Plaque Profiling

Pursuing VP Signatures & New Therapeutic Agents

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Heart Institute, Asan Medical Center, Seoul, Korea

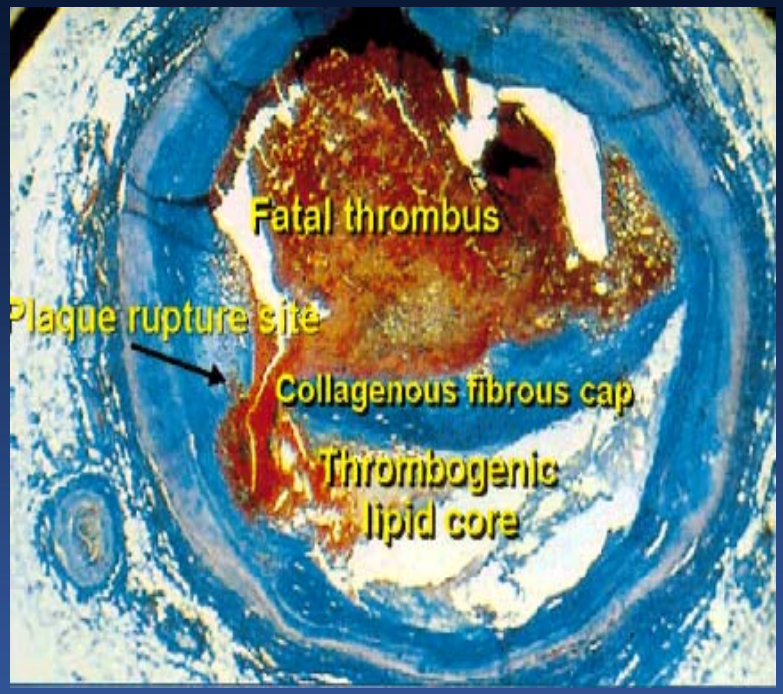
Presentation

- **Established Targets**
  - HMGCoA reductase
  - $P2Y_{12}$ receptor
- **Emerging Targets**
  - Lipid metabolism
  - ECM proteases
  - Inflammation

King of CV Medicine

Two Great Drugs

Statins and Anti-platelet Agents



THE HUMAN PROTEIN ATLAS

ABOUT & HELP

HMGCoA reductase: statins

$P2Y_{12}$ receptor: clopidogrel, prasugrel, ticagrelor

...the only proven medicine

Landmark Statin Trials

So Luxurious ...

Statin is an anti-atherosclerotic drug.

Rapid Effects of Statin Therapy

Mechanisms of early benefits are not fully understood.

Expression of HMG-CoAR in Human Coronary Plaques & Relationship to Plaque Destabilization

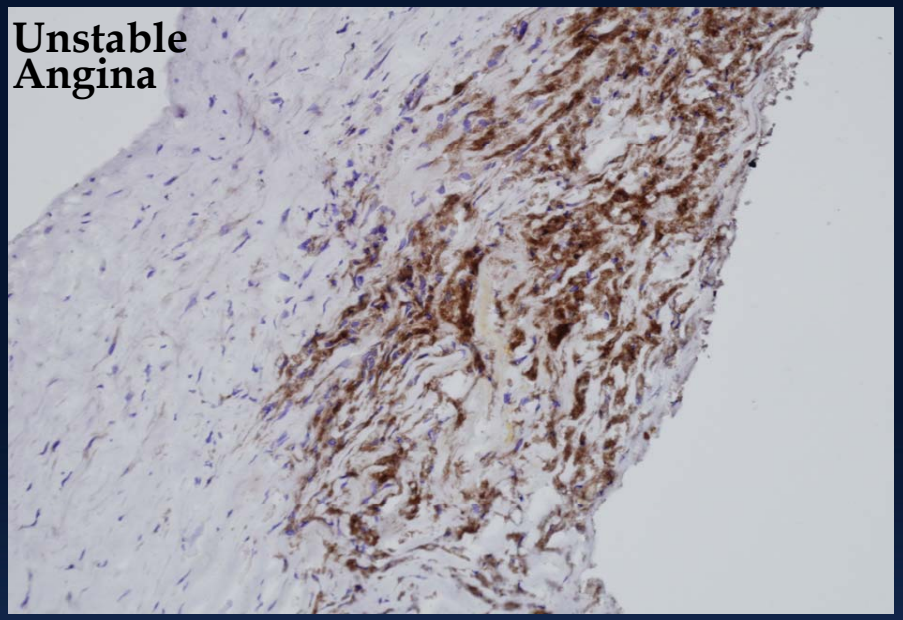
Heart 2011;97:715-20

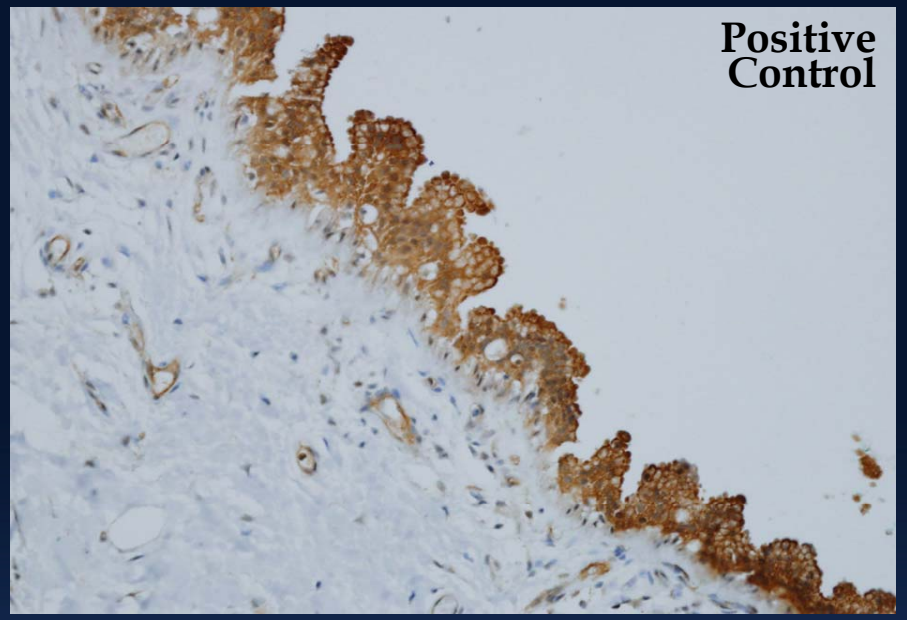
We investigated the expression of HMG-CoAR in coronary atherectomy tissues retrieved from 43 patients with unstable & stable angina, & examined the relationship of HMG-CoAR with plaque instability.

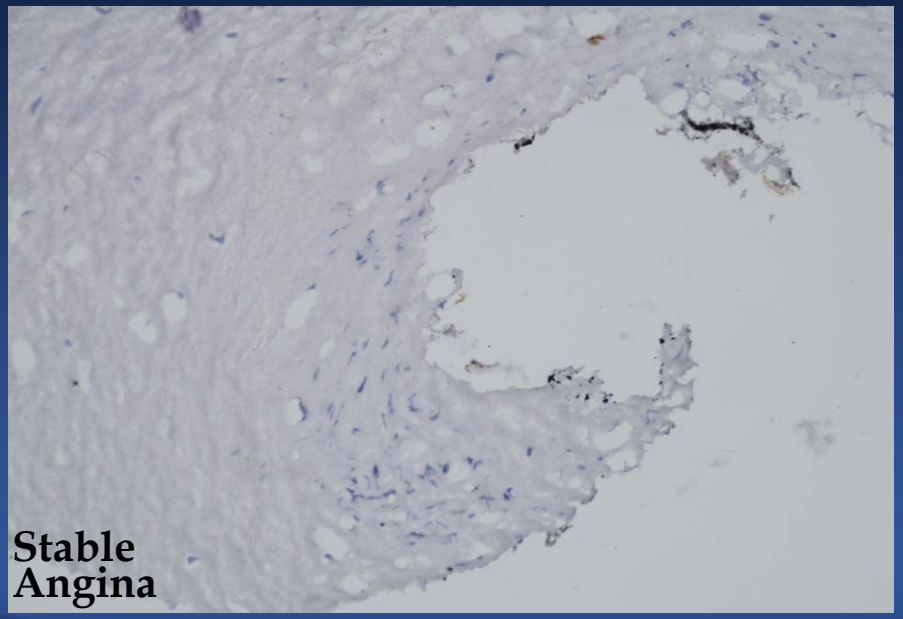
Immunohistochemistry

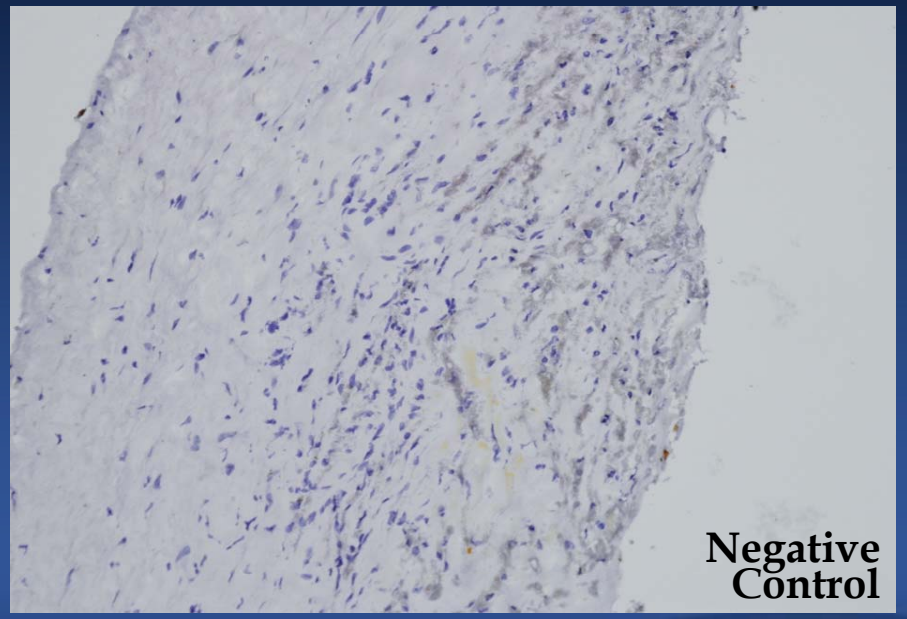
	Unstable angina (n=22)	Stable angina (n=21)	*p*-value
α-SM actin (%)	21.4±18.7	33.6±28.7	0.103
CD31 (%)	3.4±4.1	0.3±0.5	0.002
CD68 (%)	10.9±16.2	1.8±1.7	0.016
HMG-CoA R (%)	3.0±4.5	0.4±0.7	0.014

Data are shown as % positive area (immunostaining area/total plaque area×100).


