Systemic Therapy: Pharmacological Approach or Cell/Gene Therapy

Alan C. Yeung, MD

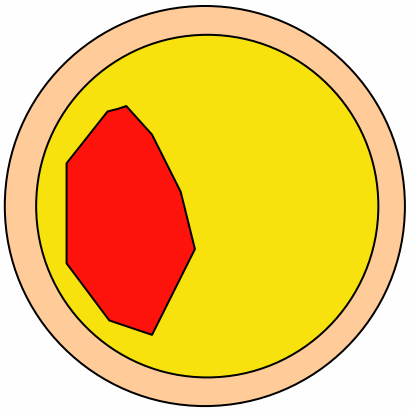
Stanford University School of Medicine

20 Billions dollars question:

- How do we find it (them) ?
- When should we look for them ?
- How do we treat them ?

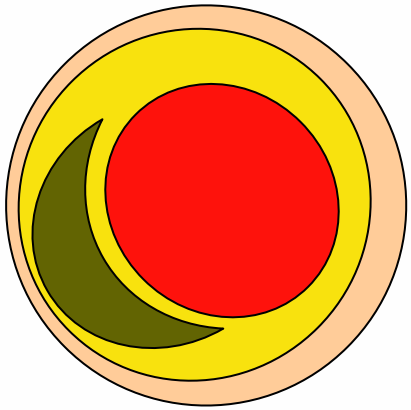
What is the Vulnerable plaque?

Stable (obstructive)



- Progressively flow-limiting
- Often causes chest pain
- Detected by angiography
- Main target of interventional therapies (angioplasty, stents)

Vulnerable (non-obstructive)

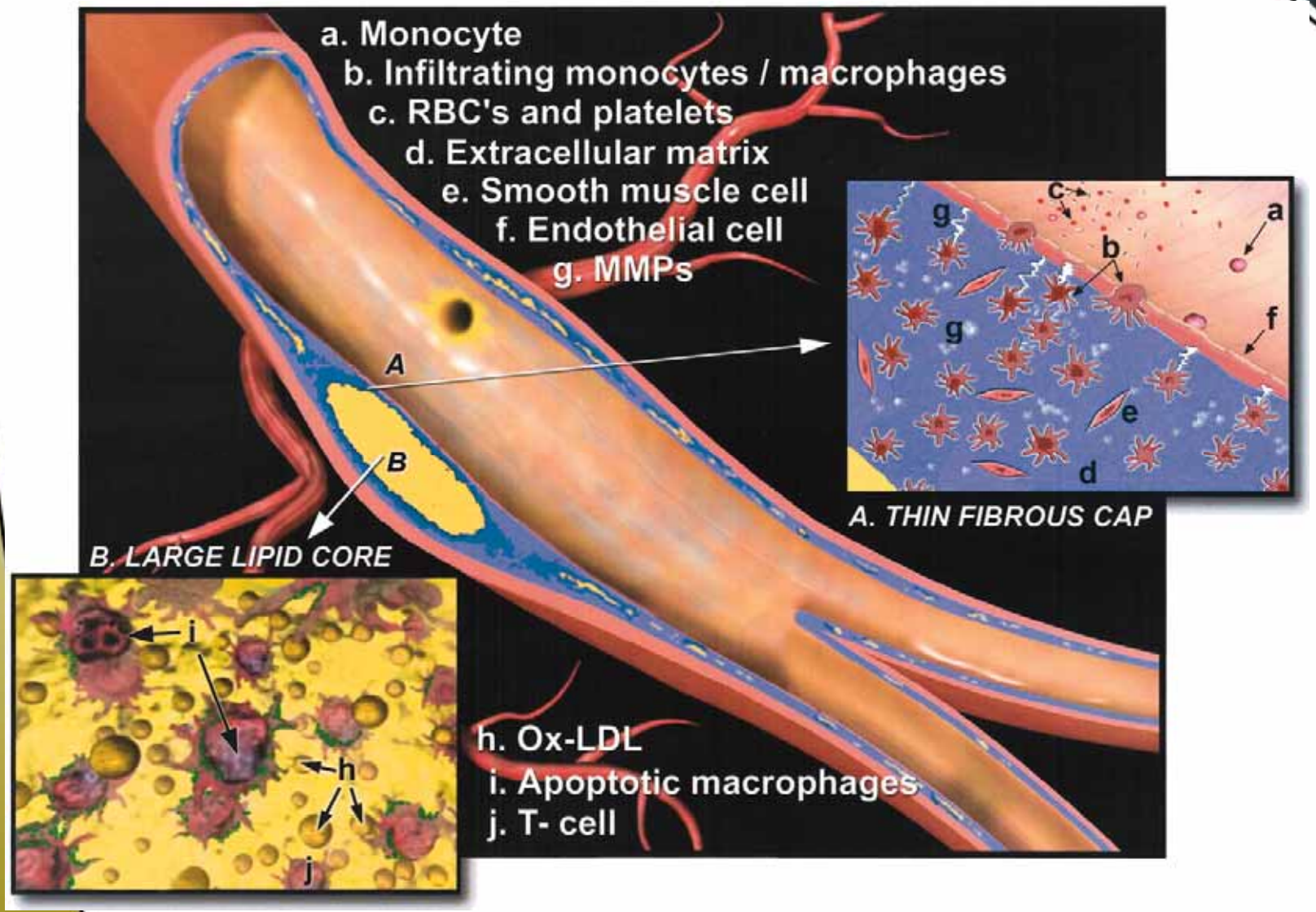


- Minimal effect on blood flow
- First symptom is often sudden death
- No established detection method
- Preventative drug therapies; directed therapies still unproven

TCFA

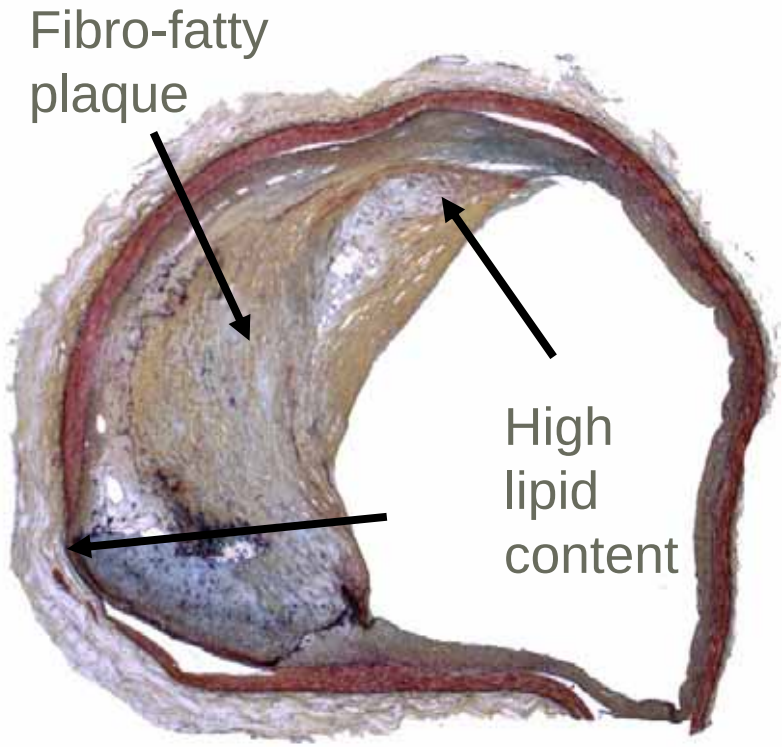
- Thin cap
  - Fibrous cap < 65 μm
  - Collagen depletion (due to loss of smooth muscle)
  - Inflammatory cells (macrophage, lymphocyte)
- Lipid rich plaque
  - Hemorrhagic, necrotic core (size > 1.0 mm^2 and/ or > 10% of the plaque area)
  - Angiogenic blood vessels into intima from the adventitia

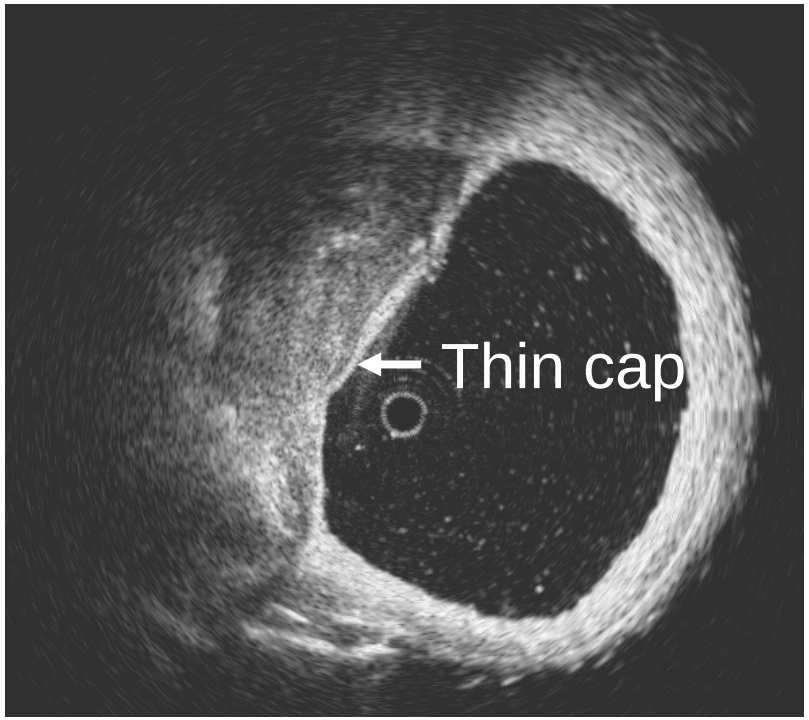
Risks of Plaque Rupture



What are the possible targets for treatment ?

(1) Thin Cap



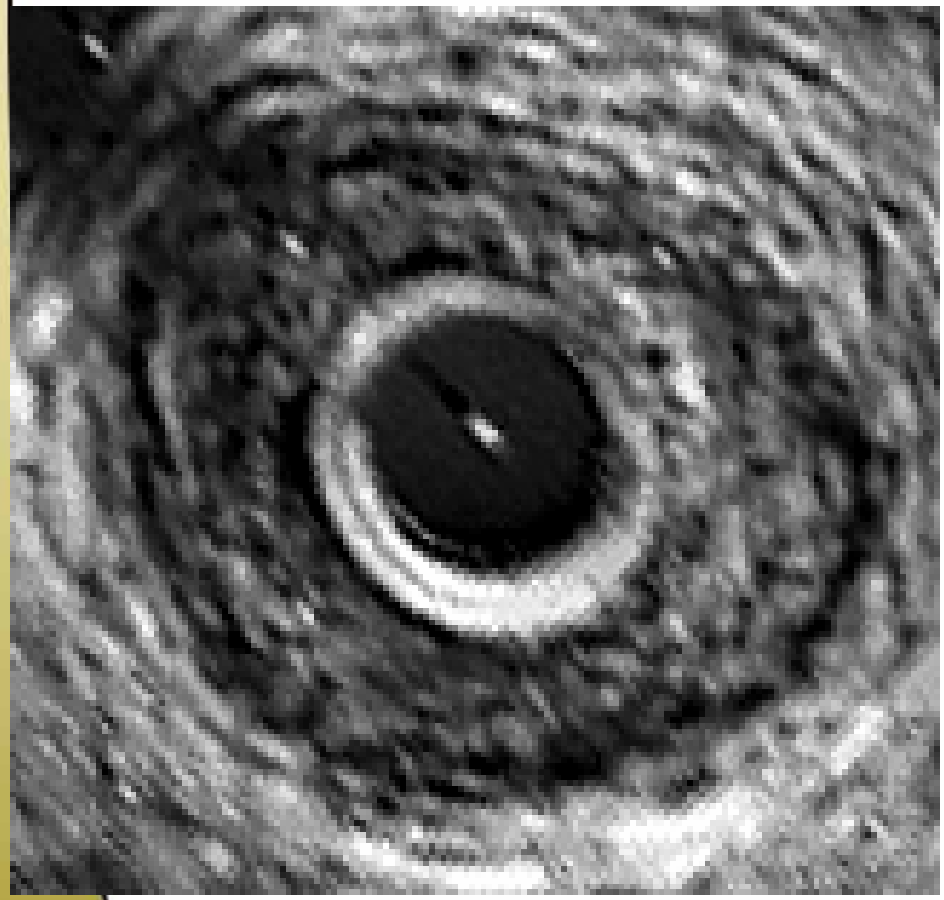


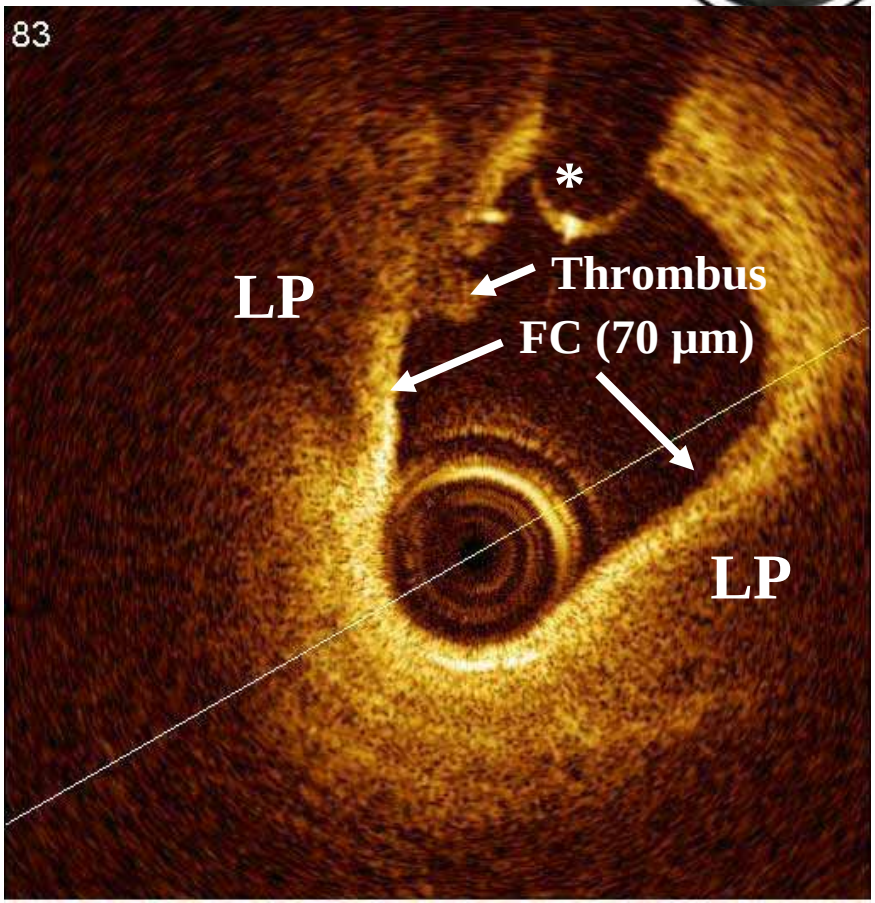
Stained histology section

OCT

Histology courtesy of E. Mont and R. Virmani, Armed Forces Institute of Pathology, Washington, DC

Thin Cap

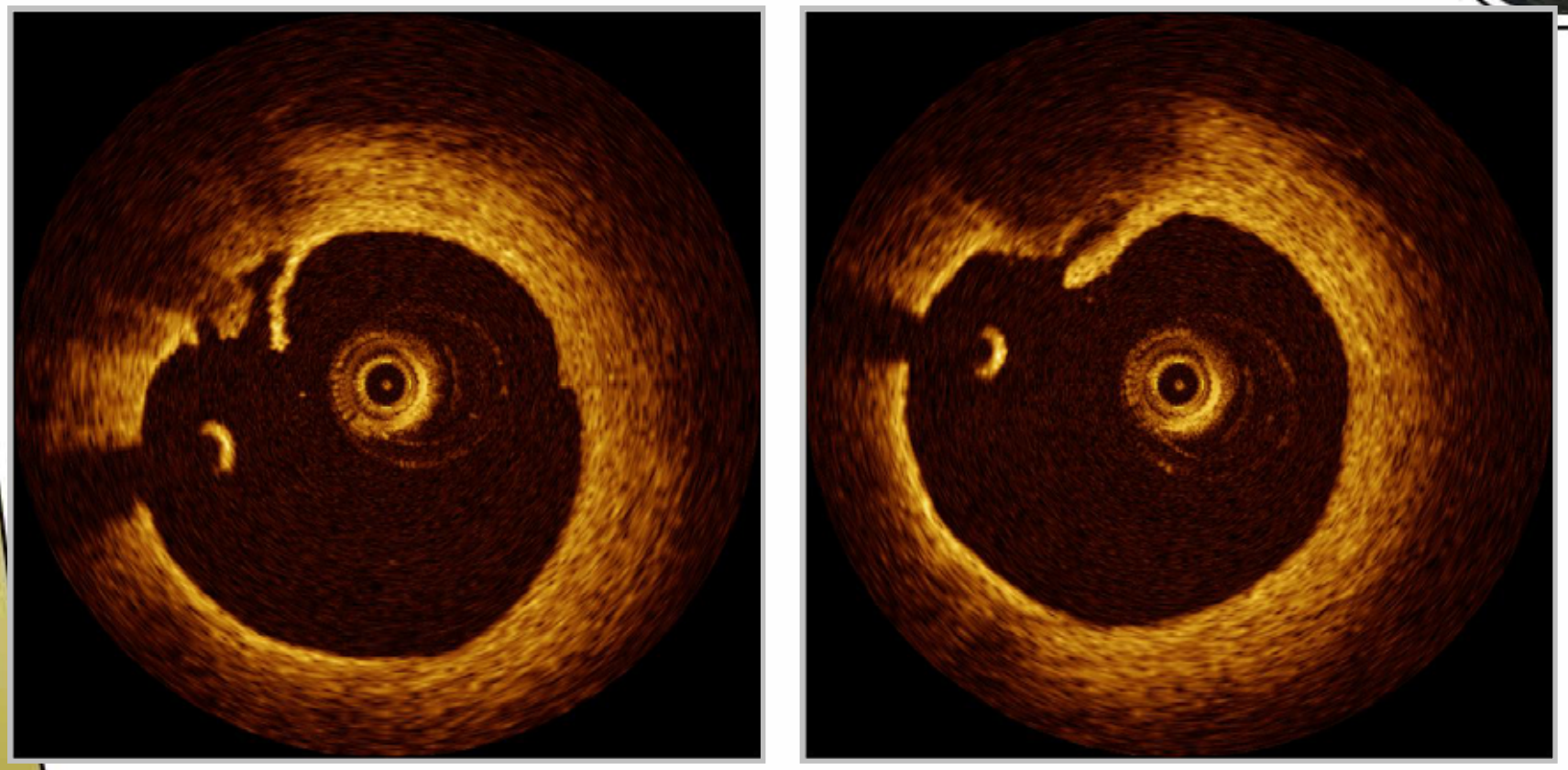




LP:Lipid pool
FC:Fibrous cap
*: Guidewire artifact

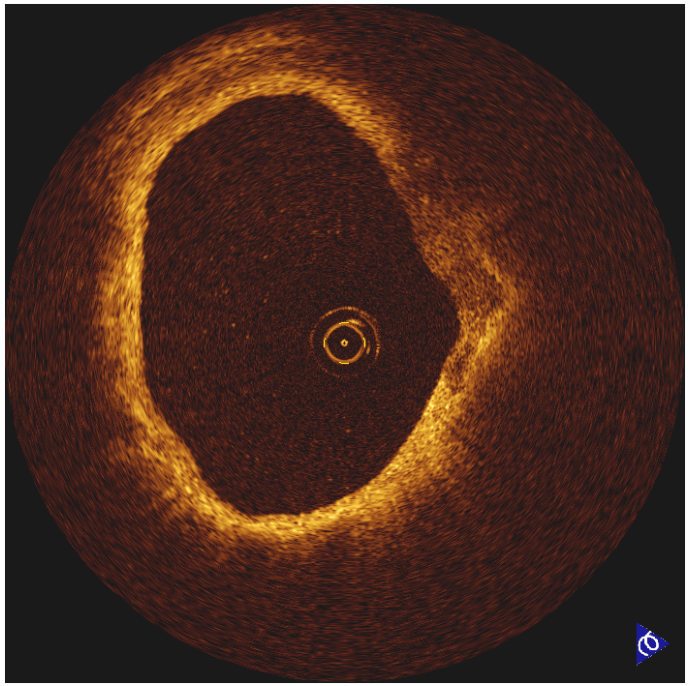
Dr. Suzuki and Dr. Katoh Toyohashi Heart Center, Japan

