Antithrombotic Therapy: Prevention and Treatment of Atherosclerosis and Atherothrombosis

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Abstract

Atherosclerosis is a multifactorial vascular disease that develops in the course of a lifetime. Numerous risk factors for atherosclerosis have been identified, mostly inflicting pro-inflammatory effects. Vessel injury, such as occurring during erosion or rupture of atherosclerotic lesions triggers blood coagulation, in attempt to maintain hemostasis (protect against bleeding). However, thrombo-inflammatory mechanisms may drive blood coagulation such that thrombosis develops, the key process underlying myocardial infarction and ischemic stroke (not due to embolization from the heart). In the blood coagulation system, platelets and coagulation proteins are both essential elements. Hyperreactivity of blood coagulation aggravates atherosclerosis in preclinical models. Pharmacologic inhibition of blood coagulation, either with platelet inhibitors, or better documented with anticoagulants, or both, limits the risk of thrombosis and may potentially reverse atherosclerosis burden, although the latter evidence is still based on animal experimentation.

Patients at risk of atherothrombotic complications should receive a single antiplatelet agent (acetylsalicylic acid, ASA, or clopidogrel); those who survived an atherothrombotic event will be prescribed temporary dual antiplatelet therapy (ASA plus a P2Y12 inhibitor) in case of myocardial infarction (6-12 months), or stroke (<6 weeks), followed by a single antiplatelet agent indefinitely. High risk for thrombosis patients (such as those with peripheral artery disease) benefit from a combination of an anticoagulant and ASA. The price of gained efficacy is always increased risk of (major) bleeding; while tailoring therapy to individual needs may limit the risks to some extent, new generations of agents that target less critical elements of hemostasis and coagulation mechanisms are needed to maintain efficacy while reducing bleeding risks.

Keywords

Anticoagulants · Antiplatelet therapy · Aspirin · Atherosclerosis · Atherothrombosis · Clopidogrel · Coagulation · Platelets · Thrombosis

1 Atherogenesis and the Role of Blood Coagulation Components

Atherosclerosis is a multifactorial vascular disease that develops in the course of a lifetime. Numerous risk factors for atherosclerosis have been identified, mostly inflicting pro-inflammatory effects, hence, the term “chronic inflammatory disease” (Ross 1999). The blood coagulation system has a primary role in maintaining hemostasis, preventing fatal bleeding (Spronk et al. 2003a). A second function relates to wound healing in a broader sense. Blood coagulation becomes activated in response to any vascular injury causing contact between the vascular matrix and/or cells with blood. This clotting process likely is meant to seal the wound surface and help to repair the underlying wound bed. Platelets, coagulation, and fibrinolysis factors, as well as fibrin are key elements in this wound healing process, and the administration of some of these components (platelets, fibrinogen) is also studied in clinical trials on wound healing of the skin (Hoffman 2018; Opneja et al. 2019). In the vasculature, pro-atherogenic changes of the vascular endothelium, involving endothelial cell (EC) activation, dysfunction, or damage (exposing subendothelial matrix), trigger the coagulation system in an attempt to vascular wound healing.

1.1 Blood Coagulation: Impact on Vascular Endothelial Cells

The blood coagulation system consists of different pathways that together provide the hemostatic plug or, in pathological situations, the thrombus. Platelets and coagulation proteins act in concert to build the fibrin-platelet clot on top of the damaged vessel wall, while the fibrinolytic system helps to limit clot formation and acts to restore blood flow upon (partial) clot lysis.

In atherogenesis, early inflammatory endothelial perturbation (Gimbrone Jr. and Garcia-Cardena 2016) may trigger activation of platelets that together with leukocyte populations provide a first thrombo-inflammatory response to injury (Fig. 1) (Messner and Bernhard 2014). Activation of platelets (but also other cells) yields extracellular vesicles (EVs) that promote thrombo-inflammatory reactions, amplifying fibrin formation (Badimon et al. 2017). Extracellular vesicles are particles that are naturally released from nearly all kinds of cells; carry a cargo of proteins, RNA, and lipids from the parent cell; and are thus considered to be key components in cell-cell communication. Repeated and/or ongoing inflammatory pressure (endogenous factors like oxidized lipoproteins, glycated end products, homocysteine, etc. and exogenous factors like smoking and other sources of particulate matter) challenges the vascular endothelium to become activated and permeable for inflammatory cells, lipids, and other toxic components (Mozaffarian et al. 2008).

Disruption of the endothelial cell barrier is induced or aggravated by pro-inflammatory cytokines, metalloproteases, and cellular enzymes like elastase and trypsin as well as by the influence of coagulation serine proteases like factor Xa and thrombin, acting on protease-activated receptors (PARs) (Coughlin 2005; Posma et al. 2016; Ruf 2018). Physiologically, PARs are expressed at the endothelial cell surface to mediate endothelial *protective* effects of activated protein C (APC), through activation of PAR1 (Mosnier et al. 2012). Besides activation of protein C, the thrombin-thrombomodulin (TM) complex also converts plasma pro-carboxypeptidase B2 (proCPB2 or thrombin-activatable fibrinolysis inhibitor, TAFI). Activated TAFI (TAFIa) inhibits plasmin formation and hence stabilizes fibrin clots by inhibiting plasmin generation and fibrinolysis (Fujiwara et al. 2012; Morser et al. 2010; Myles et al. 2003; Shao et al. 2015). TAFIa also has anti-inflammatory properties including the inactivation of pro-inflammatory mediators like bradykinin, anaphylatoxins C3a and C5a, and thrombin-cleaved OPN (Myles et al. 2003; Naito et al. 2013; Nishimura et al. 2007; Relja et al.

