Factor XI and Atrial Fibrillation: A Mismatched Pairing?

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Abstract

Factor XI (FXI) is a liver-produced coagulation zymogen that evolutionarily originated from duplication of the gene encoding for prekallikrein. It circulates in complex with high-molecular-weight kininogen, and consists of two identical subunits that bind thrombin, FXIIa and FIX. Thus, the FXI molecule has features different from other coagulation factors. Pharmacological FXI blockade using small molecules, monoclonal antibodies and antisense oligonucleotides, has been developed, with a hypothesis of a bleeding-free, effective anticoagulation. Dose-finding Phase II trials were performed for thromboprophylaxis in orthopaedic surgery, non-valvular AF and as an add-on strategy to antiplatelet drugs in acute atherothrombosis (stroke or MI). None of those studies were powered for safety or efficacy, but rather, they were used to select the optimal dose for Phase III studies. Nevertheless, their limited results were often (over)interpreted as supporting the hypothesis of the first bleeding-free anticoagulation strategy. The failure of the Phase III OCEANIC-AF trial comparing the FXI inhibitor asundexian to the FXa inhibitor apixaban in AF obliged the scientific community to reconsider the bleeding-free hypothesis and the pathophysiology of FXI. Here, the molecular, disease-related and pharmacological features of FXI were analysed to provide possible explanation(s) and hypotheses for this (temporary) failure of FXI targeting. Specifically, the authors describe the peculiar features of the molecule in the coagulation cascade, the possible mechanisms for the bypassing of FXI activity, the clinical evidence related to FXI congenital deficiency, levels measured in pro-thrombotic settings, the pathophysiology of different thromboembolic disorders and the pharmacodynamics of FXI blockade in Phase I and II studies.

Keywords

Factor XI, asundexian, milvexian, atrial fibrillation.

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Physiology of Factor XI in the Coagulation Cascade: A Bypassable Factor?

Factor XI (FXI) is a liver-produced coagulation zymogen that originated from the duplication of the KLKB1 gene encoding for prekallikrein (PK). It circulates in plasma in complex with high-molecular-weight kininogen (HK), similar to the major fraction of PK. As a result of its origin, FXI is distinctly different from other coagulation proteins, consisting of two identical subunits, each containing four apple domains (A1–A4) that bind specific enzymes, including thrombin, activated FXII (FXIIa) and FIX. In the coagulation system, FXI links FXIIa to the downstream intrinsic route, while it can independently accelerate the intrinsic cascade upon feedback activation by thrombin.[1–3] In haemostasis, FXI plays a limited and yet incompletely characterised role (see next section). In thrombosis its role may be supportive in enhancing coagulation cascade efficiency. However, its role in coagulation is ancillary, rather than essential (like FXa), given that in its absence kallikrein can directly activate FIX, bypassing FXI and, in conditions of high tissue factor (TF) levels, the extrinsic pathway directly activates FIX and FX, also obviating the need for FXIa amplification.[4]

In preclinical models, inhibiting FXI(a) prevents thrombosis in the venous and arterial circulation, including stroke models.[5–7] Interestingly, especially under arterial thrombosis conditions, activated platelet-released polyphosphates accelerate FXIIa-dependent FIX activation, bypassing FXI.[8] Under thrombo-inflammatory conditions, NETosis may further drive FXII-activated coagulation, playing a prominent role in new-onset atherothrombosis.[9,10] Whether FXI is always required to contribute to thrombosis under such arterial inflammatory circumstances remains to be determined.

FXI Levels and Phenotypes: Lessons from Human Disorders

According to the Online Mendelian Inheritance in Man definition, FXI deficiency is an autosomal bleeding disorder characterised by reduced levels of FXI in plasma (<15 IU/dl). Bleeding occurs mainly after trauma or surgery.[11]

Since the initial description of the inheritable mild-to-moderate bleeding disorder, a non-linear association between the degree and type of bleeding phenotype and the residual FXI plasma levels has been consistently reported.[12] This phenotype makes FXI deficiency different from other congenital coagulation factor deficiencies, in which there is a linear correlation between coagulation factor levels and bleeding grade.[13] Genetic studies also indicate that homozygous and heterozygous FXI deficiencies result in variable and poorly predictable bleeding diathesis, often unrelated to residual plasma antigen or activity levels, or to a specific genotype.[14] In a large cohort of 10,193 individuals tested for FXI

activity for routine screening, as compared with higher FXI activity levels, levels <50% seemed protective for venous thromboembolism (VTE; adjusted HR 0.26; 95% CI [0.08–0.84]) and for cardiovascular events, but again there was no correlation between the reduced adjusted HR and the residual FXI activity. Patients with <50% residual activity also had more gastrointestinal bleeding, but no difference in intracranial haemorrhages.[15]