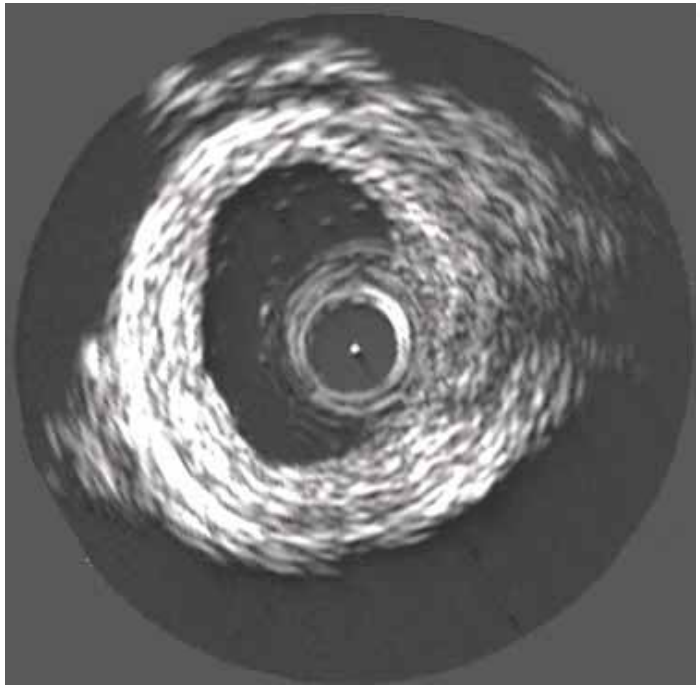
(2) Lipid Rich Plaque

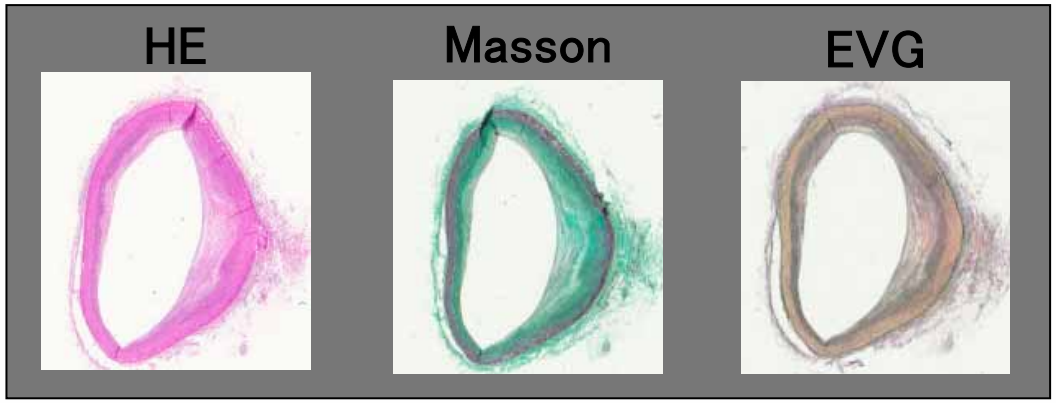


Dr. U. Gerckens and Dr. R. Müller, Herzzenturm Siegburg, Germany

Lipid rich plaque

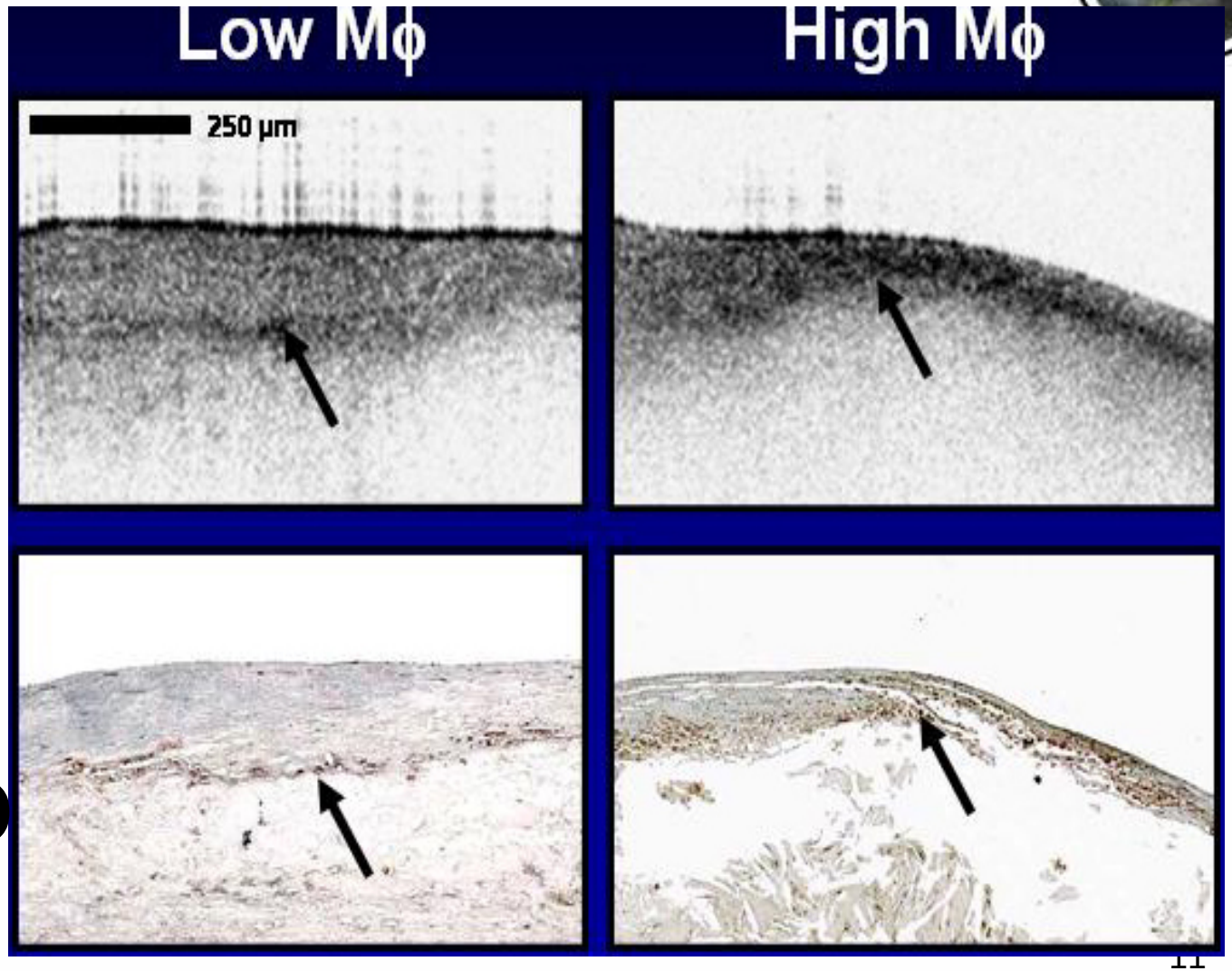






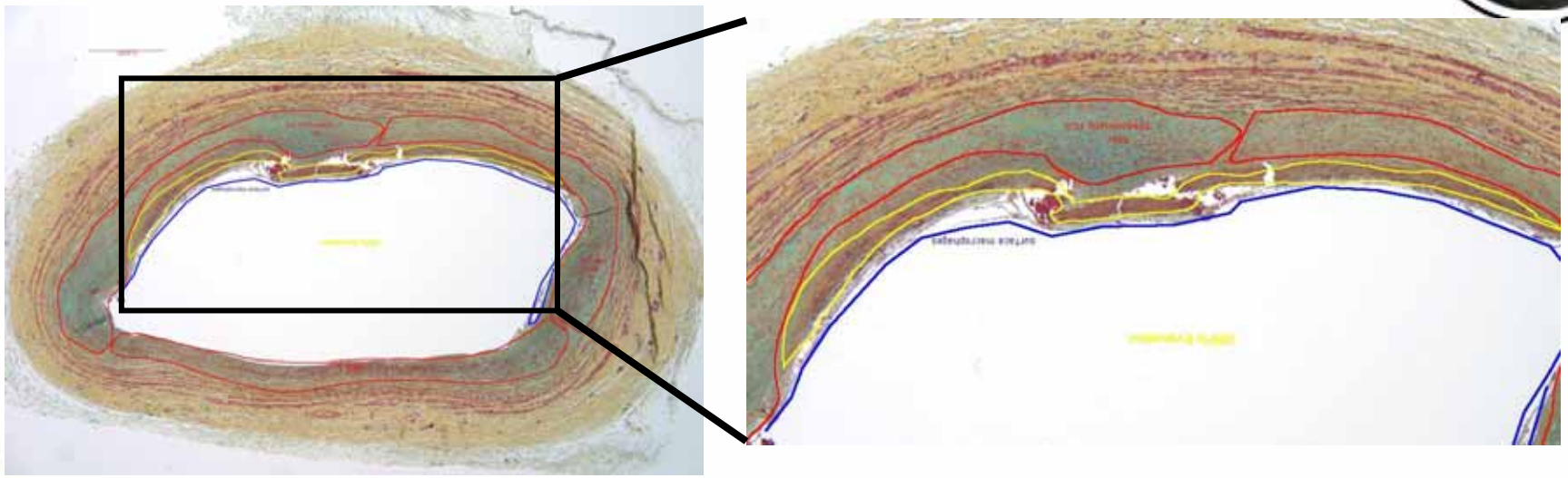
Kawasaki Medical School Hospital
Akasaka M.D. Kume M.D.

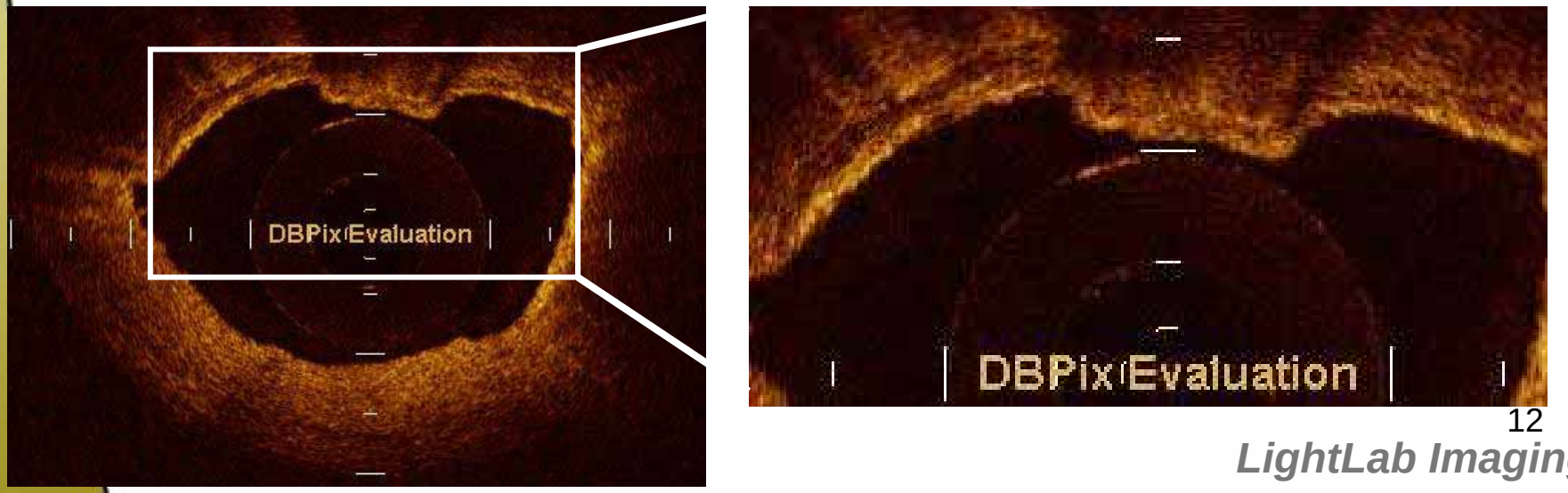
(3) Macrophage Detection



Tearney GJ, et al, Circulation. 2003 Jan 7;107(1):113-9

Macrophage Detection





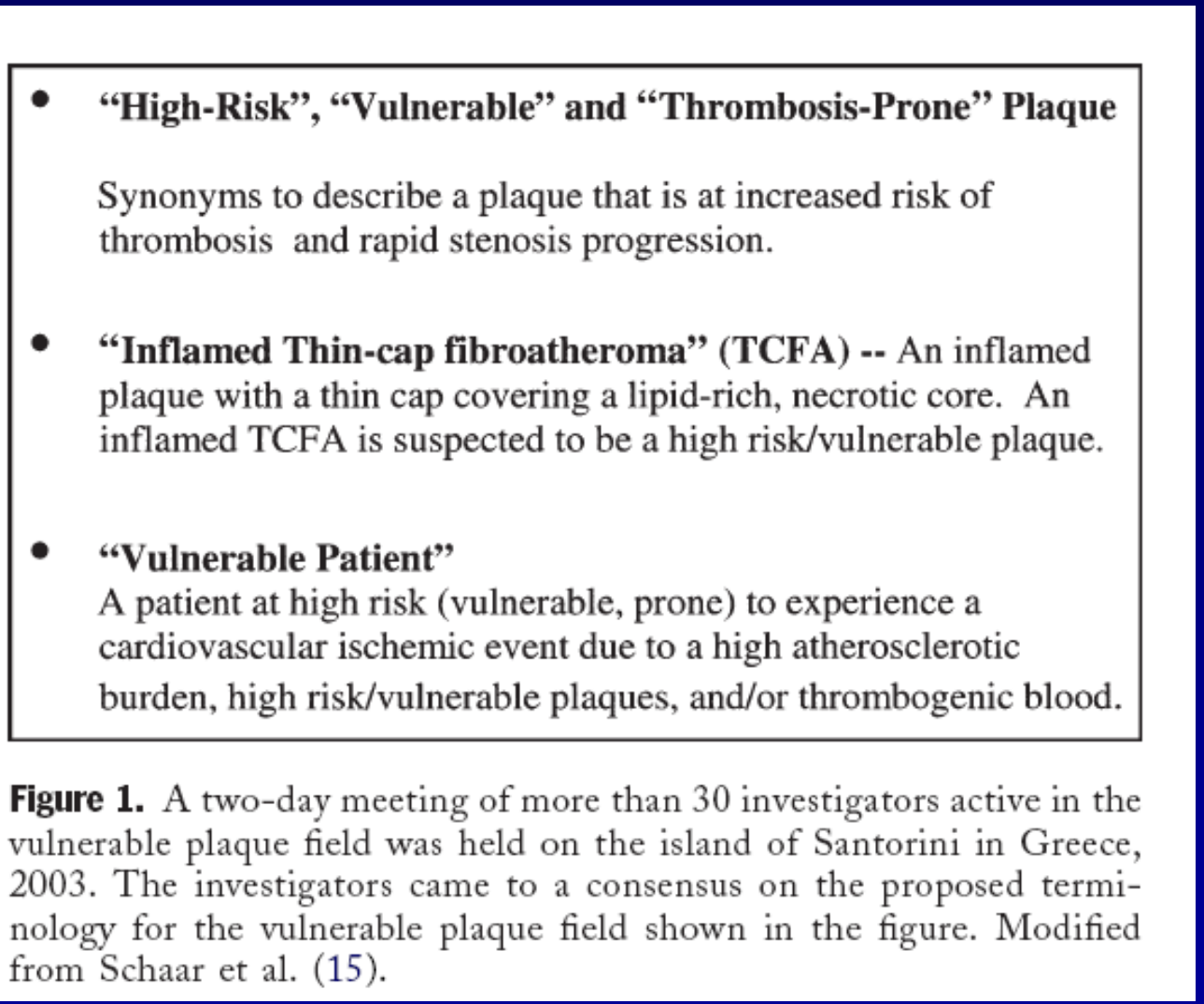
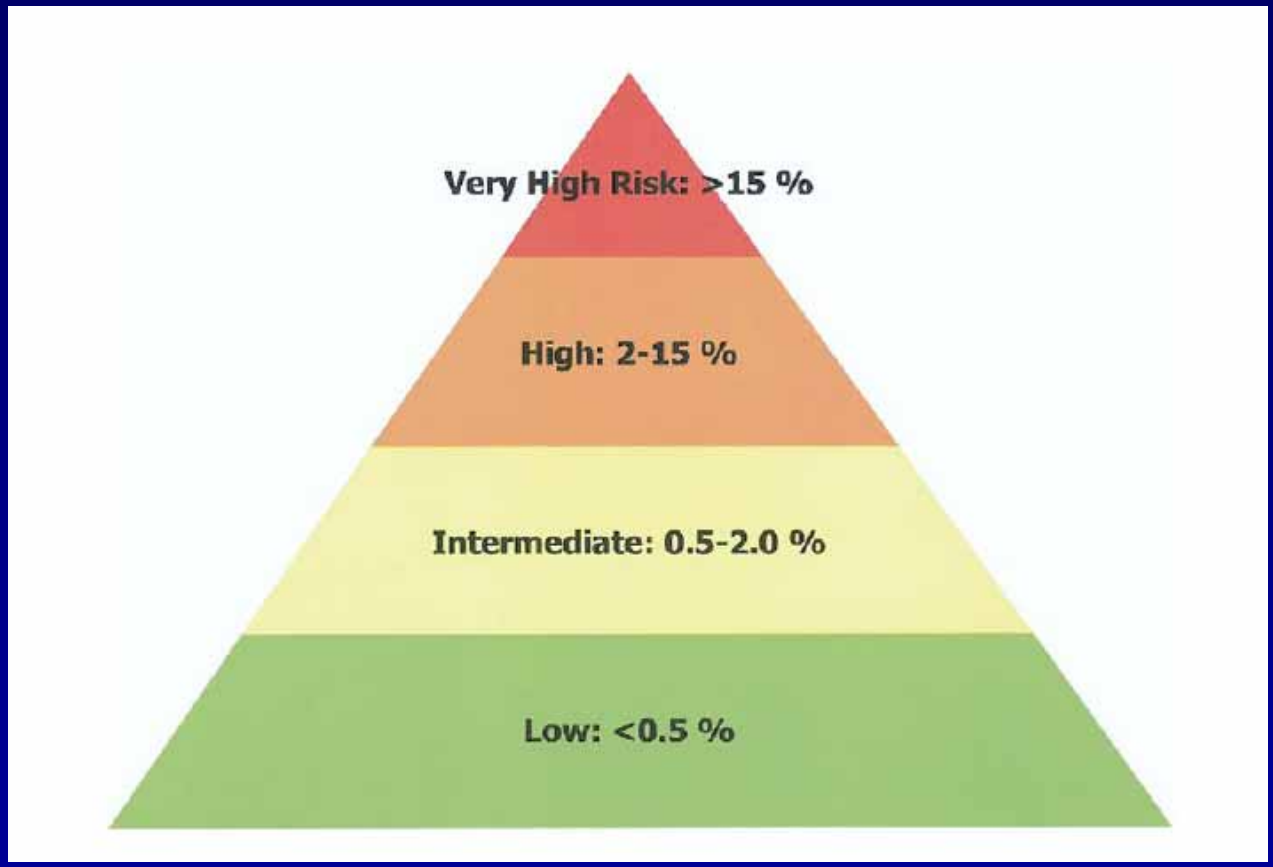
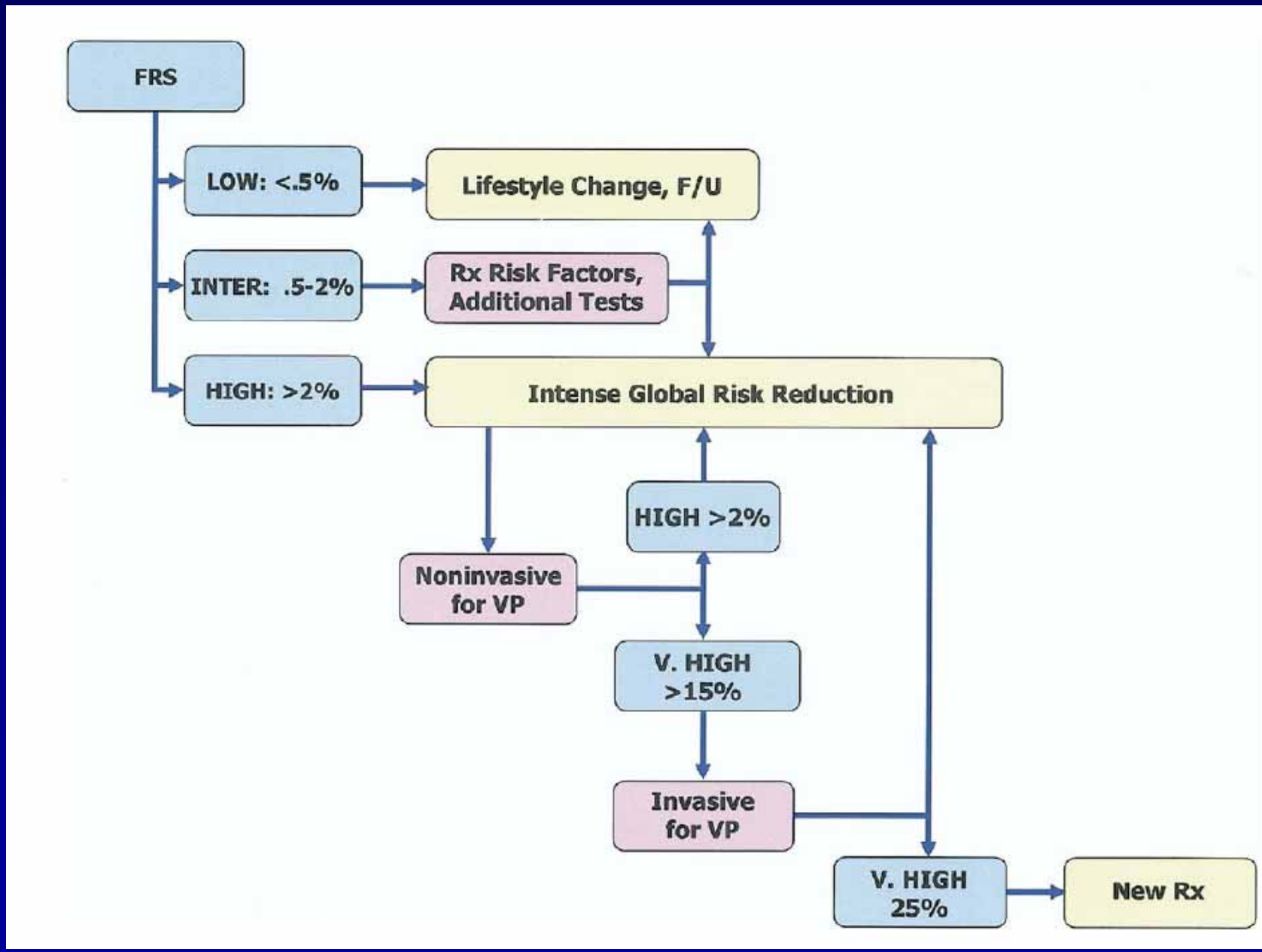


Figure 1. A two-day meeting of more than 30 investigators active in the vulnerable plaque field was held on the island of Santorini in Greece, 2003. The investigators came to a consensus on the proposed terminology for the vulnerable plaque field shown in the figure. Modified from Schaar et al. (15).