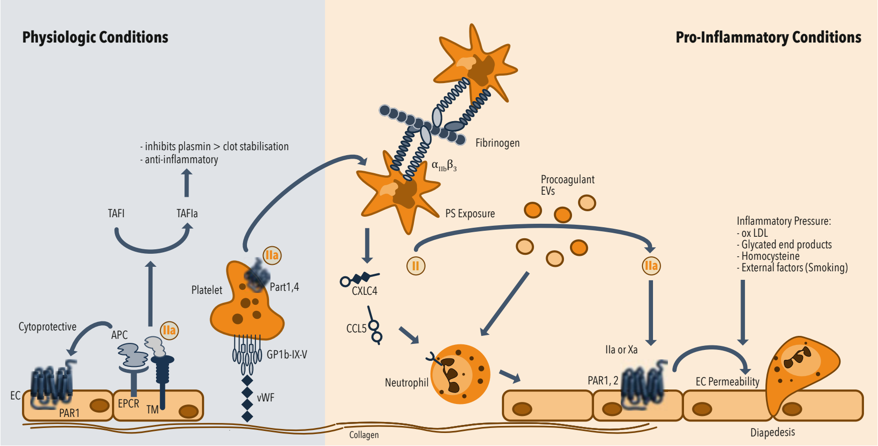

Fig. 1 Interactions of platelets, leucocytes, coagulation factors, and vascular endothelial cells in atherogenesis and atherothrombosis. Under *physiologic conditions*, thrombin (IIa) supports endothelial cell integrity through endothelial cell-mediated APC and TAFIa generation. PARs are expressed at the endothelial cell (EC) surface to mediate cytoprotective effects of APC through activation of PAR1. APC is activated by the thrombin-thrombomodulin (TM) complex, which also converts TAFI into activated TAFI (TAFIa), which has anti-inflammatory properties. Under *pro-inflammatory conditions*, several pro-inflammatory cytokines alter PAR expression patterns and downregulate the protective cellular receptors like TM and EPCR. The result is a shift to prothrombotic and offensive functions of PAR-EC interactions. Thrombin-induced PAR1 activation in combination with endogenous factors like smoking and oxidized lipoproteins challenges the vascular endothelium to become permeable for inflammatory cells. Damage of the endothelial layer results in exposure of highly reactive subendothelial proteins, such as collagen. Under arterial shear stress, vWF bound to collagen enables platelet adhesion via the glycoprotein (GP)Ib-IX-V complex. Activation of platelets yields extracellular vesicles that promote thrombo-inflammatory reactions, including attraction of leukocytes. Activated platelets deposit chemokines, such as CXCL4 and CCL5, on the inflamed endothelium, thereby recruiting leucocytes, which bind to platelets adhering to the (sub)endothelium. Platelets and leukocytes secrete pro-inflammatory and pro-angiogenic factors that further support atherogenesis. Traces of thrombin activate platelets through PAR1 and PAR4. These activated platelets provide a procoagulant phosphatidylserine (PS)-rich surface on which coagulation factors can gather, ultimately leading to the conversion of prothrombin (II) to thrombin (IIa). Thrombin-induced PAR activation induces inflammatory damage to the vascular endothelium. *EC* endothelial cells, *APC* activated protein C, *PAR* protease-activated receptor, *EPCR* endothelial cell protein C receptor, *TM* thrombomodulin, *TAFI* thrombin-activatable fibrinolysis inhibitor, *GP1b-IX-V* glycoprotein Ib-IX-V, *vWF* von Willebrand factor, *CXCL4* chemokine ligand 4, *CCL5* chemokine ligand 5, *PS* phosphatidylserine, *EV* extracellular vesicle

2013). This way, under physiologic conditions thrombin supports endothelial cell integrity through endothelial cell-mediated APC and TAFIa generation.

Under inflammatory pressure, pro-inflammatory cytokines alter PAR expression patterns and downregulate protective cellular receptors like TM and endothelial cell protein C receptor (EPCR) (Esmon 1995, 2014). The result is a shift from anticoagulant (and protective) to prothrombotic (and offensive) functions of serine protease-PAR-EC interactions (Fig. 1). In the case of PAR-1, the shift from protective (APC induced) to inflammatory (thrombin induced) is referred to as "biased" signaling (Griffin et al. 2015).

1.2 Platelets and Extracellular Vesicles

Mouse studies provide mechanistic insight in the pro-atherogenic functions of blood components including platelets, EVs, and coagulation proteins. Platelets and platelet-derived EVs are important messengers of various mediators including microRNAs that modify inflammation and atherogenesis. A range of studies provided evidence for involvement of EVs on various key pathways in atherogenesis, including inflammation, calcification, and cell trafficking (Badimon et al. 2017; Paone et al. 2019; Alique et al. 2018; Bouchareychas and Raffai 2018; Miller et al. 2016). Ongoing research is addressing ways to utilize some of these functions of EVs including transportation of microRNA for potential therapeutic purposes in atherosclerosis (Yin et al. 2015). Majority of EVs are platelet-/megakaryocyte-derived and contain cytokines, RNA species, coagulation factors, etc. EVs can interact with, e.g., leukocytes and regulate activity (Vasina et al. 2013).

Platelets roll and adhere to activated endothelium (Schulz and Massberg 2012). Platelets promote the differentiation and activation of leukocytes (Lievens and von Hundelshausen 2011). Activated platelets deposit chemokines, mainly chemokine ligand 4 (CXCL4) and chemokine ligand 5 (CCL5), on the inflamed endothelium, thereby recruiting leukocytes and exacerbating atherosclerosis (von Hundelshausen et al. 2005; Huo et al. 2003). Also, secreted chemokines attract leukocytes, which bind to platelets adhering to the (sub) endothelium. Platelets and leukocytes secrete pro-inflammatory and pro-angiogenic factors that further support atherogenesis (Semple et al. 2011). Multiple (receptor) interactions between platelets-leukocytes and platelets-vessel wall have been observed in inflammatory conditions such as atherosclerosis (Koupenova et al. 2018; Koenen 2016). Platelet-neutrophil interactions and the release of neutrophil activation products including neutrophil extracellular traps (NETs) may also trigger coagulation.

Cross talk between platelets and coagulation is another important mechanism that operates at different levels. Traces of thrombin activate platelets through PAR1, with PAR4 activation as a delayed, secondary mechanism. Activated platelets provide a phosphatidylserine (PS)-rich surface on which coagulation reactions take place (tenase and prothrombinase complex formation). Upon induction with strong agonists such as a combination of thrombin and collagen, PS exposure allows binding of Gla domain-containing coagulation factors which enhances the activity of coagulation factor complexes. This procoagulant platelet response is supported by the secretion of coagulation factors (prothrombin, Factor V, Factor 8 transcript, Factor XIII, and fibrinogen) and modified by anticoagulation factors (e.g., antithrombin, tissue factor pathway inhibitor (TFPI), protein S).

