The new era of anticoagulation: factor XI and XII inhibitors

Domenico Prisco,1,2 Irene Mattioli,1 Raffaele De Caterina,3 Alessandra Bettiol1

1Department of Experimental and Clinical Medicine, University of Florence; 2Careggi University Hospital, Florence; 3Division of Cardiology, Pisa University Hospital, and University of Pisa, Italy

ABSTRACT
The two last decades have witnessed a revolution in the field of anticoagulation, mainly due to the advent of direct anticoagulant with targeted action against single coagulation proteins. However, the residual risk of cardio- and cerebrovascular events, particularly in some critical settings, and the risk of major bleeding still represent unmet medical needs. Preclinical studies and experience from families with genetic deficiencies of factor XI or XII (FXI and FXII) allowed to identify these factors involved in the contact pathway of coagulation as potential targets for new anticoagulant approaches. To date, several pharmacological classes of FXI and FXII inhibitors have been developed, including antisense oligonucleotides, monoclonal antibodies, small molecules, natural inhibitors, and aptamers, and various molecules are currently under phase 2 or 3 clinical investigation. Particularly, promising results have been obtained in patients undergoing major orthopedic surgery, in those with end-stage kidney disease, atrial fibrillation and acute coronary syndrome. This review summarizes current knowledge on FXI and FXII inhibitors, with a particular focus on their pharmacological properties and potential clinical indications.

Introduction
The last decades have witnessed a revolution in the field of anticoagulation: after the long era of heparins and vitamin K antagonists (VKAs), direct anticoagulant drugs with a targeted action against single coagulation proteins have reached the clinical practice. Among them, the parenterally administered agents fondaparinux, targeting factor (F) Xa, and bivalirudin, targeting thrombin, have been used for the acute treatment of venous thromboembolism (VTE) and acute coronary syndromes, as well as during revascularization procedures, whereas direct oral anticoagulants (DOACs) against thrombin or activated Fxas have been widely used for the acute treatment of VTE and long-term prophylaxis of pro-thrombotic conditions. DOACs, while being non-inferior to VKAs for the prevention of stroke in atrial fibrillation and for the prevention and treatment of VTE,1,4 feature an easy handling, better compliance and more favorable safety profile, particularly in terms of bleeding risk.6 Thanks to these properties, DOACs are now preferred over traditional anticoagulants in most settings,6 although not in all clinical indications: among the latter are conditions associated to a high risk of bleeding (for example, patients candidate for dual or triple antiplatelet-anticoagulant therapy); diseases in which DOACs have reported unfavorable results (for example, in patients with mechanical heart valves or antiphospholipid antibody syndrome) or have not been adequately tested (for example, patients with extremely impaired renal or hepatic function, with extreme body weight or with sickle cell disease); or in case of possible non-negligible interactions with other drugs.8,11 Despite their wide use and indications, unmet therapeutic and prophylactic needs are still

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present in the field of anticoagulation, and the high human and economic burden associated with cardiovascular events underscore the need for new therapeutic alternatives for the management of thrombotic diseases.

In recent years, the growing knowledge of diseases associated with the congenital deficiency of factor XI and XII (FXI and FXII) has paved the way to the development of new anticoagulants directed against these two proteins of the coagulation contact pathway. This review examines current evidence on FXI and FXII inhibitors, focusing on their pharmacological features and possible therapeutic and prophylactic potentialities (Table 1).

Identification of factor XI and XII as possible therapeutic targets

FXI (also known as antihemophilic factor C) and FXII (also known as Hageman’s factor) are two circulating glycoproteins involved in the contact pathway of coagulation (Figure 1). They are synthesized in the liver and circulate in the blood as inactive zymogen that can be (auto)activated to FXIa and FXIIa in response to extracellular nucleic acids, neutrophil extracellular traps, pathogen-associated polyphosphates, or negatively charged surfaces coming in contact with the blood, such as mechanical heart valves and medical devices. FXIIa is a serine protease that activates prekallikrein, which in turn stimulates FXIIa activation via a positive feedback mechanism. FXII activation can be affected also by FXIa, which amplifies the generation of thrombin, converting FIX into its activated form.

