Discovery and Validation of Potential Genomic-Based Biomarkers for Asbestos Related Neoplasms

American Australian Mesothelioma Consortium and NYU Mesothelioma Biomarker Discovery Laboratory

Mesothelioma Biomarkers and Their Validation

- Introduction
- SMRP and Osteopontin
- Biomarkers in Progress
- US Validation Trial Update
- The Cappadochian Studies

American Australian Mesothelioma Consortium

- **NYU School of Medicine (NYU)**
  - Harvey I. Pass MD, PI
- **University of Western Australia (UWA)**
  - Bruce Robinson MD, PhD, Co-PI
- **Peter MacCallum Cancer Institute (PMCC)**
  - David Bowtell PhD
  - Andrew Holloway, PhD
- **Fujirebio Diagnostics, Inc (FDI)**

Asbestos-Related Thoracic Cancers

- **Pleural Mesothelioma**
  - 2500 in United States
  - 15-30 year latency period
  - Median Survival 6-13 months
  - Uniformly fatal when diagnosed after symptoms
  - $54 billion in asbestos-related claims and the estimated future liability ranges from $145 to $210 billion.

Mesothelioma Archives NYU

- **221MPM tumors, snap frozen**
  - 63 corresponding normal peritoneum
  - 249 sera
  - 34 plasma
  - 120 pleural effusion
  - 136 urine
  - Complete clinical demographics
- **85 Asbestos exposed**
  - All with serum, plasma, and urine
  - Complete clinical demographics
- **Over 200 lung cancers, snap frozen**
  - Corresponding normal lung
  - Corresponding serum (all); 60 with plasma
  - Complete clinical demographics
- **62 high risk for lung cancer (chemoprevention trial)**
  - All with serum and plasma
  - Complete clinical demographics

Novel Markers for Mesothelioma

- **Ready for Validation**
  - SMRP (MesoMark™)
    - Partnership with Fujirebio Diagnostics, Malvern Pennslvania
  - Osteopontin
- **Studies in Progress**
  - MMP1 and MMP9
  - HAPLN1 (CRTL-1)

Mesothelin

- **MAb K1** demonstrated selective staining of MPM tissue and cell lines
  - Pastan et al: 1992
  - Willingham et al: 1992
- The cloned cDNA from an ovarian cDNA library encoded an antigen recognized by K1:
  - a **40-kDa glycoprotein (mesothelin) present on the surface of mesothelial cells**, MPMs, and ovarian cancers with a 69 kDa precursor

8 years later….

Table 4: SAGE MESO-12: CANDIDATE CLASSIFICATION GENES

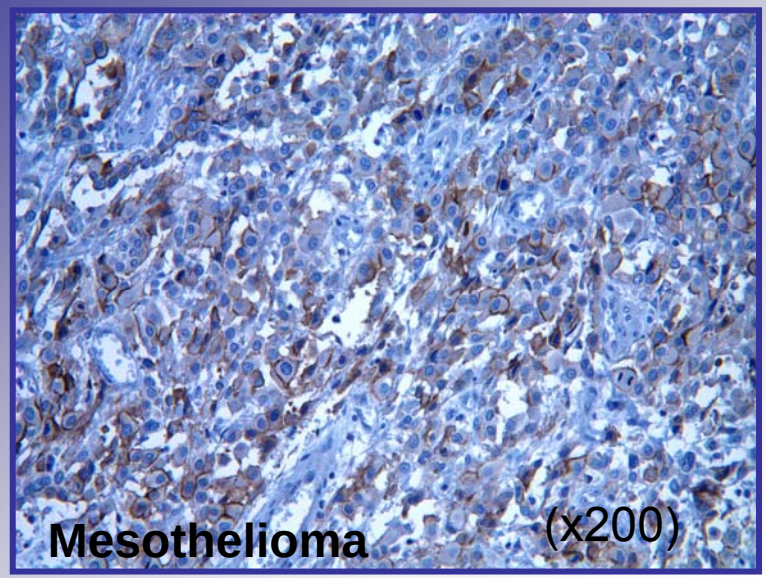
TAGS ELEVATED IN MM	FOLD ↑ MM	GENE
TCCCCTACAT	293	Intellectin
CCTCCAGCTA	112	keratin 8
CAGGCCCTGC	71	CD3Z antigen, zeta polypeptide
CAAACCATCC	59	keratin 18
GACATCAAGT	57	keratin 19
CCCCCTGCAG	49	**mesothelin**
TAGACTAGCA	46	tetraspan 3
TGTGGGAAAT	37	SLPI
AACGCGGCCA	35	macrophage migration inhibitory factor
CCGTCCAAGG	34	ribosomal protein S16
TCCCTGTTAA	34	beta-2-microglobulin
GCCGGGCCCT	32	vitronectin
TAGCAGCAAT	32	up-regulated by BCG-CWS
TTTCCCTCAA	31	protease, serine, 11 (IGF binding)
TGGTTGGTGG	29	plasmolipin
GTGCGGAGGA	26	serum amyloid A1
TAAGCTGTGC	26	duodenal cytochrome b
TTAAACAAAG	26	retinoic acid receptor responder
ACTCCTACTT	25	uroplakin 1B
GCCCCTGCTG	25	keratin 5
GCCCCTCCAG	24	claudin 15
GCCGGGTGGG	24	basigin
CCACCACCCA	23	calbindin 2,
AGCTGGATGC	22	calbindin 2, (29kD, calretinin)
ATGCTCCCTG	21	galectin 6 binding protein

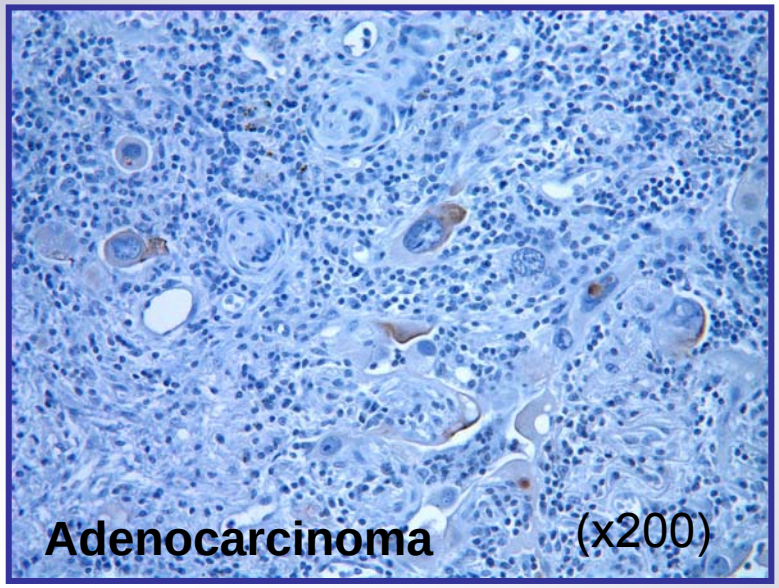
Serum Mesothelin Related Peptide (SMRP, Mesothelin Variant 1))

- Same N-terminal amino acid sequence as **mesothelin** and **megakaryocyte potentiating factor.**
- Most likely originates as a portion of the extracellular domain of **membrane-bound mesothelin**
- Non-Quantitative “sandwich ELISA” developed with antibodies 569 and 4HR

Scholler N: Proc.Natl.Acad.Sci.U.S.A, 96:11531-11536, 1999
Onda M: Clin. Canc. Res., 15: 4225-4231, 2006.

