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Apixaban, an oral direct Factor Xa inhibitor: awaiting the verdict

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For the last half-century, despite its many limitations warfarin has been the mainstay of treatment for patients with venous and arterial thromboembolic disease. During the past decade, a number of new oral anticoagulant agents have been developed that may offer an alternative to warfarin. Emerging data suggest that Factor Xa may be a target for inhibition. Apixaban is one such agent. It is a potent, selective, reversible, and orally bioavailable FXa inhibitor that demonstrates antithrombotic efficacy, with a favorable pharmacokinetic profile. At present, the safety and efficacy of apixaban for the prophylaxis and treatment of venous thromboembolism is being evaluated in Phase II and Phase III trials involving nearly 25,000 patients. Trials are also underway involving over 20,000 patients for secondary prevention after acute coronary syndromes and the prevention of stroke in patients with non-valvular atrial fibrillation. This review article discusses the discovery, pharmacokinetics, attributes, and current clinical trials of this emerging drug.

Keywords: anticoagulation, apixaban, factor Xa inhibitors, oral anticoagulants

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1. Introduction

For the last half-century, heparin and warfarin have been the mainstays of treatment for patients with venous and arterial thromboembolic disease. The administration of warfarin is limited because of its narrow therapeutic index, slow onset of therapeutic effect, numerous dietary and drug interactions, and a need for monitoring as well as dose adjustments. For over 60 years [1], warfarin has been the only available oral anticoagulant, despite its many limitations. During the past decade, new oral anticoagulant agents have been developed that may offer an attractive alternative to warfarin. The era of anticoagulation, requiring labor-intensive monitoring and treatment, may soon be ending due to a number of antithrombotic compounds currently being investigated in clinical trials [2]. Emerging data suggest that Factor Xa and thrombin are favorable targets for inhibition by new anticoagulants because of their central location in the common pathway of the coagulation cascade, blocking both intrinsic and extrinsic pathways. **Figure 1** lists many of the current investigational agents and their targeted coagulation factors.

Serine proteases play an important role in coagulation and the thrombotic process, such as venous thromboembolism, stroke and other cardiovascular disorders [3]. There is evidence to suggest that FXa may represent a better target for inhibition than thrombin [4]. In animal models, direct Factor Xa inhibitors produce less bleeding than direct thrombin inhibitors when given in doses with similar antithrombotic activity [5-10]. This is based on an understanding of the amplified nature of coagulation, where smaller doses of an anticoagulant drug are needed to block coagulation progression earlier in the sequence of reactions (i.e., one molecule of FXa catalyzes the formation of almost 1000 thrombin molecules) [11]. Secondly, some evidence suggests that direct thrombin inhibitors may be associated

