FAID 2007

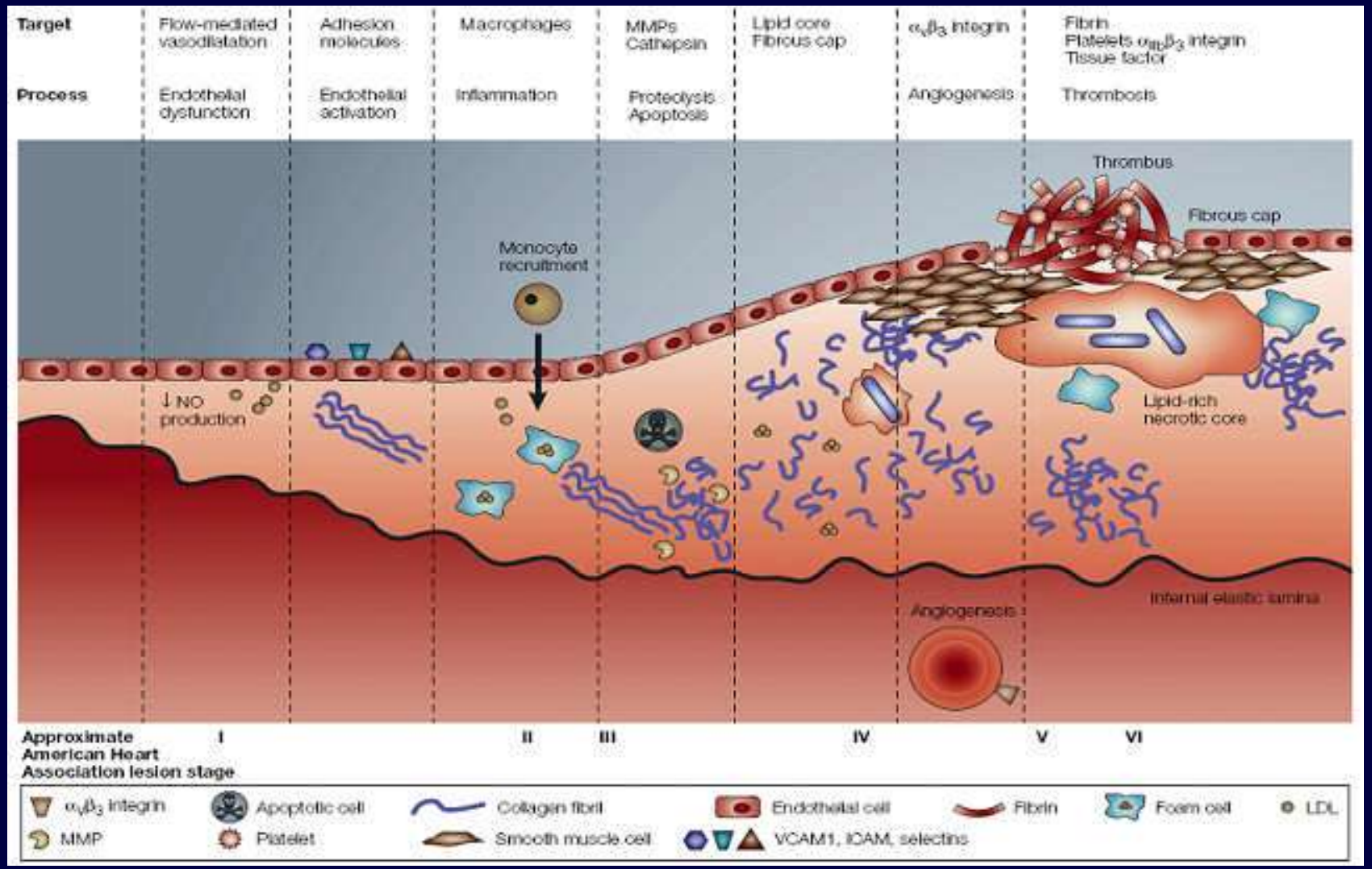
Development of contrast agents for molecular magnetic resonance imaging of vulnerable atherosclerotic plaques

Eric Lancelot and Claire Corot

Guerbet

Contrast for Life

Imaging Targets in Atherothrombosis



Choudhury RP et al, Nat Rev Drug Discov 2004

Inflammation Plays a Central Role in Atherothrombosis

- Vulnerable plaques (VP) are characterized by a large lipid core, a thin fibrous cap, and inflammatory cells at the thinnest portion of the cap surface. Matrix metalloproteinases (MMPs) secreted by macrophages lead to degradation of the cap

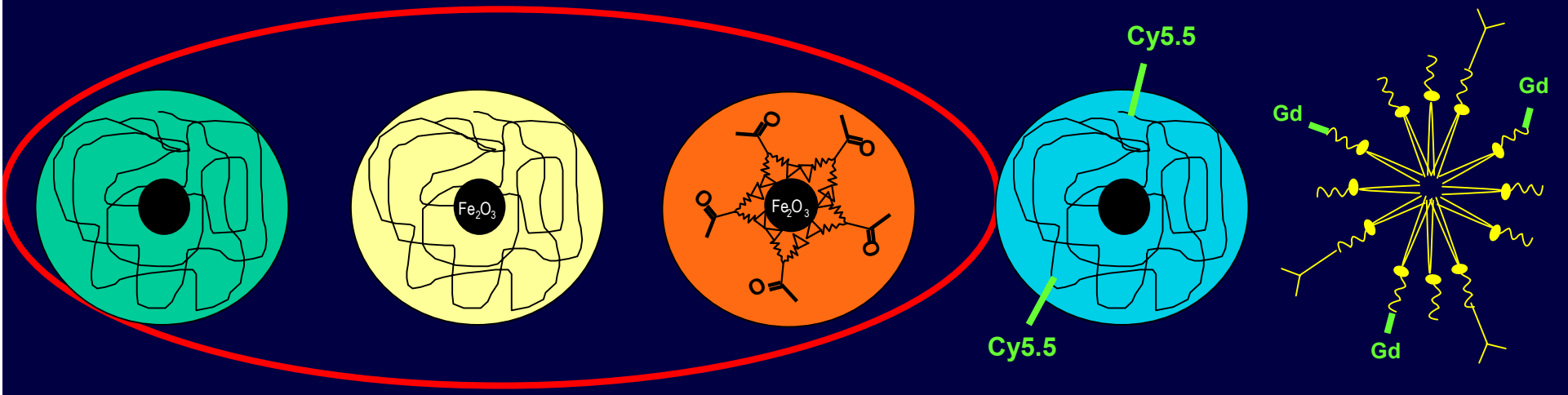
- Macrophage density
  - > in vulnerable plaques[1]
  - > in diabetic coronary arteries[2]
  - > in symptomatic carotid and coronary arteries[3,4]

- MMP expression
  - > in macrophage-rich areas[5]
  - > in atheromatous plaques[6]
  - > in symptomatic carotid plaques[7]