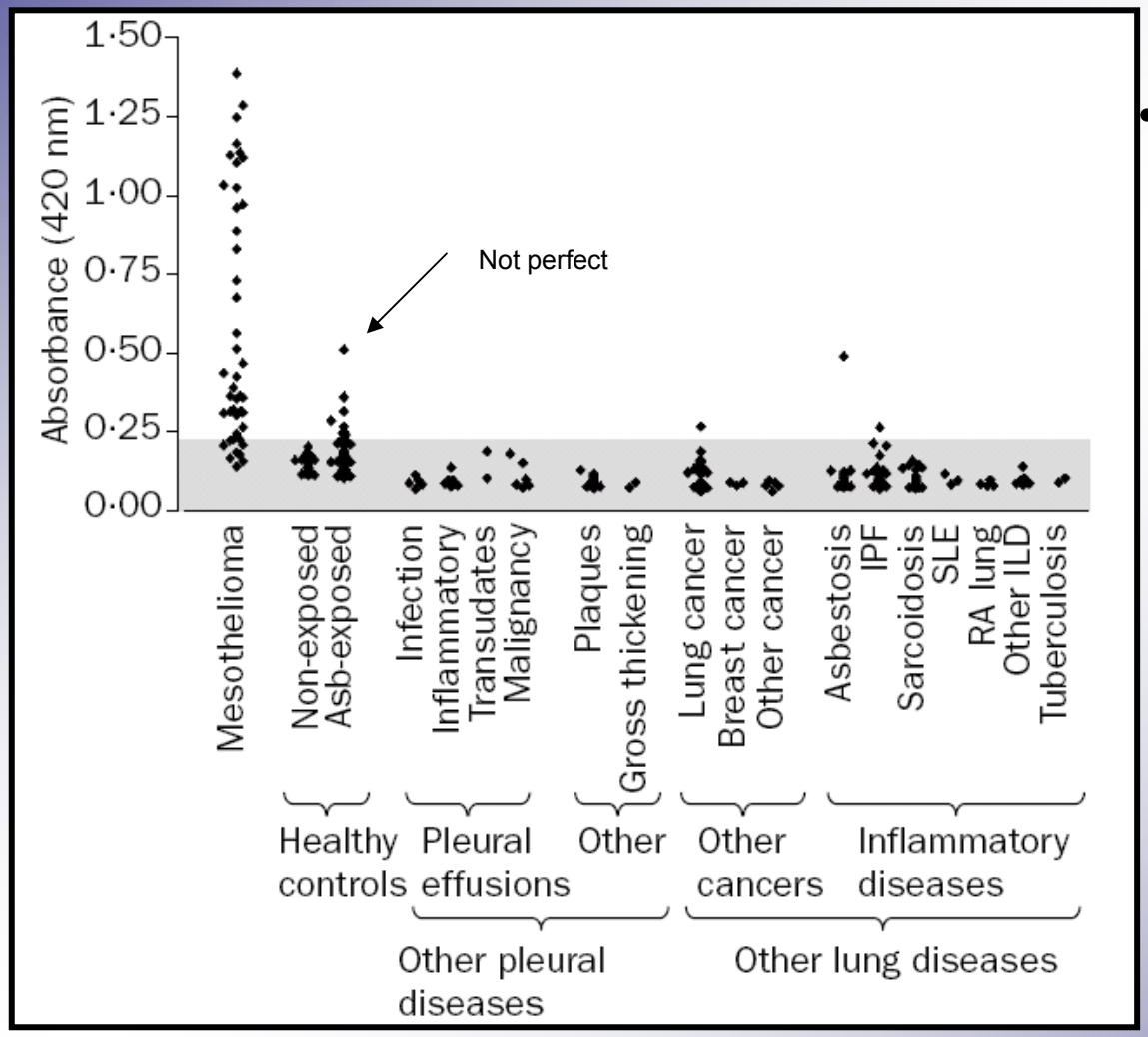
SMRP (Mesothelin Variant I) Antibody 569





- The antibody 569 stained 42/62 (68%) MPMs and 7/74 (10%) adenocarcinomas. All MPMs stained in a membranous pattern, and positive staining was seen in mainly epithelial components.

SMRP and Mesothelioma



- 84% sensitivity
  - 100% specificity when compared with other pleural diseases
  - 95% specificity when compared with other lung tumors
  - *83% when compared with people with asbestos exposure*

Robinson, B.: Lancet, *362*: 1612-1616, 2003.

Validation of SMRP in the American Cohort

Methods

- **Patient Population**
  - Serum
    - 90 MPM
    - 170 NSCLC
    - 66 Asbestos-exposed volunteers from the Center for Occupational and Environmental Medicine
    - 409 normal volunteers
  - Pleural Effusion
    - 45 MPM
    - 20 Other Cancers
    - 30 Benign
- **SMRP**
  - MesoMark™ duplicate samples
- **Statistical Analysis**
  - ROC curves
  - Kruskal-Wallis and ANOVA

Serum Demographics

	MPM (n=90)	Lung Cancer (n=170)	Asbestos (n=66)
Sex (M/F)	71/19	94/76	61/5
Age (years)	63±1 (39-84)	66±1(33-87)	64±1(36-90)
Fiber Exposure	73/90(81%)	NA	66/66 (100%)
Histology*			
Epithelial	58 (64%)	Adenocarcinoma (64%)	
Biphasic	29 (32%)	Squamous cell (33%)	
Sarcomatoid	3 (4%)	Small cell (3%)	

*Histology data available only on 120 of the 170 lung cancers

Serum SMRP

	MPM (n=90)	Lung Cancer (n=170)	Asbestos Exposed (n=66)
Mean SMRP, nM	5.67+ 0.82	1.99+0.43	0.99+0.10
Range	(0-32nM)	(0-32nM)	(0-32nM)

P<0.001

P=0.173

P<0.001

Pleural Effusion SMRP

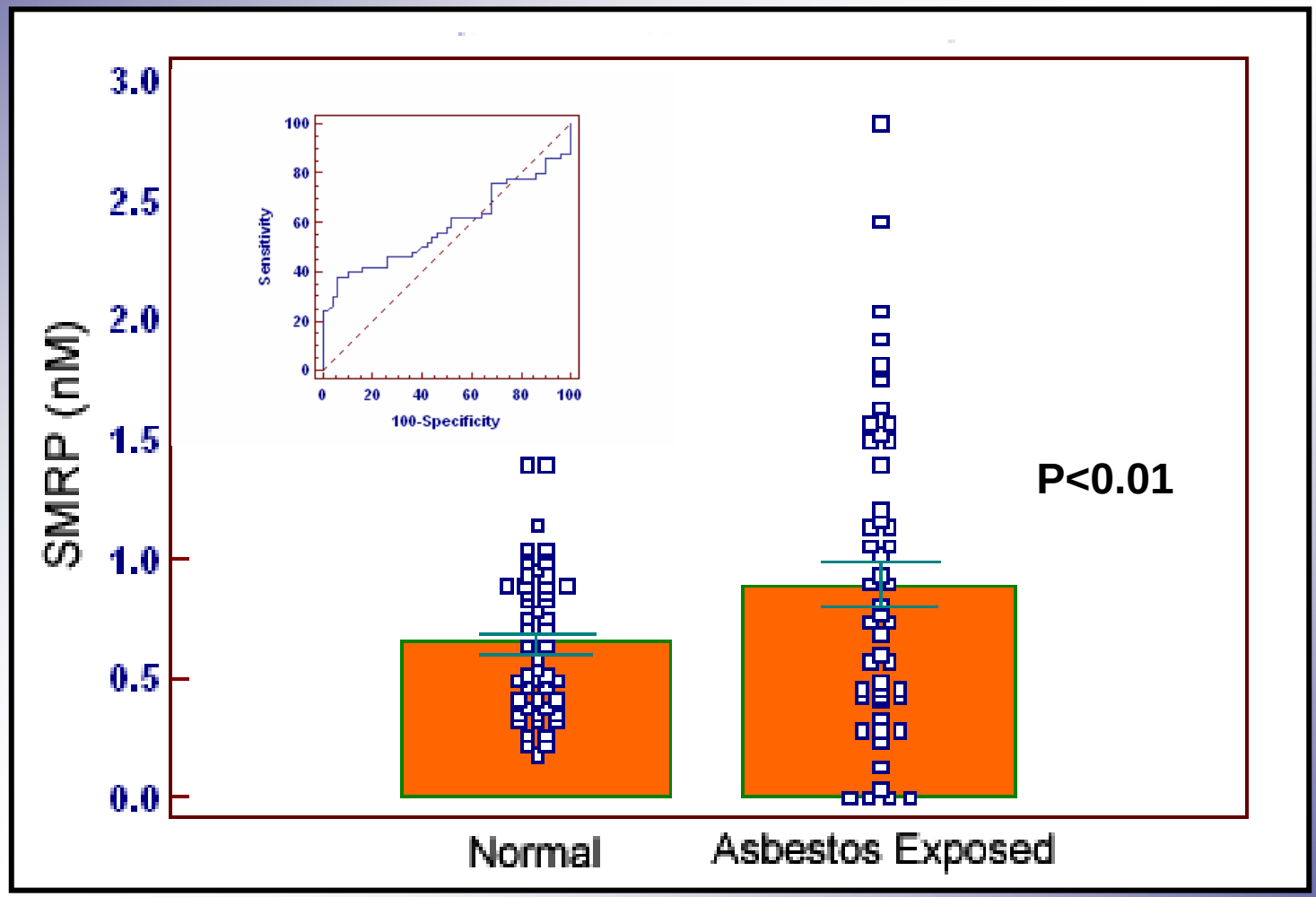
	MPM (n=45)	Other Cancers (n=20)	Benign (n=30)
Mean SMRP, nM	65.57+11.33	27.46+11.25	18.99+7.48
Range	(0-255 nM)	(0-140 nM)	(0-151 nM)

