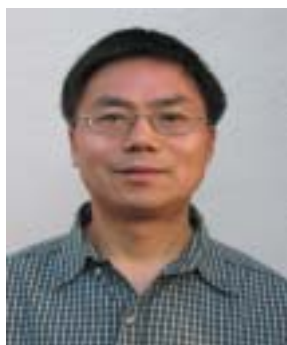


## An Overview of Antithrombotic Drug Discovery and Development

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### Abstract

*Antithrombotic drugs include anticoagulants (e.g., warfarin), antiplatelet drugs (e.g., clopidogrel) and thrombolytic agents (such as t-PA). These drugs are used in a wide range of indications for the prevention and treatment of blood clotting related diseases including acute coronary syndromes (ACS) such as heart attack, unstable angina, and myocardial infarction (MI), stroke, venous thromboembolism (VTE), and peripheral arterial occlusion. This review will focus on the issues with the existing oral antiplatelet and anticoagulant drugs and the progress made in the development of improved antithrombotic agents.*

*Both platelet aggregation and blood coagulation pathways are involved in thrombus formation (Fig. 1). In the presence of normal, non-disrupted endothelium, platelets and blood coagulation factors are not activated. However, in response to vessel injury, Von Willebrand's factor (vWF), collagen and tissue factor (TF) are exposed from endothelium, which contact with blood components and activate platelet adhesion and aggregation, as well as the intrinsic pathway of the coagulation cascade. Activation of coagulation cascade further augments platelet activation which in turn amplifies the coagulation cascade. So blood coagulation and platelet aggregation work in concert and synergism. Messing of polymeric fibrin and platelet plug renders the thrombi insoluble. Vascular injury caused by plaque rupture (such as atherosclerosis) leads to thrombi formation. Formation of thrombi in the blood vessel eventually leads to the blockage of artery or vein and causes serious damages to the tissues.*

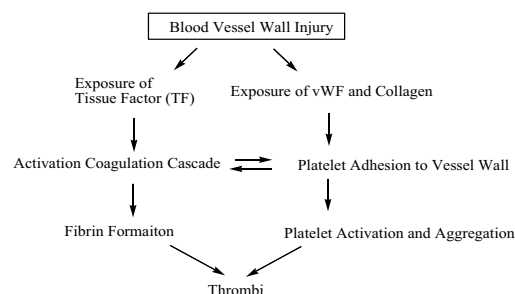


Figure 1. Illustration of Blood Coagulation and Platelet Aggregation Pathways

### I. Antiplatelet Drugs:

Under normal condition, platelets in the blood are not adhesive. Upon blood vessel wall injury, Von Willebrand's factor (vWF) is exposed from the sub-endothelium and then binds to its receptor, glycoprotein Ib-IX in the platelet membrane. Such receptor binding leads to platelet adhesion to the vessel walls, platelet activation, and subsequent platelet shape change. The activated platelets then degranulate the dense granules and release its content, ADP (adenosine diphosphate). ADP then binds to its platelet receptors (P2Y1 and P2Y12).

The binding of ADP to its receptors exposes the glycoprotein GPIIb-IIIa receptor on the platelet by conformational change. One molecule of fibrinogen in the blood will bind to two GPIIb-IIIa receptors. This cross-linking of platelet

GPIIb-IIIa receptors with fibrinogen is the final pathway of platelet aggregation and leads to blockage of blood vessels (Fig. 2). Therefore, ADP is a potent agonist of platelet aggregation and ADP receptor antagonists can prevent platelet aggregation and can be used as therapeutic agents for treatment and prevention of arterial thrombosis associated cardiovascular diseases.