→ Macrophages and MMPs = ideal biomarkers of plaque vulnerability

1. Kong YZ et al, Cardiovasc Res 2005
2. Moreno PR et al, Circulation 2000
3. Fleiner M et al, Circulation 2004
4. MacNeill BD et al, JACC 2004
5. Sluijter JPG et al, Stroke 2006
6. Dollery CM et al, Circulation 2003
7. Loftus IM, Stroke 2000

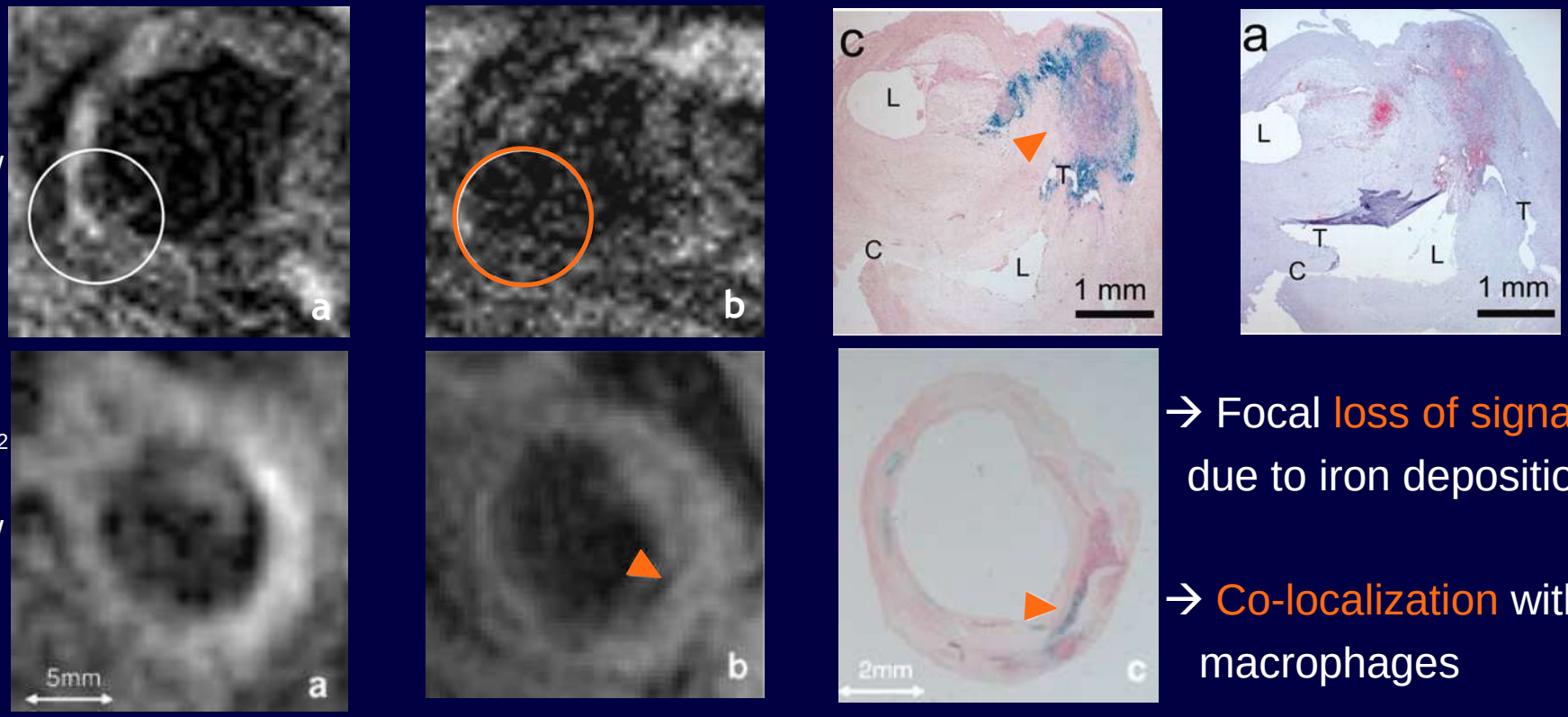
Several Contrast Agents Have Been Designed for the Detection of Macrophages by MRI in Atherosclerosis



Ferumoxtran-10	**Ferumoxytol**	**Novel USPIO**	**MFNP**	**Immunomicelles**
(USPIO)	(USPIO)	(USPIO)	($CLIO-_{47}-Cy_{5.5}$)	(Gd chelate)
Dextran coating[1]	Carboxymethyl dextran coating[1]	Monomeric coating	Dextran coating[2]	MSR-A targeting Ab[3]

1. Corot C et al, Adv Drug Delivery Rev 2006
2. Jaffer FA et al, Mol Imaging 2006
3. Lipinski MJ et al, MRM 2006

Ferumoxtran-10 Allows Non-invasive Detection of Activated Macrophages in Human Carotid Plaques



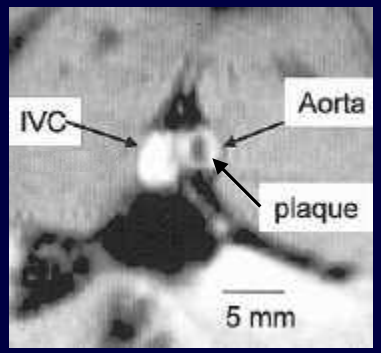
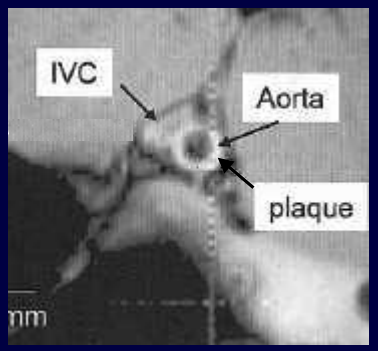
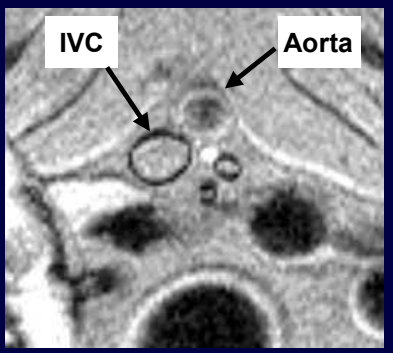
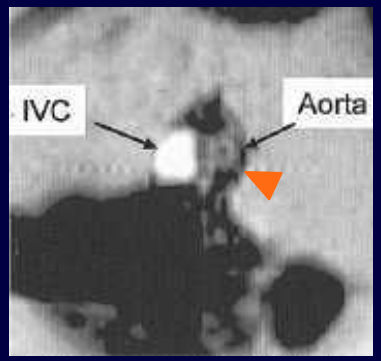
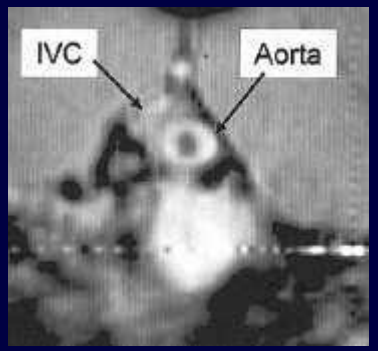
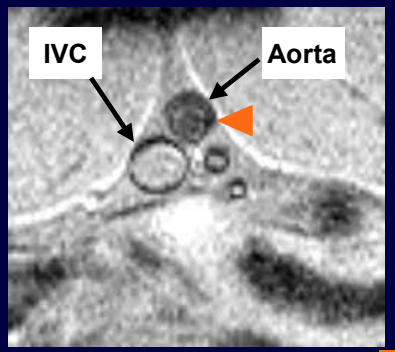
→ Focal loss of signal due to iron deposition