Platelet-derived factor V may be an important determinant of local prothrombinase formation, driving atherogenesis (Ren et al. 2017). Additional factors that are secreted by platelets are tissue factor (TF) (the origin still somewhat controversial) and protein disulfide-isomerase (PDI) that acts to de-encrypt TF in an active conformation. Polyphospate (PolyP) is secreted that may activate factor XII. Platelet-bound factor XI drives coagulation in an angiotensin-dependent manner (Kossmann et al. 2017). Another mechanism that bridges platelets and coagulation is by binding coagulation factors via the glycoprotein (Gp) complexes GPIb-V-IX, GPIIb/IIIa, and GPVI (Swieringa et al. 2018). Finally, platelets alter fibrin structure (via regulating local thrombin concentration) and mediate clot contraction. This process involves different platelet populations, including procoagulant platelets that primarily stimulate fibrin formation in the periphery of the clot, associated with clot contraction (Nechipurenko et al. 2019). In the course of this process, thrombin-activated platelets become fragmented, a process that limits further dissemination of activated platelets (Kim et al. 2019).

1.3 Antiplatelet Agents and Atherosclerosis

From the described thrombo-inflammatory mechanisms, one may infer that platelet inhibition with antiplatelet medication could potentially attenuate atherogenesis and atherosclerosis. In addition to the inhibition of thromboxane A2 (TXA2) production, aspirin (acetylsalicylic acid (ASA) being the active component) increases platelet nitric oxide (NO) synthesis, protects NO from its inactivation, and improves endothelial dysfunction (Russo et al. 2017). Aspirin also has anti-inflammatory effects, but whether the doses used to prevent platelet aggregation are sufficient to produce meaningful anti-inflammatory effects in humans remains uncertain. In mice, low-dose aspirin improves vascular inflammation and stabilizes plaques (Cyrus et al. 2002) or limits plaque severity, maybe related to reduced fractalkine levels (Liu et al. 2010). In humans, aspirin has been shown to reduce the levels of pro-inflammatory cytokines including interleukin (Il)-6 and monocyte colony-stimulating factor (Ikonomidis et al. 1999) and to protect the endothelium against inflammatory challenge (Kharbanda et al. 2002). In spite of these and other possible protective effects, the net effect on human atherosclerosis remains uncertain (Tousoulis et al. 2016). With regard to thrombosis, literature points to effects of aspirin on fibrin clot formation and stability that may contribute to its antithrombotic action (Gurbel et al. 2019).

Evidence for a role of the P2Y12 receptor in atherogenesis comes from studies in mice with a double apoE and P2Y12 deletion genotype that show reduced lesion area, increased fibrous content at the plaque site, and decreased monocyte/macrophage infiltration of the lesions in the double knockout animals as compared to control apoE$^{-/-}$ mice (Li et al. 2012; West et al. 2014). The P2Y12 inhibitor clopidogrel reduced levels of p-selectin, e-selectin, monocyte chemoattractant protein-1, and platelet-derived growth factor β, reduced macrophage and T-cell infiltration in atherosclerotic lesions, and delayed the development and progression of de novo atherosclerosis (Heim et al. 2016).

In preclinical models clopidogrel attenuated atherosclerosis (Heim et al. 2016; Afek et al. 2009; Takeda et al. 2012), although these protective effects were not evident in other studies (Schulz and Massberg 2012; West et al. 2014). Similarly, ticagrelor reduced the initiation of atherosclerosis in apoE$^{-/-}$ mice (Schirmer et al. 2012) although this effect was not observed by West et al. (West et al. 2014). Discrepancies between the positive and negative studies may be related to drug dose, timing, and duration of treatment (Nylander and Schulz 2016). Furthermore, stabilization and reduced necrotic core were seen in mice with established plaques at 20 weeks of age, for ticagrelor (Buchheiser et al. 2011).

1.4 Coagulation Proteases

Coagulation proteins are for the large part synthesized in the liver and secreted in blood after posttranslational modification. Many of the proteins are zymogens (or proenzymes) that require limited proteolysis to become fully active. It requires one or more triggers like tissue factor (TF) and amplifiers like thrombin, to activate a cascade response yielding fibrin formation at the sites of injury. The liver also makes a series of anticoagulant proteins including antithrombin and protein C that act to dampen the coagulation cascade physiologically. A fairly large number of coagulation proteins are also expressed outside the liver; this can be *constitutive* (like protein S that is for 50% synthesized in vascular EC; tissue factor pathway inhibitor (TFPI) that is partially liver, partially megakaryocyte derived; factor VIII from liver sinusoidal endothelial cells; TF expressed in fibroblasts of the arterial adventitia) or *inducible* under conditions of inflammation: an example is production of TF in blood leukocytes (monocytes/macrophages, neutrophils) and possibly platelets, vascular smooth muscle cells (VSMC), and other cell types (Grover and Mackman 2018). Other proteins like factor VII (Wilcox et al. 2003) and factor X (Sanada et al. 2017) can be expressed by VSMC and fibroblasts. Proteins like factor XI, XII, and prothrombin have been demonstrated in extravascular localizations including atherosclerotic vessels, but whether these are deposited or locally synthesized remains unknown (Borissoff et al. 2010; Wilcox 1994; Soardi et al. 1961). Of interest, coagulation enzymes including thrombin and aPC participate in the control of hematopoiesis in the bone marrow (Nguyen et al. 2018), which may be relevant for the response to stress situations including inflammation.

Within atherosclerotic lesions, coagulation proteins like TF and factors VII and X contribute to form catalytic complexes driving thrombin and fibrin formation (Borissoff et al. 2010). The local formation of fibrin and its split products are modifiers of angiogenesis and cell trafficking within the plaques (Fay 2004; Binder et al. 2017; Badimon and Vilahur 2014; Spronk et al. 2018). The important role of plasminogen activator (receptor)-plasmin and plasminogen activator

1 (PAI-1) system in regulating controlled proteolysis in atherosclerosis, with impact on inflammation (Foley 2017), falls beyond the scope of this chapter.