JACC Vol. 47, No. 8 Suppl C
April 18, 2006:C2–6

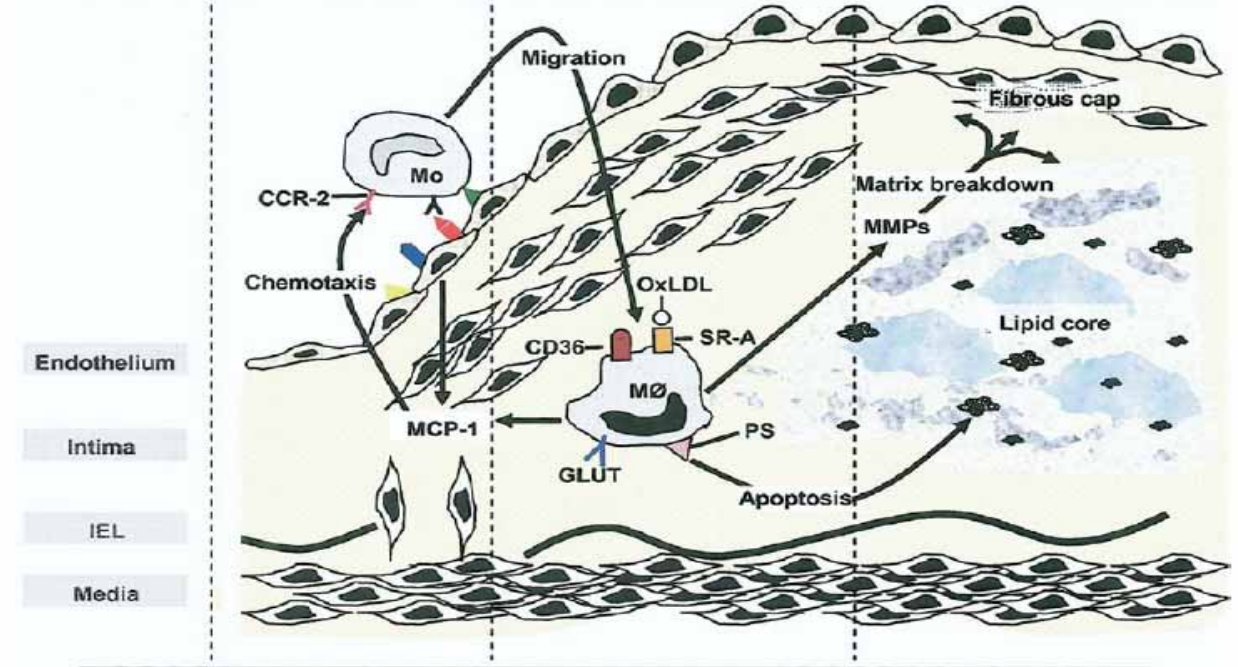


JACC Vol. 47, No. 8 Suppl C
April 18, 2006:C101–3



JACC Vol. 47, No. 8 Suppl C
April 18, 2006:C101–3

Process	Macrophage recruitment	Foam cell formation Macrophage activity	Macrophage apoptosis Matrix degradation
Target	CCR-2	OxLDL GLUT	PS MMPs
Radionuclide tracer	^{125}I-MCP-1	^{99m}Tc-oxLDL; ^{99m}Tc-MDA2 ^{18}FDG	^{99m}Tc-Annexin-A5 ^{123}I-HO-CGS 27023A



What are the possible systemic therapy?

- Thin Cap: MMP inhibitors
- Atheroma: Lipid therapy
- Inflammation: CRP reduction
- Genetic: LD

Clinical and Biochemical Results of the Metalloproteinase Inhibition with Subantimicrobial Doses of Doxycycline to Prevent Acute Coronary Syndromes (MIDAS) Pilot Trial

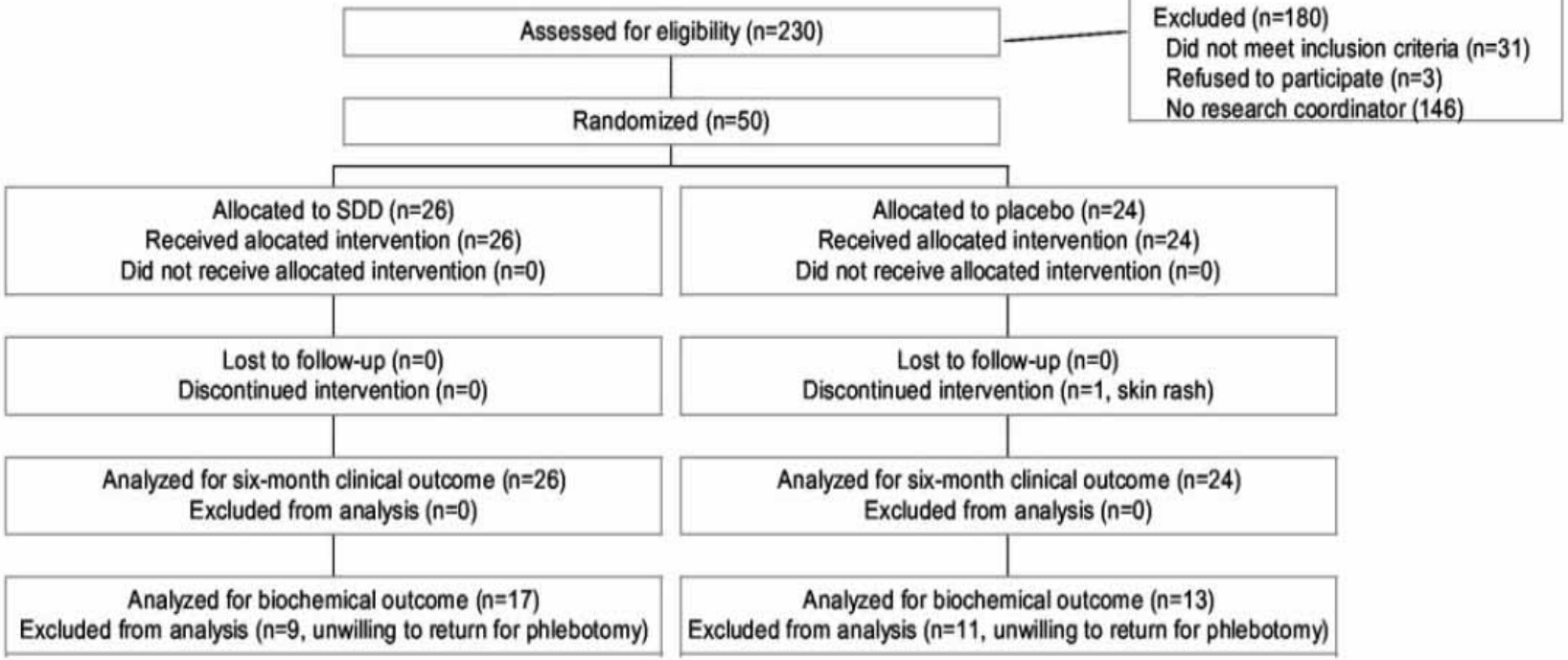


Figure 1. Flow diagram of recruitment, enrollment, treatment, and analysis of the study.

TABLE 3. Six-Month Outcomes of the Study Population

Variable	Placebo (n=24)	Doxycycline (n=26)	*P*
Sudden death (%)	0 (0)	0 (0)	
Fatal MI (%)	0 (0)	4.2 (1)	0.322
Non-fatal MI (%)	0 (0)	4.2 (1)	0.322
Unstable angina (%)	0 (0)	0 (0)	
Composite endpoint (%)	0 (0)	8.4 (2)	0.491

MI, myocardial infarction.

TABLE 4. Inflammatory Mediators, Cytokines, and Matrix Metalloproteinases in the Study Population Before and After Treatment

	Placebo (n=13)			Doxycycline (n=17)		
	Baseline	6 Months	*P*	Baseline	6 Months	*P*
hsCRP (mg/L)	5.2±0.8	4.9±0.7	0.789	4.8±0.6	2.6±0.4	0.007
IL-6 (pg/mL)	22.8±3.5	17.4±3.7	0.209	22.1±3.7	14.7±1.8	0.025
IL-10 (pg/mL)	0.9±0.4	0.5±0.5	0.156	1.3±0.6	0.5±0.3	0.313
IL-1β (pg/mL)	ND	ND		ND	ND	
TNF-α (pg/mL)	ND	ND		ND	ND	
MMP-9 protein (ng/mL)	20.4±2.8	13.6±1.5	0.086	17.9±2.7	12.8±1.4	0.235
MMP-9 92 kDa+higher MW (units)	595±82	597±61	0.982	550±103	276±79	0.011
MMP-9 92 kDa only (units)	343±45	338±50	0.945	417±76	238±56	0.028
MMP-2 activity (units)	137±32	134±35	0.983	96±21	60±21	0.248

hsCRP indicates high-sensitivity C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; ND, not detected; MW, molecular weight.

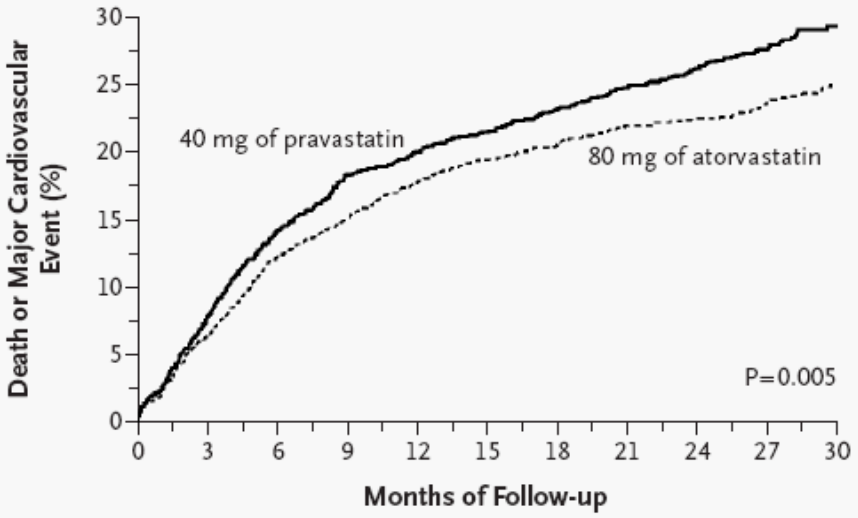
Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes

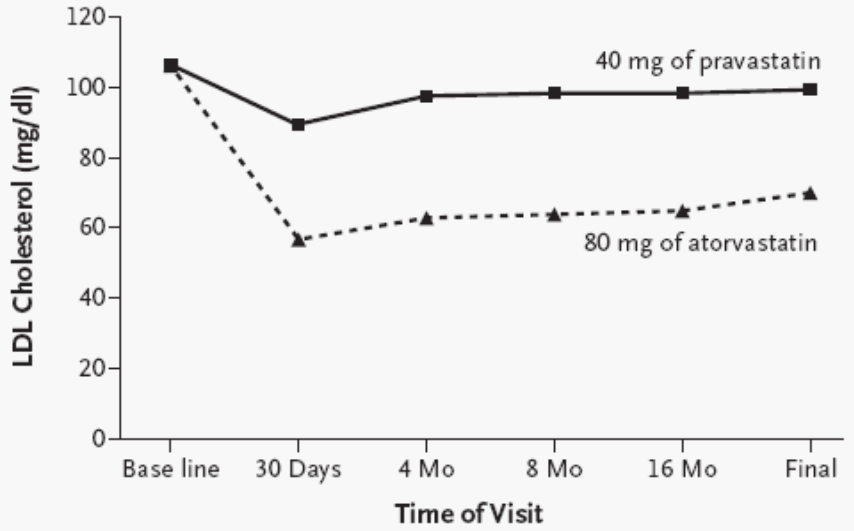
Christopher P. Cannon, M.D., Eugene Braunwald, M.D., Carolyn H. McCabe, B.S., Daniel J. Rader, M.D., Jean L. Rouleau, M.D., Rene Belder, M.D., Steven V. Joyal, M.D., Karen A. Hill, B.A., Marc A. Pfeffer, M.D., Ph.D., and Allan M. Skene, Ph.D., for the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 Investigators*

1. Cannon CP, Braunwald E, McCabe CH, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med*. 2004;350:1495-1504.

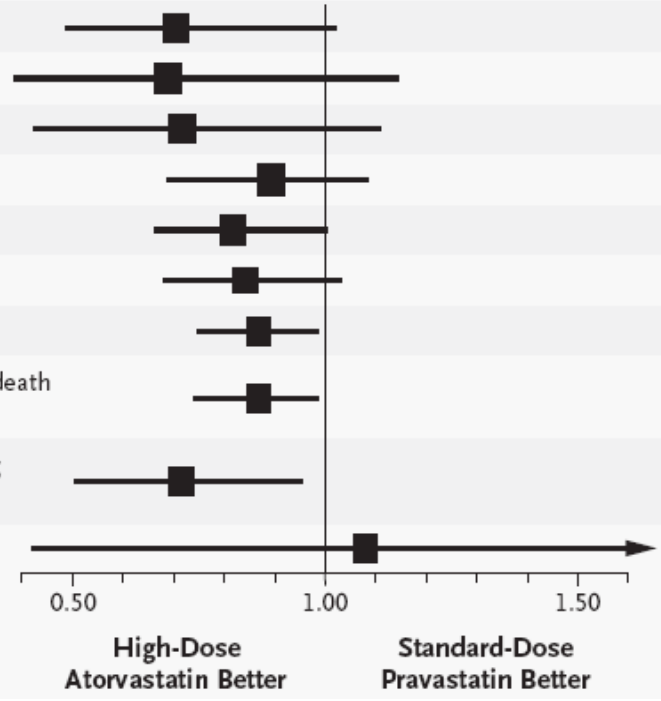
2. LaRosa JC, Grundy SM, Waters DD, et al; Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med*. 2005;352:1425-1435.

3. Pedersen TR, Faergeman O, Kastelein JJ, et al; Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study Group. High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. *JAMA*. 2005;294:2437-2445.





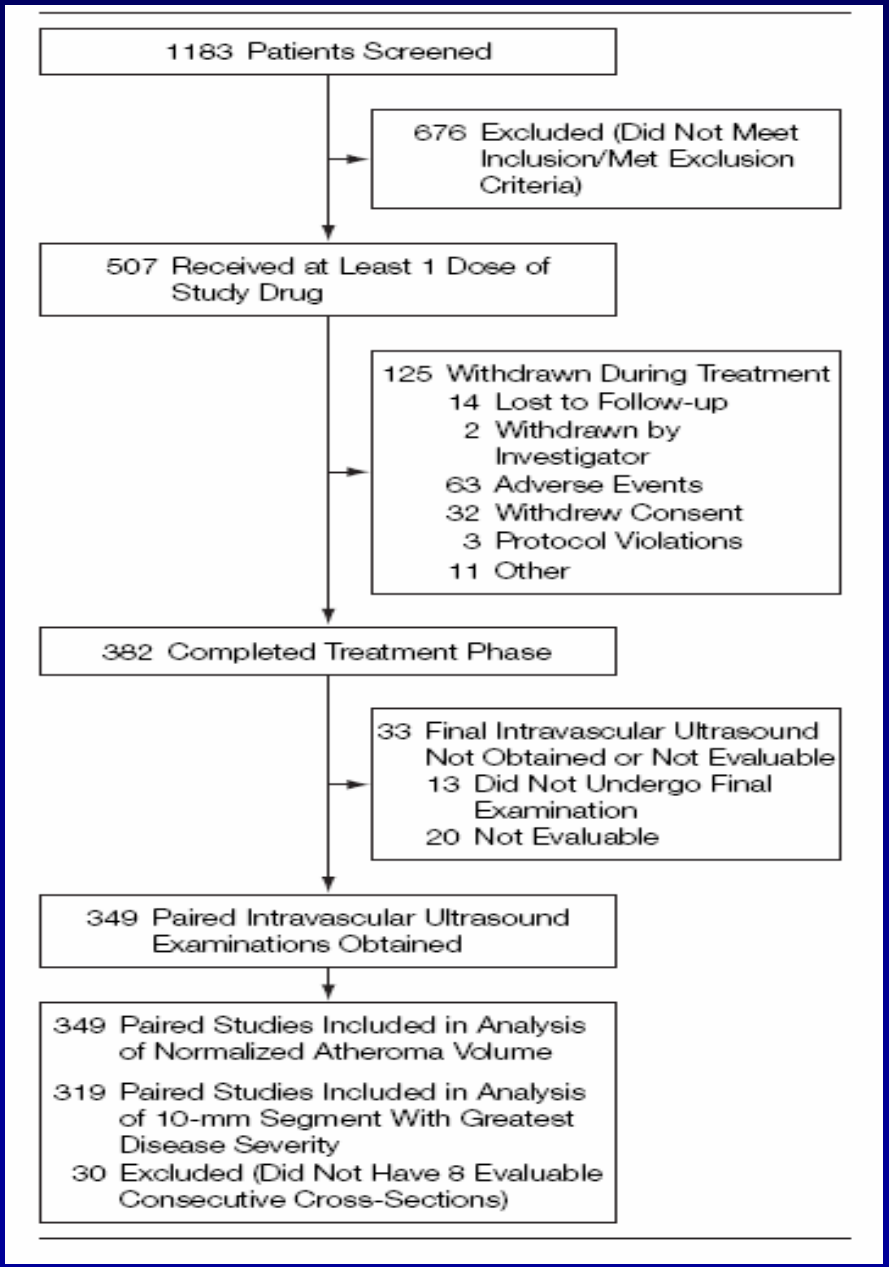
End Point	Hazard Ratio (95% CI)	Risk Reduction	2-Yr Event Rates	
			Atorvastatin	Pravastatin
		percent		
Death from any cause		28	2.2	3.2
Death from CHD		30	1.1	1.4
Death from other causes		27	1.2	1.8
MI		13	6.6	7.4
Death or MI		18	8.3	10.0
Death from CHD or MI		16	7.2	8.3
Revascularization		14	16.3	18.8
MI, revascularization, or death from CHD		14	19.7	22.3
Unstable angina requiring hospitalization		29	3.8	5.1
Stroke		−9	1.0	1.0



Cholesterol Lowering

ASTEROIDS STUDY

- Can we regress atherosclerosis ?
- Corollary: Will vulnerable plaque will also become more stable ?
- LDL of 60mg/dl



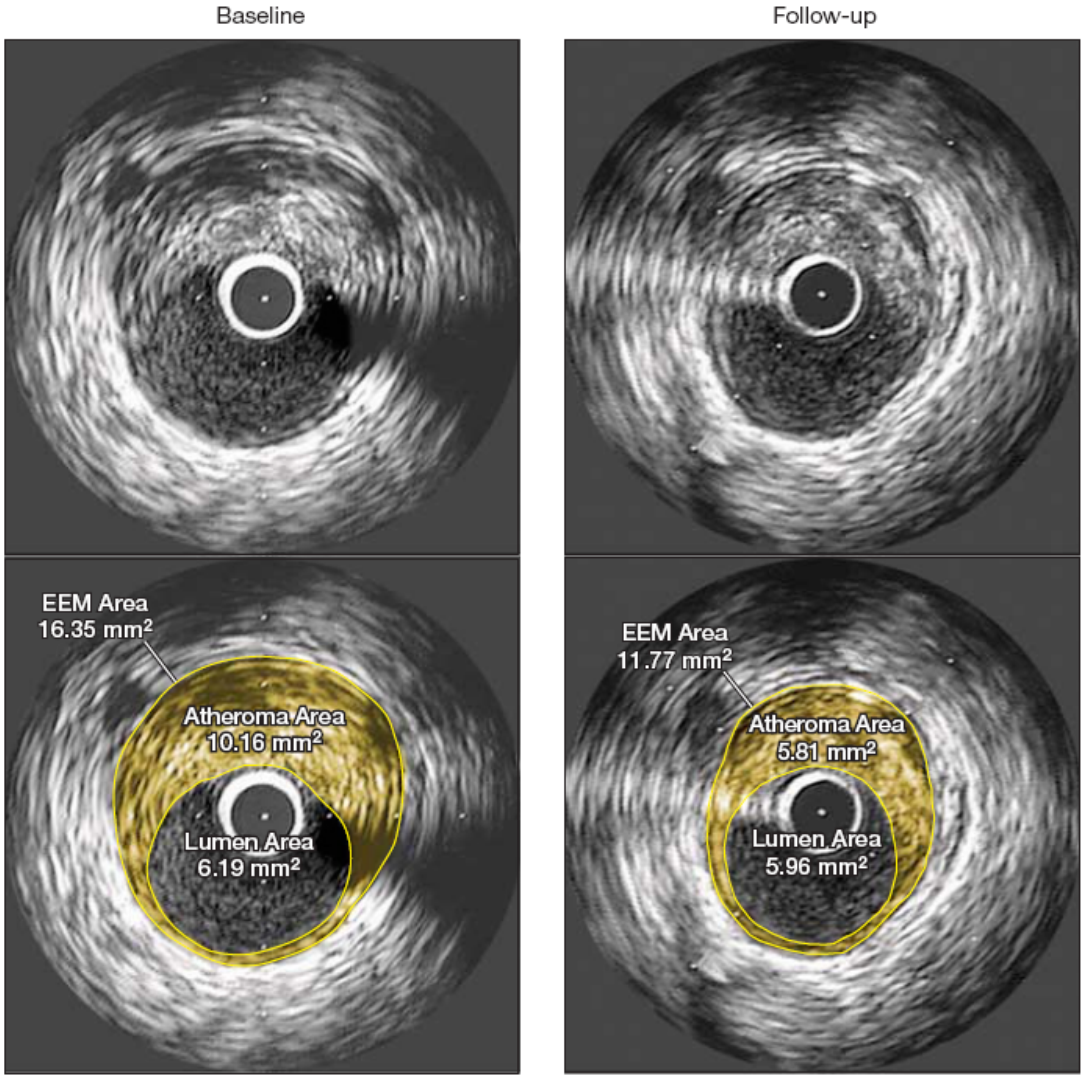
JAMA, April 5, 2006—Vol 295, No. 13

Table 3. Baseline and Follow-up Intravascular Ultrasound Results

	Baseline	Follow-up	Change	Percent Change	No. (%) With Regression
Primary efficacy parameters Percent atheroma volume (n = 349)					222 (63.6)
Mean (SD)	39.6 (8.5)	38.6 (8.5)	−0.98 (3.15)	NA	
Median (IQR)	39.9 (33.8-45.3)	38.5 (32.6-44.3)	−0.79 (−1.21 to −0.53)*†	NA	
Atheroma volume in most diseased 10-mm subsegment, mm^3 (n = 319)					249 (78.1)
Mean (SD)	65.1 (27.0)	59.0 (24.5)	−6.1 (10.1)	−8.5 (13.7)	
Median (IQR)	65.1 (45.2-82.2)	58.4 (40.6-76.3)	−5.6 (−6.82 to −3.96)*†	−9.1 (−10.83 to −7.23)*†	
Secondary efficacy parameter Normalized total atheroma volume, mm^3 (n = 349)					272 (77.9)
Mean (SD)	212.2 (81.3)	197.5 (79.1)	−14.7 (25.7)	−6.7 (11.1)	
Median (IQR)	204.7 (146.0-259.8)	186.8 (135.1-243.8)	−12.5 (−15.08 to −10.48)*‡	−6.8 (−7.82 to −5.60)*‡	

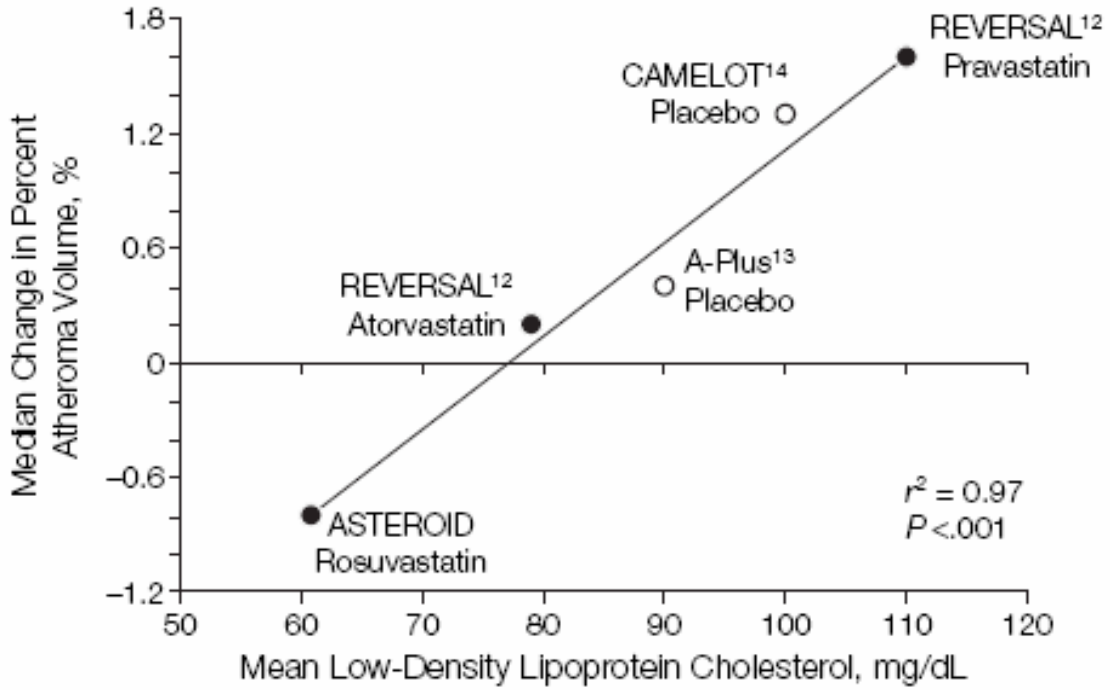
Abbreviations: IQR, interquartile range; NA, not applicable.
*$P<.001$ by Wilcoxon signed rank test for all comparisons between baseline and follow-up.
†Data in parentheses are distribution-free 97.5% confidence intervals for the median.
‡Data in parentheses are distribution-free 95% confidence intervals for the median.

