Translational Research of therapeutic angiogenesis for macrovascular complication

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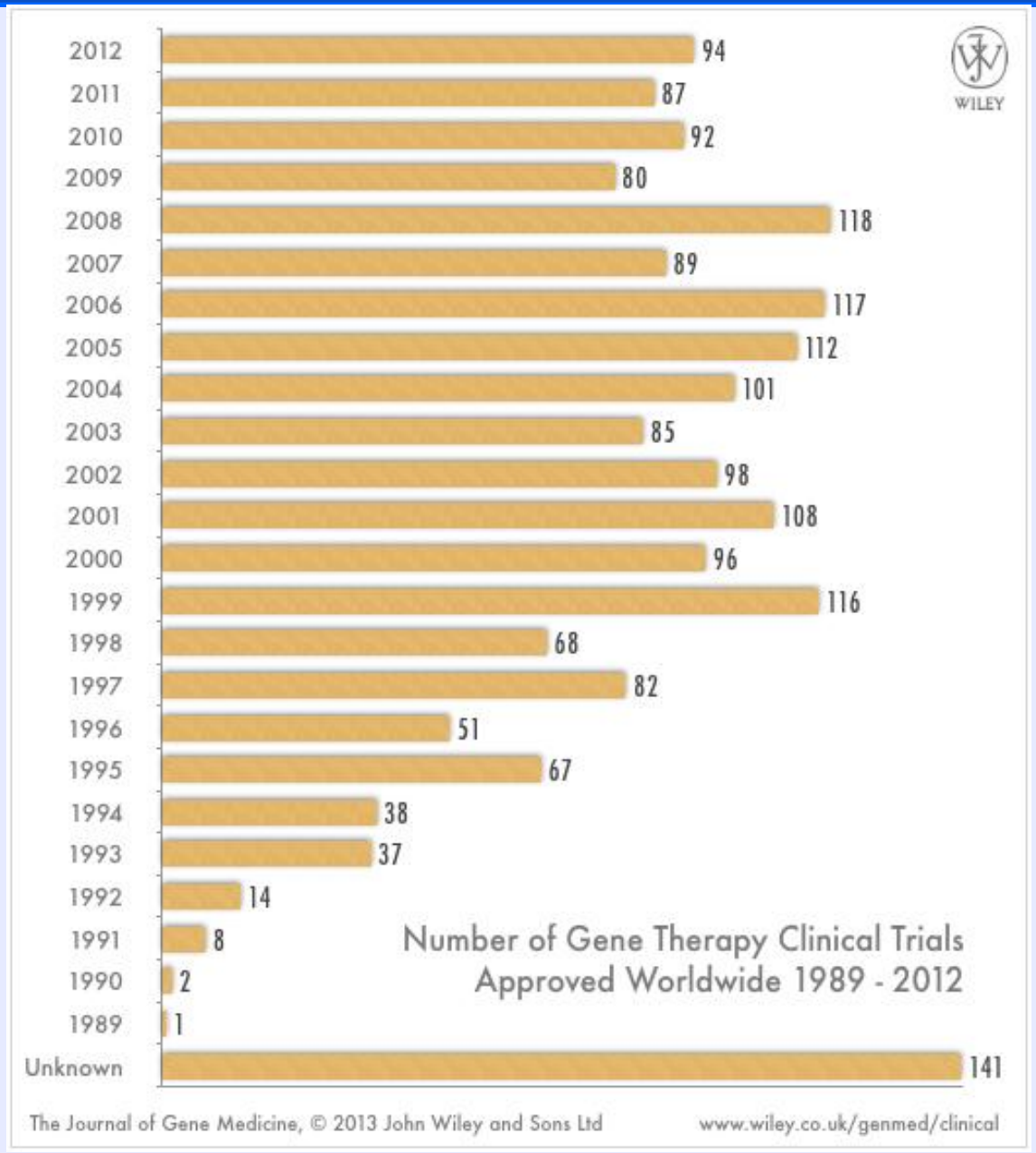
Today's my talk

1. **Recent Progress of Gene Therapy**
2. **Therapeutic angiogenesis by hepatocyte growth factor**
3. **Tranlational research of novel angiogenic peptide**
4. **New concept of vascular calcification**

Worldwide gene therapy clinical trials



First Drug as Gene Therapy

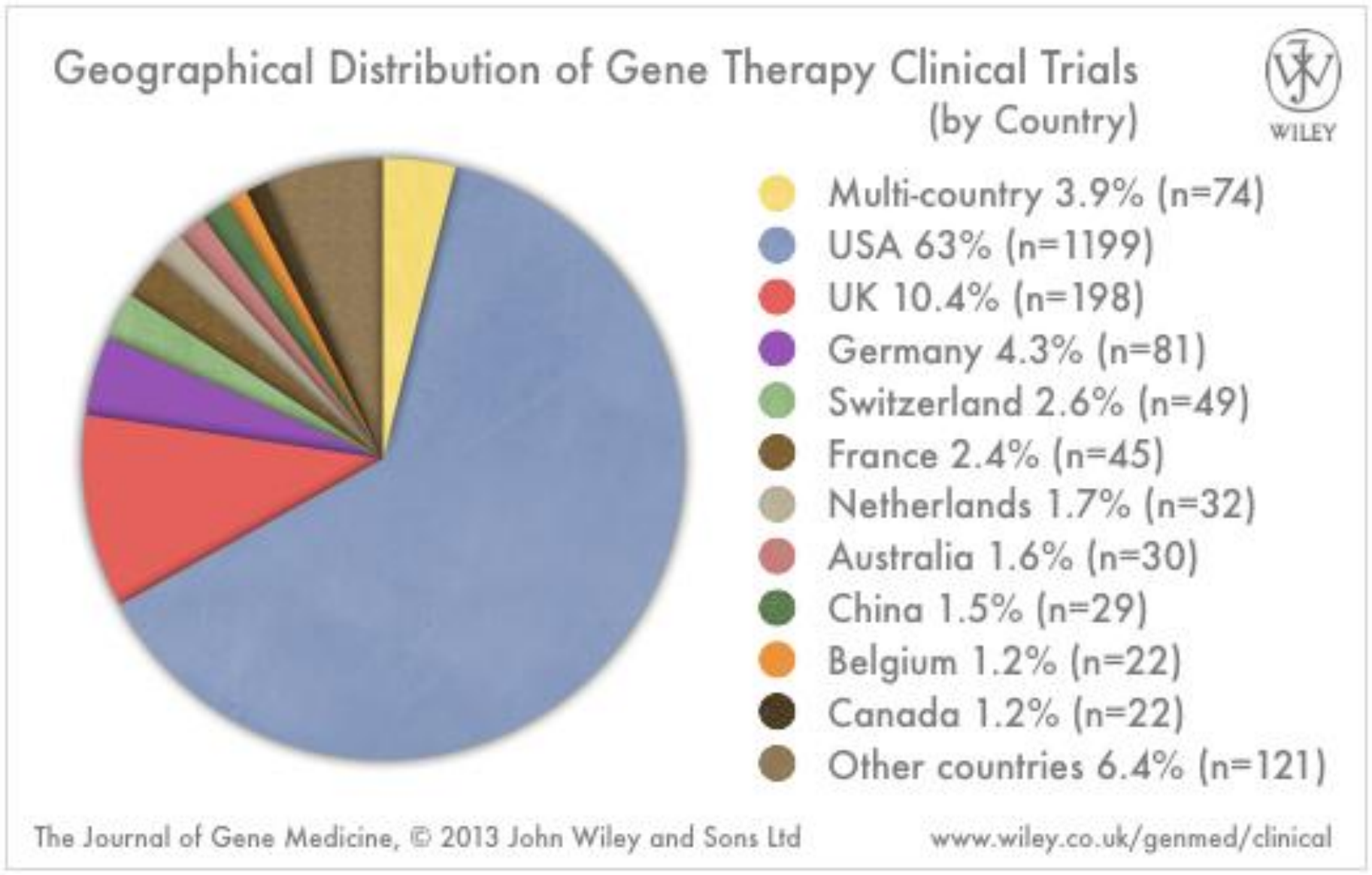
uniQure

uniQure's Glybera® First Gene Therapy Approved by European Commission

- Glybera becomes the first gene therapy approved by regulatory authorities in the Western world
- First medication approved for patients with rare metabolic disorder Lipoprotein Lipase Deficiency
- Commercial roll-out to begin second half of 2013
- Validates uniQure's unique AAV-based gene therapy platform

Amsterdam, The Netherlands – November 2, 2012 – uniQure announced today it has received approval from the European Commission for the gene therapy Glybera® (alipogene tiparvovec), a treatment for patients with lipoprotein lipase deficiency (LPLD, also called familial hyperchylomicronemia) suffering from recurring acute pancreatitis. Patients with LPLD, a very rare, inherited disease, are unable to metabolize the fat particles carried in their blood, which leads to inflammation of the pancreas (pancreatitis), an extremely serious, painful, and potentially lethal condition. The approval makes Glybera the first gene therapy approved by regulatory authorities in the Western world.

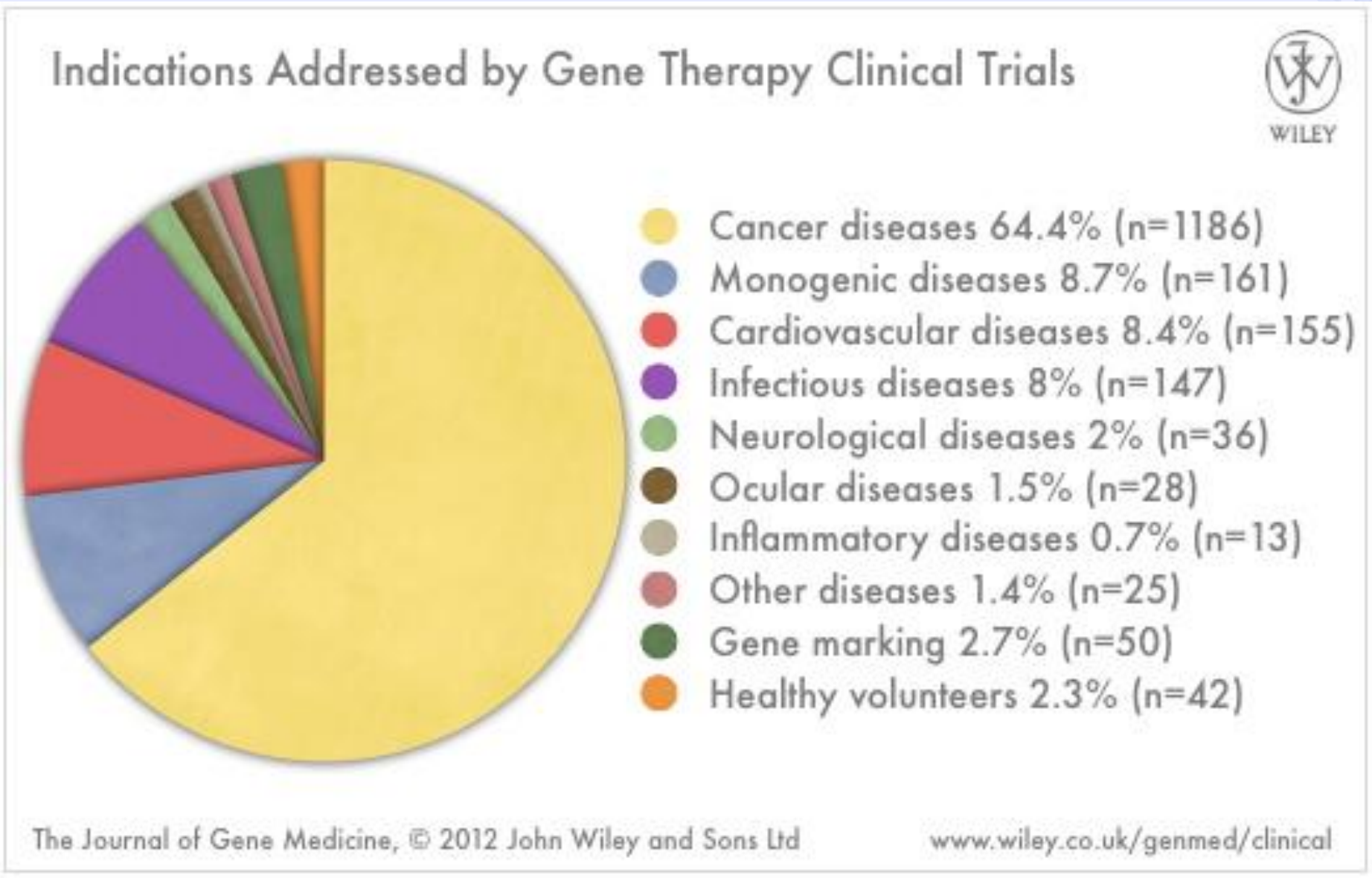
Gene therapy clinical trials in Asia



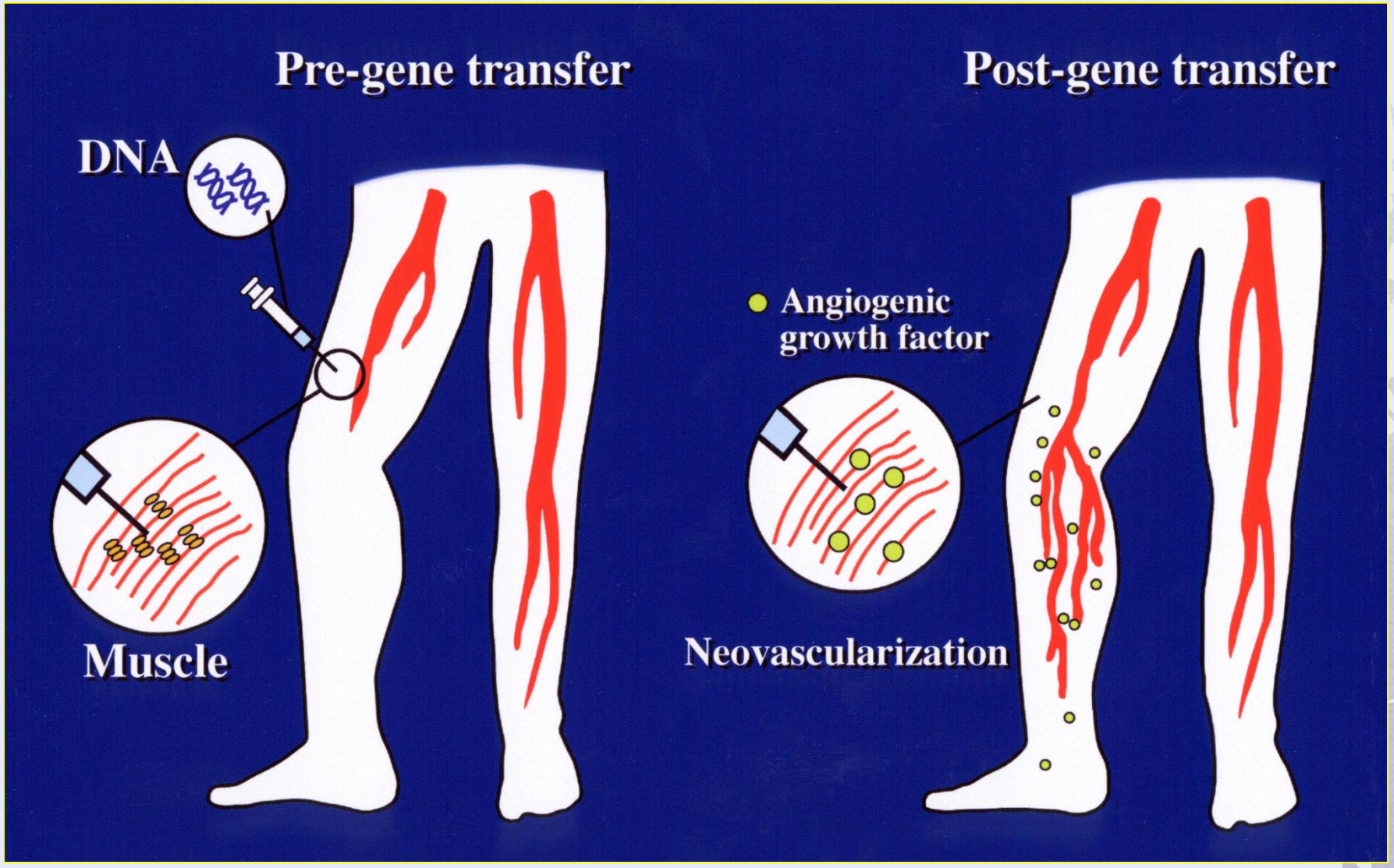
Gene therapy clinical trials in Asia

Country	Gene Therapy Clinical Trials	
	Number	%
Australia	30	1.6
Belgium	22	1.2
Canada	22	1.2
China	29	1.5
Finland	6	0.3
France	45	2.4
Germany	81	4.3
Israel	8	0.4
Italy	21	1.1
Japan	21	1.1
Netherlands	32	1.7
Norway	4	0.2
Poland	6	0.3
South Korea	14	0.7
Spain	13	0.7
Sweden	9	0.5
Switzerland	49	2.6
UK	198	10.4
USA	1199	63
Total	1902	

