

Nouvelles approches de ciblage du thrombus en imagerie

François ROUZET

- Nuclear Medicine Dpt, GH Bichat-Claude Bernard, Paris, France
- LVTS (Inserm U1148), Team 4: cardiovascular imaging
- University Paris Diderot, Sorbonne Paris Cité, France

Domaines d'application

Maladie veineuse thrombo-embolique

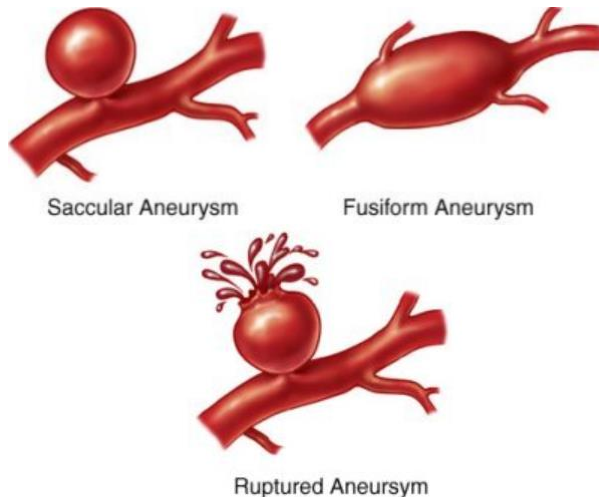
- Bilan d'extension de la maladie thrombo-embolique :
thrombose veineuse + embolie pulmonaire
 - Différencier thrombus frais instable vs. chronique organisé
 - Diagnostic de récurrence / Evaluation du potentiel évolutif
 - Evaluation précoce de l'efficacité thérapeutique
- Passer d'une approche anatomo-fonctionnelle à une approche moléculaire

Domaines d'application

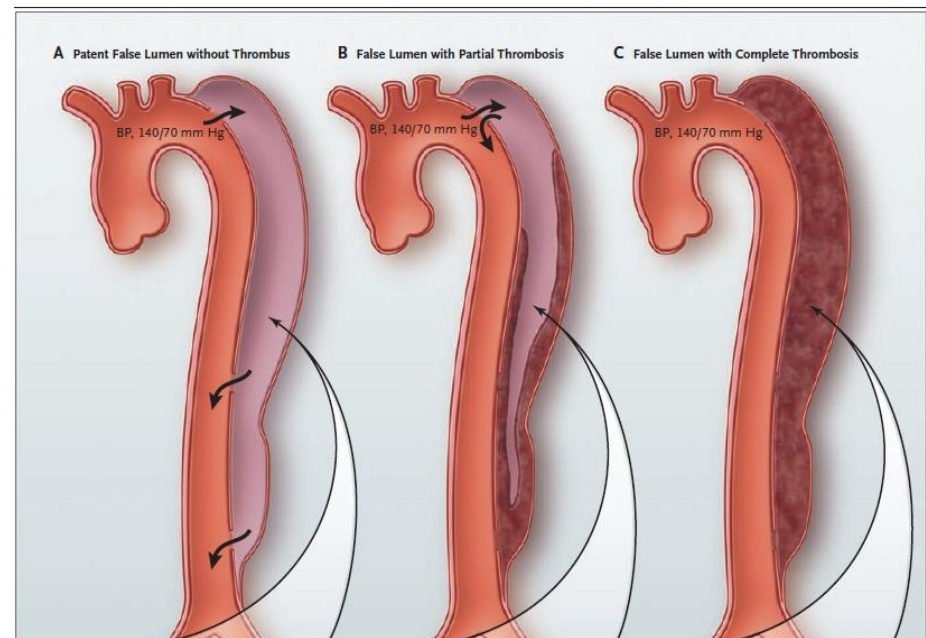
Pathologies dégénératives artérielles

Pathologie neurovasculaire

- Accident ischémique cérébral
 - Origine du thrombus
 - AVC cryptogénique
- Rupture d'anévrisme

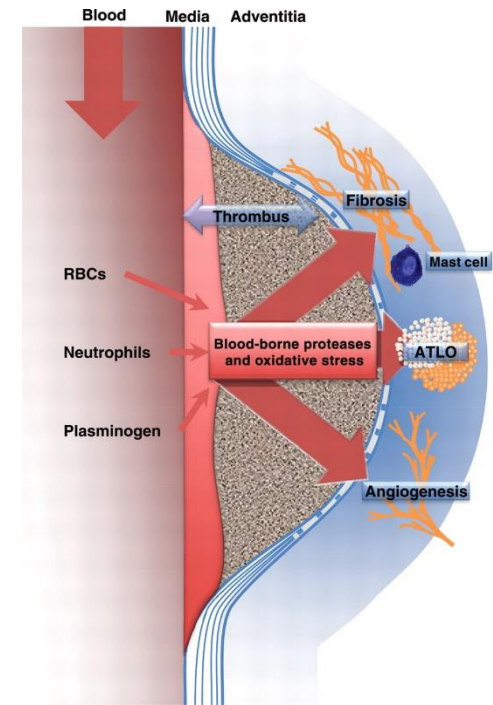
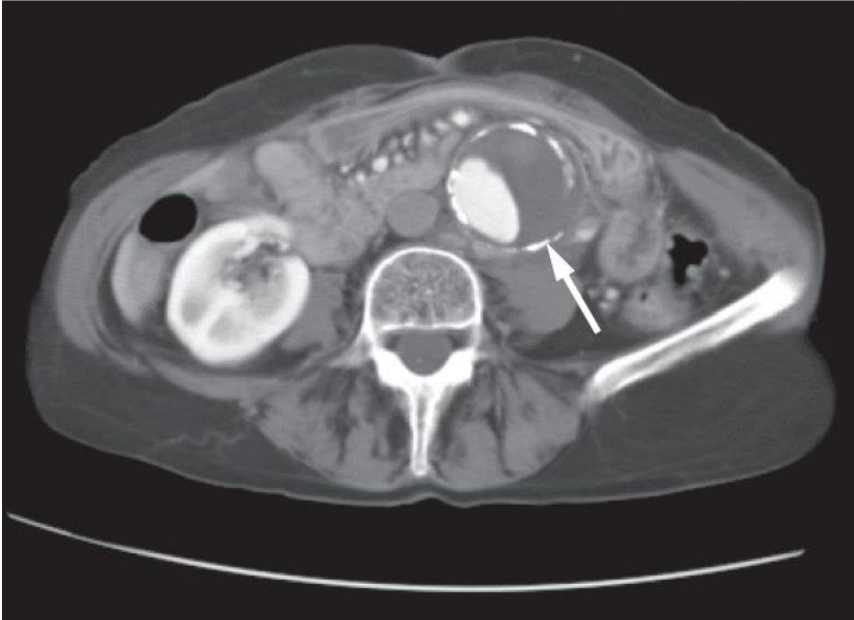


Aorte : anévrismes et dissections



Tsai TT, NEJM 2007

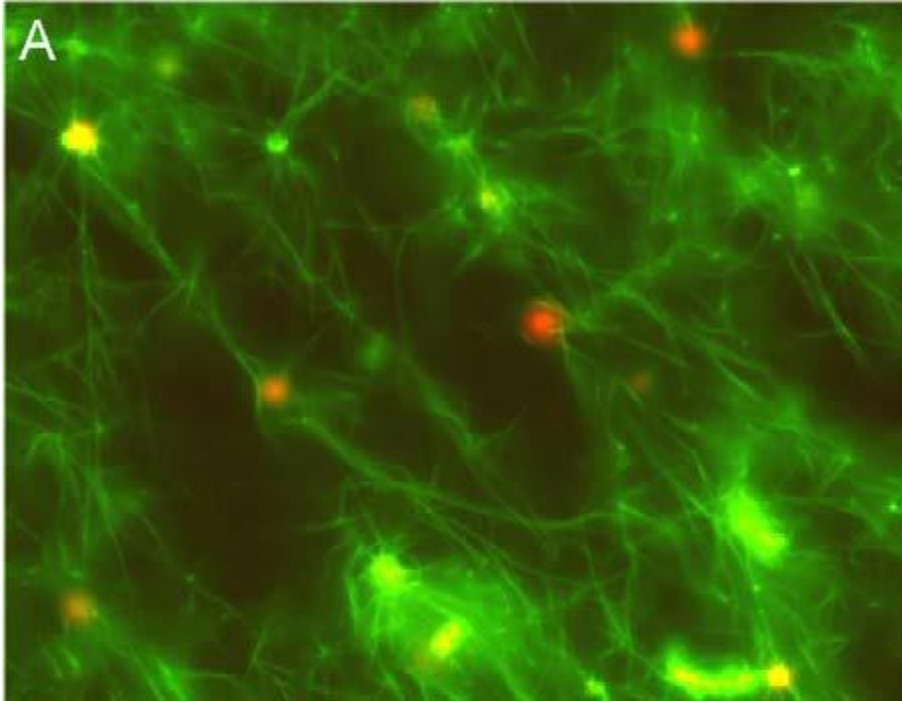
Imagerie moléculaire du thrombus



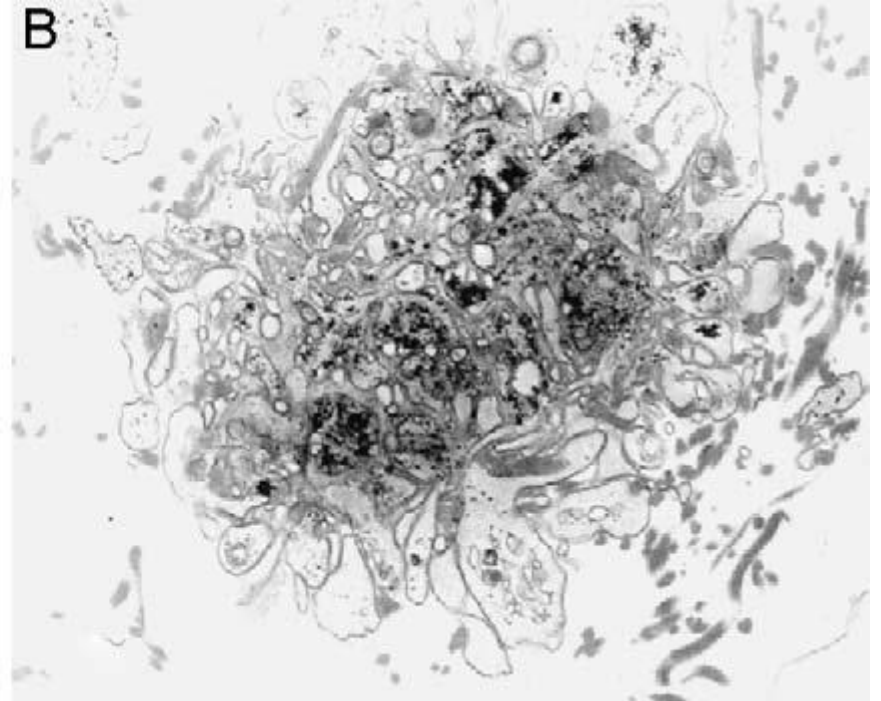
Michel J-B. et al., Cardiovasc Res 2010

- Biologie du thrombus : identification des cibles potentielles
- Imagerie moléculaire du thrombus : état des lieux et perspectives

Les composants du thrombus



Un caillot de fibrines (colorée en vert) et d'agrégats plaquettaires (en rouge), qui maintiennent les fibres de fibrine dans une organisation définie.

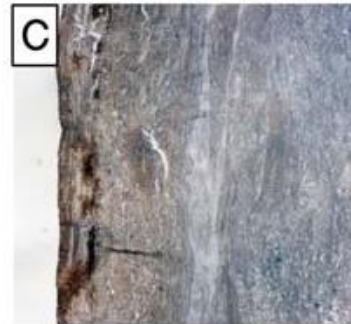


Microscopie électronique : les plaquettes forment un agrégat très compact englobé dans des fibres de fibrine.

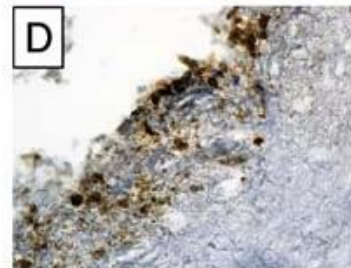
Les composants du thrombus



Hématies
+
Fibrine
(HE)



Plaquettes
(GPIb)



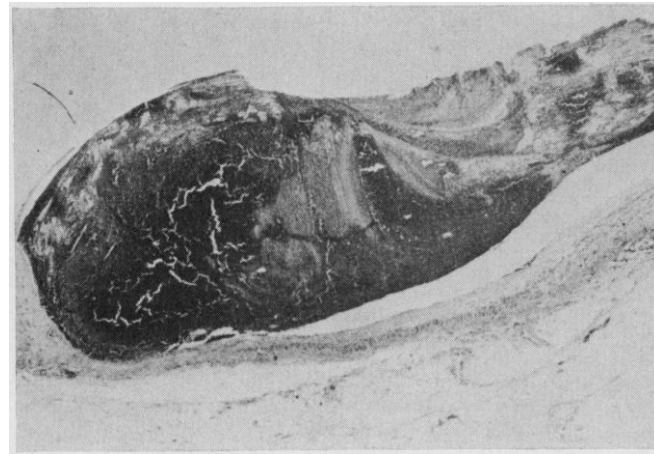
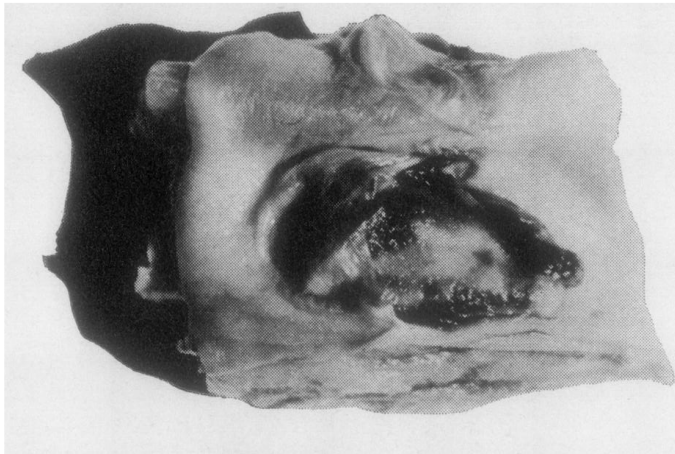
Polynucléaires
(CD66b)

Thrombus veineux : composition

Hétérogénéité de structure

Le thrombus veineux récent comprend 2 composantes :

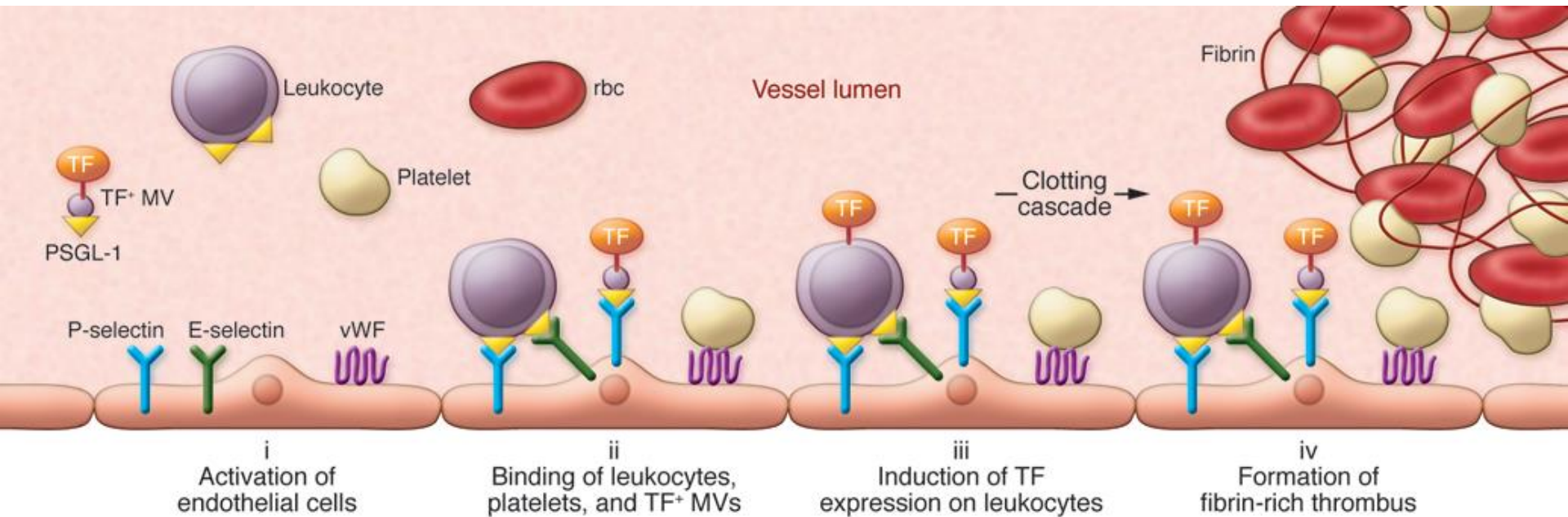
- Du **thrombus rouge** formé d'un caillot de **fibrine très riche en érythrocytes**, avec quelques **leucocytes** : site de formation initiale du thrombus au contact de la paroi veineuse.
- Des lignes ou des amas de **thrombus blanc** riche en **plaquettes et en fibrine** : zones de croissance et face interne de thrombus rouge.