JAMA, April 5, 2006—Vol 295, No. 13



The top left panel illustrates the appearance of a single cross-section at baseline intravascular ultrasound examination, while the top right panel shows the same cross-section after 24 months of treatment. The bottom 2 panels illustrate the same cross-sections, but with measurements superimposed. Atheroma area was reduced from 10.16 mm^2 to 5.81 mm^2. EEM indicates external elastic membrane.

Figure 3. Relationship Between Mean Low-Density Lipoprotein Cholesterol Levels and Median Change in Percent Atheroma Volume for Several Intravascular Ultrasound Trials



There is a close correlation between these 2 variables (r^2=0.97). REVERSAL indicates Reversal of Atherosclerosis With Aggressive Lipid-Lowering[12]; CAMELOT, Comparison of Amlodipine vs Enalapril to Limit Occurrences of Thrombosis[14]; A-Plus, Avasimibe and Progression of Lesions on Ultrasound[13]; and ASTEROID, A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden.

JAMA, April 5, 2006—Vol 295, No. 13

Hs C-Reactive Protein

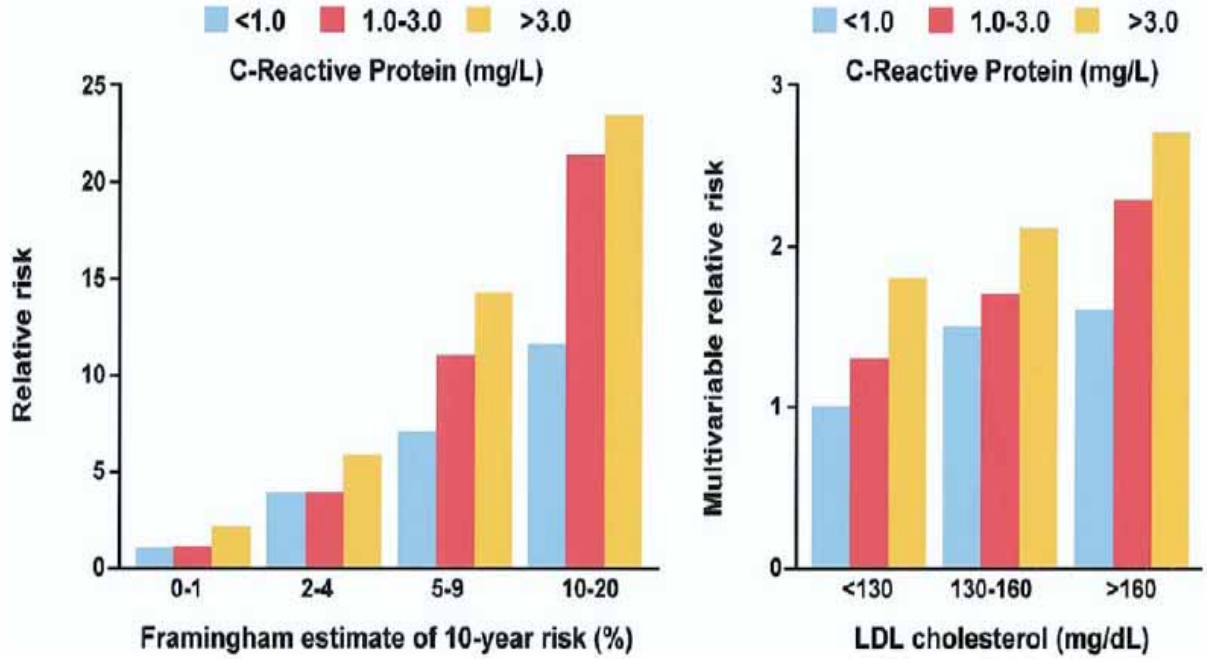
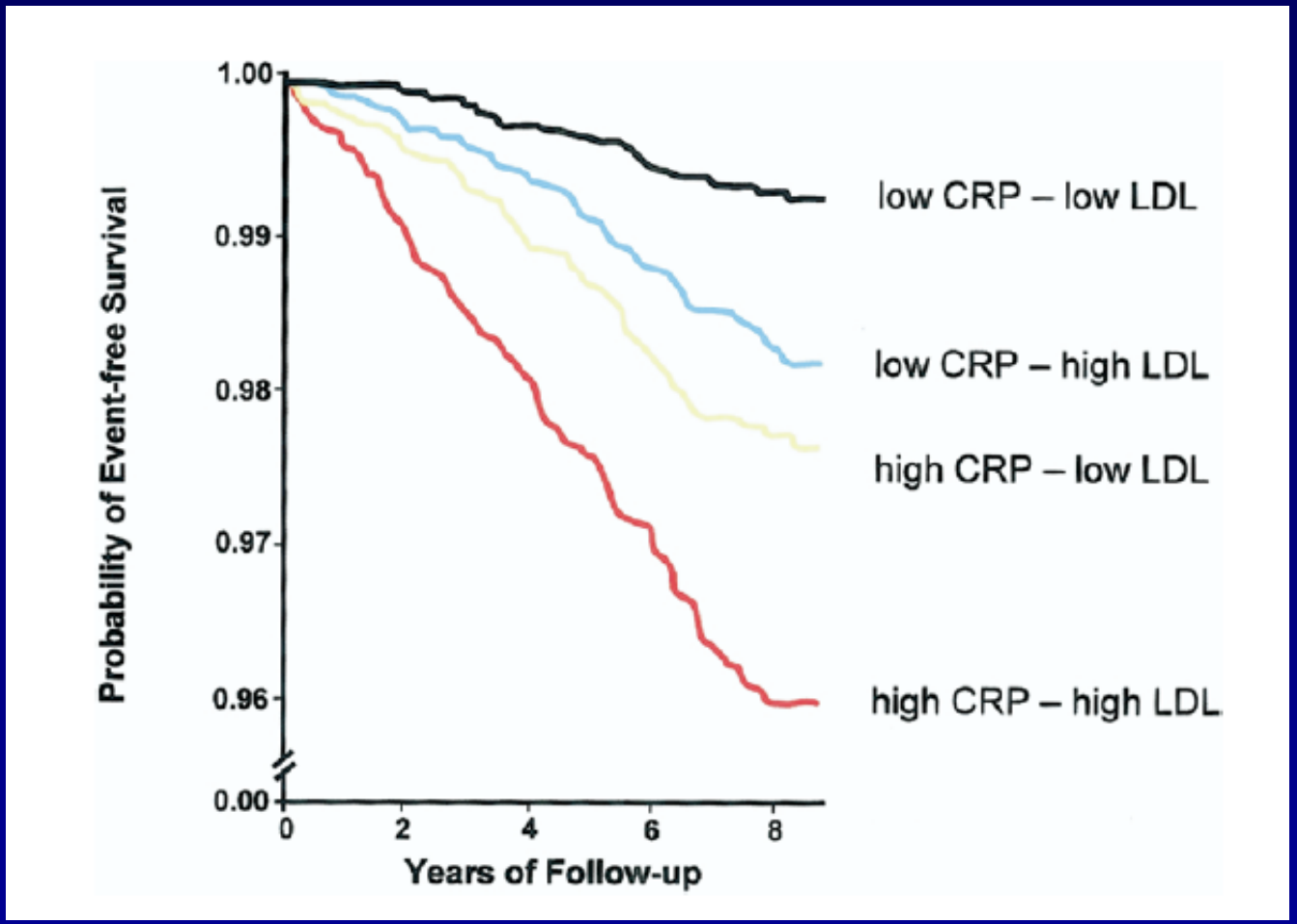


Figure 1. Additive value of high-sensitivity C-reactive protein after adjustment for traditional risk factors. Data are shown across all levels of low-density lipoprotein (LDL) cholesterol **(right)** and across all levels of calculated Framingham Risk **(left)**. Adapted, with permission, from Ridker et al. (23).

JACC Vol. 47, No. 8 Suppl C
April 18, 2006:C19–31



JACC Vol. 47, No. 8 Suppl C
April 18, 2006:C19–31

Figure 2. Cardiovascular event-free survival in apparently healthy American women according to plasma levels of low density lipoprotein (LDL)-cholesterol and high sensitivity C-reactive protein (CRP). Adapted, with permission, from Ridker et al. (23).

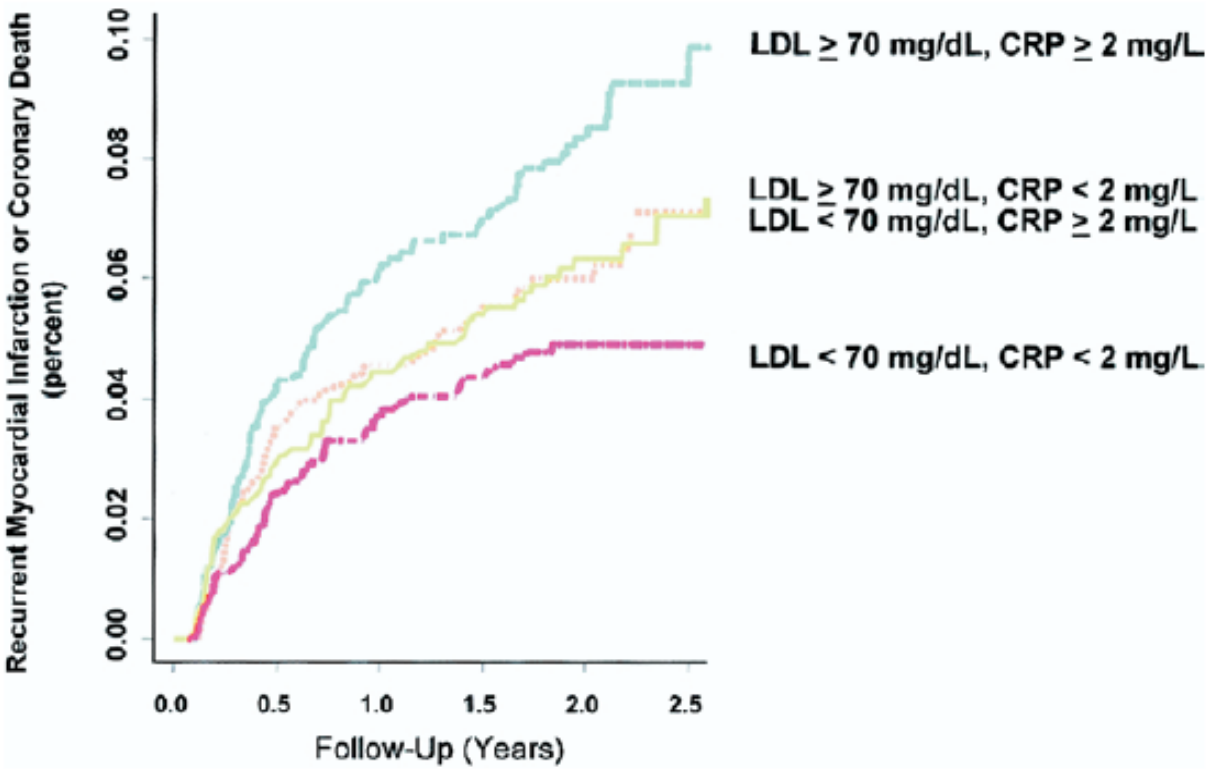
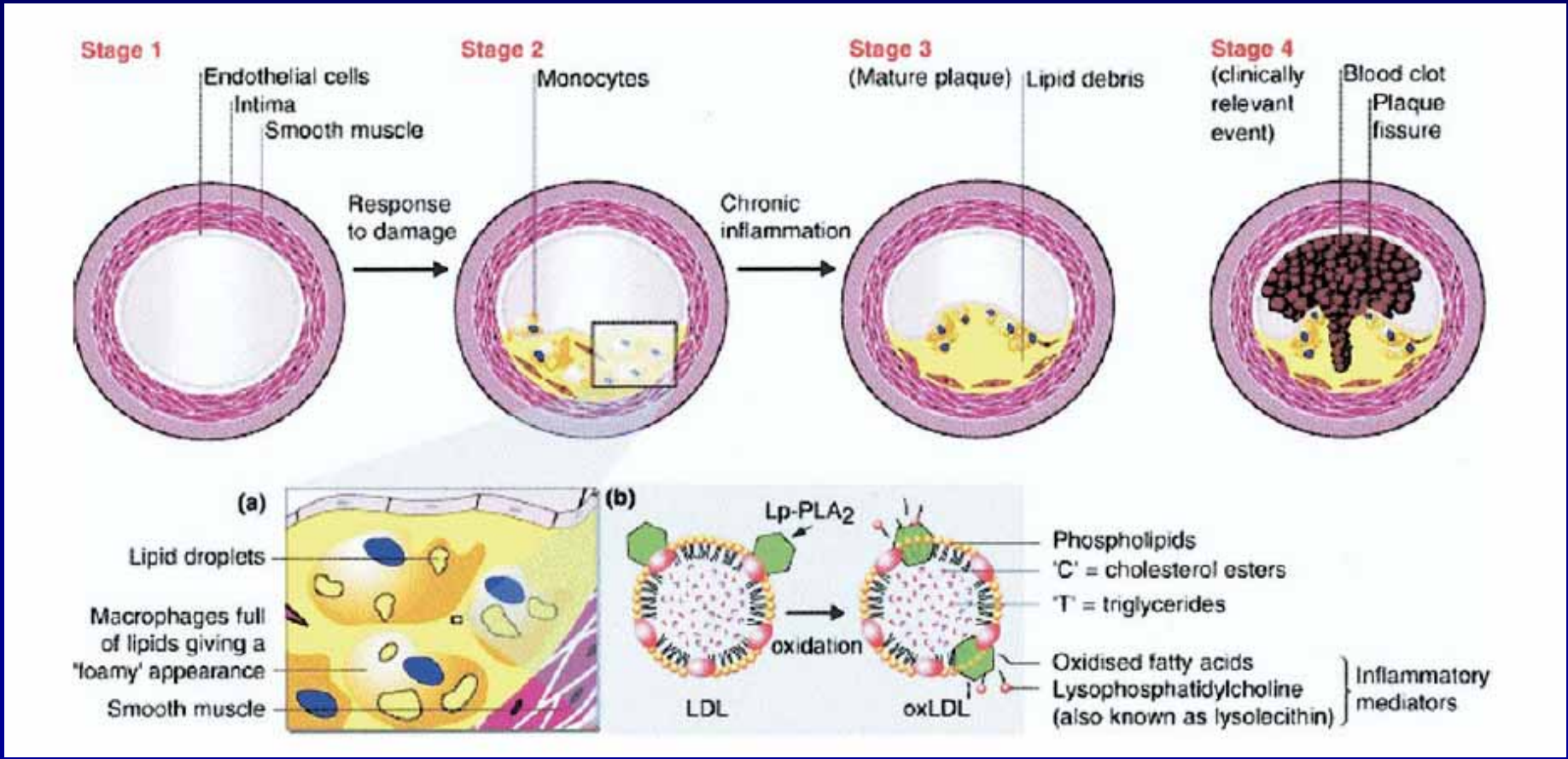


Figure 3. Rates of recurrent myocardial infarction and cardiovascular death among acute coronary syndrome patients treated with statin therapy according to achieved levels of low density lipoprotein (LDL)-cholesterol and high sensitivity C-reactive protein (CRP) in the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22 trial. Adapted, with permission, from Ridker et al. (40).


Stage 1
Endothelial cells
Intima
Smooth muscle
Response to damage
Stage 2
Monocytes
Chronic inflammation
Stage 3
(Mature plaque)
Lipid debris
Stage 4
(clinically relevant event)
Blood clot
Plaque fissure
(a)
Lipid droplets
Macrophages full of lipids giving a 'foamy' appearance
Smooth muscle
(b)
Lp-PLA2
oxidation
LDL
oxLDL
Phospholipids
'C' = cholesterol esters
'T' = triglycerides
Oxidised fatty acids
Lysophosphatidylcholine (also known as lysolecithin)
Inflammatory mediators

