The XII factor

By Amy Donner, Senior Editor

Factor XII sits atop one branch of the clotting cascade but has not been pursued for treating and preventing thrombosis because it was seen as a minor player in blood coagulation. Now, a group from the **Karolinska Institute** and **CSL Ltd.** has shown that inhibiting the factor can prevent clotting without increasing the risk of bleeding,¹ making it one of the only anticoagulants able to accomplish such a feat.

A group from **Isis Pharmaceuticals Inc.** and **McMaster University** obtained similar results with factor XII,² but the biotech maintains that blocking a downstream target, factor XI, is a better approach.

Whether blocking factor XII or factor XI will be a better strategy will probably be determined in the clinic (see Table 1, “Targeting the intrinsic pathway of coagulation and the kallikrein-kinin system”).

Passing over factors

The main reason factor XII was passed over as an anticoagulant stemmed from observational studies of patients who had a deficiency of the enzyme. Those patients had normal hemostatic capacity—the formation of fibrin-based clots—and did not have an increased risk of bleeding from either an injury or a spontaneous event.³

As a result, said Thomas Renné, almost everyone assumed that factor XII was not important for forming clots. Renné, a professor of

clinical chemistry and coagulation research at the Karolinska Institute and **Karolinska University Hospital** as well as a professor of clinical chemistry at the **University Medical Center Hamburg-Eppendorf**, suspected otherwise.

When Renné was a scientist at the **University of Wuerzburg** in 2005, he and his colleagues showed that *factor XII*-deficient mice were protected from thrombosis, which hinted that the enzyme could be a target for antithrombotic therapy.⁴

That work caught the interest of CSL, which set about exploring whether the outcomes in knockout mice could be replicated by pharmacological inhibition of factor XII.

Con Panousis, director of antibody technologies at CSL, said that the company spent several years testing small protein inhibitors of factor XII, but the compounds had off-target effects or were immunogenic.

Now, CSL and Karolinska have developed an antibody against factor XII and have provided the clearest picture to date of what happens when the target is blocked in animals.

The team screened a human Fab-based phage antibody library for compounds that could specifically inhibit the proteolytic activity of factor XIIa, which is the active form of factor XII. The group isolated

“We believe that in addition to central venous catheters, other blood-contacting medical devices, such as vascular grafts, mechanical heart valves and left ventricular assist devices, also trigger clotting. Therefore, the ASOs could be used for long-term prevention of clotting for a wide variety of medical devices.”

—Jeffrey Weitz,
McMaster University

Figure 1. Factor XIIa pathways. The intrinsic pathway of coagulation and the kallikrein-kinin system are both activated downstream of factor XII (FXII).

(I) In the intrinsic pathway, activated FXII (FXIIa) initiates an activation cascade of serine protease enzymes, including factor XIa (FXIa), factor IXa (FIXa), factor Xa (FXa) and thrombin (factor IIa; F2). Thrombin cleaves fibrinogen to form soluble fibrin, which aggregates or polymerizes to form a clot or thrombus.

(II) In the kallikrein-kinin system, FXIIa cleaves high-molecular weight (HMW) kininogen to form bradykinin. Bradykinin is degraded, and the degradation products activate bradykinin B2 receptor (BDKRB2; B2R), leading to edema.

A number of companies are developing inhibitors of the components of these two

systems (see Table 1, “Targeting the intrinsic pathway of coagulation and the kallikrein-kinin system”). Reports in *Science Translational Medicine* and *Blood* provide evidence that FXII and FXIIa can be targeted to prevent clot formation without risk of bleeding.^{1,2}

Table 1. Targeting the intrinsic pathway of coagulation and the kallikrein-kinin system. The intrinsic pathway and the kallikrein-kinin system are both activated by factor XIIa. The table below is a selected list of compounds against components of the pathways that are in clinical development or on the market.

