of principle that selective inhibition of factor Xa could provide clinically effective anticoagulation.

Antithrombin
An endogenous glycoprotein that binds covalently to thrombin and other coagulation factors, resulting in their inhibition. Antithrombin functions as a natural anticoagulant, and its inhibitory action is accelerated by heparin.

Warfarin
A vitamin K antagonist that is currently the most commonly used oral anticoagulant.

Vitamin K antagonist
A class of compounds that inhibit the vitamin K-dependent carboxylation of specific coagulation factors, resulting in decreased levels of the affected coagulation factors, leading to anticoagulation.

Therapeutic window
The interval between the lowest dose of a drug that is sufficient for clinical effectiveness and a higher dose at which adverse events or toxicity become unacceptable.

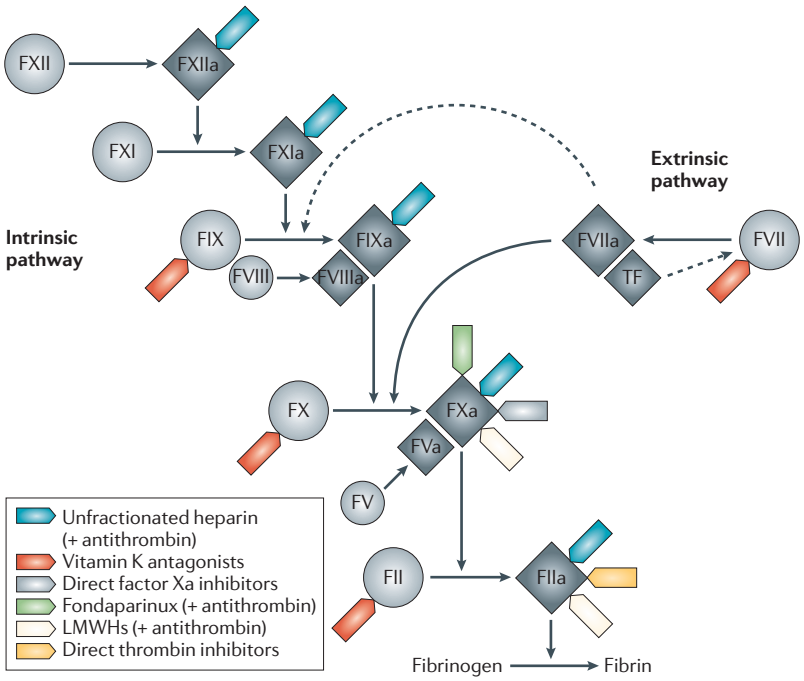


Figure 2 | **Simplified schematic for the blood coagulation cascade.** The figure identifies the target points of various anticoagulants and illustrates that factor X (FX) can be activated through either the intrinsic or the extrinsic pathway. The factor VIIa–tissue factor (TF) complex (extrinsic tenase) activates both factor IX and factor X, as well as factor VII itself (dashed arrow)[150,151]. Initiation of either pathway activates the inactive precursor, factor X, to factor Xa. This makes factor Xa a desirable intervention point for novel anticoagulants, because it occupies a central role in the blood-coagulation pathway[20]. Furthermore, in addition to initiation, both the intrinsic and extrinsic pathways lead to the propagation and amplification of coagulation through the activation of factor X. During the initiation phase of coagulation, the factor Xa produced generates some thrombin (factor IIa). This initial thrombin activates factor XI, and factors V and VIII, to factor XIa and the activated cofactors, factor Va and VIIIa, respectively. It also activates platelets (not shown), which are required for the formation of the intrinsic tenase (factor VIIIa–factor IXa) and the prothrombinase (factor Va–factor Xa) complexes. The prothrombinase complex, on the platelet surface, is substantially more efficient than free factor Xa at activating prothrombin to thrombin; the rate of thrombin formation is increased by approximately 300,000-fold over the rate with factor Xa alone[152]. Thrombin is the principal enzyme involved in the formation, growth and stabilization of thrombi. Thrombin mediates the conversion of fibrinogen to fibrin, the activation of factor XIII (which crosslinks and stabilizes fibrin), the activation of platelets and the above-mentioned feedback-activation of upstream coagulation factors, factor V, factor VIII and factor XI[151,153], resulting in the amplification of its own formation[150,154]. Because one molecule of factor Xa catalyses the formation of approximately 1,000 thrombin molecules[25,154], this amplification can be substantial. Compared with thrombin, factor Xa is thought to have fewer effects outside coagulation and, together with factor Va (as the prothrombinase complex), acts mainly to convert prothrombin to thrombin. Specific inhibition of factor Xa does not affect pre-existing thrombin but does inhibit thrombin generation[155]. Conversely, although thrombin generation is delayed in the presence of a thrombin inhibitor, the amount of thrombin generated is only reduced at higher inhibitor concentrations, albeit in a concentration-dependent manner[156]. LMWH, low-molecular-weight heparin.

Numerous investigations, performed with both direct and indirect factor Xa inhibitors, showed that inhibition of factor Xa produces antithrombotic effects by decreasing the generation of thrombin, thus diminishing thrombin-mediated activation of both coagulation and platelets without affecting the activity of existing thrombin. However, the residual thrombin generated seems to be sufficient to ensure normal systemic haemostasis, possibly because thrombin has a very high affinity for platelet receptors and minimal amounts of thrombin can provide sufficient platelet activation, thus contributing to a favourable efficacy/safety ratio[20]. On the basis of these findings, in the mid-1990s, it seemed that small-molecule, direct factor Xa inhibitors could potentially provide an advantage over the antithrombotic therapies available at that time. Several new factor Xa inhibitors are now in clinical development. These compounds, which include rivaroxaban and the other direct factor Xa inhibitors discussed later, represent new chemical entities with similar binding modes at the active site of factor Xa.

The discovery of rivaroxaban

From the first screening hit to the oxazolidinone lead. When we initiated the factor Xa programme at Bayer HealthCare in 1998, no orally active factor Xa inhibitors with sufficient antithrombotic activity were known. All known potent inhibitors that had previously been investigated contained an amidine group or other highly basic residues, which were designed to act as mimics for an arginine present in the natural substrate, prothrombin. For a long time, these mimics were thought to be a prerequisite for high binding affinity[63]. However, we found that strongly basic mimics contribute to poor oral absorption, an observation also later published by others[64,65].

High-throughput screening of approximately 200,000 compounds revealed several hits that selectively inhibited the cleavage of a chromogenic substrate by human factor Xa[63]. The most potent of these hits was on a minor impurity in a combinatorial library — a phosphonium salt with an IC_{50} of 70 nM (compound **1** in FIG. 4). We proposed that this positively charged phosphonium moiety might serve as an arginine mimic and could be interchangeable with an amidine group. This resulted in the synthesis of lead compound **2** with similar potency (IC_{50} of 120 nM)[63]. Further optimization resulted in the synthesis of compounds of the isoindolinone class, among which imidazoline **3** (FIG. 4) was the most potent (IC_{50} of 2 nM).

To achieve oral bioavailability, we explored less basic or non-basic amidine replacements. Aminopyridines, such as compound **4** (IC_{50} of 8 nM; FIG. 4), showed IC_{50} values in the low nanomolar range; however, although they were less basic, oral absorption remained insufficient. The less potent pyridylpiperazine derivative **5** (IC_{50} of 48 nM), meanwhile, revealed a significantly improved oral bioavailability of 38% in rats. Although we had demonstrated that improved oral bioavailability could be achieved using less basic amidine replacements, we were not able to meet our target of identifying factor Xa inhibitors with both high potency and sufficient oral bioavailability in the isoindolinone class of compounds. However, from these studies we did learn that broad variations in the benzylamidine part were permissible, but found that there was a very steep structure–activity relationship (SAR) for the chlorothiophene carboxamide moiety, which was already present in the lead structure.