*Sevitt S . J Clin
Pathol 1974*

Thrombus veineux : composition

Evolution temporelle : initiation de la thrombose veineuse



Thrombus veineux : composition

Evolution temporelle : involution du thrombus veineux

Table 1. Cell Types Present in the Venous Thrombus

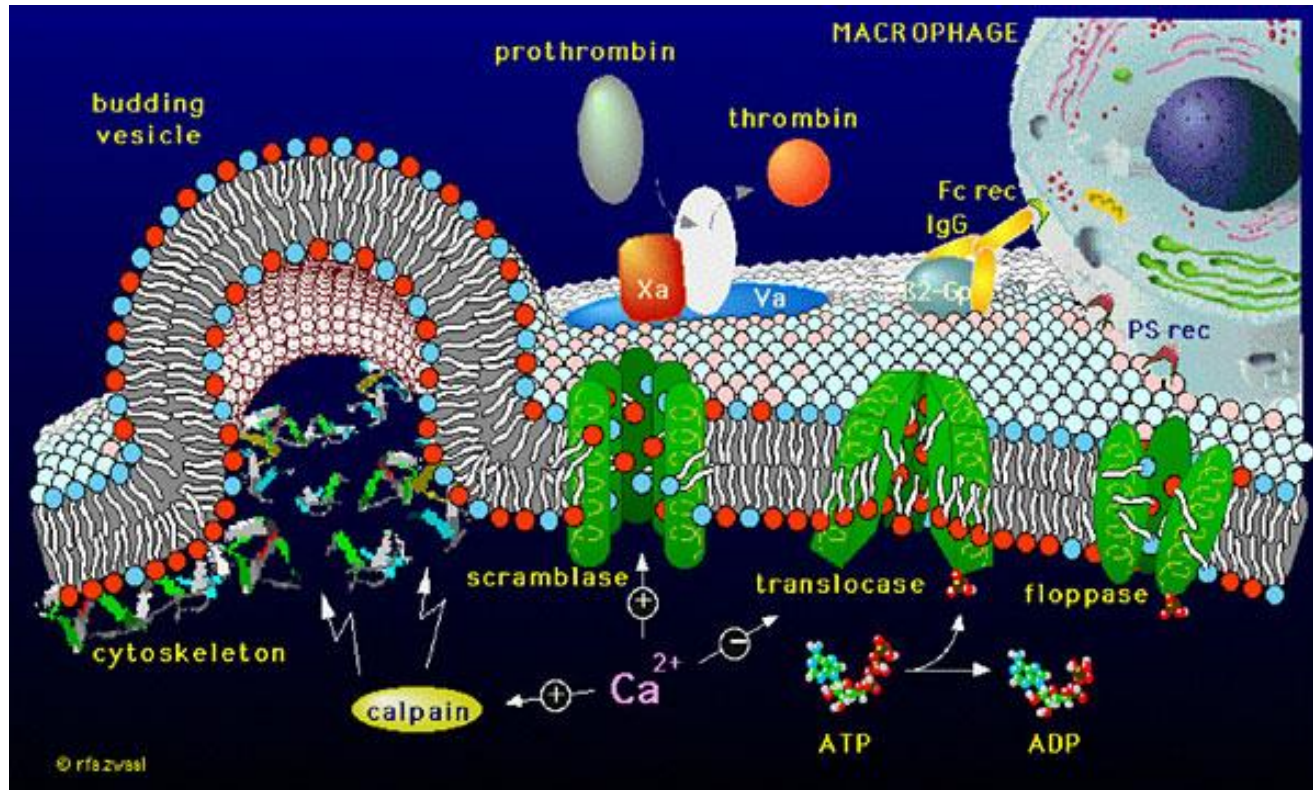
Cell Type	Marker (Antibody Clone)	Infiltration	References
Platelet	CD41 (MWRReg30)	Early	21
Neutrophil	Ly6G (NIMP-R14 or 1A8)	Early	18, 21
Macrophage	Mac2 (M3/38), F4/80 (Cl:A3-1 or BM8)	Mid-late	17, 21, 22
Endothelial	vWF (F8/86), CD31 (JC70A), Isolectin-B4 (GSL I-B4)	Mid-late	17, 23, 24

In murine models of vena cava thrombosis, early, and mid-late stages of thrombus resolution refer to days 1 to 7 and days 7 to 28 post induction, respectively.

Les cibles d'intérêt

Phosphatidylsérine (PS)

- Exposition sur les plaquettes **activées**
- Participe à la formation (avec facteurs V et X) du complexe prothrombinase

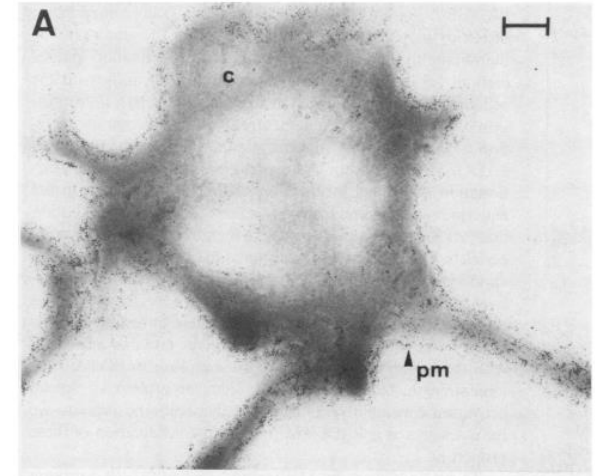
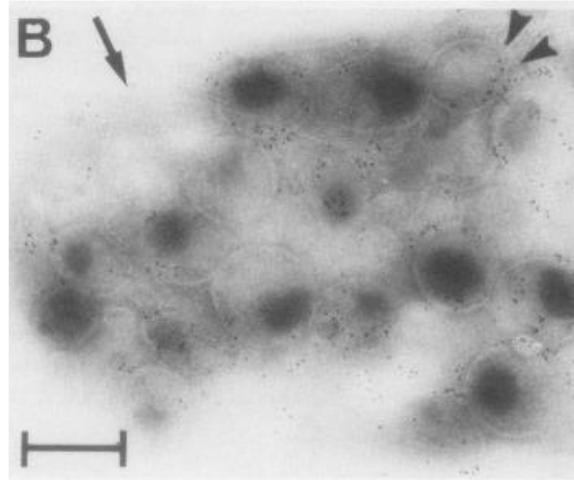


➤ **Marqueur et acteur de la coagulation**

P-sélectine

Granules α :

Immunolocalisation en MO sur coupes.

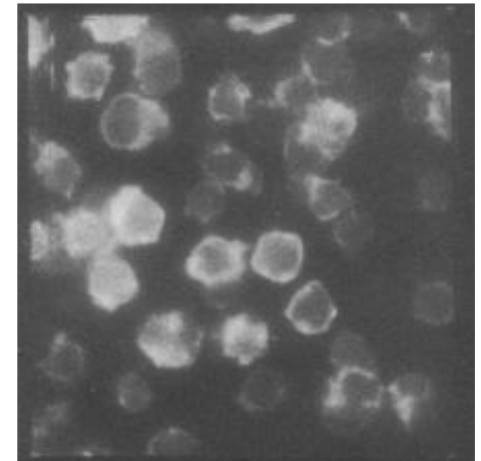
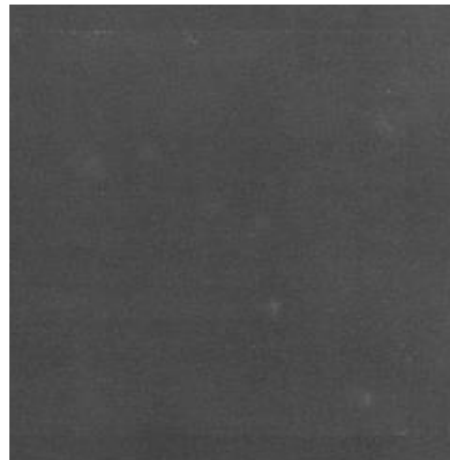


thrombine



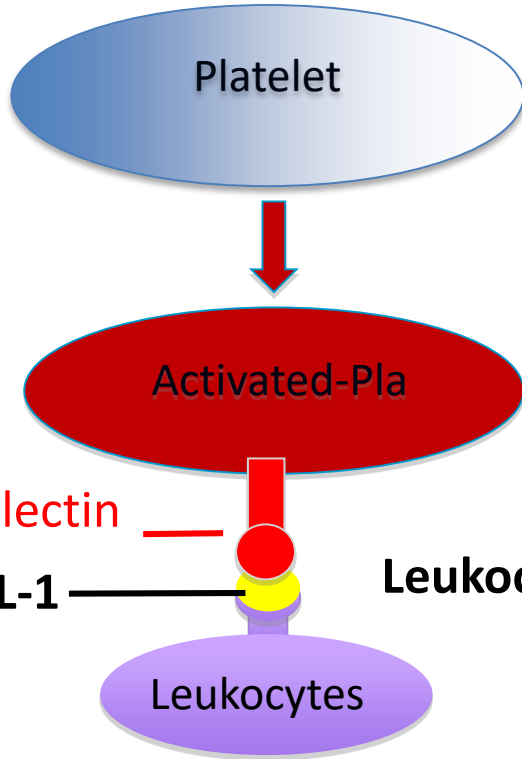
P-sélectine :

Immunofluorescence sur plaquettes entières.



P-sélectine

Coagulation



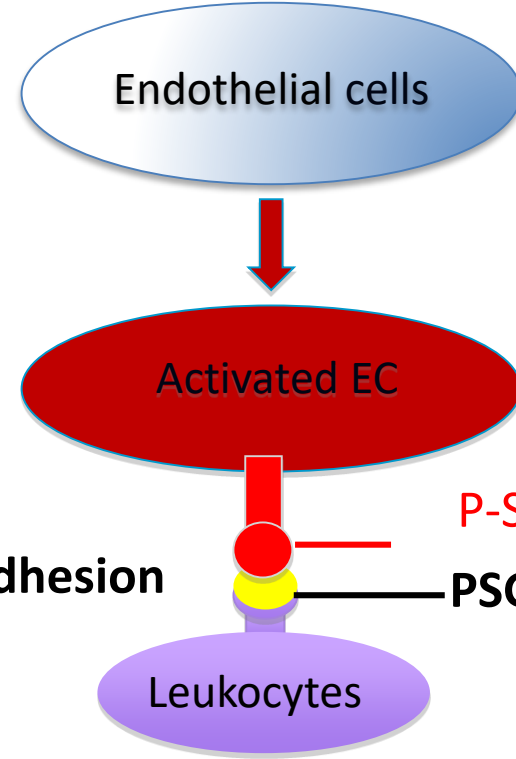
P-Selectin

PSGL-1

Leukocytes

Leukocytes rolling and adhesion

Endothelium



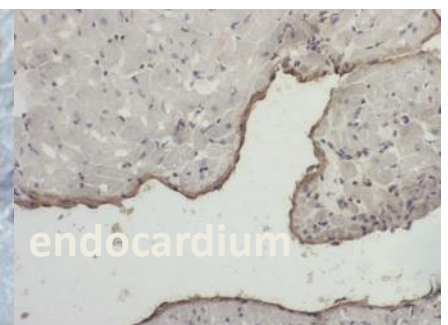
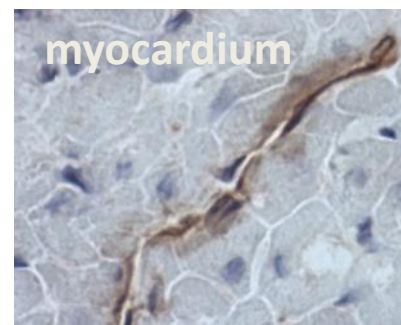
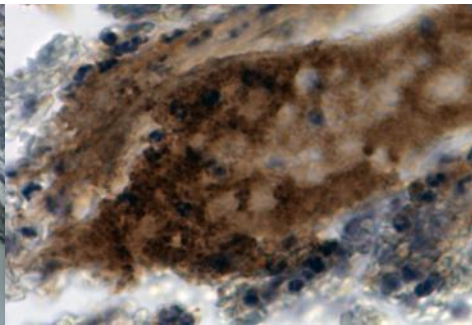
Endothelial cells