In general, hypercoagulability and/or thrombophilia has a modest but detectable effect on atherosclerosis, at least coronary artery disease (CAD), peripheral artery disease (PAD), and other manifestations of atherosclerosis (Borissoff et al. 2012; Kleinegris et al. 2013; Lowe and Rumley 2014). This association is not unequivocal, and the lack of protective effect of specific coagulation deficiencies such as in hemophilia, which is not associated with reduced burden of atherosclerosis, argues against very strong influence of coagulation activity in human atherogenesis (Biere-Rafi et al. 2012; Kamphuisen and ten Cate 2014). Nevertheless, evidence from preclinical models is quite striking (Borissoff et al. 2011). Coagulation proteases tend to aggravate atherogenesis toward atherosclerosis in mouse models of atherosclerosis, mostly apoE$^{-/-}$ mice, under pressure of a Western-type diet. In these mice, any hypercoagulable effect introduced by backcrossing apoE$^{-/-}$ mice on a specific mouse with increased procoagulant tendency such as factor V Leiden, or TM$^{\mathrm{Pro/pro}}$ mutation, results in worsening atherogenesis. In contrast, mice with a less procoagulant phenotype like FXI$^{-/-}$, FVII$^{+/-}$, or some of the FVIII$^{-/-}$ traits tend to be protected against atherosclerosis progression (Borissoff et al. 2011; Shnerb Ganor et al. 2016; Mackman 2016).

Application of direct oral anticoagulants that inhibit thrombin (dabigatran) or factor Xa (rivaroxaban) slows down atherosclerosis in apoE$^{-/-}$ mice. At least five of such studies document protection against progression of atherosclerosis, including improved plaque stability, while on dabigatran (Lee et al. 2012; Kadoglou et al. 2012; Borissoff et al. 2013; Pingel et al. 2014; Preusch et al. 2015). One protective mechanism may involve the dabigatran-mediated attenuation of pro-inflammatory M1 macrophages in the vessel wall, observed in Lldr$^{-/-}$ mice (Feldmann et al. 2019).

Detrimental effects of dabigatran exposure have also been published; in a diabetic rat model, dabigatran exposure caused increased platelet reactivity, increased coronary lipid deposition, as well as increased PAR4 expression in vessels (Scridon et al. 2019).

Rivaroxaban, the direct factor Xa inhibitor, also attenuates atherosclerosis in apoE$^{-/-}$ mice (Zhou et al. 2011; Hara et al. 2015) or even reverses existing atherosclerosis in apoE$^{-/-}$ mice (Posthuma et al. 2019). Overall, most studies suggest that applying direct oral anticoagulants (DOAC) in atherogenic mice slows down but also stabilizes atherosclerotic lesions and alter the plaque phenotype toward more (Borissoff et al. 2013) or sometimes less stability features (Seehaus et al. 2009), the latter depending on age and sex and probably additional factors like diet. The observed regression on rivaroxaban similarly showed diminished instability markers, but the actual mechanisms explaining diminished plaque volume on anticoagulation remain to be determined (Posthuma et al. 2019).

These proof-of-concept studies support important roles for coagulation proteases FXa and thrombin in driving atherogenesis and altering the phenotype in different directions. Inhibiting factor Xa to actually reverse atherosclerosis in mice raises many new questions regarding the underlying mechanisms of factor Xa-mediated cell signaling and its impact on plaque progression/regression. The protection is thought to involve reducing the impact of FXa and thrombin on PAR1 and 2 activation, respectively. The importance of these PARs in regulating atherosclerosis is emerging; recently, protection against atherosclerosis in PAR2$^{-/-}$ × apoE$^{-/-}$ mice was shown, associated with reduced activity of nuclear factor-κB (NFκB)-regulated inflammation (Hara et al. 2018). However, the importance of coagulation proteases as compared to other possible ligands remains to be demonstrated.

2 From Atherosclerosis to Atherothrombosis

During atherogenesis there are different possible stages of *atherothrombosis* development that could be characterized by combinations of acute or acute on chronic thrombus formation. The occurrence of atherothrombosis is not a single event in time; in series of autopsy studies, it was convincingly demonstrated that coronary thrombi of all stages of development can be found in patients that suddenly died (Kramer et al. 2010). These studies demonstrate that atherothrombosis is part of an ongoing process with temporary, sometimes partial occlusions, subsequent remodeling of thrombus and vessel, gradually changing the vessel wall morphology, and affecting the lumen (Mastenbroek et al. 2015).

Atherogenesis and atherosclerosis may go through several stages of disease. A first and early stage relates to “perturbation” of the vascular endothelium (Nawroth et al. 1984; de Groot et al. 1987), referring to a state of endothelial cell activation associated with a disturbance of the anticoagulant/procoagulant balance, in a pro-inflammatory and thrombotic direction. It could be imagined that this perturbation may in fact be a first trigger in specific types of arterial thrombosis that may form in absence of visible atherosclerosis with sensitive imaging of coronary arteries. A second, more frequent scenario is the formation of a thrombus based on a damaged atherosclerotic plaque. Since long, two fundamentally different scenarios, plaque erosion versus plaque rupture, are recognized (Fuster et al. 1992a, b; Arbab-Zadeh et al. 2012).

While plaque rupture was previously recognized as the dominant pathophysiologic mechanism, recent studies suggest a gradual shift toward less plaque rupture and persistently frequent plaque erosion. One hypothesis for this change in time is declining impact of smoking and increased attention for and management of cardiovascular risk factors (e.g., statins, smoking cessation) that might translate into vascular protective effects and a more stable plaque phenotype (Quillard et al. 2017; Pasterkamp et al. 2017). Currently, about 30% of ST-elevation myocardial infarction (STEMI) is thought to result from eroded plaque lesions (Libby 2013).

Although known for a long time and recognized also as a predilection site for atherothrombosis in the aforementioned autopsy studies, erosion probably triggers fundamentally different mechanisms than rupture of a vulnerable cap (Quillard et al. 2017). Plasma from patients with STEMI showed higher levels of epidermal growth factor and thrombospondin-1 in patients with intact fibrotic cap versus those with ruptured cap lesions, while interferon-inducible T-cell alpha chemoattractant (I-TAC) was lower in coronary blood from intact cap lesion subjects (Chandran et al. 2017). In thrombectomy specimens from these patients, I-TAC mRNA expression levels were markedly increased in patients with eroded lesions. Eroded lesions are characterized by fewer inflammatory cells, abundant extracellular matrix, and the presence of neutrophil extracellular traps (NETs). Eroded plaques contain more myeloperoxidase (MPO)-positive inflammatory cells; MPO is a marker of NETs. Patients with plaque erosion have more MPO in peripheral blood than those with plaque fissure (Ferrante et al. 2010). In addition, components from extracellular matrix including hyaluronan that interact with prohemostatic proteins like fibrinogen, fibrin, and fibronectin are important erosion determinants (Pedicino et al. 2018; Wight 2018). Inflammation and NETs may be complimentary mechanisms to trigger blood coagulation toward thrombus formation. Ruptured lesions typically express inflammatory cells, matrix containing oxidized lipids, and subendothelial proteins from the matrix and on inflammatory cells, including procoagulant tissue factor, factors VII and X, as well as platelets localizing and activating proteins including von Willebrand factor (vWF), collagen, and thrombospondin (Quillard et al. 2017; Pasterkamp et al. 2017). If, indeed, there are fundamental differences in the phenotypes of eroded versus ruptured lesions, it may be anticipated that thrombus formation also follows different pathways. On the other hand, it has been suggested that the main difference between eroded and ruptured plaque is the absence of direct contact between blood components and the necrotic core (Badimon and Vilahur 2014). Better insight into the mechanisms involved in atherothrombosis related to erosion or rupture of plaques is important in order to tailor antithrombotic protection in a more mechanistically founded direction. As will be discussed below, current antithrombotic management does not yet consider such differences.