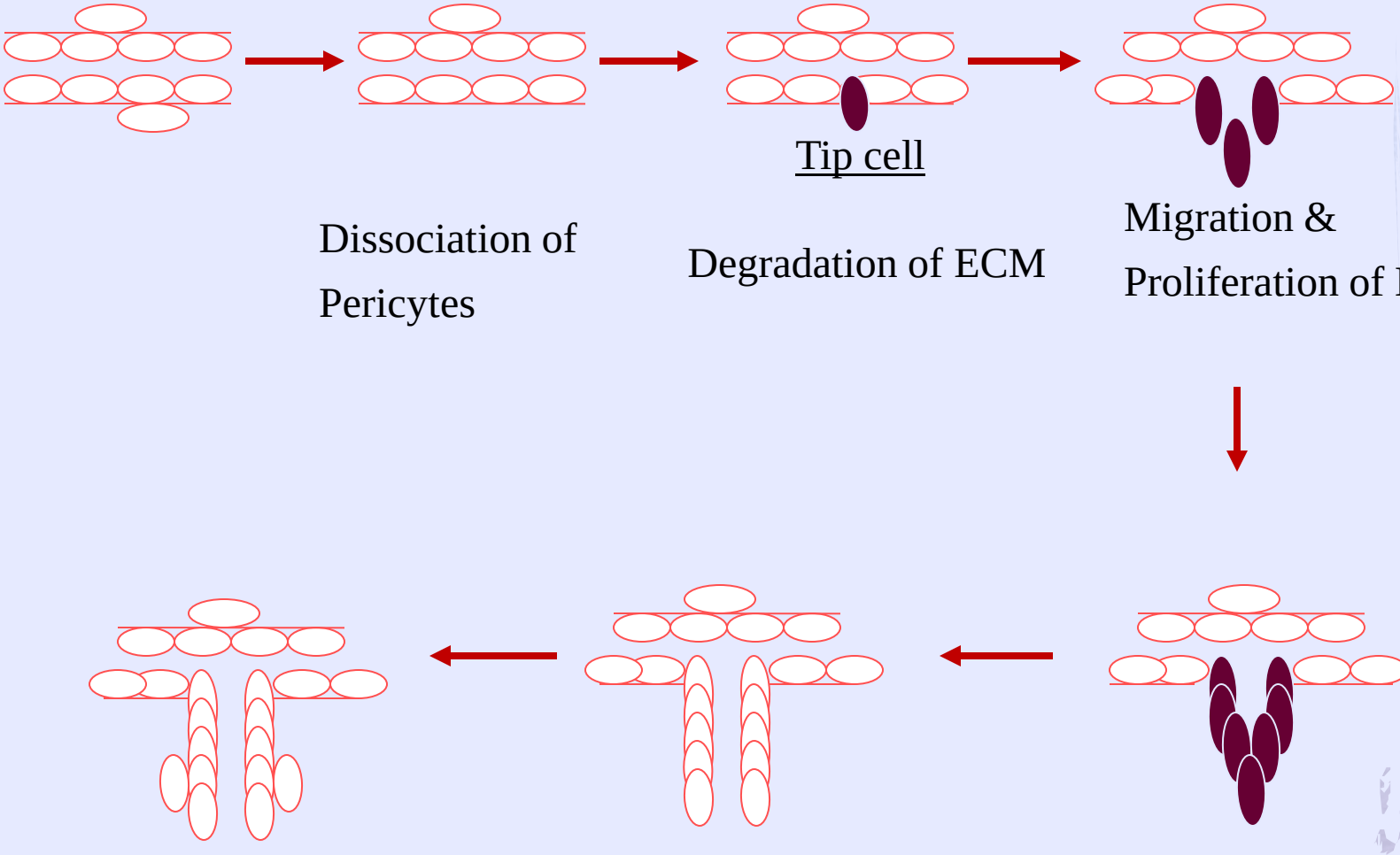
Indications of gene therapy clinical trials



Therapeutic Angiogenesis by Growth Factor



Angiogenesis by Growth Factor



VEGF Gene Therapy to Treat PAD

VEGF
GT

Before GT

4W

8W

Isner J et al. Circulation 1997

Therapeutic Angiogenesis Induced by HGF

Recombinant HGF

ASO model

rat (Hypertension 1999)

rabbit (Circulation 2000)

HGF Gene Therapy

ASO model

mice (FASEB J 2003, Circulation 2003)

rat (Gene Therapy 2001)

rabbit (Gene Therapy 2001; FASEB J 2003)

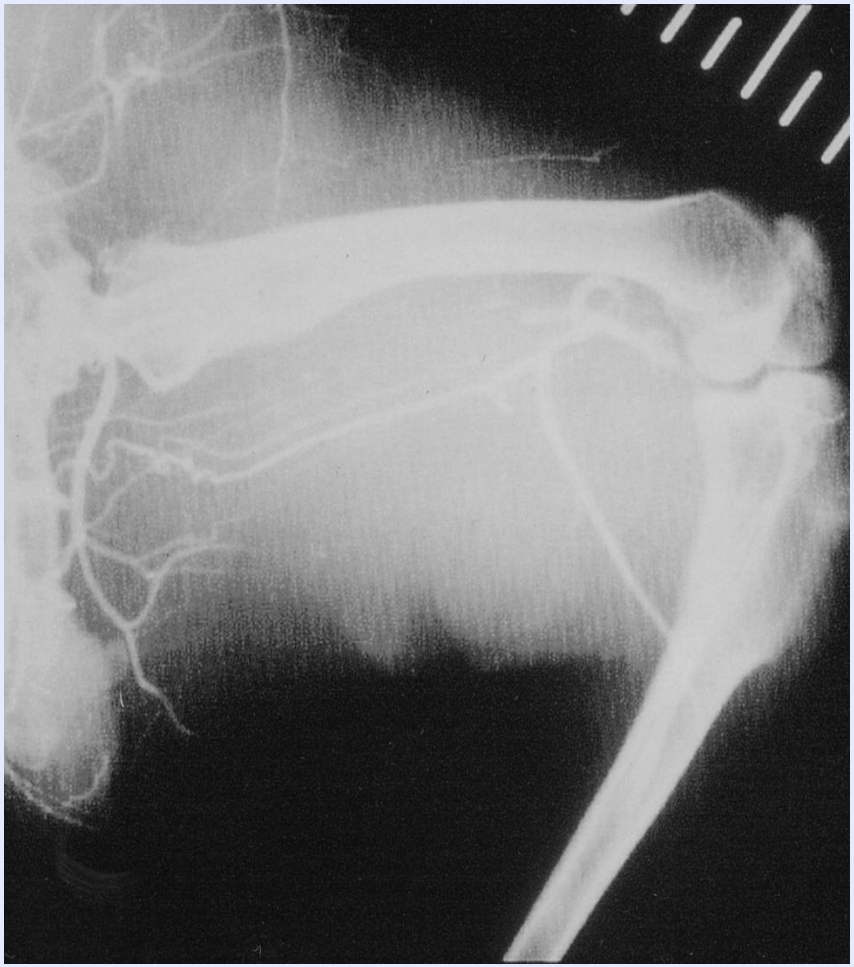
Diabetes ＋ ASO model

rat (Circulation 2002)

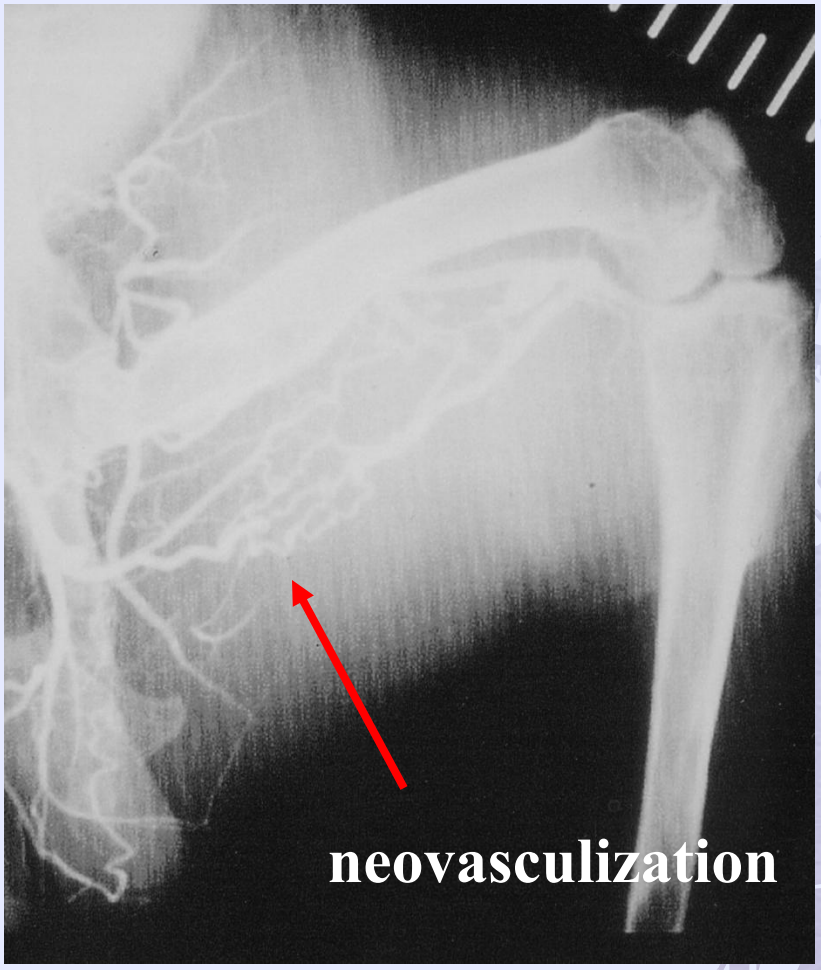
Hyperlipidemia ＋ASO model

high Lp(a) Transgenic Mice (Circulation 2002)

Therapeutic Angiogenesis by HGF Gene Transfer
Rabbit ASO Model



control



HGF

TREAT-HGF
(Clinical study for Clitical Limb Ischemia)

Inclusion criteria

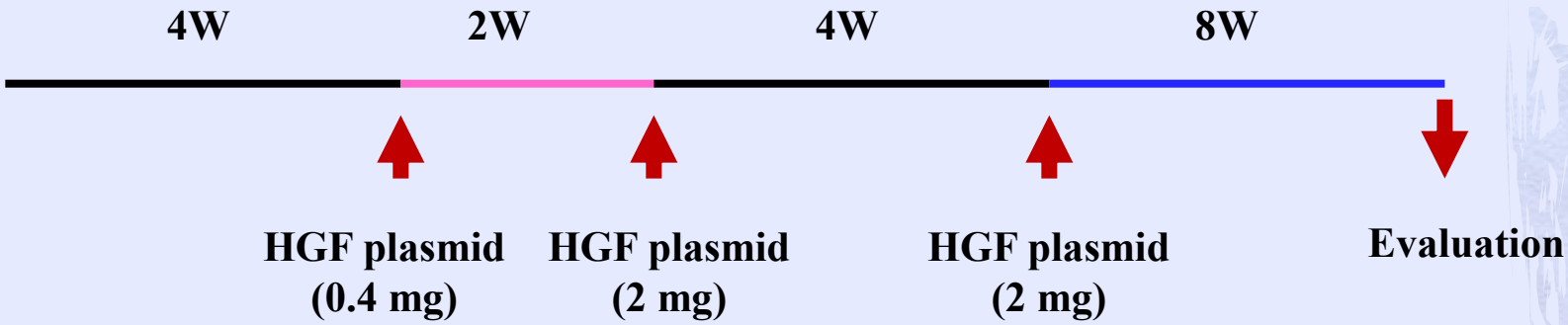
A) Sex	**Male or Female**
B) Age	**over 40 years old**
C) Clinical sympton	**Fontaine IIb/III/IV**
D) Non-invasive	**All patients must be clarified:** ***Ankle-brachial index (ABI)<0.60** ***Exercise testing: >10% ABI decrease**

Exclusion criteria

1. **Cancer including past history**
2. **Diabetic retinopathy**
3. **Alcohol or drug-dependent within 3 months**
4. **Severe livery dysfunction**
5. **Remaining life will be <1 year due to complication**
6. **Attend to other clinical trials**

TREAT-HGF

Stage 1 (Safety; n = 6)



Stage 2 (Safety; n = 16)

Efficacy

	Functional Endpoint	Clinical Endpoint
Fontaine IIb	ABI (>0.1)	Max Walking Distance (>25%)
Fontaine III	ABI (>0.1)	VAS visual analog scale (>2cm)
Fontaine IV	ABI (>0.1)	Ulcer Size (>25%)

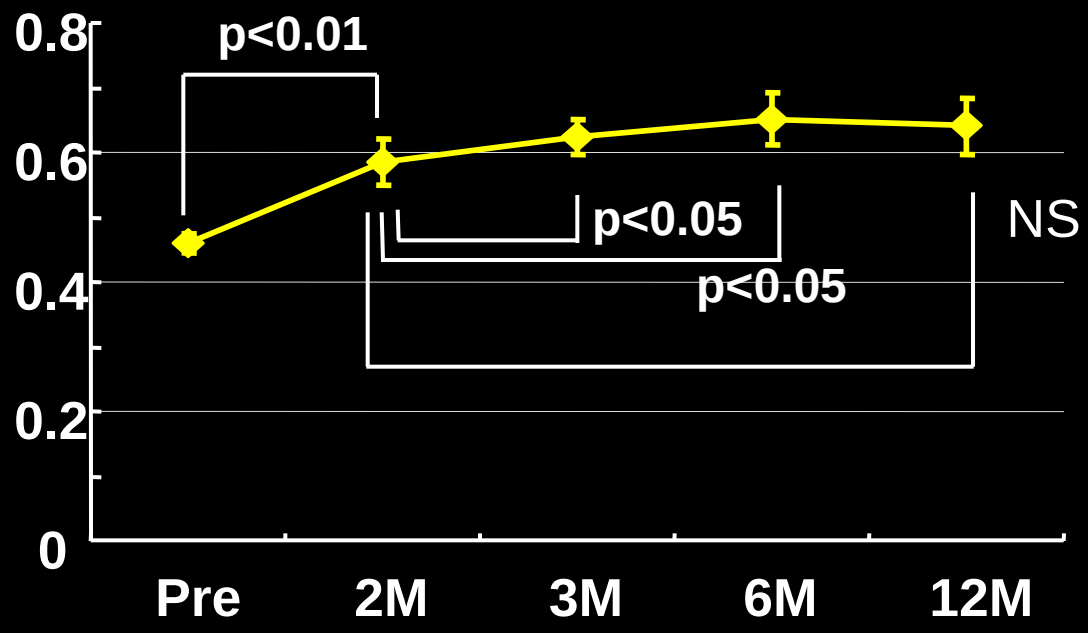
	No Change	1 EP	2 E.P.
Fontaine IIb	1	4	2
Fontaine III	1	2	1
Fontaine IV	2	4	5
total	4	10	8

18/22 Patients demonstrated 1 Endpoint Improvement.