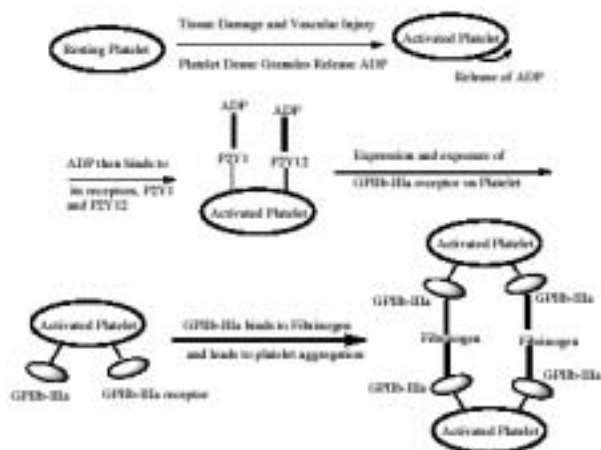


Figure 2. Platelet Aggregation Mediated by the Binding of ADP to its Receptor P2Y1 and P2Y12

There are three receptors present on the surface of platelets to which ADP can bind: P2Y1, P2Y12 and P2X. However, only P2Y1 and P2Y12 receptors appear necessary for the full platelet aggregation response. P2Y12 receptor is the clinically proven target for the development of anti-platelet agents.

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Clopidogrel (Plavix) is the most widely prescribed antiplatelet agent in clinic<sup>1</sup>. Clopidogrel itself is not ADP receptor antagonist and does not have any anti-platelet activity in vitro. Instead, clopidogrel undergoes metabolic biotransformation to exhibit its antiplatelet aggregation activity (Fig. 3). It is oxidized by cytochrome P450 in the liver to the intermediate metabolite 2-oxo-clopidogrel, which also does not have activity in vitro. Hydrolysis of 2-oxo-clopidogrel leads to the reactive metabolite, a thio reagent. This reactive metabolite then forms a covalent disulfide bond (S-S) with the extra-cellular Cys residues of ADP P2Y12 receptor on the platelet, thus

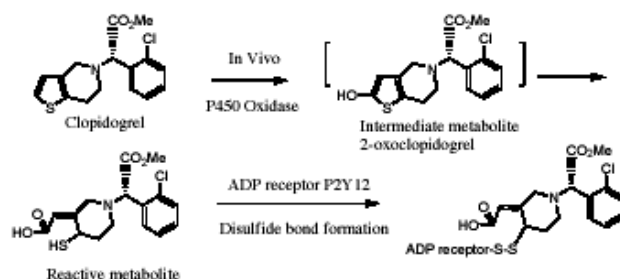


Figure 3. Clopidogrel Mechanism of Action

effectively antagonizing the P2Y12 receptor activity, since ADP can not bind to the P2Y12 receptor covalently modified by the reactive metabolite. Therefore, the half-life of antiplatelet activity of clopidogrel is dependent on the elimination of the covalently modified platelets, which has half-life of about one week.

Clopidogrel is given orally once a day with recommended loading dose of 300 mg (due to the slow onset of action), and maintenance dose of 75 mg. Clopidogrel is about 50% bioavailable as determined by its carboxylic acid metabolite and 94% of which reversibly binds to human plasma proteins. After oral administration of 75 mg for several days, about 85% of clopidogrel is rapidly hydrolyzed by esterase to the corresponding acid, inactive as ADP antagonist and no parent clopidogrel can be detected. Only 2% of clopidogrel administered is metabolized to the reactive metabolite. Dose dependent inhibition of platelet aggregation can be seen 2 hours after single oral doses of clopidogrel. Repeated doses of 75 mg clopidogrel per day inhibit ADP-induced platelet aggregation on the first day, and inhibition reaches steady state between Day 3 and Day 7. At steady state, the average inhibition level observed with a dose of 75 mg clopidogrel per day was between 40% and 60%. Platelet aggregation and bleeding time gradually return to baseline values after treatment is discontinued, generally in about 5 days (slow offset of action).

Clopidogrel has been proved to provide significant benefit for treatment and/or prevention of severe cardiovascular diseases. Its efficacy in the secondary prevention of MI, stroke and vascular events was compared to aspirin in the CAPRIE trial. Treatment with clopidogrel alone results in a risk reduction of 8.7% than aspirin alone. This is quite statistically significant and remarkable since aspirin itself already has a marked effect compared with placebo.