3 Antithrombotic Therapy: Clinical Principles and Applications

3.1 Single Antiplatelet Agents: Mode of Action and Side Effects

Aspirin is the common name for acetylsalicylic acid, a compound that acts by inhibiting prostaglandin synthesis in different cells; the relevant antithrombotic effect is thought to be mostly based on inhibition of the cyclooxygenase-1 production of thromboxane A2 in platelets (Gresele 2002). It is prescribed at doses between ±75 and 325 mg od, mostly related to regional differences (higher doses more common in the USA than Europe). Its intake results in irreversible inhibition of platelet activation and aggregation. To achieve continued effect, daily intake of aspirin is needed to suppress newly formed platelets, about 10% per day.

Clopidogrel is a prodrug that needs metabolization in the liver (Coukell and Markham 1997). It is ingested as a single dose of 75 mg/day, and the active metabolite shows a large interindividual variation in blood. This variability is partly explained by genetic polymorphisms encoding cytochrome P450 (CYP) 2C19, the hepatic enzyme involved in biotransformation of the prodrug clopidogrel to its active metabolite (Shuldiner et al. 2009). The active metabolite interferes with the binding of ADP to the P2Y12 receptor. This pharmacodynamic variation translates into variation in clopidogrel effectiveness after percutaneous coronary intervention (PCI) (Mega et al. 2010; Sibbing et al. 2009). The interindividual variation in response to clopidogrel, commonly known as clopidogrel "resistance," was first identified in patients with coronary disease, occurring up to 25% of cases in platelet function testing (depending on the test used). Given the association between clopidogrel high on-treatment platelet reactivity (HTPR) and increased incidences of major adverse cardiovascular events (MACE), several studies addressed the question whether dose adjustment based on platelet function testing would correct this problem. Unfortunately, none of the studies demonstrated that test-based adjusted clopidogrel dosing could improve clinical efficacy of such interventions (Price et al. 2011; Collet et al. 2012). In part, the variation in clopidogrel activity is "corrected" for by the development of more potent P2Y12 inhibitors with a more predictable pharmacodynamic profile including prasugrel, ticagrelor, and cangrelor.

The principal side effect of aspirin is bleeding, and the regular use of aspirin increases the risk of particularly gastrointestinal bleeding twofold (Garcia Rodriguez et al. 2016). Risk factors for bleeding should therefore be taken into account, including (recent) GI ulcer (*H. pylori* infection may be additional factor), old age, and use of interacting medication including other antiplatelet therapy (APT), NSAIDs, COX-2 selective inhibitors, oral anticoagulants, and corticosteroids. In individual decision-making, balancing the pros (risk of atherothrombotic complications) and the cons (mostly bleeding) needs to be done, and certain decision support tools like the app "Aspirin Guide" can be helpful in this regard. In general, the risk of GI bleeding with aspirin can be effectively reduced with proton pump inhibitors (PPI), better than with histamine-2 receptor antagonists (Mo et al. 2015; Szabo et al. 2017). Recently, a fixed-dose combination of aspirin and omeprazole (Yosprala) was approved by the FDA, hoping that the simultaneous intake of these agents would improve adherence by reducing gastric side effects (Veltri 2018). Although clopidogrel does not interfere with prostaglandins in gastric mucosal tissue, its use is also associated with an increased risk of bleeding, of which GI bleeds are the most common type. The standard use of PPI in patients on clopidogrel has been disputed; in fact there is evidence that its concomitant use is associated with an increased risk of MACE (Bundhun et al. 2017). Compared to clopidogrel, the newer and more potent P2Y12 inhibitors prasugrel and ticagrelor are associated with an increase in the risk of major bleeding. These agents are generally not prescribed as single APT, although ticagrelor may be used as single agent in selected PAD patients (Hiatt et al. 2017).

3.2 Primary Prevention in the Population; Selecting the Right Subject?

In patients with atherosclerotic vascular disease, antithrombotic medication has been applied since the late 1950s of the past century. Traditionally, platelets were regarded key players in atherothrombosis; hence much focus has been put on antiplatelet agents, initially mostly aspirin. Given the efficacy/safety profile, *primary prevention* with aspirin has been extensively studied. Recent studies and meta-analyses of decades of large trials refute a major role for aspirin in primary prevention. Exceptions may be subjects with diabetes in whom aspirin showed to reduce the incidence of major vascular events including myocardial infarction, at a price of increased major bleeding risk (Group ASC et al. 2018). Otherwise, primary prevention with aspirin in apparently healthy subjects may require additional risk factors like coronary calcification to yield sufficient net clinical benefit. A recent discussion paper on the pros and cons of aspirin for primary prevention in elderly subjects shows the jury is still out (McNeil et al. 2018a, b, c; Fernandes et al. 2019).

3.3 Primary Prevention in Subjects with Atherosclerosis

Primary prevention (of MACE) with aspirin or other APT is warranted in all subjects with *symptomatic* atherosclerotic disease, including angina, or symptomatic PAD. Here, the risk/benefit ratio is clearly in favor of indefinite APT. In patients with any evidence of coronary artery disease, indefinite single APT with aspirin is recommended. This policy is adjusted in case of emerging interventions like PCI; see further.