The idea that inhibiting the contact pathway of coagulation might be an effective anticoagulant approach came from the knowledge gained on hereditary diseases associated with FXI deficiency (also called hemophilia C, plasma thromboplastin antecedent deficiency, or Rosenthal disease) and FXII deficiency. FXI deficiency is a rare autosomal condition, with a prevalence of one case per 100,000 people, and a higher frequency (up to 8-9%) in some ethnic groups including Ashkenazi Jews and Iraqi Jews. Unlike the more common X-linked FVIII deficiency (hemophilia A) or FIX deficiency (hemophilia B), patients with FXI have an only slightly increased risk of bleeding, mostly associated with surgical procedures in tissues with high fibrinolytic activity (e.g., dental surgery, tonsillectomy, and prostate surgery), paired by a reduced risk of thrombosis. A study of more than 200 patients with FXI deficiency showed a significant reduction in the incidence of deep vein thrombosis (DVT) compared to the general population, while in another study on 115 patients, FXI deficiency was associated with a significantly reduced incidence of ischemic stroke, but not of myocardial infarction. Regarding FXII, studies conducted on familiar clusters of patients with moderate-to-severe FXII deficiency did not show an increase in the risk of bleeding or a reduction in the risk of thrombotic risk. Conversely, FXII up-regulation has been associated with increased mortality and FXII is known to play a central role in initiating the process of device-induced thrombosis.

Figure 1. Factors XI and XII in the coagulation cascade.
# The new era of anticoagulation: factor XI and XII inhibitors

Table 1. Pharmacodynamic and pharmacokinetic properties and clinical indications of anti-factor XI and anti-factor XII drugs that have reached phase 2 or 3 of clinical development.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Pharmacodynamics</th>
<th>Pharmacokinetics</th>
<th>Clinical indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>IONIS-FXIRX</td>
<td>2′-O-(2-methoxyethyl) (2′-O-MOE) second-generation</td>
<td>Inhibits FXI synthesis in the liver</td>
<td>Administration: subcutaneous</td>
<td>VTE prevention in surgery; ESRD</td>
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<td></td>
<td>antisense oligonucleotid</td>
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<td>Clearance: 3.12 L/hPK not affected by hemodialysis</td>
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<tr>
<td>Fesomersen</td>
<td>Triantennary N-acetyl galactosamine (GalNAc)</td>
<td>Inhibits FXI synthesis in the liver</td>
<td>Administration: subcutaneous</td>
<td>ESRD</td>
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<td></td>
<td>conjugated 2′-MOE chimeric ASO</td>
<td></td>
<td>Delivery: GalNAc conjugation facilitates drug delivery</td>
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<tr>
<td>Osocimab</td>
<td>Fully humanized monoclonal G1 antibody</td>
<td>Binds to a region adjacent to the active site of FXIa causing substantial structural changes</td>
<td>Administration: intravenous</td>
<td>VTE prevention in surgery; ESRD</td>
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<td>Mean time-to-peak plasma concentrations: 1-4 h</td>
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<td>Half-life: 30-44 d</td>
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<td>Clearance: 0.13-0.29 L/d</td>
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<tr>
<td>Abelacimab</td>
<td>Fully human monoclonal antibody</td>
<td>Binds to the catalytic domain of factor XI and locks it in the zymogen (inactive precursor) conformation</td>
<td>Administration: intravenous</td>
<td>VTE prevention in surgery; atrial fibrillation; cancer associated VTE</td>
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<td>Mean time-to-peak plasma concentrations: 1.75-2.00 h</td>
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<td>Half-life: 25-30 d</td>
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<td>Clearance: 0.006-0.012 L/d</td>
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<tr>
<td>Xisomab 3G3</td>
<td>Recombinant humanized antibody</td>
<td>Binds to the apple 2 (A2) domain of FXI and FXIa and inhibits activation of FXI by FXIIa in a concentration-dependent manner but does not inhibit thrombin-mediated activation of FXI</td>
<td>Administration: intravenous</td>
<td>ESRD; catheter-related thrombosis in cancer patients</td>
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<td></td>
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<td>Mean time-to-peak plasma concentrations: 0.08-1 h for the 0.1 mg/kg-5 mg/kg doses</td>
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<td>Half-life: 1.3 h-121.5 h for the 0.1 mg/kg-5.0 mg/kg dose</td>
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<td>Clearance: 0.094 - 0.014 L/h for the 0.5 mg/kg-5 mg/kg doses</td>
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<tr>
<td>MK-2060</td>
<td>Monoclonal Antibody</td>
<td>Designed to dually block the activation of FXI and the downstream activity of the activated protein</td>
<td>Administration: intravenous</td>
<td>ESRD</td>
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<tr>
<td>Garadacimab</td>
<td>Fully human recombinant antibody</td>
<td>Binds to the 99-, 140-, 180- and 220- loop on the beta chain of activated FXII</td>
<td>Administration: intravenous, subcutaneous</td>
<td>Catheter-related thrombosis in cancer patients; COVID-19; idiopathic pulmonary fibrosis; hereditary angioedema</td>
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<td>Mean time-to-peak plasma concentrations: 60 min for intravenous; 7 d for subcutaneous administration.</td>
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<td>Half-life: 14-20 d</td>
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<td></td>
<td></td>
<td>Clearance: 5.6-9.8 ml/h</td>
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<tr>
<td>Milvexian</td>
<td>Small molecule</td>
<td>Active-site, reversible inhibitor of human FXIa (high affinity and selectivity, Ki 0.11 nM)</td>
<td>Administration: oral</td>
<td>VTE prevention in surgery; stroke prevention; atrial fibrillation; ACS; ESRD</td>
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<td>Mean time-to-peak plasma concentrations: 3-4 h</td>
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<td>Half-life: 8.3-13.8 h</td>
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<td>Clearance: 6.9-17.8% of the administered dose is excreted within 24 h</td>
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<tr>
<td>Asundexian</td>
<td>Small molecule</td>
<td>Direct, potent, reversible FXIa inhibitor</td>
<td>Administration: oral</td>
<td>Atrial fibrillation; stroke prevention; AMI; ESRD</td>
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<td>Mean time-to-peak plasma concentrations: 2.50 h in fasting, 5.00 h in fed</td>
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<td>Half-life: 14.2 to 17.4 h</td>
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<tr>
<td>SHR2285</td>
<td>Small molecule</td>
<td>Selective inhibition of human FXIa</td>
<td>Administration: oral</td>
<td>VTE prevention in surgery</td>
</tr>
<tr>
<td>EP-7041</td>
<td>Small molecule</td>
<td>FXIa antagonist (highly selective, IC50 of 7.1 nM)</td>
<td>Administration: intravenous</td>
<td>COVID-19</td>
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<td></td>
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<td>Onset-offset of action: rapid</td>
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<td>Half-life: 45 min</td>
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</table>