Unstable
Angina


Positive
Control


Stable
Angina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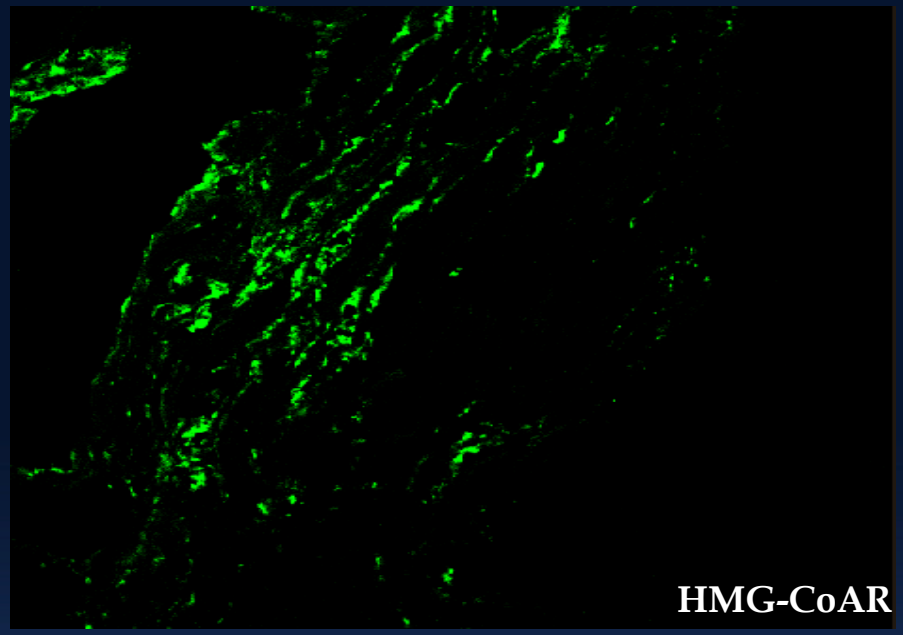
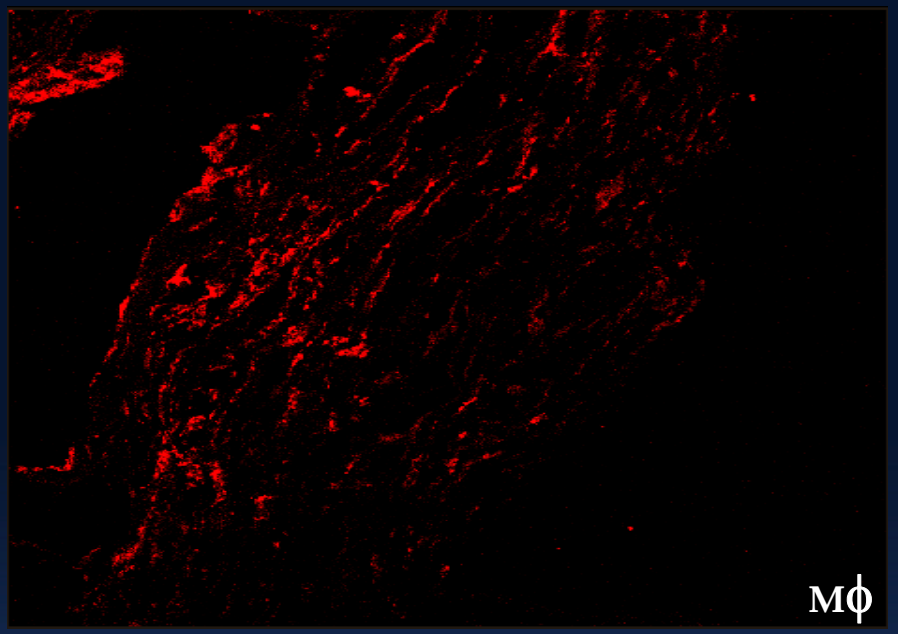
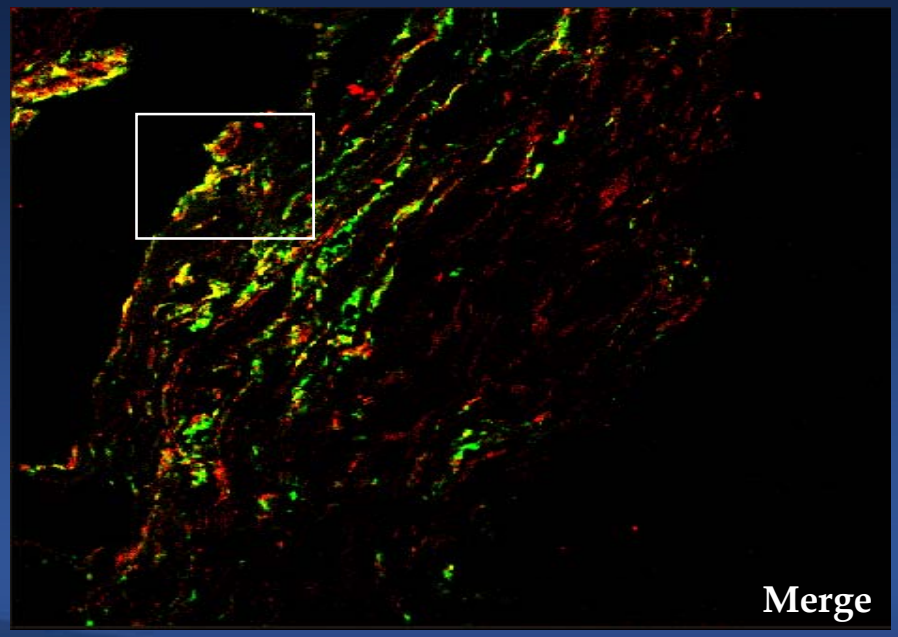
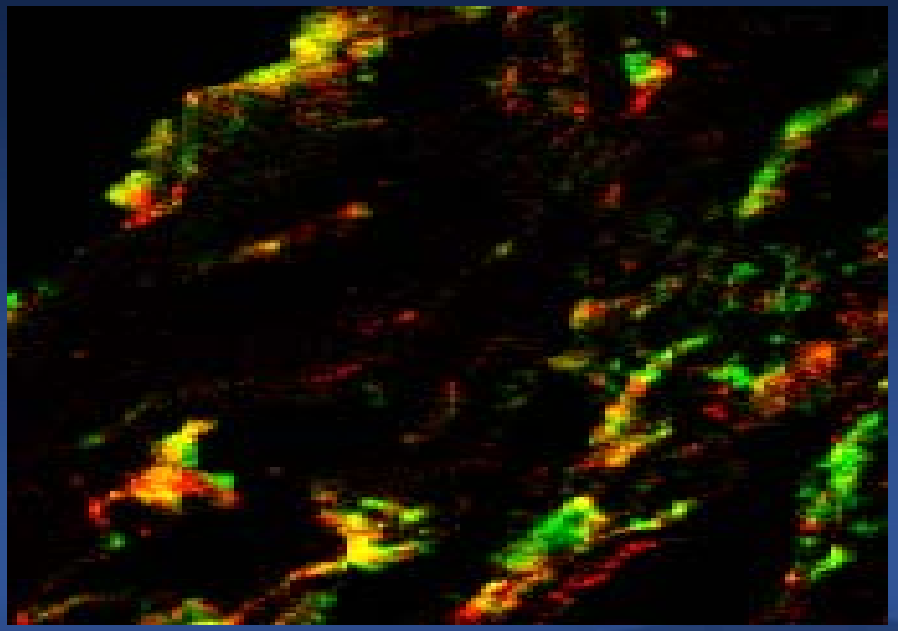
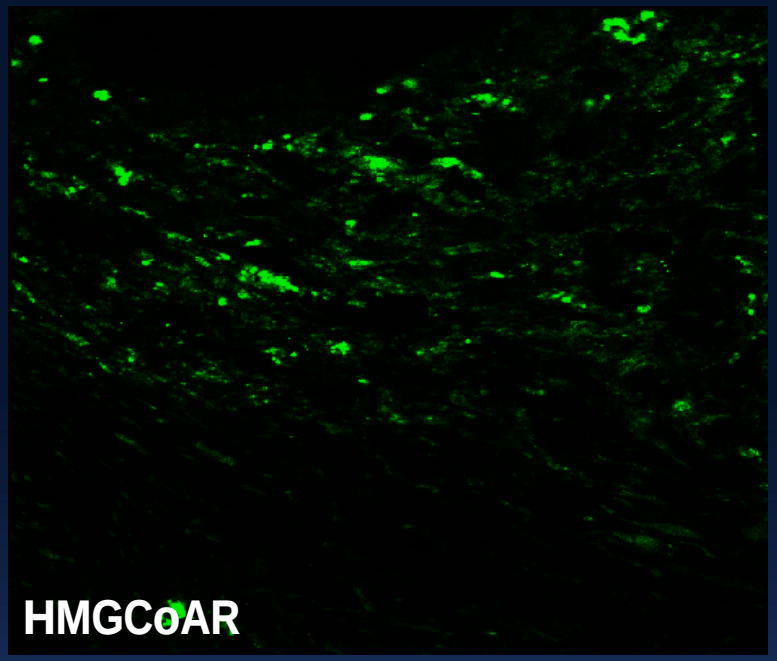
Control