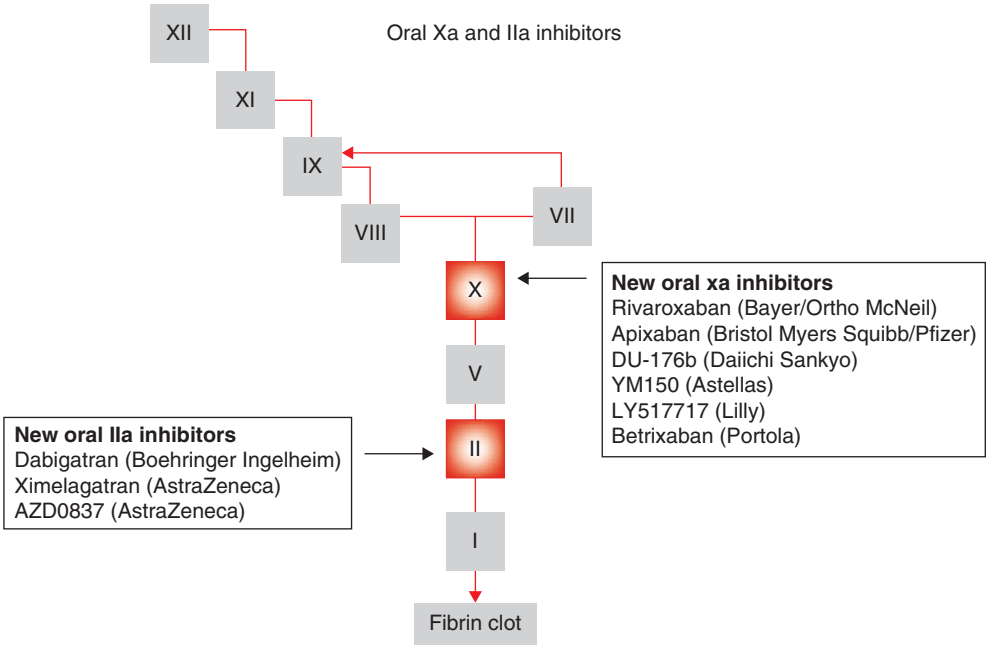

Figure 1. New anticoagulant drugs targeted to factors Xa or IIa.

with a rebound hypercoagulable state that does not appear to be associated with FXa inhibitors [12]. Thirdly, FXa inhibitors incompletely block thrombin generation, and some existing thrombin has anti-inflammatory functions that can potentially maintain hemostasis [13] as well as the potential to activate the protein C system and add to an antithrombotic potential. Lastly, *in vitro* assays have found that Factor Xa is progressively inhibited over a much wider concentration range than thrombin, suggesting that FXa inhibitors may have a wider therapeutic window than thrombin inhibitors [14]. This means that it would be easier to maintain a patient's anti-Xa concentration within the therapeutic range.

A number of direct-acting, oral Factor Xa inhibitors are in development. Apixaban is one such agent. It is a potent, selective, reversible, and orally bioavailable FXa inhibitor that demonstrates antithrombotic efficacy with a favorable pharmacokinetic profile. Apixaban not only inhibits free Factor Xa but also inactivates Factor Xa in the prothrombinase complex and Xa bound to platelets. At present, trials are underway involving about 47,000 patients examining apixaban's safety and efficacy in the prophylaxis and treatment of venous thromboembolism, and stroke prevention for patients with atrial fibrillation (AF). This review article discusses the discovery, pharmacokinetics, and current clinical trials of this emerging drug.

2. Pharmacology

Apixaban was designed as a follow-up compound to the oral, direct FXa inhibitor razaxaban. Razaxaban was a selective oral direct Factor Xa inhibitor that was discontinued based on less than optimal pharmacologic properties [15]. Efforts to identify a suitable follow-up compound to razaxaban focused on modification of the carboxamido linker. Cyclization of the carboxamido linker to the novel bicyclic tetrahydropyrazolopyridinone scaffold (see **Figure 2**), modification of the P_1 moieties, and optimization of the terminal P_4 ring proved to have exceptionally potent FXa binding activity [6]. These three modifications led to the discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl) phenyl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*c*]pyridine-3-carboxamide, also known as apixaban. Apixaban exhibits a high degree of FXa potency, selectivity, and efficacy and has an improved pharmacokinetic profile relative to razaxaban, without the bleeding risk. Apixaban is a selective inhibitor to Factor Xa with > 30,000-fold selectivity over other coagulation proteases [16]. Direct Factor Xa inhibitors bind to Factor Xa with 1:1 stoichiometry and block the interaction of Factor Xa with Factor II. It is a small molecule with a molecular weight of 460.

3. Preclinical pharmacologic studies in animal models

The preclinical pharmacokinetic and metabolic attributes of apixaban feature a small volume of distribution, a low systemic clearance, good oral bioavailability, multiple elimination pathways and minimal potential for drug–drug interactions. In the rabbit AV shunt thrombosis model, apixaban inhibited thrombus formation in a dose-dependent manner and did