F, factor; VTE, venous thromboembolism; ESRD, end stage renal disease; PK, pharmacokinetics; ACS, acute coronary syndrome; AMI, acute myocardial infarction.
Factor XI and XII inhibitors: pharmacological aspects and clinical indications

The knowledge of the X-ray crystal structure of the FXIa and FXIIa catalytic domains has enabled the rational development of several pharmacological classes of FXI and FXII inhibitors. These include antisense oligonucleotides (ASOs), monoclonal antibodies, small molecules, natural inhibitors, and aptamers (Figure 2).

Antisense oligonucleotides and double-strand oligonucleotides

ASOs are single-strand oligonucleotides that bind to a complementary mRNA molecule, thus preventing its translation and deregulating the biosynthesis of the encoded protein. The benefits of ASO include high specificity, predictable pharmacokinetics and long half-life, and the lack of drug-drug interactions. To date, two ASOs directed against the mRNA coding for FXI (IONIS-FXIRX and fesomersen), and one directed against the mRNA for FXII (ION547) have reached the clinical phases of development.

IONIS-FXIRX (also known as FXI-ASO; BAY2306001, ISIS-416858) is a 2′-O-(2-methoxyethyl) ASO that binds to the mRNA for FXI at hepatic level, thus reducing the plasma concentrations of FXI in a dose-dependent manner, with a peak after 3-4 weeks of treatment and prolonged effect for several weeks after treatment discontinuation.31 The conjugation of the anti-FXI IONIS-FXIRX with a N-acetyl galactosamine allowed to develop the more potent, second generation anti-FXI ASO fesomersen (also known as IONIS-FXI-LRX, FXI-LICA, BAY-2976217, ION-957943). Fesomersen requires less frequent administration as compared to the unconjugated molecule (once a month or less, via subcutaneous route), thus reducing the risk of drug-related reactions and accounting for a better patient compliance.