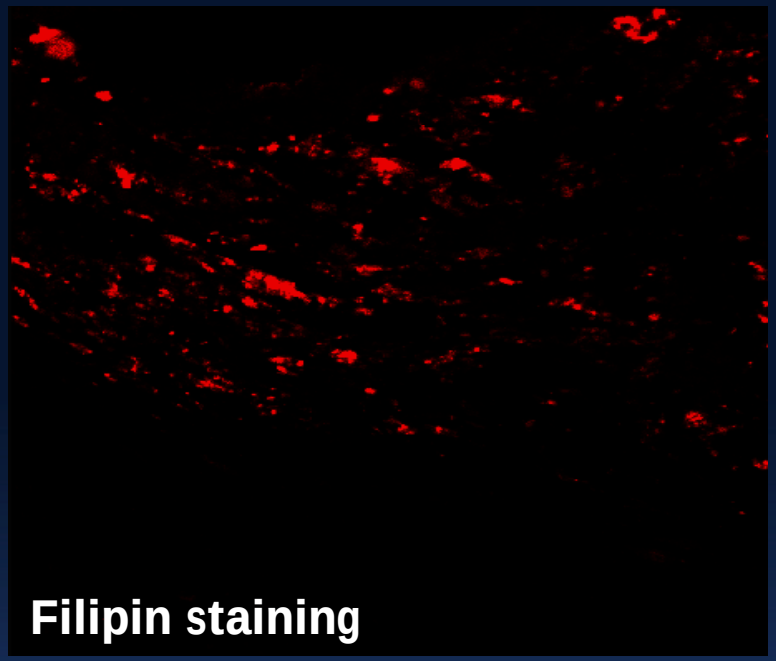

HMG-CoAR

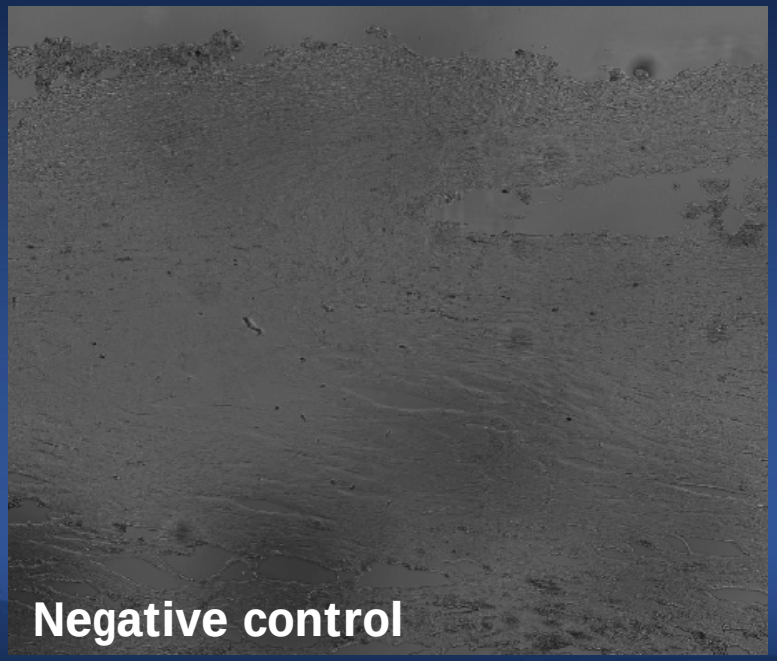

Mϕ

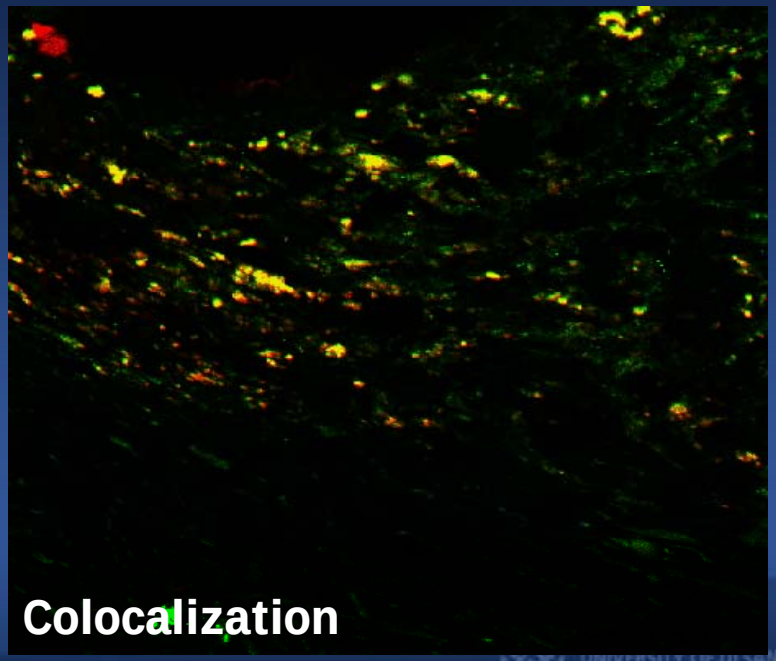

Merge



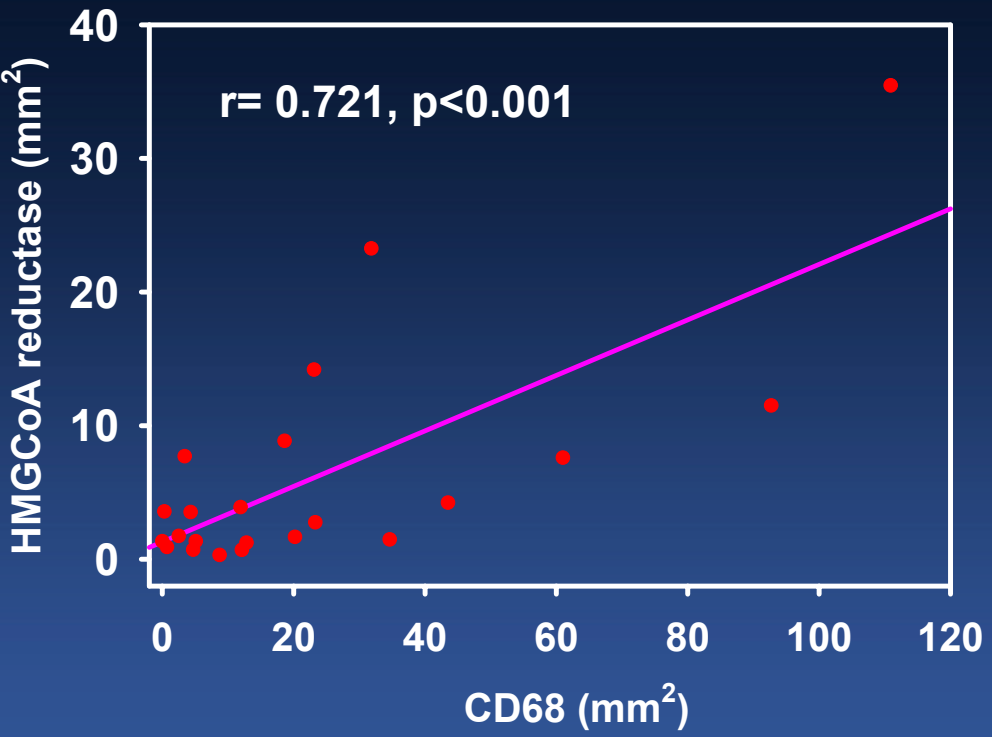

HMGCoAR


Filipin staining

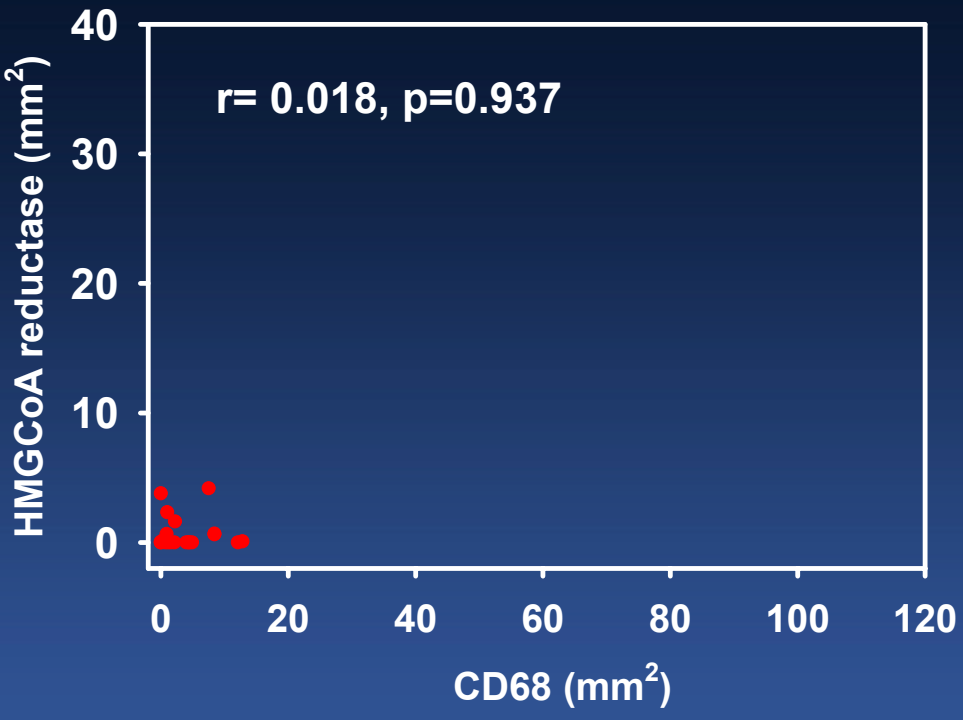

Negative control


Colocalization

Relationship Between CD68-Positive Areas & HMG-CoA Reductase-Positive Areas (mm^2)

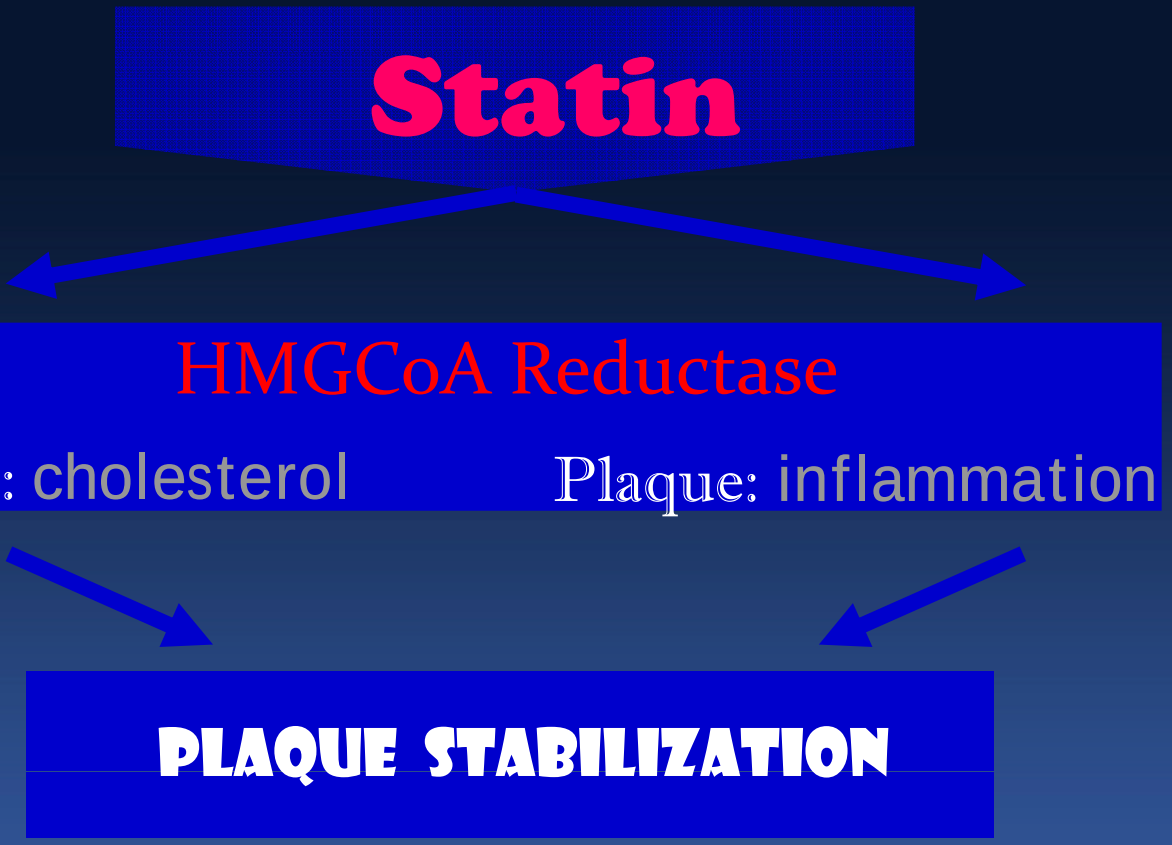


Unstable Angina



Stable Angina

Beyond Cholesterol

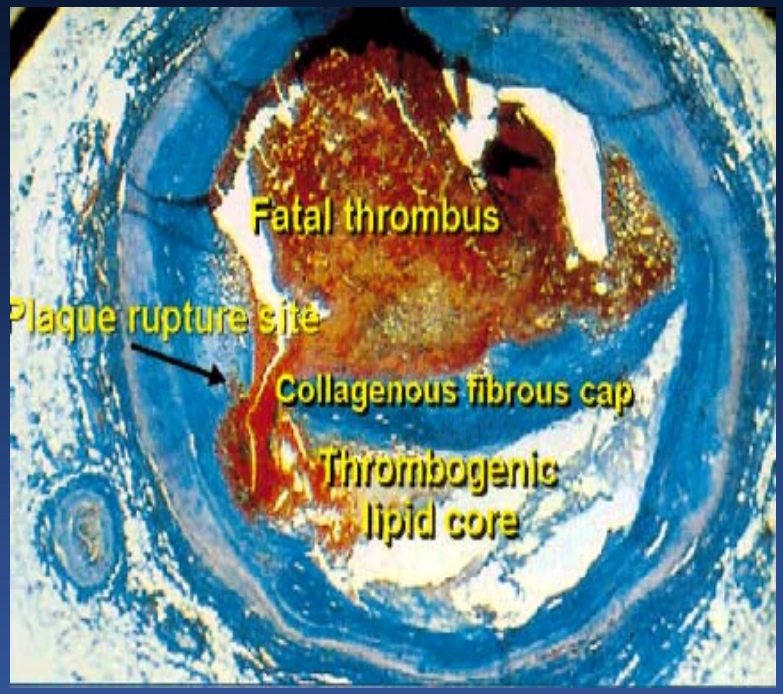


These findings support a potential role of the HMG-CoAR in the pathogenesis of ACS, and may help explain the early benefits of statin therapy in patients with ACS.