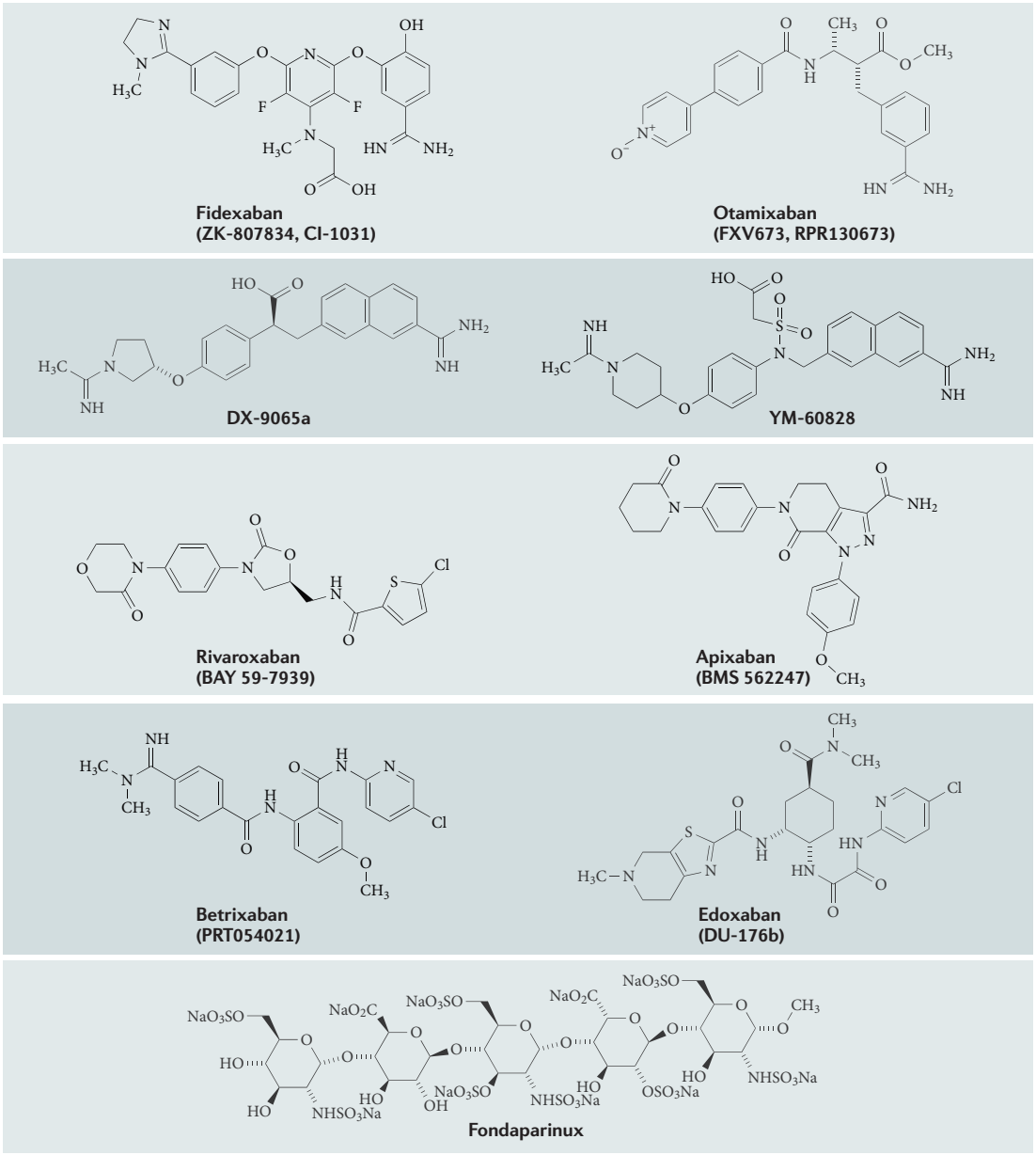


Figure 3 | **Structures of various factor Xa inhibitors.** Fidexaban, otamixaban, DX-9065a, YM-60828, rivaroxaban (Xarelto; Bayer HealthCare), apixaban (Pfizer/Bristol-Myers Squibb), betrixaban and edoxaban are shown. The structure of YM150 has not been published. Not all of these compounds are still in clinical development (for example, fidexaban and DX-9065a) but study of their structures contributed to our understanding of the structure–activity relationships and pharmacology of factor Xa inhibitors. Key features to note are the highly basic arginine mimetic amidine groups as P1 moieties in fidexaban, otamixaban, DX-9065a and YM-60828, which contribute to poor oral bioavailability. These can be contrasted with the non-basic P1 moieties: the chlorothiophene moiety in rivaroxaban, the methoxyaryl group in apixaban and the chloro-substituted pyridine rings in edoxaban and betrixaban, all of which allow improved oral bioavailability. Other synthetic factor Xa inhibitors have also been developed[26]. The structure of the synthetic indirect factor Xa inhibitor, fondaparinux (Arixtra; GlaxoSmithKline), is also shown. This is an analogue of the heparin pentasaccharide sequence required to mediate the conformational change in antithrombin and subsequent binding to factor Xa[2].

International normalized ratio
(INR). Because prothrombin time-test results vary according to the activity of the thromboplastin used, the INR conversion is used to normalize results for any thromboplastin preparation. It is valid only with vitamin K antagonists.

Thromboprophylaxis
A measure taken to prevent the development of a thrombus. It can be pharmaceutical or mechanical.

Tissue factor
A cell-membrane-bound receptor protein that is exposed to the circulating blood during vessel injury. Pre-existing factor VIIa in the blood binds to tissue factor, initiating the coagulation cascade.

Direct thrombin inhibitors
A class of anticoagulants that bind directly to thrombin and block the interaction with its substrate, fibrinogen, thereby inhibiting the generation of fibrin and clot formation.

Thrombomodulin
A membrane-bound thrombin receptor that, when bound to thrombin, functions as a cofactor in the thrombin-induced activation of protein C.

Protein C
The inactive precursor of activated protein C (APC). APC, with its cofactor protein S, inactivates factor Va and factor VIIIa, thus providing an important anticoagulant feedback function.

Factor Xa inhibitor
A class of anticoagulants that inhibit factor Xa in the coagulation cascade, either by binding directly, or indirectly through antithrombin. Inhibition of factor Xa reduces the production of thrombin.

Venous thromboembolism
(VTE). A condition in which a blood clot (thrombus) that has formed in the venous system breaks free (becoming an embolus) and migrates through the circulation to lodge in and block another blood vessel.

The failure to find a compound with sufficient potency and bioavailability could have ended the project. However, we decided instead to re-evaluate the weaker screening hits. The oxazolidinone (compound **6**; FIG. 4) was a weak factor Xa inhibitor, with an IC_{50} of 20,000 nM. Considering the importance of the chlorothiophene residue in the class of compounds studied previously, we replaced the thiophene moiety of compound **6** with a 5-chlorothiophene group, thereby creating lead compound **7** (FIG. 4). This had a >200-fold higher potency (IC_{50} of 90 nM) and did not include any basic group[63].