Concerning FXII, a recent double-blind, placebo-controlled phase 1 trial assessed the safety, tolerability, pharmacokinetics, and pharmacodynamics of the ASO ION547, in single or multiple doses, in 48 healthy volunteers, but results are not available yet (NCT04934891). Besides single-strand ASO, double-strand oligonucleotides (siRNAs) have also been developed to inhibit the translation of the mRNA encoding FXII. Among them, one of the leading compounds is ALN-F12, an oligonucleotide conjugated with N-acetylglactosamine.3233 However, to date no anti-FXII siRNA has reached the clinical phases of development.

It is worth mentioning that ASOs and siRNAs, being oligonucleotides, cannot be administered orally; moreover, because of latency for the onset of their pharmacological action, they are more likely to be used for chronic rather than acute therapeutic indications.

Clinical indications

Thromboprophylaxis in the surgical setting

Major orthopedic surgery, in particular total knee arthroplasty (TKA), is a procedure associated with a high risk of postoperative VTE, in particular of asymptomatic DVT, which can be easily detected with routine venography.34 Thus,
this clinical setting is of great interest when studying the efficacy and safety of new anticoagulant approaches.

An open-label phase 2 study assessed the safety and efficacy of the anti-FXI ASO IONIS-FXIRX (200 mg or 300 mg, administered subcutaneously) 7 times prior to TKA, and 2 times after surgery) as compared to enoxaparin (40 mg/day, from the evening before or from the day of surgery and for at least eight days post-surgery) in 300 patients undergoing elective primary unilateral TKA (NCT01713361). IONIS-FXIRX 300 mg proved superior to enoxaparin in the prevention of VTE events, assessed by mandatory bilateral venography or symptomatic event (P<0.001). As for safety, clinically relevant bleeding events occurred in 3% of patients on IONIS-FXIRX (200 or 300 mg) and 8% on enoxaparin.35

**Thromboprophylaxis in patients with chronic kidney disease**

End-stage renal disease (ESRD) is associated with a remarkably high risk of cardio- and cerebro-vascular events,34-37 paired by an increased risk of bleeding during anticoagulation.34 Moreover, hemodialysis might affect the pharmacokinetics of drugs, impairing their efficacy and safety profile. Double-blind, randomized, controlled trials showed that hemodialysis does not affect pharmacokinetics and tolerability of the anti-FXI ASO IONIS-FXIRX (200 to 300 mg, subcutaneous), which effectively reduced blood clot formation at the circuit level in ESRD patients on hemodialysis (NCT02553889; NCT03358030 – EMERALD study).38 Also, the ASO fesomersen has just completed a phase 2 study in patients with ESRD receiving hemodialysis (trial RE-THIINc ESRD for fesomersen, NCT04534114).

**Monoclonal antibodies**

To date, four monoclonal antibodies (osocimab, abelacimab, xisomab 3G3 and MK-2060) against FXI and one against FXII (garadacimab) have reached clinical phases of development. Osocimab (also known as BAY 1213790) and abelacimab (also known as MAA868) are fully human monoclonal antibodies. Osocimab binds to a region adjacent to the active site of FXIa, causing structural changes that impair its function,39 whereas abelacimab binds the catalytic domain of FXII, blocking it in the conformation of an inactivezymogen.40 Xisomab 3G3 (also known as AB023) is instead a recombinant humanized antibody that binds to the apple 2 (A2) domain of FXI and FXIIa, blocking FXIIa-mediated (but not thrombin-mediated) activation of FXI in a dose-dependent manner.41 A fourth monoclonal antibody (MK-2060), designed to dually block the activation of FXI as well as the downstream activity of the activated protein, is under clinical evaluation.