Activated EC

P-Selectin

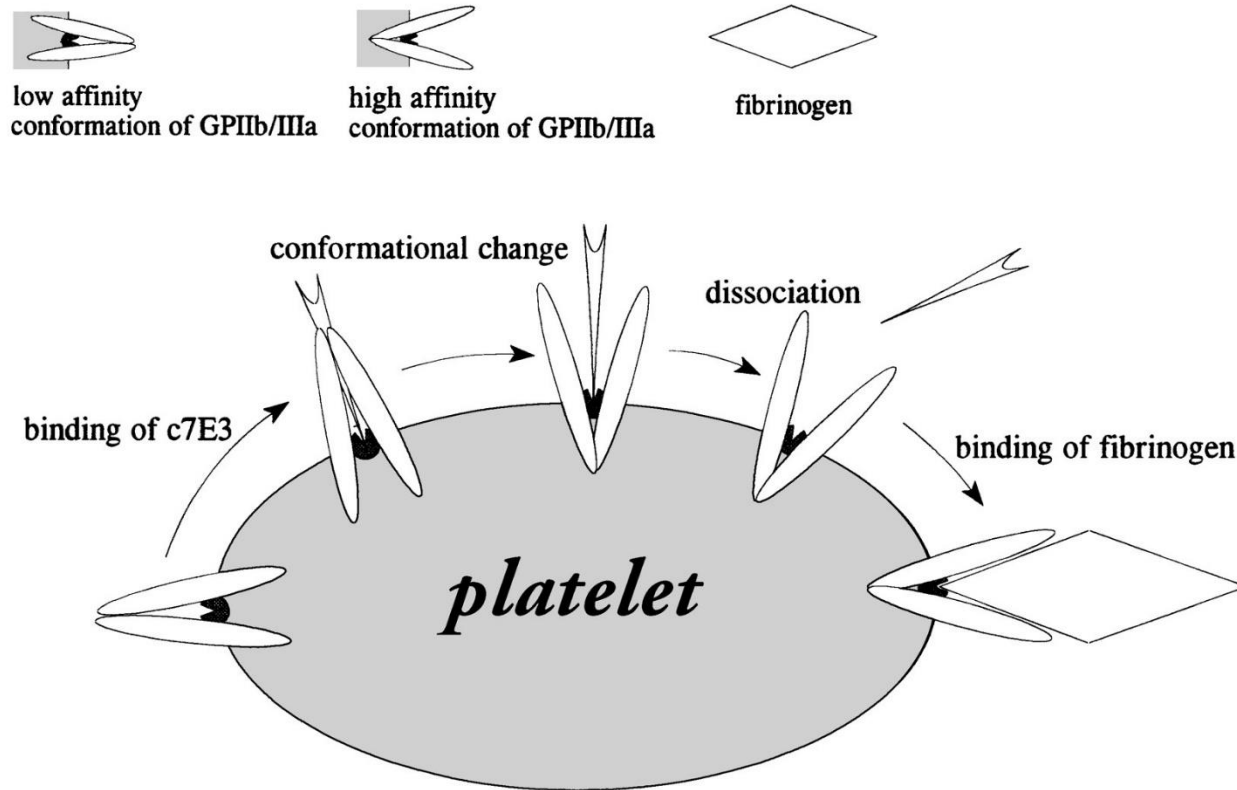
PSGL-1

Leukocytes



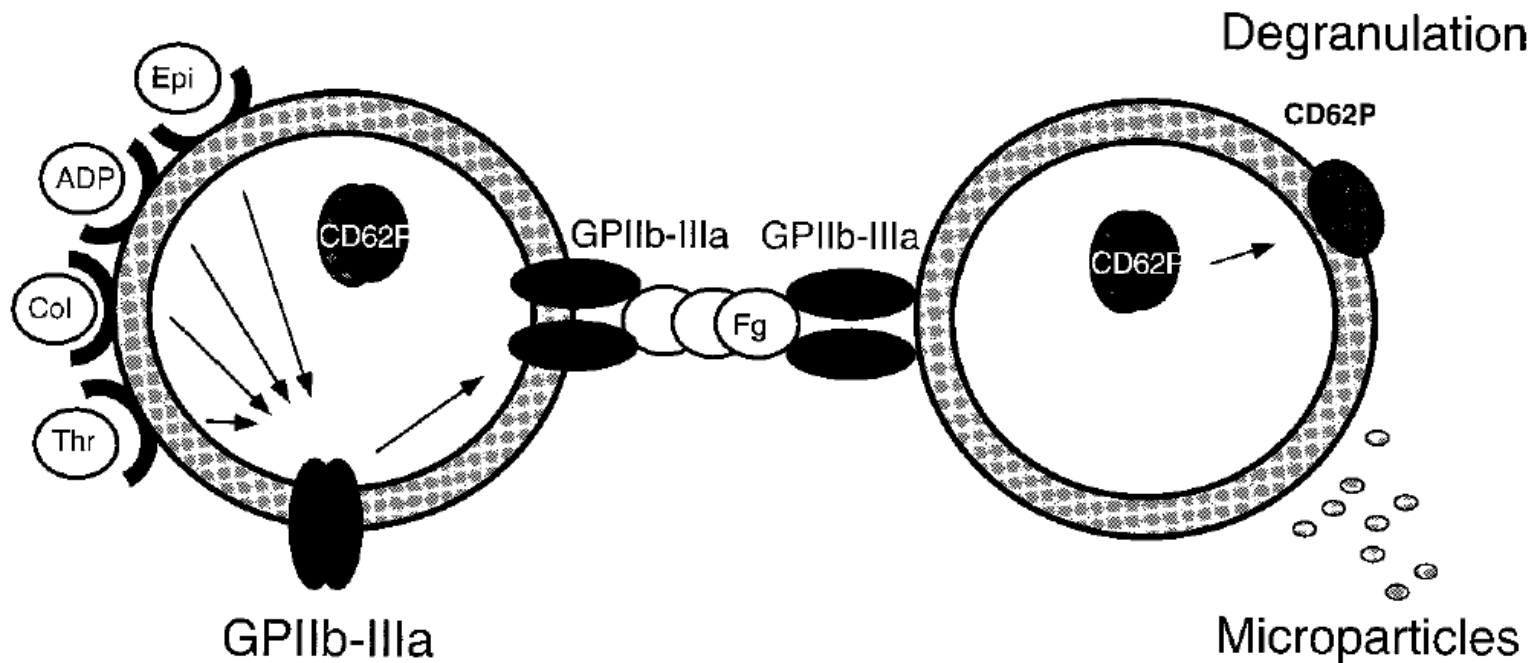
GPIIb/IIIa

Changement conformationnel : faible => forte affinité



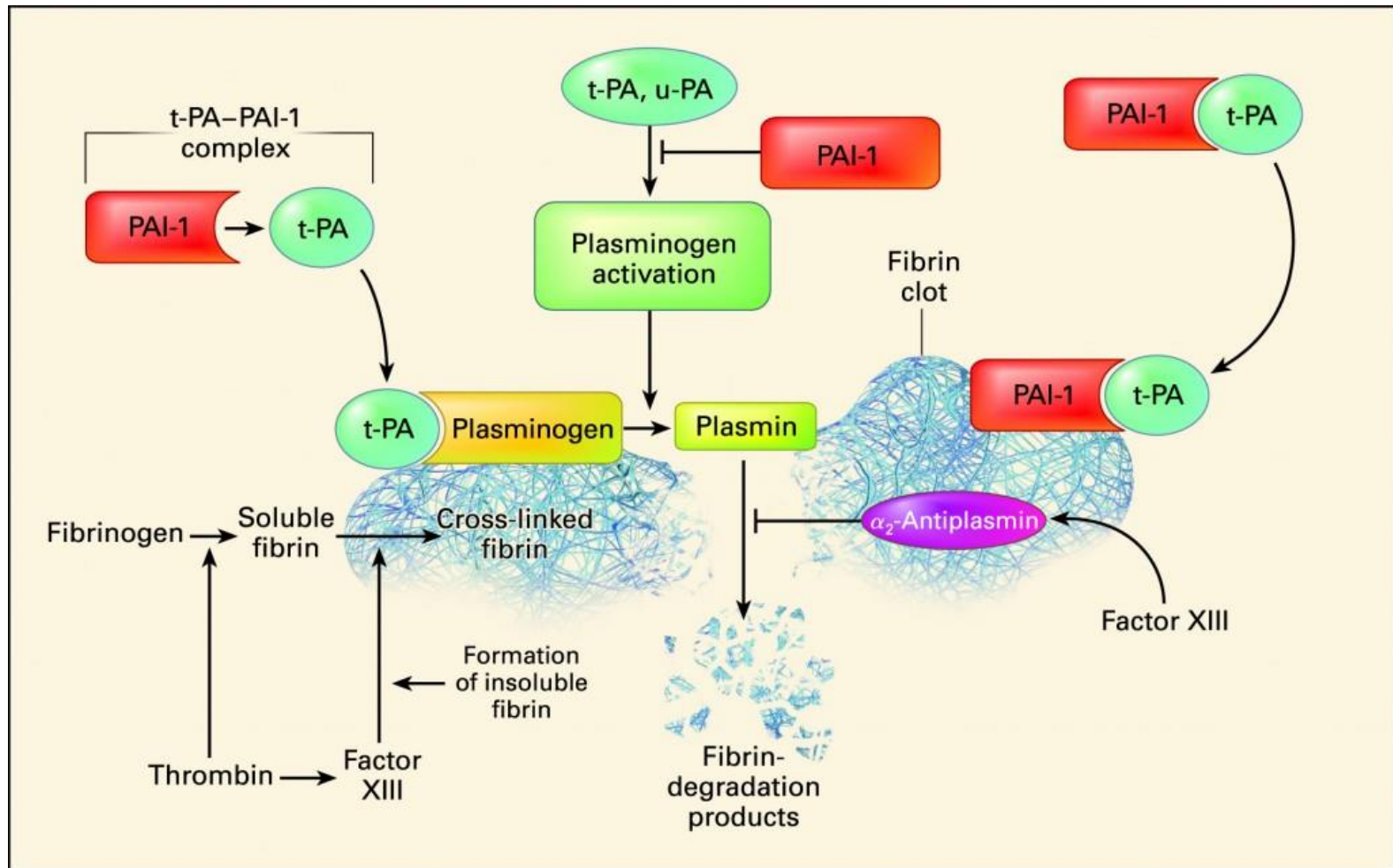
GPIIb/IIIa et Fibrinogène

Liaison du fibrinogène à la GPIIb/IIIa : agrégation plaquettaire



Plasmine

Activation du plasminogène en plasmine = Fibrinolyse

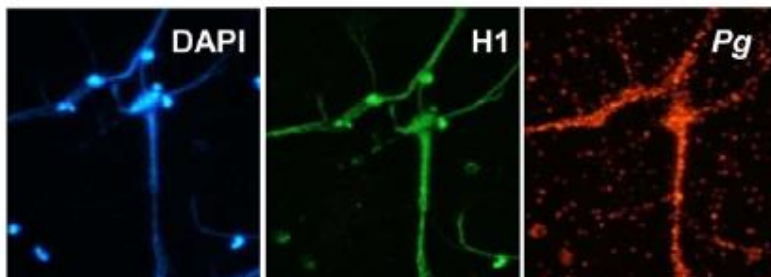


Colonisation bactérienne du thrombus

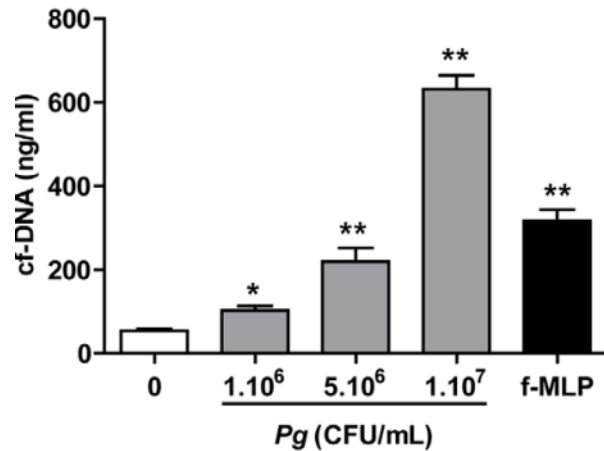
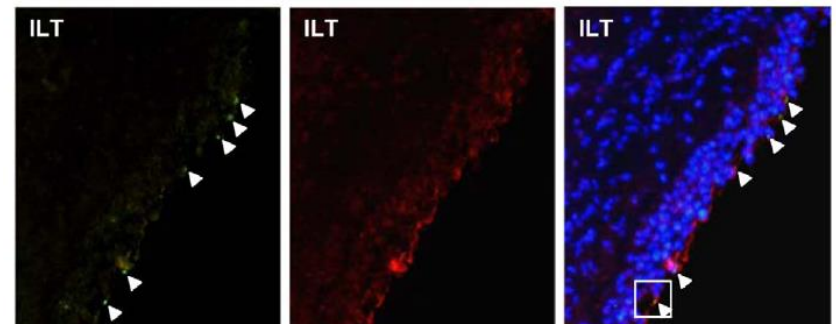
Germes faiblement pathogènes (Ex : *Porphyromonas gingivalis*)

➤ Recrutement et activation des PMNs (NETs)

Anévrysmes humains

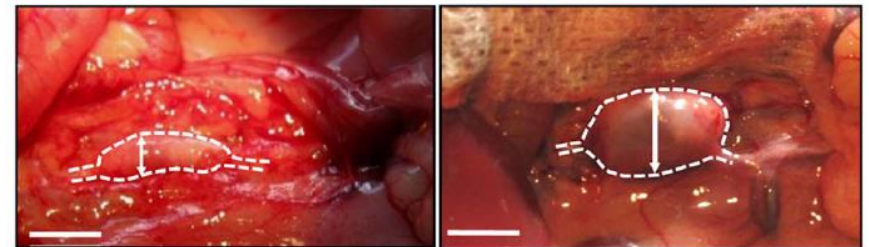


Modèle murin



Saline

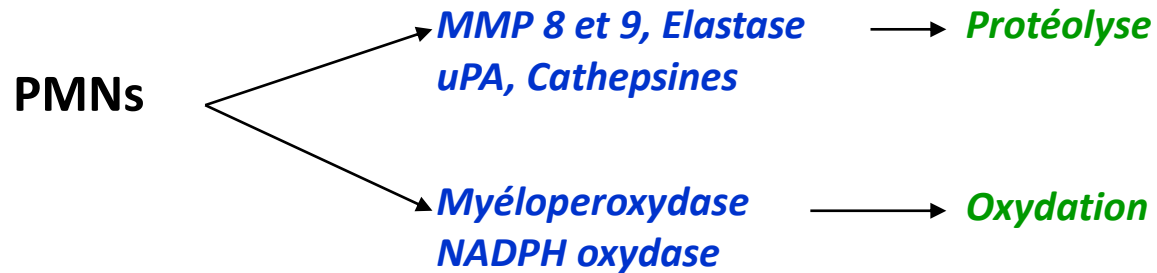
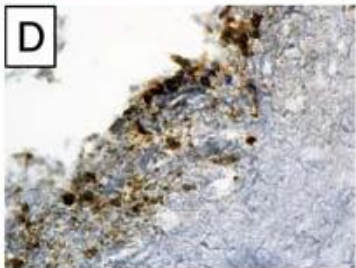
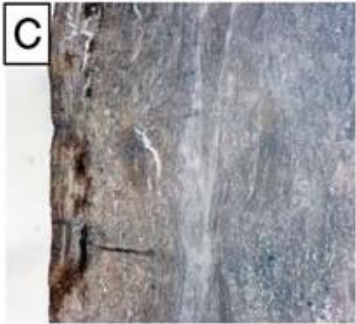
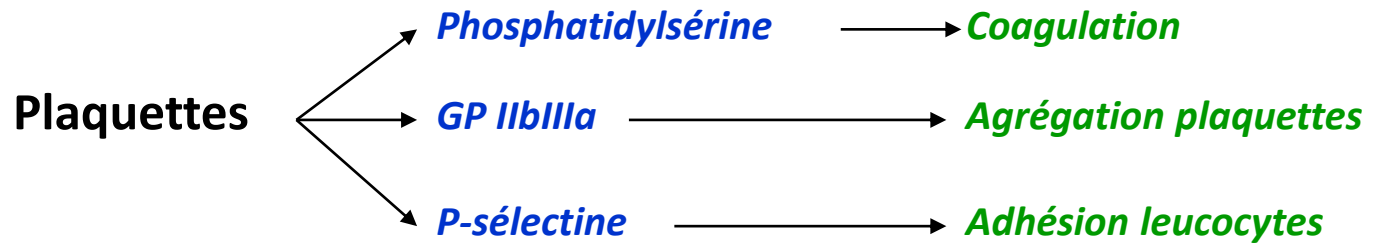
P. gingivalis



Delbosc S et al, PLoS ONE 2011

➤ Lien entre parodontopathies et athérombose

Les activités biologiques

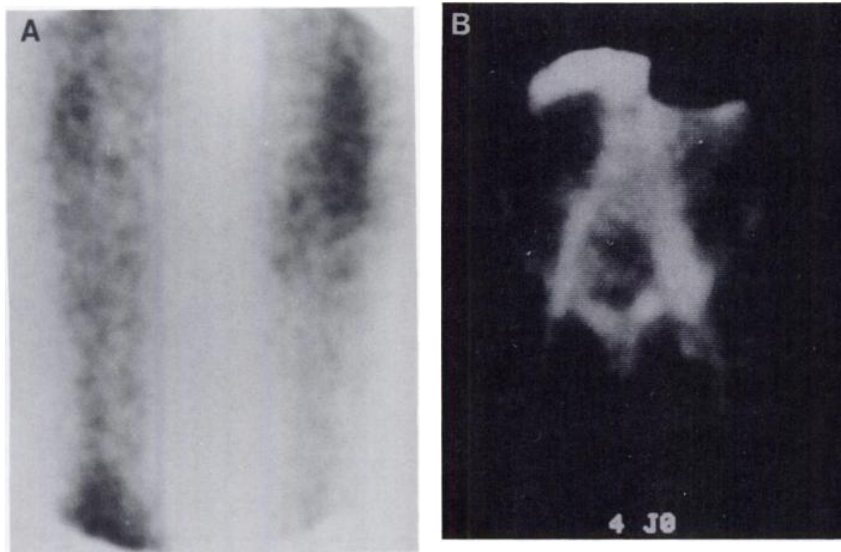


Les agents d'imagerie

Ciblage Fibrine : anticorps / SPECT

Fibrine présente à haute concentration dans les thrombi artériels et veineux mais pas dans le sang circulant

¹¹¹In-antifibrin antibodies



de Faucal F,..., Chatal JF. J Nucl Med 1991

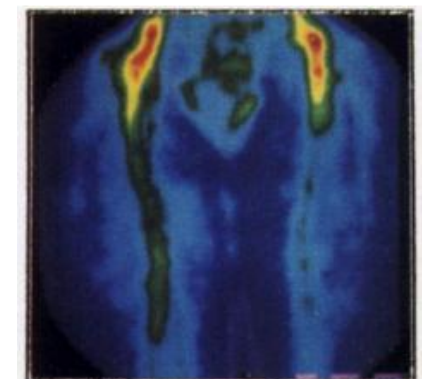
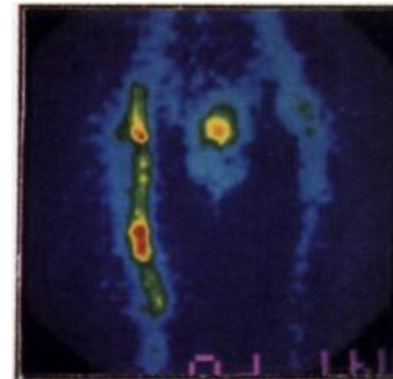
Femoropopliteal Graft