P=0.044

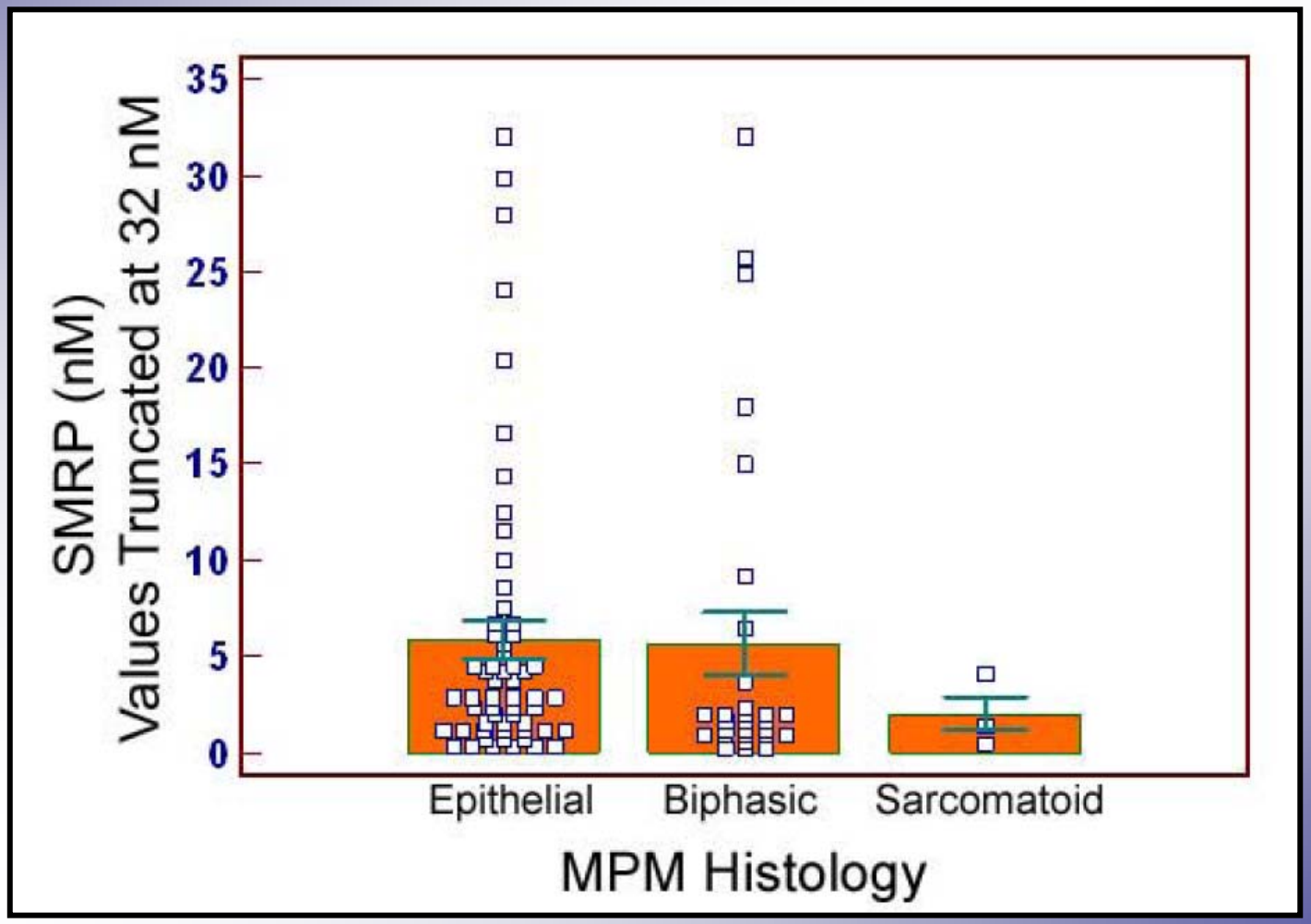
P=0.210

P<0.003

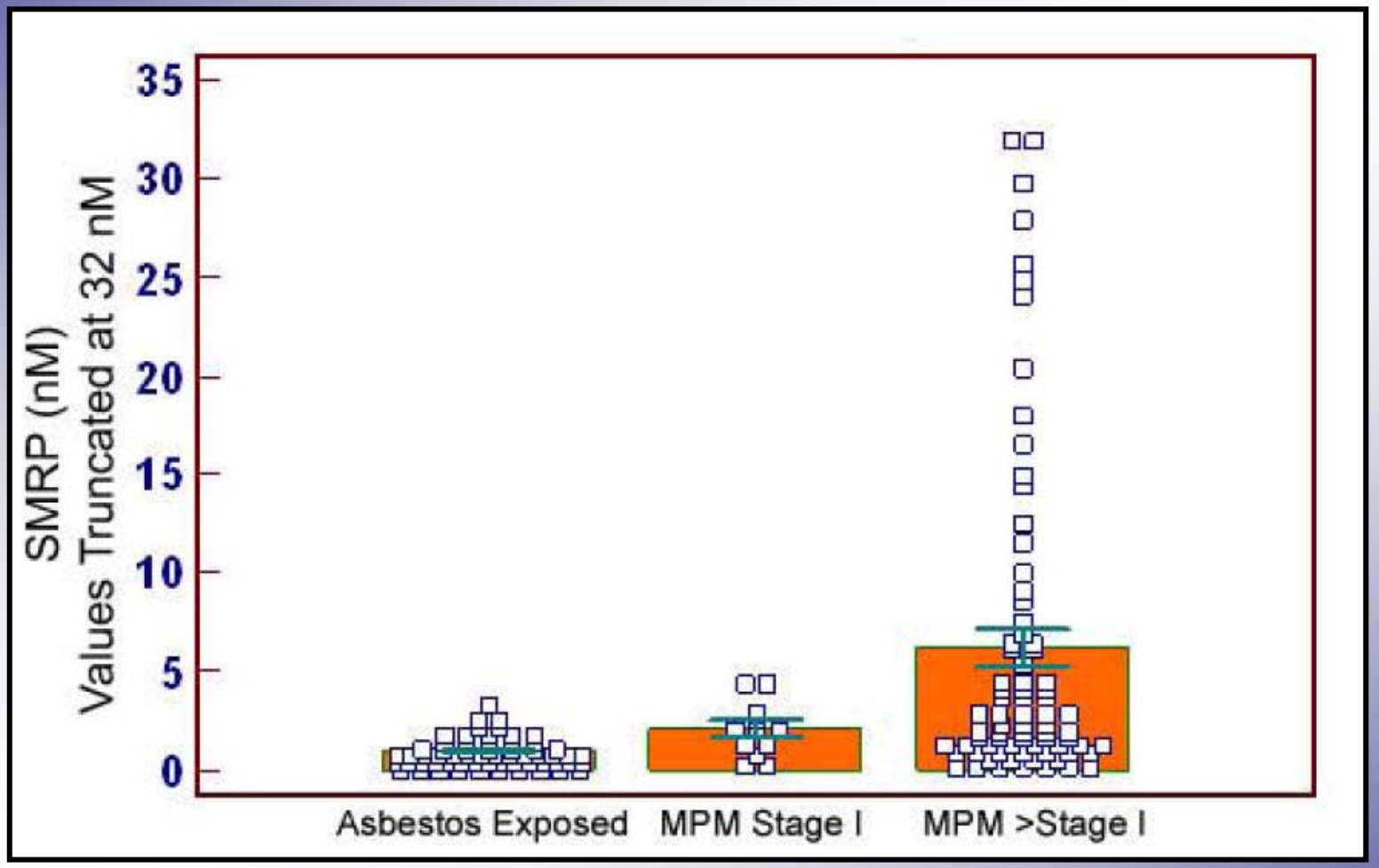
Serum SMRP:
Age/Sex Matched Controls (n=50)