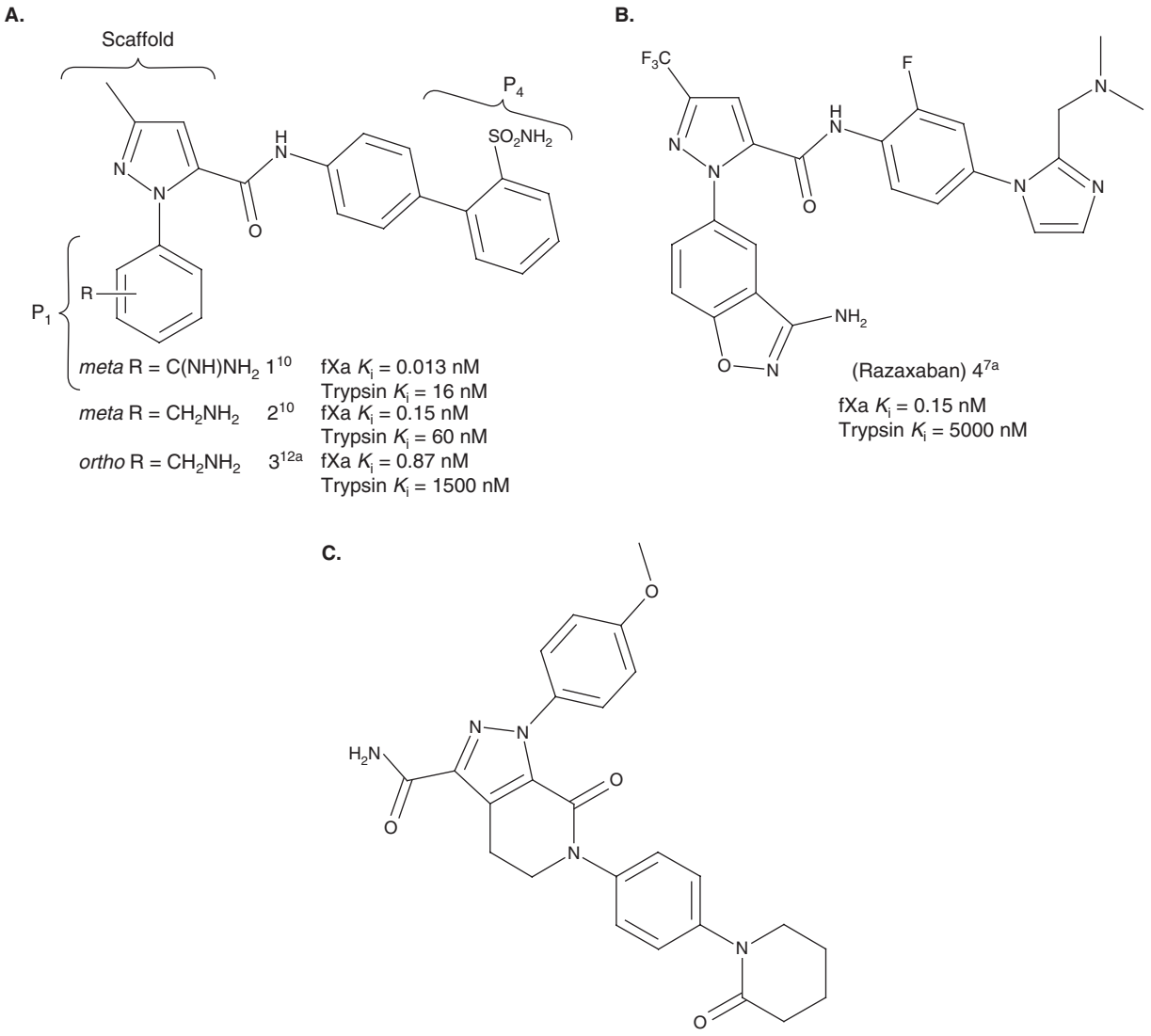

Figure 2. The pharmacology for apixaban chemical discovery (adapted from [6]) and apibaxan structure. A. Precursor molecules to razaxaban, which contains a sulfonamide group. **B.** Razaxaban structure. **C.** Apixaban structure (fXa K_i = 0.08 nM).

not affect bleeding time [9,10,16]. Apixaban has an IC_{50} value of 329 nM [6]. Apixaban is absorbed in chimpanzees, dogs and rats with a mean oral bioavailability of 51, 88 and 34%, respectively [7]. The mean volume of distribution of apixaban is 0.17, 0.29 and 0.31 l/kg in chimpanzees, dogs and rats, respectively, suggesting that apixaban is distributed (30 – 50%) to blood where the therapeutic action resides [17]. The small volume is not due to extensive plasma protein binding but possibly attributed to limited extravascular tissue distribution, given that the unbound fraction is approximately 5, 8 and 4% in chimpanzee, dog and rat serum, respectively [17]. The systemic clearance is < 3% of hepatic blood flow in chimpanzees (0.018 l/h/kg) and dogs (0.052 l/h/kg), and < 10% in rats (0.26 l/h/kg) [17]. Consistent with this low clearance, the *in vitro* metabolic clearance of apixaban is low, as indicated by the lack of significant metabolism in chimpanzee and dog liver microsomes.

