Factor XII/XIIa inhibitors: their discovery, development, and potential indications

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Abstract

Coagulation factor XII (FXII) is a S1A serine protease discovered over fifty years ago. However, its *in vivo* functions and its three-dimensional structure only started to be disclosed in the last decade. FXII was found at the crosstalk of several physiological pathways including the intrinsic coagulation pathway, the kallikrein-kinin system, and the immune response. The FXII inhibition rises as a therapeutic strategy for the safe prevention of artificial surface-induced thrombosis and in patients suffering from hereditary angioedema. The anti-FXII antibody garadacimab discovered by phage-display library technology is actually under phase II clinical evaluation for the prophylactic treatment of hereditary angioedema. The implication of FXII in neuro-inflammatory and neurodegenerative disorders is also an emerging research field. The FXII or FXIIa inhibitors currently under development include peptides, proteins, antibodies, RNA-based technologies, and, to a lesser extent, small-molecular weight inhibitors. The majority are proteins, mainly isolated from hematophagous arthropods and plants. The discovery and development of these FXII inhibitors and their potential indications will be discussed in the review.

Keywords

Serine proteinase inhibitors; drug development; antithrombotic agents; anti-inflammatory agents; contact pathway; factor XII.

Abbreviations

2'-MOE	2'-O-methoxyethyl
α1-AT Pitt	α1-antitrypsin Pittsburgh
Aβ	amyloid-beta
aPTT	activated partial thromboplastin time
ASO	antisense oligonucleotide
AT-III	antithrombin III
BbKI	*Bauhinia bauhinioides* kallikrein inhibitor
BpTI	*Bauhinia pentandra* trypsin inhibitor
BTI	Barley trypsin inhibitor
BuXI	*Bauhinia ungulata* FXa inhibitor
BvTI	*Bauhinia variegata* trypsin inhibitor
C1-Inh	C1-esterase inhibitor
CeKI	*Caesalpinia echinata* kallikrein inhibitor
cDNA	complementary DNA

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CMTI-III	*Cucurbita maxima* trypsin inhibitor III
CMTI-V	*Cucurbita maxima* trypsin inhibitor V
CTI	corn trypsin inhibitor
DIC	disseminated intravascular coagulation
EcTI	*Enterolobium contortisiliquum* trypsin inhibitor
EGF	epidermal growth factor
FnI	fibronectine type I domain
FnI-EGF	fibronectine type I domain and the second epidermal growth factor domain
FnII	fibronectine type II domain
GalNAc	N-acetylgalactosamine
HAE	hereditary angioedema
HMWK	high-molecular-weight kininogen
IL-6	interleukine 6
Inf-4	infestin-4
Ir-CPI	*Ixodes ricinus* contact phase inhibitor
LCTI-III	*Lufa cylindrical* trypsin inhibitor III
MCoTI-II	*Momordica cochinchinensis* trypsin inhibitor-II
MCTI-I	*Momordica charantia* trypsin inhibitor I
PPACK	D-phenylalanyl-prolyl-arginyl chloromethyl ketone
PS	phosphorothioate
PT	prothrombin time
r-HA-Inf-4	recombinant human albumin-fused infestin-4
RISC	RNA-induced silencing complex
SAC I	*Streptoverticillium* anticoagulant I
siRNA	small interfering RNA
TATA	1,3,5-triacryloyl-1,3,5-triazinane
TaTI	*Torresea acreana* trypsin inhibitor
TBAB	N,N',N''-(benzene-1,3,5-triyl)-tris(2-bromoacetamide)
TBMB	1,3,5-tris(bromomethyl)benzene
TcTI	*Torresea caerensis* trypsin inhibitor
uPAR	urokinase receptor

1. Introduction

Factor XII (FXII), a serine protease belonging to the S1A subfamily, is mainly produced and secreted by the liver into the circulation as a zymogen [1,2]. This liver-derived mature zymogen is a single-chain glycoprotein of 596 residues (~80 kDa) with a low proteolytic activity [3,4]. The contact with a negatively-charged surface induces its autoactivation and FXII is converted into α-FXIIa by cleaving the R353-V354 peptide bond. α-FXIIa is composed of a heavy chain of 353 residues (~50 kDa) and a light chain of 243 residues (~30 kDa). The heavy chain carries several domains needed for the binding to artificial surface, Zn^{2+}, cells, factor XI (FXI), heparin and fibrin. The light chain only consists of the catalytic domain that includes the catalytic triad (H393-D442-S544) (Figure 1) [5–7]. Plasma kallikrein further cleaves α-FXIIa at the peptide bonds R343-L344 and R334-N335 to give β-FXIIa [3,5]. The physiological inhibitors of FXIIa are mainly the serpin C1-esterase inhibitor (C1-Inh) [8] and, to a lesser extent, antithrombin III (AT-III), plasminogen activator inhibitor-1, α2-macroglobulin, α2-antiplasmin and α1-antitrypsin [3,5]. Beside this liver isoform, recent studies pointed out two new isoforms of FXII synthesized respectively by leukocytes [9] and neurons [10].
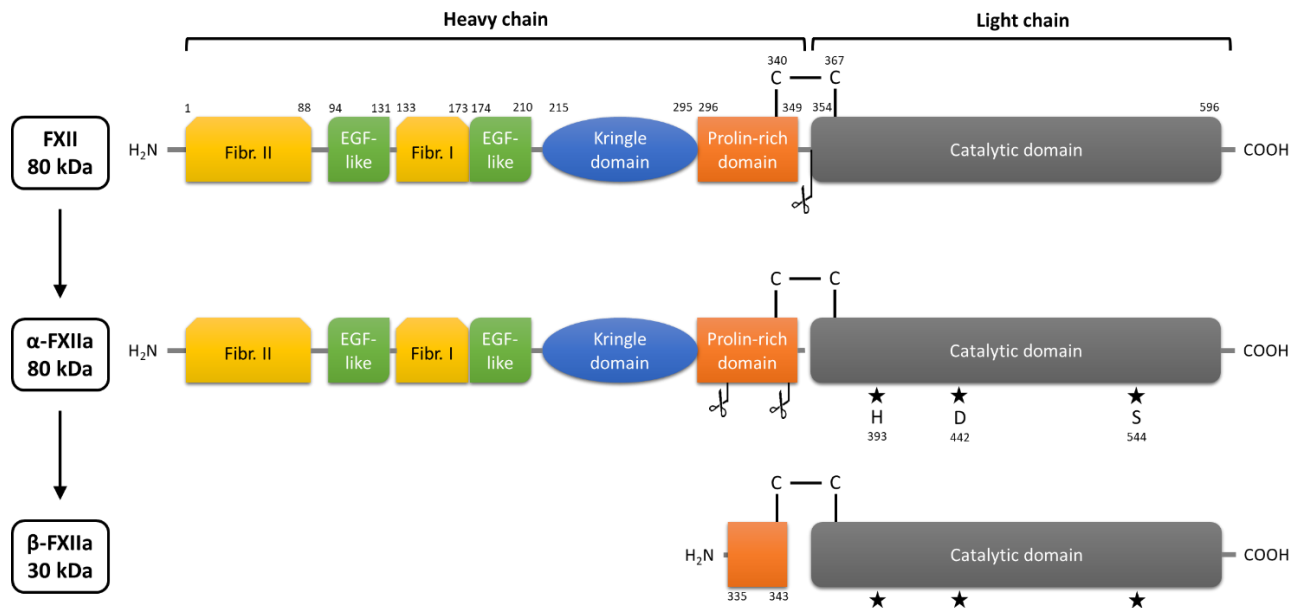
Figure 1: Structure of FXII and FXIIa. *The scissors indicate the sites of cleavage. The stars refer to the catalytic triad. The first cleavage yields the active form α-FXIIa and the second processing releases the β-FXIIa. Figure inspired by* [3,6]. *(2-column fitting image)*

Physiologically, FXII is at the crosstalk of several pathways implicated in coagulation, inflammation, and immunity (Figure 2). FXII and FXIIa also induce various direct cellular responses [5,11]. The β form of FXIIa cannot regulate all the pathways due to the loss of the heavy chain. As an example, β-FXIIa is unable to promote blood clotting because β-FXIIa lost its capacity to bind to negatively-charged surfaces and to FXI [6,12–15]. Over all, the physiological and pathological functions of the active FXII *in vivo* are diverse, not fully understood and involve complex molecular mechanisms [5].
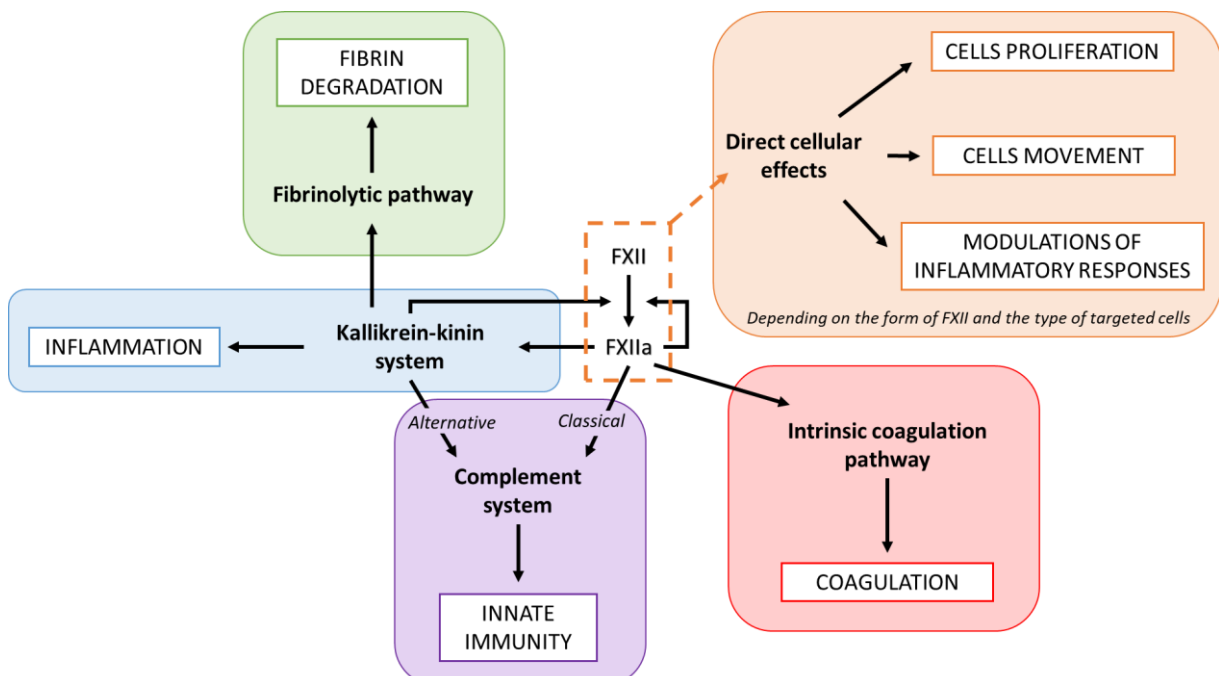

Figure 2: FXII/FXIIa, a serine protease at the crosstalk of inflammation, coagulation and innate immunity. *(1.5-column fitting image)*

Focusing on FXII/FXIIa as therapeutic target, this review (a) summarizes the actual knowledge on FXII/FXIIa three-dimensional structure, (b) highlights the potential indications of anti-FXII/FXIIa agents, and (c) describes the strategies applied for their discovery and development.

2. FXII structure

Although FXII is a protein discovered over fifty years ago, structural data are limited [16–19]. In 2013, Beringer et al. [19] resolved the 133-213 portion of the FXII heavy chain, corresponding to the fibronectine domain type I and the second epidermal growth factor domain (FnI-EGF), in its native state (PDB: 4BDX, 1.62 Å) and in a holmium-bound state (PDB: 4BDW, 2.501 Å). The overall structure of FXII FnI-EGF was found similar to the homologous tissue plasminogen activator tandem domain (PDB: 1TPG), even if the interface hydrophobic residues are not identical [19]. Previously, the fibronectin type II domain (FnII) was found important but not essential for the interaction of FXII with negatively-charged surfaces [20]. In FnI, a continuous patch of five positively charged residues (Lys146, Arg160, Lys164, His169 and Arg172) extending over 20 Å in length was highlighted and could supplement FnII. This patch could also be involved in the interaction with amyloid fibrils and cross-β structures. An alternative hypothesis is an extension of the three-stranded β-sheet. Regarding the second EGF-like domain, the steric hindrance of FnI and the lack of crucial arginine residue assume that EGF receptor stimulation by FXII is more likely via uPAR than a direct interaction [19]. In 2015, Pathak et al. [16] crystallized two constructs of the FXII light chain, termed FXIIc (PDB: 4XDE, 2.14 Å) and FXIIac (PDB: 4XE4, 2.4 Å). The constructs were His-tagged and their unpaired Cys467 was mutated to Ser to avoid potential aggregation. Their crystallization revealed a lack of oxyanion hole concurring with a zymogen-like conformation of the FXII catalytic domain. They pointed out the H1 pocket, a distinctive hydrophobic cavity in front of the S1 pocket with the Asp397 at its bottom (or Asp60A with chymotrypsin A numbering) [16]. This Asp60A was highlighted by Hamad et al. [21] as a key amino acid residue for the binding of Corn Trypsin Inhibitor (CTI), a highly potent and specific inhibitor of FXIIa [16,18,21]. In 2018, Dementiev et al. [17] published the co-crystallization of human β-FXIIa with benzamidine (PDB: 6B74, 2.323 Å) and with [3-(1-aminoisoquinolin-6-yl)phenyl]boronic acid (PDB: 6B77, 2.37 Å), a covalent inhibitor. The catalytic domain adopts a typical chymotrypsin-like serine protease active conformation. The S1 pocket, known to drive the major part of the association energy with ligands, is almost alike to that of hepatocyte growth factor activator, tissue plasminogen activator, factor Xa (FXa) and thrombin [17]. In 2019, Pathak et al. [18] reported the production and the crystal structure of recombinant His-tagged β-FXIIa (PDB: 6GT6, 2.54 Å). They also produced a construct where the N-terminal of recombinant His-tagged β-FXIIa is fused to maltose-binding protein and determined its crystal structure in complex with D-Phe-Pro-Arg-CH$_2$Cl (PPACK) (PDB: 6QF7, 4 Å). The superposition of 6B74 and 6GT6 showed a similar conformation except for the S2 pocket. Dementiev et al. [17] reported a closed configuration where Tyr99 is packed against His57 (PDB: 6B74) while Pathak et al. [18] showed a more open S2 pocket where Tyr99 is packed against Trp215 (PDB: 6GT6) and, as a results, partially occludes S3 (Figure 3). They hypothesized a transient movement of Tyr99 in the presence of substrate mimetic [18]. Unlike 6B74, the side chains of Asp60A and Gln192 does not extend into the solvent in 6GT6 (Figure 3). One explanation is the fact that 6GT6 exhibits a crystal contact. Indeed Thr-Arg preceding the His-tag of a second recombinant His-tagged β-FXIIa, which are not present in its native form, project into S2 and S1 pockets [18]. This second protein may prevent the deployment of solvent-extended amino acid side chains.
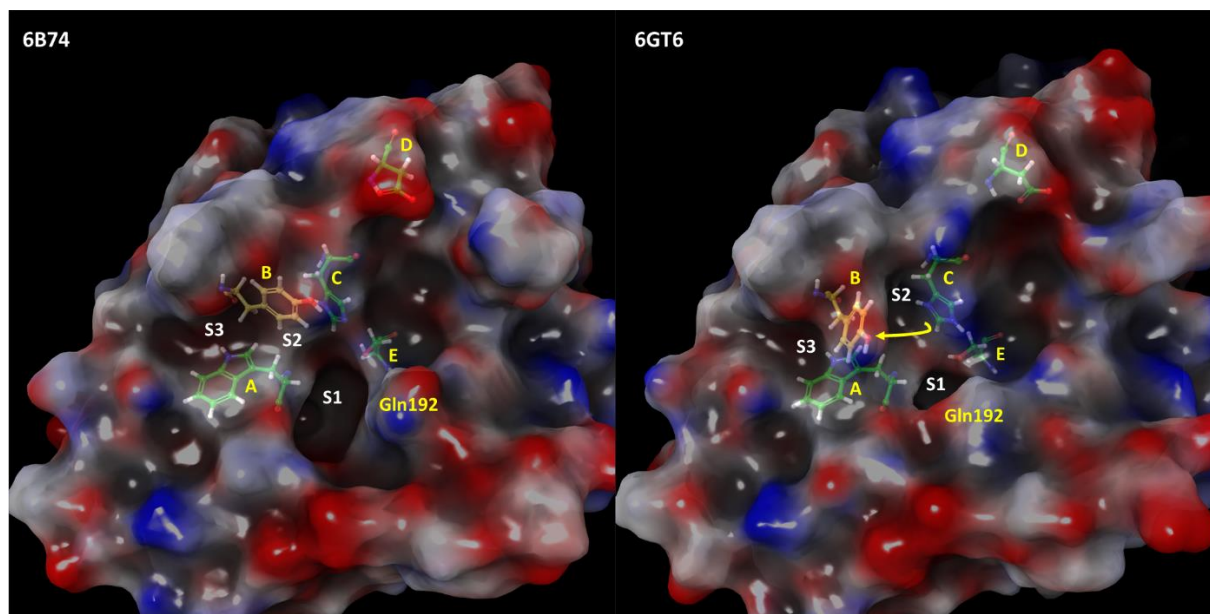
Figure 3: Structure of β-FXIIa active site. *The arrow highlights the movement of Tyr99 observed between the crystals 6B74 and 6GT6. A: Trp215. B: Tyr99. C: His57. D: Asp60A. E: Ser195.* (2-column fitting image)

3. Potential indications for anti-FXII/FXIIa agents

According to recent reviews [1,22,23], FXII-directed therapies could be useful in artificial surface-induced thrombosis and in various inflammatory disorders. The selection of indications are focused on those where there are unmet medical needs, particularly where current therapies are limited in both efficacy and safety [24]. Table 1 summarizes the potential indications for anti-FXII/FXIIa agents. The indications proposed are based either on experimental work with animals or on clinical trials. Currently, two clinical trials are ongoing with indications being (1) prophylactic treatment of hereditary angioedema (HAE) and (2) thromboprophylaxis in end-stage renal disease patients on chronic hemodialysis [25].

Table 1: Potential indications for FXII/FXIIa agents.

Indication	Agents in clinical phase	References
Prophylactic treatment of HAE attacks by preventing their onset and treatment for the rare HAE-FXII	Garadacimab§ (CSL312)	[26–28]
With **extracorporeal circuits** to prevent clotting on extracorporeal membrane oxygenator or cardio-pulmonary bypass circuits and for reducing cardiovascular events in patients on **hemodialysis**	/ xisomab 3G3§§	[4,24]
With **medical devices** to prevent clotting on mechanical heart valves, ventricular assist devices or catheters	/	[4,24]
Sepsis to mitigate the morbidity and the mortality by blocking severe inflammation without affecting hemostasis or the host immune response	/	[29]
Multiple sclerosis to redress immune balance	/	[30,31]
Alzheimer's disease treatment	/	[32–34]
Traumatic brain injury as neuroprotective	/	[35–37]

§ Garadacimab or CSL312 is an anti-FXIIa antibody.
§§ Ximomab 3G3 is an anti-FXI monoclonal antibody which inhibits its activation by FXIIa and, in consequence, acting like an anti-FXIIa agent on the coagulation pathway.
HAE: hereditary angioedema. HAE-FXII: hereditary angioedema caused by a mutation of factor XII.

3.1. In hereditary inflammatory diseases

HAE is a rare genetic disease characterized by recurrent episodes of severe subcutaneous and mucosal swellings, mostly localized on the face, the intestinal tract, and the respiratory tract. Minor trauma, infection or stress can induce an angioedema attack but most often the trigger is unknown, leading to unpredictable and life-threatening swellings [38]. Six types of HAE are recognized according to their enzymatic phenotype: 1) C1-Inh deficiency (type I HAE, about 85% of patients), 2) C1-Inh dysfunction (type II HAE), 3) FXII with an increased susceptibility to activation (HAE-FXII, also referred as type III HAE) [27,28], 4) angiopoietin-1 dysfunction [39], 5) plasminogen dysfunction [40,41] and 6) HAE with kininogen-1 gene defect [42]. In the pathophysiology of type I/II HAE and HAE-FXII, excessive bradykinin formation by overactivation of the kallikrein-kinin system is identified as a critical driver of angioedema [38]. A large panel of therapeutic options are available for the acute treatment of type I/II HAE attacks but less are available for prophylactic treatment of type I/II HAE. Actually, none of them are indicated for the treatment of HAE-FXII [26,38,43]. Preclinical data with antibodies [28,44,45] and small interfering RNA (siRNA) [46] pointed out that the inhibition of FXII alleviates edema in various mouse models. Actually, the antibody garadacimab is under phase II clinical evaluation for the prophylactic treatment of HAE (see Section 4.6.2.1.) [25].

Recently, a mutation in the kringle domain of FXII was associated with a dominantly inherited cold-induced urticarial autoinflammatory disease. This FXII mutant spontaneously activates into FXIIa, leading to an overactive contact system and an increase in inflammatory mediators. Scheffel et al. [47] termed it FXII-associated cold autoinflammatory syndrome. To prevent long-term consequences (such as amyloidosis), the pathological inflammation should be constantly controlled. In this regard, contact phase inhibitor is a promising therapeutic approach for the management of these patients [47].

3.2. In thrombosis

On the one hand, experimental studies with animals indicate that FXII is important for pathological thrombus formation but dispensable for hemostasis. This suggests that inhibiting FXII/FXIIa is an innovative strategy to develop novel and safer antithrombotic agents. On the other hand, epidemiological data are contrasted. Indeed, current epidemiological studies failed to demonstrate the role of FXII in venous thromboembolism, stroke and myocardial infarction [48–50]. In mice, FXII was not involved in the physiopathology of venous thrombosis induced by impaired natural anticoagulation [51]. However, the leading clinical perspective for FXII inhibitors resides for the safe prevention of contact-mediated thrombosis induced by blood-contacting devices [4] such as catheter [52,53], extracorporeal circuits [54] or mechanical heart valves [55–57]. This indication was tested in several animal models (Table 2).