→ Co-localization with macrophages

→ Detection of inflammation in ipsilateral (100%) and contralateral (95%) carotid arteries from symptomatic patients with severe and moderate stenosis respectively[3]

1. Kooi ME et al, Circulation 2003
2. Trivedi RA, ATVB 2006
3. Tang T et al, Stroke 2006

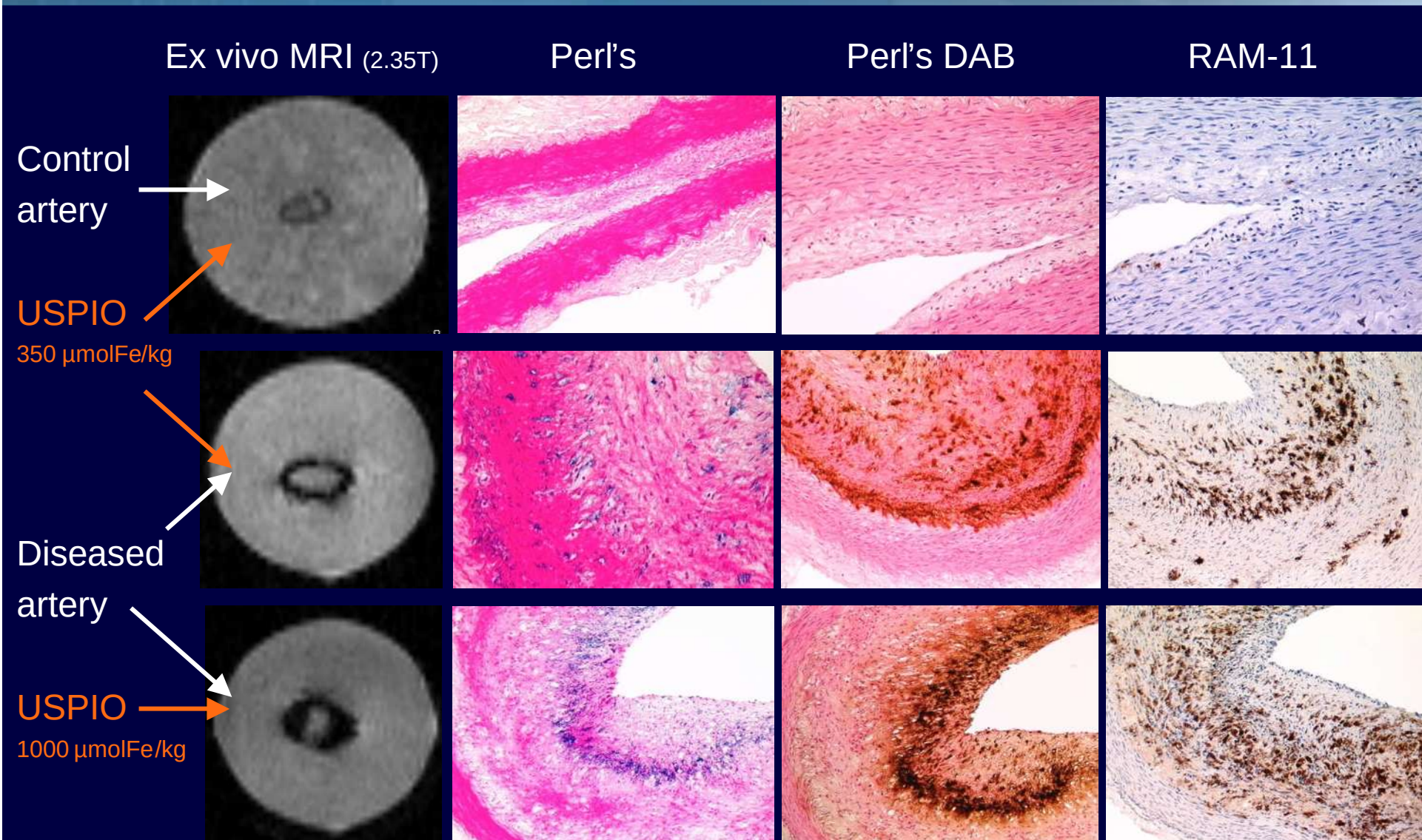
Some, but not all, USPIOs Produce Strong MR Signals in Plaques of Atherosclerotic Rabbits

	Ferumoxtran-10	Ferumoxytol	Novel USPIO
	Dose = 500 µmolFe/kg IV	Dose = 500 µmolFe/kg IV	Dose = 350 µmolFe/kg IV
	T1/2 = 48 h	T1/2 = 6 h	T1/2 = 3 h
Pre-contrast			
T2w GE 4.7T / 2.35T			
Post-contrast			