The elimination of apixaban involves multiple pathways, including renal and intestinal excretion. The biliary clearance is low in dogs, accounting for approximately 2% of the systemic clearance [17]. Apixaban shows weak activity against various P_{450} isozymes (IC_{50} > 25 μM) [6]. No glutathione adduct with apixaban was formed in dog and rat [17]. No teratogenicity was observed in rat or rabbit models [18]. In animal models, apixaban was as effective as lepirudin, and more effective than aspirin for the prevention of arterial thrombosis; it prolonged the bleeding time less than lepirudin and aspirin at antithrombotic doses [9].

The combination of apixaban and aspirin or apixaban, aspirin and clopidogrel has been studied in a rabbit model. The

study found that combining apixaban and antiplatelet agents reduced the formation of occlusive arterial thrombosis [8].

Control thrombus weight was 8.6 ± 0.9 mg. The addition of aspirin to apixaban significantly reduced the thrombus weight from 7.4 ± 0.5 to 5.3 ± 0.3, and the further addition of aspirin and apixaban to clopidogrel produced a significant additional reduction in thrombus weight from 5.3 ± 0.3 to 0.7 ± 0.1 mg. The addition of aspirin and apixaban did not increase bleeding time (BT) compared with the control. However, the combination of clopidogrel and aspirin with apixaban produced a significant increase in BT of 2.1 times control.

4. Pharmacokinetic and pharmacodynamic studies in humans

In vitro properties of apixaban show that it is a highly selective and potentially potent antithrombotic agent in human blood from healthy volunteers. Detailed kinetic analysis of apixaban inhibition of human FXa showed that it is a readily reversible competitive inhibitor with a synthetic tripeptide substrate with a K_i of 0.08 nM. K_i is a measure of how potent a drug is to produce half maximum inhibition. The human serum protein binding as measured by equilibrium dialysis for apixaban was 87% [6]. Weak affinity is observed for thrombin (K_i 3.1 μM), plasma kallikrein (K_i 3.7 μM)), and chymotrypsin (K_i 3.5 μM), trypsin (K_i > 12 μM) and all other serine proteases [6]. The unbound fraction is approximately 13% in humans [17].

The pharmacokinetic profile of apixaban is consistent with rapid oral absorption and bioavailability. It is well absorbed from the gastrointestinal tract, and peak plasma levels are achieved in about 3 h. The effective half-life is 8 – 11 h when given twice daily and 12 – 15 h for a once-daily regimen [19]. This is consistent with achievement of steady-state concentrations by day 3 with modest accumulation (1.3- to 1.9-fold for b.i.d. and 1.3- to 1.5-fold for q.d.). Lower peak-to-trough concentration ratios are observed with b.i.d. versus q.d. dosing regimens. There is no food effect on apixaban absorption following the consumption of a hig- fat, high-calorie meal [20]. Apixaban absorption is not likely to be affected by medications that alter gastric pH, based on apixaban's physical-chemical properties. Apixaban has no ionizable groups and therefore does not exhibit pH-dependent aqueous solubility. Apixaban exhibits a dual mechanism of excretion, with about 25% being excreted via the kidneys, while the remainder appears in the feces [17]. Apixaban has multiple elimination pathways that make it applicable for use in patients with renal failure and liver failure.