Serum SMRP
Mesothelioma Histology



Serum SMRP and MPM Stage

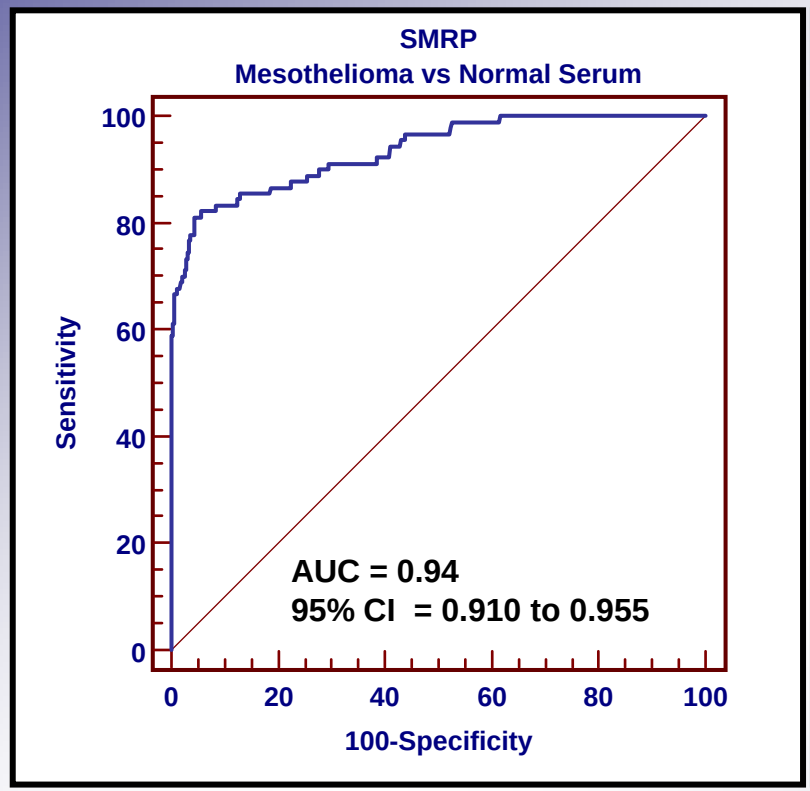


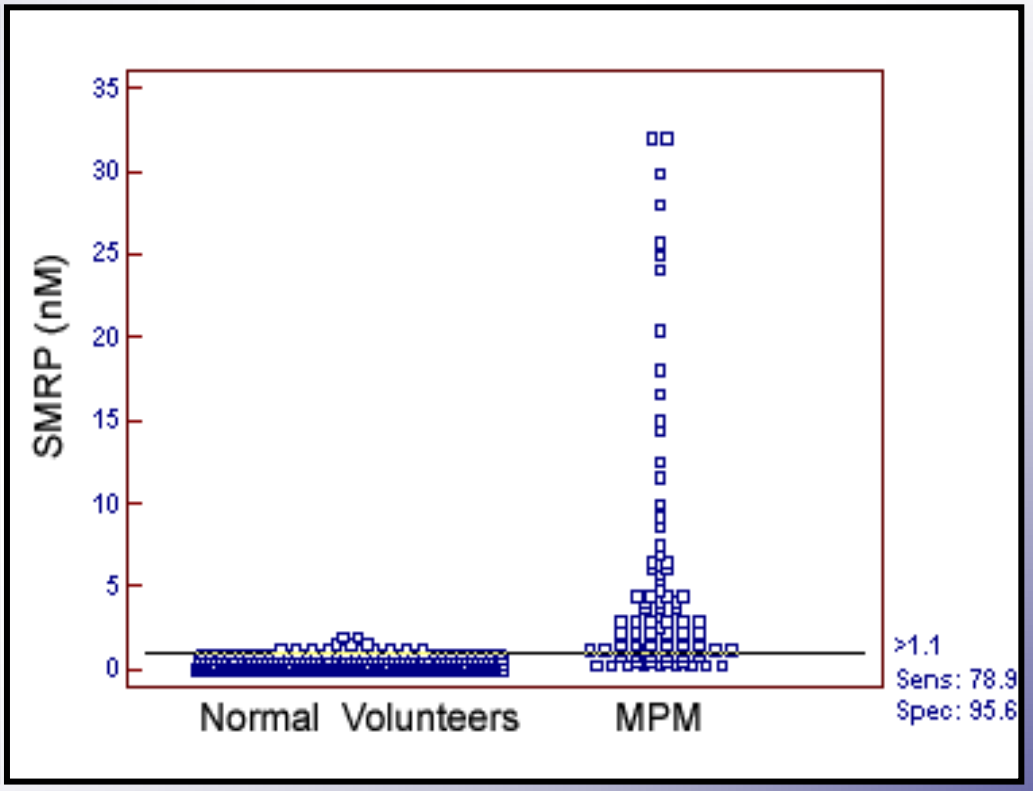
P=0.02

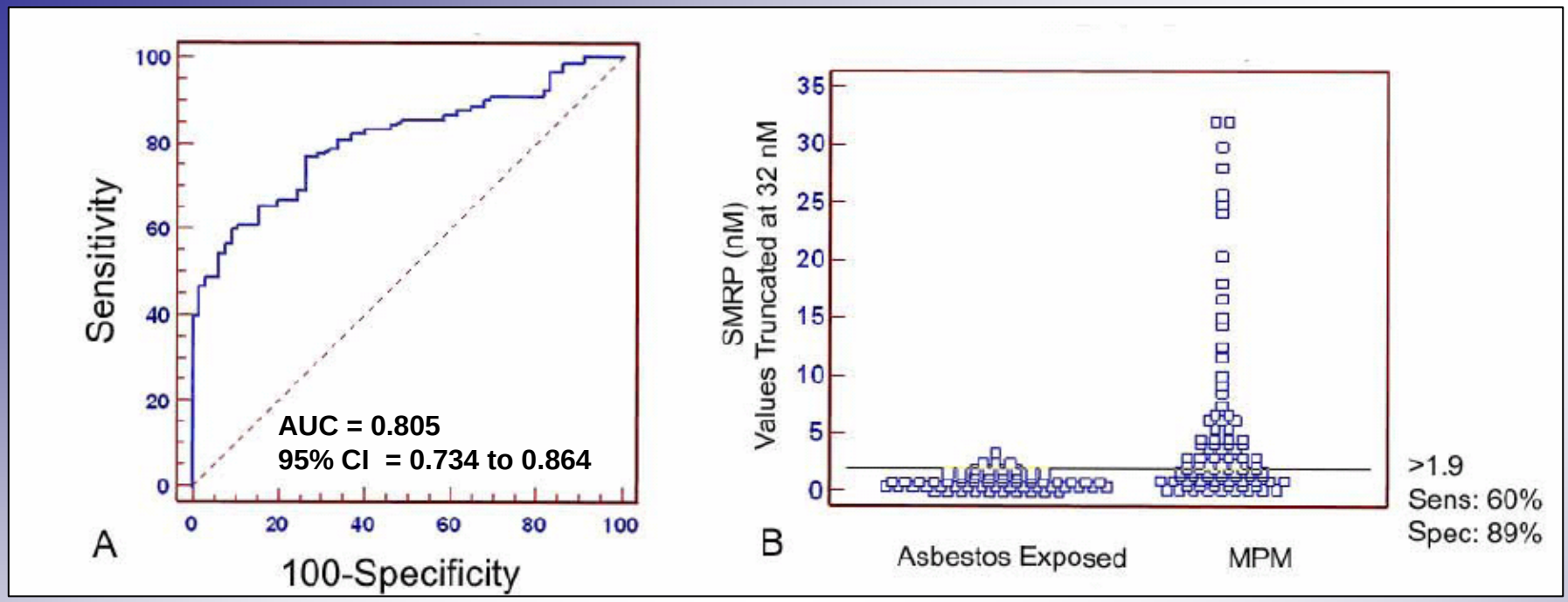
P=0.0001

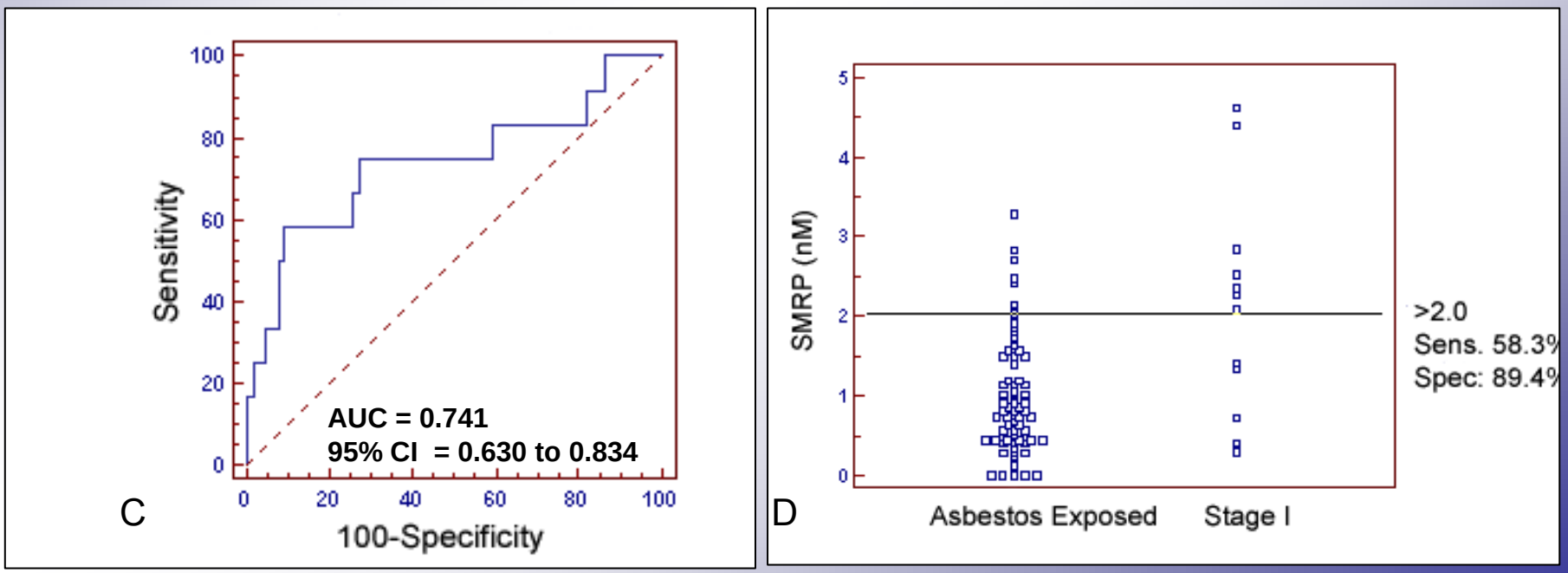
P<0.0001

Serum SMRP Performance
MPM vs Normal (n=409)






Sensitivity
100-Specificity
AUC = 0.805
95% CI = 0.734 to 0.864
A
SMRP (nM)
Values Truncated at 32 nM
Asbestos Exposed
MPM
>1.9
Sens: 60%
Spec: 89%
B


Sensitivity
100-Specificity
AUC = 0.741