→ USPIO uptake by plaque macrophages is very dependent on the type of coating

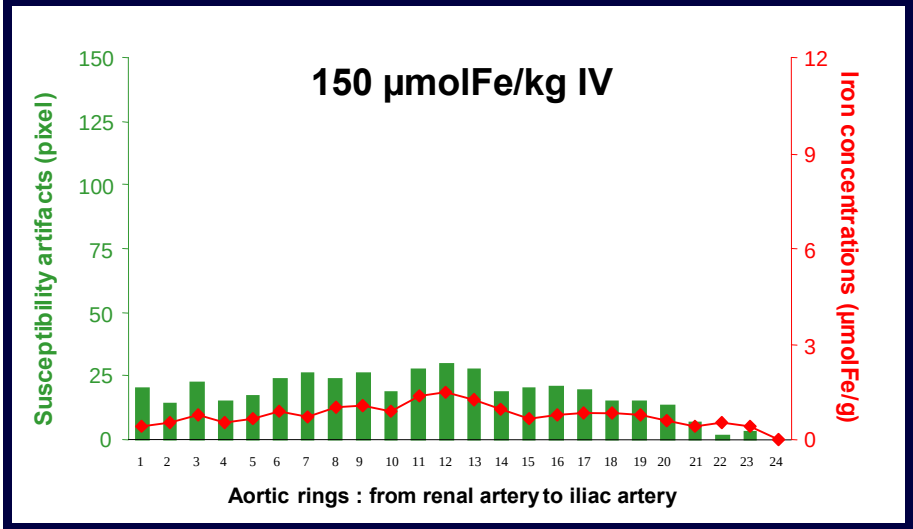
Yancy AD et al, JMRI 2005

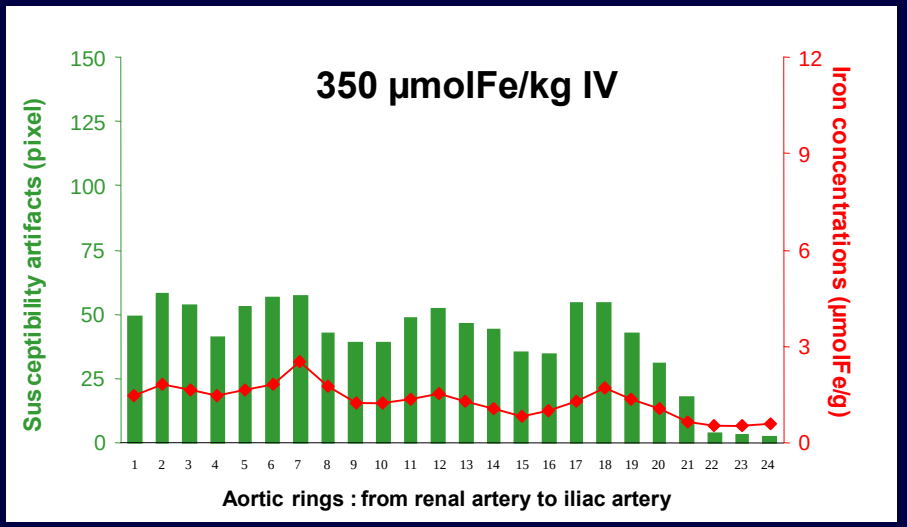
Dose-Response of a USPIO in a Rabbit Model of Atherosclerosis (1/2)

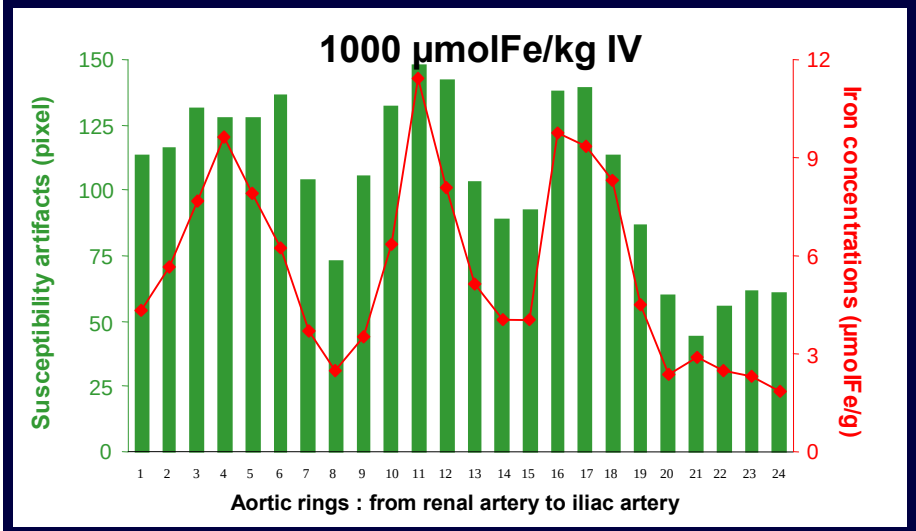


Dose-Response of a USPIO in a Rabbit Model of Atherosclerosis (2/2)

MRI (2.35T) and iron measurements were performed on aortic rings after USPIO injection







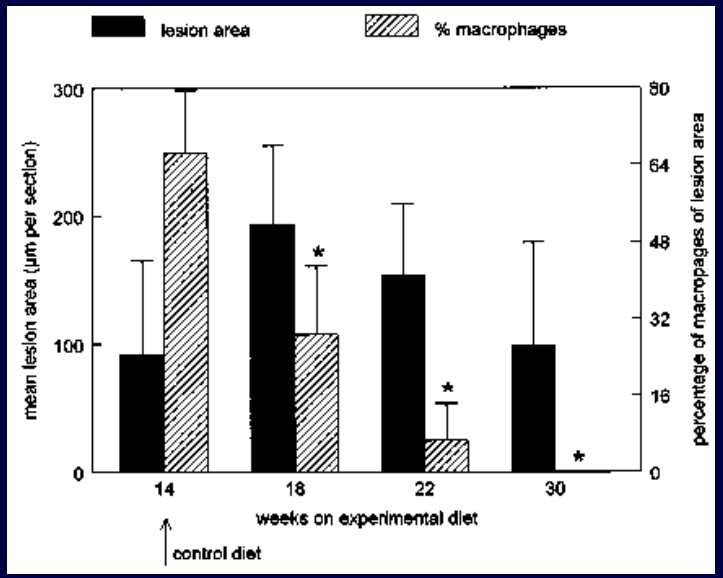
→ P904-induced magnetic susceptibility artifacts (MSA) are dose-dependent

→ MSA are closely related to iron content

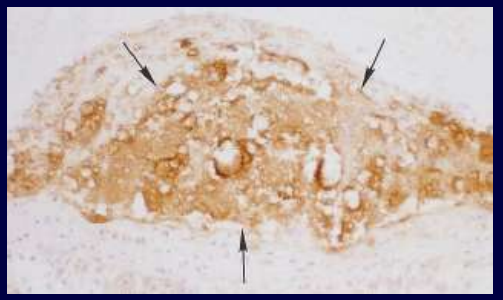
→ Artery segments with more severe lesions display higher MSA

Selection of an Animal Model with / without VP for Screening of a USPIO

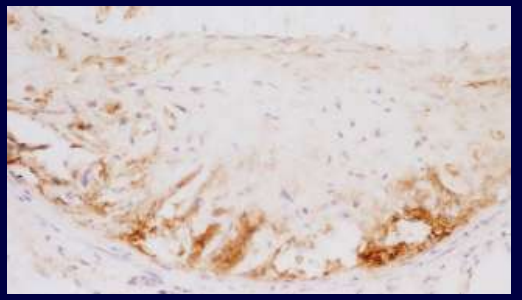
- ApoE3*Leiden mice develop atherosclerotic plaques in the aorta. The content of macrophages in these plaques can be modulated by the diet[1]



MOMA-2



High-chol. diet : 14 weeks



High-chol. diet : 14 weeks

+ Control diet : 16 weeks

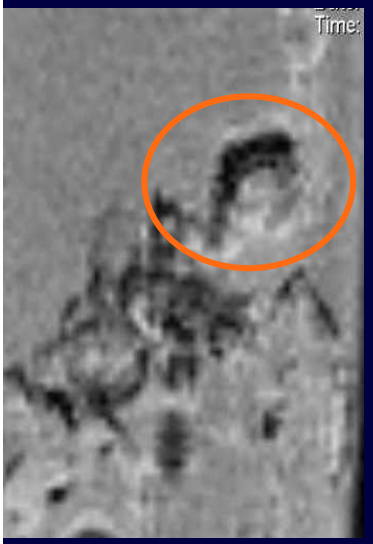
- A USPIO (1000 µmolFe/kg IV) was injected into ApoE3*Leiden mice fed with :
  - High-chol. diet for 26 weeks : progression group
  - High-chol. diet for 16 weeks + control diet for 10 weeks : regression group