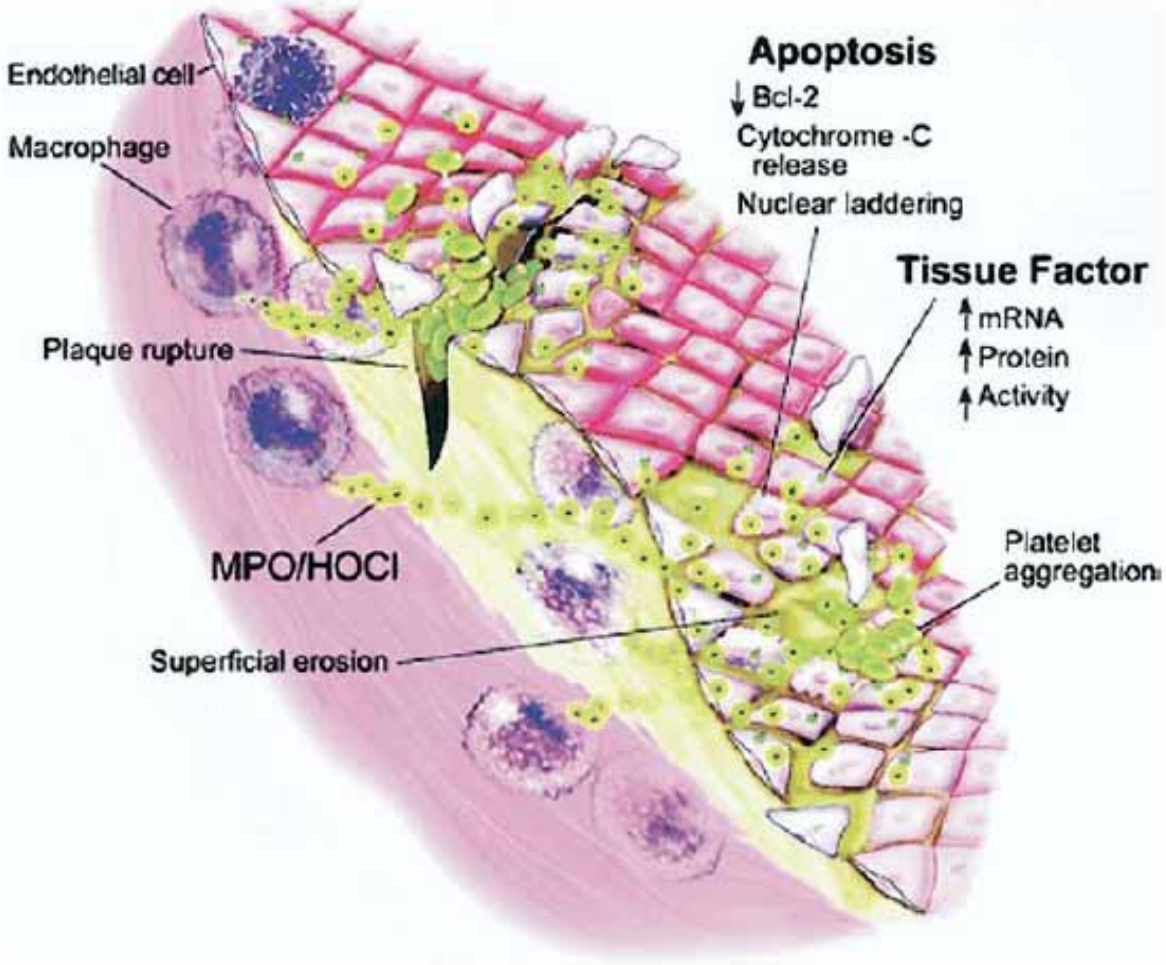
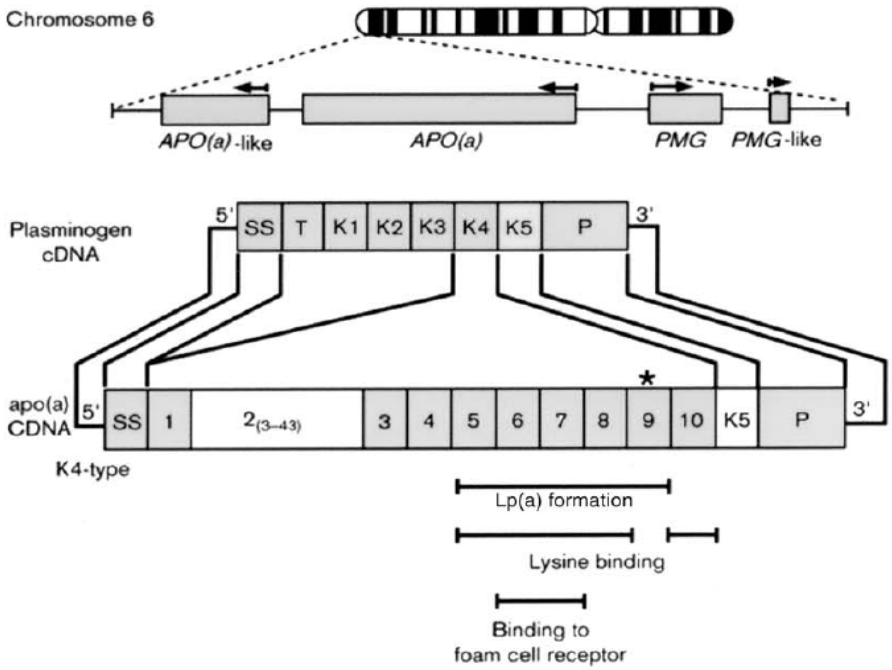
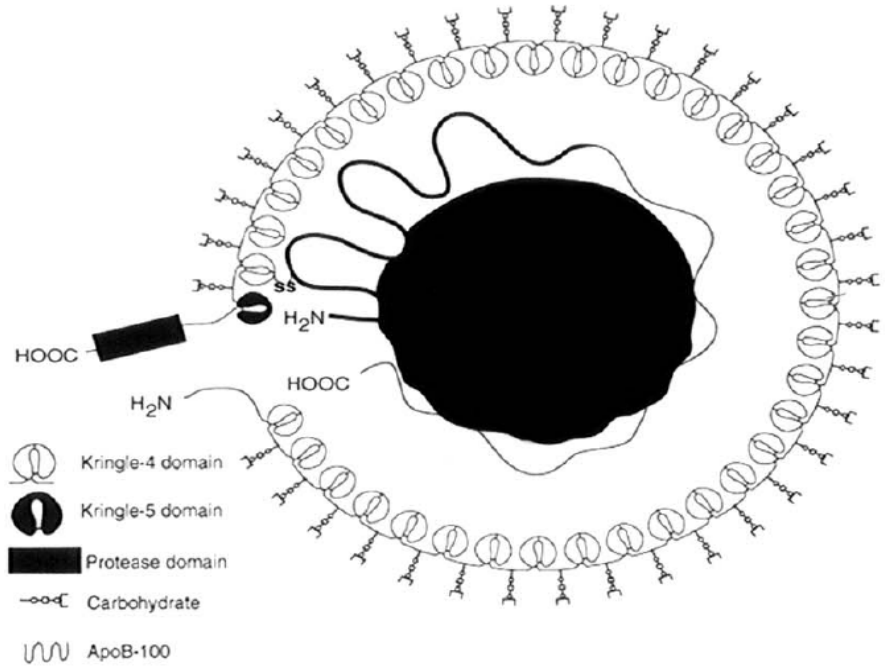


Figure 5. The role of myeloperoxidase in plaque vulnerability. Reprinted, with permission, from Hazen (66). HOCl = hypochlorous acid; MPO = myeloperoxidase; mRNA = messenger ribonucleic acid.


Chromosome 6
APO(a)-like
APO(a)
PMG
PMG-like
Plasminogen cDNA
5'
SS
T
K1
K2
K3
K4
K5
P
3'
apo(a) CDNA
5'
SS
1
2(3–43)
3
4
5
6
7
8
9
10
K5
P
3'
K4-type
Lp(a) formation
Lysine binding
Binding to foam cell receptor


SS
H2N
HOOC
HOOC
H2N
Kringle-4 domain
Kringle-5 domain
Protease domain
Carbohydrate
ApoB-100

Can we manipulate e.g. CRP ?

TABLE 2. Clinical Correlates of Log-CRP Level: Stepwise Linear Regression Model $R^2=0.26$

	Fold Change in CRP (95% CI)	Partial R^2†	*P*
Age, per 10 y	1.16 (1.12–1.21)	0.02*	<0.0001
Sex, female vs male	1.17 (1.08–1.26)	0.01*	<0.0001
Body mass index, per 5 kg/m^2	1.46 (1.42–1.51)	0.15	<0.0001
Hormone replacement therapy, yes/no	1.79 (1.62–1.97)	0.03	<0.0001
Cigarette smoking, yes/no	1.45 (1.32–1.61)	0.02	<0.0001
Total/HDL cholesterol ratio	1.06 (1.02–1.09)	0.01	<0.0001
Hypertension treatment, yes/no	1.19 (1.10–1.29)	0.01	<0.0001
Lipid-lowering therapy, yes/no	0.77 (0.71–0.85)	0.004	<0.0001
Prevalent CVD, yes/no	1.28 (1.15–1.42)	0.01	<0.0001
Triglycerides, per 100 mg/dL	1.08 (1.03–1.14)	0.003	0.0009
Systolic blood pressure, per 20 mm Hg	1.07 (1.03–1.12)	0.002	0.01
Diastolic blood pressure, per 10 mm Hg	0.95 (0.91–0.99)	0.001	0.02

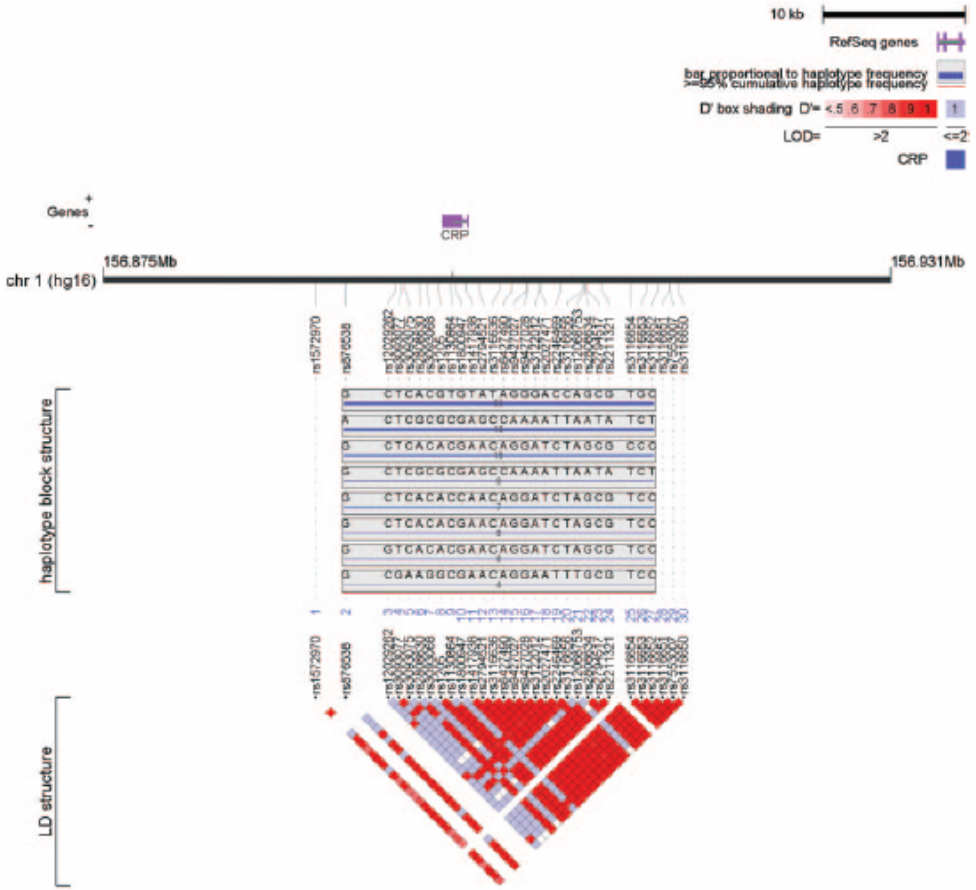
CRP indicates C-reactive protein.

*Age and sex were forced into the multivariable model.

Kathiresan et al Clinical and Genetic Correlates of Serum CRP Level

***Circulation*. 2006;113:1415-1423.**

Linkage Disequilibrium



LD structure at the *CRP* gene locus in a reference panel. Chr 1 panel depicts the *CRP* position on chromosome 1 in the human genome July 2003 assembly (hg16). Haplotype block structure panel shows that 26 SNPs fell into a single haplotype block. LD structure panel displays the LD relations between pairs of markers in the region, with each square representing the pairwise strength and significance of LD. Red indicates no or minimal evidence of historical recombination, white indicates weak LD, and blue indicates uninformative LD. Note that the triallelic SNP rs3091244 was not genotyped in the reference panel and thus is not displayed here. The chromosomal position of SNP rs3091244 falls between SNP 11 and SNP 12. Figure prepared with LocusView 2.0 (T. Petryshen et al, Broad Institute; available at: http://www.broad.mit.edu/mpg/locusview/).

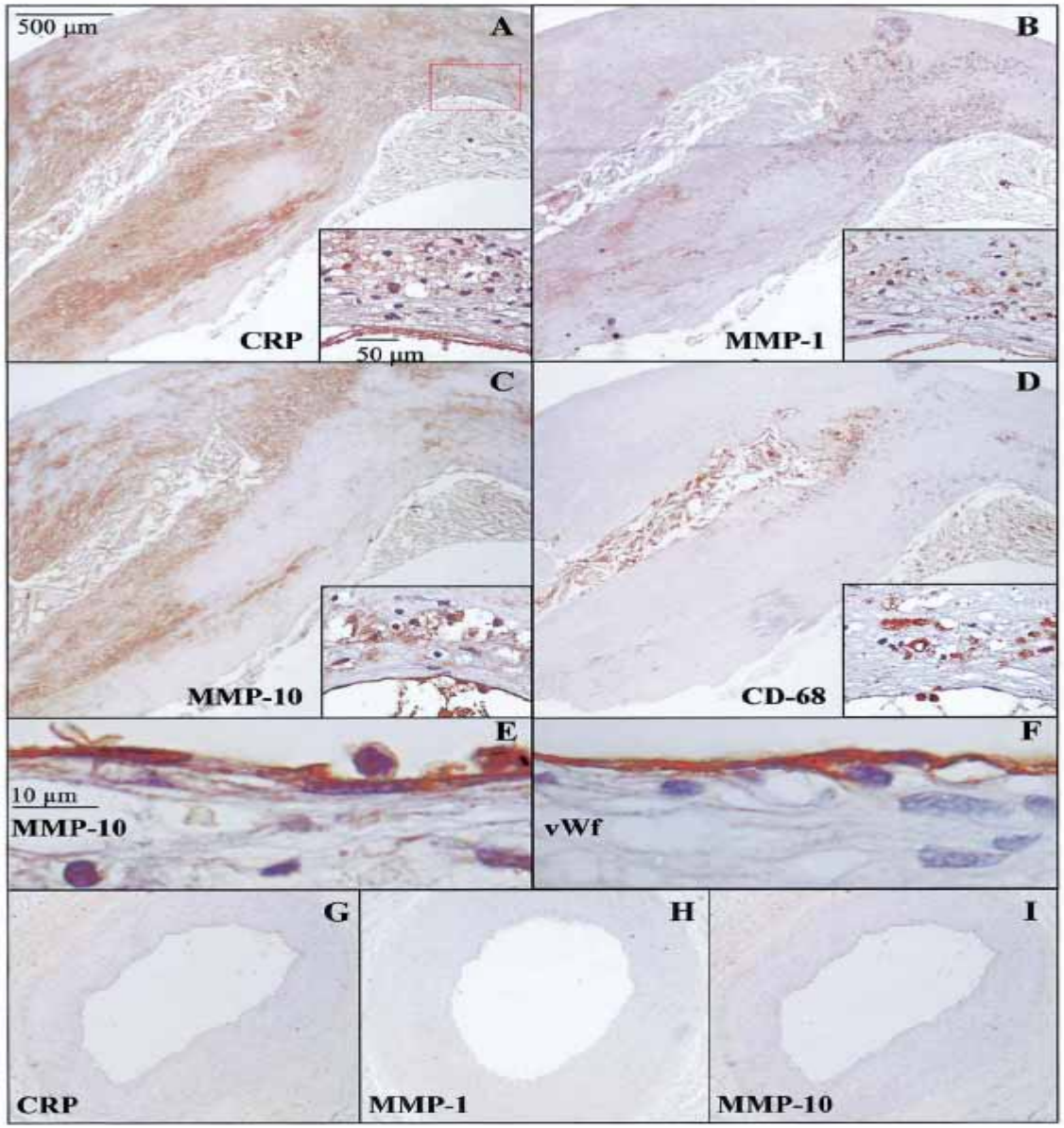


Figure 4. (A to C) Representative immunohistochemical analysis of C-reactive protein (CRP), matrix metalloproteinases (MMP)-1, and -10 in adjacent sections of human atherosclerotic plaques. Area squared in red corresponds to magnified details. Higher magnification revealed intense positive signal for CRP, MMP-1, and MMP-10 within macrophage-rich areas (CD-68 positive) (D). Magnification detail of endothelial layer (×100) showed that endothelial cell, positive for von Willebrand factor (vWf), also expressed MMP-10 (E and F). (G to I) Inmmunolocalization of CRP, MMP-1, and MMP-10 in control mammary arteries.

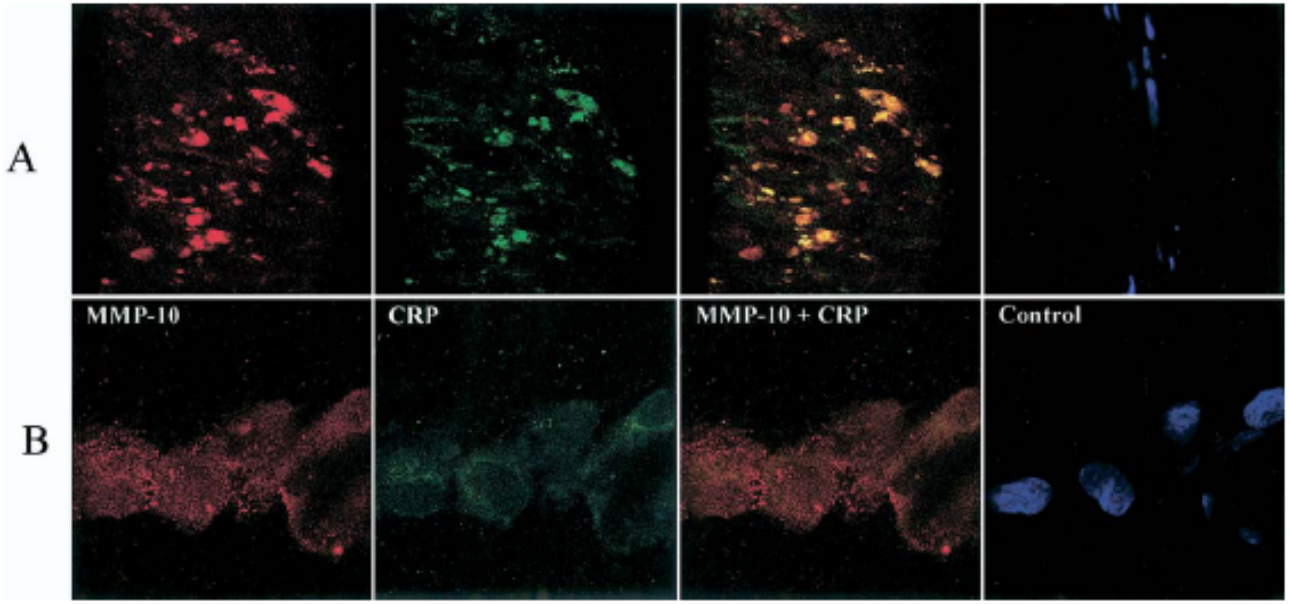
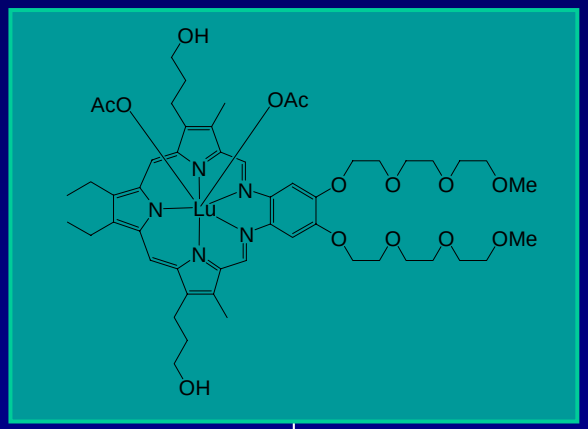


Figure 5. Confocal microscopy in advanced human atherosclerotic plaques (n = 5). Matrix metalloproteinases (MMP)-10 (red) and C-reactive protein (CRP) (green) colocalized (yellow to orange) in macrophage-rich areas (A) and endothelial layer (B). No signal was detected in the absence of the primary antibodies (control). Nuclei were counterstained with TOPRO-3 (blue).

Antrin® Photodynamic Therapy

Antrin (Motexafin Lutetium)



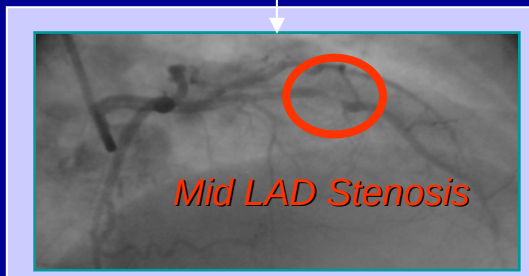
Diode Laser

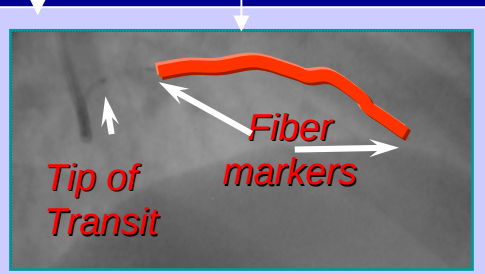
Diffusing Fiber

IV or Local Delivery

Optical Selectivity

Focal or Regional (multivessel) treatment





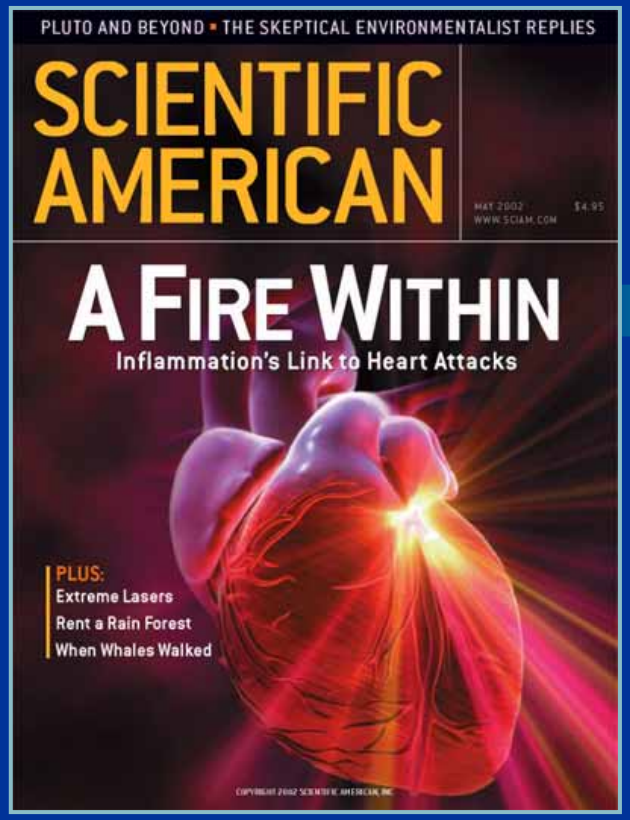
Antrin Phototherapy (PT) depletes vascular macrophages in NZW rabbit injury model, local Antrin

