Development and characterization of neutralizing monoclonal antibodies targeting the direct oral anticoagulant apixaban.

E. Maurer[1], S. Crosnier[1], C. Baltus[2], MC. Viaud-Massuard[2,3], D. Huskens[4], H. Kelchtermans[4], M. Canton[5], P. Ohlmann[1]

[1]Agro-Bio, R&D Department, 45240 La Ferté Saint Aubin, France; [2]McSAF, UFR des Sciences Pharmaceutiques, University François Rabelais, 37200 Tours, France; [3]UMR 7292 GICC Equipe 4-Innovation Moleculaire et Therapeutique, Labex SYNORG, University of Tours, Faculty of Pharmacy, 37200 Tours, France; [4] Synapse Research Institute, CARIM, 6229 EV Maastricht, the Netherlands; [5]Stago 92600 Asnières, France

Introduction

Direct oral anticoagulants (DOACs) are indicated for thromboembolism prevention and treatment[1]. Dabigatran is a thrombin inhibitor while apixaban, betrixaban, edoxaban and rivaroxaban are factor Xa (FXa) inhibitors[2]. Novel clinical research tools are required to better understand the mechanisms of action of this new generation of therapeutical molecules.

The challenge was to generate high specificity and affinity monoclonal antibodies (MAbs) against a non-immunogen hapten such as the apixaban molecule.

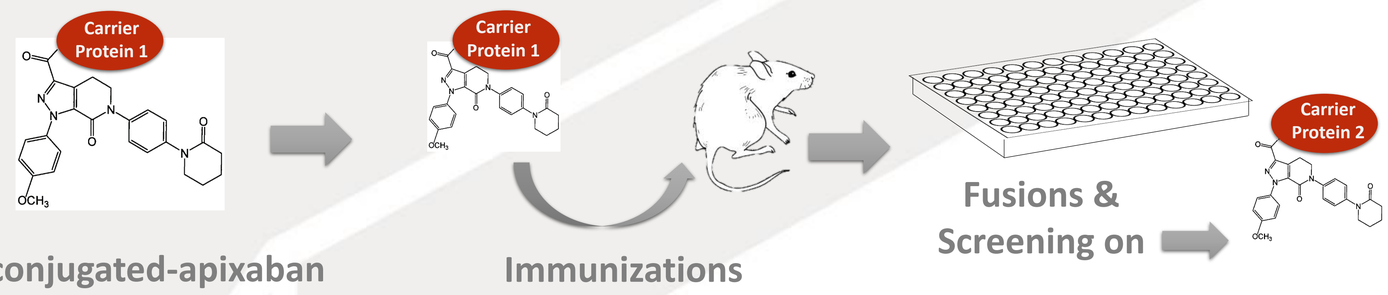
Conclusions

Our work provides evidence that these new MAbs:

- **are the first MAbs able to recognize the apixaban with a high specificity (no cross-reactivity with other FXa inhibitors) and high affinity ($10^{-6}M<K_D<10^{-9}M$).**
- **are able to neutralize the *in vitro* apixaban anticoagulant effect on FXa activity and on thrombin generation assays in apixaban spiked plasma and in plasma from Eliquis™ treated patients.**
- **could be applied to diagnostic procedure and to develop therapeutic recombinant antibody as potential antidote.**

Results

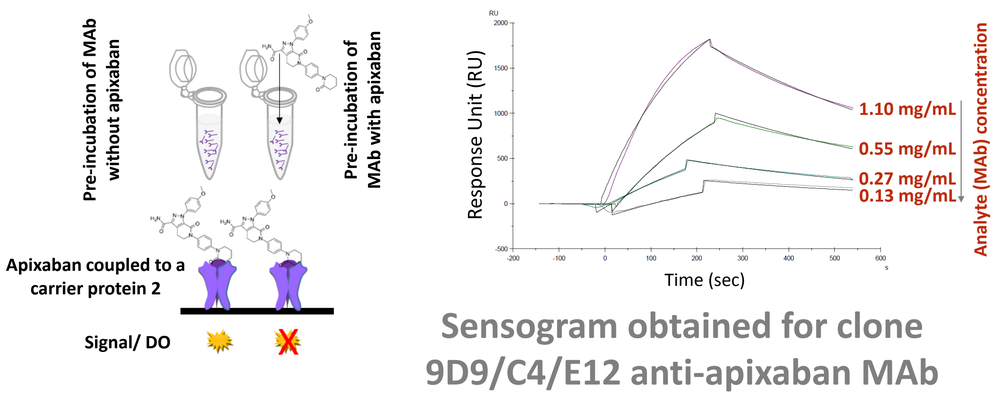
1. Generation of mouse MAbs



Bioconjugated-apixaban — Immunizations

Hybridomas obtained, after fusion of spleen cells with murine myeloma, were screened for their ability to recognize apixaban: 32 clones were obtained of which 9 were characterized.