The concept of dual anti-platelet therapy is conducted in the CURE trail. When combined with aspirin (75-320 mg/day, QD), clopidogrel (300 mg+ 75 mg/day) showed 20% relative risk reduction in heart attack, MI, stroke and cardiovascular

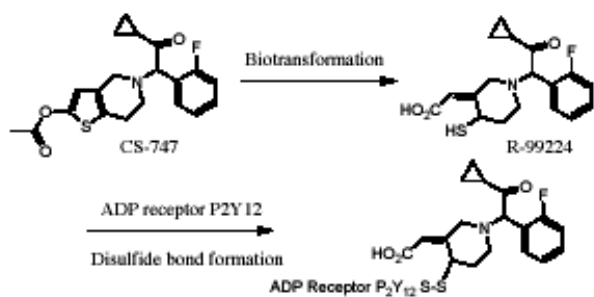
death relative to aspirin alone in patients with acute coronary syndromes (ACS) (unstable angina or non-ST segment elevation myocardial infarction) and a 31% reduction in these end-points in individuals who were undergoing angioplasty or stent placement.

Due to the unique molecule mechanism of action, clopidogrel has several disadvantages such as irreversibility, prolonged half-life (as long as the platelet in the plasma), slow onset which requires high loading dose and offset of action, and inferior inhibition of platelet aggregation (maximum 40-60% inhibition even at higher doses) and potential side effect (although rare) of thrombotic thrombocytopenic purpura (TTP).

### New ADP Receptor Antagonists in Development

Due to clopidogrel's disadvantages, small molecule and especially reversible ADP receptor P2Y<sub>12</sub> antagonists are actively pursued by pharmaceutical companies as potential clopidogrel replacement or supplement.

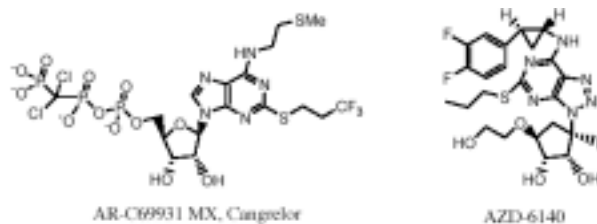
Sankyo-Lilly's CS-747 is in Phase II clinical development<sup>2</sup>. CS-747 basically mimics the biotransformation of clopidogrel but with greater potency and it also produces an active metabolite, R-99224. CS-747 is an orally active and a potent anti-platelet and anti-thrombotic agent with a rapid onset and long duration of action. It is about 10 times more potent than clopidogrel. In a rat thrombosis model, the ED<sub>50</sub> for CS-747 is 0.68 mg/kg, while it is 6.2 mg/kg for clopidogrel. AR-C69931 MX (Cangrelor), discovered by AstraZeneca, is an intravenous anti-platelet agent<sup>3,4</sup>. It acts directly and reversibly



on the P2Y<sub>12</sub> platelet receptor with K<sub>i</sub> = 0.4 nM. It inhibits platelet aggregation more effectively than clopidogrel with IC<sub>50</sub> = 0.4 nM in ADP induced platelet aggregation assay. Cangrelor caused >90% inhibition of platelet aggregation when dosed with 1-4 mg/min/kg infusion in health human volunteers and only prolong bleeding time by 1.4 fold.

Cangrelor has a very short T<sub>1/2</sub> of 10 min with very fast onset and offset of action, and thus is very suitable for acute

indications (cath lab angioplasty, cardiac surgery and acute ACS). Additionally, Cangrelor does not cause excessive bleeding complications even with >90% inhibition of platelet aggregation. This might be a major advantage over GPIIb-IIIa antagonist, such ReoPro and Integrilin. Phase II clinical trial results have been published and it is clear that cangrelor has a great potential to be developed as an intravenous antiplatelet agent in acute clinical settings with wide safety margin (efficacy and bleeding).