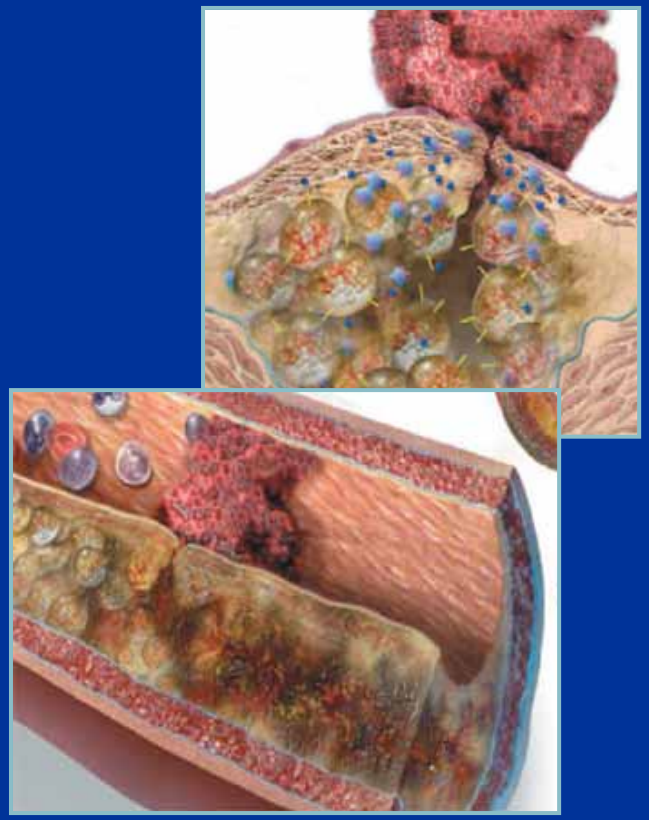
Control

Treated

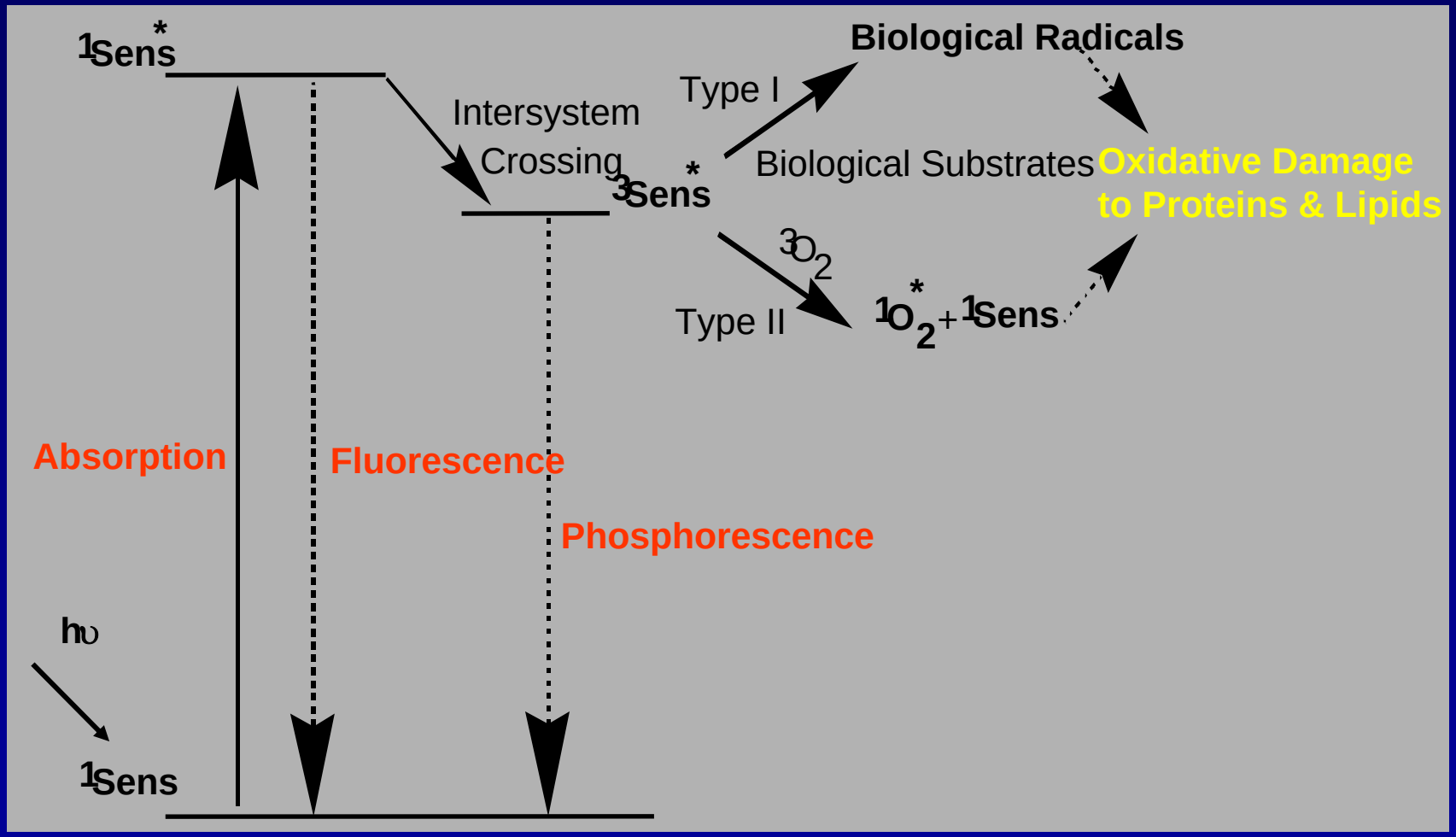
Hayase et al, Cardiovascular Research 2001, 49, 449-455.

Vulnerable Plaque





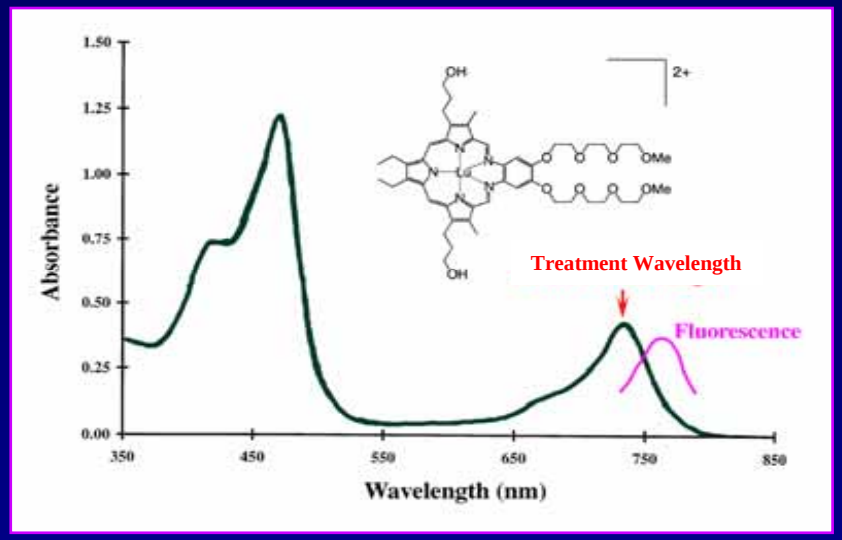
Photodynamic Therapy

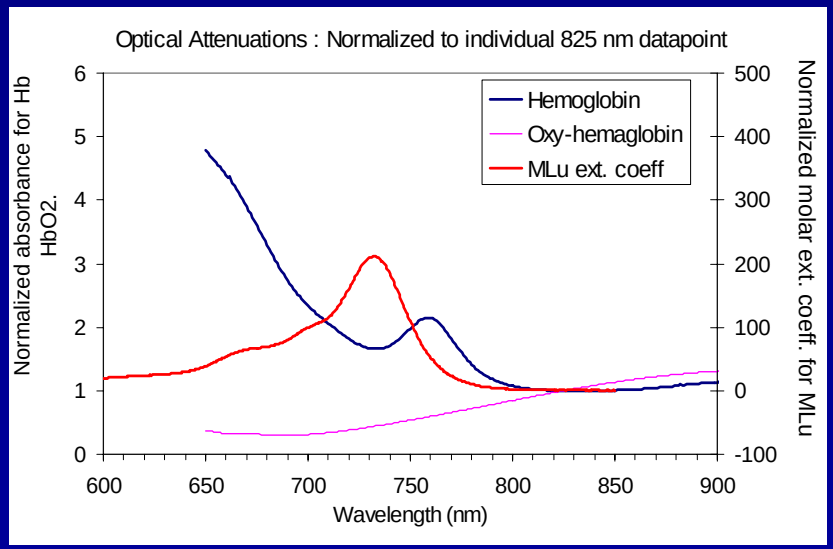


Cell death occurs only if both light and drug present

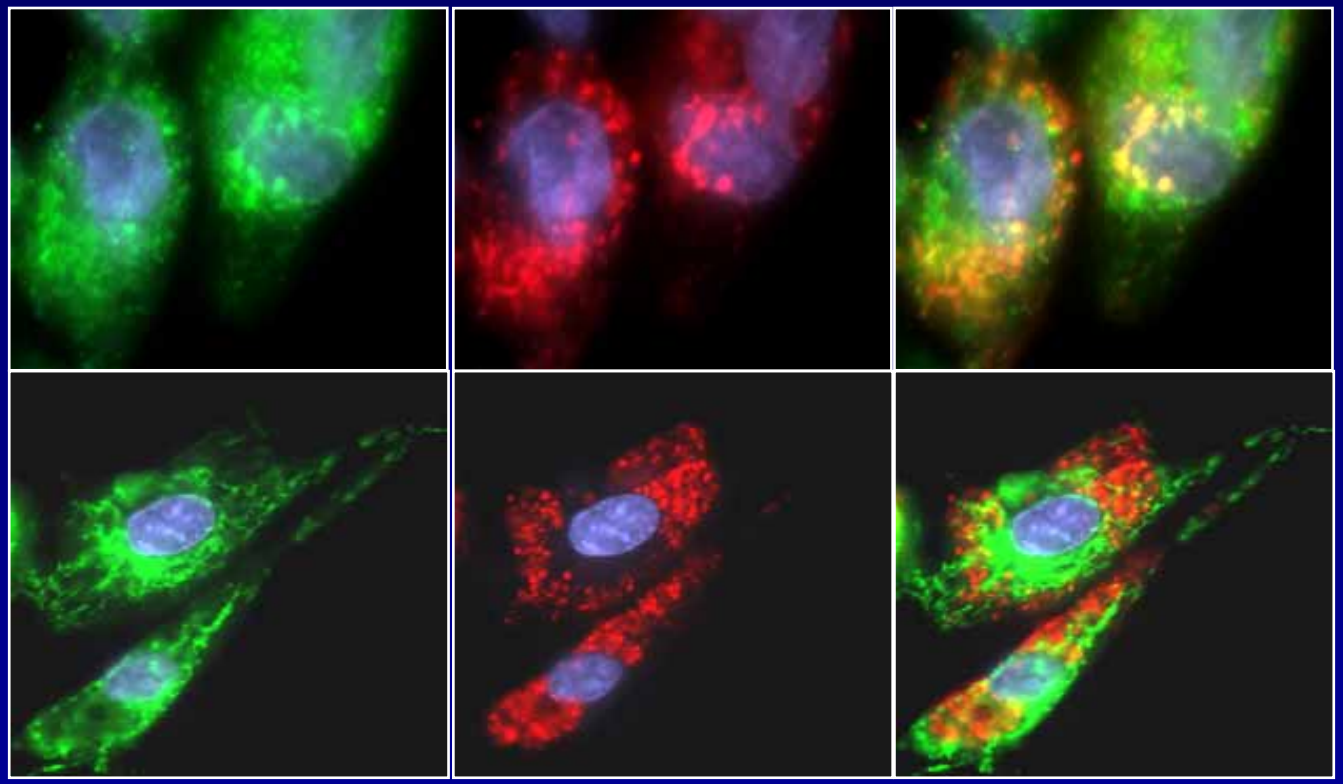
Antrin® (Motexafin Lutetium)

- Selective accumulation in atherosclerotic plaques
- Rapid clearance from plasma and normal tissues
- Light activation produces singlet O_2 (very short path length) causing cell death
- Activation at 732 nm penetrates blood and tissue better than other photosensitizers in development
- Fluorescence detected at 750 nm with 460-480 nm excitation





Intracellular Localization of Antrin



Human Macrophages

HCASMC

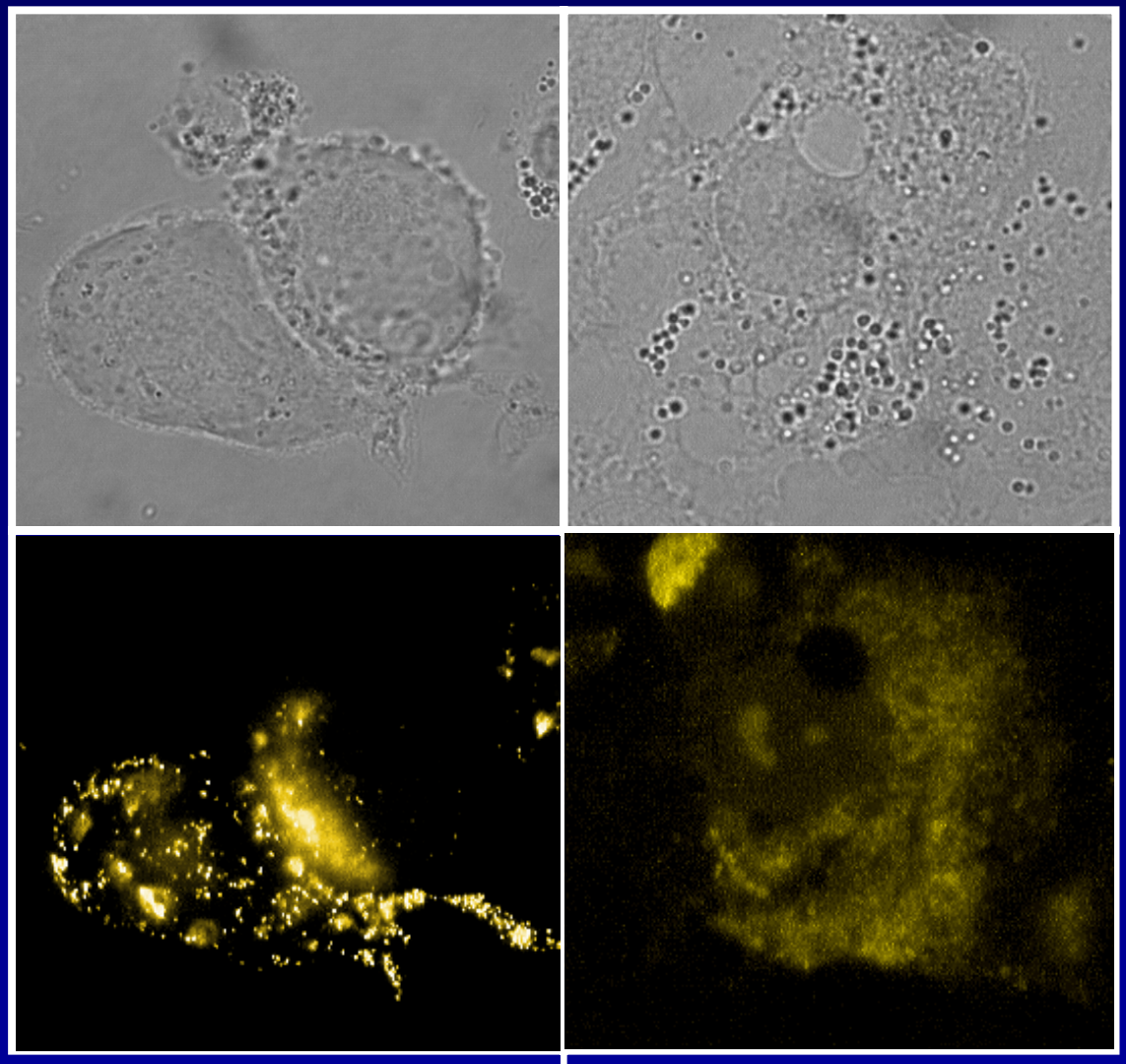
Mitochondria (green)

Antrin (red)

Overlay

Woodburn et al., ACC 2002

Lysosomal Instability after Phototherapy



THP-1 Macrophages

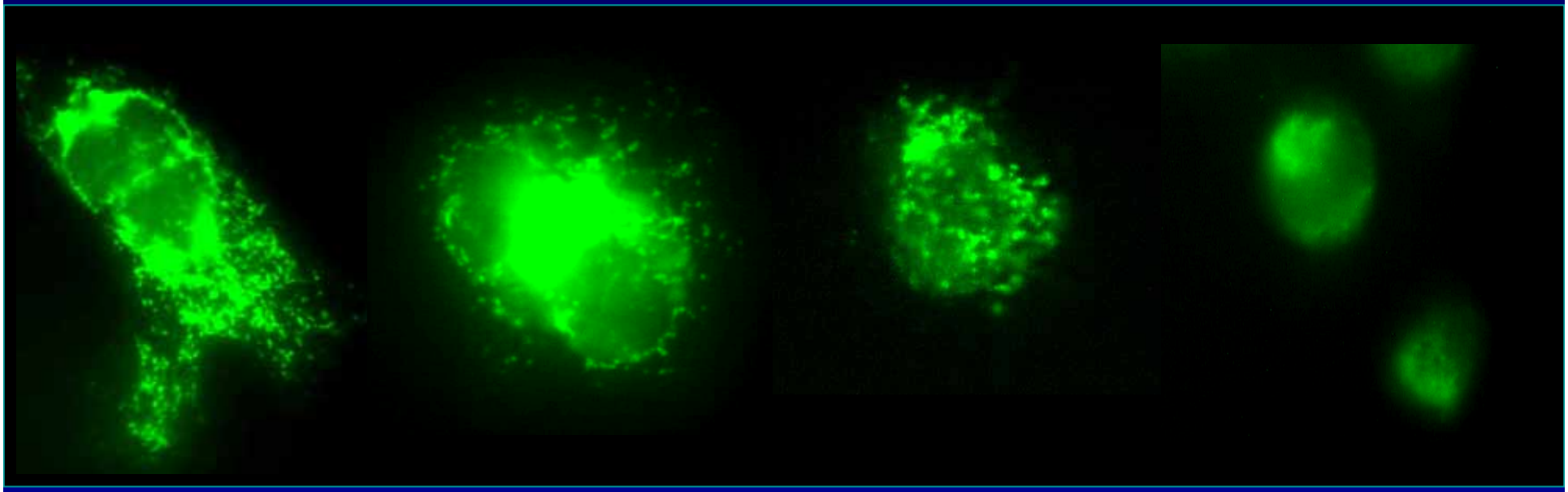
THP-1 Macrophages + Acridine Orange

Pre-treatment

Post-PDT @ LD_{50}

Woodburn et al., ACC 2002

Antrin Phototherapy Induces Apoptosis in Human Macrophages



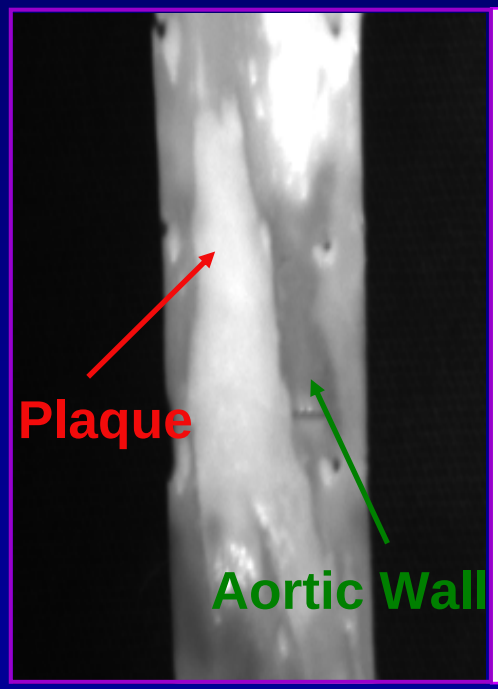
Control	20 ug/ml Antrin	2 J/cm^2 Light	Antrin + Light

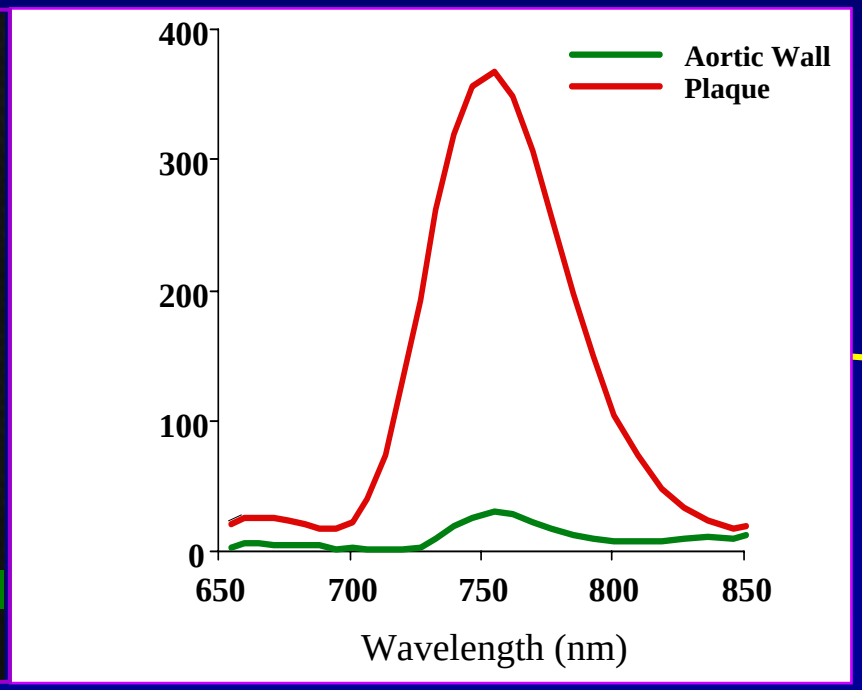
Cytochrome C immunoreactivity assay in human THP-1 cells