In - 111 Platelets
48 hours

Tc - 99 Antifibrin Antibody
10 minutes

Pelvis

Pelvis

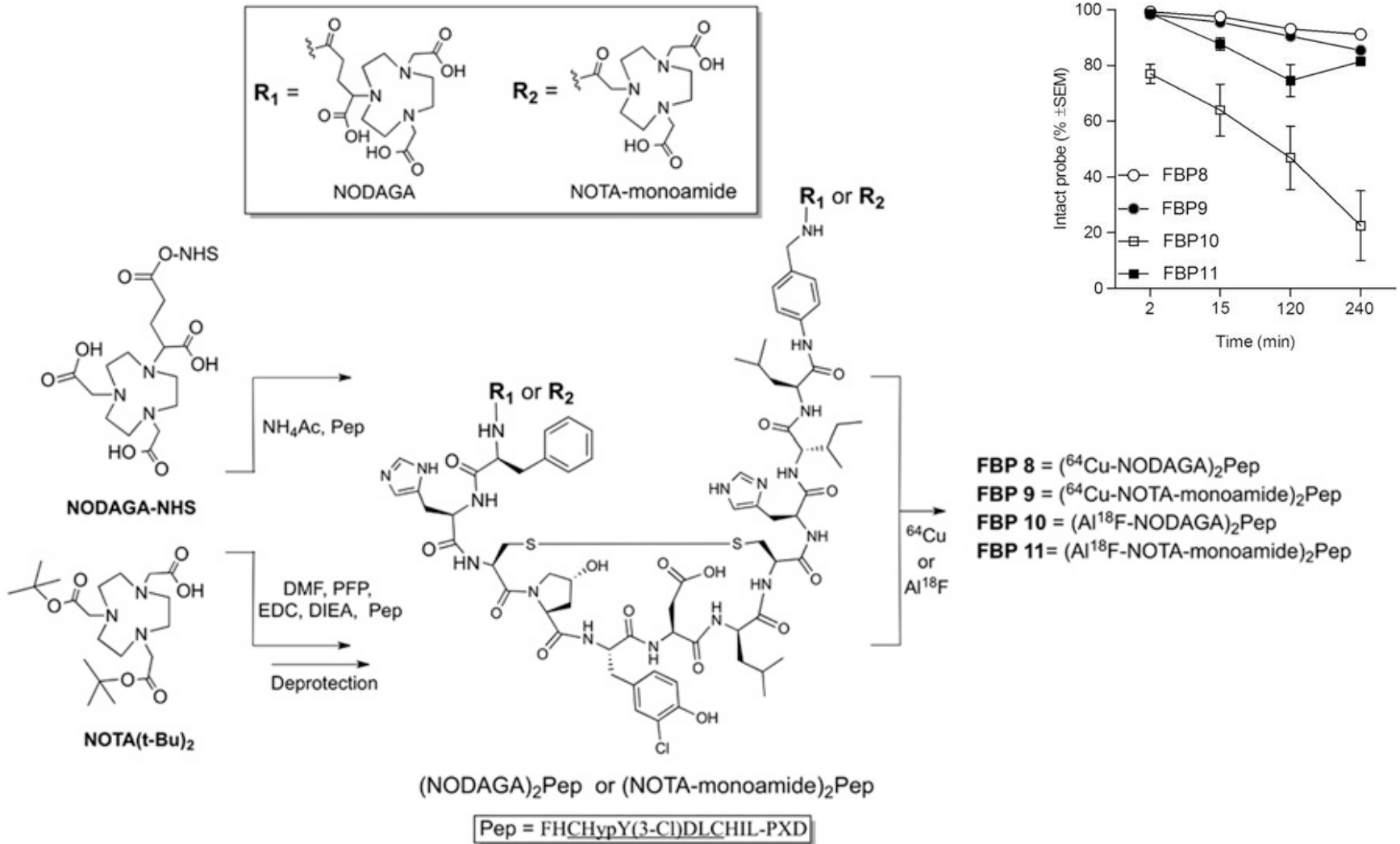


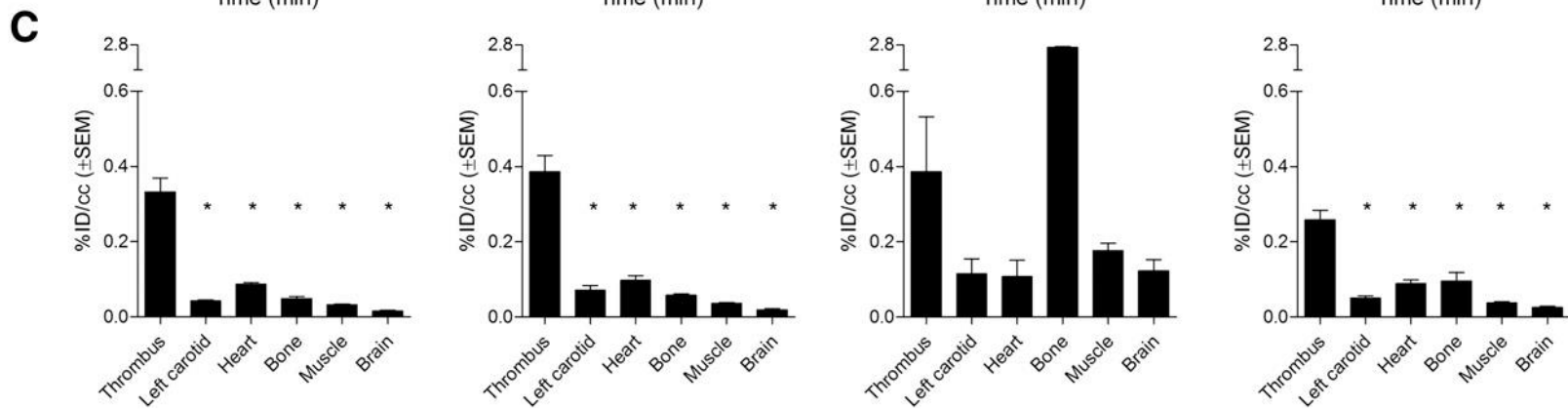
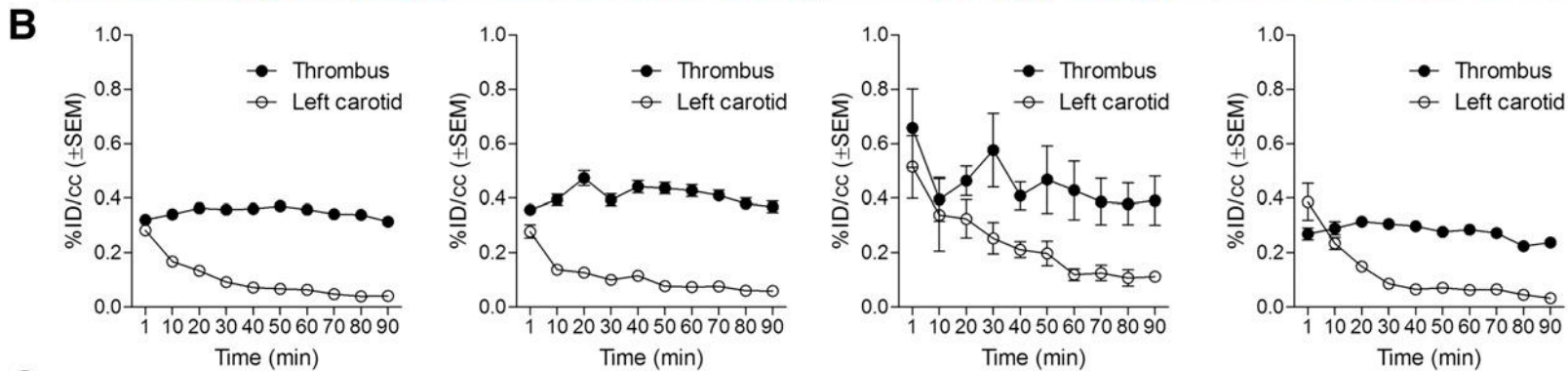
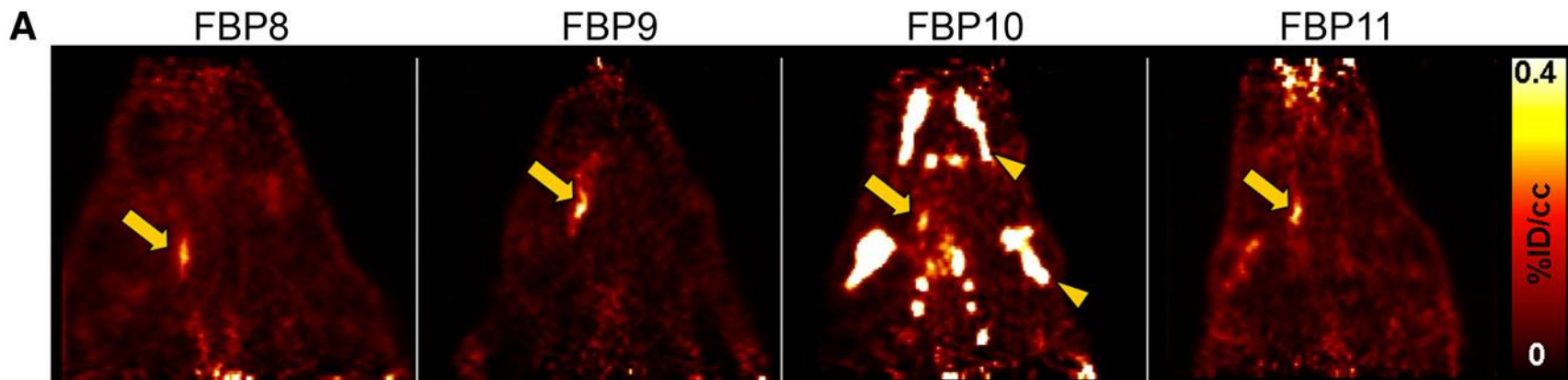
Knees

Knees

Stratton JR et al. J Nucl Med 1994

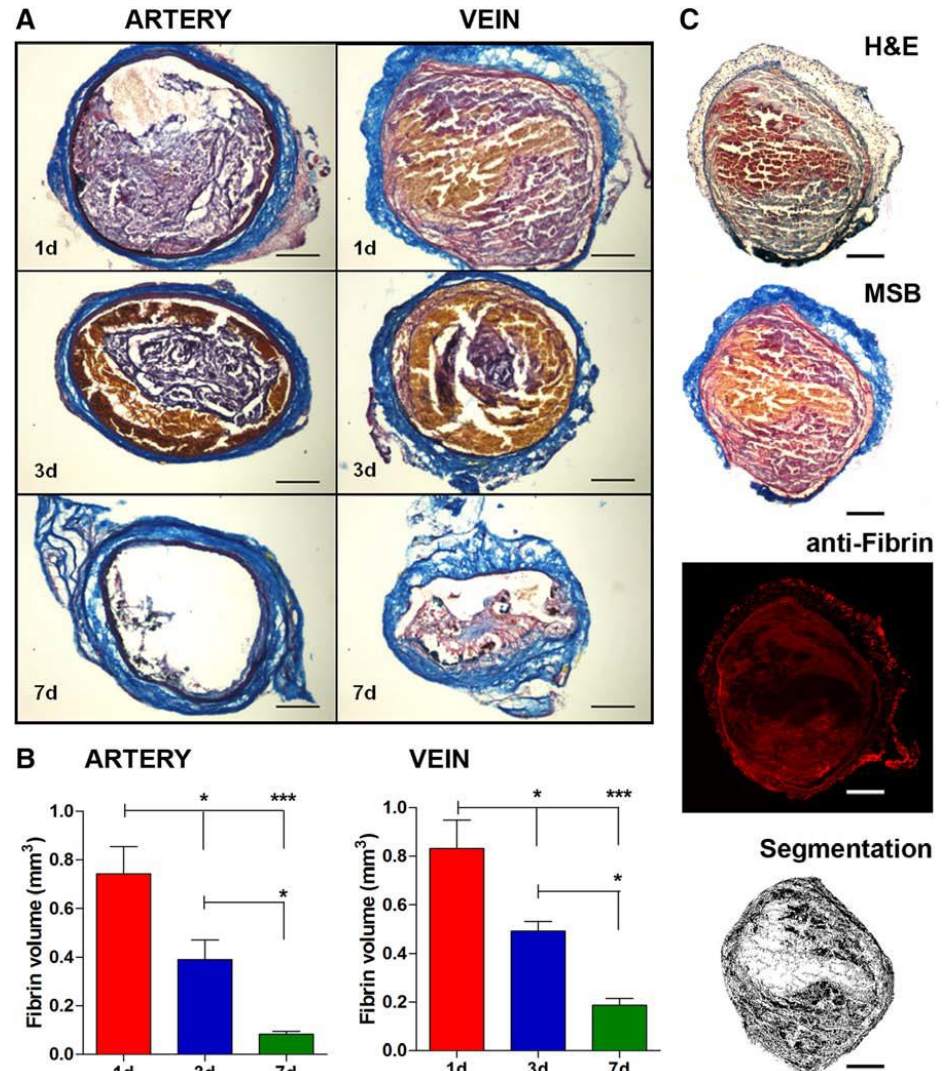
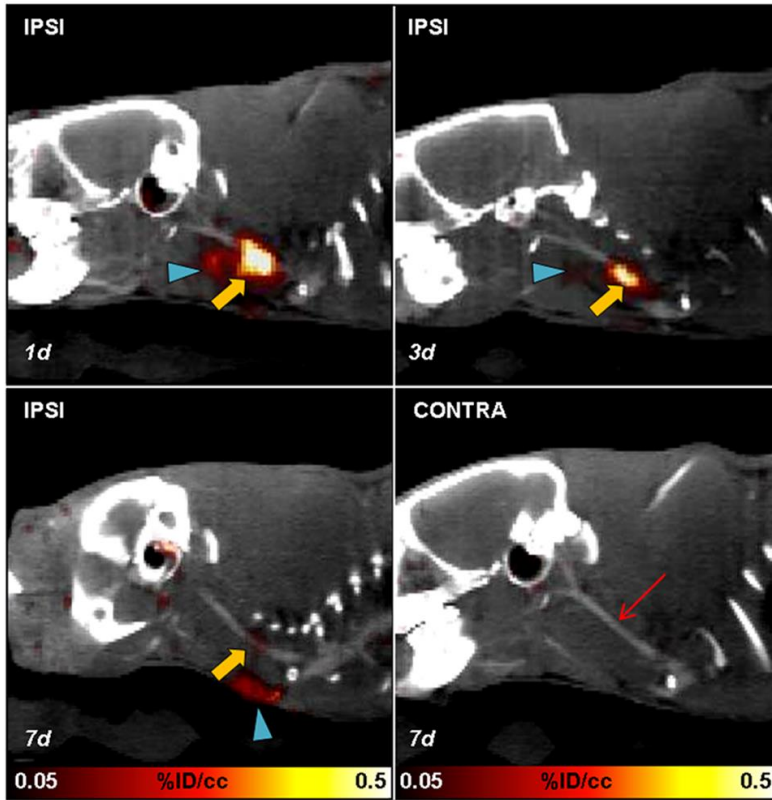
Fibrine : approche peptidique / TEP