As for FXII, garadacimab (also known as CSL312) is a fully human recombinant antibody that specifically inhibits FXIIa. In phase 1 clinical trials in healthy volunteers of various ethnic groups (NCT04580654, NCT05306275 and ACTRN12616001438448), garadacimab (3 and 10 mg/kg, intravenous or subcutaneous) induced a sustained inhibition of kallikrein activity, with a dose-dependent increase in activated partial thromboplastin time and without any change in prothrombin time, paired by a good safety and tolerability profile.42

It is worth specifying that, compared to ASOs, monoclonal antibodies have a much faster onset of action (e.g., 1-4 hours for osocimab) making them suitable also for acute indications. Also, monoclonal antibodies have a relatively long half-life (around 25-44 days), allowing monthly administrations or a single administration in the postoperative setting.39,41,43

**Clinical indications**

**Thromboprophylaxis in the surgical setting**

In the FOXTROT trial (NCT03276143), 813 patients undergoing TKA were randomized to intravenous the anti-FXI osocimab at two pre- or post-operative dosages (0.3 or 1.8 mg/kg, administered the day before surgery; or 0.3-1.8 mg/kg, given the day after surgery), or to subcutaneous enoxaparin (40 mg/day, from the night before or 6-8 hours post-surgery) or oral apixaban (2.5 mg twice daily, starting 12-24 hours post-surgery). Enoxaparin or apixaban were continued for at least 10 days or until venography. The trial results demonstrated that osocimab at the preoperative dose of 1.8 mg/kg was superior to enoxaparin, with a risk difference of 15%,44 and with a good safety profile (clinically relevant bleeding events occurred in 4.7% of patients on osocimab, 5.9% on enoxaparin and 2% on apixaban).44

Also, the anti-FXI abelacimab was investigated in an open-label phase 2 trial (ANT-005 TKA, EudraCT number 2019-003756-37) conducted on 412 patients undergoing elective TKA surgery. The trial showed that abelacimab, at doses of 75 and 150 mg, was superior to enoxaparin (40 mg/day) for VTE prevention (P<0.001),45 with bleeding events occurring in 0-2% of patients on abelacimab and 0% on heparin.45

**Thromboprophylaxis in patients with chronic kidney disease**

The anti-FXI monoclonal antibodies osocimab, xisomab 3G3 and MK-2060 were tested in phase 2 studies in patients with ESRD receiving hemodialysis (trials CONVERT NCT04523220 and NCT03787368 for osocimab; trial MK-2060-007 NCT05027074 for MK-2060; trial NCT03612856 for xisomab 3G3). To date, available results are limited to the latter trial (NCT03612856) on 24 ESRD patients on chronic hemodialysis randomized to xisomab 3G3 (0.25 or 0.5 mg/kg) or placebo, administered at the start of a normal hemodialysis procedure. Results in this clinical setting are promising both in terms of efficacy and of the safety.46

**Stroke prevention in atrial fibrillation**

Patients with atrial fibrillation are at high risk of cerebrovascular events, and DOACs are now the standard therapy for the cerebrovascular prophylaxis in this clinical setting.4 However, the use of DOACs in these patients is hampered by the residual risk of cardio- and cerebrovascular events, on one side, and by the small, but not negligible risk of severe intra- and extra-cranial bleeding,57-59 paving the way to the advent of new anticoagulant therapies. A first dose-ranging trial phase 2 trial (ANT-004) assessed the efficacy and safety of the anti-FXI abelacimab (120 or 180 mg) as compared to placebo in patients with atrial fibrillation (NCT04213807).40 Following the results of this study, the phase 2 AZALEA-TIMI 71 trial (NCT04755283) and the phase 3 LILAC-TIMI 76 trial (NCT05712200) will enroll 1200 patients with atrial fibrillation...
at moderate-to-high risk of stroke and 1900 high-risk patients, who have been deemed unsuitable for oral anticoagulation, respectively, to assess the safety and tolerability of this anti-FXI in this clinical setting.

**Device-associated thrombosis**

Medical devices, such as catheters used for diagnostic or therapeutic procedures, left ventricular assist devices or circuits for the extracorporeal membrane oxygenation, are known to be pro-thrombotic surfaces. Preclinical evidence indicates that upstream inhibition of FXIIa-induced intrinsic coagulation pathway is promising for device-induced thrombophrophylaxis. On these bases, a single-arm phase 2 clinical trial is ongoing to assess the role of the anti-FXI xisomab 3G3 for the prevention of catheter-related thrombosis in oncological patients receiving chemotherapy (NCT04465760).