1 Year Follow-up of HGF Gene Therapy

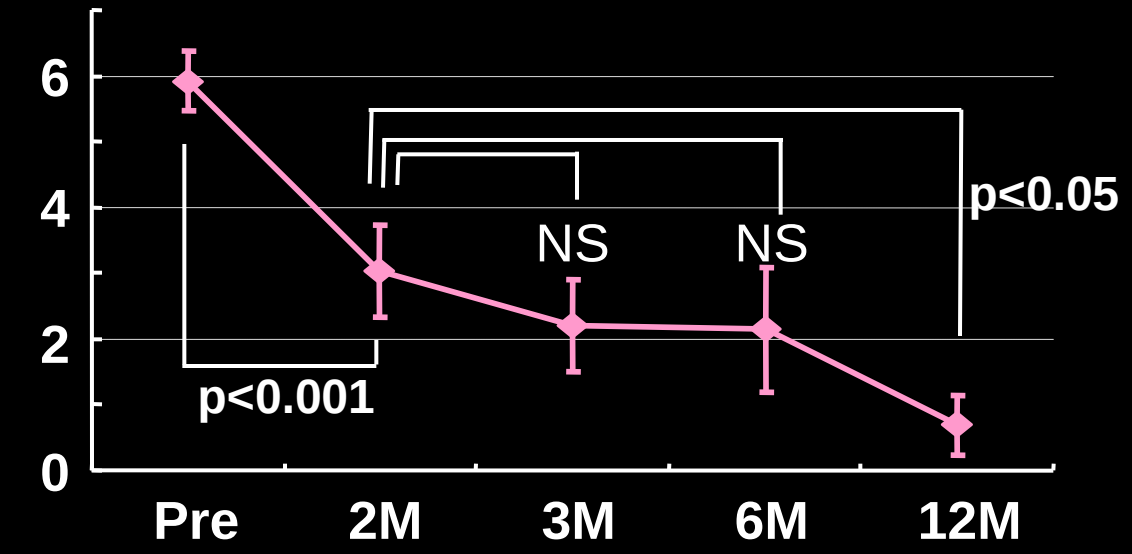
Changes in ABI

n=17



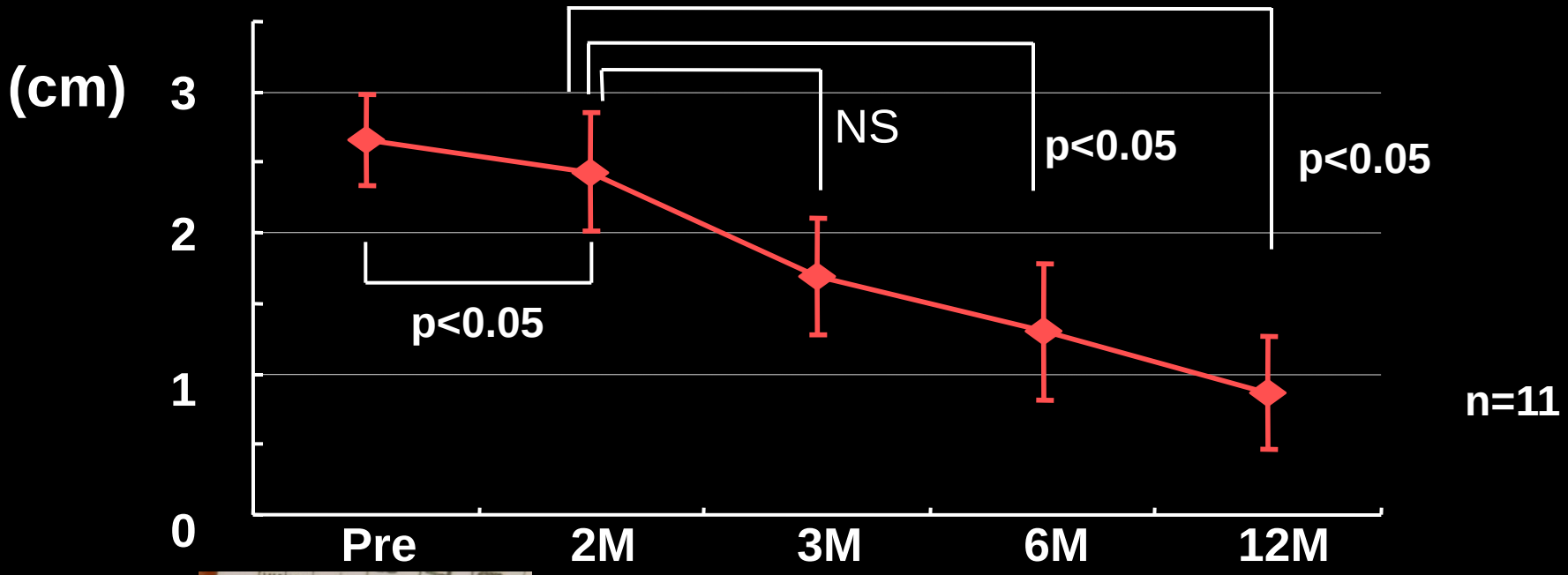
Changes in rest pain (VAS)