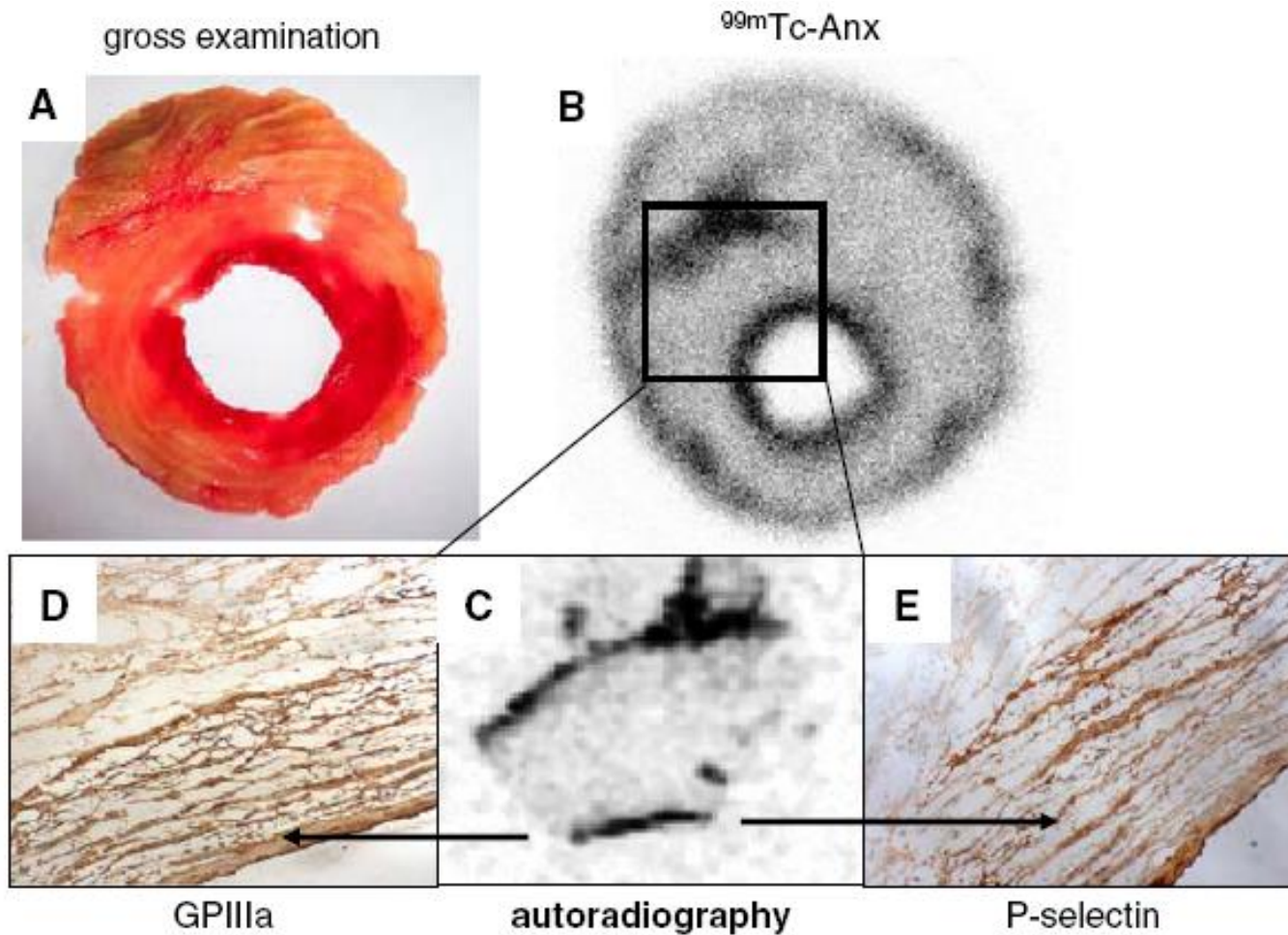
Modèle de thrombose (FeCl₃) artère carotide / veine fémorale chez le rat

⁶⁴Cu-FBP8 PET/CT



Ciblage de la PS : ^{99m}Tc -Annexine

AAA humain - *ex vivo*

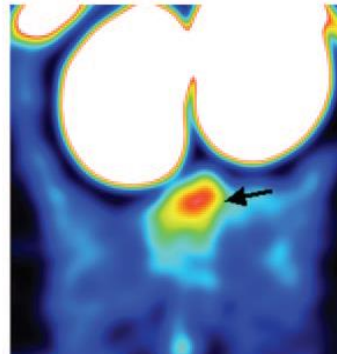


Ciblage de la PS : ^{99m}Tc -Annexine

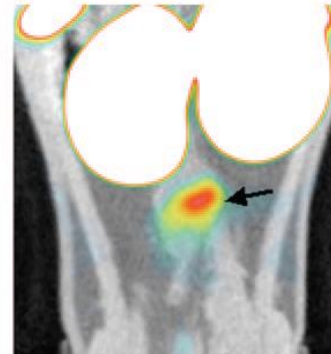
AAA chez le rat - *in vivo*



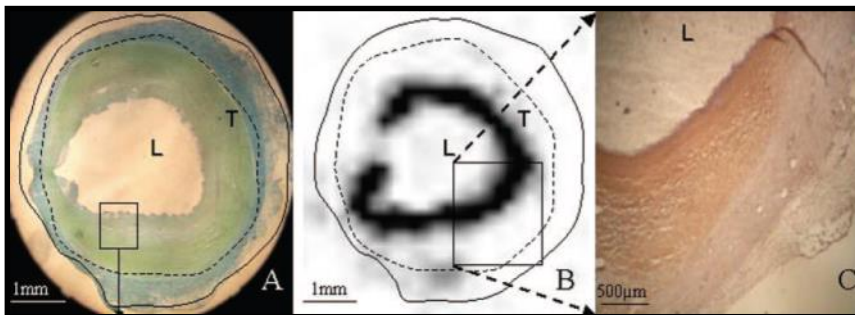
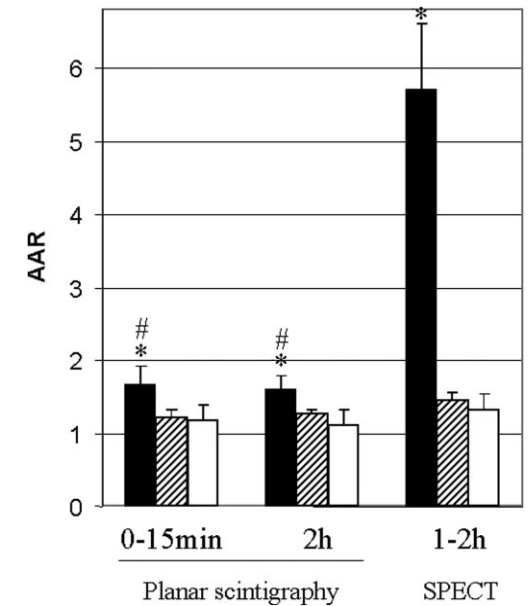
CT



SPECT



SPECT/CT



- Rats with AAA
- ▨ Elastase-perfused rats without AAA
- Sham-operated controls

Ciblage de la PS : ^{99m}Tc -Annexine

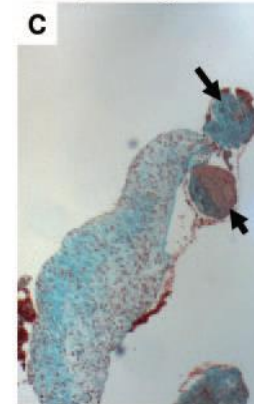
Table. Experimental Models Summary

Animal Species	Catheter Position	Group	n	Bacteria Strain
Rabbit	Left-sided	Sham	12	
		Not infected	12	
		Infected	12	<i>Enterococcus</i> JH2-2 (n=8) <i>Staphylococcus</i> HM 1054 (n=4)
	Right-sided	Not infected	4	
		Infected	9	<i>Enterococcus</i> JH2-2 (n=4) <i>Staphylococcus</i> HM 1054 (n=5)
Rat	Left-sided	Not infected	7	
		Infected	7	<i>Enterococcus</i> JH2-2 (n=7)
	Right-sided	Infected	7	<i>Staphylococcus</i> HM 1054 (n=7)

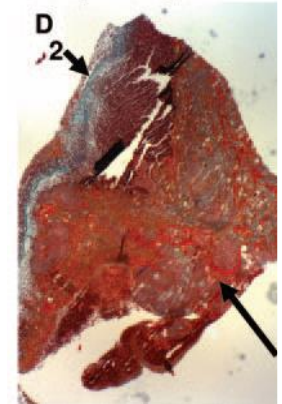
control



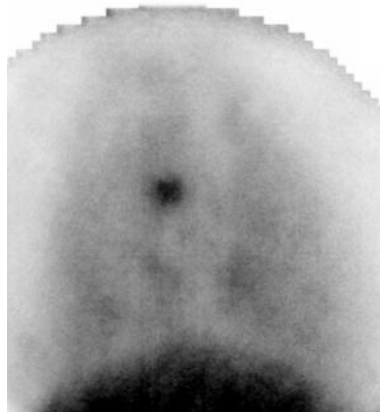
aseptic vegetation



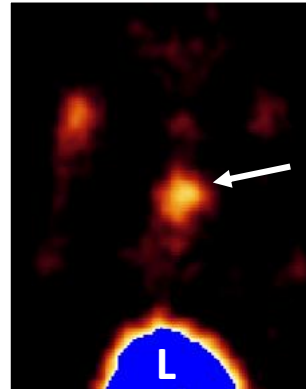
septic vegetation



Ciblage de la PS : ^{99m}Tc -Annexine

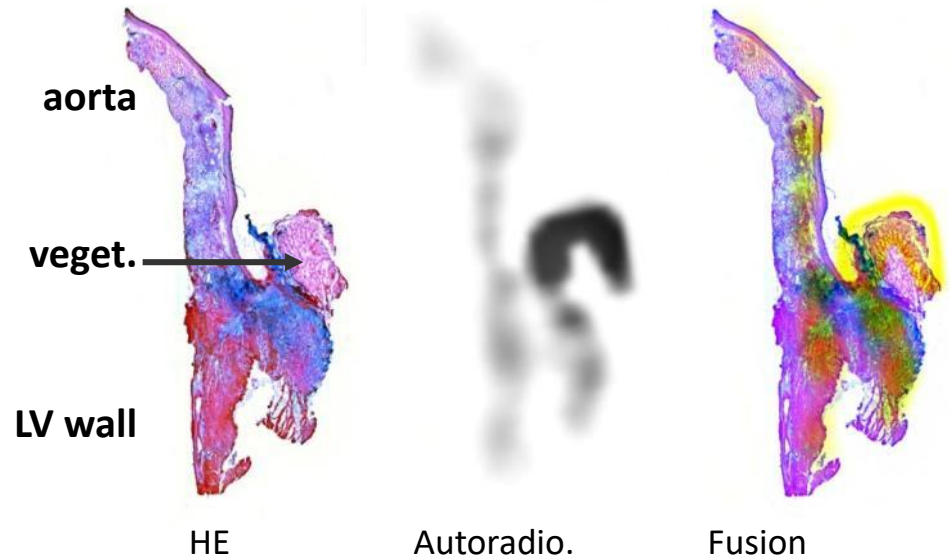
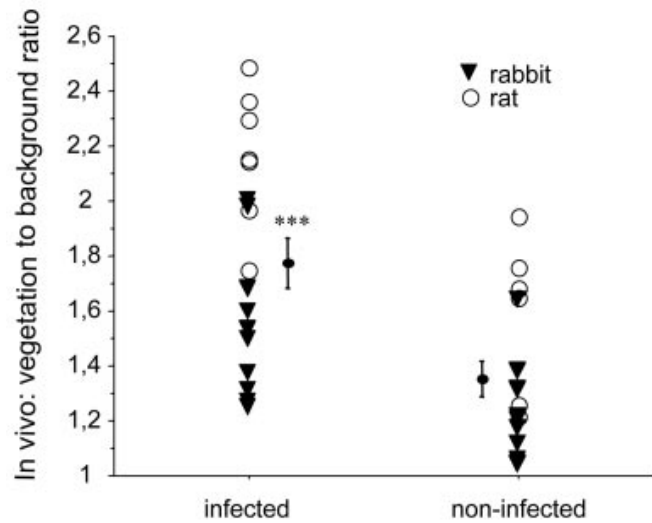


planaire



SPECT

Endocardite aortique

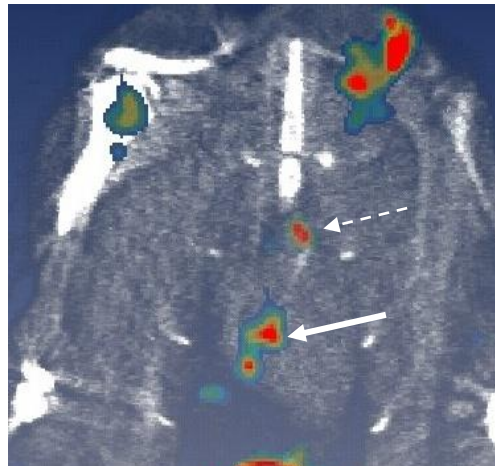


Ciblage de la PS : ^{99m}Tc -Annexine

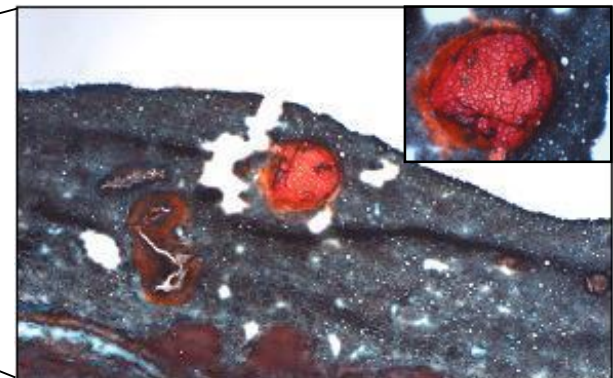
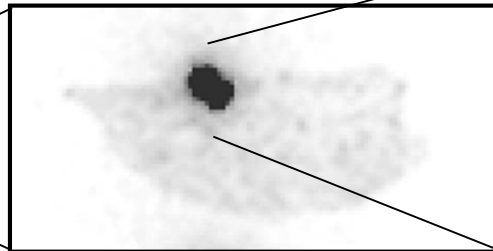
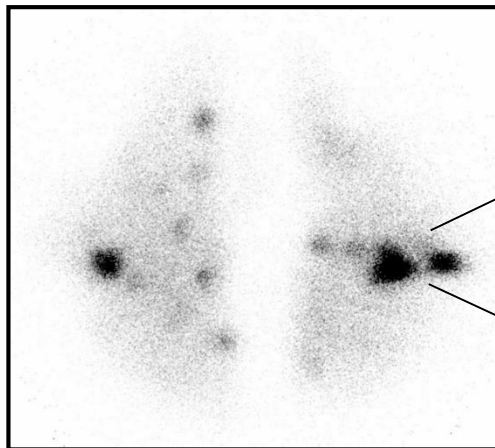
Endocardite tricuspide



Rx (lapin)