Gibels MJJ et al, Atherosclerosis 1999

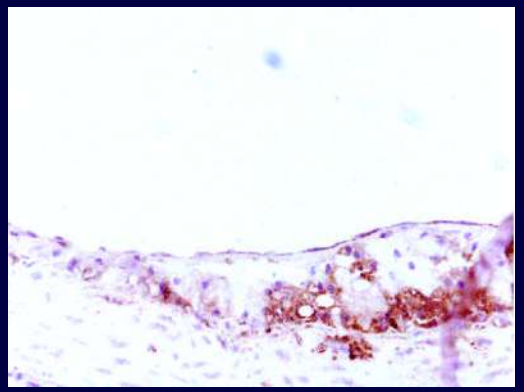
A USPIO Differentiates Mice with Macrophage-rich Plaques from Mice with Macrophage-poor Plaques

Progression group

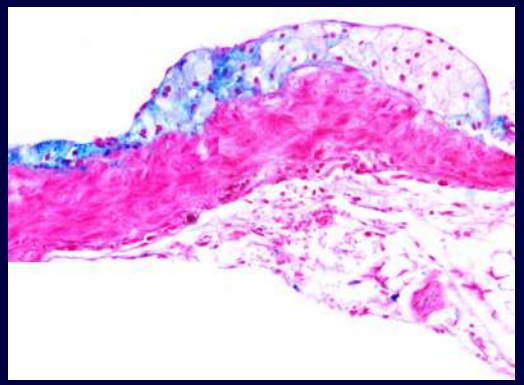
Ex vivo MRI (2.35T)



Mac-3



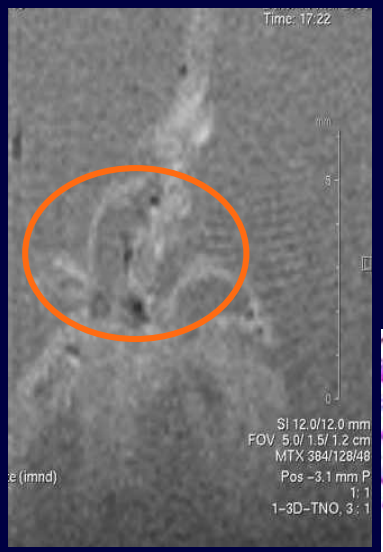
Perl's



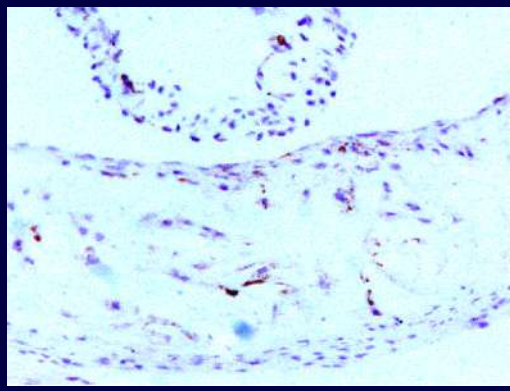
3D-MSA = 36 ± 16%

Regression group

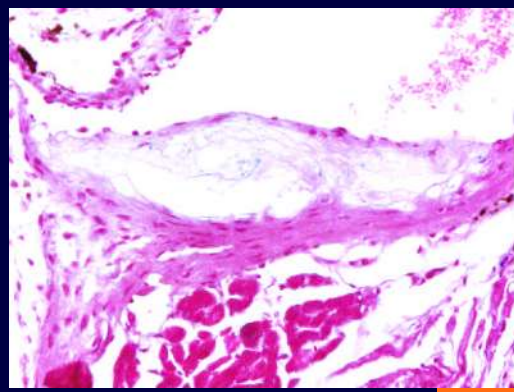
Ex vivo MRI (2.35T)



Mac-3



Perl's

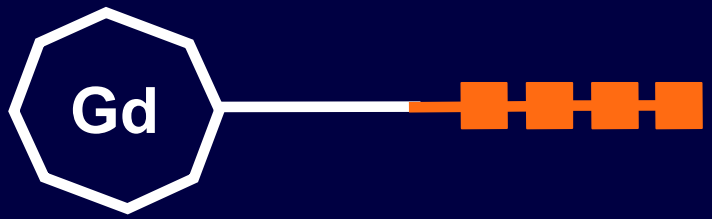


3D-MSA = 12 ± 5%

(3D-MSA = nb of pixels showing a magnetic susceptibility artifact / nb of pixels of the whole aortic arch, $p<0.05$)

MMPs, Another Interesting Target for VP Detection

- P947 is a Gd chelate combined with an MMP-inhibiting peptide



IC_{50} values (M)

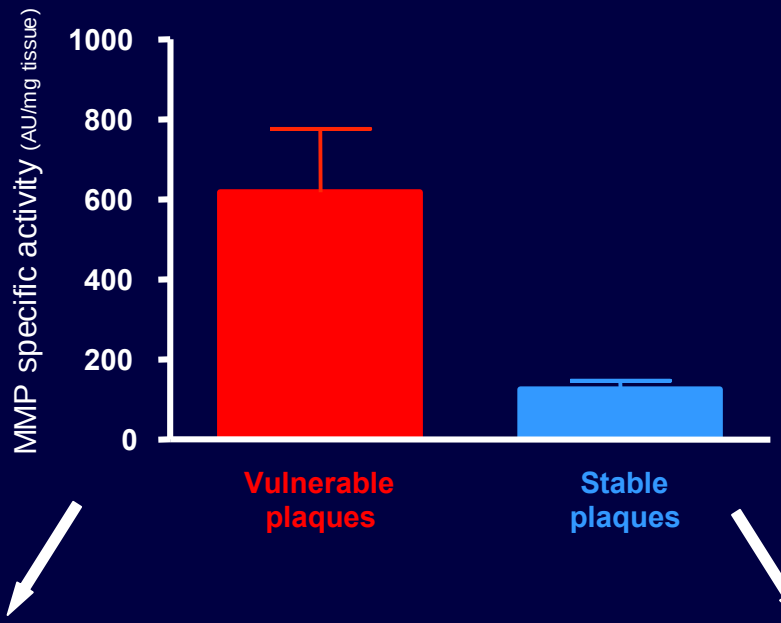
	Positive control (GM6001)	Gd-DOTA	P1135	Free peptide	P947
MMP-1	1.8E-09	> 1.0E-04	> 1.0E-03	1.0E-05	1.0E-06
MMP-2	3.2E-09	> 1.0E-03	> 1.0E-03	1.0E-05	1.0E-06
MMP-3	1.0E-08	> 6.7E-03	nd	1.5E-04 [a]	1.0E-05
MMP-8	5.8E-10	> 1.0E-04	nd	4.2E-07	1.0E-07
MMP-9	8.6E-10	> 6.7E-03	> 1.0E-03	1.7E-05	1.0E-05
MMP-13	5.2E-10	> 1.0E-04	nd	1.2E-06	1.0E-06
MMP-14	3.1E-09	> 1.0E-03	> 1.0E-03	nd	1.0E-04