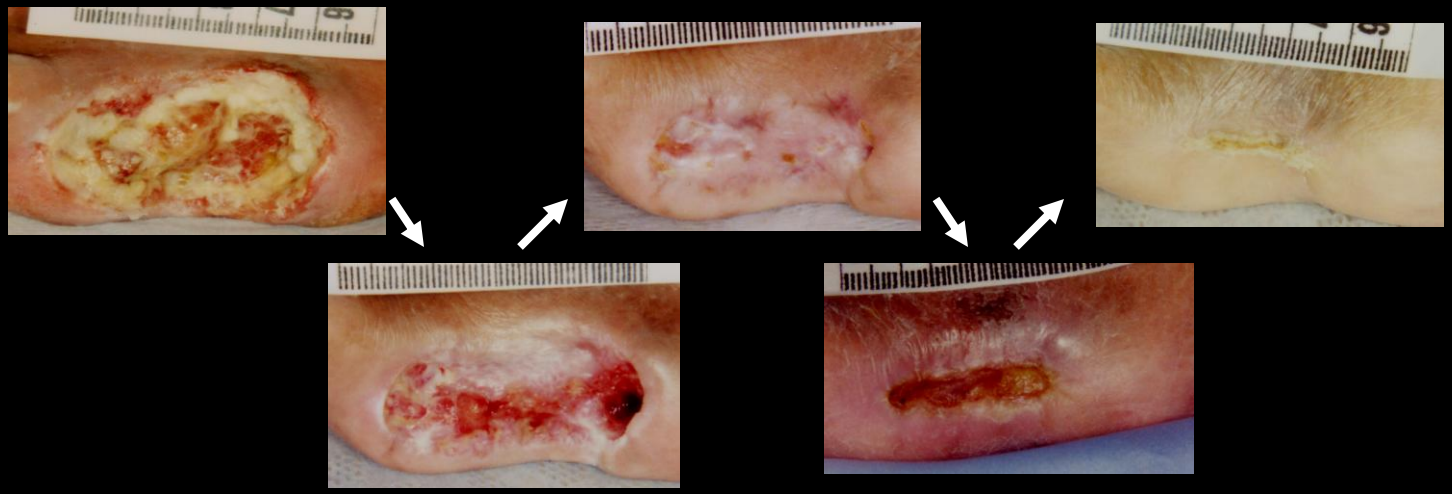
n=13



1 Year Follow-up of HGF Gene Therapy

Changes in Ulcer Size

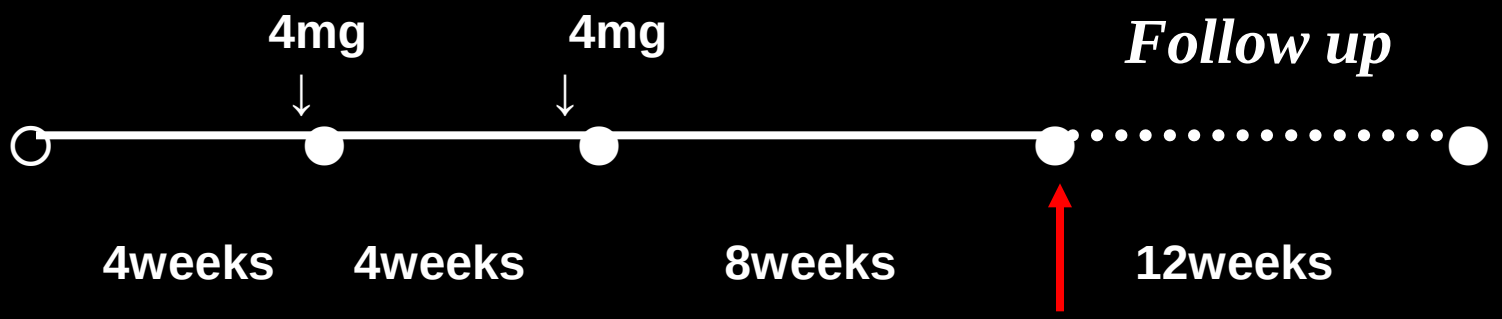




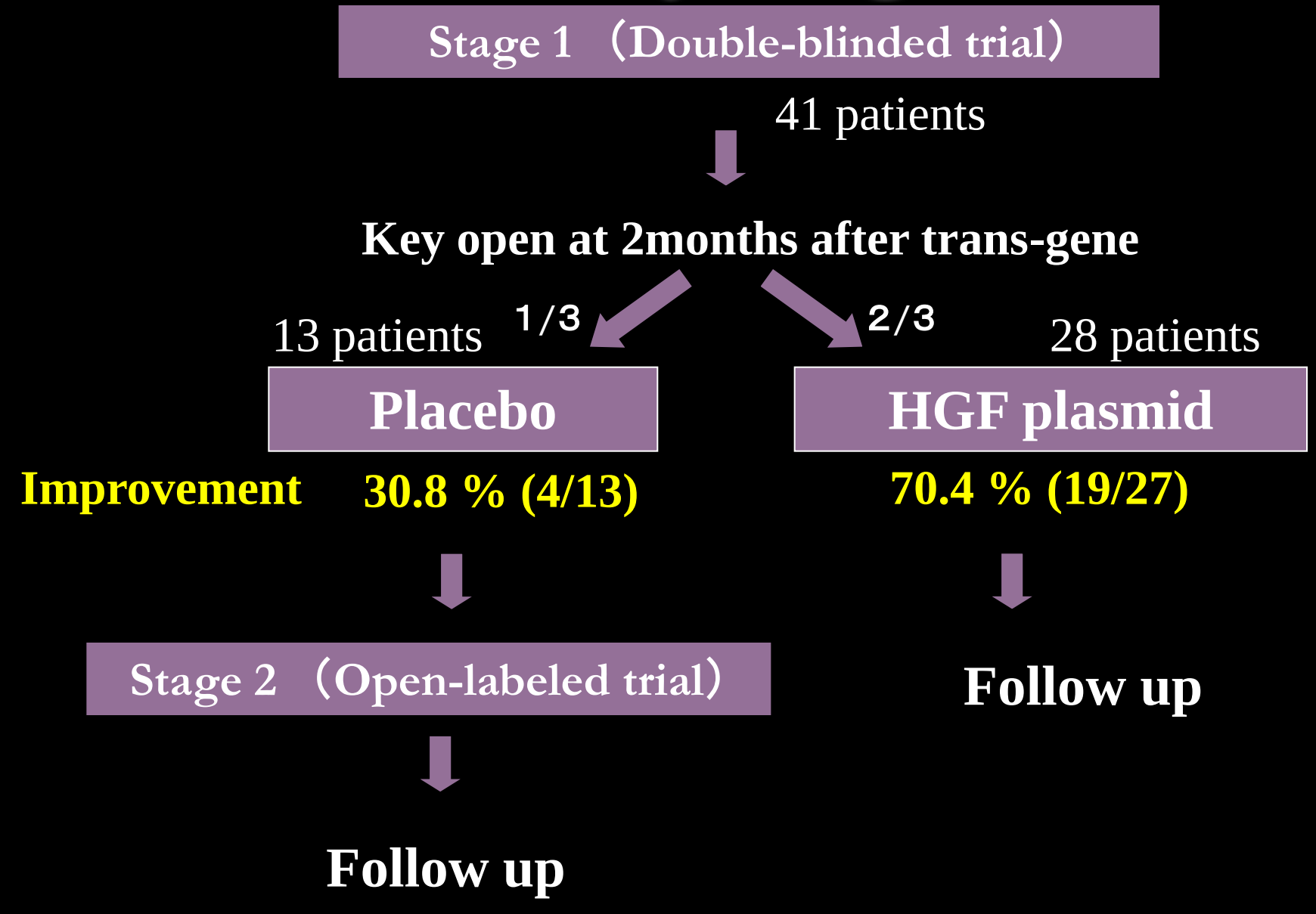
Phase III trial

PAD; double-blinded trial, 41 patients

AMG0001（HGF plasmid）

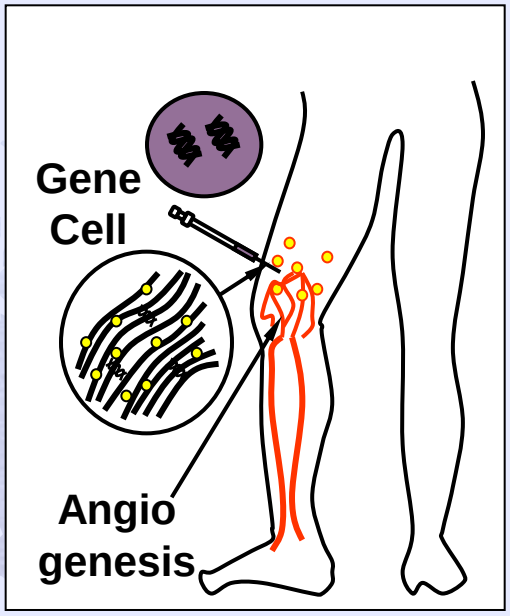


Study Design



Translational Research

Therapeutic angiogenesis



Basic

preclinical

Phase I

Phase II

Phase III

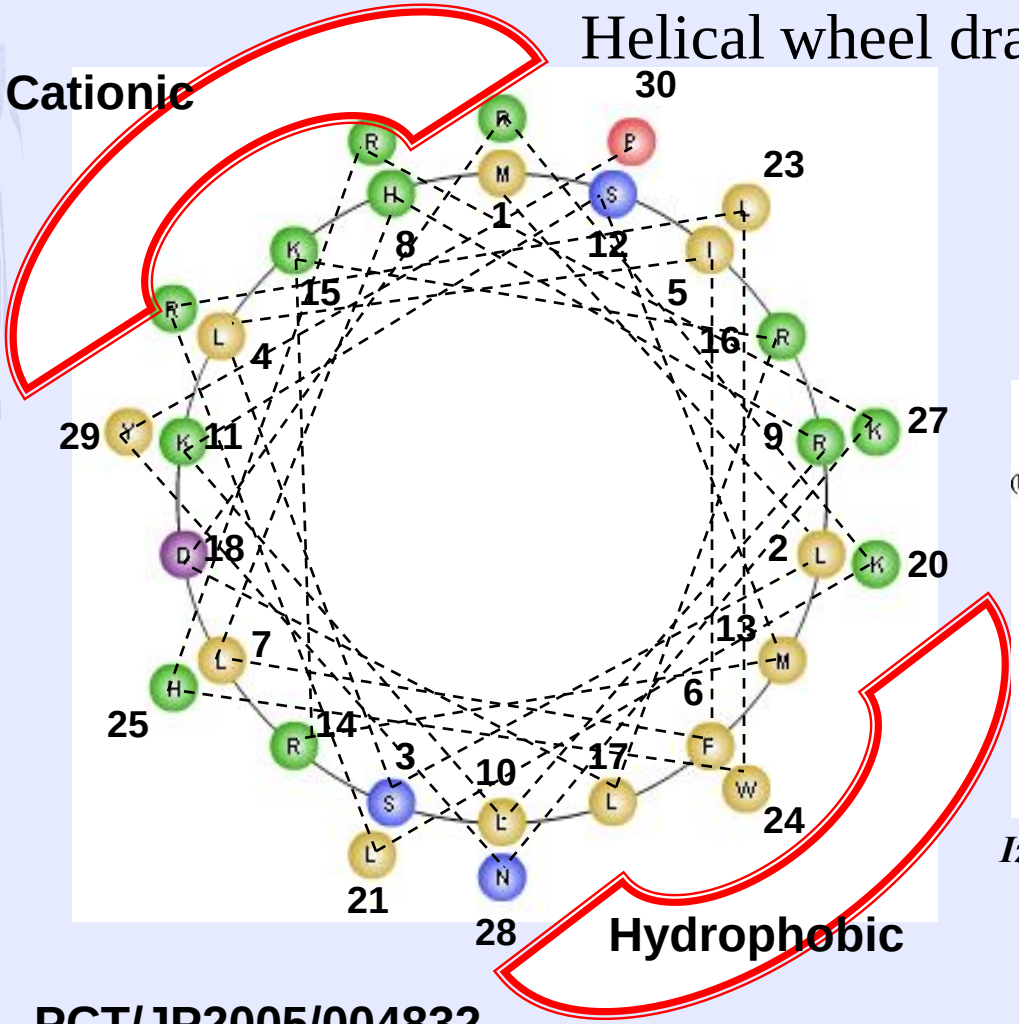
Phase IV
or
Mega trial

To identify the original therapeutic molecule

The candidate peptide forms an α-helix structure, similar to antimicrobial peptide LL-37

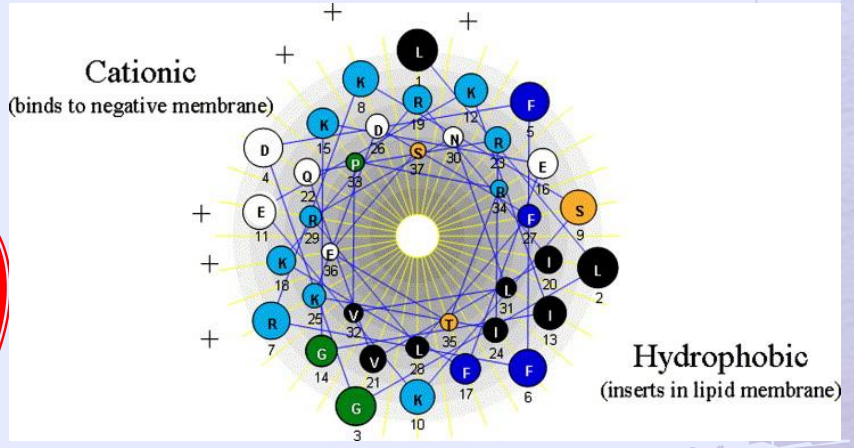
AG-30 possesses both angiogenic and anti-bacterial action.

Helical wheel drawing



Antimicrobial peptide

Cathelicidins (LL-37)



Izadpanah A, et al. J Am Acad Dermatol. 2005

PCT/JP2005/004832

Summary and Aim

AG-30 possesses both angiogenic and anti-bacterial action.

The process of wound healing requires both actions

Bacterial infection

Macro angiopathy

Toward clinical application

- **Modify AG-30 with strong action**

Basic research **FS study** **Pre-clinical study**

Seeds compound → **Functional analysis** → **Leads compound** → **Leads modification** → **Preclinical study** → **Clinical study**

Plans of Clinical Trials

Target Patients：Severe Skin Ulcer of patients
with Diabetes, Peripheral Arterial Diseases, Burger Diseases.

Inclusion Criteria：Not recovery for one month
Carring (not infection) MRSA in skin ulcer

Study Design： Open-label (no control)

Patients number : 6 (study period 2 years)

Primary End point： Safety (because of first-in-human trial)

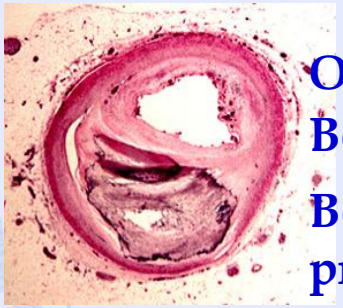
Secondary End Point：The size of skin ulcer
Quantification of bacterial culture

IRB of Osaka Univ.： Revise (2012. July 4$^{th)}$, Approve (2012.Oct. 11)

Ethical Committee of Osaka Univ. Hospital: Approve (2012. Dec.)

Start ! From 2013. January

New Concepts in Vascular Calcification



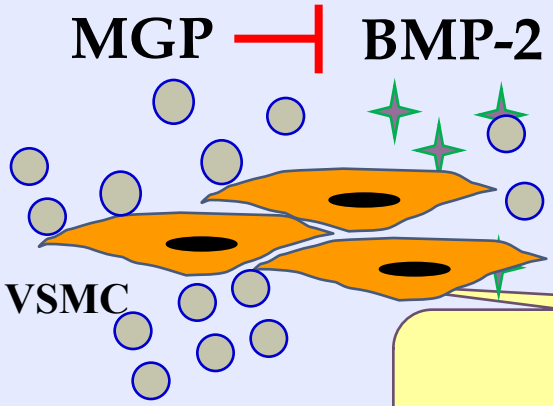
- Osteocalcin
- Bone sialoprotein
- Bone morphogenetic protein -2 and -4
- Osteopontin
- Osteonectin

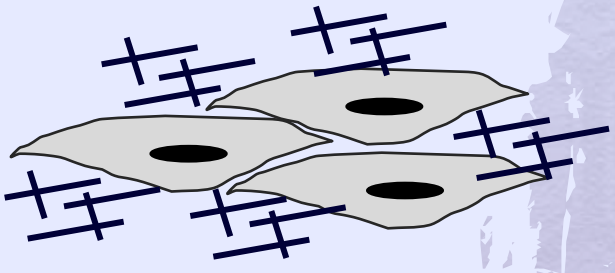
Antibody	Intimal xanthoma	Fibrous cap atheroma	Fibrocalcific plaque		
			Cartilage	Calcium	Bone
OC					
BSP					
BMP-2					
BMP-4					
OPN					
ON					

Dhore R.C et al. *ATVB.* 2001;21:1998-2003.