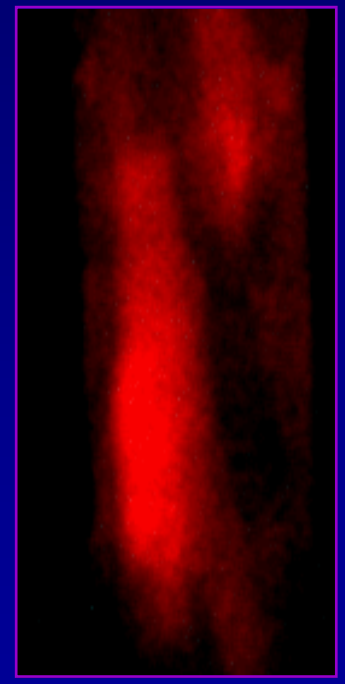
Woodburn et al., ACC 2002

Antrin Biolocalization in Atheromatous Plaque

NZW Rabbit aorta, 10 mg/kg iv administration, analysis at 24h







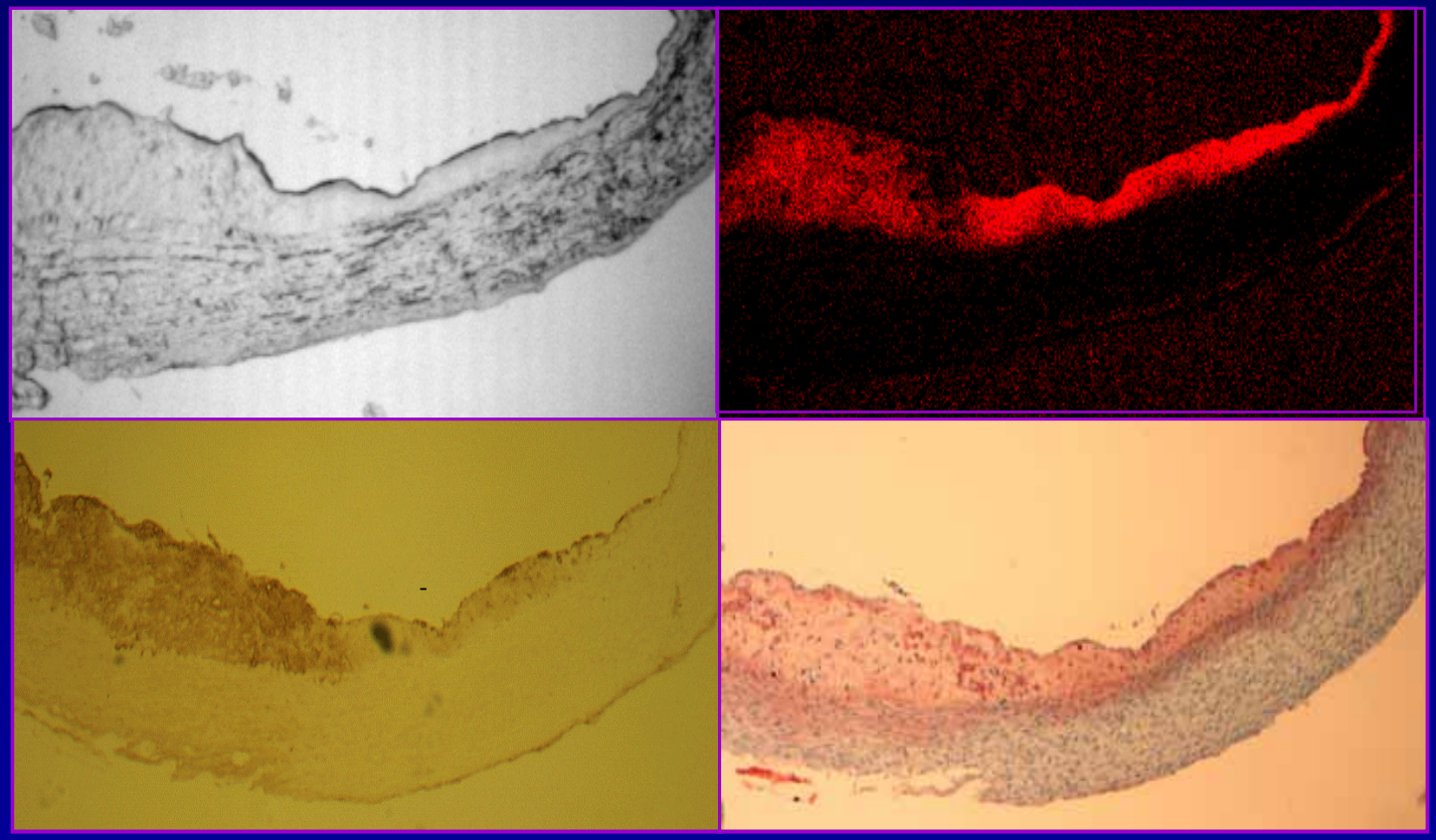
Fluorescent image

Rockson SG et al., Circulation 2000; 102:591-6

Texaphyrin Uptake in Rabbit Atheromas

B/W image

Fluorescence

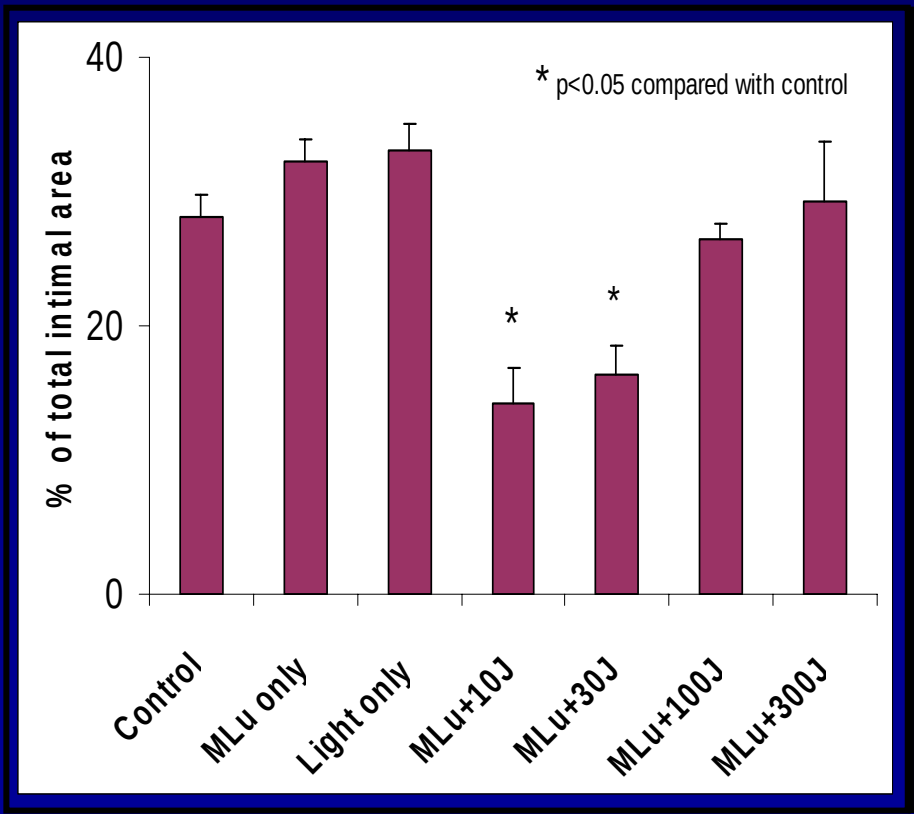


RAM-11 (macrophage)

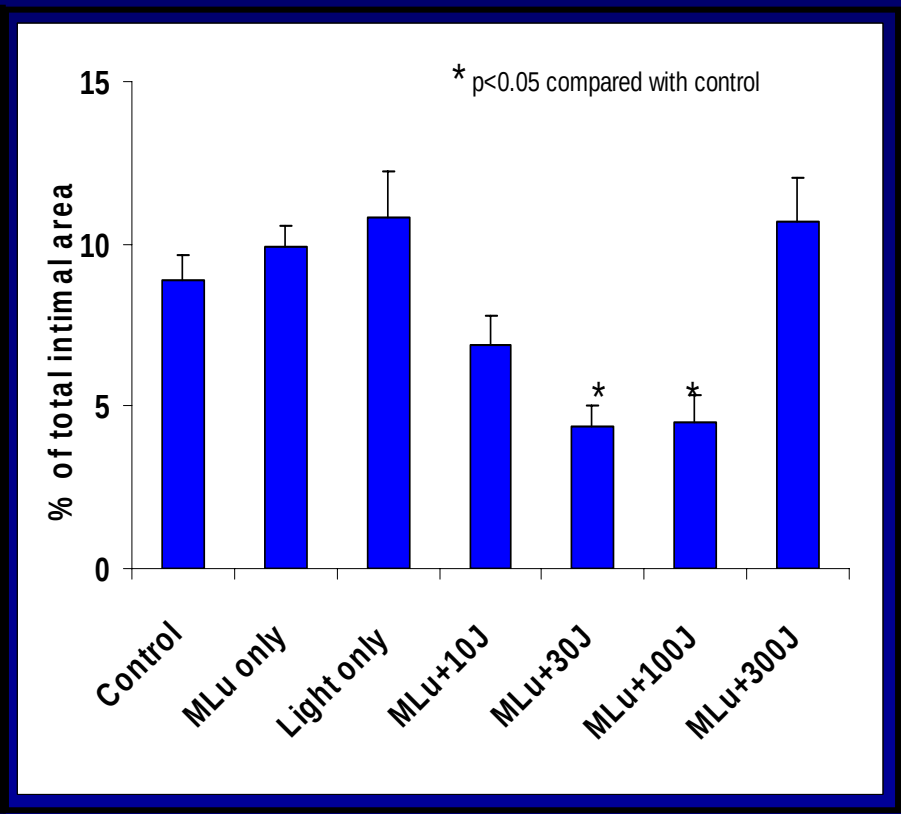
Oil Red O (neutral lipids)

Macrophage depletion in Fat-Fed New Zealand White (NZW) Rabbit; iv Antrin

Macrophage Burden as % of total intima and media



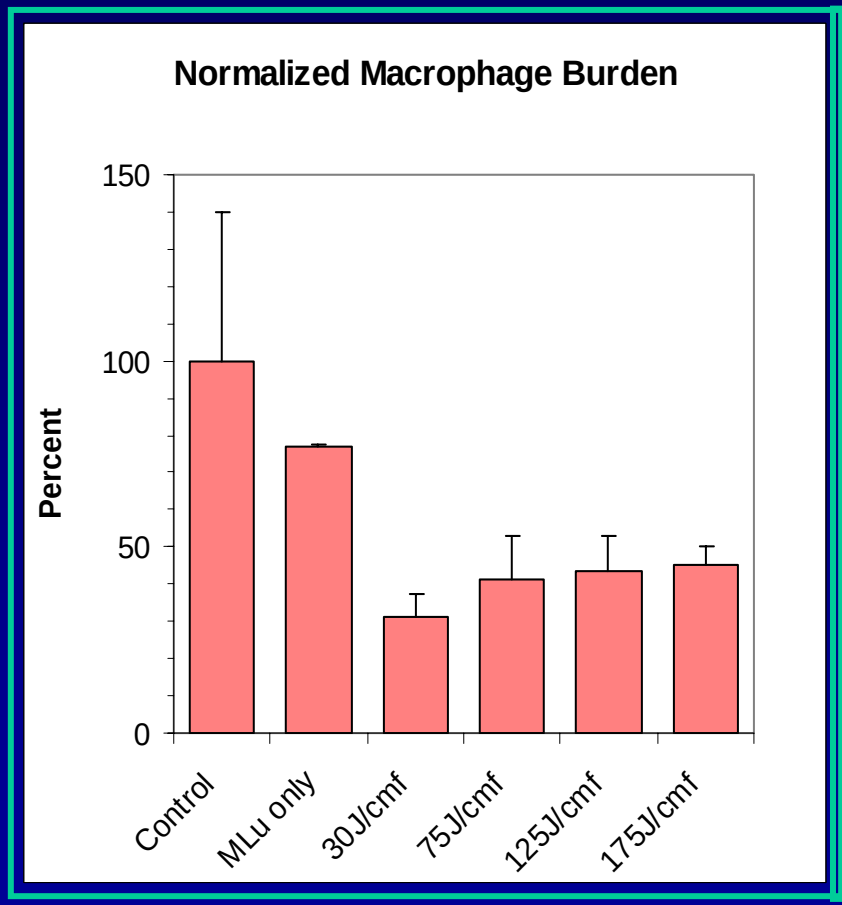
Intraballoon Illumination



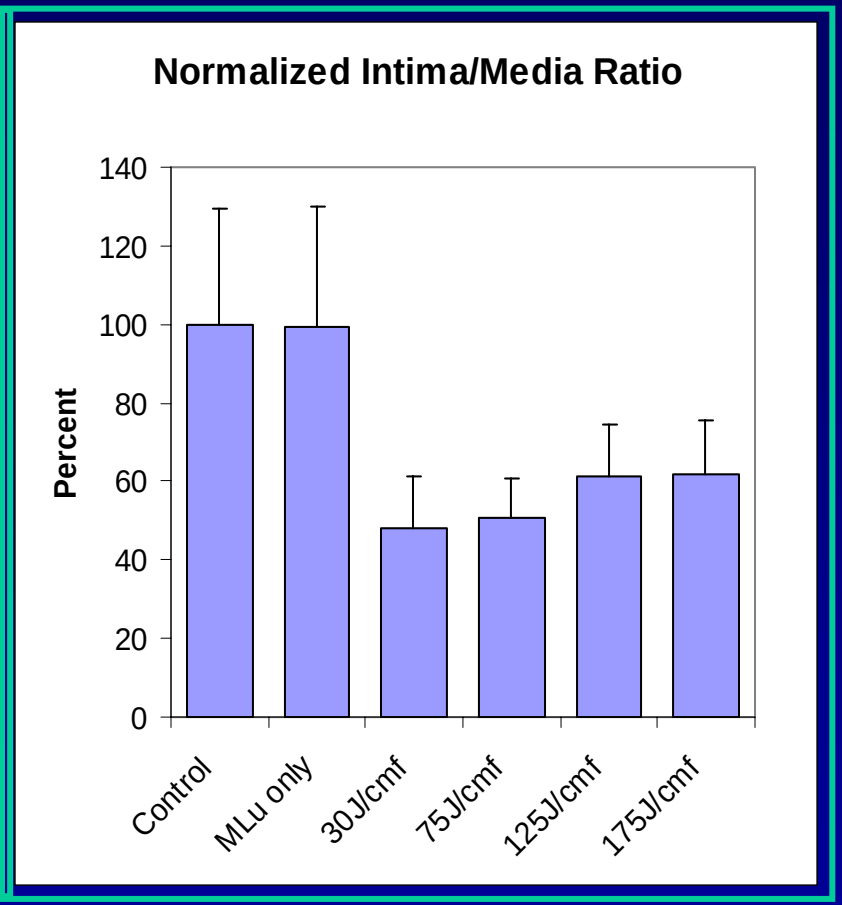
Bare Fiber Illumination

YP Sun, ACC 2003 Poster Presentation

Watanabe Hereditary Hyperlipidemic Rabbit (WHHL) Fat-Fed Model of Atherosclerosis

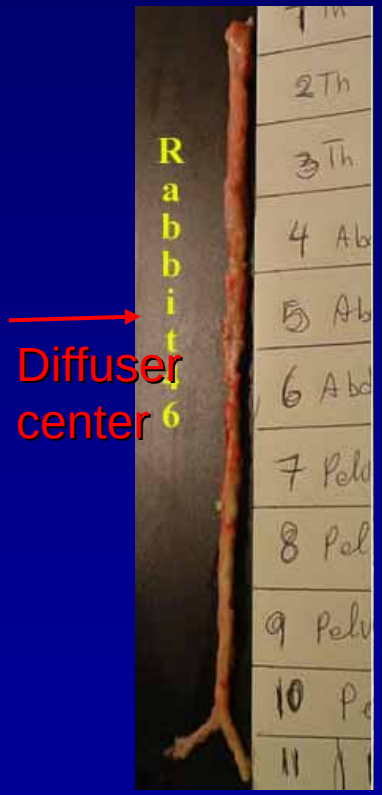


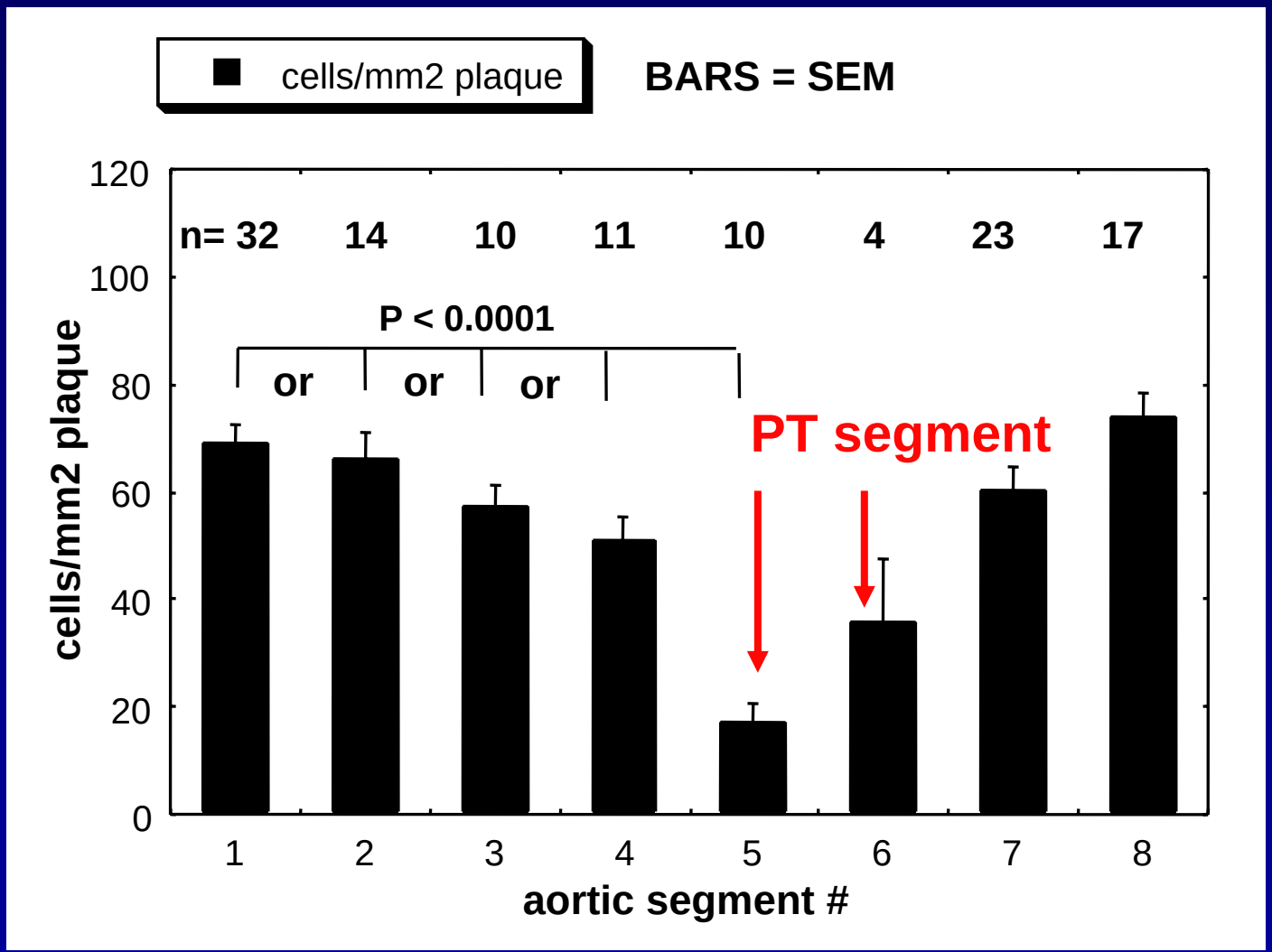
Control vs all Antrin-PT, p: 0.001~0.009



Control vs all Antrin-PT, p<0.072

Reduces cell density post-Antrin PT





NZW injury model, 7d after PDT

Hamblin, et. al, Wellman Labs, MGH, Boston

Preclinical Findings

- Antrin localizes intracellularly in mitochondria and lysosomes
- Antrin is selective to plaques rich in macrophages and neutral lipids
- Antrin Phototherapy:
  - Reduces macrophages in all rabbit models studied
  - Produces significant plaque accellularity within days post-PT
  - Downregulates cytokines involved in monocyte migration
  - Appears to maintain or increase smooth muscle area
  - Suggests some collagen remodeling of PT lesion
  - Does not traumatize normal vessel walls.
  - Potentially remodels and stabilizes unstable plaque
- Allows both focal and regional treatment of diseased vessels.

Antrin Clinical Development

Peripheral Arterial Disease (PAD)

Phase I: Dose ranging for safety. Completed.
Rockson et. al. Circulation 2000; 102:2322

Phase II: Multi-center, double-blind, randomized trial for prevention of restenosis and treatment of de novo lesion.
Study Completed. No adverse safety signals.

Coronary Arterial Disease (CAD)

Phase I: Drug and light dose escalation in subjects with CAD undergoing PCI with stent placement. Completed. Kereiakes, et. al. Circulation 2003; 103:1310

Antrin Phototherapy

Phase 1 Coronary Artery Disease Angiographic Results

- Enrollment: 79 patients
- Design: Drug and light dose escalation for safety
- Safety: No serious adverse effects
- Results: Optimum regimen identified
- Publication: **Kereiakes, et. al. Circulation 2003; 103:1310**

"The present phase 1 coronary study supports the apparent safety and tolerability of this treatment and materially extends our understanding of this emerging therapy in several ways.

Phase I CAD
Participating Investigators & Centers

Dean Kereiakes
The Lindner Center, Cincinnati

Daniel Simon
Brigham and Women's Hospital, Boston

Arthur M. Szyniszewski
Michigan Heart & Vascular, Ann Arbor

Alan Yeung
Stanford Medical Center, Palo Alto

Paul Kramer
Mid-America Heart Institute, Kansas City

Howard Herrmann
Hospital of the University of Pennsylvania

Wendy Shear
Minneapolis VA Medical Center

Jeffry Popma
QCA Core Lab, Boston, MA

Peter Fitzgerald
IVUS Core Lab, Stanford, CA

CAD Phase I Design

Dose-escalation safety trial in subjects with CAD undergoing PCI with stent placement

Eligibility: Patients With Coronary Arterial Disease

Design: IV ANTRIN Followed 18-24 Hours Later by Phototherapy.
Drug escalation (0.05 – 4 mg/kg)
Light escalation (100-600 J/cm^f)

Safety Objectives:

* Extent of restenosis in Antrin PT-treated lesions (QCA, IVUS)
* Pharmacokinetics in this CAD pop.