King of CV Medicine

Two Great Drugs

Statins and Anti-platelet Agents



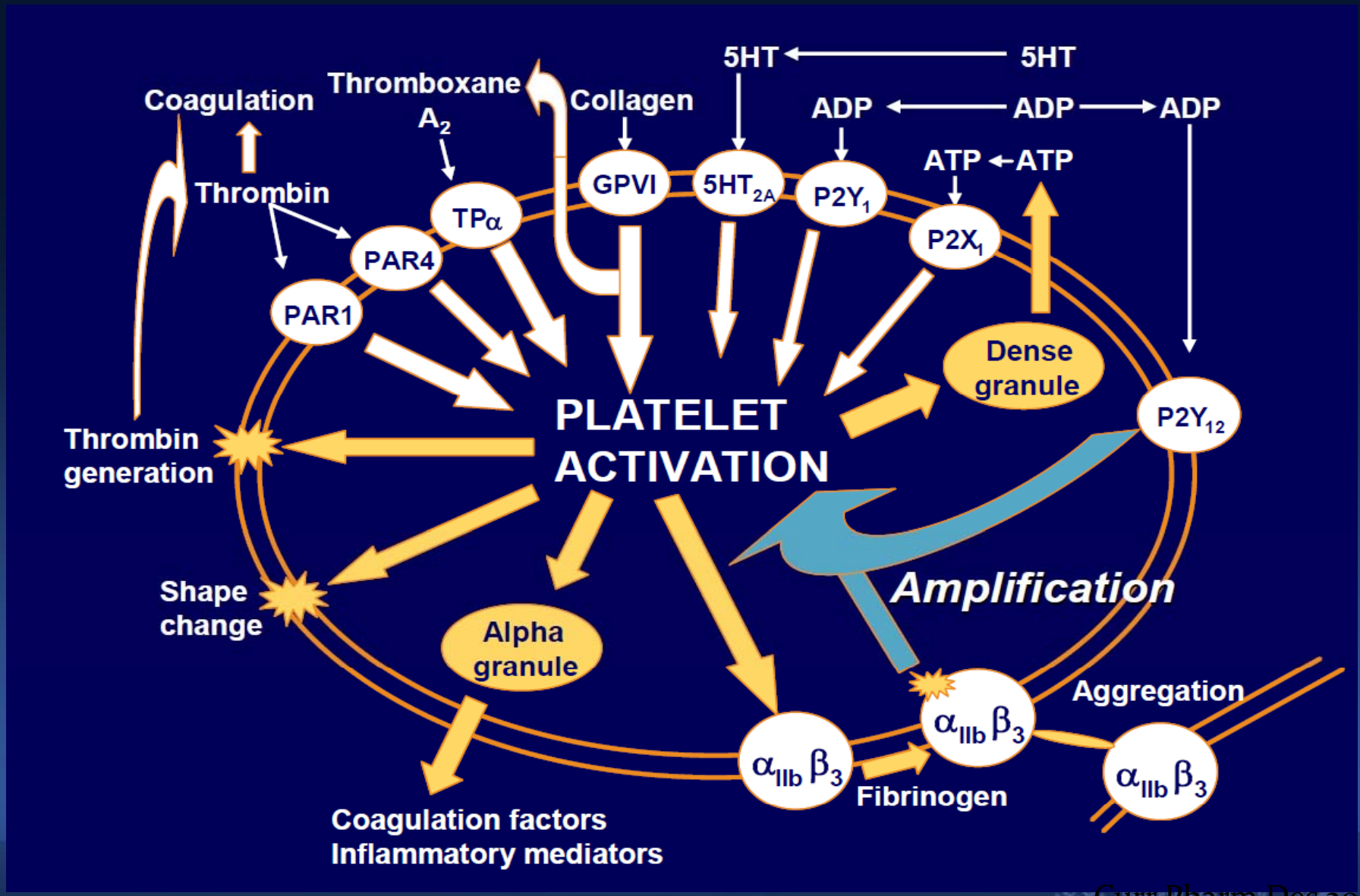
THE HUMAN PROTEIN ATLAS

ABOUT & HELP

HMGCoA reductase: statins

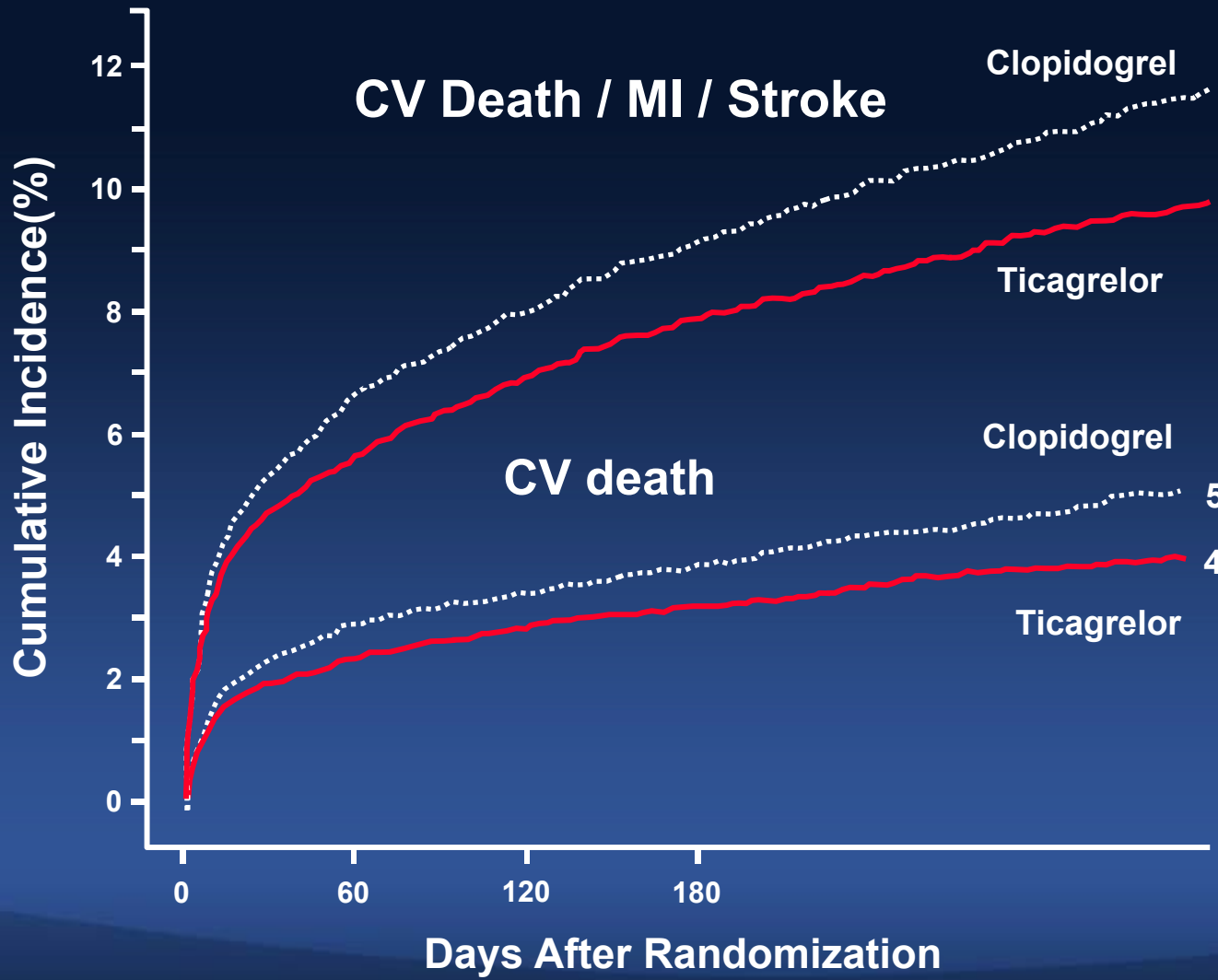
$P2Y_{12}$ receptor: clopidogrel, prasugrel, ticagrelor

$P2Y_{12}$ Receptor: A Key Player



Curr Pharm Des 2006;12:1255
Circulation 2010;121:171

PLATO: Major Outcomes



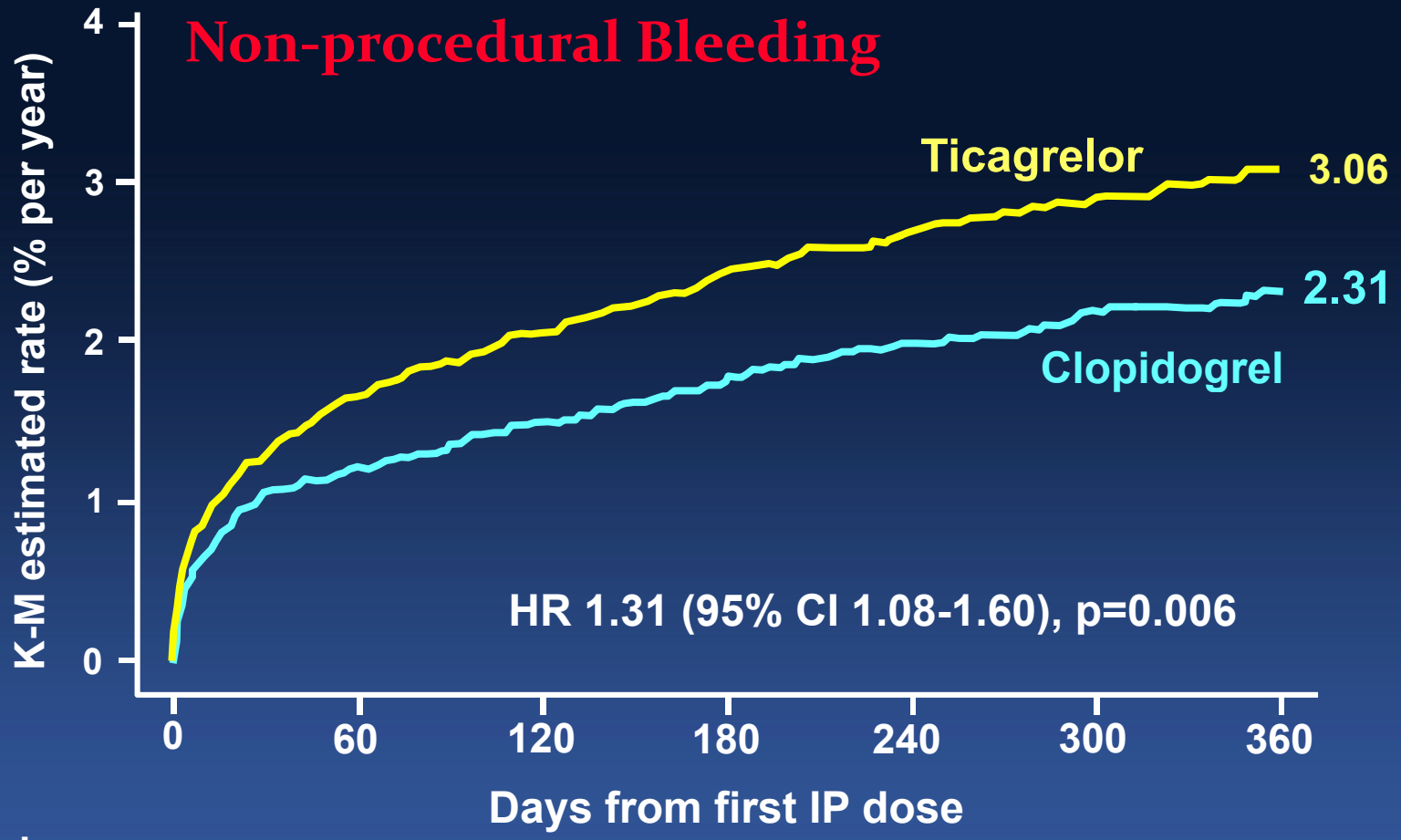
HR 0.84
(0.77-0.92)
P=.0003
NNT=54

HR 0.79
(0.69-0.91)
P=.001
NNT=90

Definite ST33%
p=0.009

Total death 22%↓
p<0.0001 (NNT70)