Early lesion → Calcification

Osteogenic pathway in action

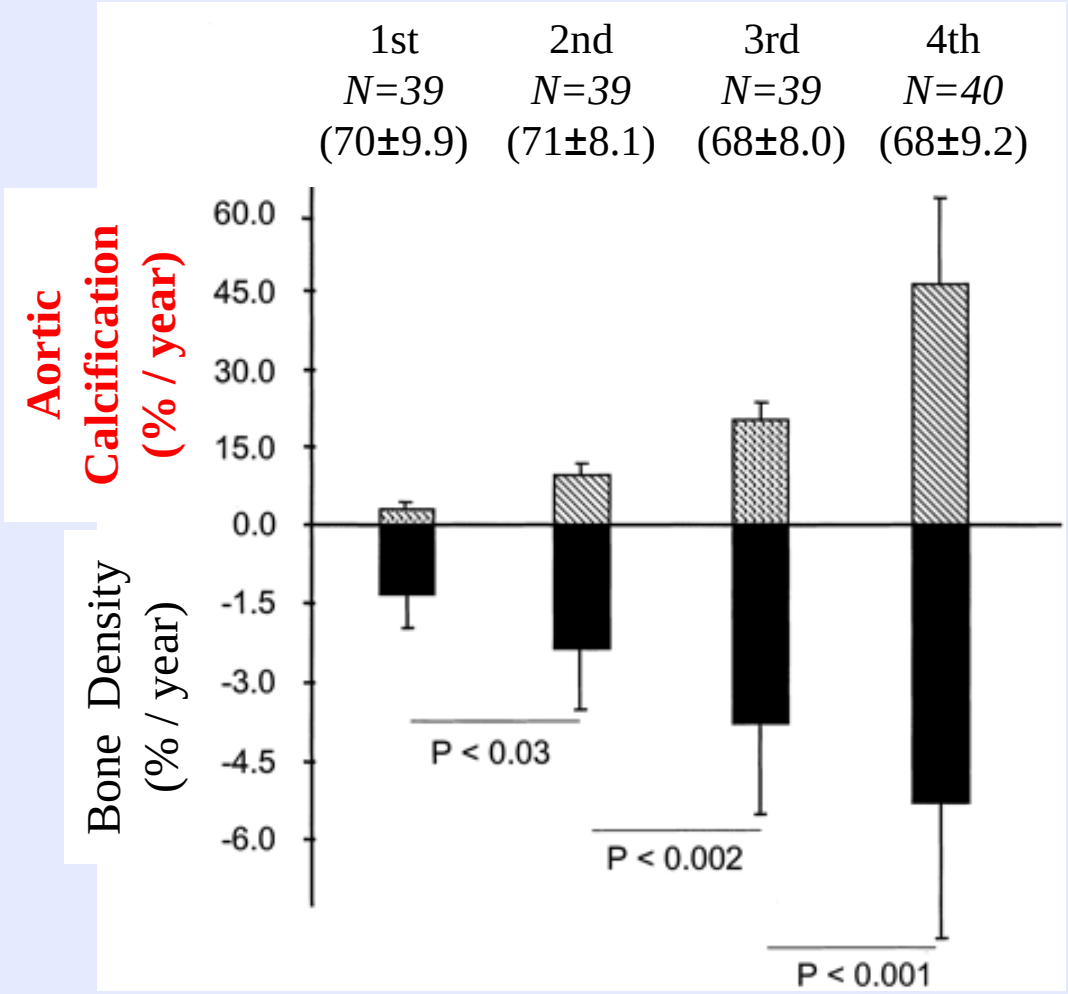




Osteoblast-like cell

Cbfa1, MSX2 → Osteocalcin (OC), Osteopontin (OPN), Alkaline phosphatase (ALP),

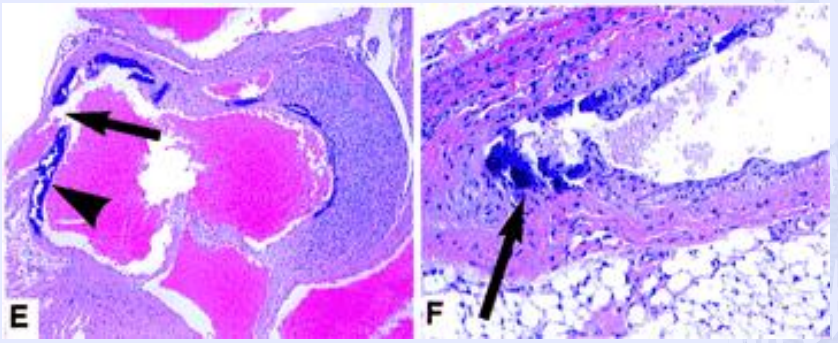
Artery and Bone Paradox

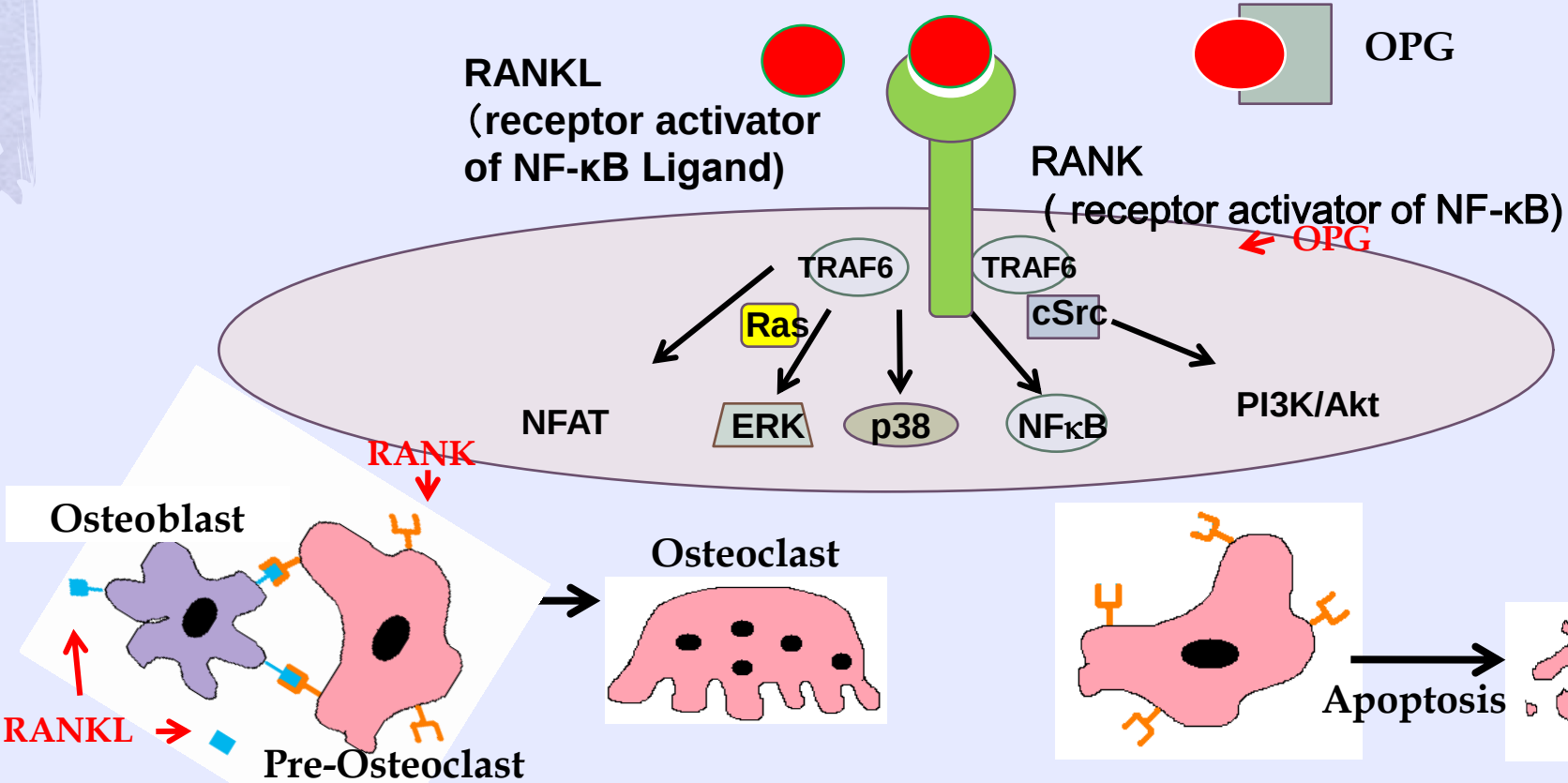


Mineralization

Schulz, E. J Clin Endocrinol Metab 2004 89: 4246-4253

Osteoprotegerin (OPG) KO mouse shows both osteoporosis and aortic calcification

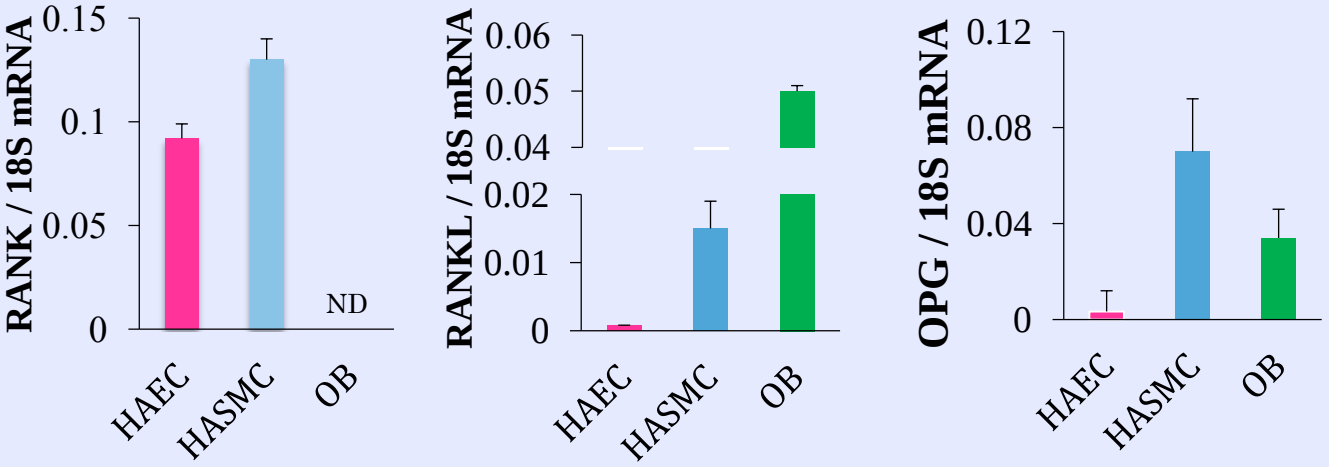




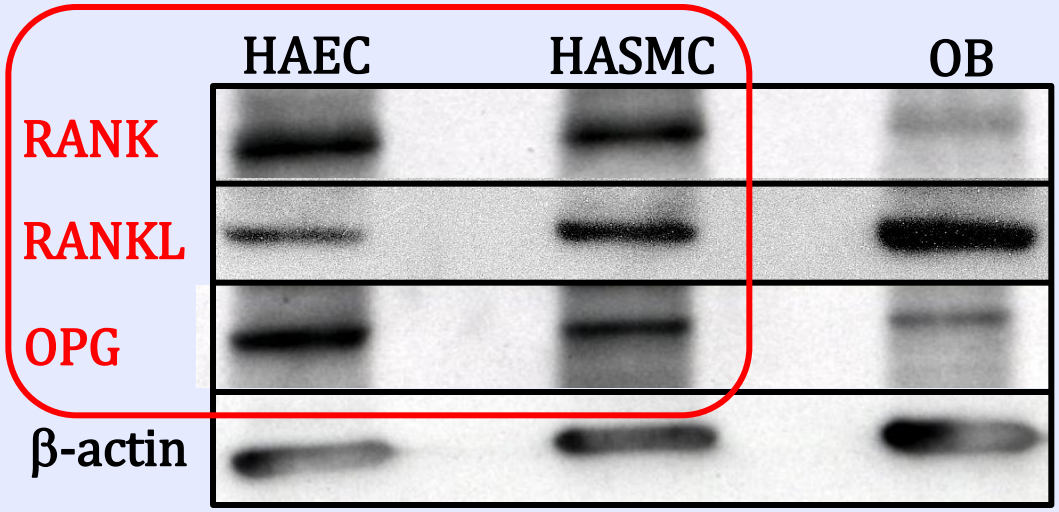
Basal Vascular Expression of RANKL System

RANK is expressed in both endothelial cells and smooth muscle cells.

A)



B)

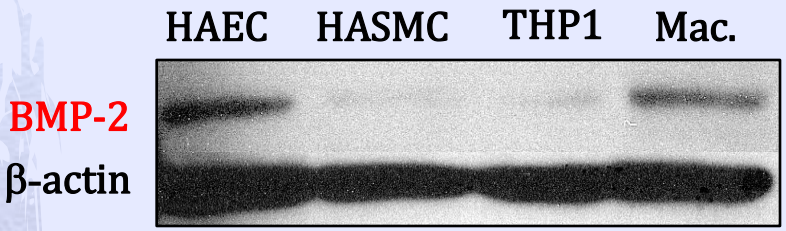


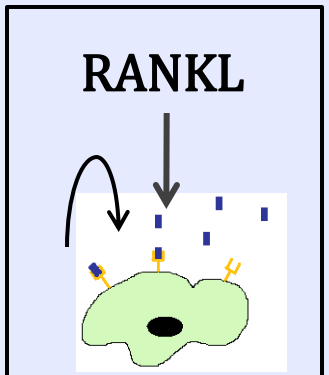
HAEC: human endothelial cell
HASMC: human aortic smooth muscle cell
OB: human osteoblast

RANKL induces BMP-2 expression

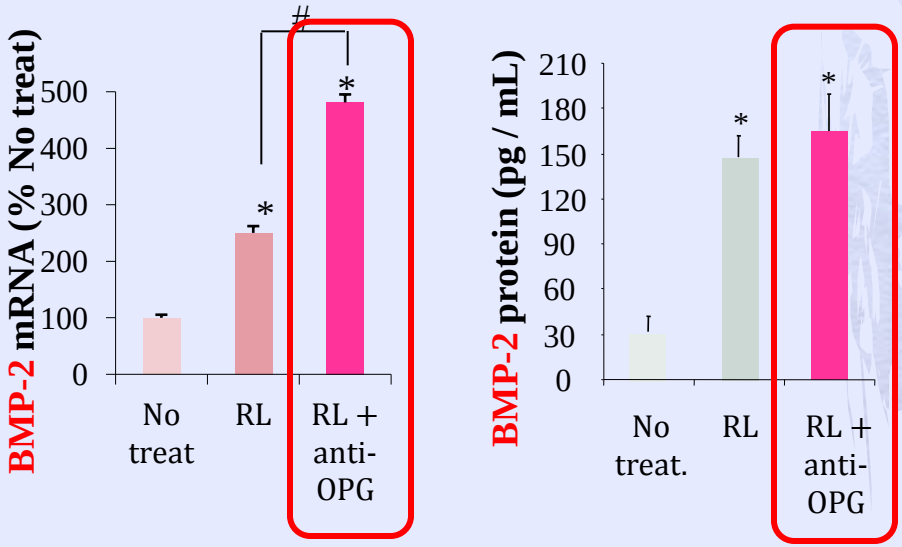
Stimulation with RANKL increased the calcification inducer BMP-2 expression only in HAECs.

A)



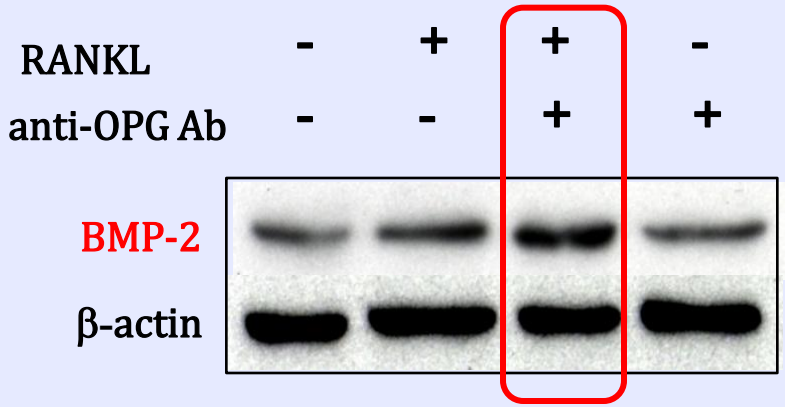


B)

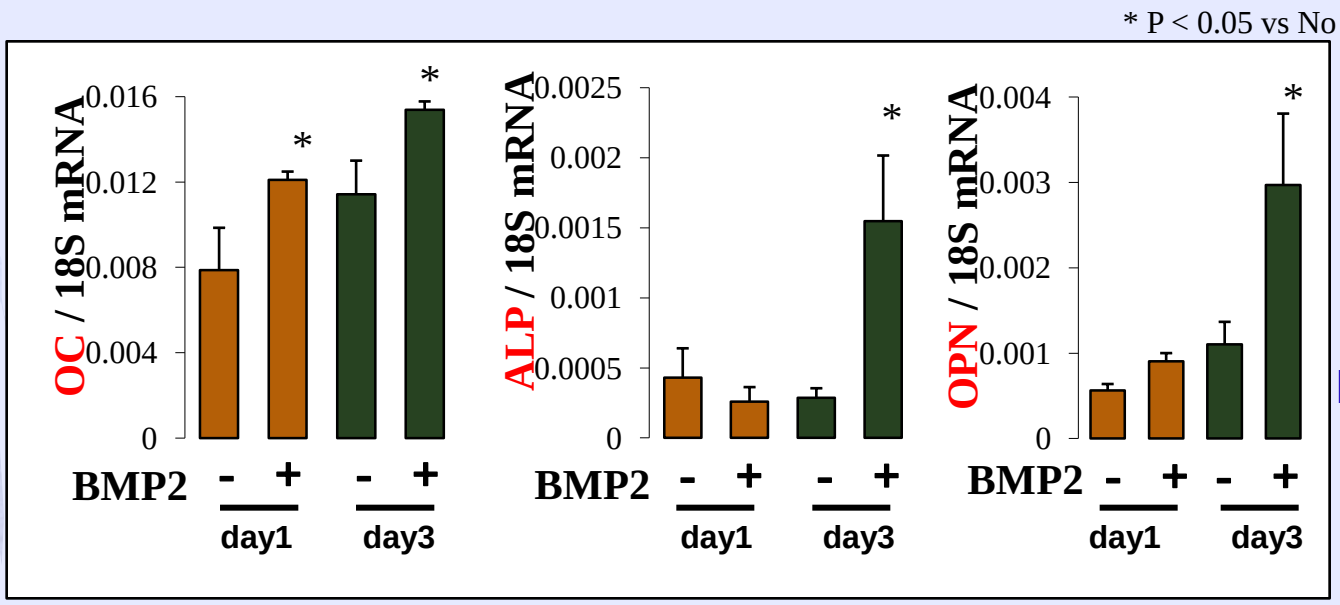


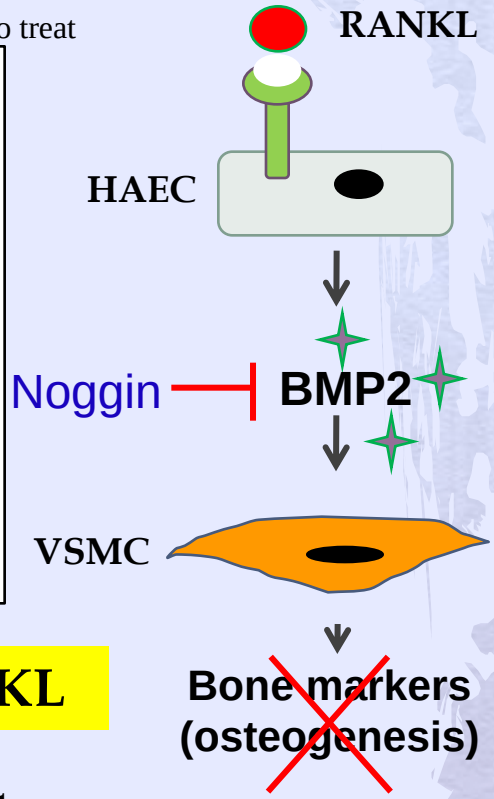
* P < 0.01 vs No treat

C)