→ P947 is a broad spectrum MMP inhibitor

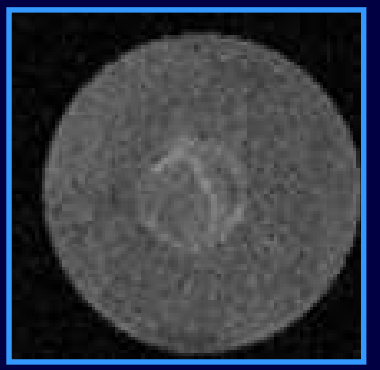
Lancelot E et al, AHA 2005

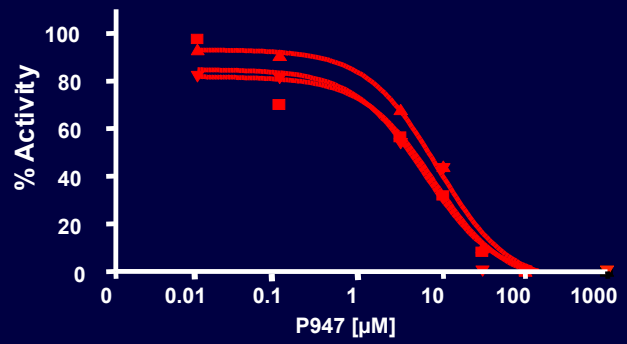
Ex Vivo, P947 Enhances MR Signal of Human Carotid Plaques in an MMP-Dependent Manner

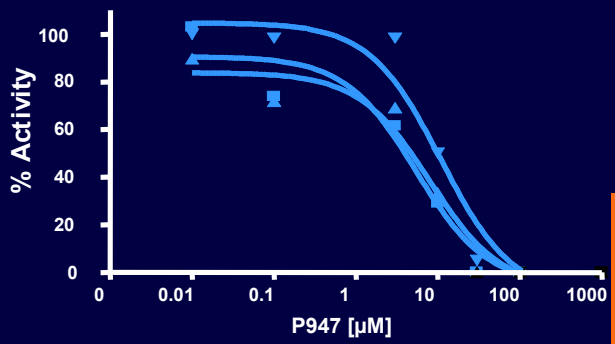
Ex vivo MRI (2.35T)



Ex vivo MRI (2.35T)