The thrombus formation and the local inflammation at the medical device sites are promoted by a series of interconnected mechanisms involving protein adsorption, platelet, and leukocyte adhesion, thrombin generation, and complement activation [58]. Regarding FXII, several *in vitro* and *in vivo* studies indicate that FXII adsorption and autoactivation on artificial surfaces are the root causes of thrombin generation and fibrin formation via the intrinsic coagulation pathway [56,58]. Activation of bound FXII also induces activation of the kallikrein-kinin and complement systems that generate inflammation [58]. By direct cellular effects, FXII and FXIIa also modulate the functions of several innate immune cells. Via an urokinase receptor (uPAR)-mediated signaling pathway on neutrophils, FXII promotes their priming, persistent inflammation at injury sites and further plasma FXIIa generation. In return, FXIIa causes neutrophils aggregation and degranulation [5,59]. Over all, FXII inhibitors could target a primary cause of contact-mediated thrombosis without increasing bleeding risk and with anti-inflammatory properties [4,60]. Actually, the antibody xisomab 3G3 completed recently a phase II clinical evaluation for thromboprophylaxis in patients with end-stage renal disease on chronic hemodialysis (See Section 4.6.1) [25].

Some authors also proposed FXII as a novel and safe target in prostate [61] and pancreatic [62] cancer-associated thrombosis. The activation of the contact system was observed in various cancers and may have a role in cancer-associated thrombotic risk by promoting thrombin generation. But the exact molecular mechanism is poorly understood and could be dependent of the cancer type [63]. In prostate cancer, some evidences indicate that tumor cells and prostasomes exposed polyphosphates on their plasma membrane, activating FXII [61].

Table 2: Preclinical evaluation of FXII inhibition on artificial surface-mediated thrombosis.

Model	FXII inhibitors	Observations	Reference
Non-human primate model			
Collagen-graft baboon thrombosis	15H8	Reduced fibrin deposition and limited platelet-rich thrombus growth downstream the graft	[64]
Collagen-graft baboon thrombosis	DX-4012	Reduced the size of the thrombus as efficiently as enoxaparin	[65]
Rabbit model			
Extracorporeal membrane oxygenation cardiopulmonary bypass system adapted for rabbits	CSL 3F7	Thromboprotection as efficient as heparin without increasing bleeding risk	[54]
Catheter-induced thrombosis	ASO	Prolonged the time to catheter occlusion by 2.2 fold	[52]
Accelerated catheter-induced thrombosis	CTI-coated catheter	Prolonged the time to occlusion by 2.5 fold	[53]
ASO: antisense oligonucleotide. CTI: corn trypsin inhibitor.			

3.3. In sepsis

Sepsis is an excessive and detrimental response to infections. The exaggerated activation of the inflammatory cascade, complement system, and coagulation pathway lead to organ hypoperfusion and death [29,66]. These systems are cross-linked and dysregulation in one of them induces perturbations in the others. At its most extreme, their systemic activation can evolve into the development of disseminated intravascular coagulation that is a life-threatening condition characterized by thrombus formation in microvasculature and hemorrhagic manifestations [29,67]. Given its implication in inflammatory and coagulopathic responses, FXII could be an attractive target in the management of sepsis complications [29]. FXII-deficiency and FXIIa inhibition have been tested in several baboon and mouse models of sepsis and the results are summarized in Table 3. In humans, a low level of FXII correlates with poor prognosis in sepsis [68]. This low level is probably due to the activation of FXII [69], demonstrated in several studies [70–73]. Taken together, further studies are needed to clarify the role of FXII and, also, to include pathogen-specific responses [5,29,74].

Table 3: Preclinical data of FXII inhibition in sepsis models.

Model	Pathogen	FXII inhibitors	Observations	Reference
Non-human primate model				
Lethal bacteremia	*Escherichia coli*	C6B7	Protects baboons from irreversible hypotension, prolongs lifetime but has no effect on DIC. Reduces complement activation, neutrophil degranulation and level of tissue plasminogen activator and IL-6	[75,76]
Lethal bacteremia	*Staphylococcus aureus*	AB023, also known as xisomab 3G3	Decreases inflammation and prevents organ failure and death	[77]
Murine model				
Lethal endotoxemia	*Escherichia coli* lipopolysaccharide	FXII-deficient mice	Attenuates early hypotensive changes but has no effect on mortality	[78]
Gram-negative pneumonia	*Klebsiella pneumoniae*	FXII-deficient mice	Improves survival and reduces bacterial outgrowth	[79]
Gram-positive pneumonia	*Streptococcus pneumoniae*	FXII-deficient mice	No protective effect	[79]

Gram-negative pneumonia	*Klebsiella pneumoniae*	ASO	No protective effect Low expression of FXII mRNA in lung tissue unaffected by ASO treatment	[80]
Cecal ligation and puncture	Polymicrobial	14E11	Enhances survival and attenuates inflammation and coagulopathy	[81]

DIC: disseminated intravascular coagulation. IL-6: interleukine 6

3.4. In neurology

The implication of coagulation factors in neurological diseases is an emerging research field. Growing evidence correlates abnormalities in coagulation pathway with both neuro-inflammation and neurodegeneration [82]. Regarding FXII, recent studies highlight its role in Alzheimer's disease and multiple sclerosis [1,3,22].

While it is generally accepted that amyloid-beta (Aβ) is a primary driver in Alzheimer's disease, the way Aβ triggers the vascular abnormalities and the neuro-inflammation remains unclear. A major finding is that Aβ can activate the contact system *in vivo* and *in vitro* in a FXII-dependent manner [32–34]. Alzheimer's disease mouse model and human plasma samples have constitutively higher levels of contact activation [1,34]. Moreover, cognitive impairment correlates with a higher level of cleaved high-molecular-weight kininogen (HMWK), concurring with higher activation of the kallikrein-kinin system (Figure 2) [83]. The HMWK cleavage was found to be mediated by FXII [34]. Alzheimer's disease mouse model depleted in plasma FXII using antisense oligonucleotide (ASO) exhibits reduced neuroinflammation, fibrin deposition and neuronal degeneration in the brain. Their cognitive decline was also alleviated [34].

Multiple sclerosis is an autoimmune disease of the central nervous system inducing inflammatory demyelinating lesions. This disease is characterized by a relapsing-remitting course in most patients. During relapse, new lesions in the central nervous system appear. Critical actors in the pathogenesis of multiple sclerosis are autoreactive CD4+ T cells, particularly CD4+ Th1 and Th17 cells [84]. Interactions of naive T cells with dendritic cells modulate their differentiation into specific effector cells (such as Th1 and Th17). In this context, Göbel et al. found that FXII regulates the cytokine profile of dendritic cells via the cell-surface receptor uPAR (also known as CD87) and, in consequence, promotes Th17 cell emergence. FXII-deficiency and inhibition of FXIIa activity with recombinant human albumin-fused infestin-4 (r-HA-Inf-4) protected mice from experimental autoimmune encephalomyelitis, the mouse model of human multiple sclerosis [30]. In plasma samples of patients with multiple sclerosis, increase level of FXII was observed, particularly during relapse [30,31]. These results further stimulate research on the contribution of FXII to the disease phenotype and progression [11,82].

Hopp et al. [35] also proposed FXII as a target in traumatic brain injury. They showed that both FXII deficiency and inhibition of FXIIa activity with r-HA-Inf-4 reduced post-traumatic inflammation and brain edema formation [36]. In addition, the two models also exhibit a better motor function and a lower neuronal cell death, leading to an improved functional outcome without increasing bleeding [35]. This potential indication has to be treated with caution as it is often difficult to translate traumatic brain injury experimental results on animals into the clinic [85].

4. Inhibitors

To describe FXII/FXIIa inhibitors, we used a two-level classification. Compounds are grouped first by their chemical nature and secondly by the way they were discovered. When it is applicable, the optimization process is also reported.

4.1. Peptides and proteins

Several peptides and proteins are reported to inhibit FXII/FXIIa. Regarding FXIIa protein inhibitors, two types can be distinguished based on their mode of inhibition: canonical inhibitors and serpins. Canonical inhibitors recognize their targets in a substrate-like manner [86]. They are divided into several families

according to structural features. Although the various families exhibit completely different global structures, all of them have the particularity to possess a protease-binding loop with a similar canonical conformation [86,87]. This loop is convex, solvent-extended and highly complementary to the concave active site of serine proteases. Disulfide bonds, hydrogen bonds and/or hydrophobic interactions maintain the canonical conformation [86,88]. The most exposed region of this loop is the reactive site P^1-$P^{1'}$ peptide bond [86]. Similarly to canonical inhibitors, serpins firstly interact with their target via a solvent-extended loop, called reactive center loop. However, after recognition, the target protease cleaves P^1-$P^{1'}$ peptide bond. Upon cleavage, the serpin spontaneously refolds and traps the target protease in a covalent complex [86,89,90].

4.1.1. Discovery from hematophagous arthropods

Several proteins with unrelated structures were discovered from blood-feeding arthropods. This is known that hematophagous arthropods produce a cocktail of bioactive compounds to survive. The inhibition of the vertebrate contact pathway is one of the most common activities found in their saliva [91,92]. The diversity in protein structure and mechanism of inhibition can be explained by the convergent evolutionary nature of hematophagy [92].

4.1.1.1.   From ticks

Ixodes ricinus

Starting with the assumption that induced-proteins of tick salivary glands are crucial for their feeding process, Leboulle et al. [93] set up a subtractive complementary DNA (cDNA) library by comparing cDNAs of 5-day blood-fed *Ixodes ricinus* salivary glands with cDNA of unfed ticks. They identified several cDNAs sequences with high homology to genes coding for immunomodulatory or anticoagulant proteins [93]. Seq7, one of these sequences, showed similarities with the second Kunitz domain of human tissue factor pathway inhibitor [93,94]. The recombinant protein, namely *Ixodes ricinus* contact phase inhibitor (Ir-CPI), showed anticoagulant properties by targeting FXIIa, factor XIa (FXIa), and kallikrein. Despite belonging to the Kunitz-type family, Ir-CPI does not interact in a substrate-like manner. It inhibits the reciprocal activation of contact factors by steric hindrance on an exosite [94]. The protein is currently under development for the safe prevention of artificial surface-induced thrombosis. Preclinical data showed a thromboprotection as effective as unfractioned heparin in three animal models. In contrast to unfractioned heparin, Ir-CPI does not induce bleeding in a pig liver bleeding model [95,96].

Haemaphysalis longicornis

In 2005, Kato et al. performed a mass sequence analysis of salivary glands cDNA from the hard tick *Haemaphysalis longicornis* at different feeding stages [97]. From this library, Kato et al. [97] identified a protein designated haemaphysalin (K_D = 2.49 ± 0.73 nM) witch prolonged activated partial thromboplastin time (aPTT) at nanomolar concentration and prothrombin time (PT) at micromolar concentration. Haemaphysalin includes two Kunitz-type domains where the C-terminal domain is more potent than the N-terminal domain [97,98]. They found that it inhibits the reciprocal activation of FXII and prekallikrein leading to a decrease in the generation of FXIa, FXIIa, and kallikrein. The suggested mode of action is an interference in the binding of FXII and HMWK with biological activating surfaces. This assumption comes from two characteristics of the interaction: (1) Zn^{2+}-dependent (2) occurring in the N-terminal region of FXII and in the D5 domain of HMWK. Zn^{2+} induces conformational modifications of FXII and HMWK to permit them to bind to activating surfaces. These interacting regions are the surface binding domains of FXII and HMWK. The PT prolongation is not explained so far [97,98].

4.1.1.2.   From triatomine insects

Triatoma infestans

a)   Infestin-4

In 2002, Campos et al. [99] isolated the protein infestin from the midgut of the blood-sucking insect *Triatoma infestans.* The native infestin has two non-classical Kazal-type domains and is a thrombin inhibitor. The analysis of the gene sequence showed that it codes for a protein with four non-classical Kazal-type domains [99,100]. The fourth domain, namely infestin-4 (Inf-4), was discovered as the only one with high inhibitory activity against FXIIa (K_i = 100 pM) [101,102]. Inf-4 comprises a short central α-helix (Asn25-Lys36), a small anti-parallel β-sheet (Val15-Gly17, Thr22-Thr23, and Leu42-Glu45) and

three disulfide bonds (PDB ID: 2ERW). After comparison with other Kazal-type inhibitors, the most dissimilar regions are the loops Ala7-Asn11 and Lys36-Leu40, the first corresponding to a portion of the protease-binding loop [101]. The active sequence of this canonical inhibitor is P^2-FRNYVPV-$P^{5'}$ where the peptide bond between P^1 Arg10 and $P^{1'}$ Asn11 forms the reactive site (Table 4) [86,103]. Inf-4 suffers from two majors drawbacks: (1) short half-time (2) off-target activity against plasmin, FXa, factor IXa, factor VIIa and thrombin [3,100,103].

Because Inf-4 acts in a substrate-like manner, one of the strategies to enhance its specificity is to mutate the active sequence to improve the matching between this loop and the active site of FXIIa [86]. Using a phage-display selection, the bound Inf-4 mutants included a Ser, Thr or Asn at the 9th position (P^2 position), an Arg at the 10th position (P^1 position) and an Arg or, less frequently, an Asn at the 11th position ($P^{1'}$ position) [101]. The amino acid residue at the P^1 position is known to penetrate the S1 pocket and to provide the major part of the association energy with the target. The interaction between the Asp189 of FXIIa and the Arg10 is therefore particularly important in the active sequence [86,88,103]. In the P^2 position of Inf-4, a Phe is observed which is very rare in the Kazal family. Thr is often preferred in this position because this amino acid can form hydrogen bonds with the $P^{1'}$ residue promoting the canonical conformation of the loop [86,87,103]. Indeed, the two most potent mutants of Inf-4, namely inf4mut15 [101] and Mutant B [103], exhibit a Thr at this P^2 position. The active sequence of inf4mut15 and Mutant B are P^2-TRRFVAV-$P^{5'}$ and P^2-TRNFVAV-$P^{5'}$ respectively (Table 4). These mutants exhibit an improved specificity towards FXIIa compared to the wild type. The strong inhibitory character of the wild type against plasmin is downgraded into at worst a weak activity with both mutants. None of them inhibits FXa [101,103]. Other coagulation factors were tested with these mutants and are described by Kolyadko et al. [103].

To enhance the pharmacokinetic properties, Inf-4 was fused to albumin. Interestingly, the r-HA-Inf-4 showed a high potency on FXIIa ($IC_{50} = 0.3 \pm 0.06$ nM for human) with at least 100-fold selectivity against other coagulation factors [100,104]. Nevertheless, at high concentration in vivo, this protein presents a modest off-target activity against FXa [104]. Barbieri et al. [105] fused albumin to inf4mut15, improving specificity, to study the utility of FXIIa inhibition in stroke prevention.

b) Triafestins

In 2007, Isawa et al. [106] identified two inhibitors of the kallikrein-kinin system belonging to the triabin family, namely triafestin-1 and triafestin-2 [106,107]. These proteins are major constituents of the saliva of *Triatoma infestans*. At nanomolar concentration, they showed an inhibition of the reciprocal activation of FXII and prekallikrein with the same characteristics as haemaphysalin. Unlike haemaphysalin, triafestins do not exert an activity against the extrinsic pathway [97,106]. Little is known on the interactions allowing its activity against FXII [107].

Triatoma dimidiata

In 2010, Kato et al. [108] performed a transcriptome-based analysis on the salivary glands of *Triatoma dimidiata*. They found two triabin-like transcripts (Td60 and Td101) on the basis of their sequence similarities [107,108]. Triabin is an exosite-binding inhibitor of thrombin found in the saliva of *Triatoma pallidipennis* [109]. Therefore, Kato et al. [108] hypotezised an anti-thrombin activity. In 2012, Ishimaru et al. [110] produced and characterized the activity of the recombinant protein of Td60, termed dimiconin. Although a high level of homology with triabin (62%), they found that dimiconin prolonged aPTT in a dose-dependant manner but did not affect PT. Their study showed that dimiconin inhibits the activation of FXII at micromolar concentration in plasma, but is inactive against FXIIa [107,110].

4.1.1.3. From flies

Two FXII inhibitors were found in the salivary glands of hematophagous flies: hamadarin from the mosquito *Anopheles stephensi* [111] and ayadualin from the sand fly *Lutzomyia ayacuchensis* [112]. These proteins are the most abundant transcripts isolated from their respective cDNA libraries [111,112]. Hamadarin, a D7 family protein, inhibits the reciprocal activation of FXII and prekallikrein with similar characteristics as triafestins [111,113]. Ayadualin exhibits a dual inhibitory effect on hemostasis. This protein inhibits platelet aggregation via its RGD sequence (a motif known to bind integrins) and the intrinsic blood coagulation pathway mostly by inhibiting the activation of FXII ($IC_{50} = 0.64$ µM) in an RGD-independent manner [112,114].

4.1.2. Discovery from microbes

Microbes are another major source of serine protease inhibitors. Their roles are not completely understood but it seems that they protect them against their own proteases or against other environmental stresses [115]. Actually, two microbial serine protease inhibitors were reported to inhibit human FXIIa in a substrate-like manner.

4.1.2.1. *From Gram-negative*

In 1983, Chung et al. [116] discovered a protein from the periplasm of *Escherichia Coli* which inhibits trypsin and other pancreatic proteases. They termed it ecotin [116]. Later, ecotin was surprisingly identified as a highly potent inhibitor of FXa, FXIIa, human kallikrein, and human leukocyte elastase (with inhibitory constants in the picomolar range). This discovery comes from the observation that *E. Coli* lysates can inhibit these proteases [117–119]. Ecotin is a dimeric protein that forms a hetero-tetramer with proteases (PDB entry 1IFG – monomeric form) [120,121]. Three distinct interfaces are involved in the hetero-tetrameric complex : (1) the substrate-like primary binding site composed by the 80's and the 50's loops (2) the antibody-like secondary binding site constituted by the 60's and the 110's loops, and (3) the dimerization site [115,120,122,123]. The active sequence of the primary interface expected to bind the catalytic site of most proteases is P^3-STMMAC-$P^{3'}$ where the peptide bond between P^1 Met84 and $P^{1'}$ Met85 forms the reactive site (Table 4) [123–125]. FXIIa was found to slowly cleave ecotin between Met84 and Met85 which is consistent with the expected active sequence [117]. The stabilization of the canonical conformation of the protease-binding loop is supported especially by a disulfide bond (Cys50-Cys87) proximal to the reactive site and several hydrogen bonds (particularly with residues P^2 Thr83 and P^3 Ser82) [123,126]. Concerning the primary interactions with proteases, there is a sub-van der Walls contact between Met84-C and the nucleophilic Ser195-Oγ. The oxygen of Met84 also faces the oxyanion hole and can form hydrogen bonds with the amides of Ser195 and Gly193 [123].

Ecotin can inhibit almost all serine proteases with a chymotrypsin-like fold. Its pan-specific character is partially attributable to its Met84. Methionine can satisfy the steric constraints of many different S1 pockets because of the inherent flexibility of its side chain [115,121,123]. In the majority of trypsin inhibitors like FXIIa, the P^1 position is typically Arg or Lys [123,125]. The idea was to mutate this position to increase potency and specificity. The substitution of Met84 by an Arg or a Lys altered the specificity of ecotin but not drastically. These mutants inhibit strongly FXa, FXIIa, and kallikrein. The inhibition of the human leukocyte elastase was largely decreased. Globally, there was an overall decrease in specificity owing to an improvement in the inhibition of thrombin, FXIa and activated protein C. Changing P^1 with an Asp or Glu also maintained the inhibition of FXa suggesting that other amino acids have significant contributions to binding [117,118,124,127,128]. This lack of specificity can be explained by the ecotin's capacity to form a hetero-tetrameric complex with target proteases. The additional interactions made by the secondary site compensate for less optimal binding at the substrate-like primary site. Therefore, the P^1 residue is less crucial for the target specificity. When the primary site is weak, the quaternary structure of ecotin changes to realize the full potential binding energy at the secondary site [121,123,126,129]. The negative cooperativity of ecotin rationalizes the pan-specificity of primary-site mutants [121]. To maximize the potential of this bidentate scaffold, the introduction of multiple mutations in two binding sites led to the variant XII-18 (V81M, T83Y, M85R, A86S, R108S, and K112S) which exhibits a high specificity towards FXIIa [130].

4.1.2.2. *From Gram-positive*

The protein *Streptoverticillium* anticoagulant I (SAC I), which was isolated from a culture fluid of *Streptoverticillium cinnamoneum*, exhibits inhibitory constants against FXIIa and kallikrein in the nanomolar range. The active sequence is P^4-ACTREWNP-$P^{4'}$ where the scissile bond is between Arg70 and Glu71 (Table 4) [131,132].

4.1.3. Discovery from plants

Plants, a common source of bioactive compounds, express abundantly peptide- and protein-based protease inhibitors to maintain their homeostasis and to serve their innate defense. Several peptide and protein families are known to inhibit serine proteases belonging to the S1 family [133].

4.1.3.1.   Kunitz-type inhibitors

From plant extracts, one two-chain Kunitz-type inhibitor, *Enterolobium contortisiliquum* trypsin inhibitor (EcTI – PDB entry 4J2Y), and five single-chain Kunitz-type inhibitors, *Bauhinia variegata* trypsin inhibitor (BvTI), *Bauhinia ungulata* FXa inhibitor (BuXI), *Bauhinia pentandra* trypsin inhibitor (BpTI), *Bauhinia bauhinioides* kallikrein inhibitor (BbKI) and *Caesalpinia echinata* kallikrein inhibitor (CeKI), displayed an activity against FXIIa in the nanomolar range (Table 4) [134–139]. BvTI and CeKI, the two most potent inhibitors of FXIIa, have an inhibitory constant reported at 21 and 0.18 nM, respectively. They also inhibit strongly kallikrein. Compared to BvTI, CeKI showed extra inhibitory activity against plasmin and FXa [137,139].

4.1.3.2.   Cereal-type inhibitors

To find a selective inhibitor of FXIIa, Hojima et al. [140] performed a screening of diverse plant extracts and found in corn kernels an inhibitor with high potency and a narrow spectrum. This inhibitor was previously isolated from opaque-2 corn seeds and named CTI.[140,141] CTI is a highly potent inhibitor of FXIIa (K_i = 0.41 ± 0.1 nM) but also inhibits trypsin (K_i = 7 ± 0.5 nM), plasmin (K_i = 5 ± 1.5 μM), FXIa (K_i between 12 and 15 μM) and activated protein C (K_i = 15.9 ± 3.8 μM) [21,103,142–144]. CTI is widely used in blood sampling to avoid the activation of the intrinsic pathway that would trouble experimental studies focused on tissue factor-mediated thrombin generation [21]. CTI is folded into two pairs of anti-parallel helices. The crystal structures reveal a canonical conformation of the 31-38 loop concurring with the assumption that it corresponds to the protease-binding loop (PDB entries 1BFA and 1BEA) [21,142]. The active sequence is P4-PRPRLPWP-P4' where the Arg34-Leu35 peptide bond forms the reactive site (Table 4) [21,143,145]. The active sequence alone is enabled to inhibit FXIIa showing important contributions of other regions [21,143]. Based on mutants' inhibitory activity against FXIIa, Arg34 at the P1 position, Trp22 of the helix α1 and Arg43 of the helix α2 are required for an efficient inhibition [21]. The docking pose generated by Hamad et al. [21] places them respectively in S1, S3 and H1 pocket of FXIIa.