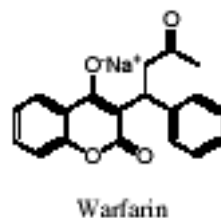


AstraZeneca has a 2<sup>nd</sup> generation, reversible and orally active ADP receptor antagonist (AZD-6140) in Phase II clinical development. It binds to P2Y<sub>12</sub> receptor with a K<sub>i</sub> value of 2 nM and IC<sub>90</sub> = 400 nM in human platelet aggregation assay. Phase I studies indicate it is a safe antiplatelet agent with predictable pharmacokinetics. AZD-6140 has F = 38 and 86% in rats and dogs. Predicted human dose is < 0.5 mg/Kg.

Inspire, Millennium, and Portola Pharmaceuticals are in the early stage of discovery and development of reversible P2Y<sub>12</sub> receptor antagonists. Several patent applications have been published.

## II. Anticoagulant Drugs

Warfarin (Coumadin) is the drug of choice as oral anticoagulant. It is used clinically for the prophylaxis and/or treatment of many thrombotic diseases such as deep venous thrombosis and DVT associated with orthopedic surgery, pulmonary embolism, myocardial infarction, thromboembolic complications (stroke) associated with atrial fibrillation and cardiac valve replacement, and to reduce the risk of death and recurrent myocardial infarction.



Warfarin works as a vitamin K antagonist. During the syntheses of blood clotting factors (prothrombin, factor VII, factor IX, and factor X) in the liver, the proteins must be post-translationally modified in order to become biologically active. The glutamate residues are carboxylated in presence of vitamin K,  $O_2$ ,  $CO_2$  and the enzyme carboxylase, to form  $\gamma$ -carboxyglutamate (Gla) residues. Vitamin K is an essential cofactor for the carboxylation process. Without vitamin K, the carboxylation does not occur and the synthesized proteins are biologically inactive. The newly-formed Gla residues chelate strongly and selectively with  $Ca^{2+}$  and the latter forms ion bridges to anionic phosphate head groups of phospholipid membrane surfaces. Binding at the phospholipids membrane surface is a critical step in the process of activation of the clotting factors (Fig. 4).

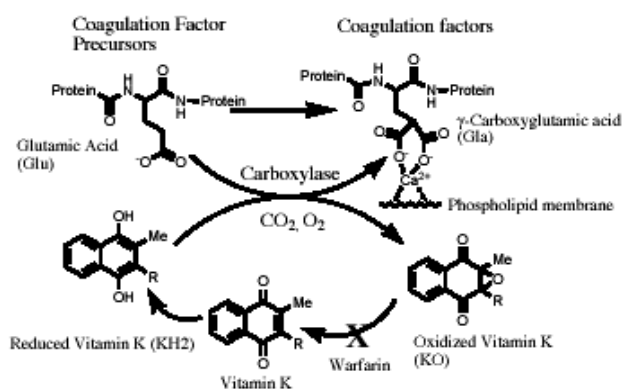


Figure 4. Warfarin Mechanism of Action

As seen in Figure 4, the carboxylation process involves vitamin K cycle. In this cycle, vitamin K is reduced by vitamin K reductase to its hydroquinone form, vitamin KH<sub>2</sub>, which then catalyzes the carboxylation process and is converted to its epoxide (KO). This is then converted back to vitamin K by vitamin KO reductase. Warfarin works by blocking the activity of the vitamin KO reductase enzyme so that vitamin KO can not be recycled back to vitamin K. This leads to a depletion of vitamin KH<sub>2</sub>, thereby limiting the carboxylation process and the production of active clotting factors. Therapeutic dose of warfarin decreases the total amount of active forms of clotting factors by 30-50%.

Warfarin was discovered through observation that livestock eating spoiled sweet clover in Wisconsin and Canada were bleeding to death. This observation led to the isolation of dicoumarol in 1940, an anticoagulant from the clover. Later in 1948, warfarin was synthesized as more potent analog and used as the ideal rat poison. In 1951, a navy recruit attempted suicide by overdosing 567 mg of warfarin but survived. His full recovery induced research into the anticoagulant potency of warfarin in human.

Warfarin was introduced commercially 1954 in man as a blood thinner. Today, warfarin is the only prescribed oral anticoagulant and is far superior to dicoumarol.