Remarkably, aspirin was not better than placebo in patients with *asymptomatic* lower extremity artery disease (LEAD), in spite of the fact that their mortality is comparable to patients with symptomatic disease (Fowkes et al. 2010). In symptomatic PAD, there is a certain preference for the P2Y12 inhibitor clopidogrel over aspirin, as the PAD subgroup in the CAPRIE trial that compared aspirin with clopidogrel in subjects at high risk for cardiovascular complications showed better antithrombotic efficacy for clopidogrel at comparable bleeding risk (Table 1) (Committee 1996). However, both aspirin and clopidogrel are still used for this indication. Stronger acting APT like ticagrelor may be used as alternative in patients with PAD in case of failure, allergy, or “resistance” to aspirin or clopidogrel, based on the EUCLID trial showing non-inferiority of ticagrelor versus clopidogrel (Hiatt et al. 2017). Table 1 presents an overview of different therapeutic strategies in symptomatic patients with stable CAD or PAD, referring to the large trials supporting these strategies.

The principle of combining APT was established in the CURE trial, comparing the efficacy and safety of aspirin plus clopidogrel with aspirin alone in the secondary prevention of MACE in patients with coronary disease (Mehta et al. 2001). Since then, dual antiplatelet therapy (DAPT) has become a cornerstone treatment in secondary prevention following PCI (see further). However, the

Table 1 Studies evaluating different antithrombotic strategies in patients with coronary artery disease (CAD) and peripheral artery disease (PAD) (adapted from Olie et al. (2018))

Therapeutic strategy	Stable coronary artery disease (CAD); outcome		Stable peripheral artery disease (PAD); outcome				Bleeding complications in PAD and CAD patients	
	MACE	**Ref.**	**MACE**	**Ref.**	**MALE**	**Ref.**	**Bleeding**	**Ref.**
Aspirin	↓	[1]	↓/–	[1, 2]	↓	[3, 4]	↑	[1-4]
MONOTHERAPY compared to aspirin								
Clopidogrel	↓	CAPRIE [5]	↓	CAPRIE [5]			↑	CAPRIE [5]
Ticagrelor			↓^{a}	EUCLID [6]	–^{a}	EUCLID [6]	↑	EUCLID [6]
Rivaroxaban 5 mg B.I.D.	–	COMPASS [7]	–	COMPASS [7]	↓	COMPASS [7]	↑↑	COMPASS [7]
COMBINATION THERAPY compared to aspirin								
Aspirin + clopidogrel	↓	CHARISMA [8, 9]	–	CHARISMA [10]	–	CHARISMA [10]	↑↑	CHARISMA [8-10]
Aspirin + ticagrelor	↓↓	PEGASUS-TIMI 54 [11]	↓↓^{b}	PEGASUS-TIMI 54 [12]	↓↓^{b}	PEGASUS-TIMI 54 [12]	↑↑	PEGASUS-TIMI 54 [11, 12]
Aspirin / DAPT + vorapaxar	↓↓↓	TRA2°P-TIMI 50 [13]	–	TRA2°P-TIMI 50 [13, 14]	↓↓	TRA2°P-TIMI 50 [13, 14]	↑↑↑	TRA2°P-TIMI 50 [13, 14]
Aspirin + VKA	↓↓↓	WARIS, ASPECT [15, 16]	–	WAVE [17]	↓	WAVE [17]	↑↑↑	WARIS, ASPECT, WAVE [15-17]
DAPT + rivaroxaban 2.5 mg B.I.D. *(post-ACS)*	↓↓↓↓	ATLAS ACS2-TIMI 51 [18]					↑↑↑	ATLAS ACS 2-TIMI 51 [18]
Aspirin + rivaroxaban 2.5 mg B.I.D.	↓↓↓↓	COMPASS [7]	↓↓↓↓	COMPASS [7]	↓↓↓↓	COMPASS [7]	↑↑	COMPASS [7]

Study population, control groups, and definition of primary efficacy and safety outcomes are highly variable between different studies. This table is based on the authors' interpretation of clinical trials and meta-analyses and is clearly not based on head-to-head comparisons of the different therapeutic strategies

(↓) to (↓↓↓↓) indicates modest to strong decrease in MACE/MALE
(↑) to (↑↑↑↑) indicates modest to strong increase in bleeding complications
(−) indicates no beneficial effect compared to aspirin monotherapy
(↓/−) indicates contradictory results
Gray boxes indicate that these results are not available and/or not applicable
MACE major adverse cardiovascular events, *MALE* major adverse limb events, *DAPT* dual antiplatelet therapy, *VKA* vitamin K antagonist, *B.I.D.* bis in die, twice a day, *ACS* acute coronary syndrome, ref. reference
Table adapted from Olie et al. (2018)

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Table 1 (continued)

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[a] No direct comparison with aspirin monotherapy but compared to clopidogrel monotherapy

[b] In patients with concomitant PAD and CAD

concept also triggered studies as to the potential benefits DAPT would offer in patients with high-risk cardiovascular disease. Large trials like CHARISMA (Bhatt et al. 2006) tested this concept, the outcome of which was however negative in failing to show improved efficacy while increasing the bleeding risk. Consequently, DAPT is not recommended in any patient for *primary* prevention of MACE, unless there are subject-specific reasons for this more potent combination.

In PAD, in general, *combined* antiplatelet therapy does not add benefit to the patient and increases bleeding risk; for this reason, it is only applied for short-term use, e.g., after endovascular interventions (Hess et al. 2017). The use of oral anticoagulants (mostly vitamin K antagonists) in therapeutic intensity is not indicated in patients with PAD, except for those that underwent venous bypass grafting (Dutch Bypass Oral Anticoagulants or Aspirin (BOA) Study Group 2000). The most recent regimen studied in patients with high-risk vascular disease, including PAD, is the combination of an anticoagulant rivaroxaban 2.5 mg bd plus low-dose aspirin, which reduced cardiovascular mortality as well as major acute limb events in patients with PAD (patients with LEAD or carotid artery disease) (Anand et al. 2018). From a mechanistic perspective, targeting platelets and the plasmatic coagulation system seems rational, given the postulated pathophysiologic mechanisms discussed above. Moreover, targeting FXa, herewith also inhibiting the formation of thrombin, reduces the potential interactions between these proteases and cellular PARs. Thus, vascular protective effects may be an additional consequence of a strategy that includes an anticoagulant (although the clinical evidence is still weak).

3.4 Secondary Prevention of Atherothrombosis in Patients with Arterial Vascular Disease

Secondary prevention with APT, following myocardial infarction or ischemic stroke (not due to cardiac embolism, thus including a spectrum of non-embolic strokes), is straightforward and based on class 1A evidence.