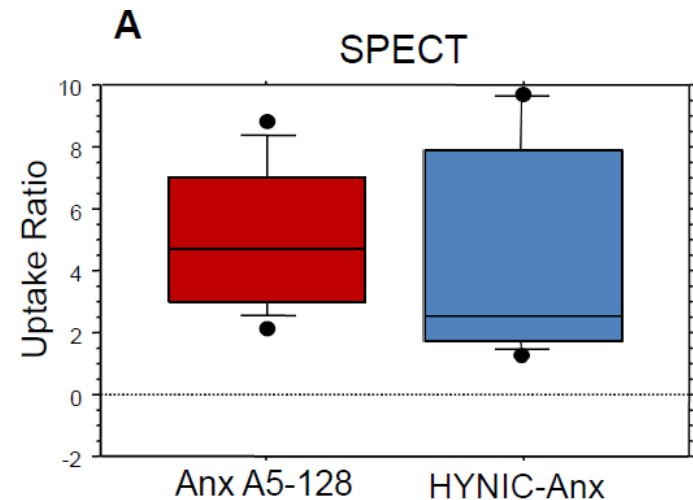
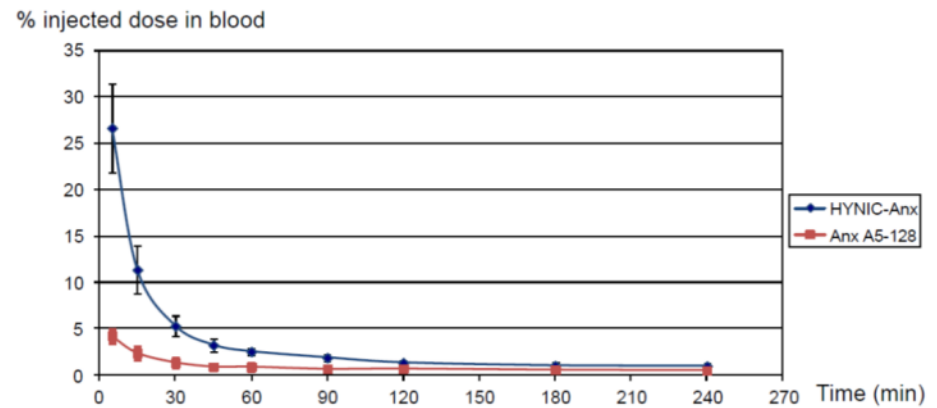
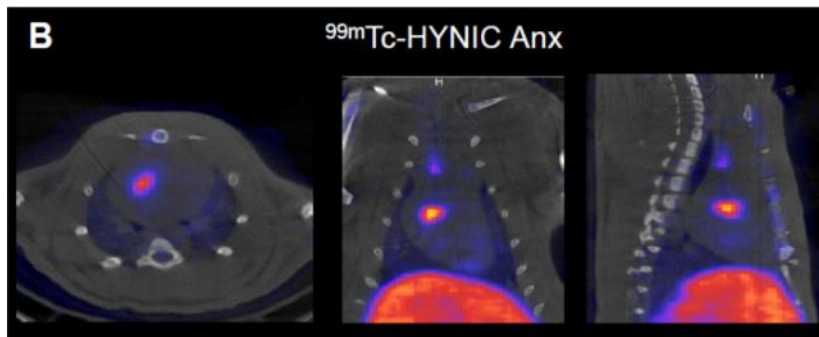
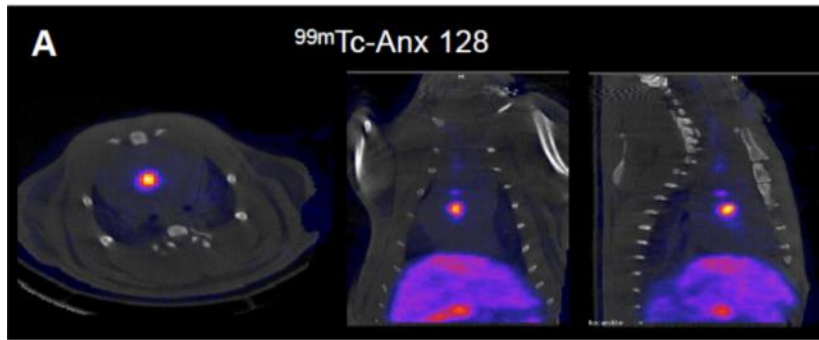
SPECT/TDM (rat)



^{99m}Tc Anx V-128

Validation de la forme GMP de l'annexine

Collaboration Atreus pharma et AAA



Etude clinique

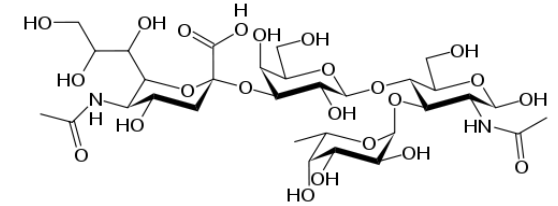
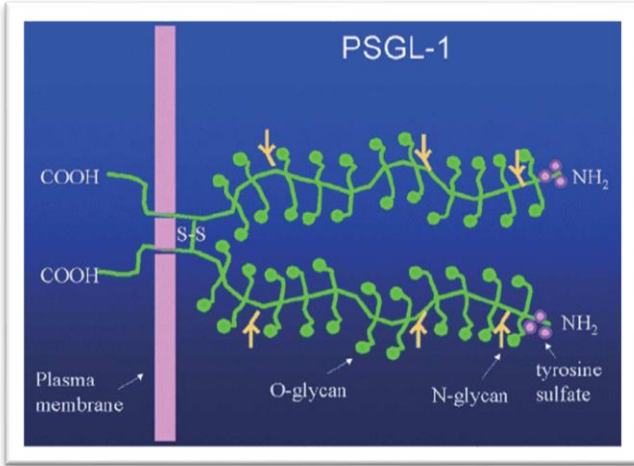
Evaluation de la rhAnnexine V-128 radiomarquée dans le diagnostic de l'endocardite infectieuse et de ses complications emboliques

- Coordonateur : Pr D. Le Guludec
- NCT02459613
- Promoteur : Inserm
- 90 patients : Endocardite infectieuse (n=60) et thrombus atrial (n=30)

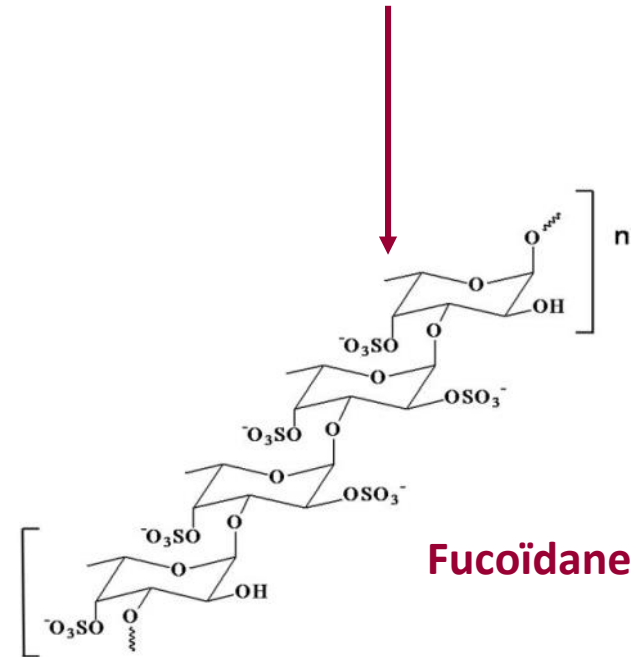


Ciblage de la P-sélectine : Le Fucoïdane

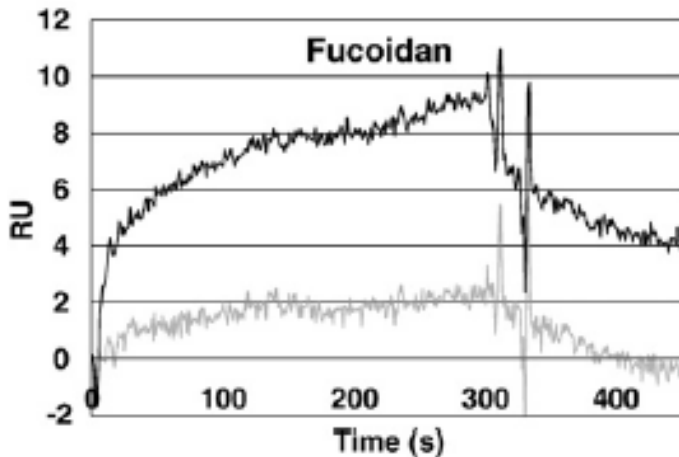
Ligand naturel : PSGL-1



Sialyl Lewis X



Fucoïdane



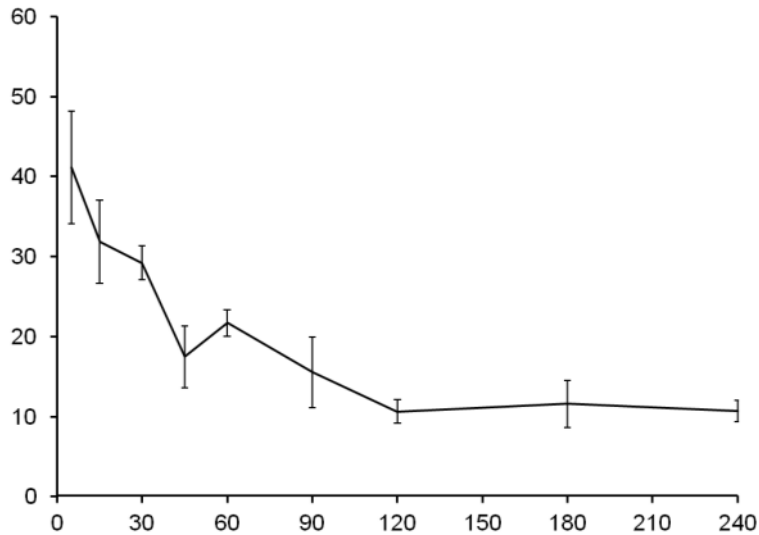
$K_D = 1,2 \text{ nM}$

Binding of Fucoïdane on P-selectin

Radiomarquage + biodistribution

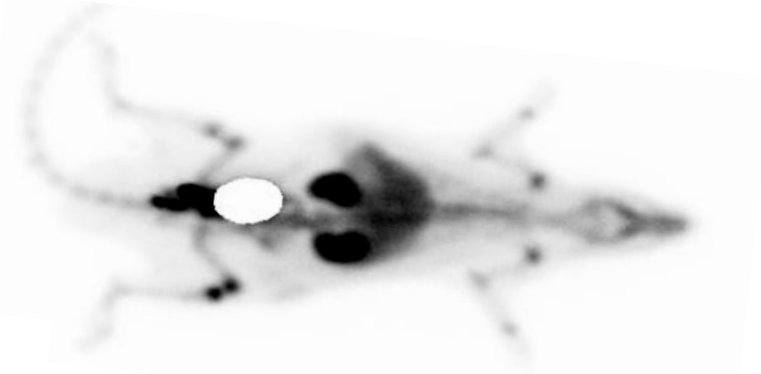
Clairance sanguine

Activité sanguine (% activité injectée)



Composante	Fraction	T _{1/2} (min)
α (rapide)	0,68	19
β (lente)	0,32	351

Biodistribution tissulaire

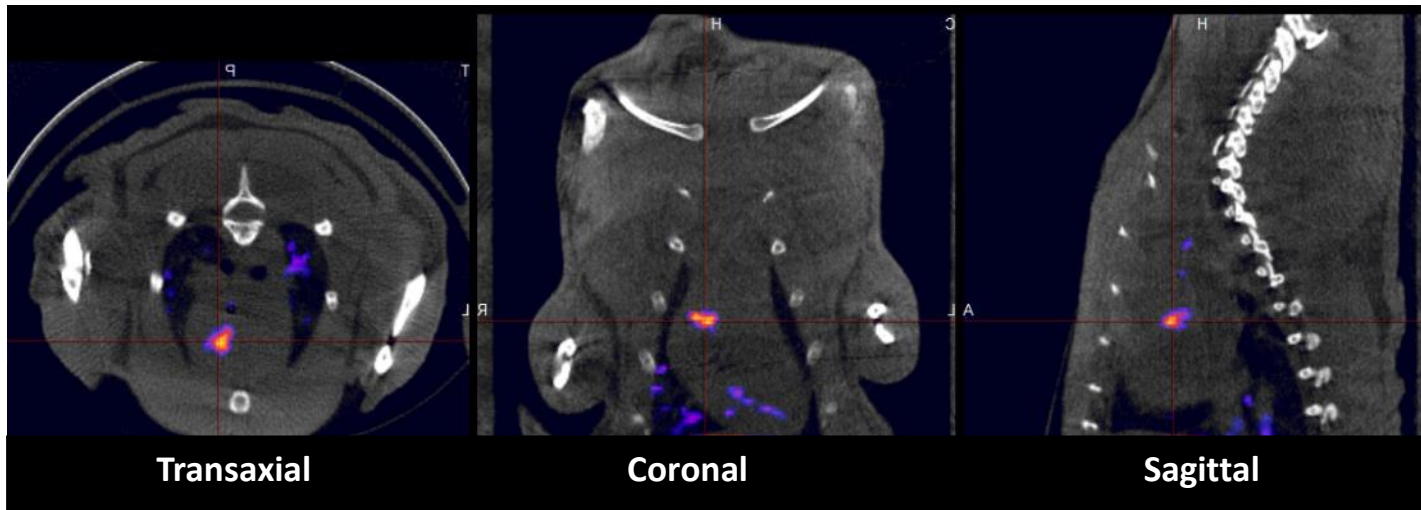


Organ	Percentage of administered dose per gram of tissue	Percentage of administered dose per organ
Blood	0.624 ± 0.042	11.73 ± 0.79
Kidneys	4.801 ± 0.681	12.17 ± 1.49
Urine*	34.171 ± 11.95	18.07 ± 5.54
Liver	0.619 ± 0.082	7.85 ± 1.10
Spleen	0.213 ± 0.055	0.182 ± 0.029
Bones	0.559 ± 0.093	16.58 ± 2.73
Muscle	0.040 ± 0.012	5.02 ± 1.53
Skin	0.164 ± 0.075	9.20 ± 4.16
Heart	0.110 ± 0.009	0.129 ± 0.027
Lungs	0.172 ± 0.028	0.270 ± 0.047
Thyroid	0.250 ± 0.100	0.005 ± 0.002
Brain	0.014 ± 0.002	0.019 ± 0.001

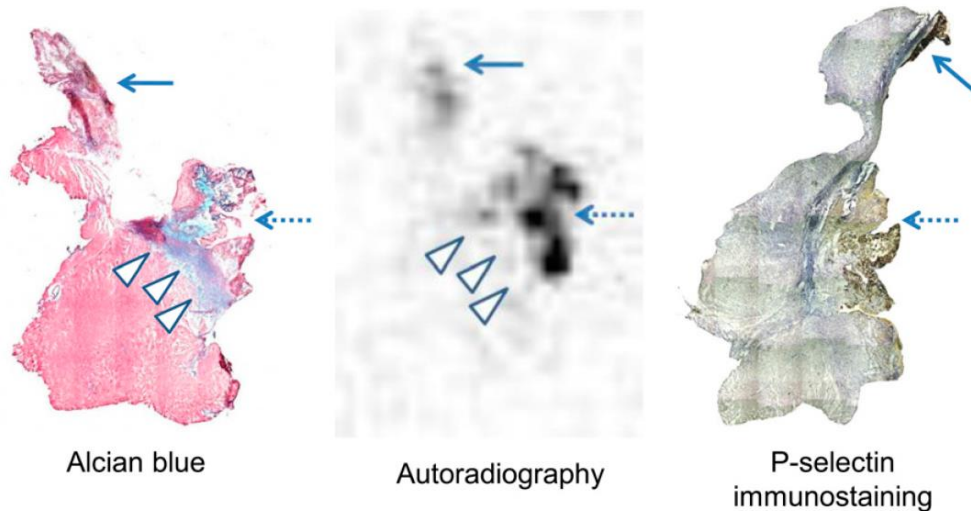
^{99m}Tc -Fucoïdane et endocardite infectieuse

Endocardite infectieuse chez le rat

SPECT/CT



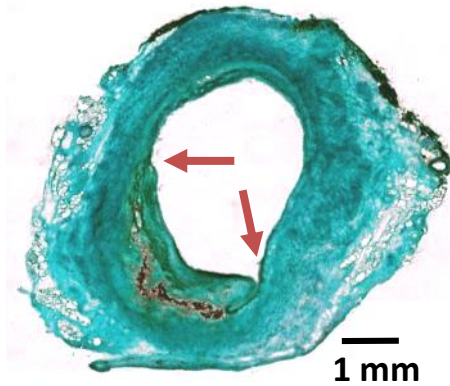
Histology



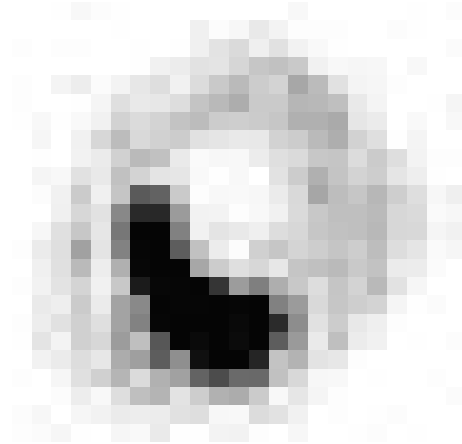
*Rouzet F et al,
J Nucl Med 2011*

Fucoïdane et thrombus anévrysmal

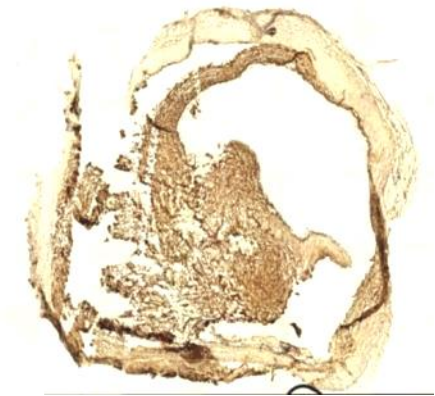
Thrombus mural d'un AAA chez le rat



Masson's trichrome

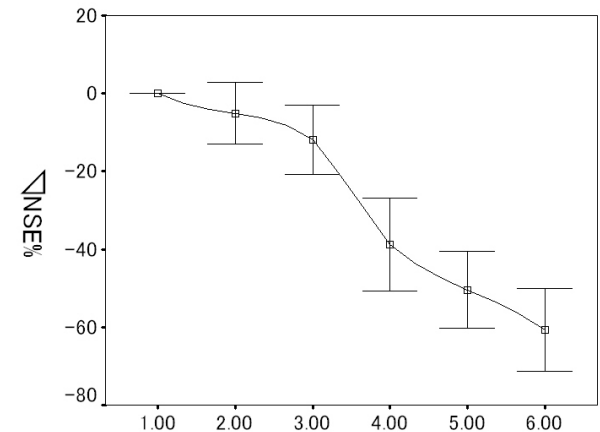
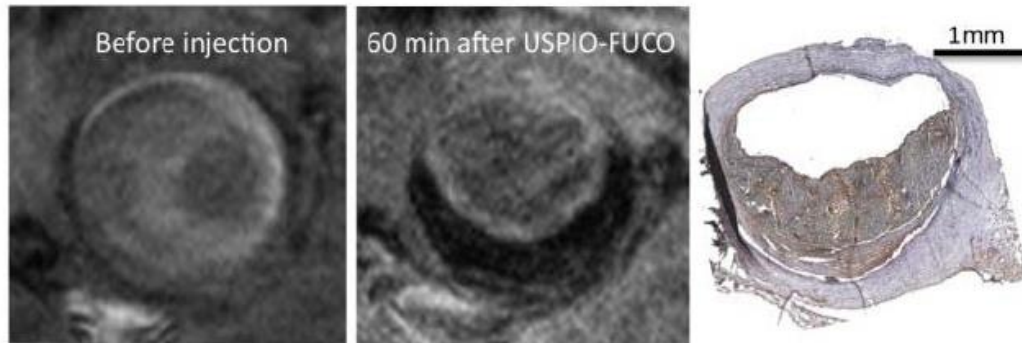