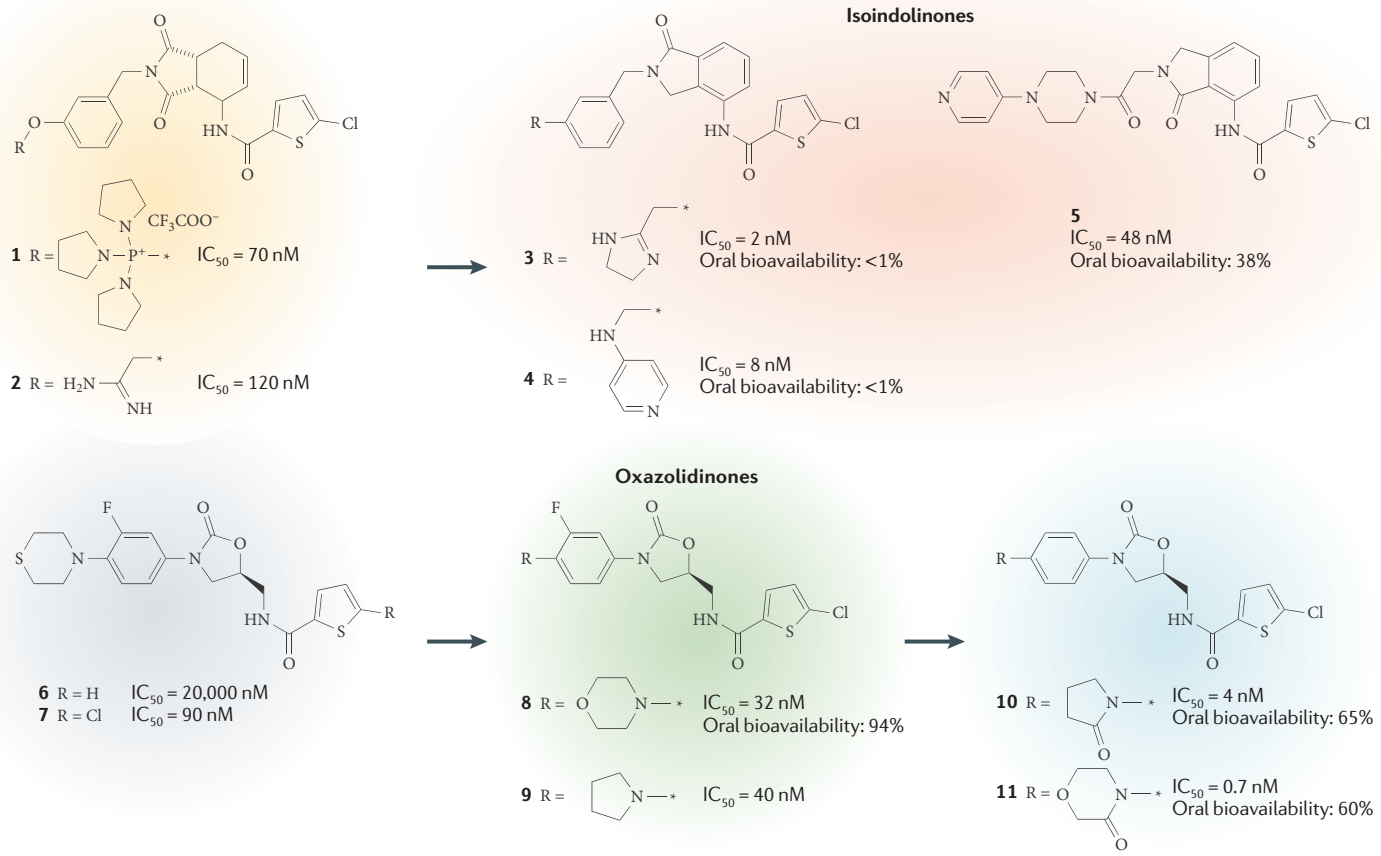


Figure 4 | **Optimization of oxazolidinone factor Xa inhibitors.** Optimization of oxazolidinone factor Xa inhibitors resulted in the discovery of rivaroxaban (compound **11**)[63]. Half-maximal inhibitory concentration (IC_{50}) values are for the inhibition of factor Xa activity; oral bioavailability is shown for rats.

On the basis of this promising lead, a medicinal chemistry programme was followed that focused on further improving the potency of the oxazolidinone class without compromising its pharmacokinetic profile.

The optimization programme leading to rivaroxaban. The starting point of these investigations was the thiomorpholine group; morpholine and pyrrolidine derivatives (compounds **8** and **9**; FIG. 4) showed some improvement in potency (IC_{50} values of 32 nM and 40 nM, respectively). Although not sufficiently potent, compound **8** was the first compound to show a favourable pharmacokinetic profile, with a high oral bioavailability of 94% in rats. An ortho-substitution led to the pyrrolidinone derivative **10**, with significantly improved potency (IC_{50} of 4 nM) and an oral bioavailability of 65%. However, the *in vivo* antithrombotic potency of this compound, evaluated in an arteriovenous shunt model in anaesthetized rats, was estimated to be too low[63]. Our knowledge of the SAR then led us to design the morpholinone residue, resulting in compound **11** (rivaroxaban; FIG. 4), for which binding to factor Xa depends on the (*S*)-configuration at the oxazolidinone core. Rivaroxaban showed increased potency *in vitro* (IC_{50} of 0.7 nM) and *in vivo* after oral administration in rats[63]. This compound also showed favourable oral bioavailability (60% in rats and 60–86% in dogs)[63].

The optimization programme was continued in order to gain a more in-depth understanding of the SAR. However, further variations evaluated at this time, such as substitution of the aryl ring or less lipophilic replacements for the chlorothiophene carboxamide moiety, did not result in further improvements[63], and the morpholinone derivative **11** (rivaroxaban) remained the most attractive candidate. The X-ray crystal structure of rivaroxaban in complex with human factor Xa[63] (BOX 1) helped us to understand its binding mode and to explain the steep SAR observed earlier. A similar binding mode has been reported for several other factor Xa inhibitors[66–70] (BOX 1).

With its combination of high binding affinity and good oral bioavailability, rivaroxaban was identified as the drug candidate for further development.

Preclinical studies

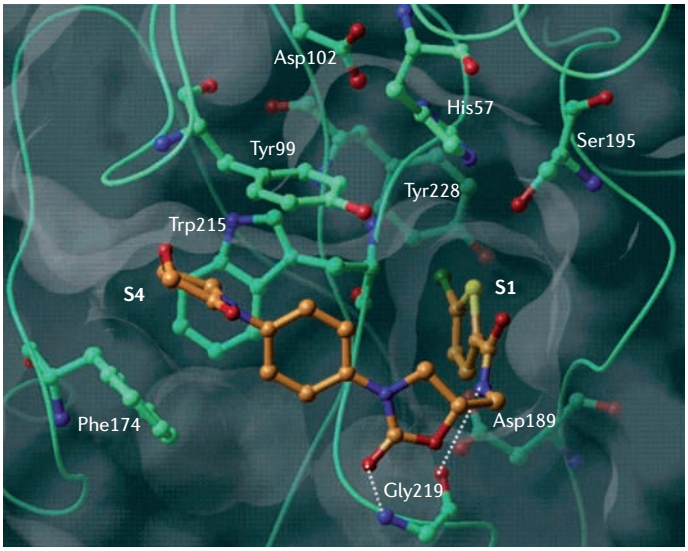
Rivaroxaban is a direct, specific factor Xa inhibitor that, unlike indirect agents such as fondaparinux, does not require a cofactor[71]. *In vitro* kinetic studies showed that the inhibition of human factor Xa by rivaroxaban was competitive (K_i of 0.4 nM), with >10,000-fold greater selectivity for factor Xa than for other serine proteases[71]. Rivaroxaban inhibits prothrombinase (IC_{50} of 2.1 nM)[71] and initial data suggest that it also inhibits clot-bound factor Xa activity (IC_{50} of 75 nM)[72]. In human plasma,

Deep-vein thrombosis
(DVT). A blood clot in a deep vein, usually in the leg. Distal DVT occurs in the calf, whereas proximal DVT occurs above the knee.