Data regarding the association between high FXI levels and thrombosis are also heterogeneous. Very high levels of FXI antigen have been associated with cerebral venous thrombosis, but a recent systematic review could not identify a clear association between high FXI levels and VTE recurrence.[16–19] Data on FXI activity and cardioembolic complications in non-valvular AF patients are rather limited and therefore its role in AF-associated coagulation disorder remains unknown.[20]

A case-cohort analysis of 30,239 participants in the REGARDS study reported no association between increased FXI levels and incident stroke or CHD.[21] Similar results have been reported in two other large cohorts in the Cardiovascular Health Study and the Atherosclerosis Risk in Communities (ARIC) study, thus questioning a pro-thrombotic role in acute atherothrombotic disorders.[22,23]

Similarly, in patients admitted with acute ischaemic stroke, although a subset had elevated levels of FXIa–inhibitor complexes, none of the measured markers for contact activation or FXIa was related to either severity of stroke or to functional outcome at 3 months.[24] In contrast, high fibrinogen, FVIII, von Willebrand factor (VWF) and glycoprotein VI (GPVI) levels were associated either with stroke severity and/or functional recovery in other patients from the same Collaboration for New Treatment in Acute Stroke (CONTRAST) consortium, suggesting that the platelet–endothelium interaction may be more important in acute stroke, with less implications for the contact system and FXI in causing organ damage.[25] In those studies, the fraction of subjects with known AF was only approximately 10%.

Measuring Factor XI in Disease and Drug Development: Is There a Reference Assay?

To study the impact of FXI in human disease, specific laboratory assays have been developed and used to probe the amount of free FXIa in plasma or assess the amount of FXIa in complex with a natural inhibitor, such as C1-inhibitor (C1-inh), alpha1-antitrypsin (α1-AT) or antithrombin (AT).

In particular, the FXIa-C1inh and FXIa-α1AT complexes have been studied in models of disease and different cardiovascular disorders. When challenged *in vivo* with an inflammatory agonist (endotoxin), humans responded with a rapid generation of FXIa with a peak at 2 hours and then a rapid decline due to ongoing inactivation by one or more serine protease inhibitors (SERPINs) against FXIa.[26] The subsequently detected complexes circulated for a prolonged period of time; half-lives of these FXIa–inhibitor complexes vary: for example, for FXIa-α1AT they vary between 95–104 minutes and 95–349 minutes.[27]

Free FXIa can be measured by enzyme capture assay, or by activated partial thromboplastin time (aPTT)-based assay, or thrombin generation assay (TGA).[20,26,28] All of these assays are quite laborious and unsuitable for routine use. Enzyme–inhibitor complex assays were used to document FXIa generation in diverse conditions such as cancer-associated VTE, acute coronary syndromes (ACS), vasculitis, COVID-19 infection and sepsis, and these data have provided insight into the involvement of contact activation, or, more specifically, FXIa formation, in relation to disease severity or outcomes. For VTE, consistent data indicate a role for FXIa measured during acute VTE in predicting the recurrence of VTE.[18] In patients with cancer-associated VTE, elevated FXIa–inhibitor complexes were linked to incident VTE and poor survival.[29]

FXIa–inhibitor complexes were positively associated with stroke in relatively young female subjects, but in middle-aged men at risk of cardiovascular complications, an inverse association was evident with FXII, but not with FXI. In patients admitted with acute stroke, acute levels of FXIa–inhibitor complexes were not associated with stroke severity or functional outcome at 3 months.[24] In contrast, biomarkers indicative of platelet, endothelial cell and inflammation (soluble GPVI, VWF, ADAMTS-13, fibrinogen, FVIII) were all linked to different aspects of stroke severity, including stroke volume and long-term functional outcomes.[25] Collectively, these data may imply that in acute stroke, there may be a contributing role for the contact system, including FXII, possibly mediated in part by FXI, but the early key checkpoint regulators may be based on platelet–endothelium inflammation interactions, in which T-cells play an important role.[30] With regard to the primary prevention of cardioembolic stroke in AF, there are few biomarker data supporting FXI as an important amplifier of thrombosis.[20] Circumstantial evidence indicated a strong correlation between FXIIa– and FXIa–inhibitor complexes in the 2nd Northwick Park Heart study, but given the negative association of FXIIa–inhibitor complexes with incident stroke, the underlying mechanisms still need to be characterised.[31]