Bleeding Tax



No. at risk							
Ticagrelor	9,235	7,641	7,247	6,979	5,496	4,067	3,698
Clopidogrel	9,186	7,718	7,371	7,134	5,597	4,147	3,764

"More Bleeding = More Death"

Impact of Bleeding on Mortality after PCI

17,393 patients from REPLACE-2, ACUITY and Horizons

Differential Expression of $P2Y_{12}$ Receptor in Culprit Plaques from Patients with AMI & Stable Angina

Am J ardiol 2011;108:799-803

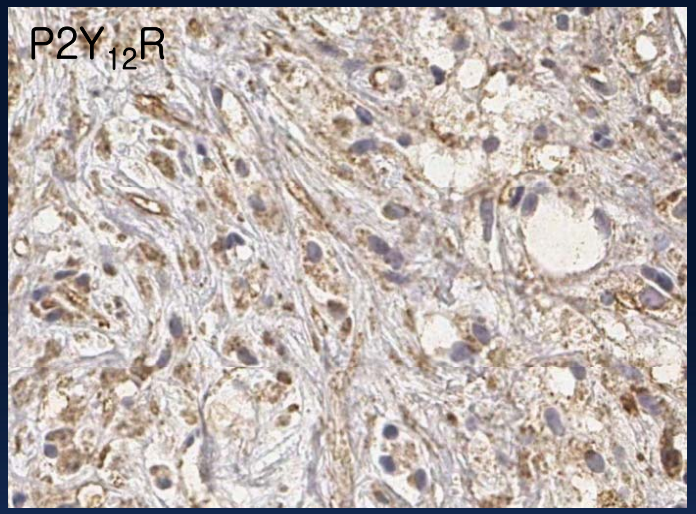
We compared the expression of P2Y12 receptors in coronary atherectomy tissues retrieved from 54 patients with AMI (n=35) or stable angina (n=19).

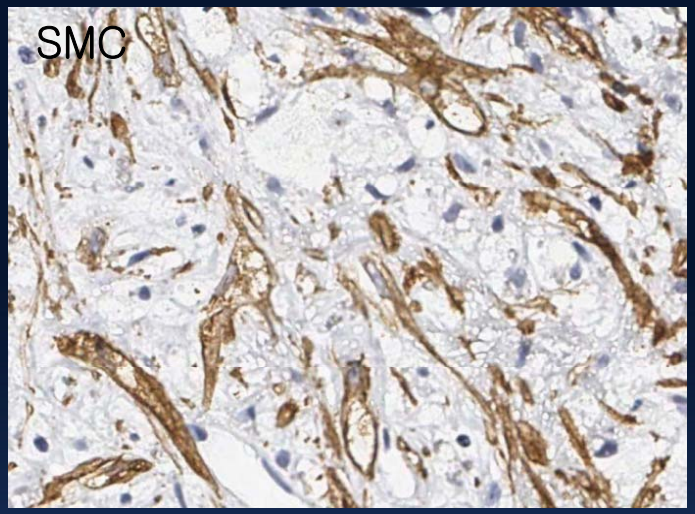
Immunohistochemistry

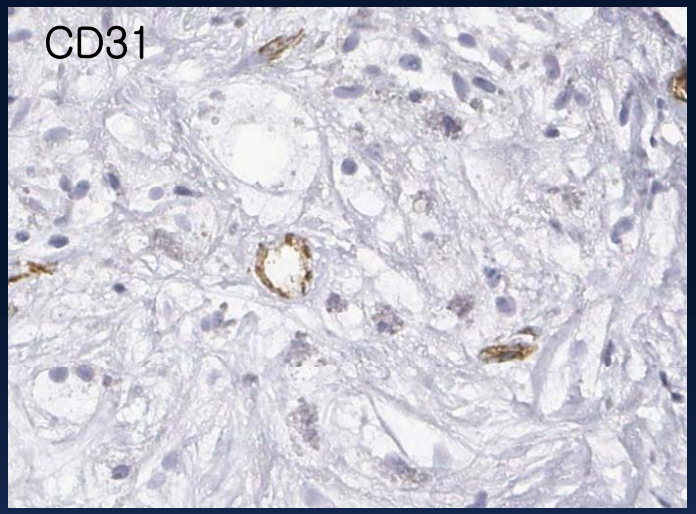
	AMI (n=35)	Stable angina (n=19)	*p*-value
α-SM actin (%)	2.9±2.7	12.3±14.4	0.011
CD31 (%)	1.1±1.6	0.2±0.2	0.001
CD68 (%)	15.5±13.6	7.0±14.6	0.038
P2Y12 Receptor (%)	1.1±0.9	0.5±0.4	<0.001

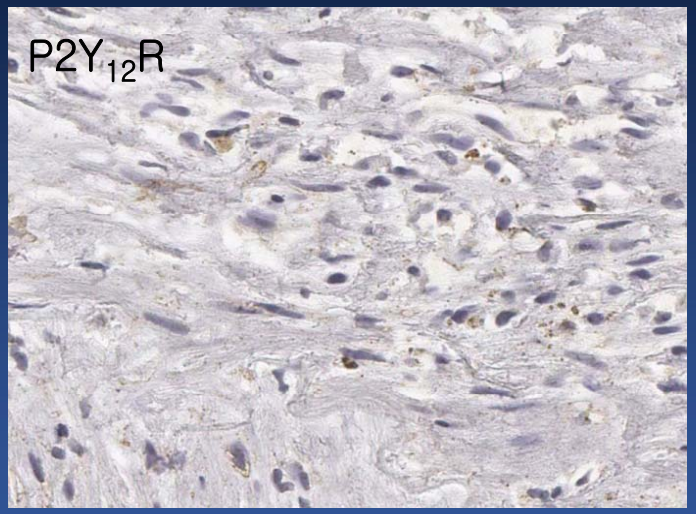
Data are shown as % positive area (immunostaining area/total plaque area×100).
AMI: STEMI 27, NSTEMI 8