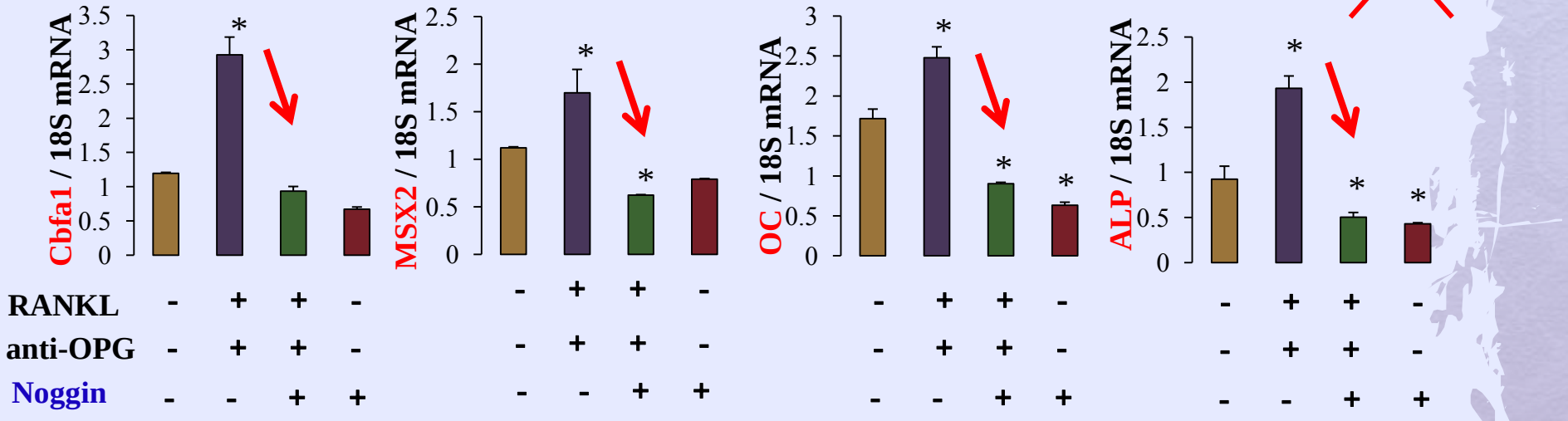


BMP-2 is a potent osteogenic inducer in HASMC :





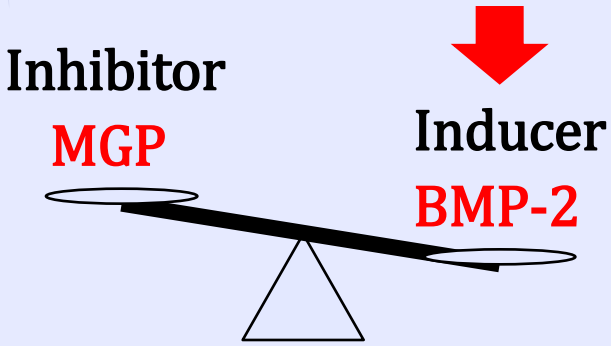
BMP-2 is the main osteogenic protein induced by RANKL



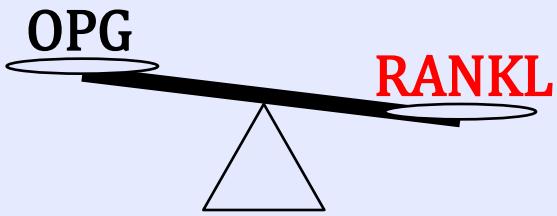
Hypothesis

Under estrogen deficiency, RANKL leads to an imbalance of pro- and anti-calcification factors in vasculature.

Vascular Calcification:

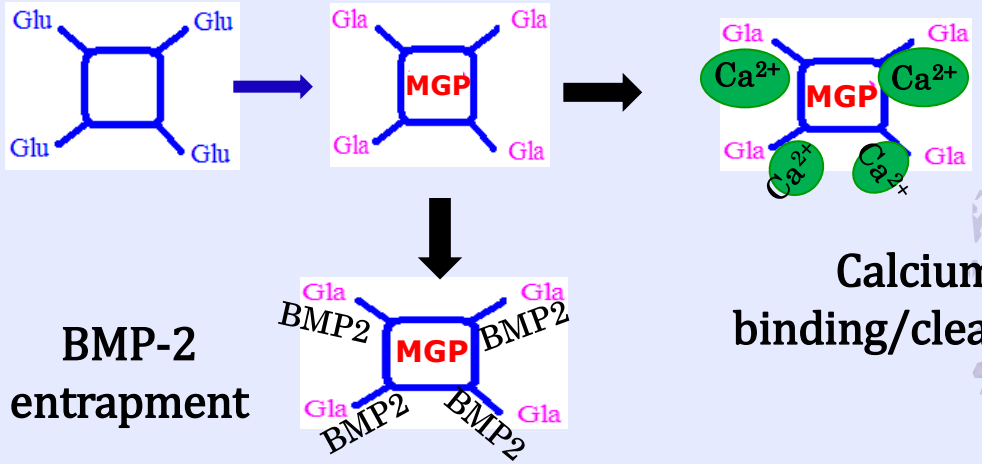


Estrogen Deficiency:



✓ Bone morphogenetic protein (BMP-2) induces aortic calcification .
(J Clin Inves. 2005; 115:1210-1220)

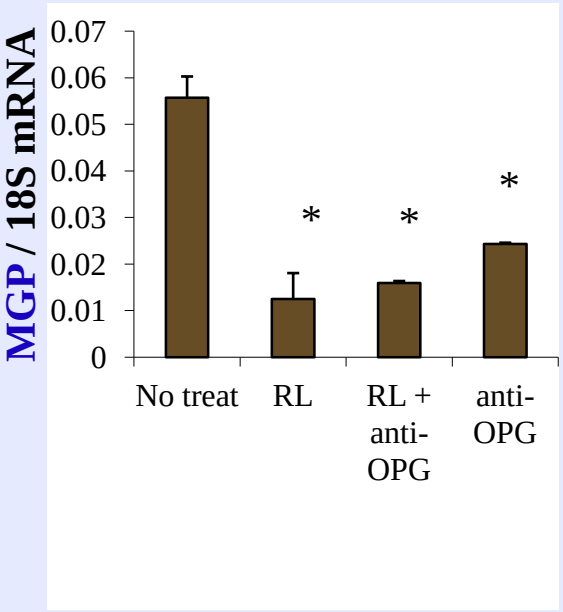
✓ VSMC produces high level of matrix Gla Protein (MGP) , and the knockout mice shows severe vascular calcification
(Nature. 1997; 386:78-81)



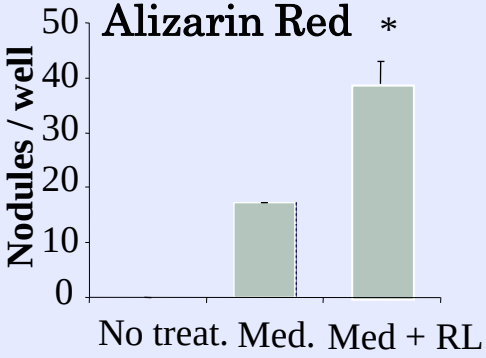
BMP-2 entrapment

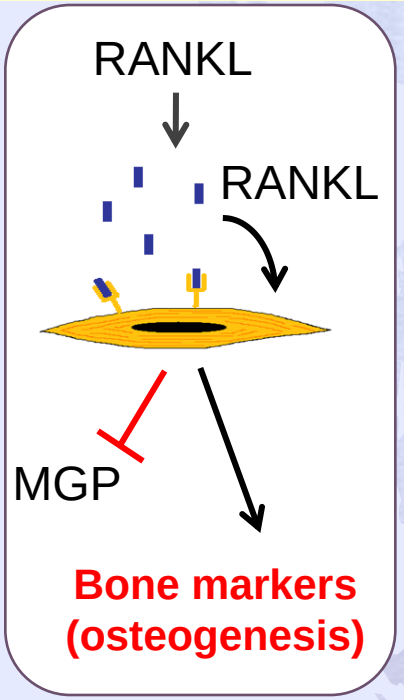
Calcium binding/clearance

RANKL increases calcification of HASMC



RANKL decreases MGP and induces bone-related protein expression in HASMC:

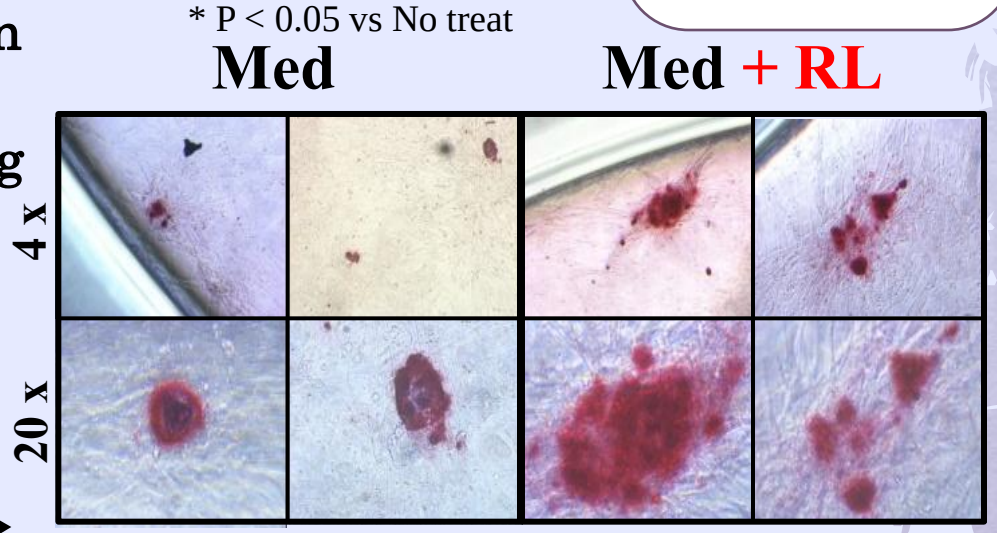




Dexamethasone,
β–Glycerophosphate,
Ascorbate…

Alizarin Red staining

* P < 0.05 vs No treat



Novel Animal Model for Aortic Calcification

ApoE KO mice with ovariectomy

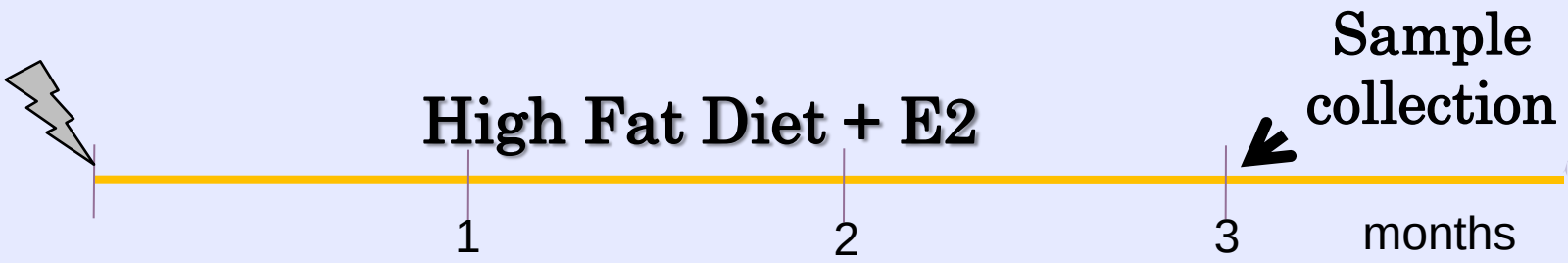
OVX/
High Fat Diet

ApoE$^{-/-}$

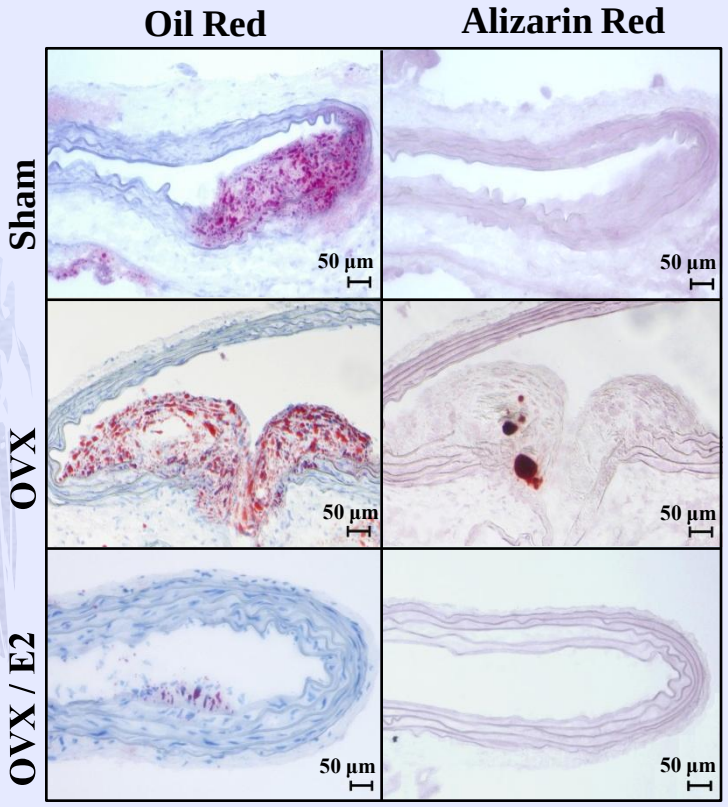
3 months

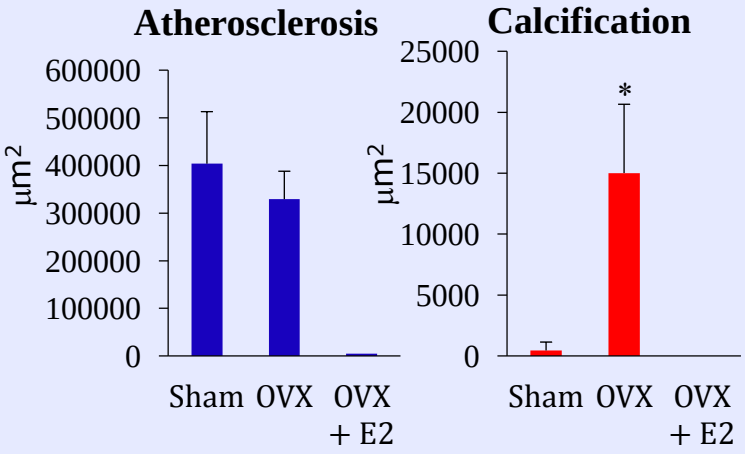
+

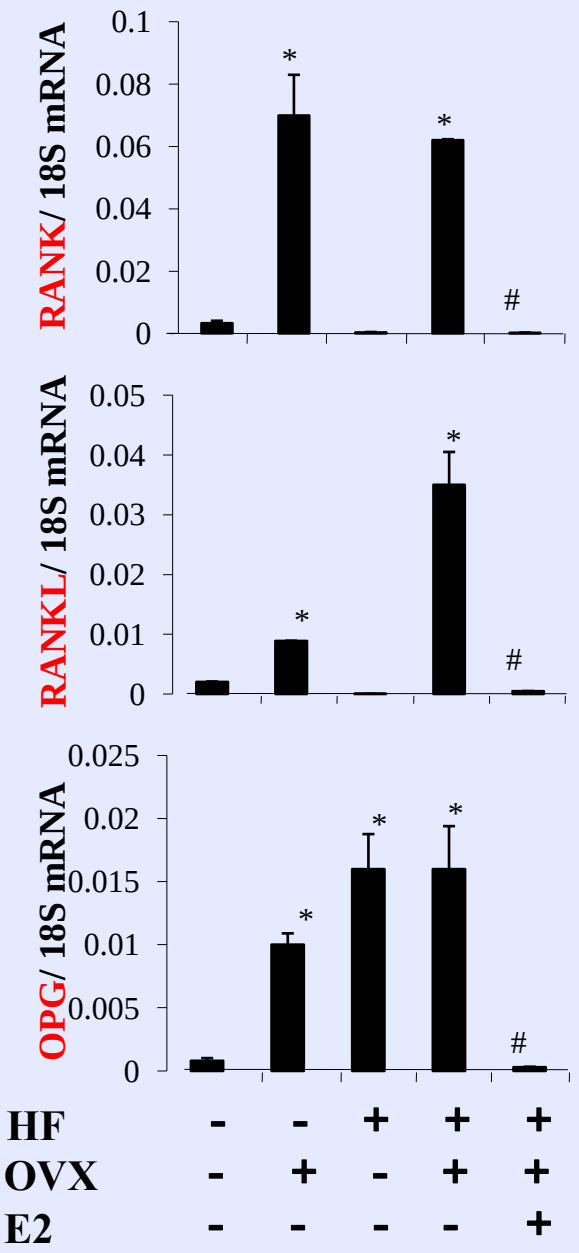
20 μg / kg/ day
17β-Estradiol (E2) by
osmotic pump