Pulmonary embolism
A blood clot or thromboembolus in a pulmonary blood vessel. Such emboli generally originate from a deep-vein thrombosis and can cause permanent lung damage, chronic pulmonary hypertension and death.

Haemostasis
The complex process that leads to the formation of a blood clot, causing bleeding to stop.

Box 1 | **Binding mode of rivaroxaban**



The figure shows the X-ray crystal structure of rivaroxaban (carbons coloured orange) in complex with human factor Xa[63]. Essential amino acids and binding pockets (S1 and S4) are indicated; hydrogen bonds are shown as dotted lines.

Two hydrogen bonds are formed between rivaroxaban and the amino acid Gly219 of factor Xa. The first, a strong interaction (2.0 Å), is from the carbonyl oxygen of the oxazolidinone core. The second, weaker interaction (3.3 Å), is from the amino group of the chlorothiophene carboxamide moiety. These two hydrogen bonds support the oxazolidinone core in directing its substituents into the S1 and the S4 subsites of factor Xa. This results in rivaroxaban forming an L-shape, a binding mode typical of synthetic, direct factor Xa inhibitors[63].

The aromatic rings of Tyr99, Phe174 and Trp215 in factor Xa define a narrow hydrophobic channel that comprises the S4 pocket. The morpholinone moiety of rivaroxaban is 'sandwiched' between Tyr99 and Phe174, while its aryl ring is oriented perpendicularly, extending across the face of Trp215. There is no direct interaction between the morpholinone carbonyl group and the factor Xa backbone; rather, this carbonyl group contributes to a planarization of the morpholinone ring, further supporting the sandwich-like arrangement[63]. Using the morpholinone moiety, as well as other six-membered rings such as lactames or pyridinones, we found new, non-basic P4 residues (see REF. 146 for patent application), yielding strongly increased binding to factor Xa. Such residues have since been used successfully for several other factor Xa inhibitors[69,147], including apixaban[148] and PD0348292 (eribaxaban)[149].

In the S1 pocket of factor Xa, the key interaction is between the chlorine substituent of the thiophene moiety and the aromatic ring of Tyr228 at the bottom of the S1 pocket. This novel interaction obviates the need for strongly basic groups, such as amidines, to achieve high factor Xa affinity, and therefore enables non-basic rivaroxaban to achieve both high potency and good oral bioavailability[63]. A similar binding mode has been found and reported for several other factor Xa inhibitors[66–70].

Figure modified, with permission, from REF. 63 © (2005) American Chemical Society.

Fibrinogen
A soluble plasma protein that, in the final phase of the coagulation process, is converted to fibrin by thrombin. Fibrin then polymerizes and forms the fibrous network base of a clot.

Oral bioavailability
The total proportion of pharmacologically active drug that enters the systemic circulation after oral administration. It is affected by both absorption and local metabolic inactivation.

Prothrombin time
A laboratory test that measures clotting time in the presence of tissue factor (thromboplastin). It is used to assess the activity of the extrinsic coagulation pathway.

Activated partial thromboplastin time
A laboratory test that measures the clotting time of plasma after contact activation. It assesses the function of the intrinsic coagulation pathway.

rivaroxaban inhibited thrombin generation — and therefore the amplification processes of coagulation — through the inhibition of factor Xa generated by either the intrinsic or the extrinsic coagulation pathways[73,74]. Thrombin generation was almost completely inhibited in platelet-rich plasma at physiologically relevant concentrations (80–100 nM) of rivaroxaban[73]. This and other studies demonstrated that rivaroxaban prolonged the initiation phase of thrombin generation, potently inhibited the physiologically relevant prothrombinase complex-bound factor Xa on the surface of activated platelets[71] and reduced the thrombin burst produced in the propagation phase[73].

In addition, preliminary work has shown that rivaroxaban inhibits thrombin generation in the presence and absence of thrombomodulin in human plasma in a concentration-dependent manner. This suggests that it does not interfere with the thrombin–thrombomodulin–APC system and, therefore, probably does not suppress APC-mediated negative feedback[75], as was shown for DX-9065a[49]. Further preliminary data suggested that rivaroxaban did not directly affect platelet aggregation in platelet-rich plasma induced by thrombin, adenosine diphosphate or collagen[76], but potently inhibited tissue-factor-induced platelet aggregation in an indirect manner, by inhibiting thrombin generation[77].

Rivaroxaban demonstrated anticoagulant effects in human plasma, with the prothrombin time being more sensitive than the activated partial thromboplastin time[71], a finding also observed with other direct factor Xa inhibitors in clinical development[78,79]. This may be because direct factor Xa inhibitors, including rivaroxaban[71], are highly effective inhibitors of the prothrombinase complex, although differences in enzyme kinetics may also be responsible[20]. A dose-dependent prolongation of prothrombin time was demonstrated *in vivo* in rat and rabbit models, with a strong correlation observed between prothrombin time and plasma concentrations of rivaroxaban (r=0.98)[71].

In vivo, rivaroxaban given prophylactically had potent and consistent antithrombotic effects in venous[71,80] and arterial[71] thrombosis models in rats and rabbits. In a rabbit treatment model, rivaroxaban reduced the accretion of radiolabelled fibrinogen into preformed clots in the jugular vein, relative to untreated controls[80]. Bleeding times in rats and rabbits were not significantly affected at antithrombotic-effective doses[71], indicating a favourable efficacy/bleeding ratio.

Although rivaroxaban has a short half-life in humans[81,82] (see the Xarelto Summary of Product Characteristics (SMPC) from the European Medicines

Agency (EMA); Further information), it may be of interest to determine whether the anticoagulant effect of rivaroxaban can be reversed, because there might be rare instances in which this would be of use — for example, a need for emergency surgery. However, it is worth noting that several established anticoagulants also lack full antidotes. For example, the anticoagulant effect of the LMWHs is only partly reversed by protamine sulphate[2] and there is no available antidote for fondaparinux (see Arixtra's prescribing information; Further information). Preliminary studies in primates and rats have been conducted to evaluate possible antidotes for rivaroxaban. These studies suggested that recombinant factor VIIa, activated prothrombin complex concentrate (FEIBA (factor VIII inhibitor bypassing activity); Baxter)[83] and prothrombin complex concentrate[84] can partially reverse, in a dose-dependent manner, the effects of high-dose rivaroxaban on bleeding time. However, it is important to note that there is no clinical experience with any of these reversal strategies (see the EMA's SMPC on rivaroxaban; Further information).

Clinical pharmacology

In Phase I and Phase II studies, rivaroxaban exhibited predictable pharmacokinetic and pharmacodynamic profiles, as follows. It is rapidly absorbed, with maximum plasma concentrations (C_{max}) occurring 2–4 hours after tablet intake[82,85]. Oral bioavailability of rivaroxaban is decreased at higher doses, possibly owing to poor solubility. However, at the currently approved 10 mg dose, oral bioavailability is 80–100%[85].