The major metabolic pathway of apixaban metabolism is the *O*-demethylation forming a phenol metabolite; it is primarily metabolized by CYP3A4 [17]. A Phase I study evaluating the effects of ketoconazole, a known potent inhibitor of CYP3A4, on apixaban pharmacokinetics found a twofold increase of apixaban [21]. These results suggest that concomitant administration of ketoconazole or other potent 3A4 inhibitors with apixaban should be avoided unless discontinued 14 days prior to the administration of apixaban. The effects of apixaban and moderate CYP3A4 inhibitors (i.e., cimetidine, diltiazem, selective serotonin receptor inhibitors) should be used with caution, but have not been studied. The effect of apixaban and the statins, which are also metabolized by 3A4, is not known. Apixaban has not been found to interact with CYP1A2, 2C19, 2C9, and 2D6 [17].

Three studies have examined the safety and tolerability of apixaban co-administered with antiplatelet agents in a limited number of healthy volunteers: CV1855002 Part B, CV185005, and CV185015 [21]. CV185002 Part B examined the interaction of apixaban 5 mg b.i.d. with aspirin 325 mg q.d. in 17 healthy subjects. CV185005 examined the interaction of apixaban (5 mg b.i.d. or 10 mg q.d.) with clopidogrel 75 mg q.d. in 35 healthy volunteers. CV185015 examined the co-administration of apixaban 20 mg q.d. with both aspirin 162 mg q.d. and clopidogrel 75 mg q.d. in 30 healthy subjects. In CV185002 Part B and CV185005, apixaban was co-administered with the antiplatelet agent following an initial lead-in period with the antiplatelet agent alone. There were no changes in INR or activated partial thromboplastin time (aPTT) beyond those attributed to apixaban alone, nor in *ex vivo* platelet aggregation measurements or bleeding time (BT) beyond those attributed to aspirin or clopidogrel alone [19]. No major bleeding events occurred in these studies. Possible interactions between apixaban and anticoagulants, including heparin, LMWH, and IIb/IIIa inhibitors, have not been evaluated in clinical trials.

Other studies have examined other possible drug interactions in likely co-medications in a clinical setting. Patients with AF, for example, may be taking digoxin for rhythm control. There was no interaction between apixaban 20 mg once daily for 10 days and the pharmacokinetic of digoxin [22]. Administration of apixaban at doses up to 50 mg once daily for 3 days had no effect on the QTc interval in healthy patients [23]. No dose-limiting adverse effects were noted.

Apixaban causes concentration-dependent prolongation of the FXa mediated clotting assays. Apixaban inhibits Factor Xa activity in a dose-dependent manner, accompanied by mild prolongations in the INR and aPTT in a concentration-dependent fashion. The human plasma concentration required to produce a doubling of the clotting time *in vitro* is 3.6 μM for prothrombin time (PT), 7.4 μM for aPTT and 0.4 μM for HepTest [13]. However, its effect on these tests is minimal at concentrations that are likely to be therapeutic. At doses that achieved similar 80% levels of thrombosis inhibition, apixaban caused less prolongation of bleeding times than warfarin [7,16]. Finally, apixaban has no effect on human platelet aggregation [9]. The mPT clotting time assay is a modification of the standard PT assay to more sensitively detect the effects of direct FXa inhibitors. For example, apixaban 10 mg q.d. produced a mPT 2.3-fold increase from baseline, 1.2-fold elevation in INR, and only 1.1-fold increase in aPTT [21]. Given the modest changes