Estrogen deficiency increases vascular calcification and RANKL

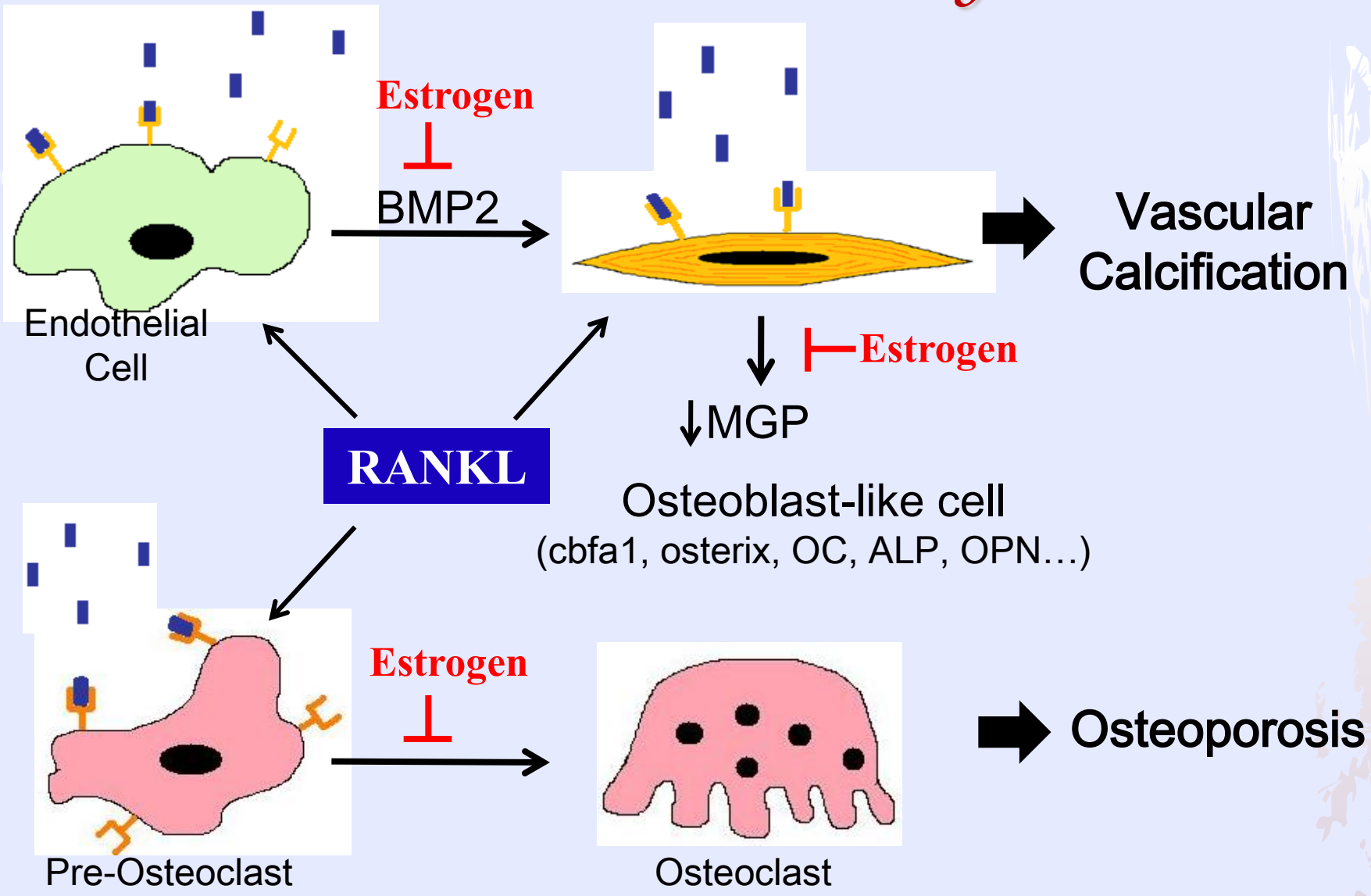






Summary1

RANKL in the Bone x Artery Paradox



JAMA®
The Journal of the American Medical Association

What is the regulator of local RANKL system in vasculature?

Calcification of the Aortic Arch

Risk Factors and Association With Coronary Heart Disease,
Stroke, and Peripheral Vascular Disease

JAMA. 2000;283:2810-2815

→ Aortic arch calcification was independently associated with older age, current smoking, and **hypertension** in both men and women.

Table 1. Distribution of Risk Factors by Presence of Aortic Arch Calcification and Sex*

Risk Factor	Aortic Arch Calcification			
	Men		Women	
	None (n = 54 824)	Present (n = 1092)	None (n = 58 803)	Present (n = 1590)
Age, y†	45.8 (11.1)	60.6 (10.8)	46.4 (10.8)	62.3 (8.8)
Body mass index, kg/m²†	25.8 (3.4)	25.7 (3.2)	24.5 (4.5)	25.2 (4.4)
Serum cholesterol, mmol/L†‡	5.9 (1.1)	6.0 (1.0)	5.9 (1.1)	6.5 (1.2)
Educational attainment				
No college	40	24	30	19
At least some college	45	68	56	75
Unknown	15	8	14	6
Race/ethnicity				
White	78	85	78	88
Black	13	10	14	9
Asian	4	2	4	1
Other/unknown	5	2	4	1
Cigarette smoking				
Never	29	28	44	54
Former	24	28	13	10
Current	39	34	34	23
Unknown	8	9	9	13
Alcohol consumption, drinks/d				
0	15	24	27	37
1-2	56	46	51	38
≥3	17	13	6	4
Unknown	12	14	19	21
Hypertension§	21	35	22	44
Diabetes‖	3	6	2	5
Family history of myocardial infarction	17	9	19	16
VDRL serologic test				
Positive	0.6	0.7	0.4	0.1
Weakly reactive	1.1	2.1	1.2	1.7

*Data based on patients enrolled in the northern California Kaiser Permanente Medical Care Program between 1964 and 1973 who had multiphasic health checkups. Values are expressed as percentages unless otherwise indicated.
†Values are expressed as mean (SD).
‡To convert cholesterol from mmol/L to mg/dL, divide by 0.0259.
§Diagnosed as having hypertension if patient had a systolic blood pressure higher than 140 mm Hg and diastolic blood pressure higher than 90 mm Hg; or if self-reported or if diagnosed by a physician; or if a patient self-reported his/her use of antihypertension medication.
‖Diagnosed as having diabetes if self-reported or if diagnosed by a physician, or if a patient self-reported his/her use of insulin or hypoglycemic agents.

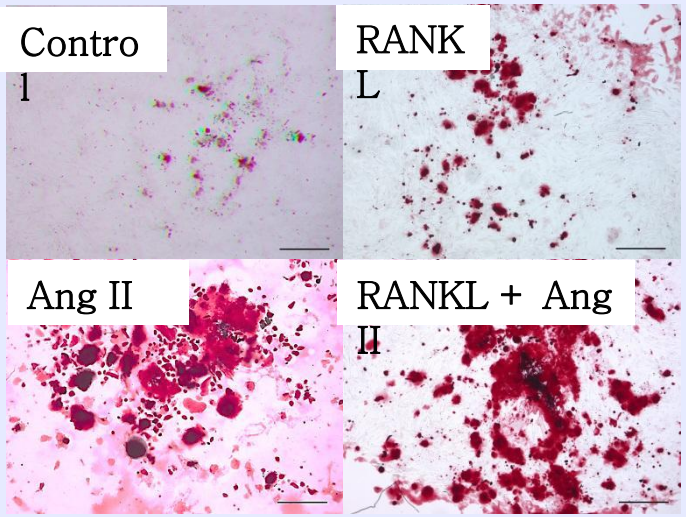
Hypothesis:

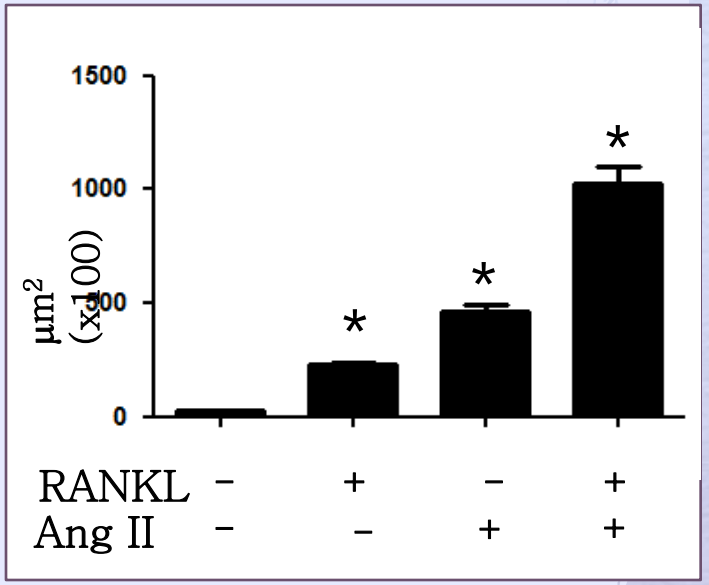
Vascular RAS is the trigger of RANKL system activation.

Ang II → ↑ RANK-RANKL → Vascular Calcification

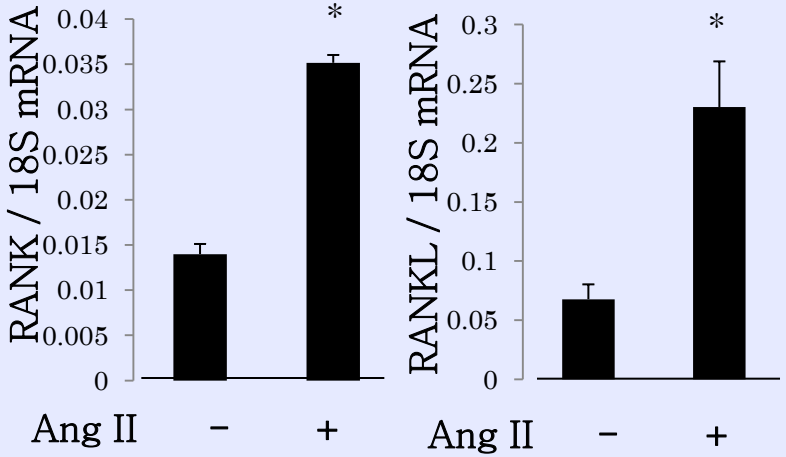
Alizarin Red Staining

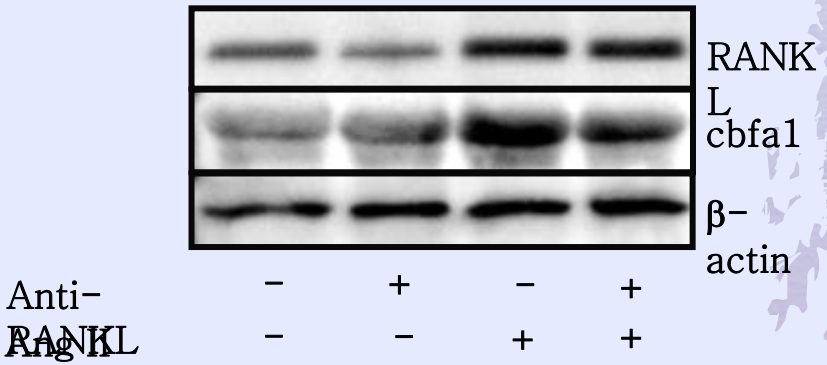
Dexamethasone,
β–Glycerophosphate,
Ascorbate…





C)





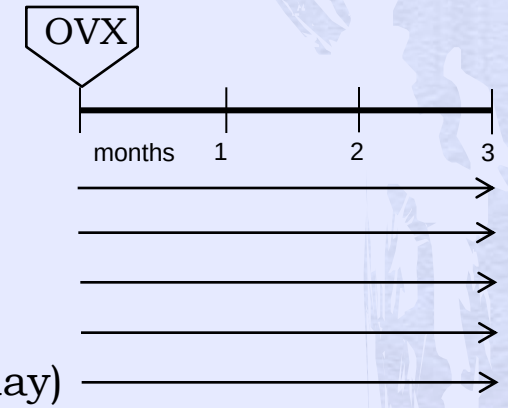
ApoE KO mice with ovariectomy

OVX/
High Fat
Diet
+AngII
3 months

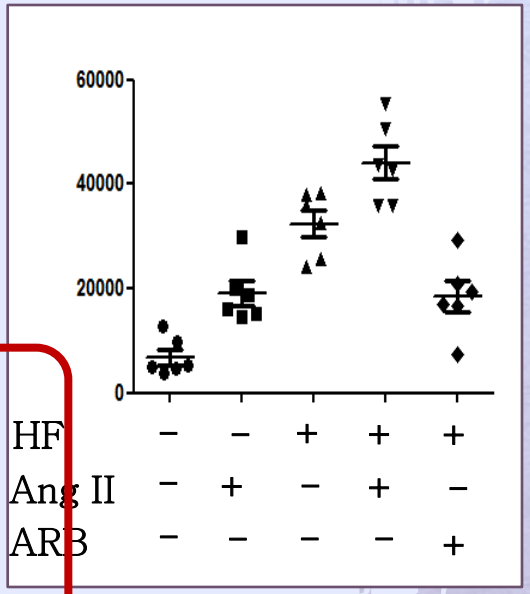
ApoE$^{-/-}$

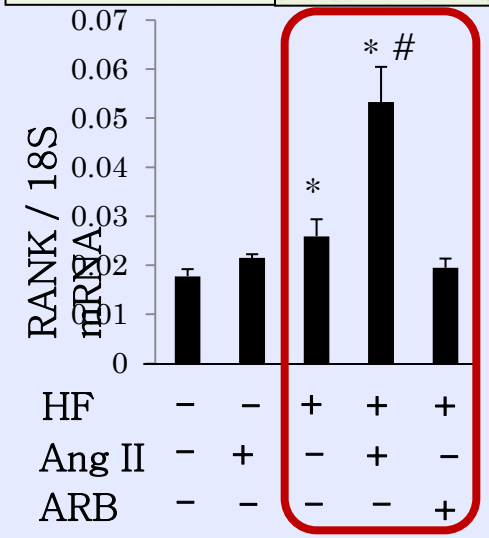
♀ApoE$^{-/-}$ mice
under OVX

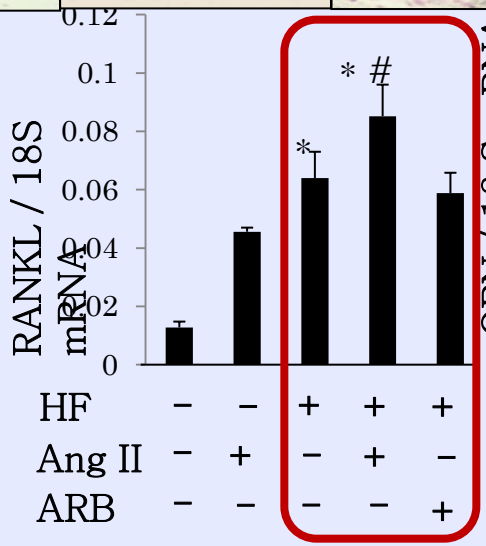
1. No treat
2. Ang II (100 ng/kg/min)
3. High Fat diet
4. High fat diet + Ang II (100 ng/kg/min)
5. High fat diet + Olmesartan (3 mg/kg/day)