95% CI = 0.630 to 0.834
C
SMRP (nM)
Asbestos Exposed
Stage I
>2.0
Sens. 58.3%
Spec: 89.4%
D

Serum SMRP for MPM vs "Asbestos"Cohorts

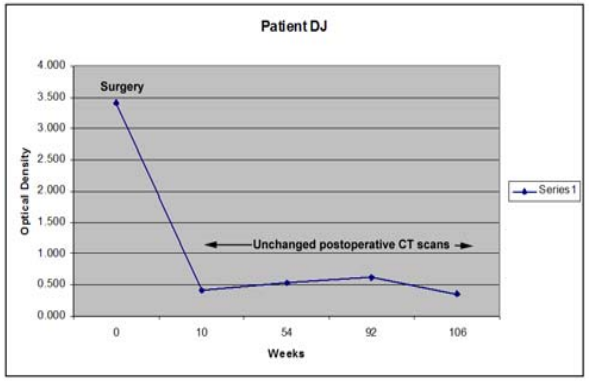
Summary

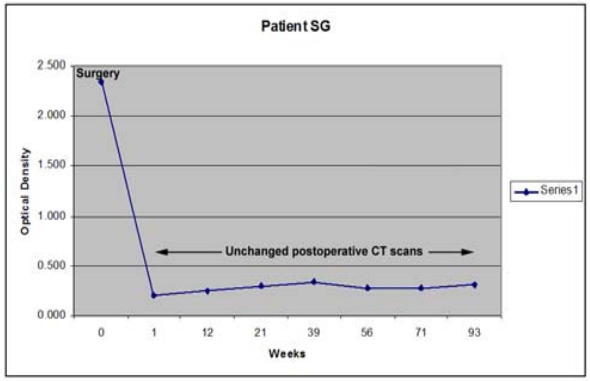
	Sensitivity	Specificity	Best Cut off
Robinson (2003)	84	83	NA
Scherpereel (2006)	80	83	0.93 nm
Present Study	60	89	1.9 nM

FDA and SMRP

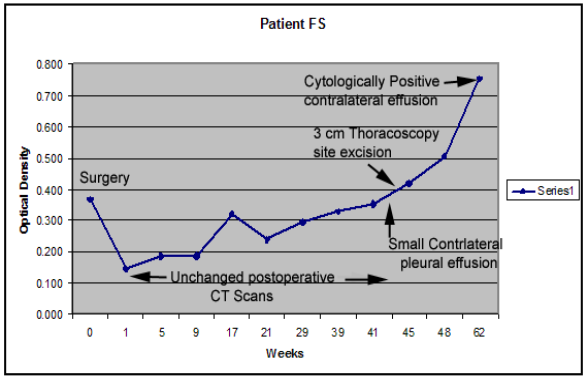
- January 2007: limited indication reference laboratory for the “monitoring” of treatment of mesothelioma