Source: BioCentury Archives

Target	Company	Compound	Status	Indication
Factor XI	Isis Pharmaceuticals Inc. (NASDAQ:ISIS)	ISIS-FXIRx (antisense oligonucleotide)	Phase II	Block coagulation
Factor IXa	Regado Biosciences Inc. (NASDAQ:RGDO)	Pegnivacogin (aptamer)	Phase II	Block coagulation
Factor Xa	Alchemia Ltd. (ASX:ACL); Dr. Reddy's Laboratories Ltd. (NYSE:RDY)	Fondared fondaparinux (small molecule)	Marketed	Deep vein thrombosis (DVT)
	Bayer AG (Xetra:BAYN); Almirall S.A. (Madrid:ALM); Johnson & Johnson (NYSE:JNJ)	Xarelto rivaroxaban (small molecule)	Marketed	DVT; venous thromboembolism (VTE); pulmonary embolism (PE); stroke
			Registration	Acute coronary syndrome (ACS)
	Bristol-Myers Squibb Co. (NYSE:BMJ); Pfizer Inc. (NYSE:PFE)	Eliquis apixaban (small molecule)	Marketed	VTE; stroke
			Registration	DVT
	Daiichi Sankyo Co. Ltd. (Tokyo:4568)	Lixiana edoxaban (small molecule)	Marketed	VTE
			Registration	Atrial fibrillation (AF); stroke; DVT; PE; VTE
	GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	Arixtra fondaparinux (small molecule)	Marketed	DVT
			Approved	ACS; PE; VTE
	Sanofi (Euronext:SAN; NYSE:SNY)	Idraparinux (polysaccharide)	Phase III	DVT; thrombosis with AF
	Portola Pharmaceuticals Inc. (NASDAQ:PTLA)	Betrixaban (small molecule)	Phase III	VTE
			Phase II	Stroke
	TeaRx LLC; Roche (SIX:ROG; OTCQX:RHHBY)	TeaRxaban (unknown)	Phase II	Thrombosis; VTE
	Green Cross Corp. (KSE:006280)	GCC4401C (small molecule)	Phase I	VTE; stroke
Thrombin (factor IIa; F2)	Asahi Kasei Pharma Corp.	Recomodulin (peptide)	Marketed	Block coagulation
	Bayer	Refludan (biologic)	Marketed	Thrombosis; thrombocytopenia
	Boehringer Ingelheim GmbH	Pradax dabigatran (small molecule)	Marketed	VTE
			Registration	Thrombosis
			Phase II	Coronary artery disease (CAD)
	Eagle Pharmaceuticals Inc. (NASDAQ:EGRX); The Medicines Co. (NASDAQ:MDCO)	Ready-to-use argatroban (small molecule)	Marketed	Thrombosis
	The Medicines Co.; AstraZeneca plc (LSE:AZN; NYSE:AZN); Grupo Ferrer Internacional S.A.	Angiomax bivalirudin (small molecule)	Marketed	ACS; angina angioplasty; percutaneous coronary intervention (PCI); thrombosis; thrombocytopenia
	Mitsubishi Tanabe Pharma Corp. (Tokyo:4508); GSK	Arganova argatroban (small molecule)	Marketed	Blood clots; thrombosis; thrombocytopenia
	AstraZeneca	AZD0837 (small molecule)	Phase II	Thrombosis
	Archemix Corp.; Arca biopharma Inc. (NASDAQ:ABIO)	ARC2172 (aptamer)	Phase I	Block coagulation
	Diakron Pharmaceutical Inc.; Orchid Chemicals & Pharmaceuticals Ltd. (BSE:524372; NSE:ORCHIDCHEM)	DP-4088 (small molecule)	Phase I	Thrombosis
Factor Xa; F2	Abbott Laboratories (NYSE:ABT)	Clivarin reviparin (low-molecular weight heparin (LMWH))	Marketed	DVT
	Leo Pharma A/S; Celgene Corp. (NASDAQ:CELG)	Innohep tinzaparin (LMWH)	Marketed	DVT
	Momenta Pharmaceuticals Inc. (NASDAQ:MNTA); Novartis AG (NYSE:NVS; SIX:NOVN)	M-enoxaparin (LMWH)	Marketed	DVT; ACS

(Continues on p. 3)

Table 1. Targeting the intrinsic pathway of coagulation and the kallikrein-kinin system (continued).

Target	Company	Compound	Status	Indication
	Pfizer; Eisai Co. Ltd. (Tokyo:4523)	Fragmin dalteparin (LMWH)	Marketed	CAD; DVT; ischemia/reperfusion injury; VTE
	Sanofi	Klexane R enoxaparin (LMWH)	Marketed	Unstable angina; non-ST segment elevation myocardial infarction (NSTEMI); DVT; myocardial infarction (MI); VTE
	Amphastar Pharmaceuticals Inc.; Actavis plc (NYSE:ACT)	Enoxaparin (LMWH)	Approved	DVT
	Merck & Co. Inc. (NYSE:MRK); Endotis Pharma	EP217609 (unknown)	Phase II	Prevent coagulation during extracorporeal circulation (ECC)
	Momenta Pharmaceuticals	M118 (LMWH)	Phase II	ACS
Fibrin	Genentech Inc. unit of Roche; Boehringer Ingelheim	Metalyse tenecteplase (biologic: enzyme)	Marketed	MI
			Phase III	Catheter clearance
			Phase II	Stroke
	Fibrex Medical Inc.; Ikaria Inc.	FX06MRI (peptide)	Phase II	Ischemia/reperfusion injury
	Grifols S.A. (Madrid:GRF; NASDAQ:GRFS)	Plasmin (biologic: enzyme)	Phase II	Peripheral vascular disease
	BioCryst Pharmaceuticals Inc. (NASDAQ:BCRX)	BCX4161 (small molecule)	Phase II	Hereditary angioedema (HAE)
Kallikrein	Dyax Corp. (NASDAQ:DYAX); Lee's Pharmaceutical Holdings Ltd. (HKSE:0950); Sigma-Tau Group; taiba Pharma LLC	Kalbitor ecallantide (peptide)	Marketed	Angioedema; HAE
	Dyax	DX-2930 (antibody)	Phase I	HAE
Bradykinin B2 receptor (BDKRB2; B2R)	Sanofi; Shire plc (LSE:SHP; NASDAQ:SHPG)	Firazyr icatibant (small molecule)	Marketed	Angioedema; HAE

an antibody, 3F7, which dose-dependently inhibited factor XIIa activity *in vitro* with an IC_{50} of 13 nM.¹

In a mouse model of thrombosis, 3F7 dose-dependently decreased occlusion rates and decreased time to occlusion compared with control antibody or saline. In the same mice, 3F7 neither increased blood loss nor prolonged bleeding.