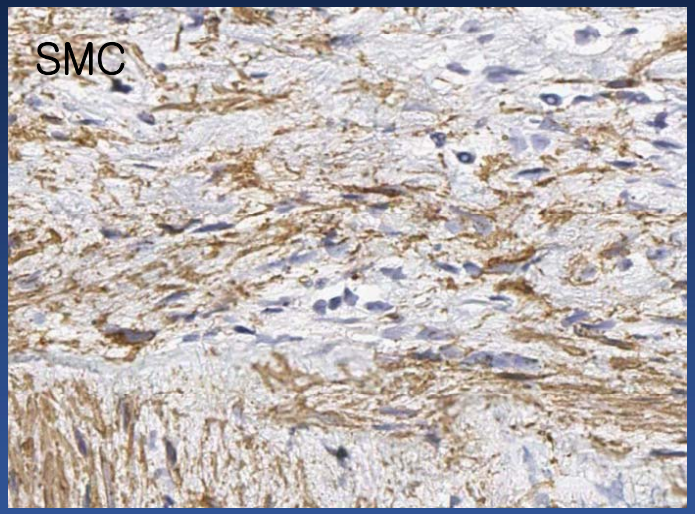
AMI

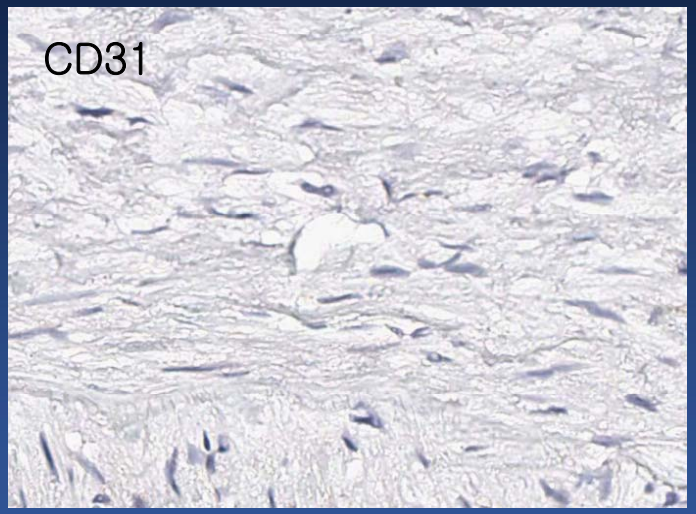






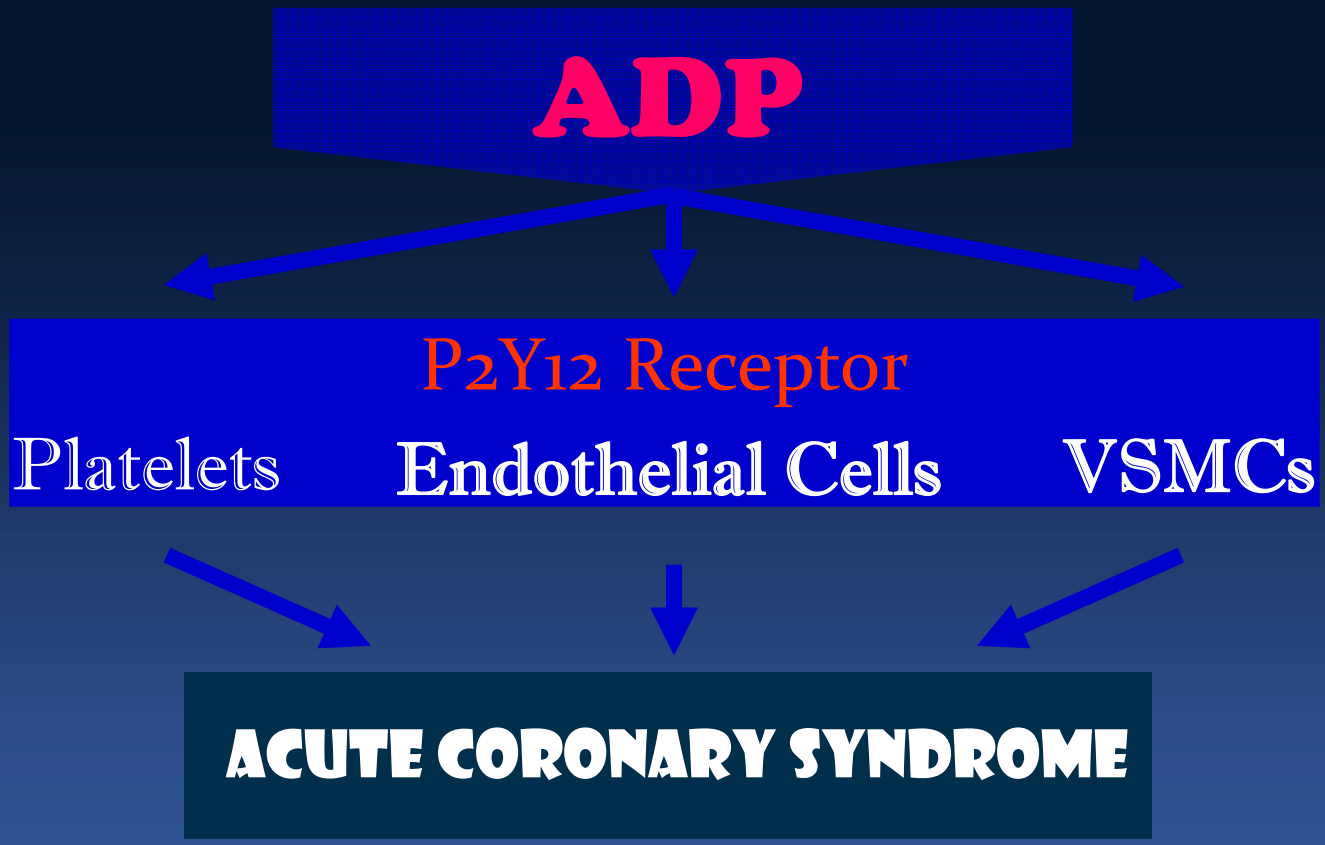






Stable angina

Beyond Platelets

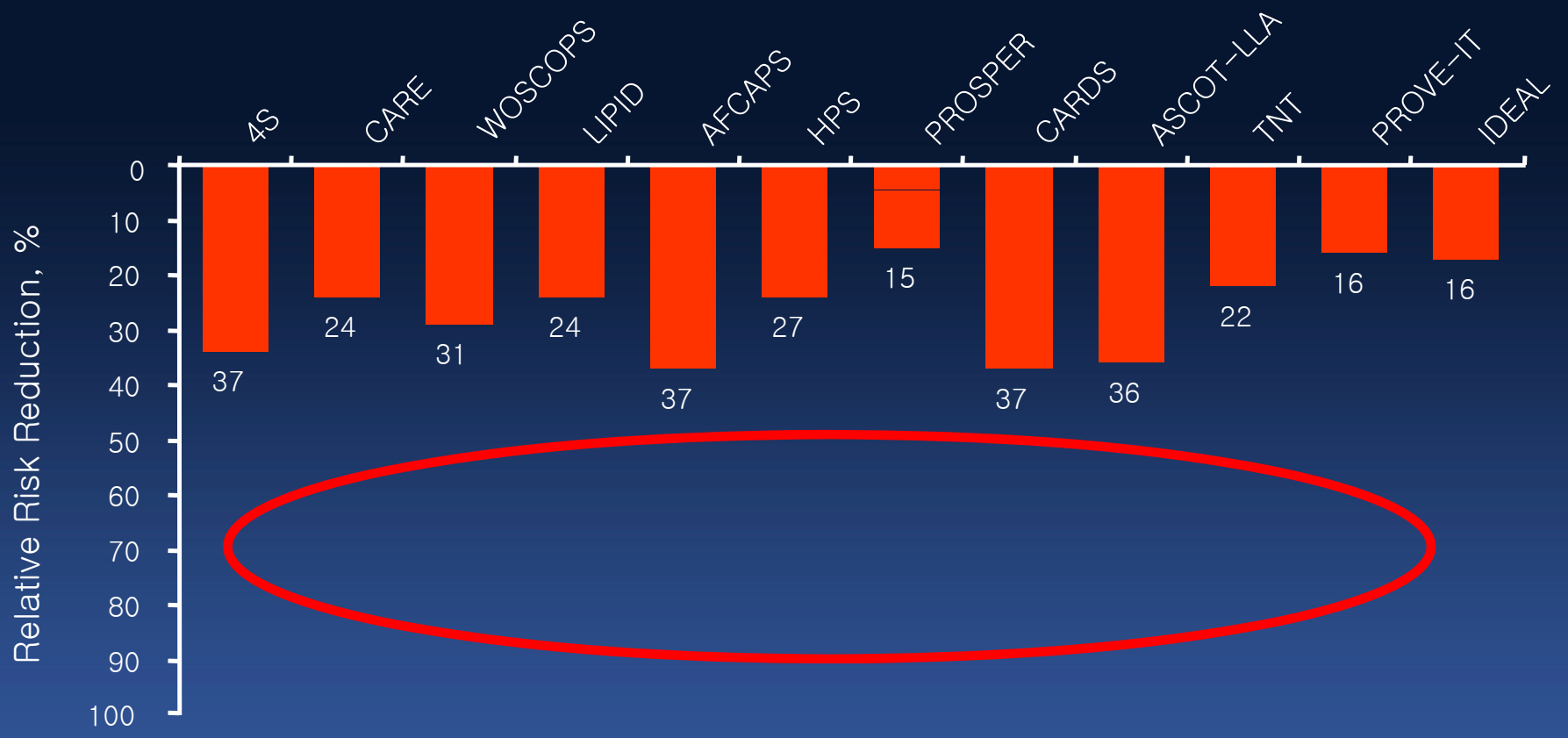


P2Y12 receptor inhibitors may have a dual anti-ischemic effect by inhibiting both platelet activation and plaque destabilization.

Presentation

- **Established Targets**
  - HMGCoA reductase
  - $P2Y_{12}$ receptor
- **Emerging Targets**
  - Lipid metabolism
  - ECM proteases
  - Inflammation

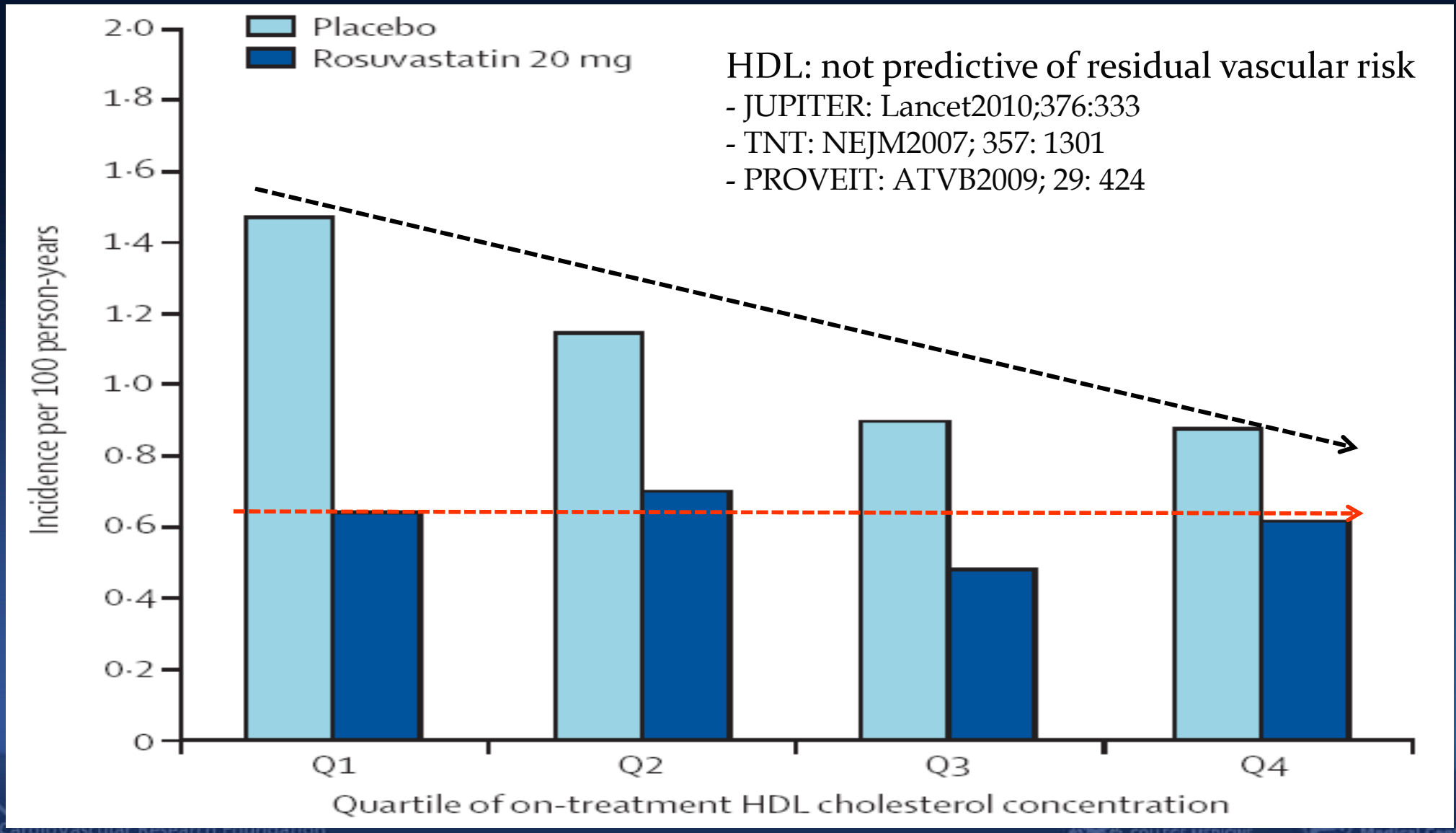
Residual CV Risk in Statin-Treated Patients



Despite optimal treatment, the residual risk of another MACE in these patients is estimated to be 70–80%.

Red Flag Sign?

On-Treatment HDL-C and CV Risk



Futile Strategies?

The Story *So Far …*

HDL-Targeted Therapies

We have reached the limit of what we can do by lowering LDL-C?.

Estrogen (↑15%): WHI trial

Fibrates (↑15%): ACCORD Lipid trial

Nicotinic acids (↑20%)

Extended release niacin: AIM-HIGH trial

Tredaptive (nicotinic acid/laropiprant) : HPS-2 trial

CETP inhibitors (↑30%-140%)

Torcetrapib: Illuminate trial (↑death: discarded)

Anacetrapib: Define trial (safe), HPS-3 (REVEAL) trial

Dalcetrapib: Dal-Plaque (promising), Dal-Outcomes trial

New Targets

A Potential Game Changer
Biologic Wows for LDL-C Lowering

PCSK9: a key regulator of the LDL receptor

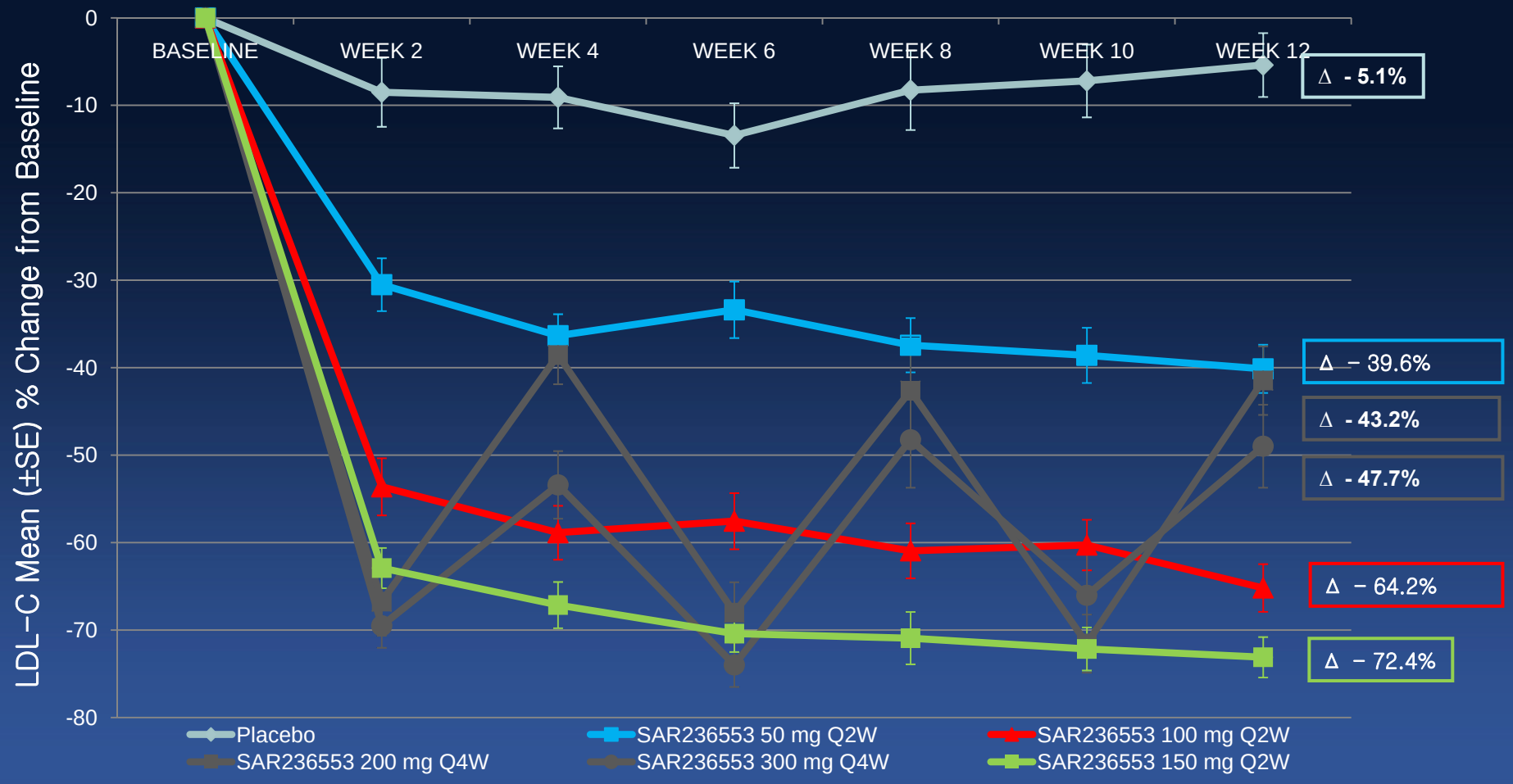
Gain-of-function mutations result in hypercholesterolemia
Loss-of-function mutations associated with low LDL-C & low prevalence of CHD events