Autoradiography



P-selectin immunostaining

Nanoparticules : USPIO-Fuco

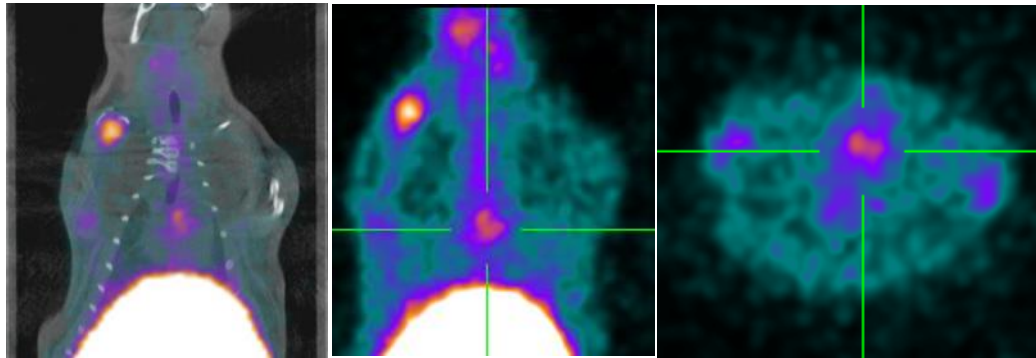


Perspectives

Nanoathero (FP7 - Coordinateur: D. Letourneur, U1148) :
développement d'un kit GMP pour le radiomarquage au ^{99m}Tc du
Fucoïdan et première injection à l'homme.

Agent d'imagerie TEP

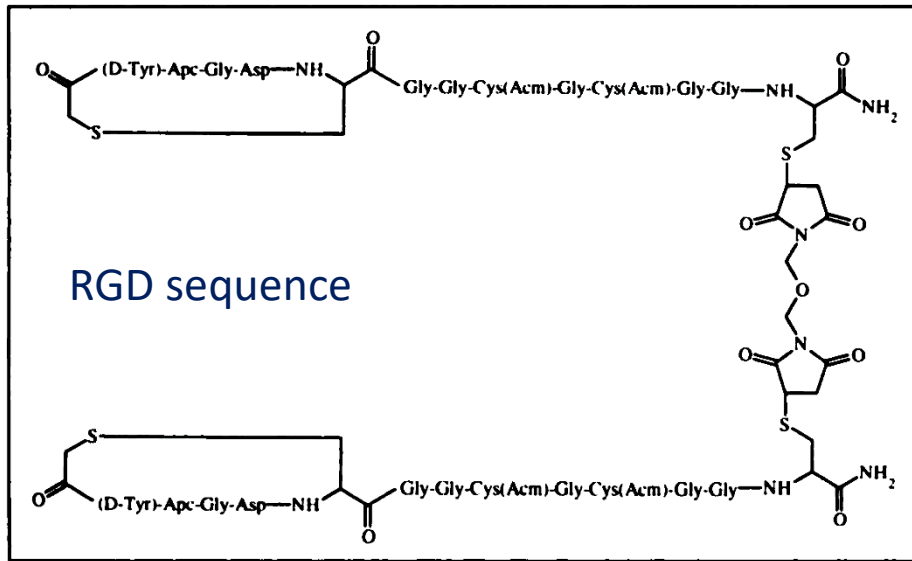
➤ Approche combinée PET/MR



^{68}Ga -Fucoïdan (Rana Ben Azzouna)

Brevet (WO/2010/116209 A1) : Fucoïdan as a ligand for P-selectin imaging.

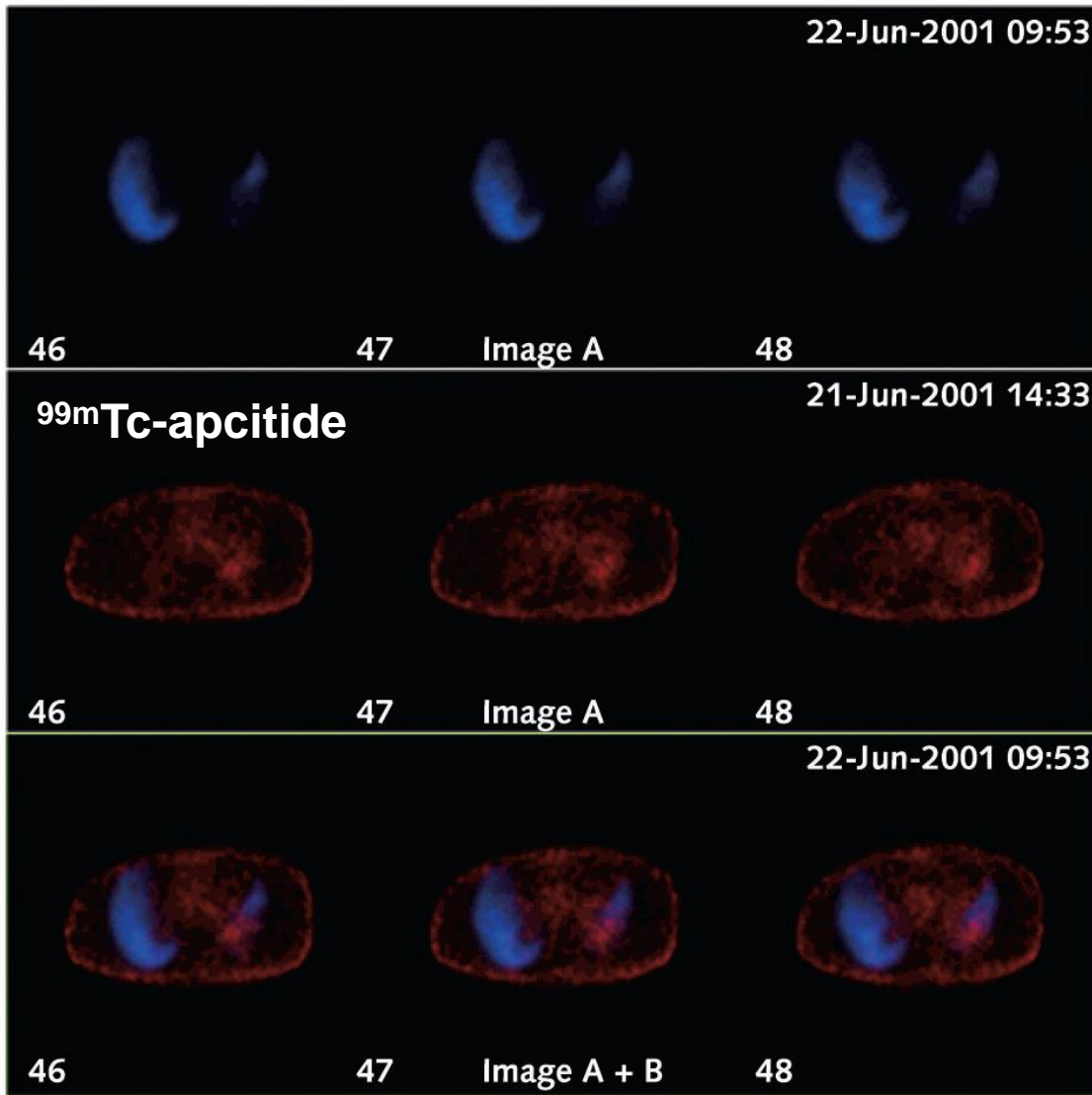
Ciblage GPIIb/IIIa : ^{99m}Tc -Apcitide



^{99m}Tc -HMPAO platelets



Thrombus of the femoral vein

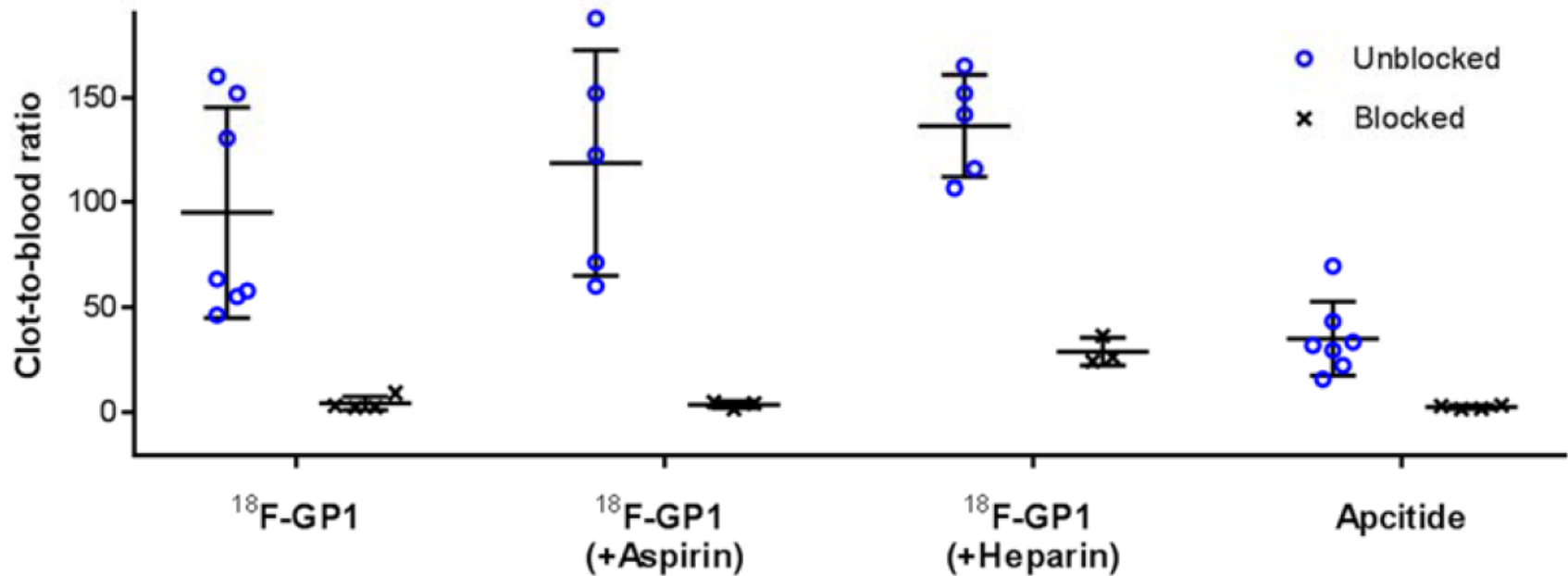


Fusion images (third row) demonstrate 2 large perfusion defects within the superior and lateral basal segments of the left lower lung lobe (lack of blue) with accumulation (shown in red) of technetium-99m apcitide within these noted perfusion defects.

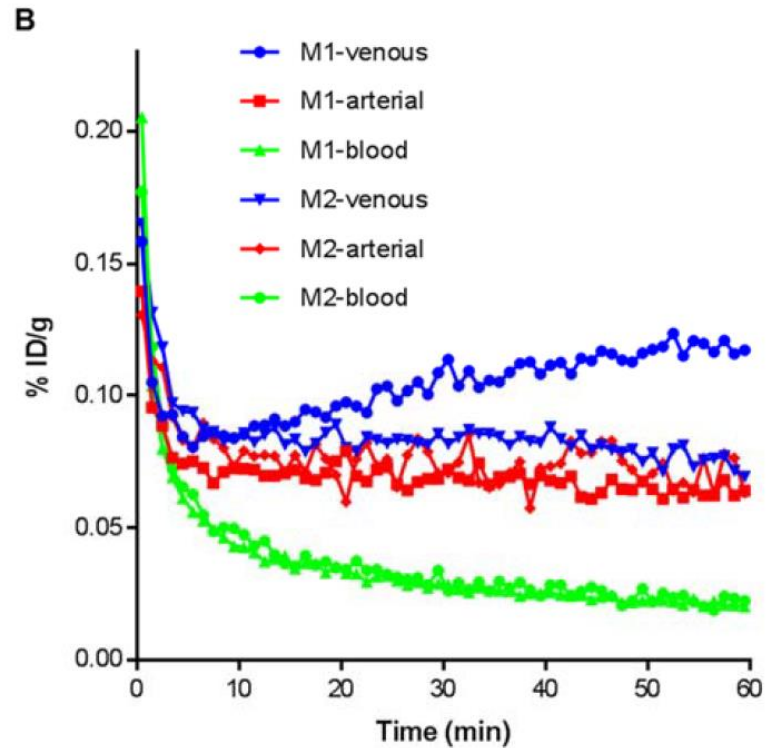
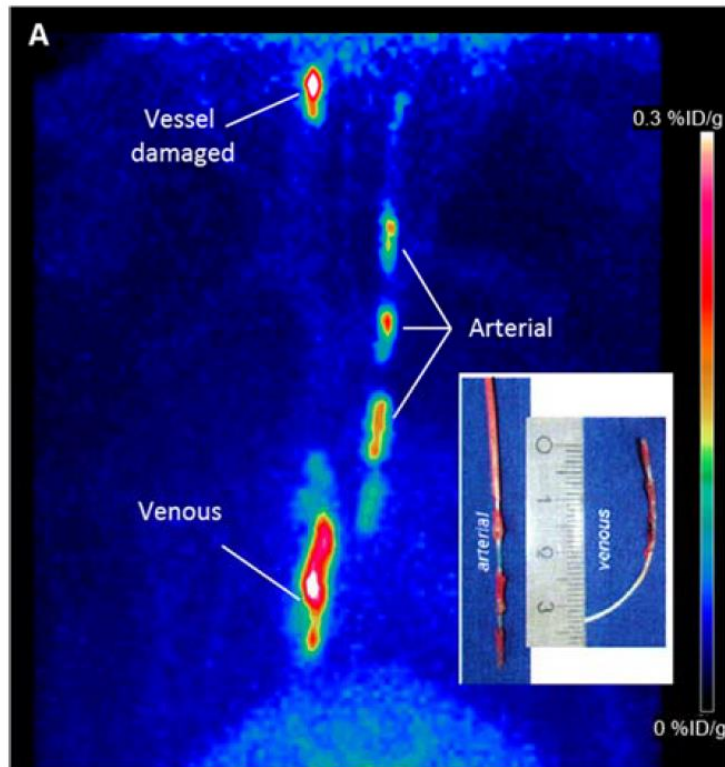
Ciblage GPIIb/IIIa en TEP : ^{18}F -GP1