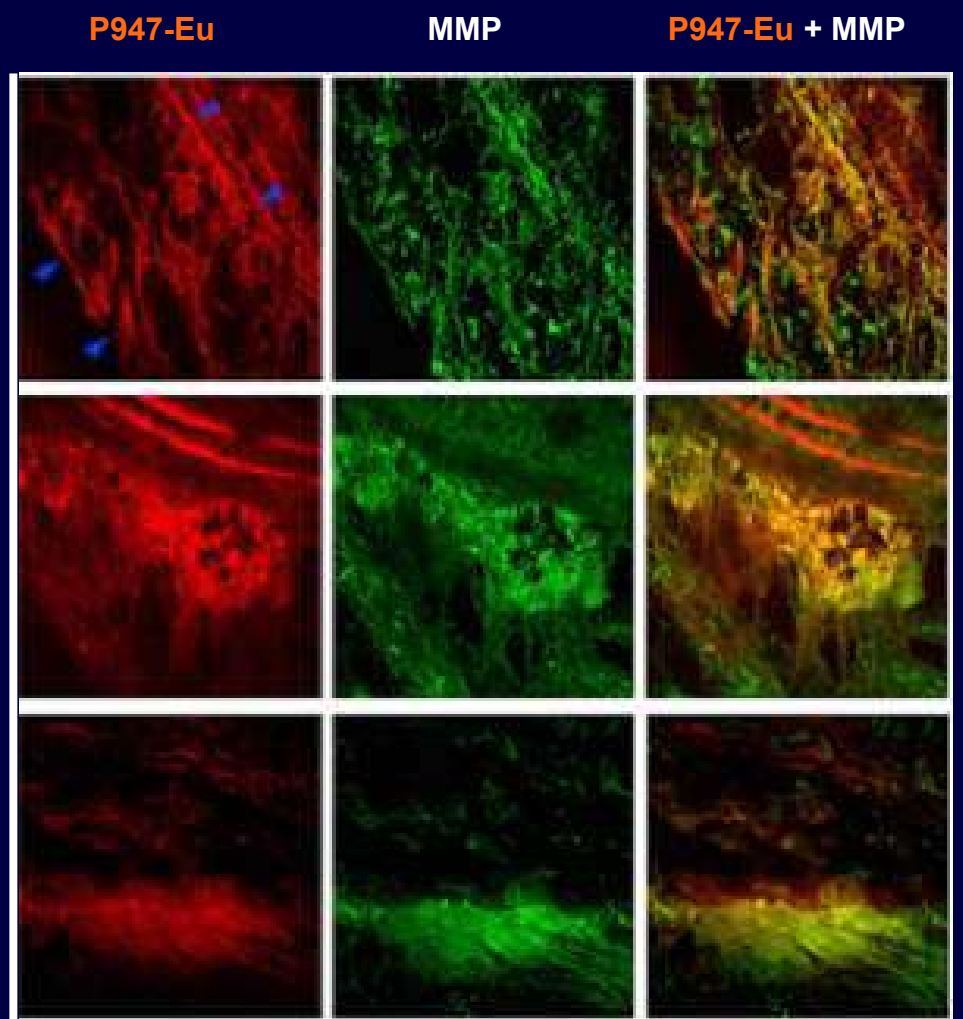






P947 Co-localizes with MMPs in ApoE-KO Mice

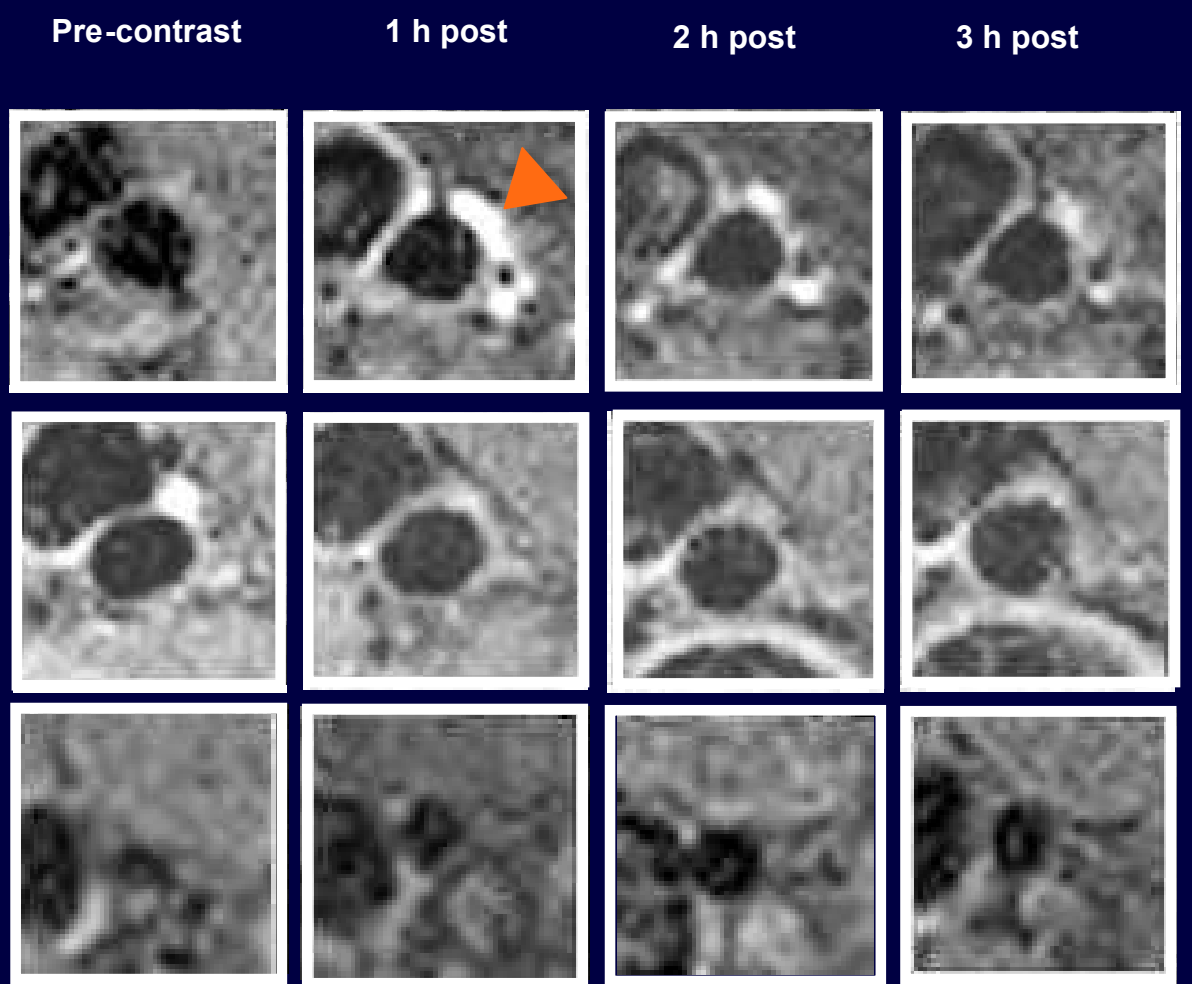
Confocal microscopy of aortic samples after P947 injection (100 µmolGd/kg IV)



Amirbekian V et al, AHA 2006

P947 Enhances MR Signal of Atherosclerotic Plaques in ApoE-KO Mice

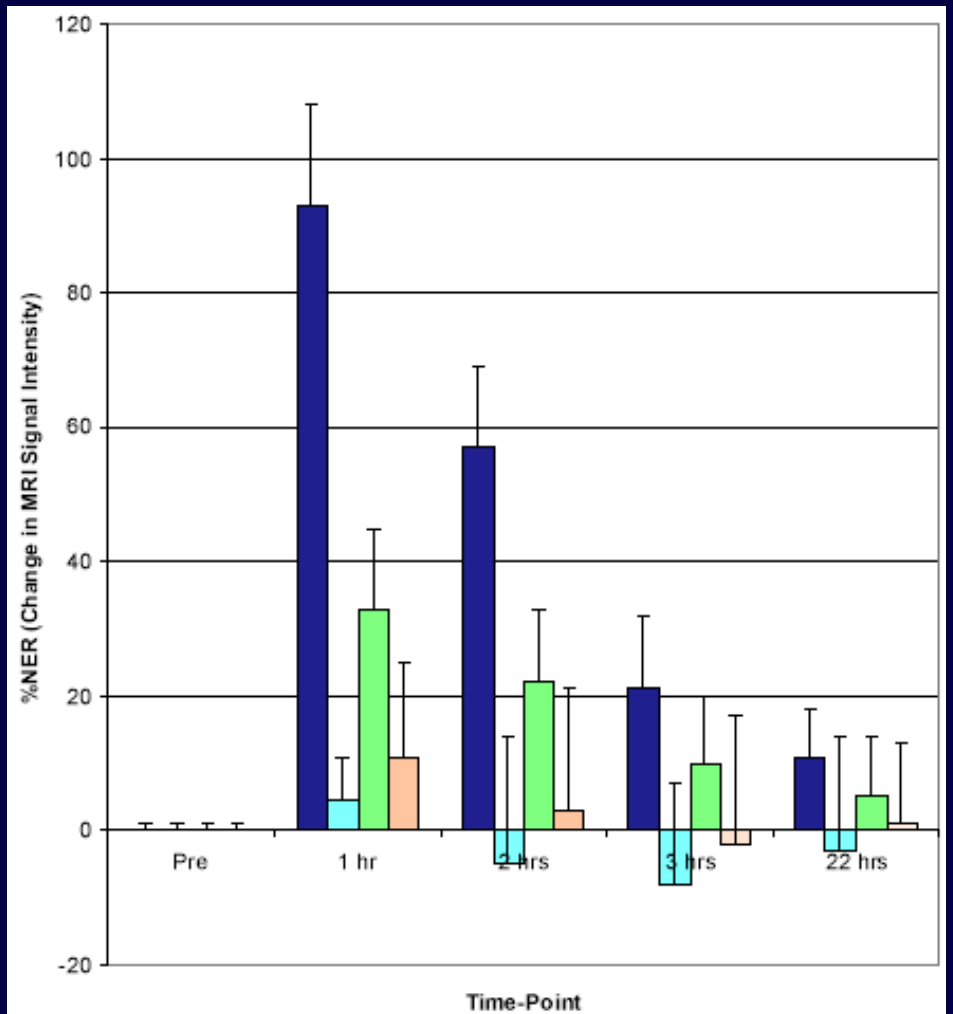
In vivo MRI images (9.4T) after contrast agent injection (100 µmolGd/kg IV)



Amirbekian V et al, AHA 2006

P947 is 3 Times More Efficient Than P1135 to Enhance Atherosclerotic Plaques in ApoE-KO Mice

MRI signal intensity measurements after contrast agent injection (100 µmolGd/kg IV)