SAR236553/REGN727 is a highly specific, fully human monoclonal antibody (mAb) to PCSK9

Company	Drug (alternative names)	Agent	Indication	Phase
Regeneron Pharmaceuticals/ Sanofi	REGN727 (SAR236553)	Human monoclonal antibody	Hypercholesterolaemia	Phase II
Amgen	AMG-145	Human monoclonal antibody	Hypercholesterolaemia	Phase II
Novartis	LGT209	Monoclonal antibody	Hypercholesterolaemia	Phase II
Pfizer	RN316 (PF-04950615)	Monoclonal antibody	Hypercholesterolaemia	Phase II
Alnylam Pharmaceuticals	ALN-PCS02	siRNA oligonucleotide	Hypercholesterolaemia	Phase I
Santaris Pharma	SPC-5001	Locked nucleic acid antisense oligonucleotide	Hypercholesterolaemia	Phase I
Adnexus Therapeutics/ Bristol-Myers Squibb	PCSK9 (Adnectin)	Fusion protein using Adnexus Therapeutics' Adnectin technology	Cardiovascular disease	Preclinical
Idera Pharmaceuticals	NA	Antisense oligonucleotide	Hypercholesterolaemia	Preclinical
Serometrix	SX-PCK9	Small peptide mimetic; LDLR antagonist	Hypercholesterolaemia	Preclinical
Shifa Biomedical Corporation	NA	Small-molecule PCSK9 modulator	Metabolic disorders	Preclinical

Adjunct to Statin

Monoclonal Antibody to PCSK9, SAR236553/REGN727, in Patients with Primary Hypercholesterolemia



It was generally safe & well tolerated. SQ every two weeks dropped LDL by 40% to 72%
There is some risk of immunity with an agent like this & we need longer-term outcome studies.

Presentation

- Established Targets
  - HMGCoA reductase
  - $P2Y_{12}$ receptor
- Emerging Targets
  - Lipid metabolism
  - ECM proteases
  - Inflammation

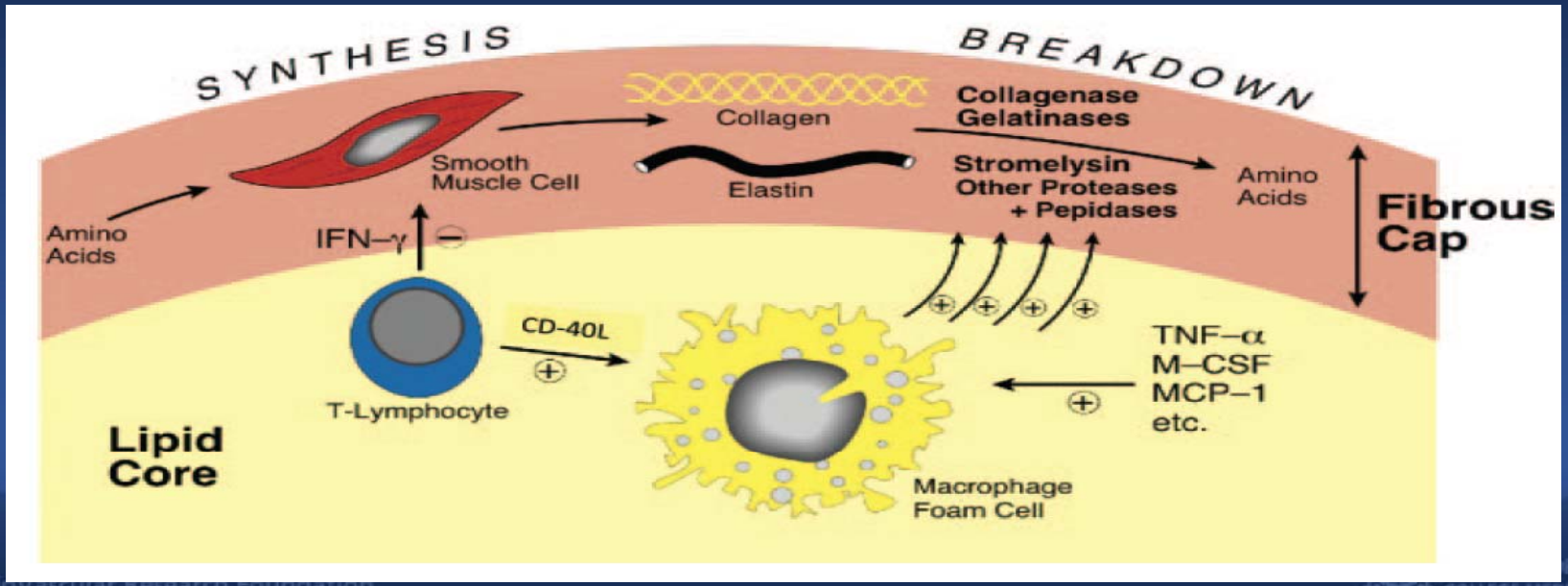
ECM Proteases Hypothesis of Plaque Rupture

Matrix metaloproteinases (MMPs)

Elevated: MMP1, MMP2, MMP3, MMP8, MMP9, MMP10, MMP13

ADAMTS proteases

Others: serine proteases (elastase), cysteine proteases (cathepsins)



Tragic Failure

MMP-Based Therapy

Matrix Metalloproteinase Inhibitors and Cancer: Trials and Tribulations

Lisa M. Coussens,[1] Barbara Fingleton,[2] Lynn M. Matrisian[2]*

MMPs have been heralded as promising targets for cancer therapy on the basis of their massive up-regulation in malignant tissues and their unique ability to degrade all components of the extracellular matrix.

Many of these trials turned out to be major failures.
Owing to serious complications, the trials were terminated early.

MMPs are vital for immune regulation. Some MMPs are the good guys in disease. To overcome the danger of off-target effects, it is essential to either thoroughly characterize the biological roles or understand the degree of cross-reactivity of inhibitors before drug development.

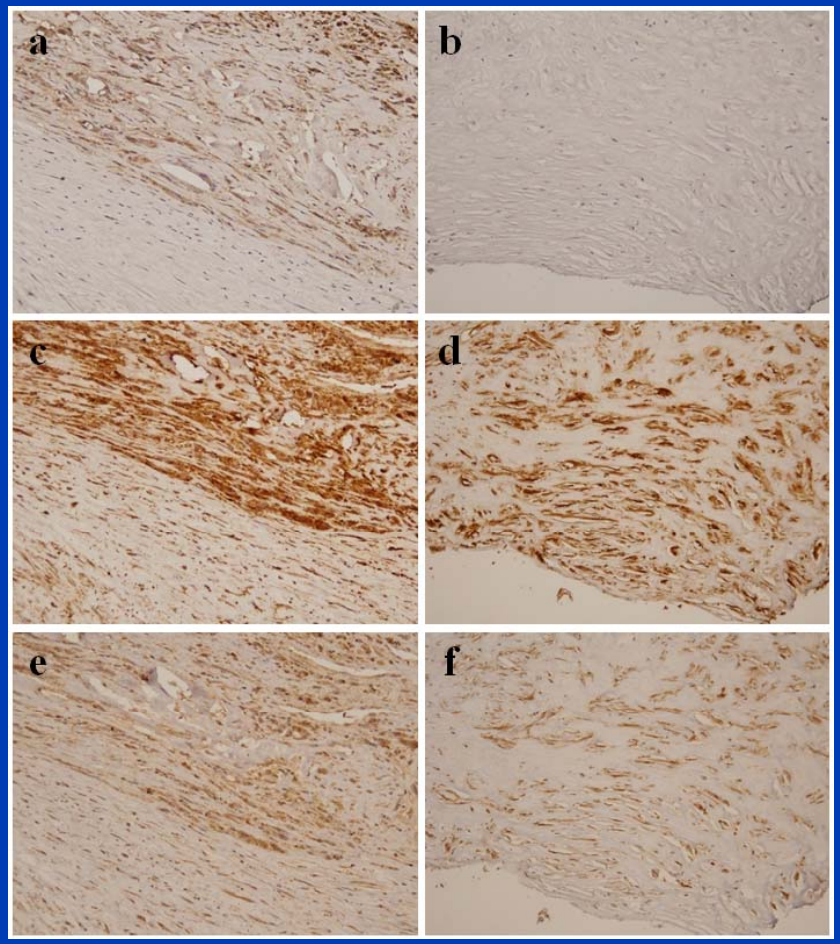
ADAMTS Proteases

AMI Stable Angina

ADMTS1

ADMTS4

ADMTS5



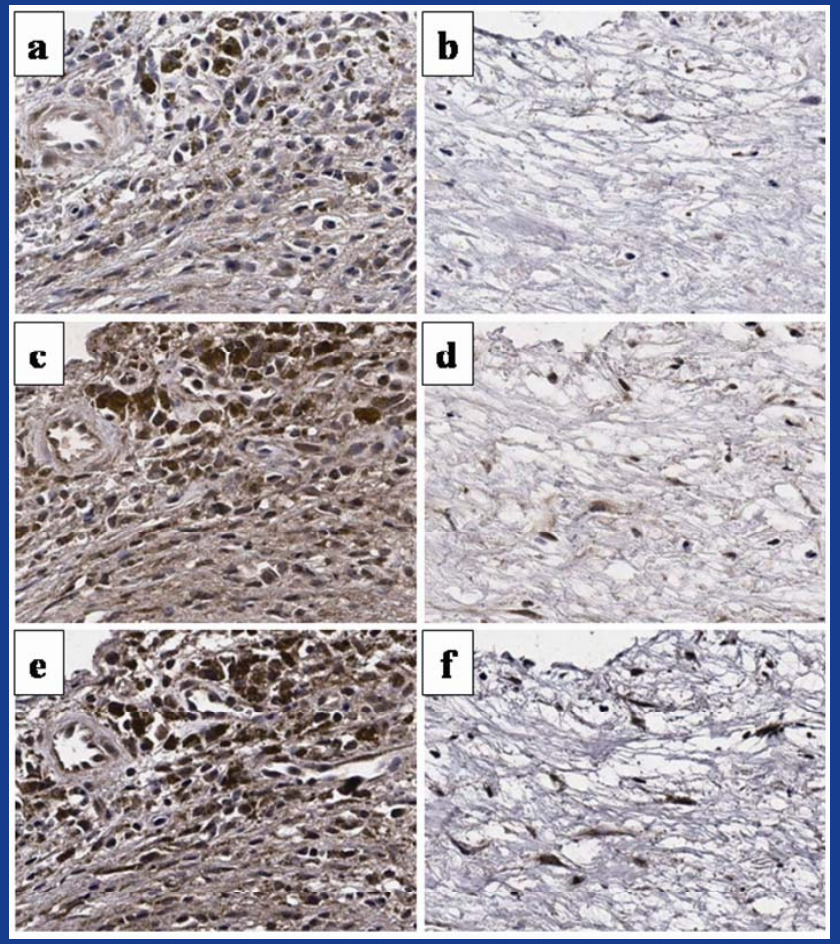
J Clin Pathol 2011;64:399

AMI Stable Angina

ADMTS2

ADMTS3

ADMTS13



J Thromb Thrombolysis 2012 (in press)

Target for future drug development

Limitations

It remains uncertain whether ADAMTS proteases are a cause or consequence of plaque instability.