In a rabbit model of cardiopulmonary bypass, 3F7 provided thromboprotection comparable to that of heparin. However, the antibody did not prolong incision-provoked bleeding.

That finding suggests that 3F7 might prevent thrombosis in acute indications like bypass surgery without increasing the risk of bleeding.

Results were published in *Science Translational Medicine*. Scientists from the **Swedish University of Agricultural Sciences** also contributed to the study.

The take-home message, the authors wrote, is that “FXIIa-driven fibrin formation is essential for pathological thrombus formation and propagation but has no function for fibrin formation during ‘normal’ hemostasis at a site of injury.”

In a related paper, a team from Isis Pharmaceuticals and McMaster University led by Jeffrey Weitz also identified factor XII as an anticoagulation target. The group showed that factor XII-directed antisense oligonucleotides (ASOs) protected against catheter-induced thrombosis in rabbits.²

Similar results were seen with antisense inhibitors of factor XI, which sits downstream of factor XII (see **Figure 1**, “**Factor XIIa**

pathways”) and had previously been shown by Isis to prevent thrombus formation without causing an increase in bleeding in mouse models of stroke.

Weitz is chair of thrombosis and a professor of medicine at McMaster University and executive director of the **Thrombosis and Atherosclerosis Research Institute**.

Results were published in *Blood*.

Factor factory

Renné said that the data on factor XII may change the classical concept of coagulation, which holds that coagulation and bleeding are inextricably bound. Indeed, marketed anticoagulants including warfarin and antiplatelet therapies are used to prevent thrombosis, but they also result in dose-dependent increases in the risk of major bleeding.

Weitz agreed but added that “factor XI may be a better target because thrombin can feed back and activate factor XI, thereby bypassing factor XIIa inhibitors.”

He did say that inhibitors of either target could have utility in a broad spectrum of indications.

“We believe that in addition to central venous catheters, other blood-contacting medical devices, such as vascular grafts, mechanical heart valves and left ventricular assist devices, also trigger clotting. Therefore, the ASOs could be used for long-term prevention of clotting for a wide variety of medical devices.”

Isis' ISIS-FXIRx, a factor XI-targeted ASO, is in Phase II testing to prevent coagulation without increasing the risk of bleeding in patients undergoing knee replacement surgery. Results are expected this year.

At least one other company thinks that it has found a way to provide anticoagulation without increasing bleeding risk. **XO1 Ltd.**'s ichorcumab, a mAb against an allosteric site on thrombin (factor IIa; F2), is in preclinical development.⁵

CSL holds patents and patent applications covering 3F7, an optimized version of the mAb and FXII inhibition in prevention of thrombi formation. The IP is not currently available for licensing for antithrombotic indications. CSL said that it is focused on inhibiting factor XII to treat hereditary angioedema (HAE) and has an antibody against the target in preclinical development for that indication.

Panousis said that factor XII is also involved in bradykinin-induced edema, which is dysregulated in HAE. CSL markets Berinert, an injectable complement 1 (C1) esterase inhibitor derived from human plasma, to treat HAE.

Donner, A. *SciBX* 7(8); doi:10.1038/scibx.2014.217
Published online Feb. 27, 2014

REFERENCES

1. Larrson, M. *et al. Sci. Transl. Med.*; published online Feb. 5, 2014; doi:10.1126/scitranslmed.3006804
Contact: Thomas Renné, Karolinska Institute, Stockholm, Sweden
e-mail: thomas@renne.net
2. Yau, J.W. *et al. Blood*; published online Feb. 5, 2014; doi:10.1182/blood-2013-12-540872
Contact: Jeffrey I. Weitz, Thrombosis and Atherosclerosis Research Institute, Hamilton, Ontario, Canada
e-mail: weitzj@taari.ca
3. Renné, T. *et al. Blood* **120**, 4296–4303 (2012)
4. Renné, T. *et al. J. Exp. Med.* **202**, 271–281 (2005)
5. Hansen, S. *BioCentury* **21**(26), A26; July 1, 2013

COMPANIES AND INSTITUTIONS MENTIONED

CSL Ltd. (ASX:CSL), Melbourne, Victoria, Australia
Isis Pharmaceuticals Inc. (NASDAQ:ISIS), Carlsbad, Calif.
Karolinska Institute, Stockholm, Sweden
Karolinska University Hospital, Stockholm, Sweden
McMaster University, Hamilton, Ontario, Canada
Swedish University of Agricultural Sciences, Uppsala, Sweden
Thrombosis and Atherosclerosis Research Institute, Hamilton, Ontario, Canada
University Medical Center Hamburg-Eppendorf, Hamburg, Germany
University of Wuerzburg, Wuerzburg, Germany
XO1 Ltd., Cambridge, U.K.