Food (a high-fat, high-calorie meal) did not affect the area under the plasma concentration–time curve (AUC) or C_{max} for the 10 mg tablet (EMA's SMPC on rivaroxaban). Rivaroxaban was safe and well tolerated across a wide dose range, with dose-proportional pharmacokinetics and pharmacodynamics. No relevant accumulation was observed at any dose level after multiple dosing in healthy subjects at steady state[82].

Rivaroxaban is metabolized by CYP450 isoforms, particularly CYP3A4, which is strongly inhibited by ketoconazole and ritonavir (see prescribing information from Janssen Pharmaceutica (ketoconazole) and Abbott (ritonavir), and the EMA's Committee of Medicinal Products for Human Use (CHMP) report for rivaroxaban; Further information). These drugs were, therefore, predicted to affect the metabolism of rivaroxaban and, because both drugs are also strong inhibitors of P-glycoprotein (P-gp) and CYP3A4 (REFS 86,87), may increase plasma concentrations of rivaroxaban. This expectation was confirmed in subsequent Phase I studies that showed a strong interaction between rivaroxaban and these two drugs. However, there was no clinically relevant interaction with clarithromycin, a strong CYP3A4 inhibitor but only a moderate inhibitor of P-gp, indicating that rivaroxaban may be used with substances that strongly inhibit only one of the two elimination pathways (see the EMA's SMPC on rivaroxaban and prescribing information from Janssen Pharmaceutica (ketoconazole) and Abbott (ritonavir)). Conversely, these findings show that concomitant use of rivaroxaban with strong inhibitors of both CYP3A4 and P-gp such as ketoconazole or HIV protease inhibitors such as ritonavir is not recommended. In addition, rivaroxaban should be used with caution if strong CYP3A4 inducers are administered concomitantly (EMA's CHMP report for rivaroxaban).

Rivaroxaban has a low propensity for drug–drug interactions with frequently used concomitant medications such as naproxen[88] and asprin[89], and no interaction with the cardiac glycoside digoxin (Lanoxin; GlaxoSmithKline) (D. Kubitza, unpublished observations).

Furthermore, dietary restrictions are not necessary at the 10 mg once-daily dose; rivaroxaban was given with and without food in the Phase III VTE-prevention studies (RECORD studies 1–4)[90–93] (EMA's SMPC on rivaroxaban).

Rivaroxaban is distributed heterogeneously to tissues and organs, and exhibits only moderate tissue affinity in rats; importantly, it does not substantially penetrate the blood–brain barrier[94]. However, like many small molecules, rivaroxaban is expected to be able to cross the placenta, although specific studies have not been published. *In vitro* and Phase I studies showed that rivaroxaban has a dual mode of elimination, with one-third eliminated unchanged by the kidneys and two-thirds metabolized by the liver to inactive metabolites, with no major or active circulating metabolites detected in plasma[95,96]. Elimination of rivaroxaban from plasma occurs with a mean terminal half-life of 7–11 hours[81,82] (EMA's SMPC on rivaroxaban). With a systemic clearance rate of approximately 10 L h^{-1}, rivaroxaban can be classified as a low-clearance drug[97,98]. Low intra-individual and moderate inter-individual pharmacokinetic variability have been observed[97,98]. In Phase I studies, the coefficient of variation for AUC ranged from 18% to 33%, and for C_{max} from 16% to 39%, for inter-individual variability. Median intra-individual variability was only 14% and 19% for AUC and C_{max}, respectively (EMA's CHMP report for rivaroxaban).

Rivaroxaban inhibited factor Xa activity in a dose-dependent manner after single dosing in healthy subjects. Rivaroxaban also inhibited thrombin generation in healthy subjects, and some parameters of thrombin generation remained inhibited for 24 hours after administration of a 30 mg dose[74]. These results offered the first indication that once-daily dosing might be feasible.

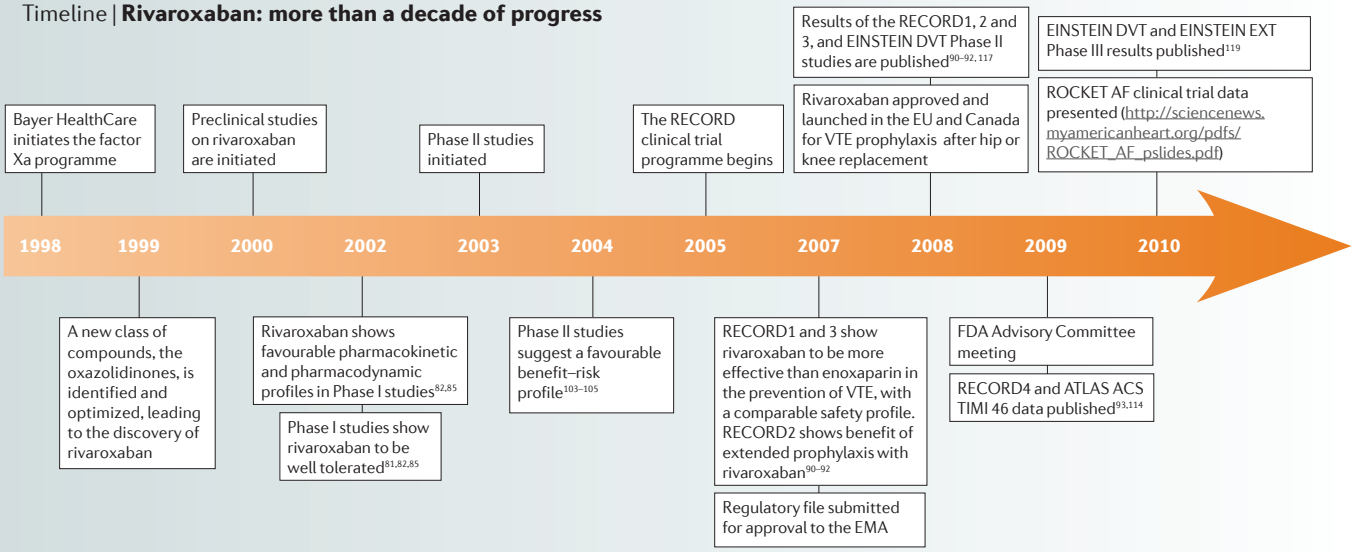
There were close correlations between pharmacokinetic and pharmacodynamic parameters[82]. Rivaroxaban plasma concentrations correlated closely with prolongation of prothrombin time and inhibition of factor Xa. Plasma concentrations correlated with prothrombin time both in healthy volunteers[82] and in patients undergoing either total hip replacement (THR) or total knee replacement (TKR) in Phase II studies[97].

Various Phase I and II studies, using total daily doses ranging from 5 mg to 80 mg, indicated that rivaroxaban could be given irrespective of age, gender[81], body weight[99] and mild (creatinine clearance: 50–79 ml min^{-1}) to moderate (creatinine clearance: 30–49 ml min^{-1}) renal impairment[100]. A further preliminary clinical study suggested that rivaroxaban can also be given irrespective of mild hepatic impairment (classified as Child–Pugh class A)[101]. Fixed doses of rivaroxaban were administered

CYP450 isoforms
Many therapeutic drugs are metabolized by cytochrome P450 (CYP450) enzymes, a 'superfamily' of related but distinct enzymes that differ in their substrate specificity.

Creatinine clearance
The rate at which the kidney clears the blood of creatinine (a waste product from muscles that is excreted at a fairly constant rate). Creatinine clearance is used as an approximation of the glomerular filtration rate.