Another member of the cereal family, Barley trypsin inhibitor (BTI), also inhibits FXIIa but with 250 times less potency and with an additional activity against kallikrein [146].

4.1.3.3.   Squash inhibitors

Squash family inhibitors are potent trypsin, chymotrypsin and elastase protease inhibitors acting in a substrate-like manner. They consist of approximatively 30 amino acids cross-linked by three disulfide bridges. These inhibitors have a rigid globular hydrophobic core with a protruded binding loop [133,147,148]. Hayashi et al. [147] isolated eight squash family inhibitors from various cucurbitaceous seeds and tested them against blood coagulation proteases. The three most active against FXIIa are *Cucurbita maxima* trypsin inhibitor III (CMTI-III), *Lufa cylindrical* trypsin inhibitor III (LCTI-III) and *Momordica charantia* trypsin inhibitor I (MCTI-I). For all of them, the reactive site is Arg-Ile and their inhibitory activity is in the nanomolar range (Table 4) [103,147]. They also have an activity against kallikrein and FXa in the micromolar range. All these peptides prolong aPTT but LCTI-III to a lesser extent [147].

Momordica cochinchinensis trypsin inhibitor-II (MCoTI-II), another member of the squash family, exhibits an atypical head-to-tail cyclized backbone [149,150]. MCoTI-II can be produced by total synthesis, possesses exceptional stability and can enter into cells. These reasons explain its wide use as molecular scaffold for drug design applications [151–154]. The wild-type MCoTI-II inhibits FXIIa (K_i = 750 ± 80 nM) but is also a potent trypsin and plasmin inhibitor [155]. To engineer MCoTI-II variants against FXIIa, Swedberg et al. [155] designed and screened a sparse matrix library of 42 tetrapeptide *para*-nitroanilide substrates, which display an Arg at P1. During the library design, they compared previous results [156,157] and pointed out some subsite preferences to limit the number of amino acid per position. The screening revealed that FXIIa prefers Phe at P2 in cooperation with Gln at P4. The modification of P4-VCPK-P1 into P4-QCFR-P1 led to a 6-fold increase in FXIIa potency (K_i = 110 ± 6 nM) but the selectivity was still limited. Based on the previous finding that only FXIIa tolerated a Trp at P2' compared to FXa, plasmin, and trypsin [158], they incorporated this mutation and obtained a variant with a large improvement in selectivity associated with a 4-fold decrease in FXIIa potency. Molecular modelling prompted the last improvement in potency and selectivity by suggesting the substitution of Gly1 into Lys1 (MCoTI-II Mutant – Table 4) [155].

4.1.3.4. Potato I – type inhibitor

After the discovery of CMTI-III, another inhibitor of FXIIa was isolated from *Cucurbita maxima* and termed CMTI-V. In contrast to the latter, CMTI-V belongs to the Potato I family which folds into a wedge shape and has the particularity to not contain a disulfide bridge [159,160]. CMTI-V inhibits trypsin and FXIIa in the nanomolar range without affecting kallikrein. The binding loop includes the amino acid residues between positions 40 and 49. The scissile bond is formed by Lys44–Asp45 (Table 4) [159,161].

4.1.3.5. Bowman-Birk inhibitors

The structural characteristic of Bowman-Birk inhibitors is a core of anti-parallel β-sheets only stabilized by multiple disulfide bridges. These inhibitors possess two reactive sites forming a double-headed structure. A loop including one reactive site protrudes on each end of the β-sheet core [86,134,160,162]. The two binding loops interact independently with proteases [86,162]. From plants, two Bowman-Birk inhibitors, namely *Torresea caerensis* trypsin inhibitor (TcTI) and *Torresea acreana* trypsin inhibitor (TaTI), showed an inhibitory activity against FXIIa in the micromolar range without affecting thrombin, kallikrein or FXa (Table 4) [134,135,163–165]. With TcTI, Tanaka et al.[165] also observed a significant increase of the aPTT but not of the PT concurring with a FXIIa inhibition.

4.1.4. Discovery from marine organisms

Besides hematophagous arthropods, microbes, and plants, marine organisms are another source of natural bioactive compounds [166–168]. Marine products generally exhibit particular scaffolds not found in terrestrial sources [168,169]. From the hydrolysate of marine organisms, two FXIIa inhibitors were reported: the 12-kDa yellowfin sole anticoagulant protein [170] and the 16-mer peptide VITPOR AI (IC$_{50}$ in plasma = 70.24 µM) [171,172].

4.1.5. Discovery from human case reports

Alpha 1-antitrypsin is a physiological serpin that strongly inhibits neutrophil elastase. Firstly discovered in the plasma of a boy who died from a bleeding disorder, the single mutation of Met-358 by an arginine (at P^1) leads to a drastic change in the inhibitory spectrum (Table 4) [89,173,174]. This protein, termed α1-antitrypsin Pittsburgh (α1-AT Pitt), is a strong inhibitor of FXIIa, kallikrein, thrombin, plasmin, and activated protein C. Compared to C1-Inh which is the only physiologically efficient plasma inhibitor of FXIIa, α1-AT Pitt is 8-fold more potent [89,175,176]. To selectively inhibit the contact activation and the bradykinin production, de Maat et al. [177] redesigned the reactive center loop of α1-AT Pitt (P^4-P$^{1'}$ RCL: AIPR/S). To improve the selectivity of the reactive center loop, they picked the sequence of the activation loop of FXII (P^4-SMTR-P^1) as it can be cleaved by FXIIa, kallikrein, FXIa and plasmin but not by thrombin, FXa or activated protein C. In addition, they performed an *in-silico* screen on previously published tripeptide libraries and selected the most promising sequence (P^4-SLLR-P^1). These two α1-AT Pitt variants (SMTR/S and SLLR/S) demonstrated increased target specificity but inhibition of thrombin, FXa and activated protein C were still significant. To reduce this residual activity, the P1' residue of both variants was substituted by a valine, the natural P$^{1'}$ residue of FXII activation loop. This replacement also lowered the potency of kallikrein and plasmin inhibition. These variants remain more potent than the endogenous inhibitor, C1-Inh [177]. Pretreatment with SMTR/V or SLLR/V variant was effective in the ferric chloride (FeCl$_3$)-induced thrombosis mice model. The variants also protected against inflammation in carrageenan-induced paw swelling model, an *in vivo* model of acute bradykinin-driven inflammation used as gold standard in the development of HAE agents. Beside, only the most potent variant (SLLR/V – Table 4) showed a protective effect against pathogenic epithelial leakage in dextran sulfate sodium-induced colitis mice model [177].

4.1.6. Discovery by phage display screening

Starting with the ascertainment that bicyclic peptides are a valuable template to develop potent and specific serine protease inhibitor, Baeriswyl et al. [178] screened two combinational libraries by phage display against human FXIIa. The peptide cyclization was performed by reaction with 1,3,5-tris(bromomethyl)benzene (TBMB) [178,179]. The first phage selection against α-FXIIa yielded high-affinity binders to many epitopes, but without efficient inhibition. To decrease the selection of exosite binders, they used β-FXIIa and, after three rounds of phage panning, they identified FXII304 (K$_i$ = 3.1 ± 0.5 µM) from the 4 X 4 bicyclic peptide library. The specificity was achieved for related proteases except for plasmin (K$_i$ = 8.3 ± 2.2 µM). The analysis of related sequence peptides revealed that the presence

of lysine at the last amino acid position was important for efficient inhibition of plasmin. The substitution of the lysine in FXII304 by glycine led to FXII401. FXII401 showed a similar FXIIa activity (K_i = 4.3 ± 1 µM) without plasmin inhibition. To improve the affinity, a sequential randomization in four amino acid positions generated FXII402 (K_i = 1.2 ± 0.2 µM) [178]. However, its potency could not be further improved. Therefore, six novel combinational libraries were generated using novel peptide cyclization reagents, namely 1,3,5-triacryloyl-1,3,5-triazinane (TATA) and N,N',N''-(benzene-1,3,5-triyl)-tris(2-bromoacetamide) (TBAB). From TATA-cyclized peptide libraries, two compounds with submicromolar activity have been found, i.e. FXII512 (K_i = 0.16 ± 0.07 µM) and FXII516 (K_i = 0.16 ± 0.08 µM). Due to higher specificity for FXIIa, FXII516 was chosen for affinity maturation. Single mutation S11R of FXII516 led to a 4-fold improvement in potency (FXII608, K_i = 44 ± 8 nM). After two supplementary rounds of mutations and screening, FXII618 (K_i = 22 ± 4 nM) was discovered (Table 4). Its specificity was confirmed by *in vitro* coagulation assay. Sequence and structure analysis revealed that FXII618 mimics protease-binding loop of CTI [144]. It is important to mention that the cyclization reagent plays a key role in the inhibitory activity. FXII618 cyclized with TATB and TBMT are less active [180]. Furthermore, the potency was improved by the introduction of unnatural amino acids. The substitution of Arg11 by (S)-β^3-homoarginine and Phe3 by 4-fluorophenylalanine generated, respectively, FXII700 (K_i = 1.5 ± 0.1 nM) and FXII800 (K_i = 0.84 ± 0.03 nM – Table 4) [181,182]. The substitution of Arg1 by norarginine in FXII800 enhanced the protease stability yielding to FXII801 (K_i = 1.63 ± 0.18 nM, > 27 000-fold selectivity, $t_{1/2}$ (plasma) = 16 ± 4 h – Table 4) [182]. Finally, to slow down the rapid renal clearance *in vivo*, Zorzi et al. [183] conjugate a heptapeptide-palmitoyl tag to FXII801 which binds to albumin. The linker between the tag and FXII801 is a long PEG chain (($NH(CH_2CH_2O)_{24}CO)_3$) grafted on the N-terminus. The resulted inhibitor, named tag-3xPEG24-FXIIa (K_i = 4 ± 0.9 nM in the presence of albumin), exhibits an extended half-life (from 13 minutes to over five hours) allowing its use for *in vivo* studies [183].

4.2. Small-molecular-weight inhibitors

For FXIIa, the development of potent and selective small synthetic inhibitors is a step behind the other strategies. Only the patent WO2019211585 disclosed the structures of selective potent FXIIa inhibitors [184]. The 3,6-disubstituted coumarins developed against FXIIa are weak but selective [185,186]. On the opposite, several compounds have been reported potent but with low or no selectivity for FXIIa [155,187–194].

4.2.1. Scaffold-based discovery

To design FXIIa inhibitors, Robert et al. [195] selected the 3,6-disubstituted coumarin scaffold. Initially discovered as mechanism-based inhibitors of α-chymotrypsin and human leukocyte elastase, the 3,6-disubstituted coumarins were also conclusive as thrombin inhibitors. All of these serine proteases belong to the S1A subfamily, motivating the choice of this scaffold against FXIIa [196–202]. After a first screening round, COU-077 popped out as a weak but selective FXIIa inhibitor (Figure 4). Based on the screening results, they synthesized novel carboxamido-coumarins by modulating the phenyl and/or the aromatic part of the coumarin ring. Among the most attractive compounds, COU-254 and COU-294 emerged as weak but selective FXIIa inhibitors [195]. Due to its higher solubility, COU-254 was selected for *in vivo* testing and failed to demonstrate efficacy in acute ischemic stroke [203]. COU-294, the most potent, was selected for further optimization. To obtain better physicochemical properties, an amino- or an oxygen-based group was introduced in the positions 3 or 6. The modulations with an amine function provided some improvement but with a cost in terms of activity. In contrast, the introduction of an oxygen-based group afforded three new compounds (ChB05, ChB06, and ChB10) having similar properties and inhibition than the reference compound (COU-294) [185]. No relevant anticoagulant activity was observable in plasma and this result was attributed to the poor activity/lipophilicity ratio of the compounds. Indeed, even if the physicochemical profile might be compatible with oral intake, their activity on FXIIa did not allow their screening at high concentrations in plasma or blood. This is why a second screening round focused on water-soluble coumarin derivatives was performed (Figure 4). One compound with a guanidine moiety (RF1), previously published as weak thrombin inhibitor [200], was found to be at least 18-fold more active on the contact factors (FXIIa, FXIa or kallikrein) than thrombin, FXa or tissue factor/factor VIIa. With the aim to improve the selectivity for FXIIa, modulations, inspired by the hits of the first screening round, were undertaken on the aromatic ring of the coumarin and the

ester group. These new compounds (DL8 and DL14) have some selectivity for FXIIa but the loss of potency led to a global loss of selectivity [186]. Clotting time assays sustained the results observed on isolated enzymes for the three compounds. In whole blood, RF1 acts as a contact inhibitor without affecting the tissue factor pathway nor the primary hemostasis. Interestingly, DL8 and DL14 have direct antiplatelet properties [186].
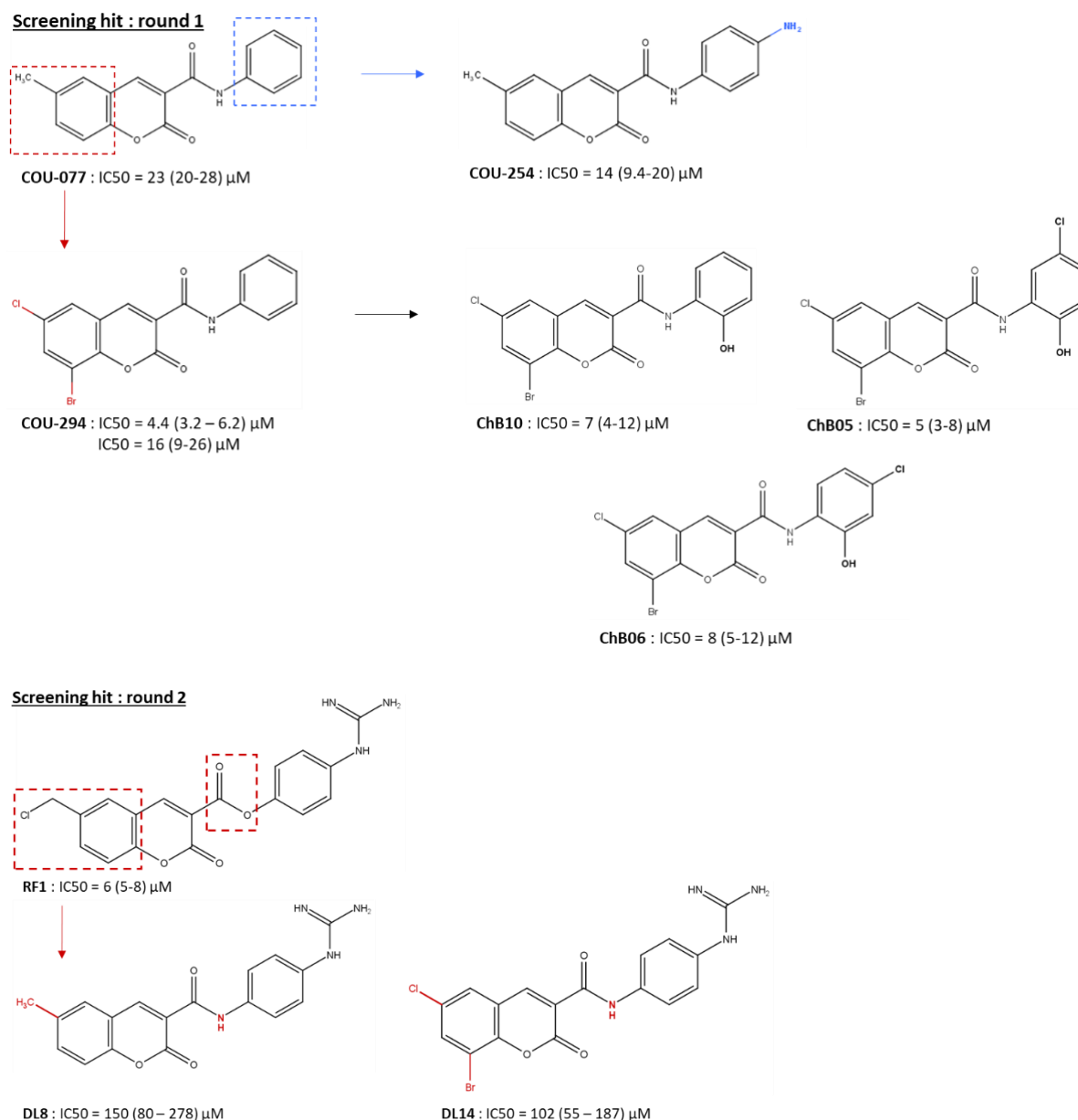

Figure 4: *Evolution of the 3,6-disubstitued coumarin scaffold.* *(2-column fitting image)*

4.2.2. Virtual high-throughput screening (vHTS)

Chen et al. [204] mine data from the α-FXIIa enzyme assay dataset recorded in PubChem (AID 728 – 82 compounds) to generate predictive classification and quantitative structure-activity relationship models. The models of FXIIa inhibition activity used in the vHTS were created by combining three ligand-based vHTS methods to maximize data utility: principal component analysis, genetic algorithm, and support vector machine. The developed models were then used to screen the PubChem Compound database (72 million) and predicted their IC_{50}. 14 candidates were then evaluated experimentally and led to a 42.9% hit-rate (6/14 compounds). The results were used to further retrain the models. In the

second screening round, a 100% hit-rate was observed (11/11 compounds). Training the models with the results from the first round alters the molecular diversity, which can produce less predictive models for the entire set but more predictive for particular scaffolds. This approach identified 17 innovative inhibitors of FXIIa with IC_{50} value below 50 μM. Among these, two scaffolds were highlighted by the authors (Figure 5) [204].
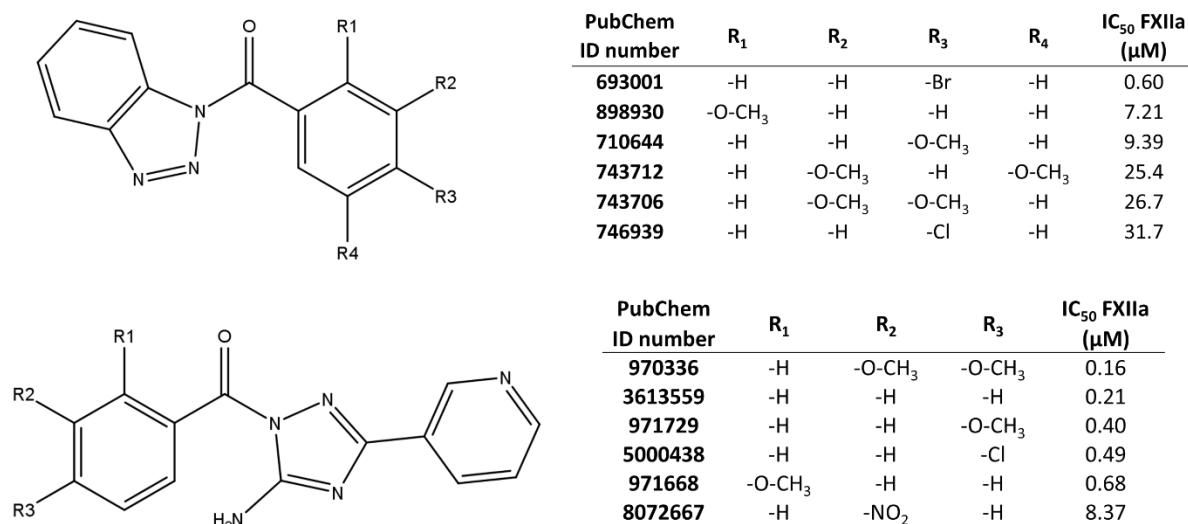

PubChem ID number	R_1	R_2	R_3	R_4	IC_{50} FXIIa (μM)
693001	-H	-H	-Br	-H	0.60
898930	-O-CH$_3$	-H	-H	-H	7.21
710644	-H	-H	-O-CH$_3$	-H	9.39
743712	-H	-O-CH$_3$	-H	-O-CH$_3$	25.4
743706	-H	-O-CH$_3$	-O-CH$_3$	-H	26.7
746939	-H	-H	-Cl	-H	31.7

PubChem ID number	R_1	R_2	R_3	IC_{50} FXIIa (μM)
970336	-H	-O-CH$_3$	-O-CH$_3$	0.16
3613559	-H	-H	-H	0.21
971729	-H	-H	-O-CH$_3$	0.40
5000438	-H	-H	-Cl	0.49
971668	-O-CH$_3$	-H	-H	0.68
8072667	-H	-NO$_2$	-H	8.37

Figure 5: *Scaffolds revealed by vHTS. (1.5-column fitting image)*

4.2.3. Substrate-guided discovery: peptidomimetic inhibitors

4.2.3.1. Transition state analog inhibitors

A traditional approach to design S1A protease inhibitors is to discover specific enzyme substrates and to turn them into transition state analog inhibitors [205]. To make this transformation, the substrate scissile bond is replaced by an electrophile, which reacts with Ser[195] to form a hemiacetal complex [205,206]. Four transition state analog inhibitors were reported with nanomolar activity against FXIIa. Among them, C921-78 (Figure 6), an FXa α-ketothiazole inhibitor, is the most potent (IC_{50} = 50 nM) [187] and Ac-QRFR-H (IC_{50} = 180 ± 10 nM) is the only one with an improved selectivity for FXIIa [155]. Ac-QRFR-H is a 4-mer peptide aldehyde inhibitor intended to evaluate the sequence to be grafted into the canonical loop of MCoTI-II (See Section 4.1.3.3.) [155]. D-Pro-Phe-Arg-CH$_2$Cl (PCK) and PPACK, which are respectively kallikrein and thrombin α-methylketone inhibitor, also exhibit nanomolar activity against FXIIa (IC_{50} = 180 and 230 nM, respectively) [194,195,207,208]. PPACK was co-crystallized with an FXIIa construct (PDB entry 6QF7). Their main finding is that Tyr99 can alter its position, generating a more open S2 pocket and a partially occluded S3 pocket (Figure 3). This conformation can rationalize the preference for the bulky Phe residue at P^2 position observed in various sequences of FXIIa inhibitors (Table 4) [18].
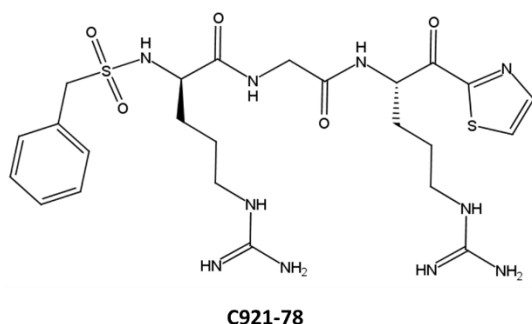

C921-78

Figure 6: Structure of C921-78. *(simple column fitting image)*

4.2.3.2. Substrate analog inhibitors

The alternative method to design inhibitors based on the substrate sequence is to suppress the scissile bond. Compared to transition state analog inhibitors, the absence of electrophile results in a loss of

inhibitory potency. But lead optimization is able to restore potency and improve selectivity [206]. The structure of several substrate analog inhibitors designed towards FXIIa are disclosed in the patent WO2019211585 but their respective activity are not mentioned [184].