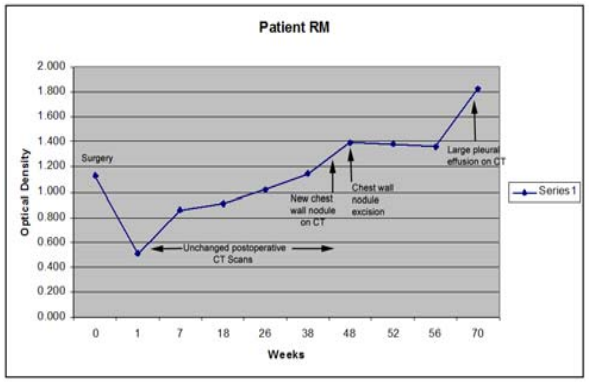
SMRP and Treatment Monitoring



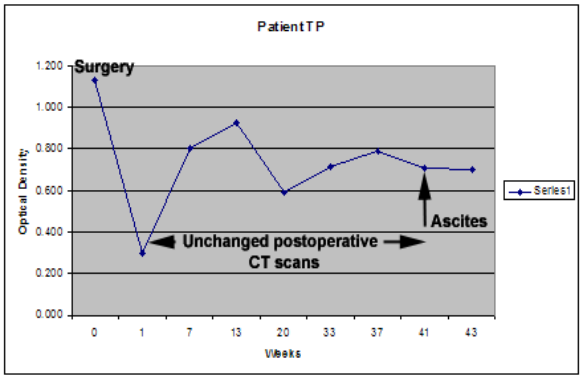


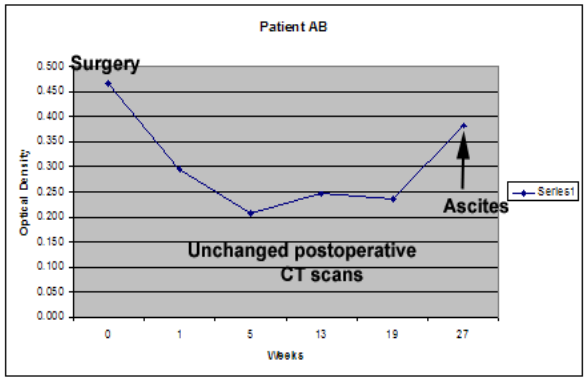
NO RECURRENCE





INTRATHORACIC RECURRENCE





INTRAABDOMINAL RECURRENCE

SMRP Conclusions

- SMRP is a reasonable single marker for mesothelioma
- The exact ranges for asbestos exposed cohorts must be studied in greater numbers of patients and in different geographies
  - ***This should be done in the context of an EDRN validation trial as an initial step***

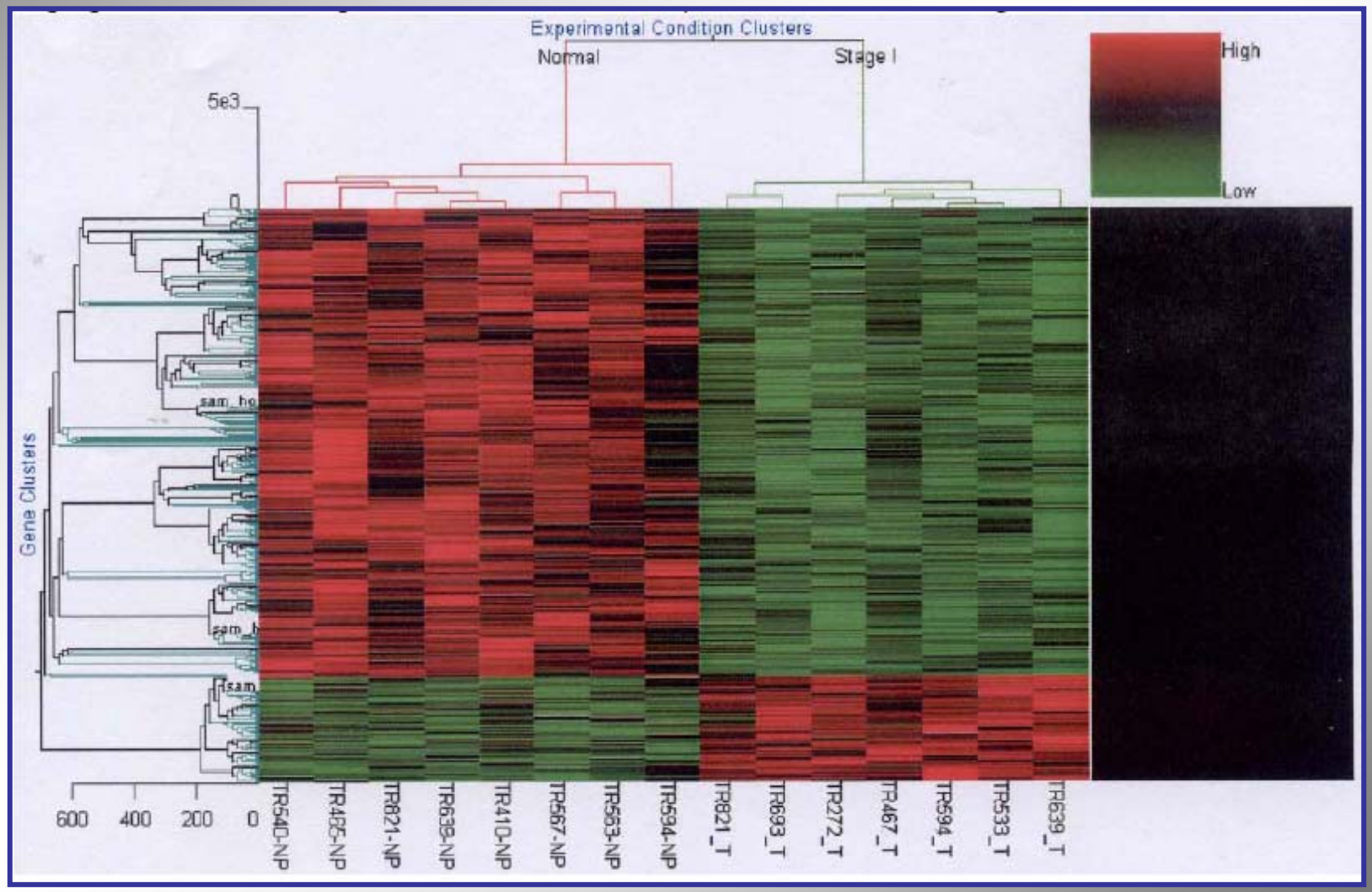
Genomic Discovery of Biomarkers

- **Hypothesis**
  - Affymetrix and Ingenuity Pathway Analyses can predict extracellular/secreted proteins which differ between normal mesothelium and early stage mesothelioma
- **Specific Aims**
  - Discover new markers in serum and plasma
  - Validate these markers using appropriate control cohorts

Methods for Discovery Differences between Normal and Mesothelioma: All Genes

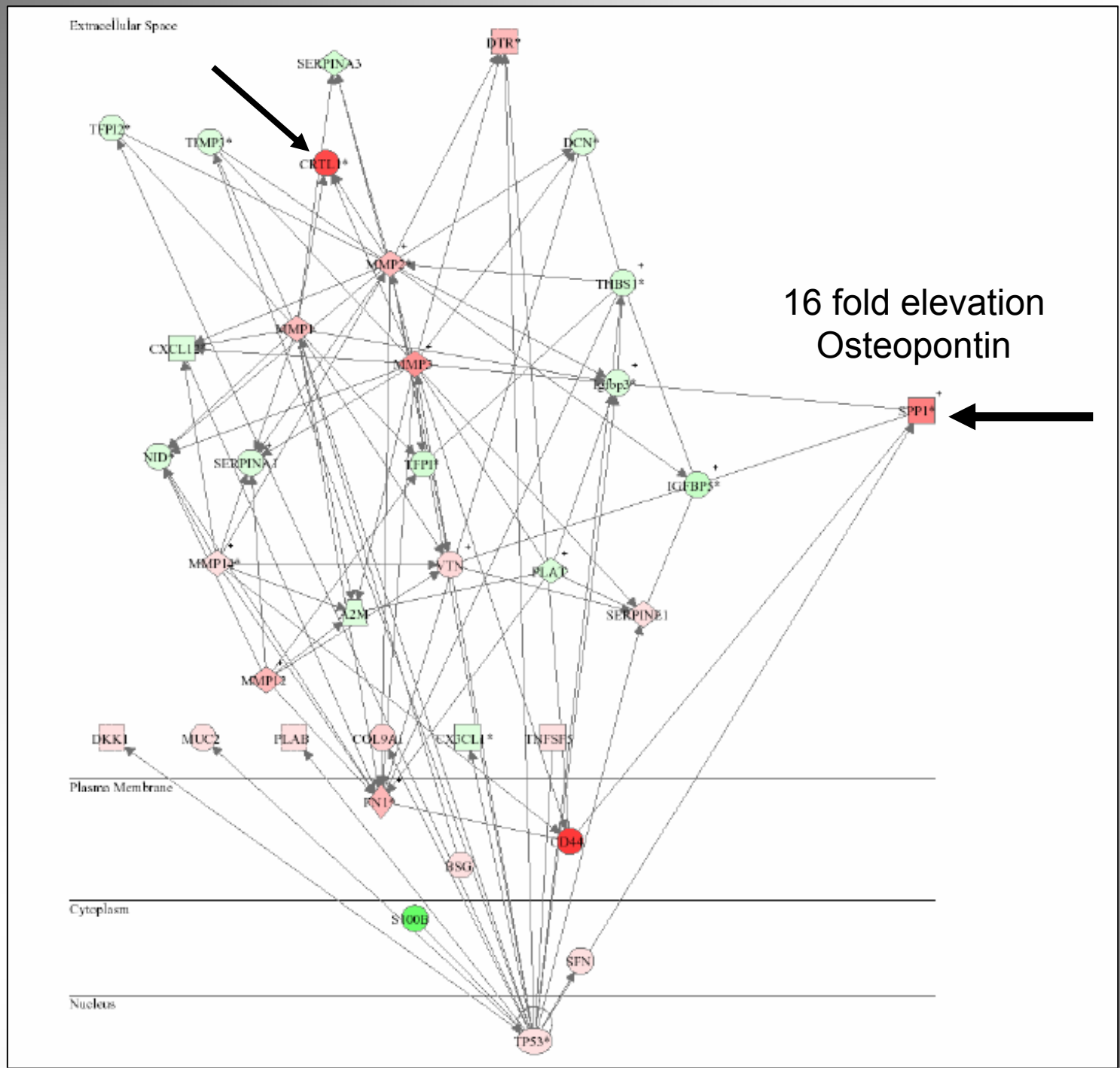
- **Specimens**
  - 8 normal peritoneum
  - 7 Stage 1 mesothelioma
- **Platform**
  - Affymetrix U133Plus
- **Analysis**
  - dCHIP crossed with SAM
    - 453 genes which were significantly different