Timeline | **Rivaroxaban: more than a decade of progress**



DVT, deep-vein thrombosis; EMA, European Medicines Agency; EU, European Union; FDA, US Food and Drug Administration; VTE, venous thromboembolism.

in Phase II studies investigating rivaroxaban for the prevention or treatment of VTE, with no restrictions on gender, or mild or moderate renal impairment[102–105]. These parameters were shown to have no clinically relevant effect on the pharmacokinetics and pharmacodynamics of rivaroxaban[97]. In addition, rivaroxaban had no effect on heart-rate-corrected QT interval prolongation[106]. Owing to its pharmacokinetic and pharmacodynamic profiles, rivaroxaban can be given at fixed doses to adult patients with no requirement for routine coagulation monitoring. However, there may be rare occasions when monitoring is of use — for example, where poor compliance is suspected or emergency surgery is required, or to confirm a possible overdose. This concern has led to the evaluation of a number of different assay types. Initial results suggest that, in light of an apparent strong correlation between the extent of factor Xa inhibition and rivaroxaban plasma concentration, chromogenic assays for factor Xa may be particularly suited for the quantification of rivaroxaban in plasma[107,108].

Clinical development of rivaroxaban

Rivaroxaban is approved for the prevention of VTE after elective hip or knee replacement in approximately 100 countries, including member states of the European Union (as of 30 September 2008) and Canada (as of 16 September 2008). Clinical development is ongoing for the prevention and treatment of thromboembolic disorders in other conditions, including the treatment of VTE, the prevention of VTE in hospitalized, medically ill patients (for example, patients hospitalized with an acute medical condition, such as cancer, heart failure or respiratory failure, or requiring intensive care), as well as the prevention of stroke in patients with atrial fibrillation and secondary prevention in patients with ACS. The TIMELINE highlights the development of rivaroxaban during the past decade.

VTE prophylaxis after total hip or knee replacement. A large, comprehensive Phase II programme involving about 2,900 patients assessed both once-daily and twice-daily dosing regimens[102–105]. Collectively, these studies demonstrated an optimal dose range of 5–20 mg per day[109], and indicated that rivaroxaban 10 mg once daily had the optimum balance of efficacy and safety, relative to enoxaparin 40 mg once daily[103] (FIG. 5).

On the basis of the results of the Phase II studies, rivaroxaban 10 mg once daily was selected for investigation in the Phase III RECORD programme, which comprised four large studies involving a total of more than 12,500 patients undergoing elective THR or TKR[90–93] (TABLE 2). In all four studies, the primary efficacy end point was the composite of any DVT, as detected by mandatory, bilateral venography, non-fatal pulmonary embolism and all-cause mortality. The primary safety end point was major bleeding, which in the RECORD programme did not include surgical-site bleeding[90–93].

The RECORD1 and 3 studies were designed to compare rivaroxaban 10 mg once daily with the standard of care — enoxaparin 40 mg once daily — both given for 31–39 days (extended prophylaxis) after THR (RECORD1)[90] or for 10–14 days after TKR (RECORD3)[92]. In both studies, rivaroxaban was more effective than enoxaparin for the prevention of VTE[90,92] (TABLE 2). Furthermore, the incidence of major bleeding was comparable and not significantly different between treatment groups[90,92]. RECORD3 also showed a significant reduction in symptomatic VTE for rivaroxaban[92].

RECORD2 investigated the efficacy and safety of extended thromboprophylaxis with rivaroxaban (35 days; range 31–39 days) compared with short-term enoxaparin treatment (10–14 days) followed by placebo in patients undergoing THR[91]. The results demonstrated that extended prophylaxis with rivaroxaban (10 mg once daily) was superior to short-term prophylaxis with enoxaparin (40 mg once daily) followed by placebo for

Chromogenic assay
An enzymatic assay in which a colour develops during the course of the reaction, which can then be measured spectrophotometrically. Colour development is reduced in the presence of an inhibitor.

Venography
Radiography of the veins after intravenous injection of a radioactive isotope or contrast dye. This can be used to confirm the presence of deep-vein thromboses.

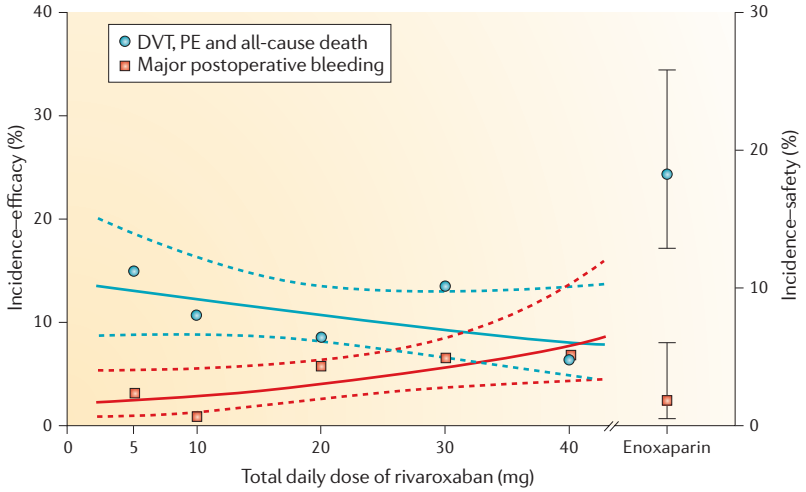


Figure 5 | **Efficacy and safety dose–response relationships.** The figure shows the dose–response relationships between rivaroxaban total daily dose and the primary efficacy end point (any deep-vein thrombosis (DVT); non-fatal pulmonary embolism (PE) and all-cause death) and primary safety end point (major bleeding) in the once-daily study investigating rivaroxaban for the prevention of venous thromboembolism after total hip replacement[103]. Solid lines show the dose–response curves (logistic regression), blue hatched lines represent 95% confidence intervals for efficacy and red hatched lines represent 95% confidence intervals for safety. Details for 40 mg enoxaparin once daily, the current European standard of care, are shown for comparison. Figure reproduced, with permission, from REF. 103 © (2006) Lippincott Williams & Wilkins.

the prevention of VTE, including symptomatic events[91] (TABLE 2). Despite rivaroxaban being given for 3 weeks longer than enoxaparin, the incidence of treatment-emergent major bleeding (that is, up to 2 days after the last dose of study medication) was <0.1% in both treatment groups. Guidelines recommend a minimum duration for prophylaxis of 10 days, and also recommend that prophylaxis be extended for up to 35 days for patients undergoing THR[110]. The RECORD2 study provided additional confirmation of the benefits of extended prophylaxis over short-term prophylaxis after THR.

RECORD4 compared the efficacy and safety of oral rivaroxaban 10 mg once daily with the commonly used North American regimen of enoxaparin 30 mg twice daily in patients undergoing TKR[93]. Rivaroxaban was significantly superior to enoxaparin for the primary efficacy end point, with no significant difference in the rates of major bleeding between the two groups (TABLE 2). So far, rivaroxaban is the only new oral anticoagulant to demonstrate superior efficacy over the greater dose intensity of the North American enoxaparin regimen[93,111,112].

A comparison of rivaroxaban with enoxaparin in these four studies showed the efficacy and safety of rivaroxaban in the prevention of VTE in patients undergoing elective THR or TKR. The superiority of rivaroxaban for the primary efficacy end point was demonstrated in all four studies. Rivaroxaban also showed a good safety profile, with a low incidence of major bleeding, comparable to that observed with enoxaparin[90–93], and no evidence of compromised liver function attributable to rivaroxaban[113]. Furthermore, the incidence of haemorrhagic wound complications (composite of excessive wound haematoma and reported surgical-site bleeding) was similar in both treatment groups[113]. Liver safety is also an important consideration in regulatory review. In a Phase II trial of rivaroxaban in patients with ACS, no patient who had received rivaroxaban for 6 months had an alanine-amino-transferase level greater than three times the upper limit of normal or total bilirubin greater than twice the upper limit of normal[114].