For the development of FXI-targeted drugs, the determination of a drug's effect on clinically meaningful coagulation assays is critical. In contrast to direct oral anticoagulants (DOACs), FXIa-inhibiting agents generally prolong aPTT, given that thromboplastin is stimulated with a contact-activating agent such as kaolin or dextran, reflecting any deficiency in the intrinsic coagulation route. For small interfering RNA (siRNA) therapies, the FXI level (antigen or mostly activity) can be measured too. For the small molecule inhibitor asundexian, the dose selection was based on an in-house validated assay for the residual, free FXIa, upon contact activation in plasma.[32] Residual FXIa was inferred as a biomarker for the inhibitory effect of asundexian and used to guide dose selection in Phase I and II studies. In daily practice, aPTT may be a method to detect the anticoagulant effects of FXIa inhibitors, given that it appears sufficiently sensitive to such agents.

In addition, FXI inhibition can be studied with a TGA, most optimally when stimulated via the contact pathway. Alternatively, FXIa inhibition is detectable in TF-stimulated plasma only at relatively low concentrations of TF, given that at higher TF concentrations, the FXI path is bypassed by direct activation of the extrinsic pathway leading to FX activation.[31] TGA was also used to study potential differences in the FXIa inhibitory potential of asundexian versus milvexian, when compared with apixaban, *in vitro*.[33]

To document the impact on downstream coagulation proteases, enzyme–inhibitor complex assays for FIXa, FXa or thrombin (F1+2 or thrombin–antithrombin complex) could be used, but these assays are not routinely available. However, they may be valuable to assess the net anticoagulant effect of FXIa inhibition. The net effect on clotting could theoretically also be probed using a D-dimer assay or another method for documenting fibrin formation, including viscoelasticity assays, such as thromboelastography or rotation thromboelastometry, although there is not sufficient data to support such methods.

Pharmacology of FXI Blockade in Phase I and II Studies: Evidence is Too Limited

In a Phase I study, doses of asundexian between 50 mg and 100 mg once daily reached a maximum plasma concentration (Cmax) at the steady state of 963 µg/l and 1,950 µg/l (approximately 30 µM), respectively, with an area under the curve (AUC) of 13,800 µg/h/l and 29,500 µg/h/l, respectively.[34] The inter-individual variability in AUC in healthy subjects varied from 11% to 33%. Clearance parameters did not change with the dose; the bioavailability was >80%, with minimal effect of food or changes in gastric pH, and a half-life of ~15 hours.[35] The aPTT at steady state was slightly more prolonged at 100 mg once daily than 50 mg once daily (aPTT ratio to baseline: 2.22 [5.96] versus 2.08 [7.24], given as the geometric mean and geometric coefficient of variation), but the lower limit of the standard deviation of data at 50 mg largely overlapped the upper limits of the 25 mg once daily dose. Based on an in-house assay developed to measure residual FXI activity, FXIa was below the limit of detection in all (n=9) healthy subjects on 100 mg once daily, and the ratio to baseline could not be calculated at Cmax or at 24 hours.[35] There was a strong inverse correlation between drug concentration and FXIa expressed as ratio to baseline and, expectedly, there was no effect on VWF.

Some *in vitro* data suggest that asundexian may be somehow less potent than milvexian (~20% based on aPTT), with a slightly weaker inhibitory potential of asundexian under low TF conditions.[33,36] However, the efficacy *in vitro*, for example, the maximum inhibition of FXIa, is similar and the high bioavailability of asundexian may compensate somehow for a slightly lower potency. Moreover, the milvexian inhibitory effects on TGA, mirroring apixaban, may suggest a less specific effect on coagulation factor(s), mimicking the DOAC inhibition.[33]

A Phase II trial on total knee arthroplasty (TKA) with siRNA in 300 patients, suggested that reducing FXI levels could prevent postoperative VTE, and, based on a very small number of events, the results were interpreted as the FXI lowering being better than low-molecular-weight heparin, with minimal risk of bleeding.[37] Interestingly, the Phase II, dose-finding trial on TKA with milvexian (25–200 mg once or twice daily) showed no clear dose-dependency of doses on aPTT prolongation or bleeding tendency. Those small, dose-finding, Phase II randomised controlled trials (RCTs) in orthopaedic surgery, albeit unsuitable to assess efficacy and safety, nevertheless raised optimism about the potential of FXI blockade as a bleeding-free antithrombotic strategy.