U133 Plus Unsupervised Clustering: Peritoneum vs Stage I MPM: All Genes Significantly Different

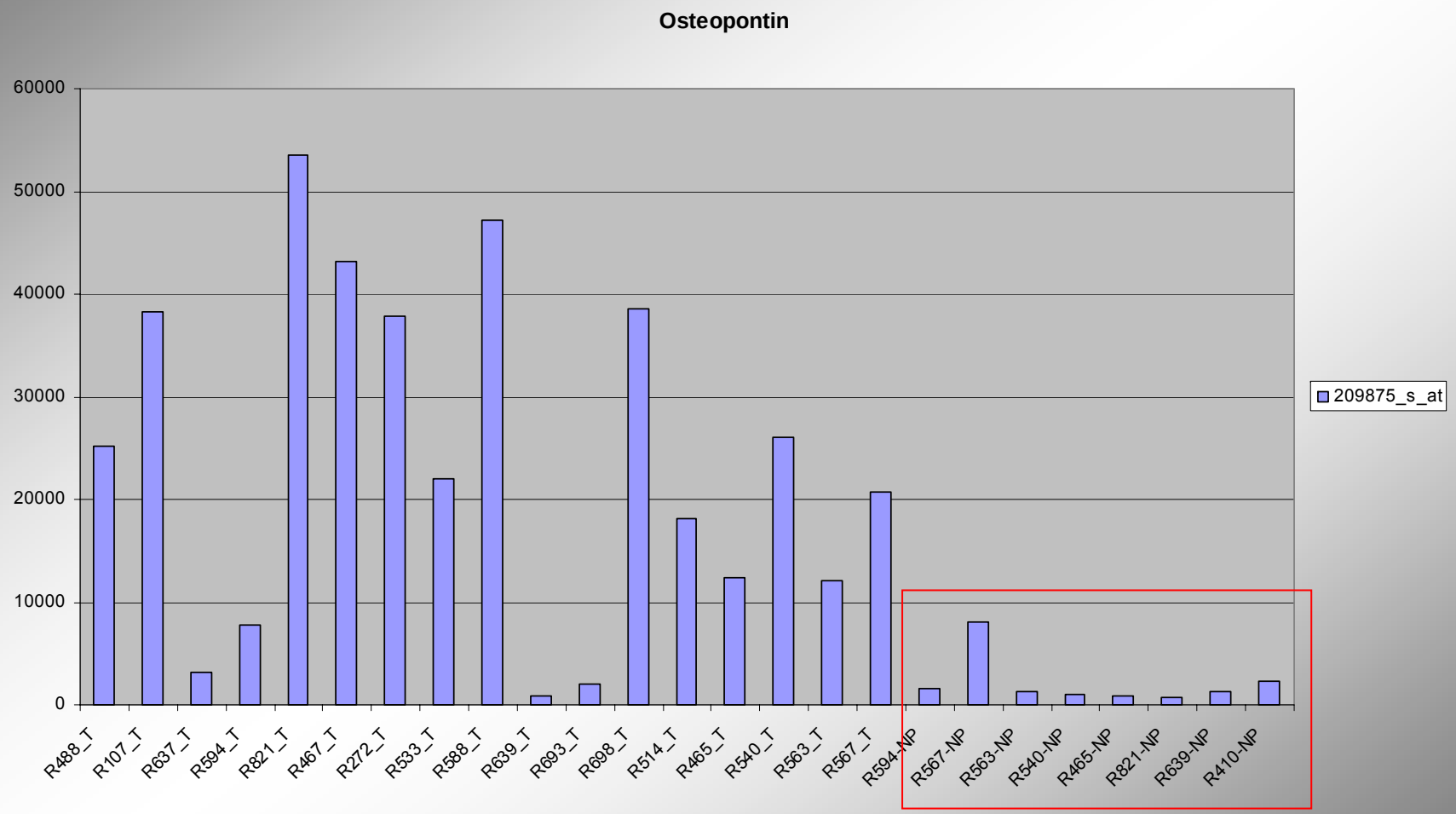


Identification of secreted proteins

- 8 NP and 7 Stage I MPM were then compared for differences in 2036 genes which code for extracellular or secreted proteins (NetAffx™)
- 669 genes were different ($p<0.01$)
- These 669 genes were then inputted into Ingenuity Pathway analyses which selected 330 genes for the analysis.
- **35 focus genes** were chosen for the networks


Extracellular Space
SERPINA3
DTR*
TFPI2*
TIMP3*
CRTL1*
DCN*
MMP2*
THBS1*
MMP1
CXCL12*
MMP3
Igfbp3*
SPP1*
NID1*
SERPINA1
TFPI1*
IGFBP5*
MMP13*
VTN
PLAT
A2M
SERPINE1
MMP12
DKK1
MUC2
PLAB
COL9A1
CX3CL1*
TNFSF5
Plasma Membrane
FN1*
CD44
BSG
Cytoplasm
S100B
SFN
Nucleus
TP53*
16 fold elevation
Osteopontin