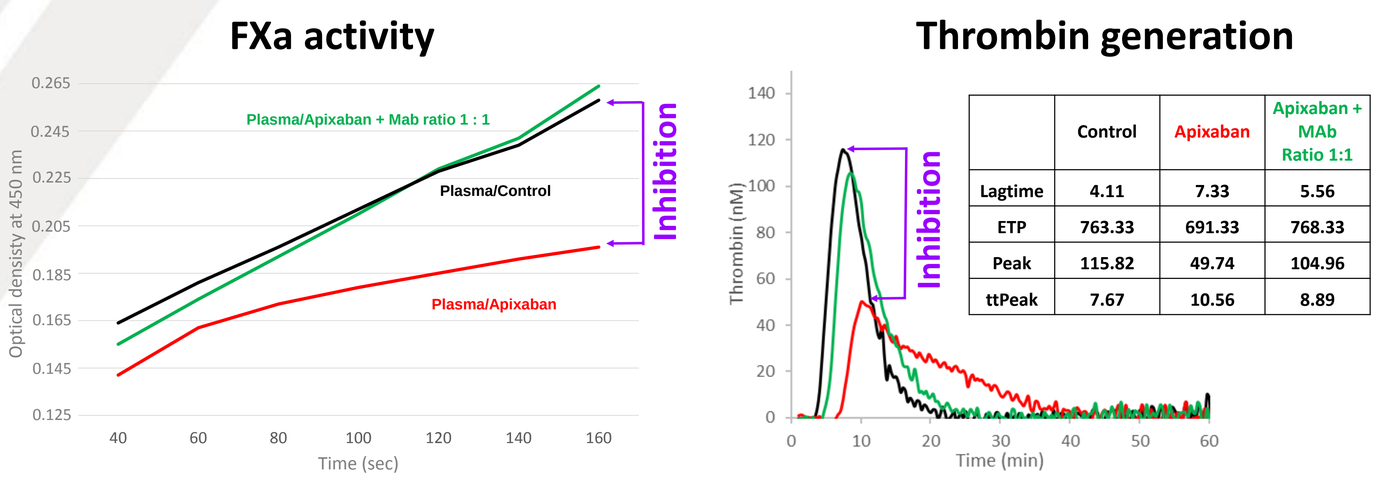
2. Characterization study



Sensogram obtained for clone 9D9/C4/E12 anti-apixaban MAb

MAbs were specific for apixaban, and presented no cross-reactivity with betrixaban, edoxaban or rivaroxaban.

3. Neutralizing ability of anti-apixaban MAbs



	Control	Apixaban	Apixaban + MAb Ratio 1:1
Lagtime	4.11	7.33	5.56
ETP	763.33	691.33	768.33
Peak	115.82	49.74	104.96
ttPeak	7.67	10.56	8.89

Curves of FXa activity or thrombin generation obtained for the clone 9D9/C4/E12 anti-apixaban MAb in PPP samples spiked with apixaban at a final concentration of 100 ng/mL (IC_{50})

4. Summary table of selected clones

Clone Name	Biacore 3000		Secretion rate µg/mL	IgG Isotype	FXa activity	Thrombin generation
	K_D (M)	K_A (M^{-1})			Ratio MAb/Apixaban	
9D9/C4/E12	$2.0\ 10^{-8}$	$5.0\ 10^{7}$	54.7	IgG1 κ	1:1	1:1
3C1/D9	$1.4\ 10^{-7}$	$6.9\ 10^{6}$	69.6	IgG1 κ	1:1	1:1
8C9/A5	$2.0\ 10^{-9}$	$5.0\ 10^{8}$	78.0	IgG1 κ	1:1	1 to 2:1
3C1/A12	$2.7\ 10^{-7}$	$3.8\ 10^{6}$	38.0	IgG1 κ	1:1	1 to 2:1
9D9/F2	$7.9\ 10^{-7}$	$5.0\ 10^{6}$	35.1	IgG1 κ	1:1	1:1
9D9/G2	$8.2\ 10^{-8}$	$1.2\ 10^{7}$	33.1	IgG1 κ	1:1	1:1

6/9 clones anti-apixaban MAbs were able *in vitro* to neutralize the anticoagulant effect of the apixaban in different coagulation tests (FXa activity and thrombin generation) in a MAb / apixaban molecular ratio range between 1:1 and 2:1.

Material and Methods

1. **Mice were immunized with bioconjugated-apixaban to carrier protein 1. Eighteen weeks after immunization spleen cells from mice presenting with the highest specific serum titers were isolated for fusion with murine myeloma cells.**
2. **Specific binding of purified MAbs to apixaban were analyzed in a competition ELISA where antibodies were pre-incubated with apixaban, betrixaban, edoxaban or rivaroxaban respectively before being added to apixaban-carrier protein 2 immobilized on 96 wells plate.**
3. **Isotypes and affinity were also determined, respectively by ELISA and by surface plasmon resonance (SPR) by using a kinetic analysis with a concentration series on a Biacore® 3000.**
4. **FXa activity (STA®-Liquid FXa Stago) and Thrombin Generation (Stago & Synapse BV) were performed in the presence and in the absence of the selected MAbs on human citrated plasmas (PPP) spiked with apixaban (concentration at IC_{50}) or on Eliquis™ treated patient plasmas.**

References

1. Guidance for the practical management of the direct oral anticoagulants (DOACs) in VTE treatment. Burnett A et al., J Thromb Thrombolysis. 2016; 41: 206-232.
2. Pros and cons of new oral anticoagulants. Bauer KA. Hematology Am Soc Hematol Educ Program. 2013; 464-70

Abstract Number: PB 1204
Topic: Management of Thromboembolism
Poster Location: Exhibition Hall 4.2