Dose-finding, Phase II studies were also conducted for asundexian and milvexian in ACS, AF and ischaemic stroke, with unclear and over-interpreted clinical outcomes. Two doses of asundexian (25 mg and 50 mg once daily) were tested in high-risk AF patients versus apixaban, the overall absolute number of events (10 major bleeding and 9 thromboses) was much lower than predicted. The same doses were tested on top of antiplatelet drug(s) in Phase II studies on stroke and MI patients (in which also 10 mg was tested). Also, in those dose-finding studies, total bleeding and thrombotic events were lower than anticipated but, still, over-elaborated. This enthusiasm was further fuelled by the AZALEA-TIMI 71 Phase II trial, using an anti-FXI monoclonal antibody, which enrolled approximately 1,200 patients randomised to three different arms (rivaroxaban, abelacimab 90 mg and 150 mg). This trial was prematurely stopped (after approximately 1 year of treatment and 50% of planned events) due to lower major and clinically relevant non-major bleeding in the two abelacimab arms, but thromboembolic complications were too low to provide any signal regarding possible efficacy.[38]

Riding this enthusiastic wave of a new 'no-thrombosis and no-bleeding' anticoagulant strategy, OCEANIC-AF, a Phase III RCT of asundexian versus apixaban in AF, was prematurely stopped due to major safety concerns. The small molecule, FXI inhibitor asundexian was associated with an approximately fourfold higher rate of thromboembolism than apixaban (HR 3.79; 95% CI [2.46–5.83]), even though the major bleeding rate was significantly lower, throwing doubt on the 'no-thrombosis and no bleeding' hypothesis.[39]

Reasons for Failure

The optimistic development of targeting FXI as an anticoagulant strategy with antithrombotic benefits at no risk of major bleeding may have had some pitfalls related to the pathophysiology of the target and/or to the specific drug development. These aspects are summarised in this section and an overview is provided in *Figure 1*.