Table 4. Amino acid sequence of peptides- and proteins-based inhibitors and substrates.

	K_i (nM)	P4	P3	P2	P1	P1'	P2'	P3'	P4'
Substrates									
PK	NA	T	S	T	R	I	V	G	G
FXII	NA	S	M	T	R	V	V	G	G
FXI	NA	I	K	P	R	I	V	G	G
S-2302	NA		P	F	R				
Physiologic inhibitors									
C1-Inh	NC	S	V	A	R	T	L	L	V
AT-III	NC	I	A	G	R	S	L	N	P
Peptides and analogs									
PCK	180		P	F	R				
PPACK	230		F	P	R				
Ac-QRFR-H	180	Q	R	F	R				
FXII618	22	R	C	F	R	L	P		
FXII800	0.84	R	C	F^{4-F}	R	L	P		
FXII801	1.63	R^{nor}	C	F^{4-F}	R	L	P		
Proteins									
Inf-4	0.1	A	C	F	R	N	Y	V	P
MutB	0.7	A	C	T	R	N	F	V	A
Inf4mut15	1.0	A	C	T	R	R	F	V	A
Ecotin	0.029	V	S	T	M	M	A	C	P
Ecotin XII-18	0.2	M	S	Y	M	R	S	C	P
SAC-I	53	A	C	T	R	E	W	N	P
EcTI	81.81	T	P	P	R	I	A	I	L
BvTI	21	A	L	P	R	S	L	F	I
BuXI	74	A	L	P	R	T	M	F	I
BpTI	80				Unknown				
BbKI	110	S	P	L	R	I	N	I	I
CeKI	0.18				Unknown				
CTI	0.41	P	R	P	R	L	P	W	P
BTI	110	Q	G	P	R	L	L	T	S
LCTI-III	3.9	I	C	P	R	I	L	M	E
CMTI-III	70	V	C	P	R	I	L	M	K
MCTI-I	13	R	C	P	R	I	L	K	Q
MCoTI-II	750	V	C	P	K	I	L	K	L
MCoTI-II Mutant	490	Q	C	F	R	I	W	K	K
CMTI-V	41	P	V	T	K	D	F	R	C
TcTI	1450	A	C	T	H	S	I	P	A
TaTI	4600	A	C	T	R	S	I	P	A
α1-AT Pitt	NC	A	I	P	R	S	I	P	P
α1-AT Pitt variant	NC	S	L	L	R	V	I	P	P

NA = not applicable, no inhibitory activity. NC = not comparable, belongs to the serpin family.
F^{4-F}= 4-fluorophenylalanine. R^{nor} = norarginine.

4.2.4. Ligand-based discovery

Starting with the discovery that hexyl ε-aminocaproate and hexyl ε-guanidinocaproate have an inhibitory activity on trypsin and plasmin [209–211], Muramatu et al. synthesized aromatic esters of ω-aminoacids [212] and of ω-guanidinoacids [213]. They found that aromatic esters are more potent than aliphatic ones on various serine proteases. Among these compounds, gabexate (also termed *p*-carbethoxy-phenyl ε-guanidinocaproate monophosphate or FOY) was one of the most potent with a K_i reported for FXIIa at 2.92 ± 0.182 µM [189,213]. Inspired by previous works [214,215], they incorporated a second aromatic ring at the guanidine side and generated p-(p'-guanidinobenzoyloxy)-phenyl derivatives [216].

Later, they synthesized derivatives with an aromatic amidine. Among these, nafamostat (also termed FUT-175) is one of the most potent low molecular-weight inhibitor against FXIIa (K_i = 105 ± 12.5 nM) even if it is not selective [189,190,217]. Finally, to improve the oral bioavailability of nafamostat, they produced analogs with a reduced basic character by modification of the guanidine or the amidine moiety [193]. One of these, sepimostat (also termed FUT-187), was described as a noncompetitive inhibitor of FXIIa with a K_i of 21 nM [192].
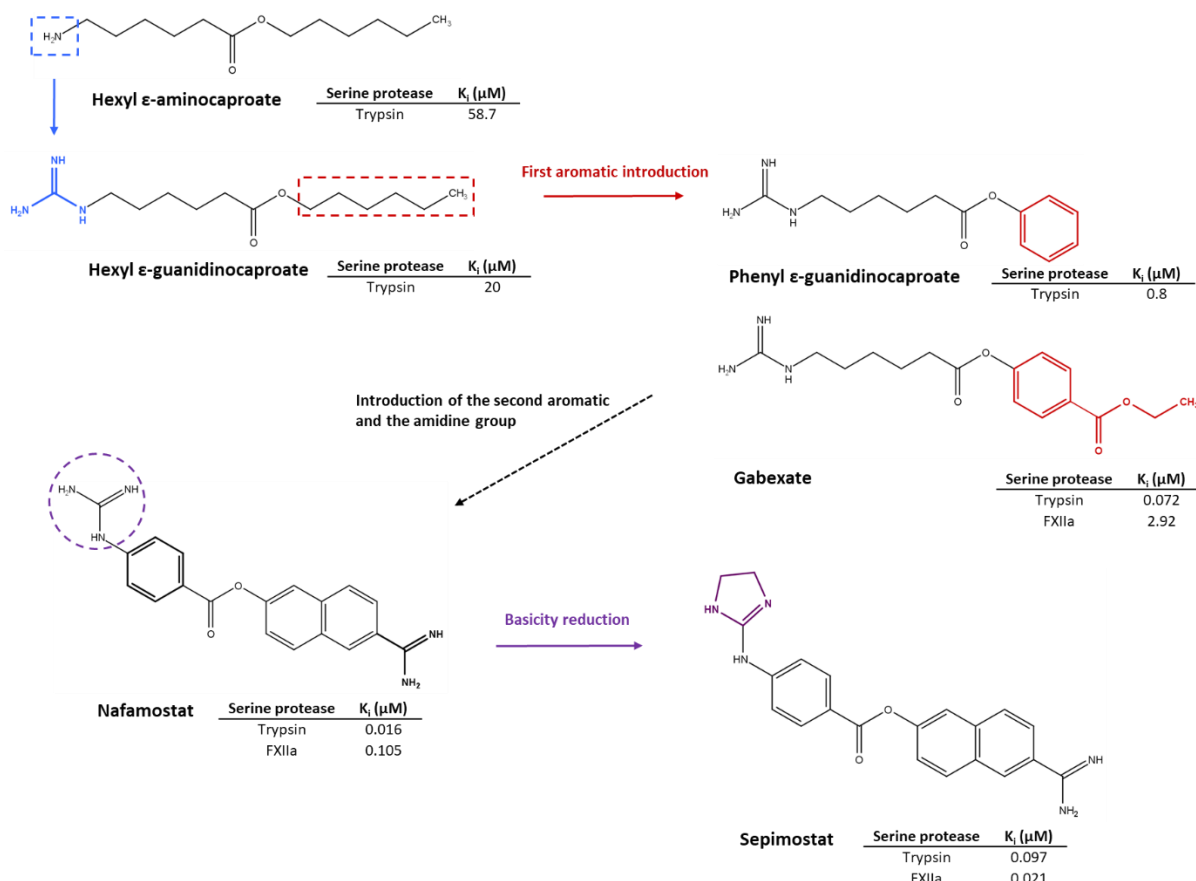
Figure 7: Development of nafamostat and sepimostat. *(2-column fitting image)*

4.3. Antisense oligonucleotides

ASOs are chemically modified single-stranded nucleic acid sequences that bind to specific regions of mRNA. This binding can induce gene suppression by different mechanisms such as RNase H activation, Ago 2 activation, RNA splicing modulation, and translation blocking [218,219]. The main restriction of the ASOs is their tissue distribution. Most ASOs are efficiently delivered into hepatocytes making this strategy suitable for FXII since this latter is mainly produced in the liver [220].

In ASO's drug discovery, the pharmacophore is separated from the so-called dianophore. While the pharmacophore is defined by the nucleotide sequence of the drug, the dianophore is the chemical and the structural architecture of the oligonucleotide that determines its physicochemical properties, and thus its pharmacokinetic and biological properties. The optimization of the specificity for the target is distinct from the tissue distribution, the half-time and the potency of the drug. ASOs are typically 18-20 nucleotides long although 13 to 15 nucleotides are generally sufficient to achieve specificity for the target [219,221–223]. When a dianophore is selected, the pharmacophore can be semi-rationally designed by screening a finite number of sequences based on the Watson-Crick base pairing complementarity. Not all sites are accessible on the RNA target leading to the need for screening. This difficulty is related to the complex secondary and tertiary structures of RNA [218,219,224].

For the design of FXII ASOs, the 2'-O-methoxyethyl phosphorothioate (2'-MOE PS) dianophore was selected. This dianophore is the best-understood class of ASOs and includes an excellent safety profile, a bioavailability demonstrated in animals and humans, and extensive clinical experience [219,221]. In

2'-MOE PS ASOs, the phosphodiester linkages were replaced by phosphorothioate bonds and the sugar groups respect a "5-10-5 design". The nucleotides on the wings contain 2'-O-methoxyethyl (2'-MOE) ribose whereas the 10 central nucleotides include desoxyribose [80,225–228]. The phosphorothioate (PS) modification largely increases nucleases resistance and supports the entry into a productive endocytic pathway mediated by the asialoglycoprotein receptor largely expressed on hepatocytes. The PS modification also provides pharmacokinetic benefits by increasing the binding to plasma proteins, which prevents rapid renal excretion [219,221,223]. The 2'-MOE modification of the sugar moiety enhances hybridization as well as resistance to nuclease degradation. The improvement in the binding affinity is related to three factors : (1) favorable electrostatic interactions with the phosphate driven by the electronegativity of the oxygens, (2) stabilization of the oligonucleotide into an RNA-like C3'-endo conformation, and (3) coordination of water molecules around the 2'-MOE which is important for the pairing stability. The enhancement in the nuclease resistance comes from the steric barrier induced by the 2'-MOE substituent combined with the shell of hydration created by the bound water [218,221,223,229]. Finally, the deoxy gap composed of the 10 central nucleotides is essential to cleave the targeted RNA. This gap mimics a DNA strand and allows the recruitment of the RNase H1. When the ASO hybrids with an RNA, the RNase H1 recognizes a DNA-RNA heteroduplex and cleaves the RNA strand [218,219,221,223]. The FXII ASO sequences mentioned are GCATGGGACAGAGATGGTGC for mice (ISIS 410944) [80,226] and GGAATGGCCATTGTCCTCGC for rabbit [228]. To select the sequence, an activity screening on cultured mouse and rabbit hepatocytes was performed, respectively. The most potent ASO was then evaluated for tolerability and efficacy *in vivo* [52,226].

Regarding thrombosis, FXII ASO treatment attenuated $FeCl_3$-induced arterial thrombosis in wild-type and histidine-rich glycoprotein knockdown mice [225,227]. In $FeCl_3$-induced inferior vena cava thrombosis and in stenosis-induced thrombosis, FXII ASO treatment reduced thrombus growth but a high FXII reduction was required to protect mice from thrombus formation. FXII ASO treatment did not affect hemostasis [225] and also attenuated catheter thrombosis [52]. In the Gram-negative pneumonia-derived sepsis caused by *Klebsiella pneumoniae*, FXII ASO treatment of mice did not have protective effect, while FXII-deficient mice had an improved survival and a reduced bacterial outgrowth [79,80]. Stroo et al.[80] explained this discrepancy by the lesser extent of FXII deficiency and/or the fact that the low expression of FXII mRNA in the lung-tissue was unaffected. Bhattacharjee et al. [226] also investigated the effect of FXII ASO treatment on the vascular permeability in mice, which is an establish test for potential HAE therapeutics. FXII ASO treatment reduced basal, captopril-induced, and C1-Inh depletion-induced vascular permeability [226].

4.4. Small interfering RNAs

siRNAs are double-stranded oligonucleotides, including a sense and an antisense strand. The sense strand facilitates the incorporation of the antisense strand into an RNA-induced silencing complex (RISC). After loading, the sense strand is discarded and the antisense strand recognizes the target mRNA to be degraded by RISC. Unlike ASOs, siRNAs are polyanions and hydrophilic which strongly impede their tissue delivery. Thus, they have to be encapsulated in nanovectors (like lipid nanoparticles) or to be chemically modified and conjugated to a ligand that induces cellular uptake [219,223]. Several siRNAs encapsulated in lipid nanoparticles were developed to knockdown totally or partially FXII expression in rodent thrombosis models [51,230–232]. It is important to mention that siF12-A demonstrated a prothrombotic effect, which was further associated with a sequence-related off-target activity [231,232].

Arrowhead Pharmaceuticals and Alnylam developed independently chemically modified anti-FXII siRNA using N-acetylgalactosamine (GalNAc) moiety as liver-targeted delivery (ARO-F12 and ALN-F12, respectively) [46,233]. Arrowhead Pharmaceuticals started its anti-FXII pipeline with ARC-F12, a preclinical program using ARC-EX1 excipient for liver delivery [234,235]. After toxicity findings, all programs including ARC-EX1 was stopped [236] and ARO-F12 emerged [233,237]. However, ARO-F12 seems to have been removed from their pipeline [238]. On the other hand, ALN-F12, a GalNAC conjugated siRNA with a DV 18 design, is actually on preclinical evaluation for thromboprophylaxis and prophylactic treatment of HAE [46,239,240].

4.5. Aptamers

Aptamers are single-stranded DNA or RNA oligonucleotides with a defined three-dimensional shape that recognizes a specific surface area of the target [241–244]. These molecules are selected from large libraries of random sequence oligonucleotides by the SELEX process (Convergent Systematic Evolution of Ligands by Exponential Enrichment). Briefly, the library is incubated with the target and aptamer-target complexes are separated from non-binding sequences. Binding aptamers are eluted and amplified. Several rounds of selection-amplification are performed until a limited subset of sequences with high affinity for the target dominates the population. Standard cloning and sequencing methods identify the sequence of selected candidates that are further optimized by truncation and chemical modifications [244–246]. Against FXII, Woodruff et al. [243] successfully applied the SELEX procedure using a modified T7 polymerase to allow the incorporation of 2'-fluoro nucleotides. They isolated R4cXII-1 (K_D = 8.9 ± 1.0 nM for FXII; K_D = 0.5 ± 0.2 nM for FXIIa), a 2'-fluoropyrimidine modified RNA aptamer. Analogous to ASO's, the 2'-modification improves the resistance to nuclease degradation [243,245,247]. R4cXII-1 interacts with the region of the FXII/FXIIa heavy chain implicated in anionic and FXI binding and, in consequence, inhibits the autoactivation of FXII and FXIIa-mediated activation of FXI, without affecting prekallikrein activation [243].

4.6. Monoclonal antibodies

The development of anti-FXII(a) monoclonal antibodies evolved with the emergence of novel technologies for their production. Two major classes can be separated: (1) murine antibodies produced by hybridoma technology and (2) human antibody isolated from phage display libraries.

4.6.1. Hybridoma technology

To generate monoclonal antibodies, mice are immunized by injection of an antigen. After extraction of B-lymphocytes from their spleen, the B-lymphocytes are fused with a myeloma cell line by chemical- or virus-induction, forming hybridomas. Each clone is separated into different culture wells and then screened. The hybridomas with desired activity and specificity are then produced *in vitro* [248–250].

The immunization of mice with FXII generated three monoclonal antibodies against FXII heavy chain (B7C9, anti-HF, P5-2-1) and two against light chain (mAb F3, OT-2) (Table 5). B7C9, anti-HF, and P5-2-1 inhibit the FXII activation at least partially. The mAb F3 interferes with interactions necessary to activate its physiological substrates whereas OT-2 completely inhibits the proteolytic activity of FXIIa. These antibodies block the coagulant activity of FXII (at least partially). OT-2 extends lifetime in lethal baboon model [251–256].

The utilization of β-FXIIa as an antigen led to C6B7, an effective blocker of human FXII coagulant activity in plasma (Table 5). C6B7 doesn't bind at the catalytic site because the amidolytic activity of FXIIa is only partially inhibited [75,257]. C6B7 was assessed in a baboon model of lethal bacteremia. The antibody protected the monkeys from FXIIa-mediated inflammatory reactions such as irreversible hypotension but did not prevent the development of disseminated intravascular coagulation [75].

Matafonov et al. [64] immunized FXII-deficient mice with FXII and FXIIa as antigens and generated the 15H8 antibody (Table 5). By binding to the EGF2 /kringle domains, 15H8 avoids the autoactivation of FXII and reduces FXIIa activation of prekallikrein. It also potentializes the activation of FXII by kallikrein or FXIa in absence of polyanion. Globally, the overall rate of activation was blunt. In the FeCl$_3$-induced thrombosis mouse model, it was able to prevent arterial thrombosis. In the collagen-graft thrombosis baboon model, 15H8 reduced fibrin deposition in the thrombogenic graft and limited platelet-rich thrombus growth downstream from the graft segment [64,258].

Finally, it is important to mention xisomab 3G3 (formerly AB023), a humanized version of the murine 14E11 antibody that completed recently a phase II trial in patients with end-stage renal disease on chronic hemodialysis (trial NCT03612856). AB023 inhibits the activation of FXI by FXIIa without affecting thrombin-induced FXI activation. Although AB023 binds to the apple 2 domain of FXI, its selective inhibition mimics an FXIIa inhibitor on the coagulation pathway [25].

4.6.2. By phage display library technology

Initially used for peptides, phage display screening was adapted to the discovery of antibodies and successfully applied against FXIIa. This technology generates fully human antibodies that abolish FXIIa enzymatic activity. One of these, CSL312, is actually on clinical trials [43,259].

4.6.2.1. CSL312

Using a standard panning protocol, Larsson et al. [54] screened a human antigen-binding fragment (Fab)-based phage antibody library. To ensure the generation of catalytic site binders, they used rHA-Inf4 as eluant during the panning process. After the expression of recombinant antibodies exposing the positive Fab clones, they tested their ability to inhibit FXIIa proteolytic activity and isolated the 3F7 antibody (IC_{50} = 13 nM; K_D = 6.2 nM) (Table 5). 3F7 completely inhibits proteolytic activity of FXIIa and is highly specific [54]. Preclinical results showed a thrombo-protection as effective as heparin without increased bleedings in extracorporeal membrane oxygenation cardiopulmonary bypass system adapted for rabbits [54]. 3F7 was also tested in a mouse model of arterial thrombosis [54] and in various murine edema models for which 3F7 has a potent and long-lasting efficacy [28,44,45]. The edema reduction was prolonged compared to traditional angioedema drugs [25]. Garadacimab (formerly CSL312), a variant of 3F7 with improved potency and affinity (K_D = 140 pM) and with additional inhibitory effect on kallikrein-kinin system, passed phase I clinical trials (trial ACTRN12616001438448) and entered into phase II for the prophylactic treatment of HAE (trial NCT03712228) (Table 5) [25,43–45].

4.6.2.2. 620I-X0177-A01

Two other human antibodies, DX-4012 (K_i = 5 pM) and D06, were isolated from phage display library (Table 5). They bind to the active site of FXIIa, blocking its proteolytic activity [65,260]. DX-4012 was assessed in collagen and tissue factor graft thrombosis models in baboons. With the reduced impact on hemostasis compared to enoxaparin, DX-4012 decreased the thrombus size formed by collagen initiation and did not have an effect on thrombus induced by tissue factor [65]. *In vitro* studies with D06 suggest that FXIIa is a major driver of thrombus formation compared to α-kallikrein. Kokoye et al. [260] also suggest that targeting both FXIIa and kallikrein could shut down more efficiently the reciprocal FXII-prekallikrein activation. With this goal in mind, the patent US 2018/0118851 describes bispecific antibodies against kallikrein and FXIIa [261]. 620I-X0177-A01, a bispecific antibody constructed by the fusion of the single-chain variable fragment of DX-4012 on the anti-kallikrein IgG DX-2930 [262], demonstrated to be more effective in contact-activated dilute plasma than their parent antibodies alone or in 1:1 combination (Table 5) [261].

Table 5: Anti-FXII(a) monoclonal antibodies.

Antibody	Type	Production	Mechanism of action	In vivo analysis	References
15H8	Murine	Immunization of FXII-deficient mice with human FXII and FXIIa	Binds to the fibronectin type I and/or kringle domain (inhibits activation)	FeCl₃ mouse model and vascular graft thrombosis primate model	[64]
B7C9	Murine	Immunization of mice with human FXII	Binds to the heavy chain of FXII (inhibits 60% activation)	/	[251]
C6B7	Murine	Immunization of mice with β-FXIIa	Binds to the catalytic domain (not at the catalytic site because inhibition not complete)	Lethal baboon model (inhibits the irreversible hypotension)	[75,257]
OT-2	Murine	Immunization of mice with FXII	Binds the light chain (complete inhibition of proteolytic activity)	Lethal baboon model (prolongs lifetime)	[255,256]

P5-2-1	Murine	Immunization of mice with human FXII	Binds to the heavy chain (inhibits activation – anionic surface binding site)	/	[253]
Anti-HF	Murine	Immunization of mice with human FXII	Binds to the heavy chain of FXII (inhibits activation)	/	[252]
mAb F3	Murine	Immunization of mice with human FXII	Binds the light chain (but not at the catalytic site because no inhibition of the amidolytic activity)	/	[254]
DX-4012	Human	Phage display against FXIIa	Inhibits proteolytic activity (K_i = 15 pM)	Collagen graft thrombosis model in baboon	[65]
Xisomab 3G3 (AB023)	Human	Humanized version of the murine 14E11 antibody	Binds to the apple 2 domain of FXI, preventing the activation of FXI by FXIIa	Phase II clinical trials	[25]
CSL 3F7	Human	Phage display against FXIIa	Abolishes FXIIa enzymatic activity	Abrogated skin edema and vascular permeability in mice Thromboprotection in ECMO system	[45,54,263]
Garadacimab (CSL 312)	Human	Variant of CSL 3F7	Abolishes FXIIa enzymatic activity	Phase II clinical trials	[43,45]
D06 (599C-x181-D06)	Human	Phage display against FXIIa	Binds the active site of FXIIa	/	[260]
620I-X0177-A01	Human	Phage display against FXIIa	Inhibits kallikrein and FXIIa (bispecific antibody)	/	[261]

ECMO: extracorporeal membrane oxygenation.