These studies were also conducted with no routine coagulation monitoring and no dose adjustment for demographic variables, consistent with preliminary results from pooled subgroup analyses[115].

Treatment of VTE. The efficacy and safety of rivaroxaban for the treatment of VTE were assessed in two Phase IIb dose-ranging studies[116,117]. These studies suggested that rivaroxaban had good efficacy and a similar safety profile, compared with standard therapy, for the treatment of acute symptomatic DVT. An initial intensified twice-daily regimen (rivaroxaban 15 mg twice daily for 3 weeks) followed by convenient 20 mg once-daily dosing for 3, 6 or 12 months was selected for investigation in Phase III studies. The efficacy and safety of rivaroxaban for the treatment of VTE are being assessed in three Phase III studies involving approximately 9,000 patients in total— EINSTEIN DVT (ClinicalTrials.gov identifier: NCT00440193), EINSTEIN PE (NCT00439777) and EINSTEIN EXT (NCT00439725).

EINSTEIN PE (pulmonary embolism) is still ongoing. However, data for EINSTEIN DVT (presented at the 2010 American Society of Hematology meeting[118] and recently published[119]) showed that rivaroxaban (15 mg twice daily for 21 days followed by 20 mg once daily) was non-inferior for the prevention of recurrent symptomatic VTE in comparison to the current standard of care (enoxaparin 1.0 mg kg^{-1} twice daily for ≥5 days followed by VKA titrated to INR 2.0–3.0). First symptomatic recurrent VTEs occurred in 2.1% of patients receiving rivaroxaban compared with 3.0% of those in the enoxaparin/VKA treatment arm. Major or non-major clinically relevant bleeding occurred in 8.1% of patients in each treatment arm[119].

In the EINSTEIN EXT trial, 1,196 patients (intention-to-treat population) who had completed 6–12 months of anticoagulant therapy for the acute index event (VTE) were randomized to an additional 6–12 months of therapy with either rivaroxaban, 20 mg once daily, or placebo[119]. Study medication was administered for a mean period of 190 days in each treatment group. Recurrent symptomatic VTE events were observed in 7.1% and 1.3% of the placebo and rivaroxaban treatment groups, respectively (hazard ratio 0.18, $P<0.0001$), a relative risk reduction of 82% with rivaroxaban[119] (hazard ratio, 0.68; 95% confidence interval, 0.44–11.04; P<0.001 for non-inferiority). Major bleeding did not occur in any placebo-treated patients and occurred in four (0.7%) rivaroxaban-treated patients, although none of these occurred in a critical location or proved fatal[119].

Thromboprophylaxis in medically ill patients. A Phase III study (NCT00571649) has been initiated to investigate the efficacy and safety of VTE prophylaxis

Index event
The acute event that leads to a patient's initial presentation. The term can also refer to the initial event resulting in a patient's inclusion in a follow-up study, such as a survey of recurrent strokes.

Table 2 | **Incidence of venous thromboembolism and bleeding events across the four RECORD* studies**

End points‡	Efficacy end point (% (*n*/*N*))			Safety end points (patients with bleeding events) (% (*n*/*N*))	
	Total VTE	Major VTE	Symptomatic VTE	Major bleeding	Major and non-major clinically relevant bleeding
RECORD1 (THR: prophylaxis administered for 35 days)					
Enoxaparin (40 mg once daily)	3.7 (58/1,558)	2.0 (33/1,678)	0.5 (11/2,206)	0.1 (2/2,224)	2.5 (56/2,224)
Rivaroxaban (10 mg once daily)	1.1 (18/1,595)	0.2 (4/1,686)	0.3 (6/2,193)	0.3 (6/2,209)	3.2 (70/2,209)
P value	<0.001	<0.001	0.22	0.18	NS§
RECORD2 (THR: extended rivaroxaban prophylaxis, 35 days, versus short-term enoxaparin, 14 days, followed by placebo)					
Enoxaparin (40 mg once daily/placebo)	9.3 (81/869)	5.1 (49/962)	1.2 (15/1,207)	<0.1 (1/1,229)	2.8 (34/1,229)
Rivaroxaban (10 mg once daily)	2.0 (17/864)	0.6 (6/961)	0.2 (3/1,212)	<0.1 (1/1,228)	3.3 (41/1,228)
P value	<0.0001	<0.0001	0.004	0.98 (REF. 157)	NR
RECORD3 (TKR: prophylaxis administered for 14 days)					
Enoxaparin (40 mg once daily)	18.9 (166/878)	2.6 (24/925)	2.0 (24/1,217)	0.5 (6/1,239)	2.7 (34/1,239)
Rivaroxaban (10 mg once daily)	9.6 (79/824)	1.0 (9/908)	0.7 (8/1,201)	0.6 (7/1,220)	3.3 (40/1,220)
P value	<0.001	0.01	0.005	0.77	0.44
RECORD4 (TKR: prophylaxis administered for 14 days)					
Enoxaparin (30 mg twice daily)	10.1 (97/959)	2.0 (22/1,112)	1.2 (18/1,508)	0.3 (4/1,508)	2.3 (34/1,508)
Rivaroxaban (10 mg once daily)	6.9 (67/965)	1.2 (13/1,112)	0.7 (11/1,526)	0.7 (10/1,526)	3.0 (46/1,526)
P value	0.012	0.124	0.187	0.11	0.18

N, total number of patients evaluable in each treatment group; NR, not reported; NS, not significant; THR, total hip replacement; TKR, total knee replacement; VTE, venous thromboembolism. *The four RECORD studies[90–93] compared rivaroxaban regimens with enoxaparin regimens (the current standard of care); summary results are shown in the table. ‡Results shown as number (*n*) of patients experiencing an event; patients could have more than one event. §*P* value not reported, but 95% confidence interval for risk difference includes zero.

with rivaroxaban 10 mg once daily (for 35 ± 4 days), compared with short-term 40 mg once-daily enoxaparin (administered for 10 ± 4 days followed by placebo), in hospitalized, medically ill patients.

Stroke prevention in non-valvular atrial fibrillation. Rivaroxaban 20 mg once daily has been compared with warfarin for the prevention of stroke and non-central-nervous-system systemic embolism in approximately 14,000 patients with non-valvular atrial fibrillation in a Phase III study (NCT00403767)[120]. Patients with moderate renal impairment (creatinine clearance: 30–49 ml min^{-1}) received a fixed dose of 15 mg once daily. It was recently reported at the 2010 American Heart Association meeting that, in the ROCKET AF study, rivaroxaban significantly reduced the risk of stroke and non-central-nervous-system thromboembolism in patients with atrial fibrillation compared to warfarin, with comparable rates of bleeding (http://sciencenews.myamericanheart.org/pdfs/ROCKET_AF_pslides.pdf).

Secondary prevention in ACS. A Phase III study investigating secondary prevention of ischaemic events in patients with ACS (NCT00809965) was started in late 2008, and is expected to enrol up to 16,000 patients. Two doses of rivaroxaban, 2.5 mg and 5 mg twice daily, are being investigated on the basis of the results of a Phase IIb study that assessed safety and efficacy in approximately 3,500 patients with recent, non-ST-elevation myocardial infarction, ST-elevation myocardial infarction or unstable angina[114]. As noted above, this trial also showed no evidence of compromised liver function in patients receiving rivaroxaban for up to 6 months.