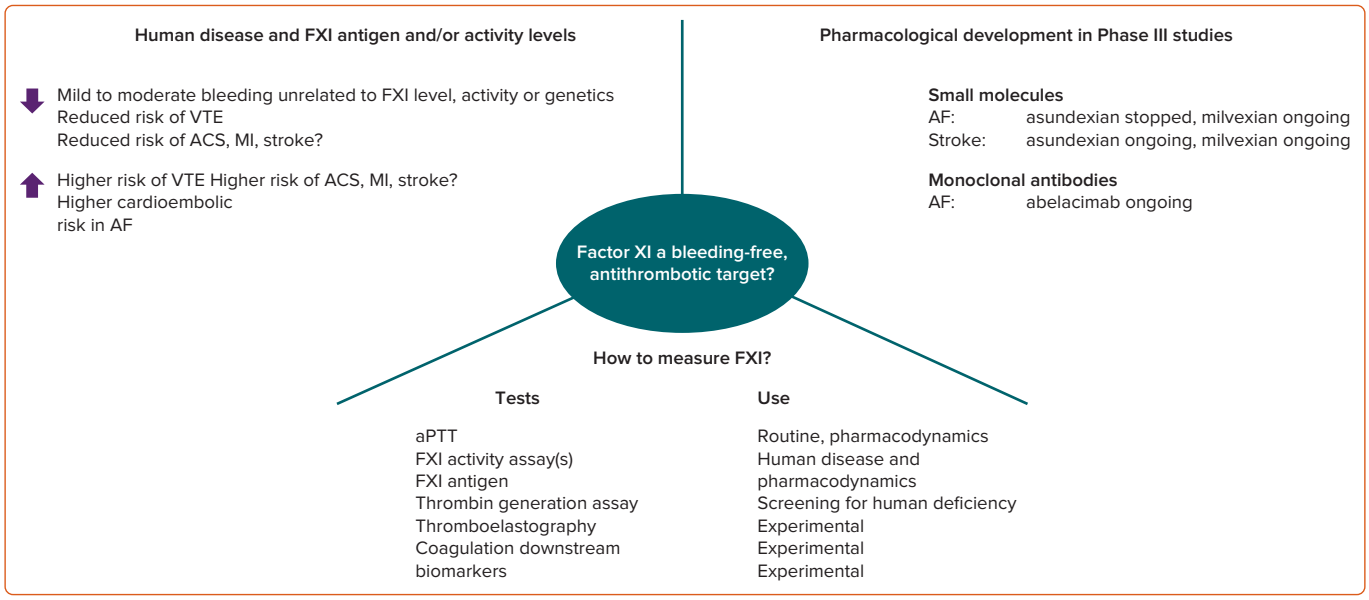
FXI as a Target

Extrapolating the few data on VTE prevention in TKA to thromboembolism prevention in AF may be over-simplistic. VTE after orthopaedic surgery with localised thrombo-inflammation of the proximal venous wall as nidus for thrombosis, cannot be extrapolated to VTE in cancer, unprovoked VTE or to AF, given that triggers and pathophysiology may be quite different. Although AF may have some similarities to VTE in its pathogenesis (mostly because anticoagulation is superior to antiplatelet therapy in preventing thrombosis), other factors, including platelet activation, blood flow and shear alteration in VTE, are different to those in systemic thromboembolism originating in the fibrillating atrium.[40] Importantly, anticoagulants that block central, converging pathways in coagulation, such as vitamin K antagonists (VKAs) or DOACs, will limit any type of coagulation-dominated thrombosis, but FXIa inhibition will only work in situations when it has an important (and prevalent) contribution to the downstream thrombin generation.

Escape mechanisms could occur upon switching from one anticoagulant to another after randomisation in the OCEANIC-AF trial.[39] While this window of hypercoagulability may have been a potential problem in other studies when switching from a VKA to a DOAC, it is unlikely that changing from a DOAC to asundexian (which similarly provides immediate effect) would provide a window of hypercoagulability unless asundexian did not sufficiently inhibit FXIa. Thus, other escape mechanisms may be more important. One mechanism could be that embolic stroke is primarily a result of TF expression-based clotting that, when the TF trigger is sufficiently strong, overrules FXI activation.[32] Alternatively, when blockade of FXIa is nearly complete, another pathway may bypass FXI, that is, kallikrein-mediated FIX activation. Although the second mechanism has been primarily shown in the absence of FXI, the direct interaction of kallikrein with FIX has been clearly demonstrated.[4] However, there are no data on the impact of this route in humans and, more importantly, in patients with AF. Consequently, there are two important routes by which coagulation can proceed, bypassing an effective blockade of FXIa. This bypassing effect may also explain the mild, variable and unpredictable bleeding tendency in congenital FXI deficiency.

Moreover, FXI may be relevant under selected conditions such as thrombo-inflammation following TKA, which is associated with abundant tissue damage and flow changes, and triggers vascular endothelium. Under such conditions, neutrophil–platelet interactions with contact factors and FXI may trigger thrombin generation most effectively.[41] Also, recent data show a strong impact of FXIa on endothelial cell integrity, which can be prevented with FXIa inhibitors and it is possible that in the surgical model, such effects may become prevalent.[42,43] In other words,

Figure 1: Relevant Features of Factor XI Deserving Further Investigation



In humans with congenital FXI deficiency, the degree and genetics of residual FXI levels are unrelated to the severity of bleeding; the role of very high or very low FXI levels in promoting or protecting from arterial thrombosis and AF is unclear. There is also a lack of validation of assays with regard to clinical outcomes and as pharmacodynamic biomarkers. Among Phase III studies targeting FXI in AF patients, asundexian was stopped for lower efficacy versus an anti-FXa agent; and trials with milvexian (versus an anti-Xa) and abelacimab (versus placebo) are currently ongoing. ACS = acute coronary syndrome; aPTT = activated partial thromboplastin time; FXI = factor XI; VTE = venous thromboembolism.

the local conditions that comprise Virchov's triad of thrombogenesis may determine whether proteins such as FXI (or FXII, PK, HK) are relevant or not. Thus, post-orthopaedic surgery VTE may not be representative of the pathophysiological conditions of a fibrillating atrium, which is characterised by very different shear, pressure and endothelial conditions.[40]

In such a scenario, individual patient characteristics and underlying disorders, FXI level, and the thrombin- and fibrin-generating potential may become additional important determinants of thrombosis. Presently, our knowledge of these mechanisms *in vivo* is limited.

Interestingly, data from the AZALEA-TIMI 71 Phase II trial are in line with OCEANIC-AF in terms of lack of bleeding, with a similar relative reduction in major bleeding, approximately 60–70% in both trials.[38] Thus, the apparent success of the AZALEA trial does not yet guarantee a successful outcome in efficacy, which will be tested in the ongoing Phase III RCT.