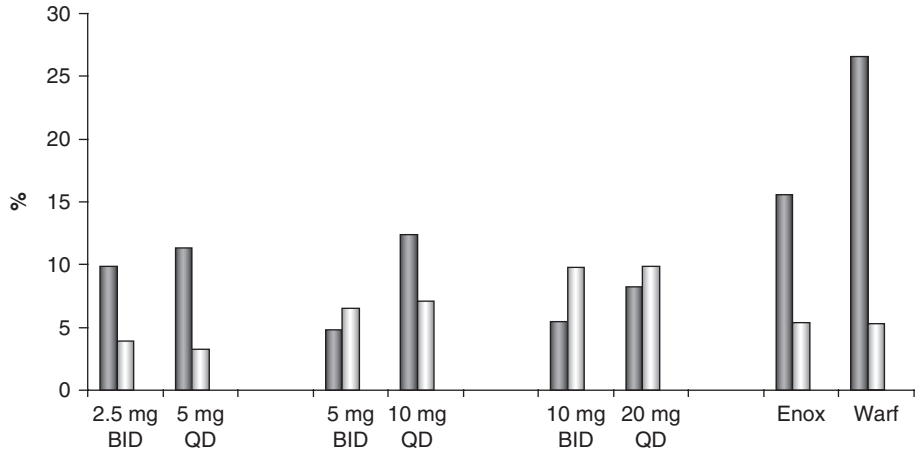

Figure 3. Incidence of adjudicated VTE plus death from any cause (dark grey bars) and total bleeding events (light bars) for b.i.d. and q.d. doses of apixaban and the comparators.
ENOX: Enoxaparin; VTE: Venous thromboembolism; Warf: Warfarin.

observed in INR and aPTT, these coagulation tests are not useful for monitoring apixaban.

5. Clinical trials

5.1 Thromboprophylaxis in orthopedic surgery

The APROPOS [24] trial was a Phase II study examining the efficacy of apixaban in preventing deep vein thrombosis (DVT) and pulmonary embolism (PE) in patients undergoing total knee replacements (TKR). The study was randomized, double-blinded, and examined 1238 patients. Patients receiving apixaban were randomized to one of six doses from 2.5 mg b.i.d. to 20 mg once daily and were compared with both enoxaparin 30 mg b.i.d. and warfarin (with a target INR of 1.8 – 3.0). Patients received treatment for 10 – 14 days, commencing 12 – 24 h after surgery with apixaban or enoxaparin, and on the evening of surgery with warfarin. The primary efficacy outcome was a composite of VTE diagnosed with mandatory venography and all-cause mortality during treatment. The primary safety outcome was major bleeding. A significantly lower incidence of DVT or PE occurred in the apixaban group than those receiving enoxaparin ($p < 0.02$) or warfarin ($p < 0.001$) (9 vs 15.6 vs 26%, respectively). At the lowest apixaban dose tested (5 mg total daily dose), the primary outcome rates for apixaban 2.5 mg b.i.d. and 5 mg q.d. were 9% (95% confidence interval [CI], 5.1 – 17.0) and 11.3% (95% CI, 5.8 – 19.4), respectively, compared with 15.6% (95% CI, 9.4 – 23.8) in the enoxaparin group and 26.6% (95% CI, 18.6 – 35.9) in the warfarin group. All apixaban groups had a lower event rate of developing DVT or PE (0 – 2.7%) than either comparator (**Figure 3**). A significant dose-related increase in the incidence of total adjudicated bleeding events was noted in the once-daily ($p = 0.01$) and twice-daily ($p = 0.02$) apixaban groups; there was no difference observed between q.d. and b.i.d. regimens. The optimal dose of apixaban was determined to be either 2.5 mg twice daily or 5 mg once daily, both of which had a promising benefit–risk profile compared with the current standards of care following TKR.

Apixaban is currently undergoing a number of Phase III studies identified in **Table 1**. At the time of this writing, ADVANCE 1 has concluded enrolment; results will be available in late 2008. The other orthopedic studies are currently ongoing.

5.2 Thromboprophylaxis in patients with medical illnesses

The ADOPT trial is a Phase III study comparing the effectiveness of apixaban to enoxaparin for the prevention of DVT in hospitalized patients (see www.clinicaltrials.gov, identifier NCT00457002). The study is randomized, double-blinded study enrolling 7502 patients. Patients receiving apixaban 2.5 mg twice daily plus placebo for 30 days will be compared to patients taking enoxaparin 40 mg subcutaneous for 6 – 14 days plus placebo for 30 days. The study is estimated to be completed in March 2009.

5.3 Thromboprophylaxis in advanced metastatic cancer

An ongoing Phase II randomized, double-blind (subject, investigator) study is currently examining the role of apixaban in preventing thromboembolic events in patients undergoing advanced or metastatic cancer treatment (www.clinicaltrials.gov, identifier NCT00320255). Demonstration of a favorable benefit–risk profile could lead to significant reduction in this serious and sometimes fatal complication of ongoing cancer and its treatment. The study is estimated to enroll 160 patients and its projected completion is October 2008. Patients in the experimental arm will receive apixaban 5 mg

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