Warfarin exists as a mixture of two chiral enantiomers. It is completely bioavailable (100%) and is almost 99% plasma protein bound. It has a long terminal  $T_{1/2}$  of one week and the effective  $T_{1/2}$  is about 20-60 hrs. Elimination of warfarin is almost entirely by metabolism. The recommended dose is in the range of 2-10 mg and INR in the range of 1.5-5 range (depending on indication).

Warfarin is a highly effective anticoagulant agent. It significantly reduced the risk of stroke by more than 60% in atrial fibrillation patients. Long-term, low-intensity warfarin therapy can reduce the risk of recurrent venous thromboembolism by 64%.

But warfarin has serious problems such as narrow therapeutic index (mechanism based), drug-drug, and drug-food interactions. Warfarin can cause serious and fatal bleeding complications if not dosed properly. Thus, it requires close and routine coagulation monitoring, and dose titration and adjustment. Additionally, warfarin has slow on-set and offset of action.

### New Anticoagulant Agents: Potential Warfarin Replacement

Development of safer anticoagulant agent with better therapeutic index has been a major quest for pharmaceutical companies due to the unmet medical needs and huge market potential.

One strategy to discover warfarin replacement is to block the blood coagulation cascade. Normally, clotting factors only circulate in the blood in their inactive forms. However, upon injury of blood vessel walls such as plaque rupture, tissue factor is exposed to the blood and this is the initial trigger of blood coagulation cascade.

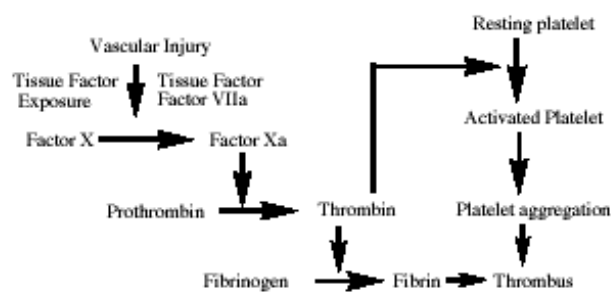


Figure 5. Blood Coagulation Cascade

As shown in Figure 5, small amount of factor VIIa circulating in the blood in combination with tissue factor exposed from endothelium will cleave inactive factor X to active Xa. Factor Xa then chops inactive prothrombin to active thrombin. Thrombin then cleaves soluble plasma fibrinogen to fibrin which undergoes cross-linkage reaction to form insoluble fibrin polymers. Additionally, thrombin is also the most potent platelet aggregation agonist.

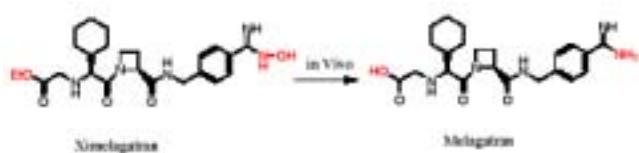
Two major strategies actively pursued by pharmaceutical industry are the development of thrombin and factor Xa inhibitors, since both thrombin and factor Xa are the key coagulation enzymes which are responsible for blood clotting formation.

### Thrombin Inhibitors:

The most advanced potential Warfarin replacement therapy is ximelagatran (Exanta) developed by AstraZeneca<sup>5</sup>. Ximelagatran is the first new oral anti-coagulant to reach late clinical development in almost 60 years since the development of warfarin. It is a double prodrug and is required to be metabolized in the liver to the active metabolite, melagatran. Melagatran is a direct and potent thrombin inhibitor and has short  $T_{1/2}$  in man.

Ximelagatran is approved in Europe already for the prevention of venous thromboembolism (VTE) for patients undergoing total hip or total knee replacement who are at high risk of venous thromboembolism.