Anti-Factor XI Drug Characteristics

The asundexian dose selected for its Phase III RCT (50 mg once daily) was based on Phase I and II dose-finding studies and specifically on standard aPTT prolongation and on the specific coagulation assay of residual FXIa. The selected dose inhibited >90–95% of the FXI activity in healthy subjects and in patients.

One hypothesis for OCEANIC-AF's failure is that the dose and, therefore, the degree of FXI activity inhibition could have been insufficient to block FXIa at a clinically meaningful level. Among antithrombotic drugs, for instance, low-dose aspirin inhibits its target by >97%, which translates into cardiovascular protection.[44] For $P2Y_{12}$ blockade, clopidogrel that inhibits ADP-induced platelet activation by approximately 40–60% has lower antithrombotic efficacy than prasugrel or ticagrelor, which blocks the ADP-induced platelet function by ≥90%.[45] Importantly, there are no data on the clinical outcomes associated with residual levels or thresholds of FXIa as assessed by aPTT, selected assays or TGA, not even from human diseases.

Unfortunately, for residual FXIa, there is no 'international normalised ratio-like' reference assay, able to predict clinical outcomes and guide FXI therapy at the moment. For TGA, FXa activity or aPTT there are no data associating thresholds or ranges of the assays with a clinical outcome (either thrombosis or bleeding) and no data to identify the reference method. Although the data on TGA are intriguing, one should be cautious in concluding that the observed effects of asundexian on TGA *in vitro* would reflect a too low dosing *in vivo* in the OCEANIC-AF trial.[33] Thus, further research is needed. Moreover, coagulation occurs on cell and platelet membranes and under dynamic flow conditions, thus *in vitro* assays, which occur in still blood and in a plastic tube, have major limitations in this respect. Similarly, platelet function testing in a plastic tube and recorded as an electric signal has never been able to guide antiplatelet drug treatment and predict thrombotic events.[46]

Given that some inter-individual variability (up to 33%) was detected in Phase I studies, a higher drug dose (100 mg once daily) may have compensated for a hypothetical lower responsiveness in a fraction of patients (10–30%) due to variability in response.

Milvexian is being tested versus apixaban in AF patients at a dose of 100 mg twice daily. This drug has a slightly shorter half-life than asundexian, its Cmax and AUC are considerably influenced by food intake, and a 200 mg dose given once daily reaches a Cmax of 1,512 ng/ml and produces a reduction of clotting FXIa of ~90% at steady state versus baseline in healthy subjects.[47,48] However, as suggested by some *in vitro* data on milvexian, too high doses could affect the selectivity of the drug, generating cross-inhibitions with other serine proteases.[33,36] Whether a twice-daily regimen with a relatively shorter-acting drug such as milvexian would be more effective than a once-daily regimen of asundexian is a theoretical issue that will not be easily solved in the absence of direct comparative studies at this stage.[49]

Abelacimab is a monoclonal antibody drug, with a very long half-life of 25–30 days that causes profound (>99%) inhibition of free FXI already at

1 hour after infusion.[48] Thus, its pharmacokinetic and pharmacodynamic characteristics are very different from the other two small molecules. Abelacimab is currently being tested versus placebo in a subset of AF patients who are unsuitable for any anticoagulant therapy.[50]

Conclusion

At the present time, the hypothesis of a bleeding-free anticoagulant agent with FXI as the target seems rather too simplistic, at least for thromboembolism prevention in AF. Misinterpretation of Phase II studies, still-unknown aspects of human disease, the incorrect assumption that VTE and AF coagulation dysfunctions share similar pathways and pathophysiology, and the limitations and unknown features of coagulation biomarkers and assays to describe a drug's pharmacodynamics in relation to clinical outcomes, drug characteristics and dose may share some responsibility in this failure.

The ongoing RCTs of other molecules targeting FXI in AF and stroke will help to answer those questions. The clinical validation of coagulation assays and biomarkers and the possible pharmacokinetic analyses of the OCEANIC-AF trial will be of the utmost importance to untie these knots. Also, the analysis of downstream coagulation markers, such as FIXa-AT, prothrombin fragment 1+2 and thrombin–antithrombin complex, in patients with and without events will be crucial. Although unfortunate, the failure of the OCEANIC-AF contributed to improving knowledge, prompted a re-think of pathophysiological hypotheses and paths of drug development, and shaped new therapeutic settings for targeting FXI. ❑

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