5. Conclusion

Neglected for a long time, FXII regains interest due to the wide diversity of its functions. FXII is implicated in multiple physiological pathways and induces specific cellular responses according to its form and the structure of the multi-protein receptor complex. Anti-FXII/FXIIa therapies could answer unmet medical needs such as the safe prevention of thrombosis in patients exposed to blood-contacting devices. The FXII/FXIIa inhibition also appears as a therapeutic strategy in patients suffering from hereditary angioedema and as an emerging research field in neuro-inflammatory and neurodegenerative disorders. Actually, there is a large panel of anti-FXII/FXIIa agents such as peptides, proteins, antibodies, and RNA-based technologies. Nevertheless, a highly potent and selective small-molecular-weight inhibitor is still missing. The recent elucidation of its three-dimensional structure will probably help to fill this gap.

6. Acknowledgements

[1]   A.H. Schmaier, E.X. Stavrou, Factor XII - What's important but not commonly thought about, Res. Pract. Thromb. Haemost. 3 (2019) 599–606. https://doi.org/10.1002/rth2.12235.

[2]   N.D. Rawlings, A.J. Barrett, P.D. Thomas, X. Huang, A. Bateman, R.D. Finn, The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database, Nucleic Acids Res. 46 (2018) D624–D632. https://doi.org/10.1093/nar/gkx1134.

[3]   H. Weidmann, L. Heikaus, A.T. Long, C. Naudin, H. Schlüter, T. Renné, The plasma contact system, a protease cascade at the nexus of inflammation, coagulation and immunity, Biochim. Biophys. Acta - Mol. Cell Res. 1864 (2017) 2118–2127. https://doi.org/10.1016/j.bbamcr.2017.07.009.

[4]   B. Tillman, D. Gailani, Inhibition of Factors XI and XII for Prevention of Thrombosis Induced by Artificial Surfaces, Semin. Thromb. Hemost. 44 (2018) 060–069. https://doi.org/10.1055/s-0037-1603937.

[5]   M. Didiasova, L. Wujak, L. Schaefer, M. Wygrecka, Factor XII in coagulation, inflammation and beyond, Cell. Signal. 51 (2018) 257–265. https://doi.org/10.1016/j.cellsig.2018.08.006.

[6]   E. Stavrou, A.H. Schmaier, Factor XII: What does it contribute to our understanding of the physiology and pathophysiology of hemostasis & thrombosis, Thromb. Res. 125 (2010) 210–215. https://doi.org/10.1016/j.thromres.2009.11.028.

[7]   S. de Maat, C. Maas, Factor XII: form determines function, J. Thromb. Haemost. 14 (2016) 1498–1506. https://doi.org/10.1111/jth.13383.

[8]   G.S. Salvesen, J.J. Catanese, L.F. Kress, J. Travis, Primary structure of the reactive site of human C1-inhibitor., J. Biol. Chem. 260 (1985) 2432–6.

[9]   E.X. Stavrou, C. Fang, K.L. Bane, A.T. Long, C. Naudin, E. Kucukal, A. Gandhi, A. Brett-Morris, M.M. Mumaw, S. Izadmehr, A. Merkulova, C.C. Reynolds, O. Alhalabi, L. Nayak, W.-M. Yu, C.-K. Qu, H.J. Meyerson, G.R. Dubyak, U.A. Gurkan, M.T. Nieman, A. Sen Gupta, T. Renné, A.H. Schmaier, Factor XII and uPAR upregulate neutrophil functions to influence wound healing, J. Clin. Invest. 128 (2018) 944–959. https://doi.org/10.1172/JCI92880.

[10]   D. Zamolodchikov, Y. Bai, Y. Tang, J.R. McWhirter, L.E. Macdonald, N. Alessandri-Haber, A Short Isoform of Coagulation Factor XII mRNA Is Expressed by Neurons in the Human Brain, Neuroscience. 413 (2019) 294–307. https://doi.org/10.1016/j.neuroscience.2019.05.040.

[11]   N. Ziliotto, F. Bernardi, D. Jakimovski, R. Zivadinov, Coagulation Pathways in Neurological Diseases: Multiple Sclerosis, Front. Neurol. 10 (2019) 1–21. https://doi.org/10.3389/fneur.2019.00409.

[12]   R.S. Woodruff, B. Sullenger, R.C. Becker, The many faces of the contact pathway and their role in thrombosis, J. Thromb. Thrombolysis. 32 (2011) 9–20. https://doi.org/10.1007/s11239-011-0578-5.

[13]   E. Kenne, T. Renné, Factor XII: a drug target for safe interference with thrombosis and inflammation, Drug Discov. Today. 19 (2014) 1459–1464. https://doi.org/10.1016/j.drudis.2014.06.024.

[14]   B.A. McMullen, K. Fujikawa, Amino acid sequence of the heavy chain of human α-factor XIIa (activated Hageman factor), J. Biol. Chem. 260 (1985) 5328–5341.

[15]   J. Shan, M. Baguinon, L. Zheng, R. Krishnamoorthi, Expression, refolding, and activation of the catalytic domain of human blood coagulation factor XII, Protein Expr. Purif. 27 (2003) 143–149. https://doi.org/10.1016/S1046-5928(02)00608-3.

[16]   M. Pathak, P. Wilmann, J. Awford, C. Li, B.K. Hamad, P.M. Fischer, I. Dreveny, L. V. Dekker, J. Emsley, Coagulation factor XII protease domain crystal structure, J. Thromb. Haemost. 13 (2015) 580–591. https://doi.org/10.1111/jth.12849.

[17]   A. Dementiev, A. Silva, C. Yee, Z. Li, M.T. Flavin, H. Sham, J.R. Partridge, Structures of human plasma β-factor XIIa cocrystallized with potent inhibitors, Blood Adv. 2 (2018) 549–558. https://doi.org/10.1182/bloodadvances.2018016337.

[18]   M. Pathak, R. Manna, C. Li, B.G. Kaira, B.K. Hamad, B.D. Belviso, C.R. Bonturi, I. Dreveny,

P.M. Fischer, L. V. Dekker, M.L.V. Oliva, J. Emsley, Crystal structures of the recombinant β-factor XIIa protease with bound Thr-Arg and Pro-Arg substrate mimetics, Acta Crystallogr. Sect. D Struct. Biol. 75 (2019) 578–591. https://doi.org/10.1107/S2059798319006910.

[19] D.X. Beringer, L.M.J. Kroon-Batenburg, The structure of the FnI-EGF-like tandem domain of coagulation factor XII solved using SIRAS, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 69 (2013) 94–102. https://doi.org/10.1107/S1744309113000286.

[20] F. Citarella, H. te Velthuis, M. Helmer-Citterich, C. Hack, Identification of a Putative Binding Site for Negatively Charged Surfaces in the Fibronectin Type II Domain of Human Factor XII, Thromb. Haemost. 84 (2000) 1057–1065. https://doi.org/10.1055/s-0037-1614171.

[21] B.K. Hamad, M. Pathak, R. Manna, P.M. Fischer, J. Emsley, L. V. Dekker, Assessment of the protein interaction between coagulation factor XII and corn trypsin inhibitor by molecular docking and biochemical validation, J. Thromb. Haemost. 15 (2017) 1818–1828. https://doi.org/10.1111/jth.13773.

[22] K.F. Nickel, A.T. Long, T.A. Fuchs, L.M. Butler, T. Renné, Factor XII as a Therapeutic Target in Thromboembolic and Inflammatory Diseases, Arterioscler. Thromb. Vasc. Biol. 37 (2017) 13–20. https://doi.org/10.1161/ATVBAHA.116.308595.

[23] K. Göbel, S. Eichler, H. Wiendl, T. Chavakis, C. Kleinschnitz, S.G. Meuth, The Coagulation Factors Fibrinogen, Thrombin, and Factor XII in Inflammatory Disorders—A Systematic Review, Front. Immunol. 9 (2018). https://doi.org/10.3389/fimmu.2018.01731.

[24] J.I. Weitz, J.C. Fredenburgh, Factors XI and XII as Targets for New Anticoagulants, Front. Med. 4 (2017) 1–6. https://doi.org/10.3389/fmed.2017.00019.

[25] E.P. DeLoughery, S.R. Olson, C. Puy, O.J.T. McCarty, J.J. Shatzel, The Safety and Efficacy of Novel Agents Targeting Factors XI and XII in Early Phase Human Trials, Semin. Thromb. Hemost. 45 (2019) 502–508. https://doi.org/10.1055/s-0039-1692439.

[26] H. Farkas, Hereditary angioedema: examining the landscape of therapies and preclinical therapeutic targets, Expert Opin. Ther. Targets. 23 (2019) 457–459. https://doi.org/10.1080/14728222.2019.1608949.

[27] H. Philippou, Heavy chain of FXII: not an innocent bystander!, Blood. 133 (2019) 1008–1009. https://doi.org/10.1182/blood-2019-01-895110.

[28] J. Björkqvist, S. de Maat, U. Lewandrowski, A. Di Gennaro, C. Oschatz, K. Schönig, M.M. Nöthen, C. Drouet, H. Braley, M.W. Nolte, A. Sickmann, C. Panousis, C. Maas, T. Renné, Defective glycosylation of coagulation factor XII underlies hereditary angioedema type III, J. Clin. Invest. 125 (2015) 3132–3146. https://doi.org/10.1172/JCI77139.

[29] V. Raghunathan, J. Zilberman-Rudenko, S.R. Olson, F. Lupu, O.J.T. McCarty, J.J. Shatzel, The contact pathway and sepsis, Res. Pract. Thromb. Haemost. 3 (2019) 331–339. https://doi.org/10.1002/rth2.12217.

[30] K. Göbel, S. Pankratz, C.-M. Asaridou, A.M. Herrmann, S. Bittner, M. Merker, T. Ruck, S. Glumm, F. Langhauser, P. Kraft, T.F. Krug, J. Breuer, M. Herold, C.C. Gross, D. Beckmann, A. Korb-Pap, M.K. Schuhmann, S. Kuerten, I. Mitroulis, C. Ruppert, M.W. Nolte, C. Panousis, L. Klotz, B. Kehrel, T. Korn, H.F. Langer, T. Pap, B. Nieswandt, H. Wiendl, T. Chavakis, C. Kleinschnitz, S.G. Meuth, Blood coagulation factor XII drives adaptive immunity during neuroinflammation via CD87-mediated modulation of dendritic cells, Nat. Commun. 7 (2016) 11626. https://doi.org/10.1038/ncomms11626.

[31] N. Ziliotto, M. Baroni, S. Straudi, F. Manfredini, R. Mari, E. Menegatti, R. Voltan, P. Secchiero, P. Zamboni, N. Basaglia, G. Marchetti, F. Bernardi, Coagulation Factor XII Levels and Intrinsic Thrombin Generation in Multiple Sclerosis, Front. Neurol. 9 (2018) 1–8. https://doi.org/10.3389/fneur.2018.00245.

[32] D. Zamolodchikov, Z.-L. Chen, B.A. Conti, T. Renné, S. Strickland, Activation of the factor XII-driven contact system in Alzheimer's disease patient and mouse model plasma, Proc. Natl. Acad. Sci. 112 (2015) 4068–4073. https://doi.org/10.1073/pnas.1423764112.

[33]   D. Zamolodchikov, T. Renné, S. Strickland, The Alzheimer's disease peptide β-amyloid promotes thrombin generation through activation of coagulation factor XII, J. Thromb. Haemost. 14 (2016) 995–1007. https://doi.org/10.1111/jth.13209.

[34]   Z.-L. Chen, A.S. Revenko, P. Singh, A.R. MacLeod, E.H. Norris, S. Strickland, Depletion of coagulation factor XII ameliorates brain pathology and cognitive impairment in Alzheimer disease mice, Blood. 129 (2017) 2547–2556. https://doi.org/10.1182/blood-2016-11-753202.

[35]   S. Hopp, C. Albert-Weissenberger, S. Mencl, M. Bieber, M.K. Schuhmann, C. Stetter, B. Nieswandt, P.M. Schmidt, C.-M. Monoranu, I. Alafuzoff, N. Marklund, M.W. Nolte, A.-L. Sirén, C. Kleinschnitz, Targeting coagulation factor XII as a novel therapeutic option in brain trauma, Ann. Neurol. 79 (2016) 970–982. https://doi.org/10.1002/ana.24655.

[36]   S. Hopp, M.W. Nolte, C. Stetter, C. Kleinschnitz, A.-L. Sirén, C. Albert-Weissenberger, Alleviation of secondary brain injury, posttraumatic inflammation, and brain edema formation by inhibition of factor XIIa, J. Neuroinflammation. 14 (2017) 39. https://doi.org/10.1186/s12974-017-0815-8.

[37]   C. Albert-Weissenberger, S. Hopp, B. Nieswandt, A.-L. Sirén, C. Kleinschnitz, C. Stetter, How is the formation of microthrombi after traumatic brain injury linked to inflammation?, J. Neuroimmunol. 326 (2019) 9–13. https://doi.org/10.1016/j.jneuroim.2018.10.011.

[38]   M. Maurer, M. Magerl, I. Ansotegui, E. Aygören-Pürsün, S. Betschel, K. Bork, T. Bowen, H. Balle Boysen, H. Farkas, A.S. Grumach, M. Hide, C. Katelaris, R. Lockey, H. Longhurst, W.R. Lumry, I. Martinez-Saguer, D. Moldovan, A. Nast, R. Pawankar, P. Potter, M. Riedl, B. Ritchie, L. Rosenwasser, M. Sánchez-Borges, Y. Zhi, B. Zuraw, T. Craig, The international WAO/EAACI guideline for the management of hereditary angioedema-The 2017 revision and update, Allergy. 73 (2018) 1575–1596. https://doi.org/10.1111/all.13384.

[39]   V. Bafunno, D. Firinu, M. D'Apolito, G. Cordisco, S. Loffredo, A. Leccese, M. Bova, M.P. Barca, R. Santacroce, M. Cicardi, S. Del Giacco, M. Margaglione, Mutation of the angiopoietin-1 gene (ANGPT1) associates with a new type of hereditary angioedema, J. Allergy Clin. Immunol. 141 (2018) 1009–1017. https://doi.org/10.1016/j.jaci.2017.05.020.

[40]   K. Bork, K. Wulff, L. Steinmüller-Magin, I. Braenne, P. Staubach-Renz, G. Witzke, J. Hardt, Hereditary angioedema with a mutation in the plasminogen gene, Allergy. 73 (2018) 442–450. https://doi.org/10.1111/all.13270.

[41]   A. Recke, E.G. Massalme, U. Jappe, L. Steinmüller-Magin, J. Schmidt, Y. Hellenbroich, I. Hüning, G. Gillessen-Kaesbach, D. Zillikens, K. Hartmann, Identification of the recently described plasminogen gene mutation p.Lys330Glu in a family from Northern Germany with hereditary angioedema, Clin. Transl. Allergy. 9 (2019) 9. https://doi.org/10.1186/s13601-019-0247-x.

[42]   K. Bork, K. Wulff, H. Rossmann, L. Steinmüller-Magin, I. Brænne, G. Witzke, J. Hardt, Hereditary angioedema cosegregating with a novel kininogen 1 gene mutation changing the N-terminal cleavage site of bradykinin, Allergy. 74 (2019) 2479–2481. https://doi.org/10.1111/all.13869.

[43]   F. Perego, M.A. Wu, A. Valerieva, S. Caccia, C. Suffritti, A. Zanichelli, L. Bergamaschini, M. Cicardi, Current and emerging biologics for the treatment of hereditary angioedema, Expert Opin. Biol. Ther. 19 (2019) 517–526. https://doi.org/10.1080/14712598.2019.1595581.

[44]   H. Cao, M. Biondo, H. Lioe, S. Busfield, V. Rayzman, B. Nieswandt, K. Bork, L.C. Harrison, P. Auyeung, H. Farkas, D. Csuka, M. Pelzing, S. Dower, M.J. Wilson, A. Nash, M.W. Nolte, C. Panousis, Antibody-mediated inhibition of FXIIa blocks downstream bradykinin generation, J. Allergy Clin. Immunol. 142 (2018) 1355–1358. https://doi.org/10.1016/j.jaci.2018.06.014.

[45]   Z. (Helen) Cao, M. Biondo, V. Rayzman, M. Hardy, A. McDonald, S. Busfield, M.W. Nolte, M. Wilson, A. Nash, C. Panousis, Development and Characterization of an Anti-FXIIa Monoclonal Antibody for the Treatment of Hereditary Angioedema, J. Allergy Clin. Immunol. 135 (2015) AB194. https://doi.org/10.1016/j.jaci.2014.12.1570.

[46]   J. Liu, J. Qin, A. Borodovsky, T. Racie, A. Castoreno, M. Schlegel, M.A. Maier, T. Zimmerman,

K. Fitzgerald, J. Butler, A. Akinc, An investigational RNAi therapeutic targeting Factor XII (ALN-F12) for the treatment of hereditary angioedema, RNA. 25 (2019) 255–263. https://doi.org/10.1261/rna.068916.118.

[47]   J. Scheffel, N.A. Mahnke, Z.L.M. Hofman, S. de Maat, J. Wu, H. Bonnekoh, R.J. Pengelly, S. Ennis, J.W. Holloway, M. Kirchner, P. Mertins, M.K. Church, M. Maurer, C. Maas, K. Krause, Cold-induced urticarial autoinflammatory syndrome related to factor XII activation, Nat. Commun. 11 (2020) 179. https://doi.org/10.1038/s41467-019-13984-8.

[48]   A. Girolami, S. Ferrari, E. Cosi, B. Girolami, M.L. Randi, Thrombotic events in severe FXII deficiency in comparison with unaffected family members during a long observation period, J. Thromb. Thrombolysis. 47 (2019) 481–485. https://doi.org/10.1007/s11239-019-01819-8.

[49]   A. Girolami, S. Ferrari, E. Cosi, M.L. Randi, Heterozygous FXII deficiency is not associated with an increased incidence of thrombotic events: Results of a long term study, Blood Cells, Mol. Dis. 77 (2019) 8–11. https://doi.org/10.1016/j.bcmd.2019.03.001.

[50]   N.S. Key, Epidemiologic and clinical data linking factors XI and XII to thrombosis, Hematology. 2014 (2014) 66–70. https://doi.org/10.1182/asheducation-2014.1.66.

[51]   M. Heestermans, S. Salloum-Asfar, T. Streef, E.H. Laghmani, D. Salvatori, B.M. Luken, S.S. Zeerleder, H.M.H. Spronk, S.J. Korporaal, D. Kirchhofer, G.T.M. Wagenaar, H.H. Versteeg, P.H. Reitsma, T. Renné, B.J.M. van Vlijmen, Mouse venous thrombosis upon silencing of anticoagulants depends on tissue factor and platelets, not FXII or neutrophils, Blood. 133 (2019) 2090–2099. https://doi.org/10.1182/blood-2018-06-853762.

[52]   J.W. Yau, P. Liao, J.C. Fredenburgh, A.R. Stafford, A.S. Revenko, B.P. Monia, J.I. Weitz, Selective depletion of factor XI or factor XII with antisense oligonucleotides attenuates catheter thrombosis in rabbits, Blood. 123 (2014) 2102–2107. https://doi.org/10.1182/blood-2013-12-540872.

[53]   J.W. Yau, A.R. Stafford, P. Liao, J.C. Fredenburgh, R. Roberts, J.L. Brash, J.I. Weitz, Corn trypsin inhibitor coating attenuates the prothrombotic properties of catheters in vitro and in vivo, Acta Biomater. 8 (2012) 4092–4100. https://doi.org/10.1016/j.actbio.2012.07.019.

[54]   M. Larsson, V. Rayzman, M.W. Nolte, K.F. Nickel, J. Bjorkqvist, A. Jamsa, M.P. Hardy, M. Fries, S. Schmidbauer, P. Hedenqvist, M. Broome, I. Pragst, G. Dickneite, M.J. Wilson, A.D. Nash, C. Panousis, T. Renne, A Factor XIIa Inhibitory Antibody Provides Thromboprotection in Extracorporeal Circulation Without Increasing Bleeding Risk, Sci. Transl. Med. 6 (2014) 222ra17-222ra17. https://doi.org/10.1126/scitranslmed.3006804.

[55]   A. Aimo, R.P. Giugliano, R. De Caterina, Non–Vitamin K Antagonist Oral Anticoagulants for Mechanical Heart Valves, Circulation. 138 (2018) 1356–1365. https://doi.org/10.1161/CIRCULATIONAHA.118.035612.

[56]   I.H. Jaffer, J.C. Fredenburgh, J. Hirsh, J.I. Weitz, Medical device-induced thrombosis: what causes it and how can we prevent it?, J. Thromb. Haemost. 13 (2015) S72–S81. https://doi.org/10.1111/jth.12961.

[57]   I.H. Jaffer, A.R. Stafford, J.C. Fredenburgh, R.P. Whitlock, N.C. Chan, J.I. Weitz, Dabigatran is Less Effective Than Warfarin at Attenuating Mechanical Heart Valve-Induced Thrombin Generation, J. Am. Heart Assoc. 4 (2015) e002322. https://doi.org/10.1161/JAHA.115.002322.

[58]   I.H. Jaffer, J.I. Weitz, The blood compatibility challenge. Part 1: Blood-contacting medical devices: The scope of the problem, Acta Biomater. 94 (2019) 2–10. https://doi.org/10.1016/j.actbio.2019.06.021.