Combined APT (dual (DAPT) antiplatelet therapy) is indicated in all settings of acute coronary syndrome, with or without PCI with stent placement (DAPT for 6–12 months, longer in selected cases) (Roffi et al. 2016; Authors/Task Force et al. 2014; Amsterdam et al. 2014). In acute ischemic stroke, DAPT is only indicated for a very limited duration, up to 6 weeks, due to the observed increased bleeding risk (Wang et al. 2013; Johnston et al. 2018). Following these time windows, single APT will be continued indefinitely, comprising of aspirin in most patients with CAD and aspirin or, more commonly, clopidogrel in patients after atherothrombotic stroke (Hackam and Spence 2019).

In most combined APT regimens, aspirin remains an element; in patients with CAD, DAPT comprises aspirin plus a P2Y12 inhibitor, prasugrel or ticagrelor (unless contraindicated clopidogrel, a weaker P2Y12 inhibitor, is used in combination with aspirin). Although the thienopyridine prasugrel is a prodrug like clopidogrel, it only requires a single oxidation step to form its active metabolite, and it seems to be not affected by genetic variations in CYP enzymes. Compared to clopidogrel its use is associated with an increase in the risk of major bleeding, especially among those with age ≥75 years or body weight <60 kg, and is contraindicated in patients with prior stroke (Wiviott et al. 2007). Ticagrelor, an oral, direct acting, reversible P2Y12 receptor antagonist, provides faster and greater platelet inhibition with less patient-to-patient variation (Wallentin et al. 2009).

Despite intensification of APT in recent years, an approximately 10% risk for recurrent ischemic events at 1 year after coronary events still remains (Wiviott et al. 2007; Wallentin et al. 2009). With blockade of the TXA2 pathway and the P2Y12 receptor, platelets can still be activated by thrombin, via the PAR1 and PAR4 receptor on their surface (Olie et al. 2019). Vorapaxar is an orally administered, competitive PAR1 antagonist that blocks thrombin-mediated platelet activation via PAR1, without inhibiting other modes of thrombin activity, such as fibrin formation, protein C activation, and PAR4 activation. On top of standard antiplatelet therapy (consisting of aspirin in almost all cases and a thienopyridine or dipyridamole in a significant proportion), addition of vorapaxar led to significant reduction in rates of ischemic cardiovascular events in patients with stable CAD or PAD, but at the price of increased major bleeding, limiting its use in clinical practice (Morrow et al. 2012). Non-platelet-mediated effects of PAR inhibition on the vascular endothelium have been speculated on, since PAR1 is also present on endothelial cells and VSMCs, where it mediates mitogenic effects (Posma et al. 2016). Thus, PAR1 inhibition might be effective in reducing vascular remodeling and consecutive progression of atherosclerosis.

In patients with atherosclerotic disease, oral anticoagulants were not routinely applied until recently. One reason for not using anticoagulants was the assumption that atherothrombosis primarily is a platelet-dependent phenomenon, given the efficacy data of all APT trials. A second reason to refrain from anticoagulants was the concern of cholesterol embolization that could follow anticoagulant-associated instability and plaque rupture; however, the evidence for a causal effect of anticoagulants is poor.

Early trials with vitamin K antagonists (van Bergen et al. 1994; Smith et al. 1990) in CAD patients had provided proof of principle that inhibition of coagulation may be of additional benefit in atherothrombotic disease, although in daily practice, their use was practically abandoned due to markedly increased bleeding and more effective protection by DAPT. Nonetheless, after the introduction of direct oral anticoagulants (DOACs), the role of these anticoagulants in secondary prevention of ischemic events was re-evaluated in several trials (Olie et al. 2018). Addition of low-dose rivaroxaban (2.5 mg twice daily) on top of APT reduced major adverse cardiac events both in patients with acute coronary syndrome (in the ATLAS-ACS-2 TIMI 51 trial (Mega et al. 2012)) and in patients with stable CAD or PAD (in the COMPASS trial (Eikelboom et al. 2017)). In COMPASS also cardiovascular mortality was reduced in patients receiving the combination of vascular doses of rivaroxaban (2.5 mg bd) and aspirin as compared to either agent alone, which means a breakthrough in the efficacy of antithrombotic management (Coppens et al. 2019).

4 The Effects of Antithrombotic Therapy on the Vessel Wall and Atherogenesis: Clinical Relevance?

Platelets are pivotal in atherogenesis, but the impact of antiplatelet agents beyond inhibition of platelet activation and aggregation (including the procoagulant role platelets play in catalyzing phospholipid-dependent coagulation reactions) remains controversial. As discussed, low-dose aspirin may have local anti-inflammatory effects in the vessel wall, but evidence for diminished atherosclerosis related to aspirin intake is not present.

Human studies revealed several anti-inflammatory effects and reduction in platelet CD40 ligand and CD62, respectively, associated with clopidogrel (less evident in those with clopidogrel "resistance") and prasugrel, both thienopyridines (summarized in (Nylander and Schulz 2016)). Ticagrelor had anti-inflammatory effects including reduced formation of platelet-neutrophil aggregates in inflammation models and more profound reduction in Il-6 in a human sepsis model as compared to clopidogrel, suggesting that stronger inhibition of the P2Y12 receptor (ticagrelor) may provide greater anti-inflammatory effect (Nylander and Schulz 2016). Off-target effects of ticagrelor on endothelial function and vascular biomarkers have been speculated on, but a recent study comparing ticagrelor to prasugrel and clopidogrel found no evidence for any non-platelet-mediated effects in post-acute coronary syndrome patients (Ariotti et al. 2018).

The previous generation of oral anticoagulants, the vitamin K antagonists (VKA), has defined effects on the vasculature due to inhibition of carboxylation of specific vitamin K-dependent proteins like matrix Gla protein, a protein involved in the inhibition of both medial and intimal calcification. Ample mouse studies support these effects, where VKA rapidly induces calcification that can be partially prevented by additional vitamin K administration (Spronk et al. 2003b). Whether the VKA achieved inhibition of FXa and thrombin generation also in part affects atherosclerosis remains difficult to dissect, because of the overwhelming effect of calcification. Whether VKA affects plaque *stability* in humans is still uncertain. Observational studies suggest more plaque instability in patients on VKA due to intraplaque hemorrhage particularly upon prolonged exposure and higher intensity of anticoagulation (Mujaj et al. 2018; Li et al. 2014). However, in the Rotterdam study, the effects were comparable for anticoagulants (VKA) and aspirin, so the specificity and causal contribution of anticoagulation versus antiplatelet effects remain unproven. More direct evidence for effects of inhibiting coagulation protease needs to come from studies with DOACs. Two studies that either randomized patients to rivaroxaban or warfarin (Lee et al. 2018) or performed propensity score matching yielding three populations that used DOAC, warfarin, or no anticoagulants (Plank et al. 2018) were recently published. Both studies show diminished calcification and less instability features in patients on DOAC versus VKA. These somewhat preliminary data seem to point to a possibly favorable impact of DOAC (hence, FXa/thrombin inhibition) on atherosclerosis and the vessel wall.