- Développement : Bayer AG - Piramal Imaging GmbH
- GP1 : inhibiteur de la GPIIb/IIIa, analogue du Tirofiban
- IC_{50} pour GPIIb/IIIa: 20 nM

Contraste sang/thrombus frais



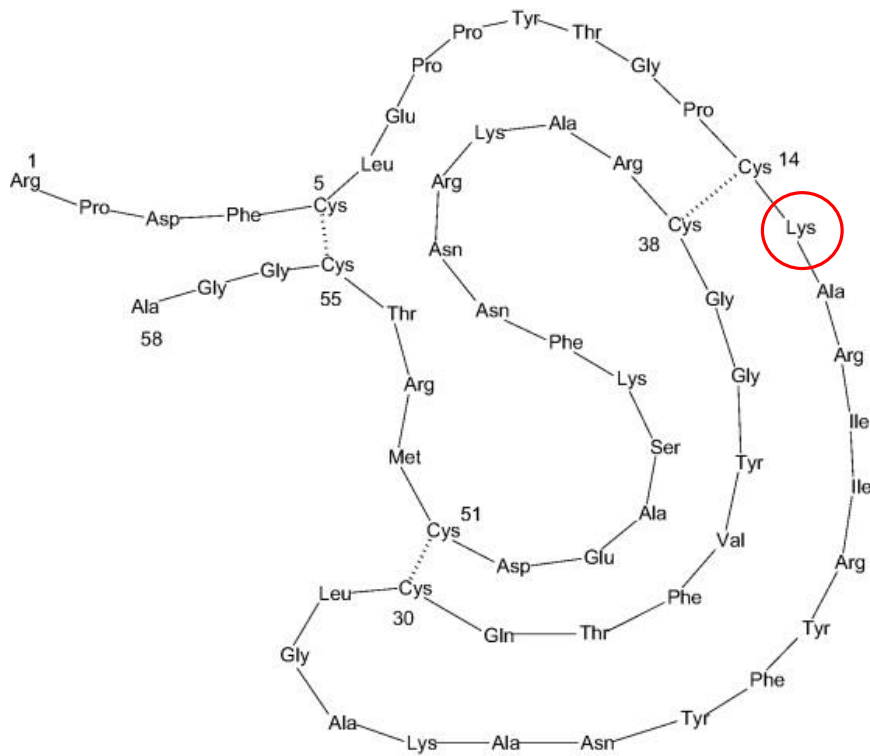
Ciblage GPIIb/IIIa en TEP : ^{18}F -GP1



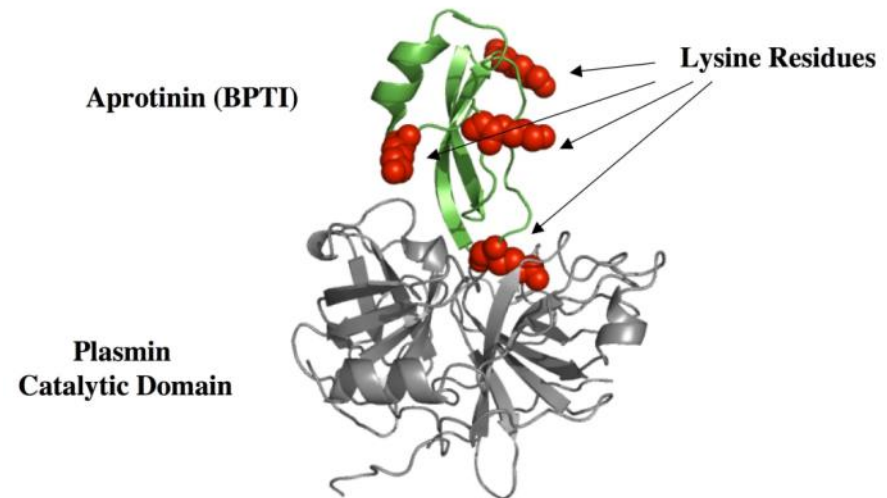
- Etude de première injection à l'homme en cours (NCT02469376)
- Anévrisme Aorte Abdominale / Thrombose veineuse profonde

Ciblage de la plasmine : ^{99m}Tc -aprotinine

Aprotinine : inhibiteur des sérine protéases à large spectre

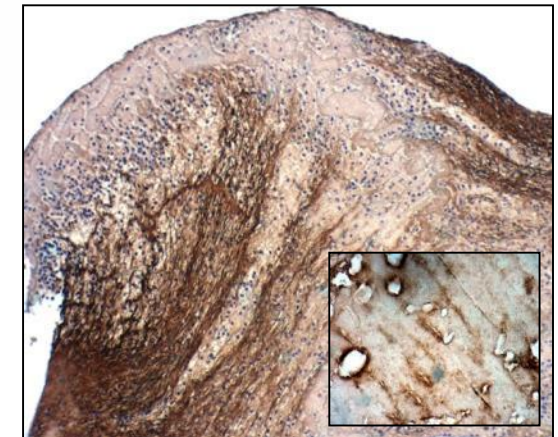
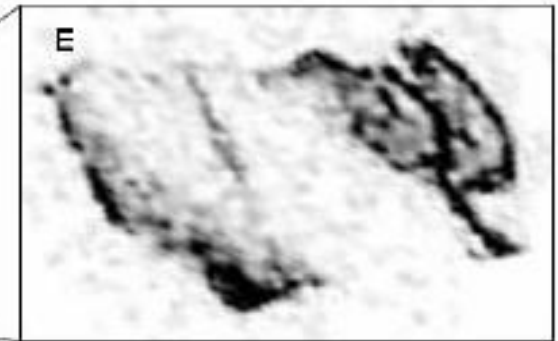
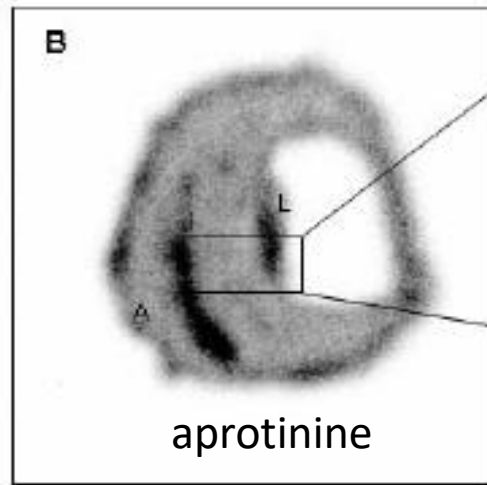
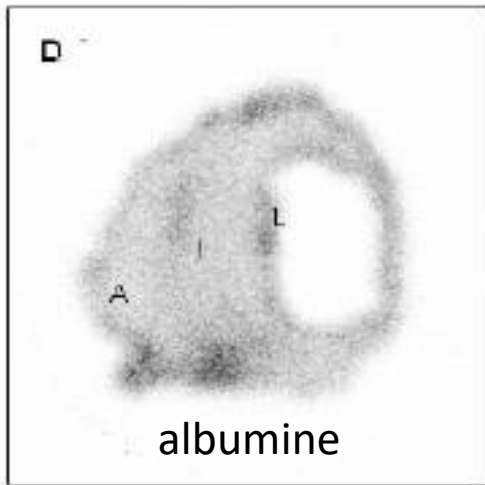


Poids moléculaire : 6511 Da



Ciblage de la plasmine : ^{99m}Tc -aprotinine

Topologie de l'activité plasminergique

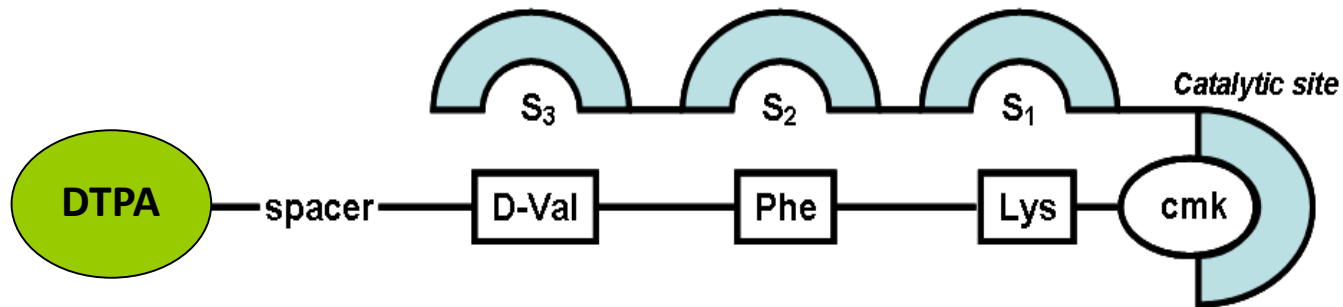


AAA humain - *ex vivo*

Plasminogène/plasmine

Ligand peptidique de la plasmine : PL704

Inhibiteur irréversible et sélectif de la Plasmine (VFK-cmk)

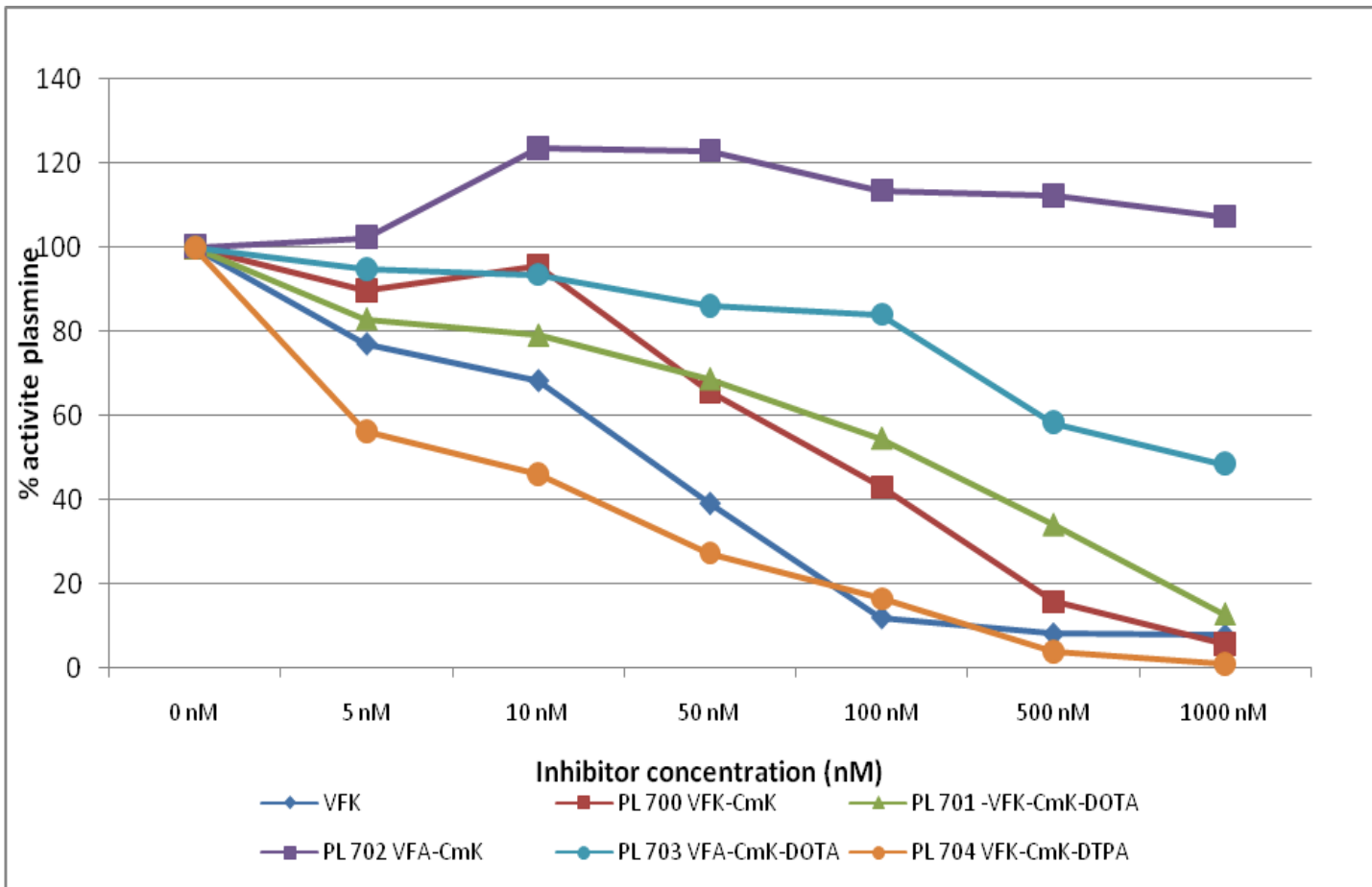


PL704 = DTPA-VFK-cmK (MW: 971 Da)

Brevet (WO2012072819A1). Agents for the molecular imaging of serine-protease in human pathologies.

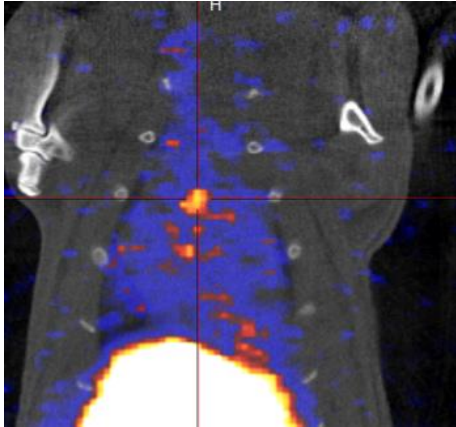
Affinité pour la plasmine

Pouvoir inhibiteur de l'activité plasmine

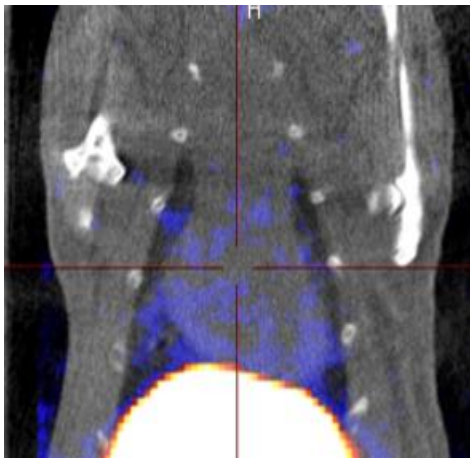


PL704 : Sélectivité du signal

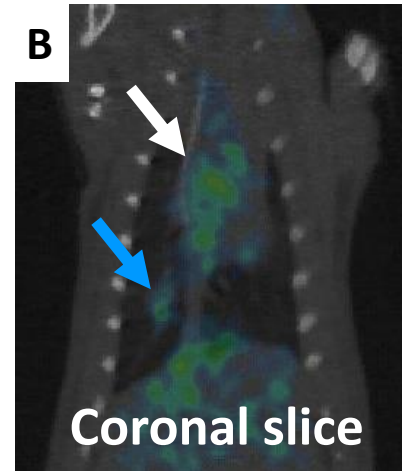
^{99m}Tc -PL704



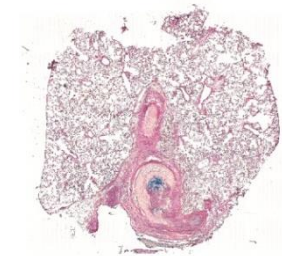
**Pré-injection VFK-cmk,
puis ^{99m}Tc -PL704**



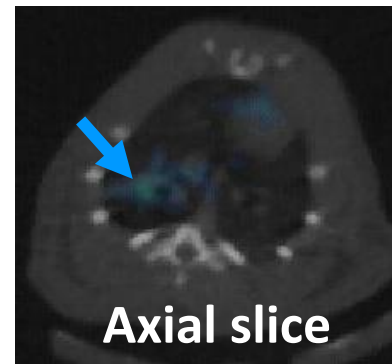
Right-sided endocarditis



Lung sample



Hematoxylin-Eosin



Autoradiography

En résumé

- Plusieurs cibles pertinentes
- Processus :
 - activation plaquettaire : formation / croissance
 - thrombolyse : résorption / risque embolique
- Nombreux échecs
 - renouvellement permanent des composants du thrombus
 - surface de l'interface sang/thrombus (problème si occlusif)
- Nouveaux candidats pour translation clinique

Contraintes / Indications

- Diagnostic rapide (½ vie courte)
- Coût faible
- Modalité
 - Facilité d'accès (échographie)
 - Corps entier (MN)
 - Résolution spatiale (TDM, IRM)
 - Sensibilité (MN)