[59]   T. Renné, E.X. Stavrou, Roles of Factor XII in Innate Immunity, Front. Immunol. 10 (2019) 1–9. https://doi.org/10.3389/fimmu.2019.02011.

[60]   N.C. Chan, J.I. Weitz, Antithrombotic Agents, Circ. Res. 124 (2019) 426–436. https://doi.org/10.1161/CIRCRESAHA.118.313155.

[61]   K.F. Nickel, L. Labberton, A.T. Long, F. Langer, T.A. Fuchs, E.X. Stavrou, L.M. Butler, T. Renné, The polyphosphate/factor XII pathway in cancer-associated thrombosis: novel

perspectives for safe anticoagulation in patients with malignancies, Thromb. Res. 141 (2016) S4–S7. https://doi.org/10.1016/S0049-3848(16)30353-X.

[62] E. Campello, A. Ilich, P. Simioni, N.S. Key, The relationship between pancreatic cancer and hypercoagulability: a comprehensive review on epidemiological and biological issues, Br. J. Cancer. 121 (2019) 359–371. https://doi.org/10.1038/s41416-019-0510-x.

[63] E. Campello, M.W. Henderson, D.F. Noubouossie, P. Simioni, N.S. Key, Contact System Activation and Cancer: New Insights in the Pathophysiology of Cancer-Associated Thrombosis, Thromb. Haemost. 118 (2018) 251–265. https://doi.org/10.1160/TH17-08-0596.

[64] A. Matafonov, P.Y. Leung, A.E. Gailani, S.L. Grach, C. Puy, Q. Cheng, M. Sun, O.J.T. McCarty, E.I. Tucker, H. Kataoka, T. Renné, J.H. Morrissey, A. Gruber, D. Gailani, Factor XII inhibition reduces thrombus formation in a primate thrombosis model, Blood. 123 (2014) 1739–1746. https://doi.org/10.1182/blood-2013-04-499111.

[65] S. Mason, J.A. Kenniston, A. Nixon, D.J. Sexton, S.R. Comeau, B. Adelman, A monoclonal antibody inhibitor of factor XIIa, US20190002584 A1, 2019.

[66] F. Lupu, R.S. Keshari, J.D. Lambris, K. Mark Coggeshall, Crosstalk between the coagulation and complement systems in sepsis, Thromb. Res. 133 (2014) S28–S31. https://doi.org/10.1016/j.thromres.2014.03.014.

[67] S. Oehmcke, H. Herwald, Contact system activation in severe infectious diseases, J. Mol. Med. 88 (2010) 121–126. https://doi.org/10.1007/s00109-009-0564-y.

[68] R.A. Pixley, S. Zellis, P. Bankes, R.A. DeLa Cadena, J.D. Page, C.F. Scott, J. Kappelmayer, E.G. Wyshock, J.J. Kelly, R.W. Colman, Prognostic value of assessing contact system activation and factor V in systemic inflammatory response syndrome, Crit. Care Med. 23 (1995) 41–51. https://doi.org/10.1097/00003246-199501000-00010.

[69] M. Bachler, C. Niederwanger, T. Hell, J. Höfer, D. Gerstmeyr, B. Schenk, B. Treml, D. Fries, Influence of factor XII deficiency on activated partial thromboplastin time (aPTT) in critically ill patients, J. Thromb. Thrombolysis. 48 (2019) 466–474. https://doi.org/10.1007/s11239-019-01879-w.

[70] J.H. Nuijens, C.C.M. Huijbregts, M. Cohen, G.O. Navis, A. de Vries, A.J.M. Eerenberg, J.C. Bakker, C.E. Hack, Detection of Activation of the Contact System of Coagulation In Vitro and In Vivo: Quantitation of Activated Hageman Factor-C1-Inhibitor and Kallikrein-C1-Inhibitor Complexes by Specific Radioimmunoassays, Thromb. Haemost. 58 (1987) 778–785. https://doi.org/10.1055/s-0038-1645969.

[71] J. Nuijens, C. Huijbregts, A. Eerenberg-Belmer, J. Abbink, R. Strack van Schijndel, R. Felt-Bersma, L. Thijs, C. Hack, Quantification of plasma factor XIIa-C1(-)-inhibitor and kallikrein-C1(-)-inhibitor complexes in sepsis, Blood. 72 (1988) 1841–1848. https://doi.org/10.1182/blood.V72.6.1841.bloodjournal7261841.

[72] W.A. Wuillemin, M. Minnema, J.C. Meijers, D. Roem, A.J. Eerenberg, J.H. Nuijens, H. ten Cate, C.E. Hack, Inactivation of factor XIa in human plasma assessed by measuring factor XIa-protease inhibitor complexes: major role for C1-inhibitor., Blood. 85 (1995) 1517–26.

[73] W.A. Wuillemin, K. Fijnvandraat, B.H.F. Derkx, M. Peters, W. Vreede, H. ten Cate, C.E. Hack, Activation of the Intrinsic Pathway of Coagulation in Children with Meningococcal Septic Shock, Thromb. Haemost. 74 (1995) 1436–1441. https://doi.org/10.1055/s-0038-1649961.

[74] B.N. Jukema, S. de Maat, C. Maas, Processing of Factor XII during Inflammatory Reactions, Front. Med. 3 (2016) 1–7. https://doi.org/10.3389/fmed.2016.00052.

[75] R.A. Pixley, R. De La Cadena, J.D. Page, N. Kaufman, E.G. Wyshock, A. Chang, F.B. Taylor, R.W. Colman, The Contact System Contributes to Hypotension but Not Disseminated Intravascular Coagulation in Lethal Bacteremia, J. Clin. Invest. 91 (1993) 61–68.

[76] P. Jansen, R. Pixley, M. Brouwer, I. de Jong, A. Chang, C. Hack, F.J. Taylor, R. Colman, Inhibition of factor XII in septic baboons attenuates the activation of complement and fibrinolytic systems and reduces the release of interleukin-6 and neutrophil elastase, Blood. 87 (1996)

2337–2344. https://doi.org/10.1182/blood.V87.6.2337.bloodjournal8762337.

[77]   R. Silasi, R.S. Keshari, C. Lupu, W.J. Van Rensburg, H. Chaaban, G. Regmi, A. Shamanaev, J.J. Shatzel, C. Puy, C.U. Lorentz, E.I. Tucker, D. Gailani, A. Gruber, O.J.T. McCarty, F. Lupu, Inhibition of contact-mediated activation of factor XI protects baboons against S aureus–induced organ damage and death, Blood Adv. 3 (2019) 658–669. https://doi.org/10.1182/bloodadvances.2018029983.

[78]   T. IWAKI, D. CRUZ-TOPETE, F.J. CASTELLINO, A complete factor XII deficiency does not affect coagulopathy, inflammatory responses, and lethality, but attenuates early hypotension in endotoxemic mice, J. Thromb. Haemost. 6 (2008) 1993–1995. https://doi.org/10.1111/j.1538-7836.2008.03142.x.

[79]   I. Stroo, S. Zeerleder, C. Ding, B. Luken, J. Roelofs, O. de Boer, J. Meijers, F. Castellino, C. van 't Veer, T. van der Poll, Coagulation factor XI improves host defence during murine pneumonia-derived sepsis independent of factor XII activation, Thromb. Haemost. 117 (2017) 1601–1614. https://doi.org/10.1160/TH16-12-0920.

[80]   I. Stroo, C. Ding, A. Novak, J. Yang, J.J.T.H. Roelofs, J.C.M. Meijers, A.S. Revenko, C. van 't Veer, S. Zeerleder, J.R. Crosby, T. van der Poll, Inhibition of the extrinsic or intrinsic coagulation pathway during pneumonia-derived sepsis, Am. J. Physiol. Cell. Mol. Physiol. 315 (2018) L799–L809. https://doi.org/10.1152/ajplung.00014.2018.

[81]   E.I. Tucker, N.G. Verbout, P.Y. Leung, S. Hurst, O.J.T. McCarty, D. Gailani, A. Gruber, Inhibition of factor XI activation attenuates inflammation and coagulopathy while improving the survival of mouse polymicrobial sepsis, Blood. 119 (2012) 4762–4768. https://doi.org/10.1182/blood-2011-10-386185.

[82]   S. Lorenzano, M. Inglese, T. Koudriavtseva, Editorial: Role of Coagulation Pathways in Neurological Diseases, Front. Neurol. 10 (2019) 1–3. https://doi.org/10.3389/fneur.2019.00791.

[83]   H. Yamamoto-Imoto, D. Zamolodchikov, Z.-L. Chen, S.L. Bourne, S. Rizvi, P. Singh, E.H. Norris, F. Weis-Garcia, S. Strickland, A novel detection method of cleaved plasma high-molecular-weight kininogen reveals its correlation with Alzheimer's pathology and cognitive impairment, Alzheimer's Dement. Diagnosis, Assess. Dis. Monit. 10 (2018) 480–489. https://doi.org/10.1016/j.dadm.2018.06.008.

[84]   B. Segal, The Diversity of Encephalitogenic CD4+ T Cells in Multiple Sclerosis and Its Animal Models, J. Clin. Med. 8 (2019) 120. https://doi.org/10.3390/jcm8010120.

[85]   K.K. Jain, Neuroprotection in Traumatic Brain Injury, in: Handb. Neuroprotection, Springer Protocols Handbooks, New York, NY, 2019: pp. 281–336. https://doi.org/10.1007/978-1-4939-9465-6_4.

[86]   D. Krowarsch, T. Cierpicki, F. Jelen, J. Otlewski, Canonical protein inhibitors of serine proteases, Cell. Mol. Life Sci. 60 (2003) 2427–2444. https://doi.org/10.1007/s00018-003-3120-x.

[87]   W. Apostoluk, J. Otlewski, Variability of the canonical loop conformations in serine proteinases inhibitors and other proteins, Proteins Struct. Funct. Genet. 32 (1998) 459–474. https://doi.org/10.1002/(SICI)1097-0134(19980901)32:4<459::AID-PROT5>3.0.CO;2-B.

[88]   J. Otlewski, M. Jaskólski, O. Buczek, T. Cierpicki, H. Czapińska, D. Krowarsch, A.O. Smalas, D. Stachowiak, A. Szpineta, M. Dadlez, Structure-function relationship of serine protease-protein inhibitor interaction., Acta Biochim. Pol. 48 (2001) 419–428. https://doi.org/10.18388/abp.2001_3926.

[89]   W. Sanrattana, C. Maas, S. de Maat, SERPINs—From Trap to Treatment, Front. Med. 6 (2019) 1–8. https://doi.org/10.3389/fmed.2019.00025.

[90]   B.M. Scott, W.P. Sheffield, Engineering the serpin α 1 -antitrypsin: A diversity of goals and techniques, Protein Sci. 29 (2020) 856–871. https://doi.org/10.1002/pro.3794.

[91]   C.Y. Koh, C. Modahl, N. Kulkarni, R.M. Kini, Toxins Are an Excellent Source of Therapeutic Agents against Cardiovascular Diseases, Semin. Thromb. Hemost. 44 (2018) 691–706.

https://doi.org/10.1055/s-0038-1661384.

[92]   B. Arcà, J.M. Ribeiro, Saliva of hematophagous insects: a multifaceted toolkit, Curr. Opin. Insect Sci. 29 (2018) 102–109. https://doi.org/10.1016/j.cois.2018.07.012.

[93]   G. Leboulle, C. Rochez, J. Louahed, B. Ruti, M. Brossard, A. Bollen, E. Godfroid, Isolation of Ixodes ricinus salivary gland mRNA encoding factors induced during blood feeding., Am. J. Trop. Med. Hyg. 66 (2002) 225–233. https://doi.org/10.4269/ajtmh.2002.66.225.

[94]   Y. Decrem, G. Rath, V. Blasioli, P. Cauchie, S. Robert, J. Beaufays, J.-M. Frère, O. Feron, J.-M. Dogné, C. Dessy, L. Vanhamme, E. Godfroid, Ir-CPI, a coagulation contact phase inhibitor from the tick Ixodes ricinus, inhibits thrombus formation without impairing hemostasis, J. Exp. Med. 206 (2009) 2381–2395. https://doi.org/10.1084/jem.20091007.

[95]   E. Godfroid, Y. Decrem, L. Vanhamme, A. Bollen, G. Leboulle, Identification and molecular characterisation of proteins, expressed in the Ixodes ricinus salivary glands, US9212216 B2, 2015.

[96]   V. Pireaux, J. Tassignon, S. Demoulin, S. Derochette, N. Borenstein, A. Ente, L. Fiette, J. Douxfils, P. Lancellotti, M. Guyaux, E. Godfroid, Anticoagulation With an Inhibitor of Factors XIa and XIIa During Cardiopulmonary Bypass, J. Am. Coll. Cardiol. 74 (2019) 2178–2189. https://doi.org/10.1016/j.jacc.2019.08.1028.

[97]   N. Kato, S. Iwanaga, T. Okayama, H. Isawa, M. Yuda, Y. Chinzei, Identification and characterization of the plasma kallikrein-kinin system inhibitor, haemaphysalin, from hard tick, Haemaphysalis longicornis, Thromb. Haemost. 93 (2005) 359–367. https://doi.org/10.1160/TH04-05-0319.

[98]   N. Kato, T. Okayama, H. Isawa, M. Yuda, Y. Chinzei, S. Iwanaga, Contribution of the N-Terminal and C-Terminal Domains of Haemaphysalin to Inhibition of Activation of Plasma Kallikrein-Kinin System, J. Biochem. 138 (2005) 225–235. https://doi.org/10.1093/jb/mvi123.

[99]   I.T.N. Campos, R. Amino, C.A.M. Sampaio, E.A. Auerswald, T. Friedrich, H.-G. Lemaire, S. Schenkman, A.S. Tanaka, Infestin, a thrombin inhibitor presents in Triatoma infestans midgut, a Chagas' disease vector: gene cloning, expression and characterization of the inhibitor, Insect Biochem. Mol. Biol. 32 (2002) 991–997. https://doi.org/10.1016/S0965-1748(02)00035-8.

[100] I. Hagedorn, S. Schmidbauer, I. Pleines, C. Kleinschnitz, U. Kronthaler, G. Stoll, G. Dickneite, B. Nieswandt, Factor XIIa Inhibitor Recombinant Human Albumin Infestin-4 Abolishes Occlusive Arterial Thrombus Formation Without Affecting Bleeding, Circulation. 121 (2010) 1510–1517. https://doi.org/10.1161/CIRCULATIONAHA.109.924761.

[101] I.T.N. Campos, T.A.C.B. Souza, R.J.S. Torquato, R. De Marco, A.M. Tanaka-Azevedo, A.S. Tanaka, J.A.R.G. Barbosa, The Kazal-type inhibitors infestins 1 and 4 differ in specificity but are similar in three-dimensional structure, Acta Crystallogr. Sect. D Biol. Crystallogr. 68 (2012) 695–702. https://doi.org/10.1107/S0907444912009067.

[102] I.T.N. Campos, A.M. Tanaka-Azevedo, A.S. Tanaka, Identification and characterization of a novel factor XIIa inhibitor in the hematophagous insect, Triatoma infestans (Hemiptera: Reduviidae), FEBS Lett. 577 (2004) 512–516. https://doi.org/10.1016/j.febslet.2004.10.052.

[103] V.N. Kolyadko, S. V. Lushchekina, T.A. Vuimo, S.S. Surov, R.A. Ovsepyan, V.A. Korneeva, I.I. Vorobiev, N.A. Orlova, L. Minakhin, K. Kuznedelov, K. V. Severinov, F.I. Ataullakhanov, M.A. Panteleev, New Infestin-4 Mutants with Increased Selectivity against Factor XIIa, PLoS One. 10 (2015) e0144940. https://doi.org/10.1371/journal.pone.0144940.

[104] Y. Xu, T.-Q. Cai, G. Castriota, Y. Zhou, L. Hoos, N. Jochnowitz, C. Loewrigkeit, J. Cook, A. Wickham, J. Metzger, M. Ogletree, D. Seiffert, Z. Chen, Factor XIIa inhibition by Infestin-4: in vitro mode of action and in vivo antithrombotic benefit, Thromb. Haemost. 111 (2014) 694–704. https://doi.org/10.1160/TH13-08-0668.

[105] C.M. Barbieri, X. Wang, X. Zhou, A.M. Ogawa, K. O'Neil, W. Wu, G. Castriota, D.A. Seiffert, D. Gutstein, Factor XIIa: New insights on chemical tractability and target indications from a potent and selective tool inhibitor, Arterioscler. Thromb. Vasc. Biol. 36 (2016) A523.

[106] H. Isawa, Y. Orito, N. Jingushi, S. Iwanaga, A. Morita, Y. Chinzei, M. Yuda, Identification and characterization of plasma kallikrein-kinin system inhibitors from salivary glands of the blood-sucking insect Triatoma infestans, FEBS J. 274 (2007) 4271–4286. https://doi.org/10.1111/j.1742-4658.2007.05958.x.

[107] M. Hernández-Vargas, C. Santibáñez-López, G. Corzo, An Insight into the Triabin Protein Family of American Hematophagous Reduviids: Functional, Structural and Phylogenetic Analysis, Toxins (Basel). 8 (2016) 44. https://doi.org/10.3390/toxins8020044.

[108] H. Kato, R.C. Jochim, E.A. Gomez, R. Sakoda, H. Iwata, J.G. Valenzuela, Y. Hashiguchi, A repertoire of the dominant transcripts from the salivary glands of the blood-sucking bug, Triatoma dimidiata, a vector of Chagas disease, Infect. Genet. Evol. 10 (2010) 184–191. https://doi.org/10.1016/j.meegid.2009.10.012.

[109] P. Fuentes-Prior, C. Noeske-Jungblut, P. Donner, W.-D. Schleuning, R. Huber, W. Bode, Structure of the thrombin complex with triabin, a lipocalin-like exosite-binding inhibitor derived from a triatomine bug, Proc. Natl. Acad. Sci. 94 (1997) 11845–11850. https://doi.org/10.1073/pnas.94.22.11845.

[110] Y. Ishimaru, E.A. Gomez, F. Zhang, L. Martini-Robles, H. Iwata, T. Sakurai, K. Katakura, Y. Hashiguchi, H. Kato, Dimiconin, a novel coagulation inhibitor from the kissing bug, Triatoma dimidiata, a vector of Chagas disease, J. Exp. Biol. 215 (2012) 3597–3602. https://doi.org/10.1242/jeb.074211.

[111] H. Isawa, M. Yuda, Y. Orito, Y. Chinzei, A Mosquito Salivary Protein Inhibits Activation of the Plasma Contact System by Binding to Factor XII and High Molecular Weight Kininogen, J. Biol. Chem. 277 (2002) 27651–27658. https://doi.org/10.1074/jbc.M203505200.

[112] H. Kato, E.A. Gomez, M. Fujita, Y. Ishimaru, H. Uezato, T. Mimori, H. Iwata, Y. Hashiguchi, Ayadualin, a novel RGD peptide with dual antihemostatic activities from the sand fly Lutzomyia ayacuchensis, a vector of Andean-type cutaneous leishmaniasis, Biochimie. 112 (2015) 49–56. https://doi.org/10.1016/j.biochi.2015.02.011.

[113] E. Calvo, B.J. Mans, J.F. Andersen, J.M.C. Ribeiro, Function and Evolution of a Mosquito Salivary Protein Family, J. Biol. Chem. 281 (2006) 1935–1942. https://doi.org/10.1074/jbc.M510359200.

[114] H. Kato, R.C. Jochim, E.A. Gomez, H. Uezato, T. Mimori, M. Korenaga, T. Sakurai, K. Katakura, J.G. Valenzuela, Y. Hashiguchi, Analysis of salivary gland transcripts of the sand fly Lutzomyia ayacuchensis, a vector of Andean-type cutaneous leishmaniasis, Infect. Genet. Evol. 13 (2013) 56–66. https://doi.org/10.1016/j.meegid.2012.08.024.

[115] B.. Harish, K.B. Uppuluri, Microbial serine protease inhibitors and their therapeutic applications, Int. J. Biol. Macromol. 107 (2018) 1373–1387. https://doi.org/10.1016/j.ijbiomac.2017.09.115.

[116] C.H. Chung, H.E. Ives, S. Almeda, A.L. Goldberg, Purification from Escherichia coli of a periplasmic protein that is a potent inhibitor of pancreatic proteases., J. Biol. Chem. 258 (1983) 11032–11038.

[117] J.S. Ulmer, R.N. Lindquist, M.S. Dennis, R.A. Lazarus, Ecotin is a potent inhibitor of the contact system proteases factor XIIa and plasma kallikrein, FEBS Lett. 365 (1995) 159–163. https://doi.org/10.1016/0014-5793(95)00466-M.

[118] J.L. Seymour, R.N. Lindquist, M.S. Dennis, B. Moffat, D. Yansura, D. Reilly, M.E. Wessinger, R.A. Lazarus, Ecotin Is a Potent Anticoagulant and Reversible Tight-Binding Inhibitor of Factor Xa, Biochemistry. 33 (1994) 3949–3958. https://doi.org/10.1021/bi00179a022.

[119] M.J. Lauwereys, A.-M.V.R. Lambeir, Ecotin as a factor Xa, XIa, and XIIa inhibitor, US5585259, 1996.

[120] S.A. Gillmor, T. Takeuchi, S.Q. Yang, C.S. Craik, R.J. Fletterick, Compromise and accommodation in ecotin, a dimeric macromolecular inhibitor of serine proteases, J. Mol. Biol. 299 (2000) 993–1003. https://doi.org/10.1006/jmbi.2000.3812.

[121] C.T. Eggers, S.X. Wang, R.J. Fletterick, C.S. Craik, The role of ecotin dimerization in protease

inhibition, J. Mol. Biol. 308 (2001) 975–991. https://doi.org/10.1006/jmbi.2001.4754.

[122]   L. Jin, P. Pandey, R.E. Babine, J.C. Gorga, K.J. Seidl, E. Gelfand, D.T. Weaver, S.S. Abdel-Meguid, J.E. Strickler, Crystal Structures of the FXIa Catalytic Domain in Complex with Ecotin Mutants Reveal Substrate-like Interactions, J. Biol. Chem. 280 (2005) 4704–4712. https://doi.org/10.1074/jbc.M411309200.

[123]   M.E. Mcgrath, S.A. Gillmor, R.J. Fletterick, Ecotin: Lessons on survival in a protease-filled world, Protein Sci. 4 (2008) 141–148. https://doi.org/10.1002/pro.5560040201.