5 Novel Antiplatelet and Anticoagulant Targets

In spite of the available array of antithrombotic agents, there is still a need for further improvement. One of the critical downsides of all antithrombotic agents is a risk of bleeding, linked to the potency of the drug. Thus, more effective antiplatelet agents like prasugrel or ticagrelor will generally inflict more bleeding risk than the less potent P2Y12 inhibitor clopidogrel. Combinations of APT like in DAPT or even triple therapy will also show increased bleeding risk as compared to single agents.

Although the current class of DOAC has a better safety profile with regard to intracranial bleeding as compared to VKA, there is still a substantial risk of other clinically relevant bleeding complications, including gastrointestinal bleeds. Combined anticoagulant and APT therapy also has increased bleeding potential, and even the relatively low doses of rivaroxaban in the COMPASS regimen, combined with aspirin, increase the risk of major bleeding (Eikelboom et al. 2017).

With existing antiplatelet drugs, a therapeutic ceiling seems to be reached, whereby increased potency is offset by elevated bleeding risk. Ongoing research on developing new antiplatelet drugs therefore focuses on identifying targets that inhibit thrombosis while maintaining hemostasis. Developing thrombi at sites of endothelial injury are now known to be composed of two distinct regions: the hemostatic plug (composed of highly activated platelets and rich in fibrin) and the propagating platelet thrombus (composed of platelets in a low activation state) (Stalker et al. 2013). The latter region of the propagating thrombus is regulated by phosphatidylinositol 3-kinase-β (PI3Kβ), glycoprotein (GP)IIb/IIIa outside-in signaling, and activation of protein disulfide-isomerase (PDI). Inhibition of these factors that regulate thrombus propagation seems to protect against thrombotic occlusion while preserving hemostasis. Therefore, novel agents include PI3Kβ inhibitors, PDI inhibitors, conformation-specific targeting of activated GPIIb/IIIa, and selective inhibition of GPIIb/IIIa outside-in signaling (McFadyen et al. 2018).

Other candidate drugs include inhibitors of the GPIb-vWF axis, novel PAR1 and PAR4 inhibitors, and blockade of platelet GPVI-mediated adhesion pathways. The GPIb-IX-V receptor binds to vWF during injury and under conditions of high shear stress, allowing early platelet adhesion to the subendothelium. Therefore, various inhibitors of this axis have been developed. Although two antibodies against vWF (ARC1779 and caplacizumab) have demonstrated to have antithrombotic effects, their development has been halted owing to an increased incidence of bleeding (Markus et al. 2011; Muller et al. 2013). However, additional agents, directly targeting GPIb or the vWF binding domain, are under development. As discussed, the use of the currently available PAR-1 antagonist vorapaxar is limited by substantially increased rates of bleeding. Besides orthosteric antagonists like vorapaxar, another class of PAR1 inhibitors, called paramodulins, has been developed. These target the cytoplasmic face of PAR1, contrary to blocking the ligand-binding site like vorapaxar, which inhibits all signaling downstream of the PAR1 receptor. This may allow paramodulins to selectively block platelet and endothelial cell activation mediated by PAR1 while maintaining the cytoprotective signaling pathways in endothelial cells (Aisiku et al. 2015). As thrombin activates platelets via both PAR1 and PAR4, also the PAR4 receptor is currently under investigation as a target for platelet antagonism (McFadyen et al. 2018). Another promising target is the interaction between GPVI and collagen. The observation that GPVI is platelet-specific, in combination with the fact that patients with GPVI deficiency usually suffer from only a mild bleeding phenotype, has led to strategies targeting GPVI. Phase II trials, studying the anti-GPVI agent Revacept in patients with stable CAD and symptomatic carotid stenosis, are currently underway (Majithia and Bhatt 2019).

In recent years, the contribution of the proteins of the contact system (factors VIII, IX, XI, and XII, prekallikrein, and high-molecular-weight kininogen) to the process of atherothrombosis has gained more attention. Factor XII-deficient humans have a normal hemostatic capacity, while animal models have revealed an important role of factor XIIa-driven coagulation in arterial thrombosis (Kuijpers et al. 2014). Furthermore, factor XIIa contributes to inflammation through the activation of the inflammatory bradykinin-producing kallikrein-kinin system (Nickel et al. 2017; Long et al. 2016). Thus, pharmacological inhibition of factor XII(a) may not only be a safer therapeutic strategy (by inhibition of thrombosis while preserving hemostasis) but also has additional beneficial anti-inflammatory and anti-atherogenic effects. Currently, factor XII(a) and its activator polyphosphate are being studied as potential targets for prevention of thrombosis. However, factor XIIa also stimulated the fibrinolytic pathway (Long et al. 2016), and inhibition may thus have potential prothrombotic side effects. Moreover, when thrombosis is initiated by TF exposure, small amounts of thrombin generated by extrinsic tenase have the potential to activate FXI, thereby bypassing FXII inhibition. Therefore, FXI inhibition may be a better target than FXII inhibition. Furthermore, besides attenuation of coagulation, factor XI deprivation has also been shown to slow down atherogenesis in apoE/factor XI double knockout mice (Shnerb Ganor et al. 2016). Several potential strategies to target FXI are currently under investigation, including antisense oligonucleotides (ASOs) that reduce hepatic synthesis of FXI, monoclonal antibodies that suppress FXIa generation and inhibit FXIa activity, and aptamers that block the binding site and small molecules that bind reversibly to the active site of FXIa and inhibits its activity (Weitz and Chan 2019). Clinical phase 2 studies with FXI-directed ASOs, monoclonal antibodies against FXIa, and an oral FXIa inhibitor have been performed (Buller et al. 2015) or are currently underway (Weitz and Chan 2019).

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