The practical benefits of ximelagatran include: fixed dose oral administration (twice daily); rapid onset of action; no need for dose titration; no need for routine blood coagulation monitoring; and lack of drug/drug and drug/food interactions



In Phase III THRIVE trial (treatment of VTE), ximelagatran was shown to provide superior efficacy to warfarin in preventing total venous thromboembolism (VTE) and all-cause mortality (22.5 per cent ximelagatran vs. 31.9 per cent warfarin,  $p < 0.001$ ), with no significant difference in bleeding compared with dose-adjusted warfarin

In the largest ever Phase III stroke prevention SPORTIF trial in AF patients, it is also effective in preventing stroke in AF patients. The results show that fixed dose twice daily 36 mg

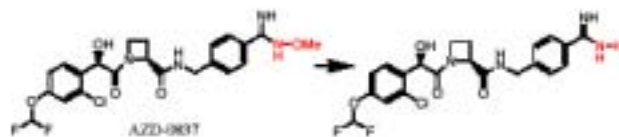
oral ximelagatran compares favorably with dose-adjusted warfarin in preventing stroke and systemic

embolic events in patients with atrial fibrillation (AF). A statistically significant relative risk reduction (RRR) of 41% was demonstrated for ximelagatran compared with well-controlled warfarin in patients.

Based on these clinical data, ximelagatran is a promising antithrombotic agent to replace warfarin due to the safety window and lack of routine coagulation monitoring. However, ximelagatran suffers from some drawbacks such as prodrug nature, twice a day dosing regime, transient liver enzyme elevation and potential drug-drug interactions.

AstraZeneca also has a backup compound (AZD-0837) in Phase I development. AZD-0837 is a mono-prodrug (methoxy-amidine). However, no clinical data have yet been reported.

Merck, Johnson and Johnson, and GlaxoSmithKline all have thrombin inhibitors in early stage clinical development.



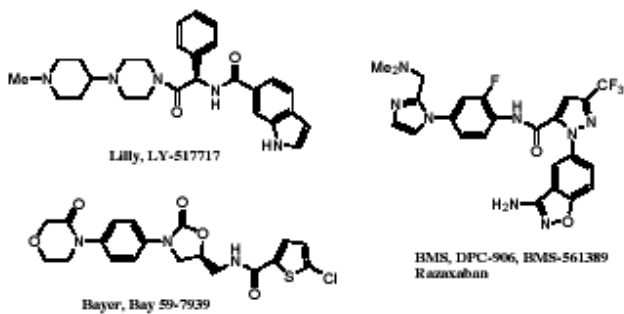
### Factor Xa inhibitors:

There are also great hopes for orally active inhibitors of factor Xa; a serine protease that plays a central role in the process of coagulation and platelet activation.

Pharmaceutical companies have pursued oral factor Xa inhibitors for almost 10 years and it is only until recent that promising compounds have been progressed into Phase II clinical trial.

Bayer's BAY 597939, Lilly's 517717, Bristol-Myers Squibb's DPC-906 (Razaxaban), and Millennium's MLN1021 are in the early stage of clinical development.

Promising Phase II clinical data for Razaxaban has been reported. Results showed that razaxaban administered at 25 mg twice daily had similar safety compared with the standard treatment, enoxaparin 30 mg twice daily. Razaxaban at 25-mg dose bid PO has potential for increased efficacy and similar safety compared to current standard treatment. However, at >50 mg does, it caused excess bleeding



complications. Bayer also disclosed the PK/PD profiles of Bay 59-7939 in human. It has predictable pharmacokinetics, offers predictable anticoagulation and is well tolerated with a rapid onset of action. The drug achieved dose-dependent effects, with close correlation between pharmacokinetic (PK) and pharmacodynamic (PD) parameters. However, it has short  $T_{1/2}$  and suitable for twice a day dosing. Lilly's LY-517717's preclinical data are promising as once a day anticoagulant agent. But it is significantly less potent than DPC-906 and/or Bay 59-7939.

### Conclusion:

Antithrombotic drug discovery efforts to replace warfarin and clopidogrel remain as one of the most challenging missions in the pharmaceutical industry. But as evidenced from successful Exanta story, persistence will eventually prevail in developing new anticoagulant and anti-platelet drugs with much better safety and/or efficacy profiles by targeting the specific ADP receptor P2Y<sub>12</sub> and enzymes (thrombin and factor Xa).

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