Another phase 2 trial (NCT04281524) was meant to assess the efficacy and safety of the anti-FXII garadacimab as prophylactic antithrombotic treatment in cancer patients with peripherally inserted central venous catheter (PICC; trial NCT04281524), however this trial was suspended by decision of the pharmaceutical company.

**Thromboprophylaxis in the oncological setting**

Thromboprophylaxis is a relevant challenge in the oncological setting: DOACs are progressively replacing low-molecular-weight heparin, but the high risk of bleeding, particularly gastrointestinal, observed in these patients still represents an unmet medical need that can potentially be overcome with FXI inhibitors. On these bases, two phase 2 trials are evaluating the safety and efficacy of the anti-FXI abelacimab as compared to dalteparin (MAGNOLIA trial, NCT05171075) and to apixaban (ASTER trial, NCT05171049) for the prevention of cancer related VTE.

**Thromboprophylaxis in patients with severe COVID-19 infection**

A phase 2 trial has just been completed on the anti-FXII garadacimab in combination with standard therapy for thromboprophylaxis in 124 patients with severe COVID-19 infection. Preliminary results indicate that tracheal intubation or death prior to tracheal intubation occurred in 22.2% and 26.2% of patients on garadacimab and placebo, respectively, whereas all-cause mortality occurred in 17.5% and 18.0% of patients, respectively (NCT04440590).

**Other indications**

Various phase 2 trials assessed the anti-FXII garadacimab in various clinical setting, including idiopathic pulmonary fibrosis (NCT05130970) and the prophylaxis of hereditary angioedema (HAE) attacks (NCT03712228; NCT04656418; NCT04739059). To date, only the first of these trials on HAE is completed, and the preliminary results coming from 44 patients indicate that the mean time normalized number of HAE attacks per month during the first period of treatment ranged between 0.05 and 0.48 for the different garadacimab dosages, as compared to 4.24 in the placebo group.

**Small molecules**

Differently from ASOs and monoclonal antibodies, synthetic molecules have a low molecular weight (max 900 Dalton), which confers them a rapid onset/offset of action and makes them suitable for enteral administration. Regarding anti-FXI, four small molecules, milvexian, milvexian (also known as BMS-986177 or JNJ-70033093), asundexian (BAY 2433334), SHR2285 and EP-7041 have reached clinical phase 2 or 3 of development. Milvexian and asundexian are reversible, highly selective inhibitors of FXIIa and can be administered orally. From a pharmacokinetic point of view, they display a rapid peak plasma concentration (2-4 hours) and a relatively short half-life (8-16 hours). Notably, the pharmacokinetics of asundexian has been characterized also in patients with impaired hepatic or renal function (NCT05419663, NCT03196206).

SHR2285 is suitable for oral administration, similarly to ONO-7684, while two additional small molecules (EP-7041 and BMS-962212) have been developed for intravenous administration. Indeed, the parenteral use ensures a very rapid onset of action (about 45 minutes-2 hours), making them promising therapies for acute clinical uses.

Concerning anti-FXII, various small molecules belonging to the classes of N-acetylated 1H-1,2,4-triazol-5-amines and N-acetylated 1H-pyrazol-3-amines have been developed to inhibit FXIIa in the presence of thrombin and other coagulation factors. Notably, in vivo studies have shown not only an anticoagulant effect, but also a possible role of these molecules in the treatment of immune-mediated pathologies, such as autoimmune encephalomyelitis (patent no. WO2017123518) or collagen-induced arthritis (patent no. WO2019108565). Among the small molecules directed against FXII molecules there are also peptidomimetics, a series of (S)-piperazin-2-carboxamides characterized by various chemical substitutions, which account for different pharmacokinetic and pharmacodynamic profiles.

**Clinical indications**

**Thromboprophylaxis in the surgical setting**

In the AXIOMATIC TKR trial (NCT03891524), 1242 patients undergoing elective TKA were randomized to one of seven postoperative regimens of the anti-FXI milvexian (25-200 mg, once or twice daily orally) or enoxaparin (40 mg/day, subcutaneously). VTE was reported in 21-8% of patients on milvexian 25-200 mg twice daily, compared to 21% of those on 25-200 mg, once or twice daily orally, and 25-7% of those on 25-200 mg once daily, compared to 21% of those randomized to enoxaparin. Clinically relevant bleeding events occurred in 1% and 2% of patients on milvexian and enoxaparin, respectively.