P947 in ApoE-KO mice

Dotarem^{R} in ApoE-KO mice

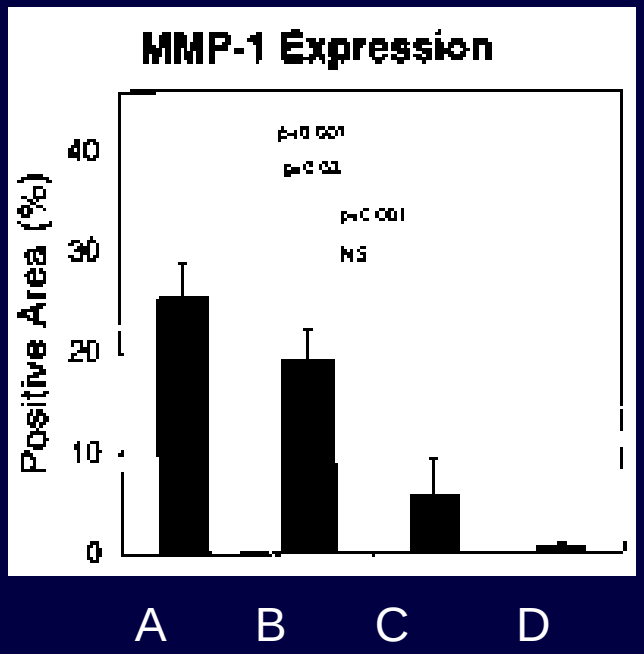
P1135 in ApoE-KO mice

P947 in WT mice

Amirbekian V et al, AHA 2006

Selection of an Animal Model with / without VP for P947 Screening

- Rabbits develop atherosclerotic plaques in the aorta after high-cholesterol diet + balloon injury. The content of MMPs in plaques can be modulated by the diet[1]



A : 4 months of high-cholesterol diet

B : A + 16 months of high-cholesterol diet

C : A + 8 months of low-cholesterol diet

D : A + 16 months of low-cholesterol diet

- P947 (50 µmolGd/kg IV) was injected into atherosclerotic rabbits fed with :
  - High-cholesterol diet for 8 months : progression group
  - High-cholesterol diet for 4 months + control diet for 4 months : regression group

Aikawa M et al, Circulation 1998

P947 Discriminates MMP-rich Plaques from MMP-poor Plaques in Atherosclerotic Rabbits

T1w, SE, 1.5T

50 µmolGd/kg

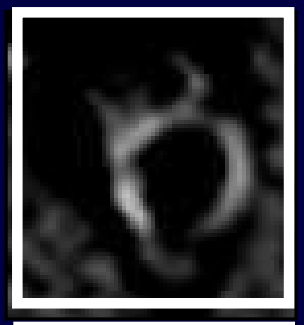
4 months Pre-contrast

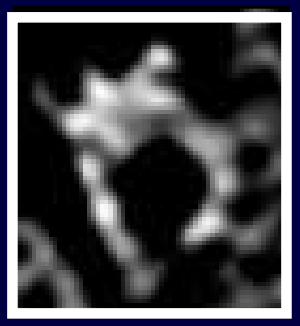
4 months Post-P947

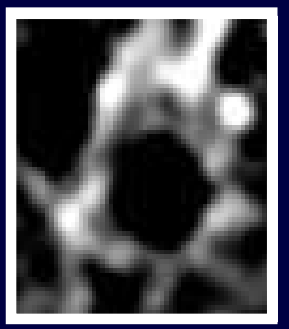
8 months Post-P947

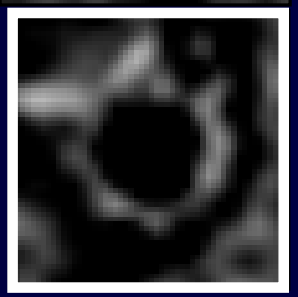
Progression group

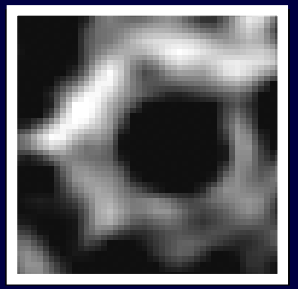
Regression group

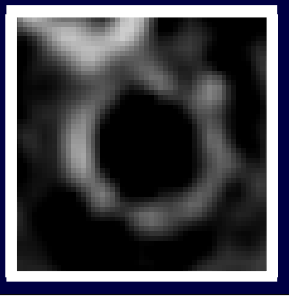


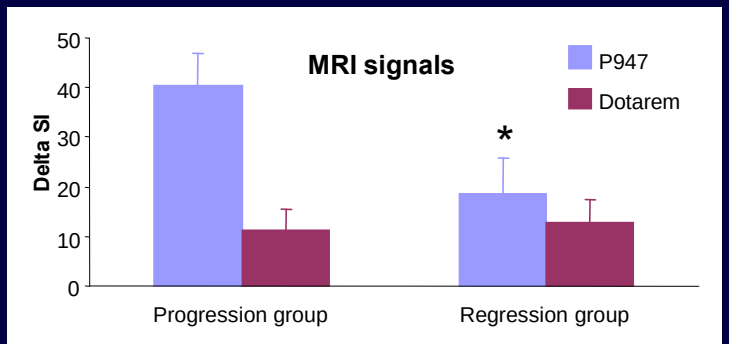


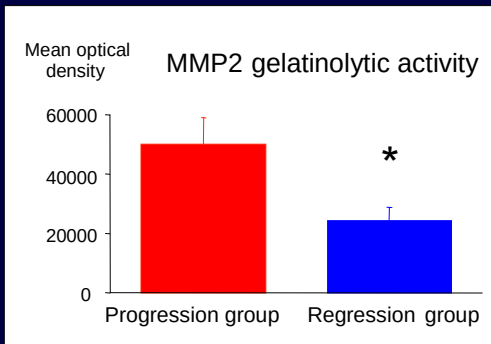












Hyafil F et al, AHA 2006

Conclusions

- A USPIO or P947 may be used to monitor macrophage content or MMP activity by MRI in atherosclerotic plaques
- It is very probable that some contrast agent will be commercially available for MRI detection of VP within the next 5-10 years
- However, several important questions have still to be solved :
  - → What is the target population : carotid or coronary atherosclerotic patients ?
  - → What is the most important need : identify high-risk patients, predict events, monitor treatment efficacy?
  - → What is the most prefered contrast agent (Gd or USPIO) : diagnostic efficiency, easiness to use ?
  - → What is the best MRI protocol : sequence, timing, image acquisition, image post-processing ?
  - → Is spatial resolution a mandatory requirement for VP detection in coronary arteries ?
- Clinical studies are necessary to validate the concepts of VP detection

Thanks to all my colleagues from the GUERBET Research department

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- → Dr. J. Gillard et al. (Cambridge, UK)
- → Dr. JB. Michel et al. (Paris, France)

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