Other direct factor Xa inhibitors in development

Below, we discuss other direct factor Xa inhibitors that are in advanced clinical development (Phase III).

Apixaban. Apixaban (developed by Pfizer/Bristol-Myers Squibb) is a small-molecule, oral, direct factor Xa inhibitor (FIG. 3) that selectively and reversibly inhibits both free factor Xa (K_i of 0.08 nM) and prothrombinase activity[79,121]. Another study reported that apixaban reacts rapidly with factor Xa (k_{on} 2×10^7 M^{-1} s^{-1}) with high-affinity binding (K_i of 0.3 nM at 37 °C)[122]. Preclinical studies have shown that apixaban was well absorbed in chimpanzees, dogs and rats; mean oral bioavailability was 51% (chimpanzees), 88% (dogs) and 34% (rats)[79]. The drug is currently

being evaluated in a number of thromboembolic indications, including the prevention and treatment of VTE, the prevention of stroke in patients with atrial fibrillation and the prevention of cardiovascular events in ACS.

In the Phase III programme for the prevention of VTE after major orthopaedic surgery, apixaban did not meet the prespecified criteria for non-inferiority compared with enoxaparin 30 mg twice daily (NCT00371683) for VTE prevention after TKR[111]. However, a second study (NCT00452530) conducted in patients undergoing TKR demonstrated superior efficacy for apixaban against enoxaparin 40 mg once daily. Clinically relevant bleeding (major or non-major) occurred in fewer patients given apixaban, although the differences were not significant[123]. A third study (NCT00423319), presented as an abstract, assessed extended prophylaxis with apixaban versus enoxaparin (40 mg once daily) in patients undergoing THR and also demonstrated superior efficacy for apixaban with similar rates of major and non-major clinically relevant bleeding[124].

A Phase II trial in patients with ACS (NCT00313300) assessed efficacy and safety in patients taking apixaban compared with placebo[125]. The developing companies recently reported that a subsequent Phase III trial (NCT00831441) in patients with ACS investigating whether apixaban (5 mg twice daily) is superior to placebo has been halted (press release, Bristol-Myers Squibb; Further information). In addition, other apixaban trials are ongoing for VTE prevention in patients with acute medical illness (NCT00457002), or recently completed for malignant disease (NCT00320255), as well as Phase III trials for the treatment of VTE (NCT00633893, NCT00643201) and trials for the prevention of stroke in patients with atrial fibrillation (NCT00412984, NCT00496769)[126,127]. Data for the AVERROES trial (NCT00496769) were recently presented at the European Society of Cardiology Congress. Patients with atrial fibrillation who have either been demonstrated to be or were expected to be unsuitable for treatment with VKAs received apixaban 5 mg twice daily or aspirin 81–324 mg per day. Preliminary results showed that the primary end point of stroke or systemic embolism occurred at a significantly lower rate in patients receiving apixaban, with an absolute risk reduction of approximately 2% versus aspirin[128].

Edoxaban. Edoxaban (developed by Daiichi Sankyo) is an oral, direct and specific factor Xa inhibitor with a K_i of 0.56 nM (FIG. 3); K_i values for other coagulation factors are >10,000-fold higher[78]. In healthy volunteers, peak plasma levels of edoxaban were observed at 1.5 hours after a single oral dose, corresponding to the maximum inhibition of factor Xa activity; and *ex vivo* thrombus formation was reduced at 1.5 hours and 5 hours after administration[129].

Phase II trials in patients undergoing TKR[130] or THR[131] have been completed, as well as a Phase II trial for stroke prevention in atrial fibrillation[132], and two Phase III trials are now under way. One is designed to compare two different doses of edoxaban with warfarin for stroke prevention in patients with atrial fibrillation (NCT00781391) and another is evaluating edoxaban for the secondary prevention of recurrent VTE in patients with acute symptomatic proximal DVT or pulmonary embolism (NCT00986154).

YM150. YM150 (developed by Astellas) is also an oral, direct factor Xa inhibitor (K_i of 31 nM). Preliminary data show that both YM150 and its major metabolite YM-222714 (K_i of 20 nM) have antithrombotic effects at doses that do not prolong template bleeding time in animal models of venous and arterial thrombosis[133]. Phase II trials for VTE prevention after THR[134] or TKR (NCT00408239) have been completed, as has a warfarin-controlled Phase II trial for stroke prevention in patients with atrial fibrillation (NCT00448214). Three Phase III trials are now in progress. One will assess once-daily and twice-daily doses of YM150 against enoxaparin for VTE prevention in patients undergoing elective hip replacement (NCT00902928). In another Phase III trial, YM150 is being compared with mechanical prophylaxis for the prevention of VTE after major abdominal surgery (NCT00942435). A third study is being conducted in Japan to evaluate YM150 for the prevention of VTE in the 28 days following hospitalization for an acute medical illness (NCT01028950).

Outlook

For more than 65 years, VKAs have been the only available oral anticoagulants, and although effective, the need for dose adjustment and periodic coagulation monitoring considerably complicates their clinical management. New and improved anticoagulants could potentially address the shortcomings associated with the current standard of care and it should be noted that other approaches, besides the development of oral, direct factor Xa inhibitors, are also being evaluated. For example, the parenteral direct factor Xa inhibitor, otamixaban (developed by Sanofi–Aventis), has completed Phase II trials for short-term use in non-ST-elevation ACS[135] and in percutaneous coronary intervention[136]. The encouraging results obtained in these studies have been followed by an ongoing Phase III trial in patients with unstable angina or non-ST elevation myocardial infarction undergoing an invasive intervention (NCT01076764). Conversely, idraparinux, and its successor idrabiotaparinux[137] (Sanofi–Aventis; both now discontinued) are parenterally administered pentasaccharides (indirect factor Xa inhibitors) with very long half-lives[138] that were developed to permit once-weekly dosing in indications requiring long-term or chronic therapy[139,140].

In addition, direct thrombin inhibitors are also in development, most notably dabigatran (Pradaxa/Pradax; Boehringer Ingelheim), which, like rivaroxaban, has completed several Phase III thrombosis prevention and treatment studies[141–144] and has been approved in member states of the European Union and other countries for the prevention of VTE after elective hip or knee replacement. Dabigatran is also in trials for a number of other indications, as is AZD0837 (developed by AstraZeneca), which is in Phase II[145]. Although indirect comparisons, based on meta-analyses, can be conducted, direct comparative trials are required for a comprehensive evaluation of one particular drug regimen versus another.

In the future, it is anticipated that long-term anticoagulant therapy will favour oral agents with a wide therapeutic window and a predictable anticoagulant response that do not require routine coagulation monitoring or dose adjustment. Emerging data suggest that direct factor Xa inhibitors are both effective antithrombotic agents for short-term use and promising agents for long-term usage. As they have shown a predictable pharmacological profile, are given orally and do not require routine coagulation monitoring, these new agents may also facilitate patient compliance and adherence to clinical guidelines. Thus, they are likely to improve anticoagulation treatment in thromboembolic disorders and reduce the burden associated with long-term therapy, thereby providing important alternative options for the management of these conditions.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

FURTHER INFORMATION
ClinicalTrials.gov: http://clinicaltrials.gov
Bayer HealthCare Xarelto (rivaroxaban) Summary of Product Characteristics: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000944/WC500057108.pdf
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Rocket AF study: http://sciencenews.myamericanheart.org/pdfs/ROCKET_AF_pslides.pdf

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