Also, the anti-FXI small molecule SHR2285 is currently undergoing a phase 2 trial which compares multiple oral administrations of SHR2285 to enoxaparin in 500 patients undergoing elective TKA (NCT05203705).

**Stroke prevention, particularly in patients with atrial fibrillation**

In the PACIFIC-AF trial (NCT04218266), 753 patients with atrial fibrillation (median age 74 years, CHA2DS2-VASC ≥ 2 for men and ≥ 3 for women) were randomized to the anti-FXI...
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of patients on asundexian, and 2% of patients on placebo. This clinically relevant non-major bleeding events occurred in 3-4% asundexian and 19% on placebo (P=0.80). Major and/or detected on follow-up MRI occurred in 19-22% of patients on symptomatic ischemic stroke and/or occult cerebral infarction therapy. At 26 weeks, the composite outcome of recurrent orally) or placebo, in combination with routine antiplatelet from the onset, to asundexian (10 mg, 20 mg, or 50 mg/day, (LIBREXIASTROKE trial, NCT05702034).

In another phase 2 trial (PACIFIC Stroke trial, NCT04304508), more than 1800 patients with acute ischemic non-cardioembolic stroke were randomized, within 48 hours of the onset, to asundexian (10 mg, 20 mg, or 50 mg/day, orally) or placebo, in combination with routine antiplatelet therapy. At 26 weeks, the composite outcome of recurrent symptomatic ischemic stroke and/or occult cerebral infarction detected on follow-up MRI occurred in 19-22% of patients on asundexian and 19% on placebo (P=0.80). Major and/or clinically relevant non-major bleeding events occurred in 3-4% of patients on asundexian, and 2% of patients on placebo. This trial is followed by the ongoing phase 3 OCEANIC Stroke trial on more than 9000 patients (NCT05686070).

Regarding secondary cerebrovascular prevention, the randomized, double-blind phase 2 AXIOMATIC SSP trial (NCT03766581) included 2366 patients with acute non-cardioembolic ischemic stroke or high-risk transient ischemic attack (TIA), randomized within 48 hours of the event, to receive one of 5 doses of the anti-FXI milvexian (200 mg, 100 mg or 50 mg twice daily, or 25 mg or 25 daily) or placebo in combination with traditional antiplatelet therapy (single or dual; clopidogrel 75 mg/day for the first 21 days and aspirin 100 mg/day). The results, presented at the 2022 Congress of the European Society of Cardiology (ESC), indicate that, for the primary efficacy endpoint (the composite of recurrent stroke or asymptomatic cerebral infarction on magnetic resonance imaging), no dose-response relationship was detected for milvexian. Milvexian was associated with a lower risk of symptomatic ischemic stroke (but not of asymptomatic cerebral infarction) by approximately 30% for all doses except the 200 mg twice daily dose, for which no benefit was shown. Major bleeding events occurred in 0.6% of patients on placebo, 0.6% on milvexian 25 mg and 1.6% on milvexian 100 mg twice daily.

The encouraging results of this trial paved the way for the ongoing phase 3 trials on 15500 patients with atrial fibrillation randomized to milvexian or apixaban (LIBREXIA-AF, NCT05757869), and on 15000 patients randomized to milvexian or placebo after an acute ischemic stroke or high-risk TIA (LIBREXIASTROKE trial, NCT05702034).

**Secondary prevention in patients with acute coronary syndrome**

The standard secondary prophylactic therapy for patients with acute coronary syndrome (ACS) is based on the dual antiplatelet therapy with aspirin and a P2Y12 receptor inhibitor. However, the risk of recurrence in these patients remains high, while the use of DOACs (namely rivaroxaban in combination with aspirin and antiplatelet therapy) was associated with a high risk of major intra- or extra-cranial bleeding.