Primary Outcome Variables

* Dose-limiting toxicities associated with Antrin Injection and/or illumination
* Phototherapy-related procedural adverse events
* Death, Stroke, CK or CK-MB > 3 x ULN

CAD Phase I Design

Secondary Outcome Variables

* Late lumen loss/index (QCA; IVUS) at 6 months
* Angiographic restenosis rate (> 50%)
* TLR, TVR, TVF
* Pharmacokinetics

Inclusion Criteria:

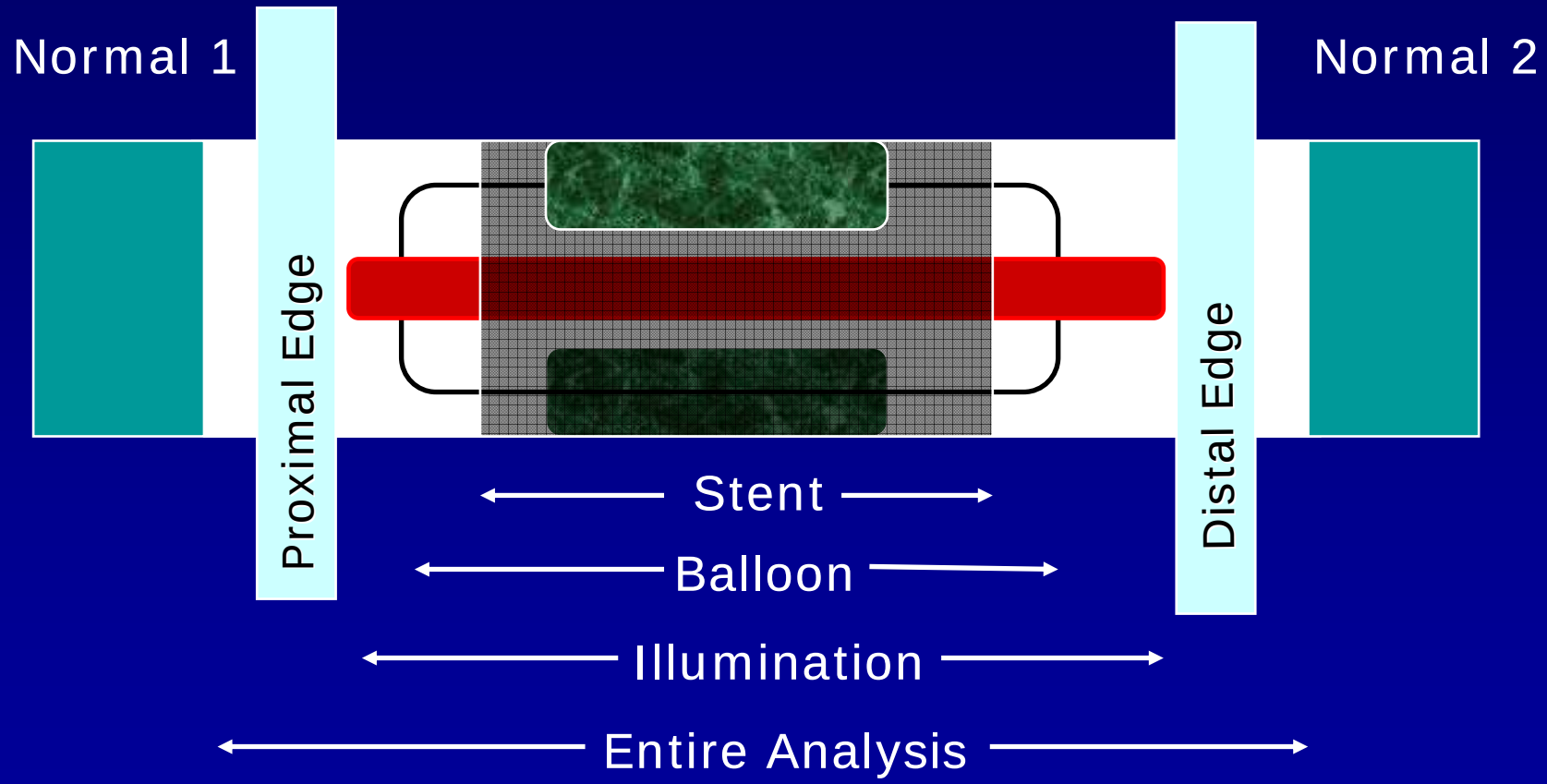
* Target lesion stenosis >50% needing PCI
* Target lesion for PCI $\leq$ 30 mm long
* Men or women $\geq$ 18 yrs old
* Give informed consent

Exclusion Criteria:

* Target lesions with previously placed stent
* Target lesions involving left main or ostial left anterior descending arteries

Angiographic Analysis Plan

BWH Angiography Core Lab – J. Popma



CAD Phase I Study Demographics (n=79)

Median Age, yrs	64.0 (43-85)
Men, %	70.9%
Diabetes, %	20.3%
Prior PCI, %	16.5%
Prior MI, %	51.9%
NYHA Class I, %	31.6%
NYHA Class II, %	68.4%

CAD Phase I Study
Target Vessel Characteristics

Target Vessel, %

- LAD 40 %
- LCX 28 %
- RCA 32 %

Stent Diameter (mm; mean) 3.33

Stent Length (mm; mean) 19.1

CAD Phase I Study
Procedure and Device Performance

Device Success*	100%
Procedural Success**	96.2%
• Interrupted Illumination	0%
• Fiber could not be delivered	0%
• Bailout procedure [left main disease; angioplasty dissection]	3.8% (n=3)
GP IIb/IIIa Use	51%

**Successful delivery of the illuminating fiber when attempted*

***No in-hospital MACE*

CAD Phase I Study
Preliminary Acute Safety (30 days)

Emergent CABG	0 %
Death	0 %
Stroke	0 %
Total CK Elevation (>3xULN)	1.3 %
Total CK-MB Elevation (>3xULN)	10.3 %
Target vessel revascularization	1.3 %
Stent Thrombosis	0 %

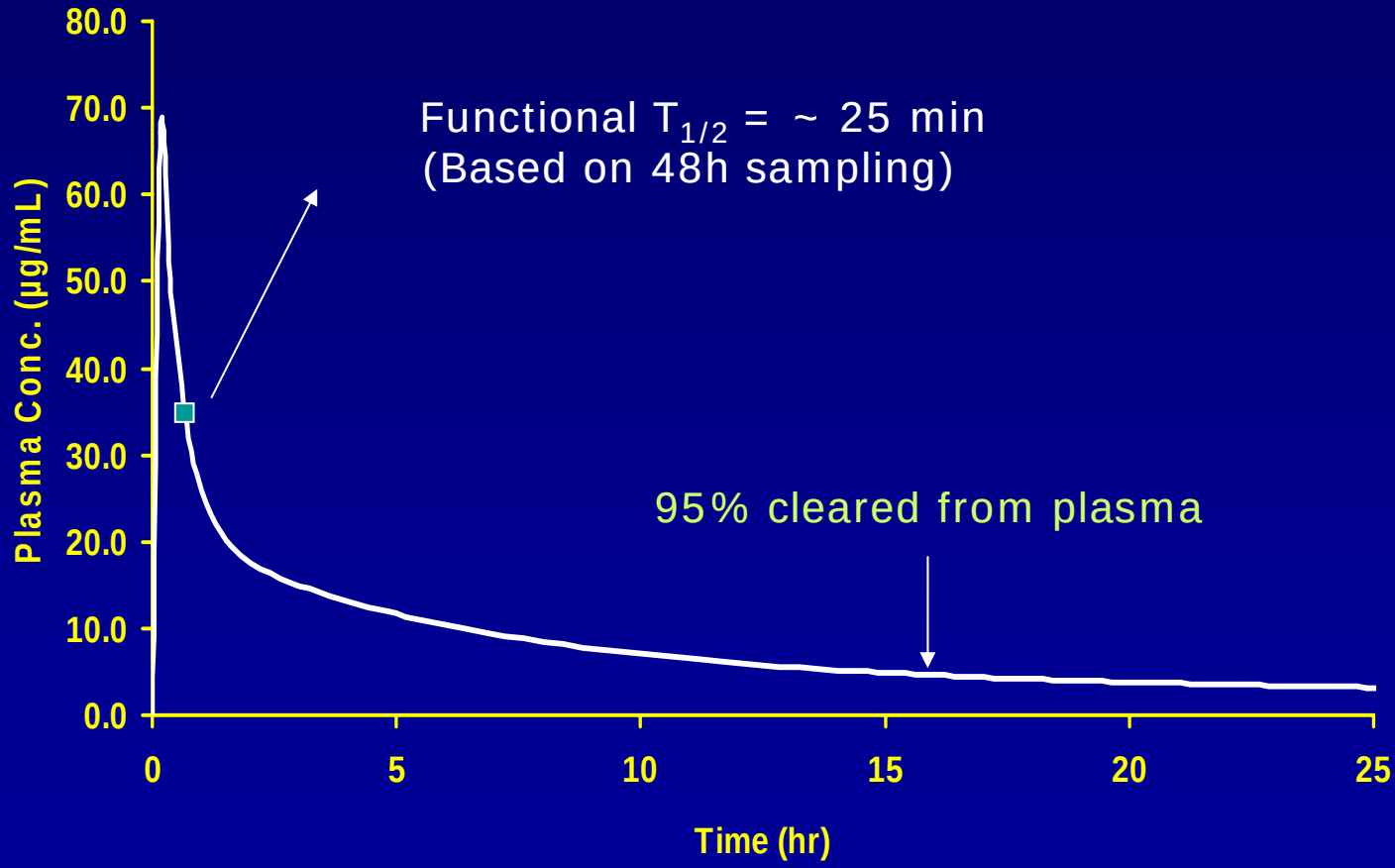
Antrin CAD Phase I Study Infusion Related Events

Dose (mg/kg)	N	Peripheral Paresthesia*	Rash*
0.05	5	1 (20.0%)	0 (0)
0.15	5	0 (0%)	0 (0)
0.5	6	0 (0%)	0 (0)
1	6	1 (16.7%)	1 (16.7)
2	21	10 (47.6%)	3 (14.3)
3	26	14 (53.8%)	5 (19.2)
4	10	6 (60.0%)	3 (30.0)

* Rashes were not phototoxic reactions. Duration of paresthesias and rashes ranged from 0-46 days, and 0-51 days, respectively. All were mild to moderate in severity

Antrin (MLu) Pop Pharmacokinetics Rapid Clearance from Plasma

Across all drug doses: 0.05-4 mg/kg

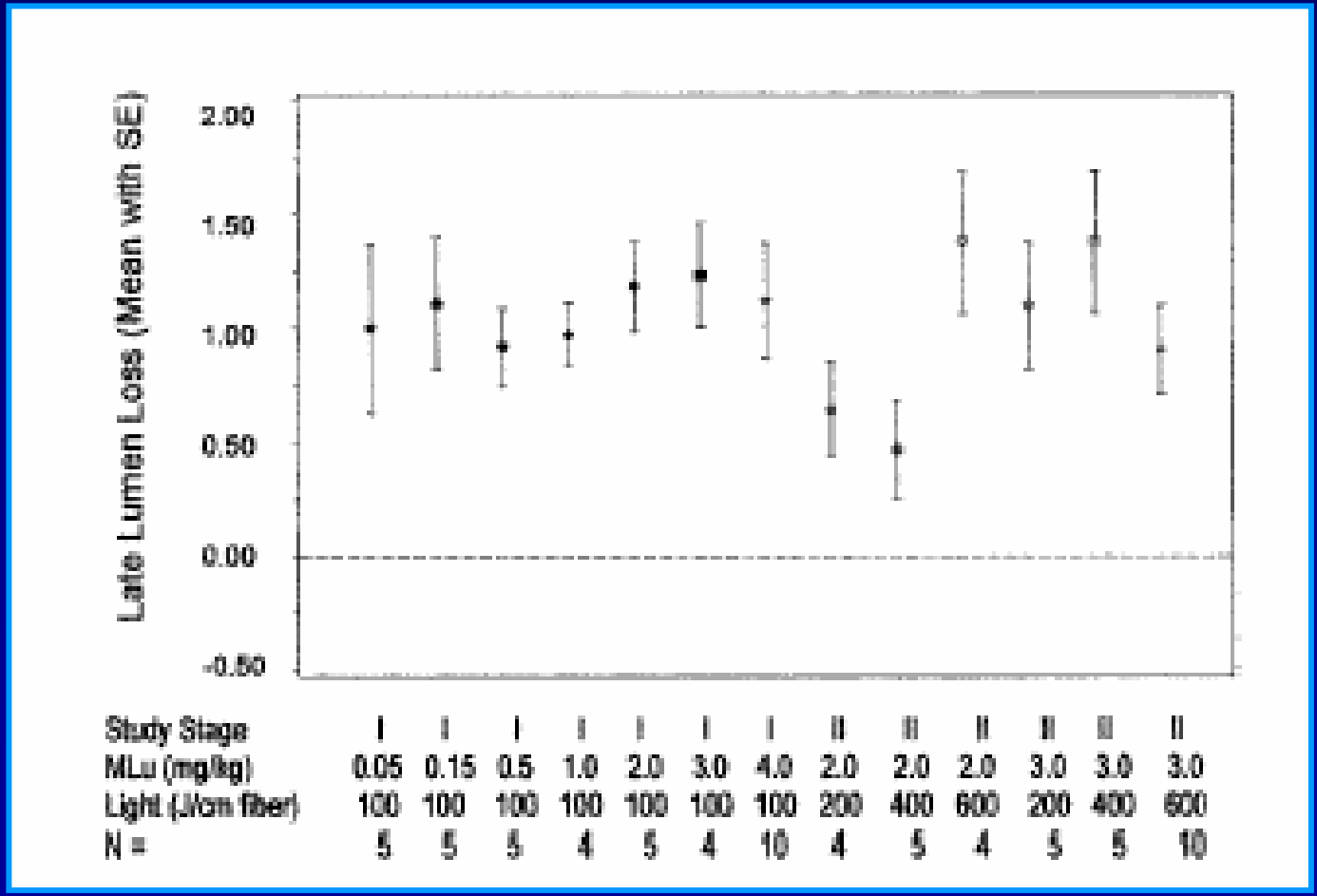


Follow-Up Angiography
Quantitative Results

There were no clinically significant differences between the stent, balloon injury, illumination and analysis segments

	Overall % (95% CI)
Stent Segment (Binary Restenosis)	24/71, 33.8 % (23.0, 46.0)
Stent Segment MLD (mm)	1.75
Stent Segment Late lumen loss (mm)	1.02
Edge segments	2/70 (2.9 %) (0.3, 9.9)
Pre-PCI % stenosis (median)	66.0 %
Post-PCI % stenosis (median)	6.8 %
Pre / Post- ref. vessel diameter (mean)	2.93 / 2.94 mm

Mean (SE) late lumen loss by quantitative coronary angiography stratified by study stage, MLu dose, and light fluence.



Qualitative Assessment by IVUS (N=39)

- Incomplete apposition

  Preserved incomplete apposition: 2

  Resolved incomplete apposition: 1

  Late incomplete apposition: 0

- Stent edge dissection

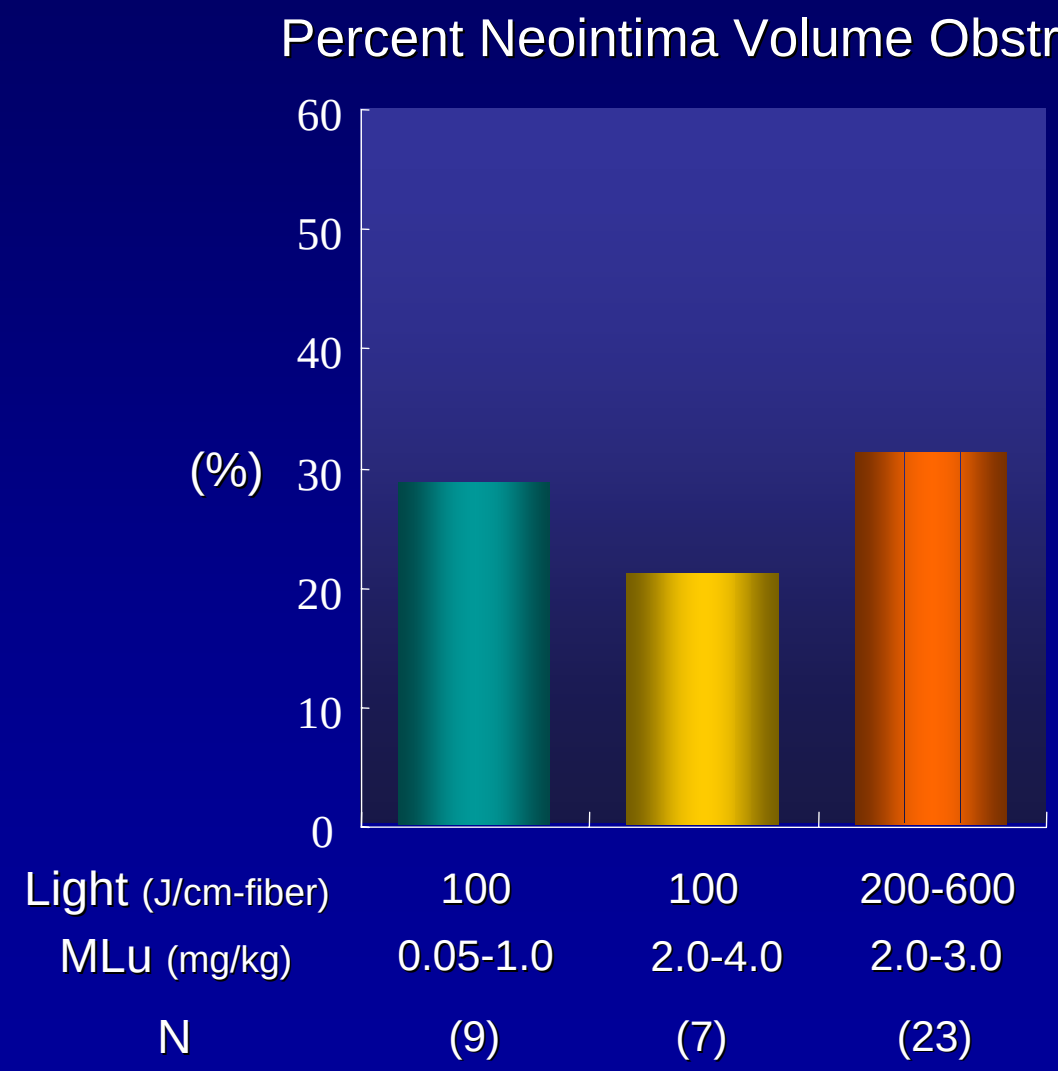
Baseline	6-month follow-up
4	0

- Intraluminal thrombus

Baseline	6-month follow-up
0	0

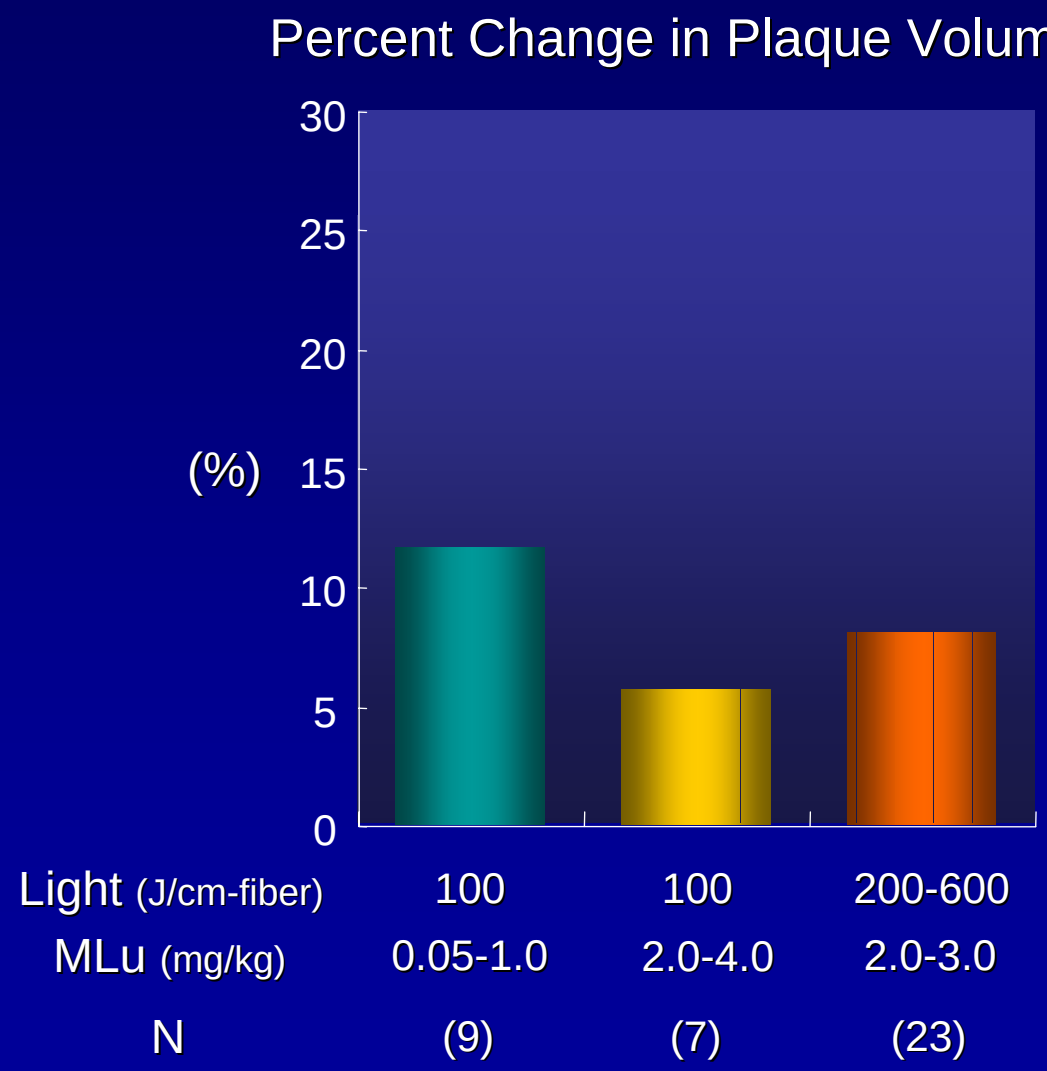
Hongo, et. al., manuscript under preparation

Quantitative Assessment by IVUS (N=39)



Hongo, et. al., manuscript under preparation

Quantitative Assessment by IVUS (N=39)



Hongo, et. al., manuscript under preparation

Safety Summary - CAD

- ANTRIN Phototherapy is feasible, well-tolerated and safe in >250 trial patients to date.
  - **No drug/light dose-limiting toxicities**
  - **Self-limited paresthesias with > 2.0 mg/kg**
  - **Successful and safe intravascular light delivery**
- Absence of late incomplete stent apposition with PT
- Very low incidence of geographical miss
- No evidence of deleterious edge effects
- No reported treatment-related aneurysms
- No observed subacute stent thrombosis or proliferative fibrosis within the reference segment