[124]   R.A. Lazarus, M.S. Dennis, J.S. Ulmer, Pharmaceutical compositions containing ecotin and homologs thereof, US6113896, 2000.

[125]   M.E. McGrath, W.M. Hines, J.A. Sakanari, R.J. Fletterick, C.S. Craik, The sequence and reactive site of ecotin: A general inhibitor of pancreatic serine proteases from Escherichia coli, J. Biol. Chem. 266 (1991) 6620–6625.

[126]   D.H. Shin, H.K. Song, S.W. Suh, I.S. Seong, C.S. Lee, C.H. Chung, Crystal structure analyses of uncomplexed ecotin in two crystal forms: Implications for its function and stability, Protein Sci. 5 (1996) 2236–2247. https://doi.org/10.1002/pro.5560051110.

[127]   R.A. Lazarus, M.S. Dennis, J.S. Ulmer, Pharmaceutical administration of ecotin homologs, US5843895, 1998.

[128]   R.A. Lazarus, M.S. Dennis, J.S. Ulmer, DNA encoding ecotin homologs, US5719041, 1998.

[129]   S.Q. Yang, C.-I. Wang, S.A. Gillmor, R.J. Fletterick, C.S. Craik, Ecotin: a serine protease inhibitor with two distinct and interacting binding sites, J. Mol. Biol. 279 (1998) 945–957. https://doi.org/10.1006/jmbi.1998.1748.

[130]   A.A. Stoop, C.S. Craik, Engineering of a macromolecular scaffold to develop specific protease inhibitors, Nat. Biotechnol. 21 (2003) 1063–1068. https://doi.org/10.1038/nbt860.

[131]   M. Tanabe, T. Asano, N. Moriya, H. Sugino, A. Kakinuma, Isolation and Characterization of Streptoverticillium Anticoagulant (SAC), a Novel Protein Inhibitor of Blood Coagulation Produced by Streptoverticillium cinnamoneum subsp. cinnamoneum, J. Biochem. 115 (1994) 743–751. https://doi.org/10.1093/oxfordjournals.jbchem.a124405.

[132]   M. Tanabe, K. Kawahara, T. Asano, K. Kato, A. Kakinuma, Primary Structure and Reactive Site of Streptoverticillium Anticoagulant (SAC), a Novel Protein Inhibitor of Blood Coagulation Produced by Streptoverticillium cinnamoneum subsp. cinnamoneum, J. Biochem. 115 (1994) 752–761. https://doi.org/10.1093/oxfordjournals.jbchem.a124406.

[133]   R. Hellinger, C.W. Gruber, Peptide-based protease inhibitors from plants, Drug Discov. Today. 24 (2019) 1877–1889. https://doi.org/10.1016/j.drudis.2019.05.026.

[134]   C.A.M. Sampaio, M.L. V. Oliva, M.U. Sampaio, I.F.C. Batista, N.R. Bueno, A.S. Tanaka, E.A. Auerswald, H. Fritz, Plant serine proteinase inhibitors. Structure and biochemical applications on plasma kallikrein and related enzymes, Immunopharmacology. 32 (1996) 62–66. https://doi.org/10.1016/0162-3109(96)00073-2.

[135]   C.A.M. Sampaio, M.L. V. Oliva, A.S. Tanaka, M.U. Sampaio, Proteinase inhibitors in brazilian leguminosae, Mem. Inst. Oswaldo Cruz. 86 (1991) 207–209.

[136]   M. Oliva, S. Andrade, M. Juliano, R. Sallai, R. Torquato, M. Sampaio, V. Pott, C. Sampaio, Kinetic Characterization of Factor Xa Binding Using a Quenched Fluorescent Substrate Based on the Reactive Site of Factor Xa Inhibitor from Bauhinia ungulata Seeds, Curr. Med. Chem. 10 (2003) 1085–1093. https://doi.org/10.2174/0929867033457548.

[137]   M.L. Vilela Oliva, M.U. Sampaio, Bauhinia Kunitz-type proteinase inhibitors: structural characteristics and biological properties, Biol. Chem. 389 (2008) 1007–1013. https://doi.org/10.1515/BC.2008.119.

[138]   I.F.C. Batista, M.C. Nonato, M.R. Bonfadini, L.M. Beltramini, M.L. V. Oliva, M.U. Sampaio, C.A.M. Sampaio, R.C. Garratt, Preliminary crystallographic studies of EcTI, a serine proteinase inhibitor from Enterolobium contortisiliquum seeds, Acta Crystallogr. Sect. D Biol. Crystallogr.

57 (2001) 602–604. https://doi.org/10.1107/S0907444901001810.

[139] I. Cruz-Silva, A.J. Gozzo, V.A. Nunes, A.K. Carmona, A. Faljoni-Alario, M.L. V. Oliva, M.U. Sampaio, C.A.M. Sampaio, M.S. Araujo, A proteinase inhibitor from Caesalpinia echinata (pau-brasil) seeds for plasma kallikrein, plasmin and factor XIIa, Biol. Chem. 385 (2004) 1083–1086. https://doi.org/10.1515/BC.2004.140.

[140] Y. Hojima, J. V. Pierce, J.J. Pisano, Hageman factor fragment inhibitor in corn seeds: Purification and characterization, Thromb. Res. 20 (1980) 149–162. https://doi.org/10.1016/0049-3848(80)90381-3.

[141] M.J. Swartz, H.L. Mitchell, D.J. Cox, G.R. Reeck, Isolation and Characterization Opaque-2 Corn Seeds, J. Biol. Chem. 252 (1977) 8105–8107.

[142] C.A. Behnke, V.C. Yee, I. Le Trong, L.C. Pedersen, R.E. Stenkamp, S.-S. Kim, G.R. Reeck, D.C. Teller, Structural Determinants of the Bifunctional Corn Hageman Factor Inhibitor: X-ray Crystal Structure at 1.95 Å Resolution, Biochemistry. 37 (1998) 15277–15288. https://doi.org/10.1021/bi9812266.

[143] V.A. Korneeva, M.M. Trubetskov, A. V. Korshunova, S. V. Lushchekina, V.N. Kolyadko, O. V. Sergienko, V.G. Lunin, M.A. Panteleev, F.I. Ataullakhanov, Interactions Outside the Proteinase-binding Loop Contribute Significantly to the Inhibition of Activated Coagulation Factor XII by Its Canonical Inhibitor from Corn, J. Biol. Chem. 289 (2014) 14109–14120. https://doi.org/10.1074/jbc.M114.553735.

[144] V. Baeriswyl, S. Calzavarini, S. Chen, A. Zorzi, L. Bologna, A. Angelillo-Scherrer, C. Heinis, A Synthetic Factor XIIa Inhibitor Blocks Selectively Intrinsic Coagulation Initiation, ACS Chem. Biol. 10 (2015) 1861–1870. https://doi.org/10.1021/acschembio.5b00103.

[145] W.C. Mahoney, M.A. Hermodson, B. Jones, D.D. Powers, R.S. Corfman, G.R. Reeck, Amino acid sequence and secondary structural analysis of the corn inhibitor of trypsin and activated Hageman factor, J. Biol. Chem. 259 (1984) 8412–8416.

[146] G.L. Chong, G.R. Reeck, Interaction of trypsin, β-factor XIIa, and plasma kallikrein with a trypsin inhibitor isolated from barley seeds: A comparison with the corn inhibitor of activated hageman factor, Thromb. Res. 48 (1987) 211–221. https://doi.org/10.1016/0049-3848(87)90418-X.

[147] K. Hayashi, T. Takehisa, N. Hamato, R. Takano, S. Hara, T. Miyata, H. Kato, Inhibition of Serine Proteases of the Blood Coagulation System by Squash Family Protease Inhibitors, J. Biochem. 116 (1994) 1013–1018. https://doi.org/10.1093/oxfordjournals.jbchem.a124621.

[148] W. Bode, H.J. Greyling, R. Huber, J. Otlewski, T. Wilusz, The refined 2.0 Å X-ray crystal structure of the complex formed between bovine β-trypsin and CMTI-I, a trypsin inhibitor from squash seeds (Cucurbita maxima), FEBS Lett. 242 (1989) 285–292. https://doi.org/10.1016/0014-5793(89)80486-7.

[149] J.-F. Hernandez, J. Gagnon, L. Chiche, T.M. Nguyen, J.-P. Andrieu, A. Heitz, T. Trinh Hong, T.T.C. Pham, D. Le Nguyen, Squash Trypsin Inhibitors from Momordica cochinchinensis Exhibit an Atypical Macrocyclic Structure, Biochemistry. 39 (2000) 5722–5730. https://doi.org/10.1021/bi9929756.

[150] M.E. Felizmenio-Quimio, N.L. Daly, D.J. Craik, Circular Proteins in Plants, J. Biol. Chem. 276 (2001) 22875–22882. https://doi.org/10.1074/jbc.M101666200.

[151] K. Gray, S. Elghadban, P. Thongyoo, K.A. Owen, R. Szabo, T.H. Bugge, E.W. Tate, R.J. Leatherbarrow, V. Ellis, Potent and specific inhibition of the biological activity of the type-II transmembrane serine protease matriptase by the cyclic microprotein MCoTI-II, Thromb. Haemost. 112 (2014) 402–411. https://doi.org/10.1160/TH13-11-0895.

[152] T. Mahatmanto, Review seed biopharmaceutical cyclic peptides: From discovery to applications, Biopolymers. 104 (2015) 804–814. https://doi.org/10.1002/bip.22741.

[153] P. Thongyoo, C. Bonomelli, R.J. Leatherbarrow, E.W. Tate, Potent Inhibitors of β-Tryptase and Human Leukocyte Elastase Based on the MCoTI-II Scaffold, J. Med. Chem. 52 (2009) 6197–

6200. https://doi.org/10.1021/jm901233u.

[154] J.E. Swedberg, H.A. Ghani, J.M. Harris, S.J. de Veer, D.J. Craik, Potent, Selective, and Cell-Penetrating Inhibitors of Kallikrein-Related Peptidase 4 Based on the Cyclic Peptide MCoTI-II, ACS Med. Chem. Lett. 9 (2018) 1258–1262. https://doi.org/10.1021/acsmedchemlett.8b00422.

[155] J.E. Swedberg, T. Mahatmanto, H. Abdul Ghani, S.J. de Veer, C.I. Schroeder, J.M. Harris, D.J. Craik, Substrate-Guided Design of Selective FXIIa Inhibitors Based on the Plant-Derived Momordica cochinchinensis Trypsin Inhibitor-II (MCoTI-II) Scaffold, J. Med. Chem. 59 (2016) 7287–7292. https://doi.org/10.1021/acs.jmedchem.6b00557.

[156] D.N. Gosalia, W.S. Denney, C.M. Salisbury, J.A. Ellman, S.L. Diamond, Functional phenotyping of human plasma using a 361-fluorogenic substrate biosensing microarray, Biotechnol. Bioeng. 94 (2006) 1099–1110. https://doi.org/10.1002/bit.20927.

[157] D.N. Gosalia, C.M. Salisbury, J.A. Ellman, S.L. Diamond, High Throughput Substrate Specificity Profiling of Serine and Cysteine Proteases Using Solution-phase Fluorogenic Peptide Microarrays, Mol. Cell. Proteomics. 4 (2005) 626–636. https://doi.org/10.1074/mcp.M500004-MCP200.

[158] S.J. de Veer, C.K. Wang, J.M. Harris, D.J. Craik, J.E. Swedberg, Improving the Selectivity of Engineered Protease Inhibitors: Optimizing the P2 Prime Residue Using a Versatile Cyclic Peptide Library, J. Med. Chem. 58 (2015) 8257–8268. https://doi.org/10.1021/acs.jmedchem.5b01148.

[159] R. Krishnamoorthi, Y. Gong, M. Richardson, A new protein inhibitor of trypsin and activated hageman factor from pumpkin (Cucurbita maxima) seeds, FEBS Lett. 273 (1990) 163–167. https://doi.org/10.1016/0014-5793(90)81075-Y.

[160] K. S. Bateman, M. N.G. James, Plant Protein Proteinase Inhibitors: Structure and Mechanism of Inhibition, Curr. Protein Pept. Sci. 12 (2011) 341–347. https://doi.org/10.2174/138920311796391124.

[161] J. Liu, Y. Gong, O. Prakash, L. Wen, I. Lee, J.-K. Huang, R. Krishnamoorthi, NMR studies of internal dynamics of serine proteinase protein inhibitors: Binding region mobilities of intact and reactive-site hydrolyzed Cucurbita maxima trypsin inhibitor (CMTI)-III of the squash family and comparison with those of counterparts of CMTI, Protein Sci. 7 (1998) 132–141. https://doi.org/10.1002/pro.5560070114.

[162] F.M. Grosse-Holz, R.A.L. van der Hoorn, Juggling jobs: roles and mechanisms of multifunctional protease inhibitors in plants, New Phytol. 210 (2016) 794–807. https://doi.org/10.1111/nph.13839.

[163] A.S. Tanaka, M.U. Sampaio, C.A. Sampaio, M.L. Oliva, Purification and preliminary characterization of Torresea cearensis trypsin inhibitor., Brazilian J. Med. Biol. Res. 22 (1989) 1069–1071.

[164] A.S. Tanaka, M.U. Sampaio, R. Mentele, E.A. Auerswald, C.A.M. Sampaio, Sequence of a new Bowman-Birk inhibitor fromTorresea acreana seeds and comparison withTorresea cearensis trypsin inhibitor (TcTI2), J. Protein Chem. 15 (1996) 553–560. https://doi.org/10.1007/BF01908537.

[165] A.S. Tanaka, M.U. Sampaio, S. Marangoni, B. de Oliveira, J.C. Novelle, M.L. V. Oliva, E. Fink, C.A.M. Sampaio, Purification and Primary Structure Determination of a Bowman-Birk Trypsin Inhibitor from Torresea cearensis Seeds, Biol. Chem. 378 (1997) 273–282. https://doi.org/10.1515/bchm.1997.378.3-4.273.

[166] T. Li, T. Ding, J. Li, Medicinal Purposes: Bioactive Metabolites from Marine-derived Organisms, Mini-Reviews Med. Chem. 19 (2018) 138–164. https://doi.org/10.2174/1389557517666170927113143.

[167] P. Shinde, P. Banerjee, A. Mandhare, Marine natural products as source of new drugs: a patent review (2015–2018), Expert Opin. Ther. Pat. 29 (2019) 283–309. https://doi.org/10.1080/13543776.2019.1598972.

[168] A.M.S. Mayer, K.B. Glaser, C. Cuevas, R.S. Jacobs, W. Kem, R.D. Little, J.M. McIntosh, D.J. Newman, B.C. Potts, D.E. Shuster, The odyssey of marine pharmaceuticals: a current pipeline perspective, Trends Pharmacol. Sci. 31 (2010) 255–265. https://doi.org/10.1016/j.tips.2010.02.005.

[169] H. Malve, Exploring the ocean for new drug developments: Marine pharmacology, J. Pharm. Bioallied Sci. 8 (2016) 83. https://doi.org/10.4103/0975-7406.171700.

[170] N. Rajapakse, W. Jung, E. Mendis, S. Moon, S. Kim, A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation, Life Sci. 76 (2005) 2607–2619. https://doi.org/10.1016/j.lfs.2004.12.010.

[171] P. Indumathi, A. Mehta, A novel anticoagulant peptide from the Nori hydrolysate, J. Funct. Foods. 20 (2016) 606–617. https://doi.org/10.1016/j.jff.2015.11.016.

[172] A.A. Syed, K.L. Venkatraman, A. Mehta, An anticoagulant peptide from Porphyra yezoensis inhibits the activity of factor XIIa: In vitro and in silico analysis, J. Mol. Graph. Model. 89 (2019) 225–233. https://doi.org/10.1016/j.jmgm.2019.03.019.

[173] J.H. Lewis, R.M. Iammarino, J.A. Spero, U. Hasiba, Antithrombin Pittsburg : An α1-Antitrypsin Variant Causing Hemorrhagic Disease, Blood. 51 (1978) 129–138.

[174] R. Huber, R.W. Carrell, Implications of the three-dimensional structure of .alpha.1-antitrypsin for structure and function of serpins, Biochemistry. 28 (1989) 8951–8966. https://doi.org/10.1021/bi00449a001.

[175] C.F. Scott, R.W. Carrell, C.B. Glaser, F. Kueppers, J.H. Lewis, R.W. Colman, Alpha-1-antitrypsin-Pittsburgh. A potent inhibitor of human plasma factor XIa, kallikrein, and factor XIIf., J. Clin. Invest. 77 (1986) 631–634. https://doi.org/10.1172/JCI112346.

[176] M. Schapira, M.A. Ramus, S. Jallat, D. Carvallo, M. Courtney, Recombinant alpha 1-antitrypsin Pittsburgh (Met 358----Arg) is a potent inhibitor of plasma kallikrein and activated factor XII fragment., J. Clin. Invest. 77 (1986) 635–637. https://doi.org/10.1172/JCI112347.

[177] S. de Maat, W. Sanrattana, R.K. Mailer, N.M.J. Parr, M. Hessing, R.M. Koetsier, J.C.M. Meijers, G. Pasterkamp, T. Renné, C. Maas, Design and characterization of α1-antitrypsin variants for treatment of contact system–driven thromboinflammation, Blood. 134 (2019) 1658–1669. https://doi.org/10.1182/blood.2019000481.

[178] V. Baeriswyl, S. Calzavarini, C. Gerschheimer, P. Diderich, A. Angelillo-Scherrer, C. Heinis, Development of a Selective Peptide Macrocycle Inhibitor of Coagulation Factor XII toward the Generation of a Safe Antithrombotic Therapy, J. Med. Chem. 56 (2013) 3742–3746. https://doi.org/10.1021/jm400236j.

[179] A. Angelini, L. Cendron, S. Chen, J. Touati, G. Winter, G. Zanotti, C. Heinis, Bicyclic Peptide Inhibitor Reveals Large Contact Interface with a Protease Target, ACS Chem. Biol. 7 (2012) 817–821. https://doi.org/10.1021/cb200478t.

[180] H. van de Langemheen, V. Korotkovs, J. Bijl, C. Wilson, S.S. Kale, C. Heinis, R.M.J. Liskamp, Polar Hinges as Functionalized Conformational Constraints in (Bi)cyclic Peptides, ChemBioChem. 18 (2017) 387–395. https://doi.org/10.1002/cbic.201600612.

[181] J. Wilbs, S.J. Middendorp, C. Heinis, Improving the Binding Affinity of in-Vitro-Evolved Cyclic Peptides by Inserting Atoms into the Macrocycle Backbone, ChemBioChem. 17 (2016) 2299–2303. https://doi.org/10.1002/cbic.201600336.

[182] S.J. Middendorp, J. Wilbs, C. Quarroz, S. Calzavarini, A. Angelillo-Scherrer, C. Heinis, Peptide Macrocycle Inhibitor of Coagulation Factor XII with Subnanomolar Affinity and High Target Selectivity, J. Med. Chem. 60 (2017) 1151–1158. https://doi.org/10.1021/acs.jmedchem.6b01548.

[183] A. Zorzi, S.J. Middendorp, J. Wilbs, K. Deyle, C. Heinis, Acylated heptapeptide binds albumin with high affinity and application as tag furnishes long-acting peptides, Nat. Commun. 8 (2017) 16092. https://doi.org/10.1038/ncomms16092.

[184]   H. Philippou, R. Foster, C. Fishwick, C. Revill, I. Yule, R. Taylor, A. Naylor, P.S. Fallon, S. Crosby, A. Hopkins, M.R. Stewart, N.L. Winfield, FACTOR XIIa INHIBITORS, WO/2019/211585, 2019.

[185]   C. Bouckaert, S. Serra, G. Rondelet, E. Dolušić, J. Wouters, J.-M. Dogné, R. Frédérick, L. Pochet, Synthesis, evaluation and structure-activity relationship of new 3-carboxamide coumarins as FXIIa inhibitors, Eur. J. Med. Chem. 110 (2016) 181–194. https://doi.org/10.1016/j.ejmech.2016.01.023.

[186]   C. Bouckaert, S. Zhu, J.W.P. Govers-Riemslag, M. Depoorter, S.L. Diamond, L. Pochet, Discovery and assessment of water soluble coumarins as inhibitors of the coagulation contact pathway, Thromb. Res. 157 (2017) 126–133. https://doi.org/10.1016/j.thromres.2017.07.015.

[187]   U. Sinha, P. Ku, J. Malinowski, B.Y. Zhu, R.M. Scarborough, C.K. Marlowe, P.W. Wong, P.H. Lin, S.J. Hollenbach, Antithrombotic and hemostatic capacity of factor Xa versus thrombin inhibitors in models of venous and arteriovenous thrombosis, Eur. J. Pharmacol. 395 (2000) 51–59. https://doi.org/10.1016/S0014-2999(00)00219-3.

[188]   A. Stürzebecher, D. Dönnecke, A. Schweinitz, O. Schuster, P. Steinmetzer, U. Stürzebecher, J. Kotthaus, B. Clement, J. Stürzebecher, T. Steinmetzer, Highly Potent and Selective Substrate Analogue Factor Xa Inhibitors ContainingD-Homophenylalanine Analogues as P3 Residue: Part 2, ChemMedChem. 2 (2007) 1043–1053. https://doi.org/10.1002/cmdc.200700031.

[189]   E.-P. Pâques, J. Römisch, Comparative study on the in vitro effectiveness of antithrombotic agents, Thromb. Res. 64 (1991) 11–21. https://doi.org/10.1016/0049-3848(91)90201-7.

[190]   Y. Hitomi, N. Ikari, S. Fujii, Inhibitory Effect of a New Synthetic Protease Inhibitor (FUT-175) on the Coagulation System, Haemostasis. 15 (1985) 164–168. https://doi.org/10.1159/000215139.

[191]   G. TANS, T. JANSSEN-CLAESSEN, J. ROSING, J.H. GRIFFIN, Studies on the effect of serine protease inhibitors on activated contact factors. Application in amidolytic assays for factor XIIa, plasma kallikrein and factor XIa, Eur. J. Biochem. 164 (1987) 637–642. https://doi.org/10.1111/j.1432-1033.1987.tb11174.x.

[192]   K. NAKAMURA, A. JOHMURA, M. ODA, Y. INO, H. UCHIYAMA, H. OHTANI, H. MIYAZAKI, M. KURUMI, Y. AKIZAWA, T. OKA, Inhibitory Effects of Sepimostat Mesilate (FUT-187) on the Activities of Trypsin-like Serine Proteases in Vitro, YAKUGAKU ZASSHI. 115 (1995) 201–212. https://doi.org/10.1248/yakushi1947.115.3_201.