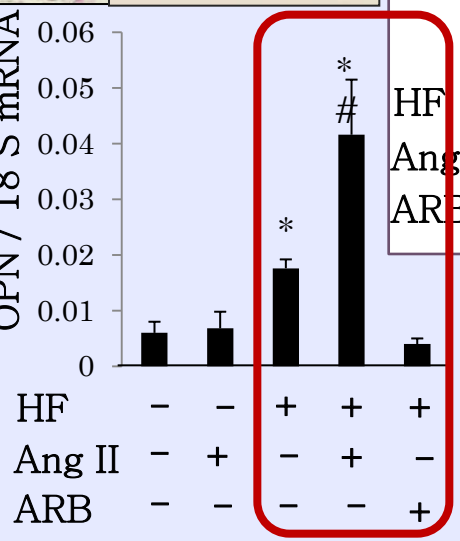


Von Kossa Staining





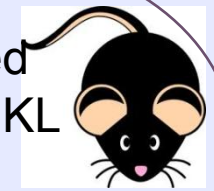




Effect of AngII in OPG KO mice

Unopposed RANK-RANKL signaling

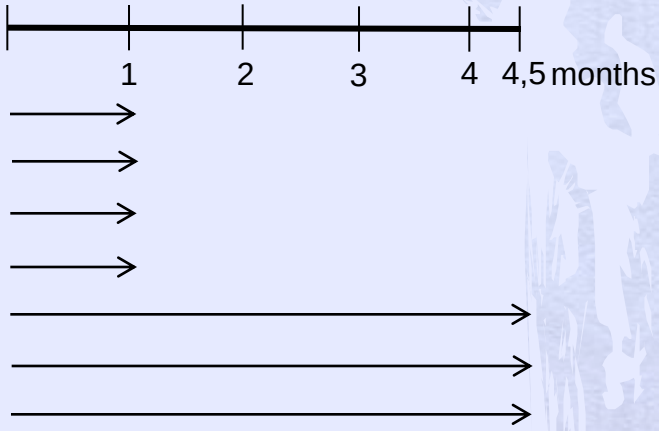
+AngII



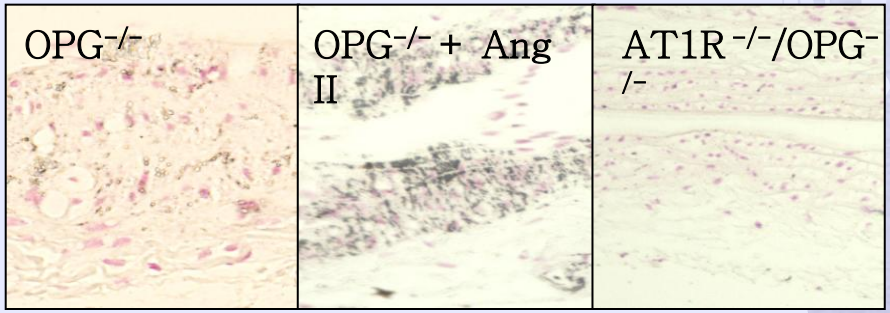
$OPG^{-/-}$

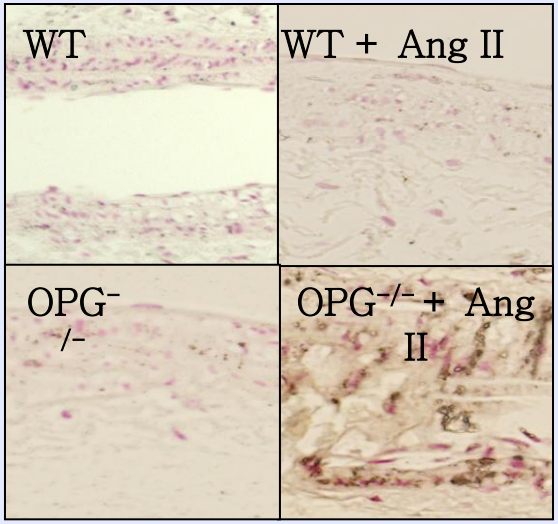
♂WT, $OPG^{-/-}$, $AT1R^{-/-}/OPG^{-/-}$ mice

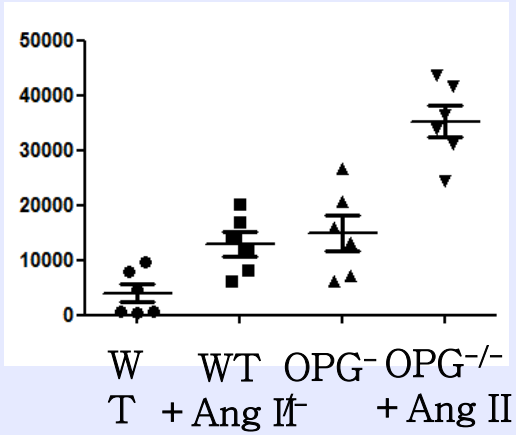
1. WT
2. WT + Ang II (100 ng/kg/min)
3. $OPG^{-/-}$
4. $OPG^{-/-}$ + Ang II (100 ng/kg/min)
5. $OPG^{-/-}$
6. $OPG^{-/-}$ + Ang II (100 ng/kg/min)
7. $AT1R^{-/-}/OPG^{-/-}$

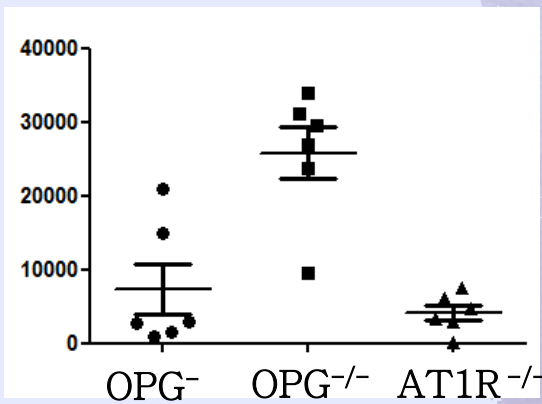


Von Kossa Staining



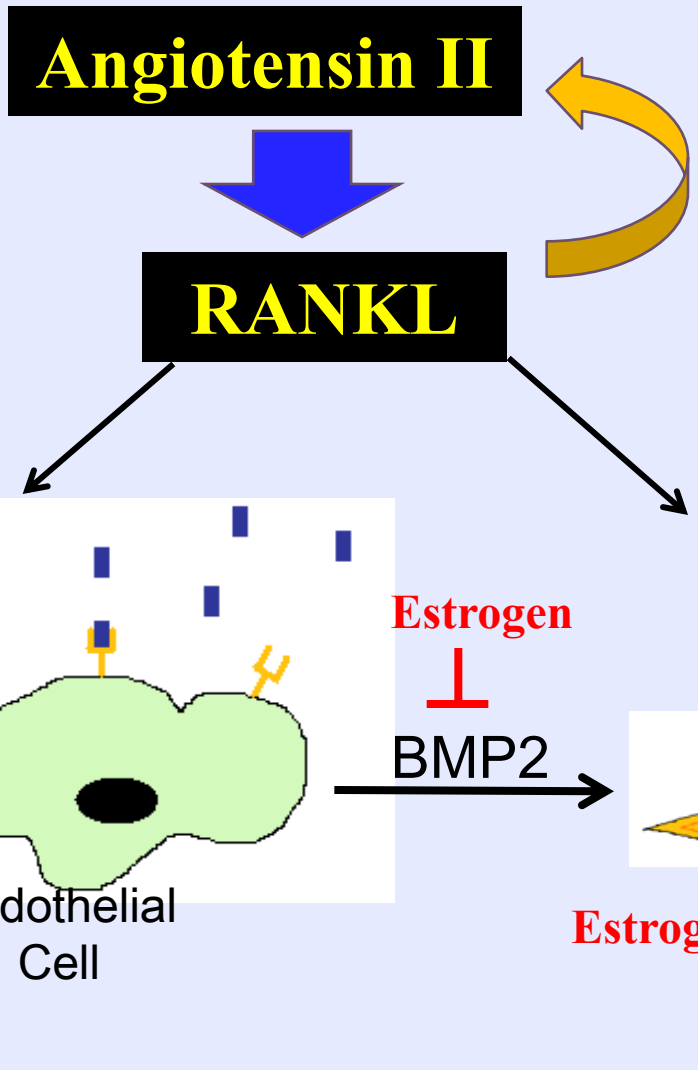


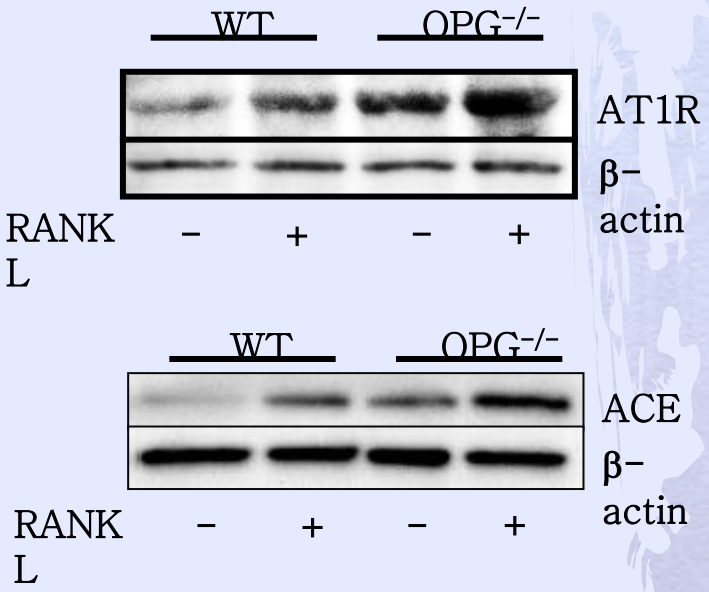




Summary

RAS activates RANKL system, and conversely, unopposed RANKL stimulation activates local RAS, and this vicious cycle aggravates vascular calcification



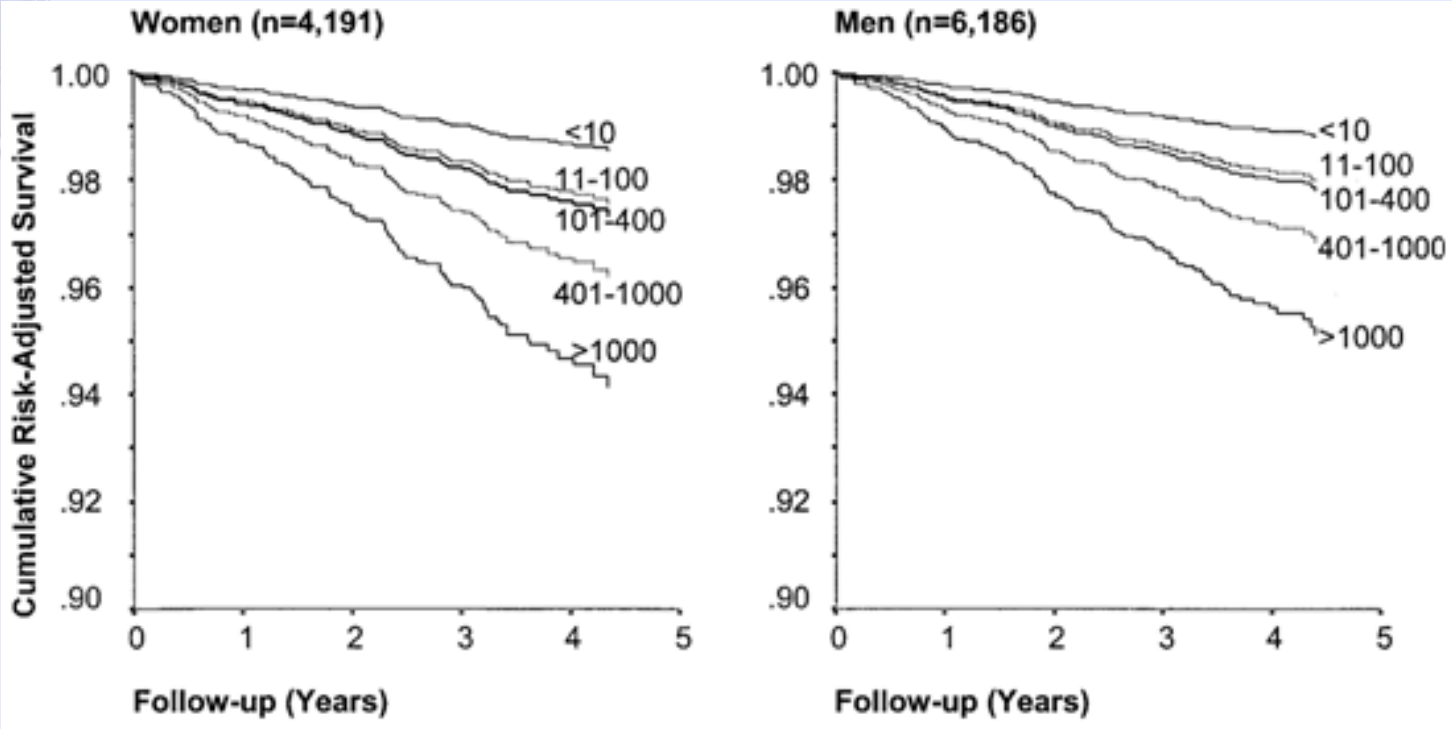


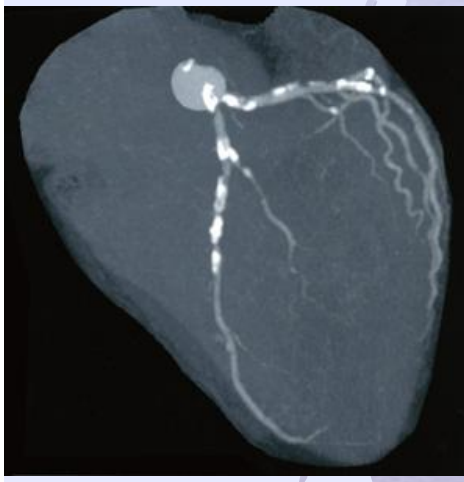
Vascular Calcification and Serum OPG

Biomarkers of the Osteoprotegerin Pathway

Clinical Correlates, Subclinical Disease, Incident Cardiovascular Disease, and Mortality

ATVB 2010; 30: 1849-1854.





Raggi P. et al, Journal of Women's Health 13; 3 2004

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