The phase 2 PACIFIC-AMI trial (NCT04304534) assessed the safety and efficacy of the anti-FXI asundexian for the secondary prevention of acute myocardial infarction (MI) in 1601 patients with recent MI (approximately half of them STEMI), randomized within 5 days of hospitalization to asundexian 10, 20 or 50 mg/day or placebo for 6-12 months, in addition to a dual antiplatelet therapy with aspirin and a P2Y12 inhibitor. During a median follow-up of 368 days the composite efficacy outcome (cardiovascular death, recurrent MI, ischemic or hemorrhagic stroke, and/or stent thrombosis) occurred in 6.8-5.5% of treated patients on asundexian 10-50 mg, respectively, and in 5.5% of patients on placebo. The composite safety outcome (bleeding type 2, 3, or 5, according to the Bleeding Academic Research Consortium, BARC classification) was reported in 7.6-10.5% of patients on asundexian 10-50 mg, respectively, and in 9.0% of patients on placebo. These data support future studies of asundexian (50 mg/day) for secondary prevention of MI, including the upcoming phase 3 trial OCEANIC-AMI.

Also, milvexian is undergoing a phase 3 placebo-controlled trial in patients with recent ACS (LIBREXIA-ACS, NCT05754957).

**Thromboprophylaxis in patients with chronic kidney disease**

The anti-FXI small molecules milvexian and asundexian were also tested in clinical studies on patients with ESRD receiving hemodialysis (trials NCT03196206, NCT03000673 for milvexian; trial NCT04510987 for asundexian). The results available for the trial NCT03000673 showed that a single dose of milvexian (60 mg) was safe and well tolerated also in subjects with moderate or severe renal impairment, and that the half-life increased from 13.8 h in subjects normal renal function to 18.0 h and 17.7 h in those with moderate or severe renal impairment.

**Thromboprophylaxis in patients with severe COVID-19 infection**

The anti-FXI small molecule EP-7041 is under clinical evaluation as a thromboprophylactic therapy in COVID-19 patients admitted to the intensive care unit (NCT05040776).

**Natural inhibitors**

Alongside synthetic molecules, there are also natural inhibitors of FXI and FXII derived from nematodes (acaNAP10), snakes (fassxiator), bats (desmolaris), ticks (boofilin and the contact phase inhibitor of Ixodes ricinus, Ir-CPI), Triatoma infestans (like infestin and the synthetic version obtained by the fusion with recombinant human albumin, rHA-infestin-4) and Cucurbita Maxima pumpkin seeds (like thermostable inhibitors of contact activation 1, TICA1). Ir-CPI is the only natural inhibitor of both FXI and FXII to isolated from the salivary glands of the tick Ixodes Ricinus. In a phase 1, double-blind, placebo-controlled trial, the pharmacokinetic, pharmacodynamic, safety and tolerability profile of Ir-CPI was investigated in healthy male volunteers (NCT04653766). Not only exogenous or synthetic natural
proteins, but also endogenous macromolecules, including serine protease inhibitors are undergoing preclinical investigation as potential inhibitors of FXIIa.70

Aptamers

Aptamers are short oligonucleotides of single-strand DNA or RNA isolated from complex nucleic acid libraries using an iterative in vitro selection procedure called systematic evolution of ligands by exponential enrichment. Among them, Factor ELeven Inhibitory APtamer, FELIAP, is an single-strand DNA aptamer designed to inhibit the activity of FXIa by binding at or near its active site with a high affinity,71 and was considered a lead compound for the development of other anti-FXIa aptamers, including two RNA aptamers (11.16 and 12.7).72 Despite the interest in these molecules, the currently existing aptamers have important pharmacokinetic limitations, and their renal clearance makes them unattractive for clinical development.73

Conclusions

Considerable progresses have been made in the field of thromboprophylaxis, particularly with the advent of DOACs. However, the risk of major bleeding associated with their use remain a clinically relevant challenge, and some therapeutic settings (e.g., thromboprophylaxis for mechanical heart valves, severe renal or hepatic function impairment, or extreme body weight) represent unsolved medical needs.

Inhibition of the contact coagulation pathway at the level of the FXI or FXII is a new concept in the field of anticoagulation, to uncouple thrombosis and hemostasis. To date, several phase 2 and 3 clinical trials have been conducted or initiated on FXI thromboprophylaxis, particularly with the advent of DOACs. Important pharmacokinetic limitations, and their renal clearance remain a clinically relevant challenge, and some therapeutic

References