[193]   T. NAKAYAMA, S. TAIRA, M. IKEDA, H. ASHIZAWA, M. ODA, K. ARAKAWA, S. FUJII, Synthesis and Structure-Activity Study of Protease Inhibitors. V. Chemical Modification of 6-Amidino-2-naphthyl 4-Guanidinobenzoate., Chem. Pharm. Bull. (Tokyo). 41 (1993) 117–125. https://doi.org/10.1248/cpb.41.117.

[194]   M. Silverberg, A.P. Kaplan, Enzymatic activities of activated and zymogen forms of human Hageman factor (factor XII)., Blood. 60 (1982) 64–70.

[195]   S. Robert, C. Bertolla, B. Masereel, J.-M. Dogné, L. Pochet, Novel 3-Carboxamide-coumarins as Potent and Selective FXIIa Inhibitors, J. Med. Chem. 51 (2008) 3077–3080. https://doi.org/10.1021/jm8002697.

[196]   C. Doucet, L. Pochet, N. Thierry, B. Pirotte, J. Delarge, M. Reboud-Ravaux, 6-Substituted 2-Oxo-2 H -1-benzopyran-3-carboxylic Acid as a Core Structure for Specific Inhibitors of Human Leukocyte Elastase †, J. Med. Chem. 42 (1999) 4161–4171. https://doi.org/10.1021/jm990070k.

[197]   L. Pochet, C. Doucet, G. Dive, J. Wouters, B. Masereel, M. Reboud-Ravaux, B. Pirotte, Coumarinic derivatives as mechanism-based inhibitors of α-chymotrypsin and human leukocyte elastase, Bioorg. Med. Chem. 8 (2000) 1489–1501. https://doi.org/10.1016/S0968-0896(00)00071-7.

[198]   L. Pochet, C. Doucet, M. Schynts, N. Thierry, N. Boggetto, B. Pirotte, K.Y. Jiang, B. Masereel, P. de Tullio, J. Delarge, M. Reboud-Ravaux, Esters and Amides of 6-(Chloromethyl)-2-oxo-2 H -1-benzopyran-3-carboxylic Acid as Inhibitors of α-Chymotrypsin: Significance of the "Aromatic" Nature of the Novel Ester-Type Coumarin for Strong Inhibitory Activity, J. Med. Chem. 39

(1996) 2579–2585. https://doi.org/10.1021/jm960090b.

[199] L. Pochet, R. Frederick, B. Masereel, Coumarin and Isocoumarin as Serine Protease Inhibitors, Curr. Pharm. Des. 10 (2004) 3781–3796. https://doi.org/10.2174/1381612043382684.

[200] R. Frédérick, C. Charlier, S. Robert, J. Wouters, B. Masereel, L. Pochet, Investigation of mechanism-based thrombin inhibitors: Implications of a highly conserved water molecule for the binding of coumarins within the S pocket, Bioorganic Med. Chem. Lett. 16 (2006) 2017–2021. https://doi.org/10.1016/j.bmcl.2005.12.070.

[201] R. Frédérick, S. Robert, C. Charlier, J. de Ruyck, J. Wouters, B. Pirotte, B. Masereel, L. Pochet, 3,6-Disubstituted Coumarins as Mechanism-Based Inhibitors of Thrombin and Factor Xa, J. Med. Chem. 48 (2005) 7592–7603. https://doi.org/10.1021/jm050448g.

[202] R. Frédérick, S. Robert, C. Charlier, J. Wouters, B. Masereel, L. Pochet, Mechanism-based thrombin inhibitors: Design, synthesis, and molecular docking of a new selective 2-oxo-2H-1-benzopyran derivative, J. Med. Chem. 50 (2007) 3645–3650. https://doi.org/10.1021/jm061368v.

[203] P. Kraft, T. Schwarz, L. Pochet, G. Stoll, C. Kleinschnitz, COU254, a specific 3-carboxamide-coumarin inhibitor of coagulation factor XII, does not protect mice from acute ischemic stroke, Exp. Transl. Stroke Med. 2 (2010) 5. https://doi.org/10.1186/2040-7378-2-5.

[204] J.J.F. Chen, D.P. Visco, Identifying novel factor XIIa inhibitors with PCA-GA-SVM developed vHTS models, Eur. J. Med. Chem. 140 (2017) 31–41. https://doi.org/10.1016/j.ejmech.2017.08.056.

[205] B.-Y. Zhu, W. Huang, T. Su, C. Marlowe, U. Sinha, S. Hollenbach, R. Scarborough, Discovery of Transition State Factor Xa Inhibitors as Potential Anticoagulant Agents, Curr. Top. Med. Chem. 1 (2001) 101–119. https://doi.org/10.2174/1568026013395425.

[206] J.B.M. Rewinkel, A.E.P. Adang, Towards the Ideal Orally Active Thrombin Inhibitor, Curr. Pharm. Des. 5 (1999) 1043–1075.

[207] C. Kleinschnitz, G. Stoll, M. Bendszus, K. Schuh, H.-U. Pauer, P. Burfeind, C. Renné, D. Gailani, B. Nieswandt, T. Renné, Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis, J. Exp. Med. 203 (2006) 513–518. https://doi.org/10.1084/jem.20052458.

[208] C. Kettner, E. Shaw, Inactivation of Trypsin-Like Enzymes with Peptides of Arginine Chloromethyl Ketone, Methods Enzymol. 80 (1981) 826–842. https://doi.org/10.1016/S0076-6879(81)80065-1.

[209] M. MURAMATU, T. ONISHI, S. MAKINO, S. FUJII, Y. YAMAMURA, Inhibition of Caseinolytic Activity of Plasmin by Various Synthetic Inhibitors, J. Biochem. 57 (1965) 402–406. https://doi.org/10.1093/oxfordjournals.jbchem.a128094.

[210] M. MURAMATU, T. ONISHI, S. MAKINO, S. FUJII, Y. YAMAMURA, Inhibition of Fibrinolytic Activity of Plasmin by Various Synthetic Inhibitors, J. Biochem. 57 (1965) 450–453. https://doi.org/10.1093/oxfordjournals.jbchem.a128100.

[211] M. MURAMATU, S. FUJII, Inhibitory Effects of ω-Guanidino Acid Esters on Trypsin, Plasmin, Thrombin and Plasma Kallikrein, J. Biochem. 64 (1968) 807–814. https://doi.org/10.1093/oxfordjournals.jbchem.a128963.

[212] M. Muramatu, S. Fujii, Inhibitory effects of ω-amino acid esters on trypsin, plasmin, plasma kallikrein and thrombin, Biochim. Biophys. Acta - Enzymol. 242 (1971) 203–208. https://doi.org/10.1016/0005-2744(71)90100-8.

[213] M. Muramatu, S. Fujii, Inhibitory effects of ω-guanidino acid esters on trypsin, plasmin, plasma kallikrein and thrombin, Biochim. Biophys. Acta - Enzymol. 268 (1972) 221–224. https://doi.org/10.1016/0005-2744(72)90218-5.

[214] M. Mares-Guia, E. Shaw, The specific inactivation of trypsin by ethyl p-guanidinobenzoate., J. Biol. Chem. 242 (1967) 5782–8.

[215] T. Chase, E. Shaw, Comparison of the Esterase Activities of Trypsin, Plasmin, and Thrombin on Guanidinobenzoate Esters. Titration of the Enzymes, Biochemistry. 8 (1969) 2212–2224. https://doi.org/10.1021/bi00833a063.

[216] Y. Tamura, M. Hirado, K. Okamura, Y. Minato, S. Fujii, Synthetic inhibitors of trypsin, plasmin, kallikrein, thrombin, C1r, and C1 esterase, BBA - Enzymol. 484 (1977) 417–422. https://doi.org/10.1016/0005-2744(77)90097-3.

[217] S. Fujii, Y. Hitomi, New Synthetic Inhibitors of Clr, Clesterase, Thrombin, Plasmin, Kallikrein and Trypsin, Japanese J. Clin. Chem. 10 (1981) 248–252. https://doi.org/10.14921/jscc1971b.10.3_248.

[218] C.F. Bennett, E.E. Swayze, RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 259–293. https://doi.org/10.1146/annurev.pharmtox.010909.105654.

[219] S.T. Crooke, J.L. Witztum, C.F. Bennett, B.F. Baker, RNA-Targeted Therapeutics, Cell Metab. 27 (2018) 714–739. https://doi.org/10.1016/j.cmet.2018.03.004.

[220] B.F. Tillman, A. Gruber, O.J.T. McCarty, D. Gailani, Plasma contact factors as therapeutic targets, Blood Rev. 32 (2018) 433–448. https://doi.org/10.1016/j.blre.2018.04.001.

[221] J.K. Watts, The Medicinal Chemistry of Antisense Oligonucleotides, in: N. Ferrari, R. Seguin (Eds.), Oligonucleotide-Based Drugs Ther., First Edit, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2018: pp. 39–90. https://doi.org/10.1002/9781119070153.ch2.

[222] A. Khvorova, J.K. Watts, The chemical evolution of oligonucleotide therapies of clinical utility, Nat. Biotechnol. 35 (2017) 238–248. https://doi.org/10.1038/nbt.3765.

[223] C.I.E. Smith, R. Zain, Therapeutic Oligonucleotides: State of the Art, Annu. Rev. Pharmacol. Toxicol. 59 (2019) 605–630. https://doi.org/10.1146/annurev-pharmtox-010818-021050.

[224] P.H. Hagedorn, B.R. Hansen, T. Koch, M. Lindow, Managing the sequence-specificity of antisense oligonucleotides in drug discovery, Nucleic Acids Res. 45 (2017) 2262–2282. https://doi.org/10.1093/nar/gkx056.

[225] A.S. Revenko, D. Gao, J.R. Crosby, G. Bhattacharjee, C. Zhao, C. May, D. Gailani, B.P. Monia, A.R. MacLeod, Selective depletion of plasma prekallikrein or coagulation factor XII inhibits thrombosis in mice without increased risk of bleeding, Blood. 118 (2011) 5302–5311. https://doi.org/10.1182/blood-2011-05-355248.

[226] G. Bhattacharjee, A.S. Revenko, J.R. Crosby, C. May, D. Gao, C. Zhao, B.P. Monia, A.R. MacLeod, Inhibition of Vascular Permeability by Antisense-Mediated Inhibition of Plasma Kallikrein and Coagulation Factor 12, Nucleic Acid Ther. 23 (2013) 175–187. https://doi.org/10.1089/nat.2013.0417.

[227] T.T. Vu, J. Zhou, B.A. Leslie, A.R. Stafford, J.C. Fredenburgh, R. Ni, S. Qiao, N. Vaezzadeh, W. Jahnen-Dechent, B.P. Monia, P.L. Gross, J.I. Weitz, Arterial thrombosis is accelerated in mice deficient in histidine-rich glycoprotein, Blood. 125 (2015) 2712–2719. https://doi.org/10.1182/blood-2014-11-611319.

[228] J.W. Yau, P. Liao, J.C. Fredenburgh, A.R. Stafford, A.S. Revenko, B.P. Monia, J.I. Weitz, Selective depletion of factor XI or factor XII with antisense oligonucleotides attenuates catheter thrombosis in rabbits, Blood. 123 (2014) 2102–2107. https://doi.org/10.1182/blood-2013-12-540872.

[229] M. Egli, P.S. Pallan, Crystallographic studies of chemically modified nucleic acids: A backward glance, Chem. Biodivers. 7 (2010) 60–89. https://doi.org/10.1002/cbdv.200900177.

[230] T.Q. Cai, W. Wu, M.K. Shin, Y. Xu, N. Jochnowitz, Y. Zhou, L. Hoos, R. Bentley, W. Strapps, A. Thankappan, J.M. Metzger, M.L. Ogletree, M. Tadin-Strapps, D.A. Seiffert, Z. Chen, Factor XII full and partial null in rat confers robust antithrombotic efficacy with no bleeding, Blood Coagul. Fibrinolysis. 26 (2015) 893–902. https://doi.org/10.1097/MBC.0000000000000337.

[231] M. Heestermans, A. de Jong, S. van Tilburg, P.H. Reitsma, H.H. Versteeg, H.M. Spronk,

B.J.M. van Vlijmen, Use of "C9/11 Mismatch" Control siRNA Reveals Sequence-Related Off-Target Effect on Coagulation of an siRNA Targeting Mouse Coagulation Factor XII, Nucleic Acid Ther. 29 (2019) 218–223. https://doi.org/10.1089/nat.2018.0767.

[232]   M. Heestermans, S. Salloum-Asfar, D. Salvatori, E.H. Laghmani, B.M. Luken, S.S. Zeerleder, H.M.H. Spronk, S.J. Korporaal, G.T.M. Wagenaar, P.H. Reitsma, B.J.M. van Vlijmen, Retraction note: role of platelets, neutrophils, and factor XII in spontaneous venous thrombosis in mice, Blood. 131 (2018) 2996. https://doi.org/10.1182/blood-2018-05-854596.

[233]   Z. Li, RNAi Subcutaneous Delivery Platform Development and ARO-F12, in: Asia TIDES, Kyoto, 2017.

[234]   Y. Huang, Preclinical and Clinical Advances of GalNAc-Decorated Nucleic Acid Therapeutics, Mol. Ther. - Nucleic Acids. 6 (2017) 116–132. https://doi.org/10.1016/j.omtn.2016.12.003.

[235]   S. Melquist, D. Wakefield, H. Hamilton, Q. Chu, A. Almeida, L. Almeida, M. Walters, J. Montez, J. Hegge, J. Klein, C. Hazlett, T. Milarch, S. Bertin, A. Andersen, E. Doss, R. Schmidt, L. Goth, S. Ferger, D. Rozema, J. Hamilton, D. Lewis, S. Kanner, Targeting Factor 12 (F12) with a Novel RNAi Delivery Platform As a Prophylactic Treatment for Hereditary Angioedema (HAE), J. Allergy Clin. Immunol. 137 (2016) AB251. https://doi.org/10.1016/j.jaci.2015.12.905.

[236]   A.M. Turner, J. Stolk, R. Bals, J.D. Lickliter, J. Hamilton, D.R. Christianson, B.D. Given, J.G. Burdon, R. Loomba, J.K. Stoller, J.H. Teckman, Hepatic-targeted RNA interference provides robust and persistent knockdown of alpha-1 antitrypsin levels in ZZ patients., J. Hepatol. 69 (2018) 378–384. https://doi.org/10.1016/j.jhep.2018.03.012.

[237]   S. Melquist, T. Pei, H. Hamilton, Q. Chu, C. Schienebeck, C. Chapman, C. Hagen, M. Walters, T. Milarch, Z. Trilling, A. Andersen, G. Christensen, J. Casper, C. Klas, J. Hegge, Z. Li, S. Kanner, Factor XII RNAi-based therapeutic as a prophylactic anti-thrombotic therapy, Arterioscler. Thromb. Vasc. Biol. 37 (2017).

[238]   Arrowhead Pharmaceuticals, Pipeline - Arrowhead Pharmaceuticals, Inc., (2019). https://arrowheadpharma.com/pipeline/ (accessed July 3, 2020).

[239]   J. Liu, J. Qin, B.C. Cooley, A. Akinc, J. Butler, J. Qin, Reduction of Hepatic Factor XII Expression in Mice by ALN-F12 Inhibits Thrombosis without Increasing Bleeding Risk, in: ISTH, 2017.

[240]   D.J. Foster, C.R. Brown, S. Shaikh, C. Trapp, M.K. Schlegel, K. Qian, A. Sehgal, K.G. Rajeev, V. Jadhav, M. Manoharan, S. Kuchimanchi, M.A. Maier, S. Milstein, Advanced siRNA Designs Further Improve In Vivo Performance of GalNAc-siRNA Conjugates, Mol. Ther. 26 (2018) 708–717. https://doi.org/10.1016/j.ymthe.2017.12.021.

[241]   G. Mayer, The chemical biology of aptamers, Angew. Chemie - Int. Ed. 48 (2009) 2672–2689. https://doi.org/10.1002/anie.200804643.

[242]   C. Wilson, A.D. Keefe, Building oligonucleotide therapeutics using non-natural chemistries, Curr. Opin. Chem. Biol. 10 (2006) 607–614. https://doi.org/10.1016/j.cbpa.2006.10.001.

[243]   R.S. Woodruff, Y. Xu, J. Layzer, W. Wu, M.L. Ogletree, B.A. Sullenger, Inhibiting the intrinsic pathway of coagulation with a factor XII-targeting RNA aptamer, J. Thromb. Haemost. 11 (2013) 1364–1373. https://doi.org/10.1111/jth.12302.

[244]   E. Dausse, S. Da Rocha Gomes, J.J. Toulmé, Aptamers: a new class of oligonucleotides in the drug discovery pipeline?, Curr. Opin. Pharmacol. 9 (2009) 602–607. https://doi.org/10.1016/j.coph.2009.07.006.

[245]   P. Sundaram, H. Kurniawan, M.E. Byrne, J. Wower, Therapeutic RNA aptamers in clinical trials, Eur. J. Pharm. Sci. 48 (2013) 259–271. https://doi.org/10.1016/j.ejps.2012.10.014.

[246]   M. Blind, M. Blank, Aptamer Selection Technology and Recent Advances, Mol. Ther. - Nucleic Acids. 4 (2015) e223. https://doi.org/10.1038/mtna.2014.74.

[247]   K.D. Kovacevic, J.C. Gilbert, B. Jilma, Pharmacokinetics, pharmacodynamics and safety of aptamers, Adv. Drug Deliv. Rev. 134 (2018) 36–50. https://doi.org/10.1016/j.addr.2018.10.008.

[248] J.K.H. Liu, The history of monoclonal antibody development - Progress, remaining challenges and future innovations, Ann. Med. Surg. 3 (2014) 113–116. https://doi.org/10.1016/j.amsu.2014.09.001.

[249] F. Breedveld, Therapeutic monoclonal antibodies, Lancet. 355 (2000) 735–740. https://doi.org/10.1016/S0140-6736(00)01034-5.

[250] S.R. Samoilovich, C.B. Dugan, A.J.L. Macario, Hybridoma technology: new developments of practical interest, J. Immunol. Methods. 101 (1987) 153–170. https://doi.org/10.1016/0022-1759(87)90147-5.

[251] R.A. Pixley, L.G. Stumpo, K. Birkmeyer, L. Silver, R.W. Colman, A monoclonal antibody recognizing an icosapeptide sequence in the heavy chain of human factor XII inhibits surface-catalyzed activation, J. Biol. Chem. 262 (1987) 10140–10145.

[252] E.J. Small, J.A. Katzmann, R.P. Tracy, O.D. Ratnoff, G.J. Goldsmith, B. Everson, A monoclonal antibody that inhibits activation of human Hageman factor (factor XII), Blood. 65 (1985) 202–210.

[253] H. Saito, T. Ishihara, H. Suzuki, T. Watanabe, Production and characterization of a murine monoclonal antibody against a heavy chain of Hageman factor (factor XII), Blood. 65 (1985) 1263–1268.

[254] J.H. Nuijens, C.C.M. Hijbregts, A.J.M. Eerenberg-Belmer, J.C.M. Meijers, B.N. Bouma, C.E. Hack, Activation of the contact system of coagulation by a monoclonal antibody directed against a neodeterminant in the heavy chain region of human coagulation factor XII (Hageman factor), J. Biol. Chem. 264 (1989) 12941–12949.

[255] J.H. Nuijens, C. Huijbregts, C.E. Hack, Inhibitors of factor XII activation and applications thereof, WO9117258, 1991.

[256] D.M. Dors, J.H. Nuijens, C.C.M. Huijbregts, C.E. Hack, A Novel Sensitive Assay for Functional Factor XII Based on the Generation of Kallikrein-C1-Inhibitor Complexes in Factor XII-Deficient Plasma by Glass-Bound Factor XII, Thromb. Haemost. 67 (1992) 644–648. https://doi.org/10.1055/s-0038-1648516.

[257] R.A. Pixley, Monoclonal antibodies to the light chain region of human factor XII and methods of preparing and using the same, WO8911865, 1989.

[258] A. Matafonov, A.E. Gailani, S.L. Grach, P.Y. Leung, Q. Cheng, Y. Geng, O.J.T. McCarty, E.I. Tucker, T. Renne, J.H. Morrissey, A. Gruber, D. Gailani, Antibodies to Human Factor XII with Antithrombotic Properties, Blood. 120 (2012) 1106–1106. https://doi.org/10.1182/blood.V120.21.1106.1106.

[259] M.L. Chiu, G.L. Gilliland, Engineering antibody therapeutics, Curr. Opin. Struct. Biol. 38 (2016) 163–173. https://doi.org/10.1016/j.sbi.2016.07.012.

[260] Y. Kokoye, I. Ivanov, Q. Cheng, A. Matafonov, S.K. Dickeson, S. Mason, D.J. Sexton, T. Renné, K. McCrae, E.P. Feener, D. Gailani, A comparison of the effects of factor XII deficiency and prekallikrein deficiency on thrombus formation, Thromb. Res. 140 (2016) 118–124. https://doi.org/10.1016/j.thromres.2016.02.020.

[261] S.R. Comeau, A. Nixon, N. Kastrapeli, J.A. Kenniston, G.P. Conley, S. Mason, A.P. Lindberg, K. Kopacz, B. Adelman, Bispecific antibodies against plasma kallikrein and factor XII., WO2016109774A1, 2018.

[262] J.A. Kenniston, R.R. Faucette, D. Martik, S.R. Comeau, A.P. Lindberg, K.J. Kopacz, G.P. Conley, J. Chen, M. Viswanathan, N. Kastrapeli, J. Cosic, S. Mason, M. DiLeo, J. Abendroth, P. Kuzmic, R.C. Ladner, T.E. Edwards, C. TenHoor, B.A. Adelman, A.E. Nixon, D.J. Sexton, Inhibition of Plasma Kallikrein by a Highly Specific Active Site Blocking Antibody, J. Biol. Chem. 289 (2014) 23596–23608. https://doi.org/10.1074/jbc.M114.569061.

[263] M. Worm, E.C. Köhler, R. Panda, A. Long, L.M. Butler, E.X. Stavrou, K.F. Nickel, T.A. Fuchs, T. Renné, The factor XIIa blocking antibody 3F7 : a safe anticoagulant with anti-inflammatory activities, Ann. Transl. Med. 3 (2015) 247. https://doi.org/10.3978/j.issn.2305-5839.2015.09.07.