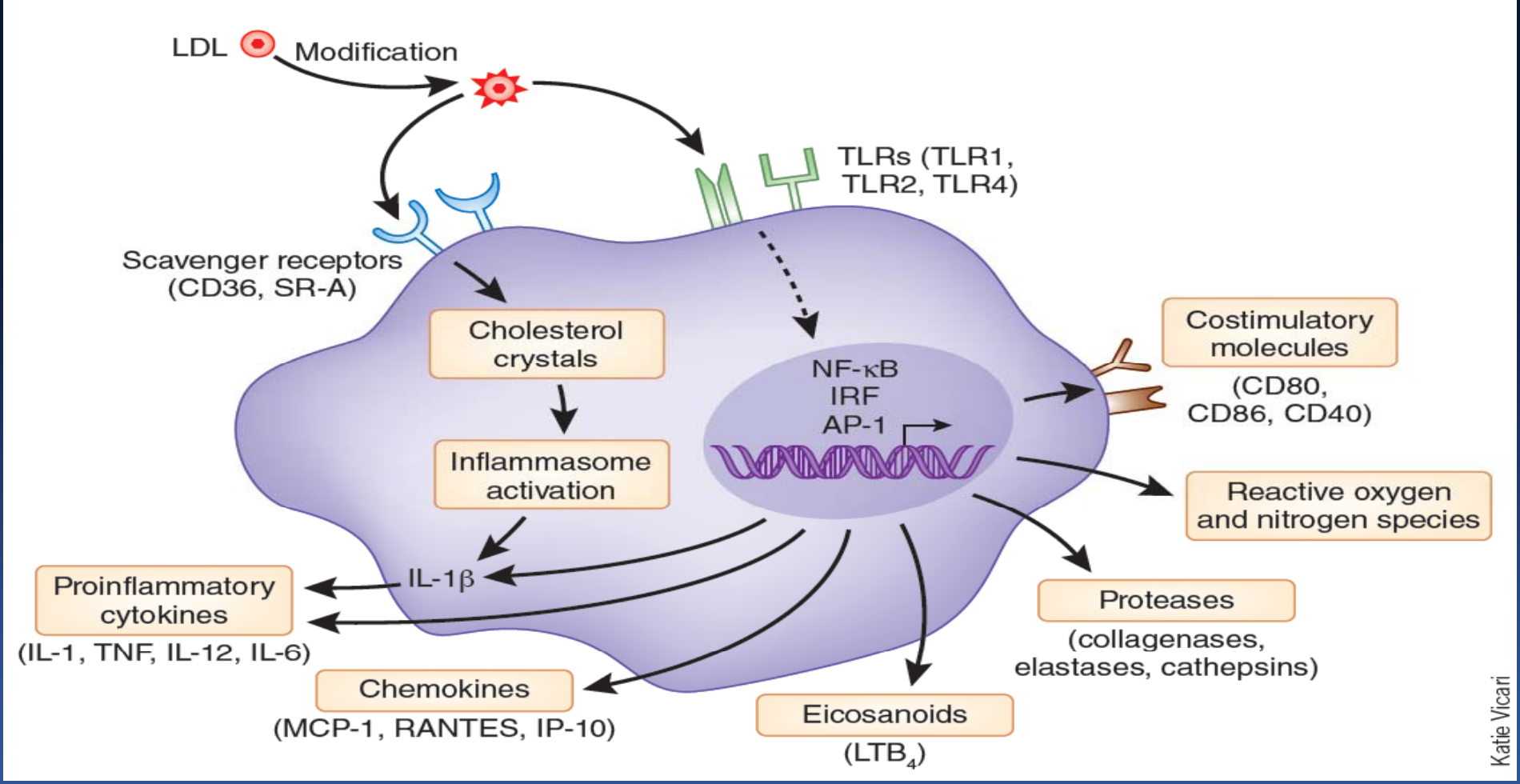
You can see firefighters at every flaming building, but they did not set the blazes.

Presentation

- Established Targets
  - HMGCoA reductase
  - $P2Y_{12}$ receptor
- Emerging Targets
  - Lipid metabolism
  - ECM proteases
  - Inflammation

Inflammation

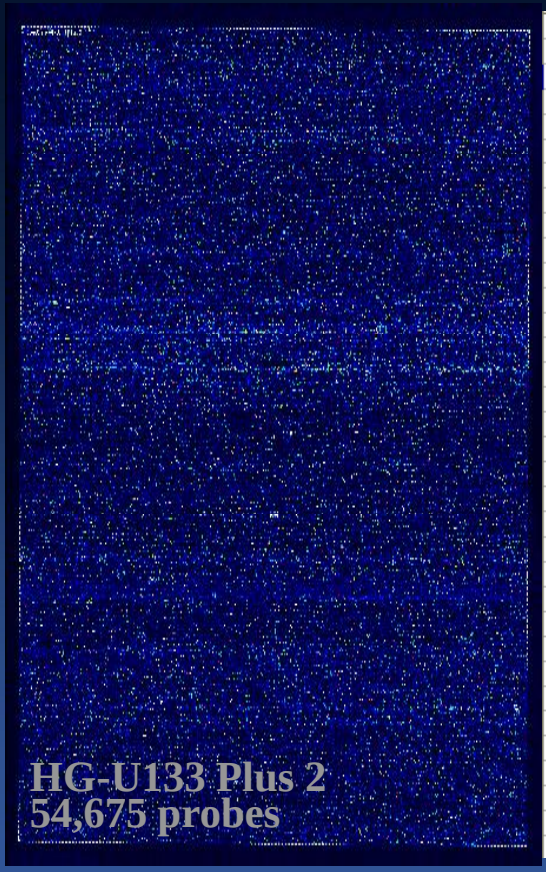
A Key Player



The contribution of inflammation to the development of atherosclerotic plaques is now well-understood and several promising targets have been identified.

Inflammatory Genes at Ruptured Plaques

The proper study of mankind is man.



HYPERLINKED GO CATEGORY	TOTAL GENES	CHANGED GENES	P-Value	FALSE DISCOVERY RATE
GO:0002376_immune_system_process	684	147	5.52E-45	0
GO:0006955_immune_response	530	127	5.97E-44	0
GO:0006952_defense_response	459	93	5.32E-26	0
GO:0050896_response_to_stimulus	1851	219	2.46E-25	0
GO:0009605_response_to_external_stimulus	506	85	2.99E-18	0
GO:0006954_inflammatory_response	255	55	6.42E-17	0
GO:0009611_response_to_wounding	351	64	1.10E-15	0
GO:0006935_chemotaxis	130	36	5.11E-15	0
GO:0042330_taxis	130	36	5.11E-15	0
GO:0007626_locomotory_behavior	161	39	4.62E-14	0
GO:0006968_cellular_defense_response	62	21	3.89E-11	0
GO:0007155_cell_adhesion	611	79	8.80E-11	0
GO:0022610_biological_adhesion	611	79	8.80E-11	0
GO:0002504_antigen_processing_and_presentation_of_pe	13	10	1.61E-10	0
GO:0042221_response_to_chemical_stimulus	386	57	3.15E-10	0
GO:0019882_antigen_processing_and_presentation	42	16	1.15E-09	0
GO:0007610_behavior	259	42	4.57E-09	0
GO:0030595_leukocyte_chemotaxis	18	10	1.86E-08	0
GO:0045321_leukocyte_activation	160	30	2.74E-08	0
GO:0001775_cell_activation	182	32	4.67E-08	0
GO:0001816_cytokine_production	94	21	1.58E-07	0
GO:0002250_adaptive_immune_response	72	18	2.00E-07	0
GO:0002460_adaptive_immune_response_based_on_soma	72	18	2.00E-07	0
GO:0007154_cell_communication	2977	240	2.03E-07	0
GO:0007165_signal_transduction	2678	220	2.06E-07	0
GO:0050900_leukocyte_migration	22	10	2.20E-07	0
GO:0030593_neutrophil_chemotaxis	10	7	3.10E-07	0
GO:0002443_leukocyte_mediated_immunity	71	17	8.46E-07	0
GO:0046649_lymphocyte_activation	139	25	8.94E-07	0
GO:0042108_positive_regulation_of_cytokine_biosynthetic	44	13	1.28E-06	0
GO:0042089_cytokine_biosynthetic_process	69	16	2.80E-06	0
GO:0045727_positive_regulation_of_protein_biosynthetic_	54	14	2.85E-06	0
GO:0042107_cytokine_metabolic_process	70	16	3.43E-06	0

Inflammation is caused by complex interactions involving multiple cell types & multiple mediators. It is not yet clear which targets will yield the greatest effect in atherosclerosis.

Anti-inflammatory Drugs

Cooling Down Burning Plaques

PLA2 inhibitors (↓ oxidized LDL, multiple isozymes)

Darapladib (Lp-PLA2): STABILITY trial (Phase 3)

Varespladib (sPLA2): VISTA-16 trial (Phase 3)

Experimental Drugs

Antiinflammatory agents

- Methotrexate: Cardiovascular Inflammation Reduction Trial

- Others:

Veliflapon (DG-031); DeCODE genetics	FLAP	Leukotriene	Inhibits leukotrienes; reduces leukocyte activity in plaque	No clinical morbidity and mortality; Phase II biomarker study	Phase II
Atreleuton (VIA-2291); VIA Pharmaceuticals	5-LO	Leukotriene	Inhibits leukotrienes; reduces leukocyte activity in plaque	No clinical MACE; Phase II biomarker; imaging; preclinical models	Phase II
MLN1202; Millennium Pharmaceuticals	CCR2	CCL2 –CCR2 pathway	Inhibits monocyte trafficking into atherosclerotic plaque	No clinical MACE; biomarkers; preclinical models	Phase II
Academic	Methotrexate	Antiproliferative	Antiproliferative agent blocks inflammatory response	Rheumatoid arthritis; high level of atherosclerosis; biomarkers	Phase III (CIRT)
Unspecified	TNF	TNF pathway	TNF-mediated effects	Retrospective clinical outcomes	Unknown
Academic	IL-1	IL-1 pathway	IL-1 mediated effects	Genetic; biomarkers	Phase II (MRC-ILA-HEART)
Roche; RO4905417	P-selectin	Cellular migration — endothelial cells	Blocks monocyte and/or leukocyte penetration into intimal space	Preclinical models	Phase II

It is not yet clear which anti-inflammatory targets will yield the greatest effect in preventing, reversing or delaying this process.

Summary

- There are no therapies specifically to target the inflammatory component of atherosclerosis.
- There is a critical need to identify key markers & local mediators of the atherosclerotic process so that new therapeutics can be developed.