Actual Expression for OPN in MPM

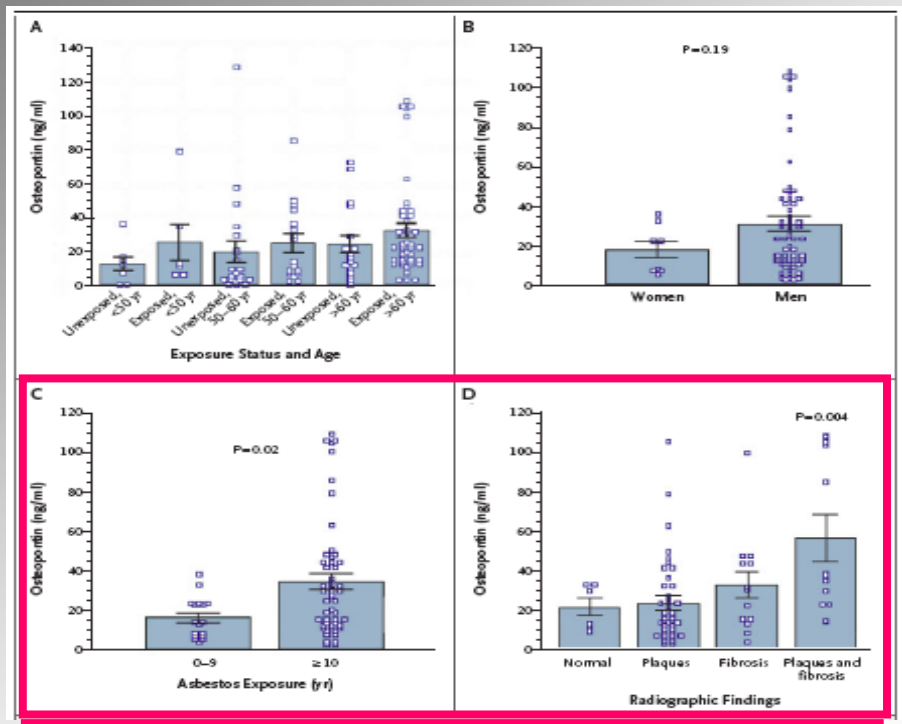


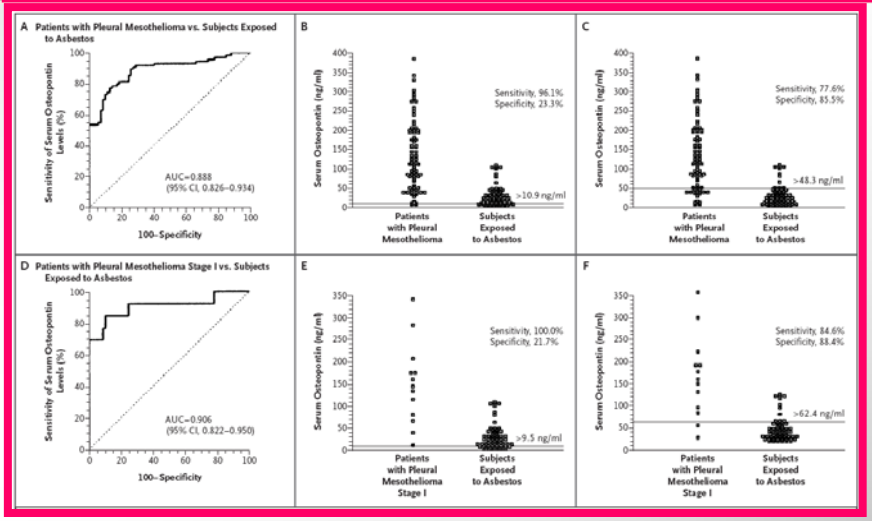
Osteopontin Levels and Environmental Cancers: Test Populations

- 48 normal sera
- 66 asbestos-exposed
- 72 mesothelioma sera

What happens to Osteopontin in Asbestos Exposed Individuals?

Published Data: Serum OPN and MPM





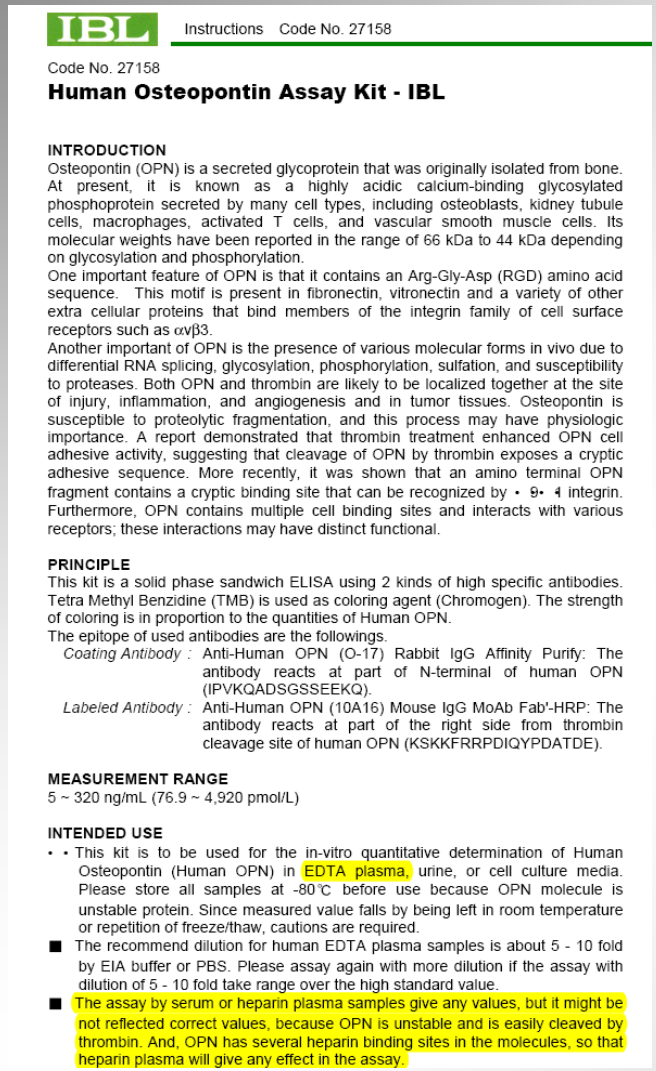
- **Serum OPN rises with duration of exposure and severity of radiographic asbestos changes**
- **Promising distinction between asbestos exposed individuals and mesotheliomas**

Pass H, Lott D, Lonardo F, et al:
Asbestos Exposure, Pleural Mesothelioma, and Serum Osteopontin Levels. NEJM 2005:353;1564-1573

Osteopontin New Initiatives

- **Is this reproducible in plasma?**
- **Can you distinguish MPM from lung cancer?**

Why Plasma?



IBL Instructions Code No. 27158

Code No. 27158

Human Osteopontin Assay Kit - IBL

INTRODUCTION

Osteopontin (OPN) is a secreted glycoprotein that was originally isolated from bone. At present, it is known as a highly acidic calcium-binding glycosylated phosphoprotein secreted by many cell types, including osteoblasts, kidney tubule cells, macrophages, activated T cells, and vascular smooth muscle cells. Its molecular weights have been reported in the range of 66 kDa to 44 kDa depending on glycosylation and phosphorylation.

One important feature of OPN is that it contains an Arg-Gly-Asp (RGD) amino acid sequence. This motif is present in fibronectin, vitronectin and a variety of other extra cellular proteins that bind members of the integrin family of cell surface receptors such as αvβ3.

Another important of OPN is the presence of various molecular forms in vivo due to differential RNA splicing, glycosylation, phosphorylation, sulfation, and susceptibility to proteases. Both OPN and thrombin are likely to be localized together at the site of injury, inflammation, and angiogenesis and in tumor tissues. Osteopontin is susceptible to proteolytic fragmentation, and this process may have physiologic importance. A report demonstrated that thrombin treatment enhanced OPN cell adhesive activity, suggesting that cleavage of OPN by thrombin exposes a cryptic adhesive sequence. More recently, it was shown that an amino terminal OPN fragment contains a cryptic binding site that can be recognized by • 9• 4 integrin. Furthermore, OPN contains multiple cell binding sites and interacts with various receptors; these interactions may have distinct functional.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of Human OPN.

The epitope of used antibodies are the followings.

Coating Antibody : Anti-Human OPN (O-17) Rabbit IgG Affinity Purify: The antibody reacts at part of N-terminal of human OPN (IPVKQADSGSSEEKQ).

Labeled Antibody : Anti-Human OPN (10A16) Mouse IgG MoAb Fab'-HRP: The antibody reacts at part of the right side from thrombin cleavage site of human OPN (KSKKFRRPDIQYPDATDE).

MEASUREMENT RANGE

5 ~ 320 ng/mL (76.9 ~ 4,920 pmol/L)

INTENDED USE

• • This kit is to be used for the in-vitro quantitative determination of Human Osteopontin (Human OPN) in EDTA plasma, urine, or cell culture media. Please store all samples at -80℃ before use because OPN molecule is unstable protein. Since measured value falls by being left in room temperature or repetition of freeze/thaw, cautions are required.

■ The recommend dilution for human EDTA plasma samples is about 5 - 10 fold by EIA buffer or PBS. Please assay again with more dilution if the assay with dilution of 5 - 10 fold take range over the high standard value.

■ The assay by serum or heparin plasma samples give any values, but it might be not reflected correct values, because OPN is unstable and is easily cleaved by thrombin. And, OPN has several heparin binding sites in the molecules, so that heparin plasma will give any effect in the assay.

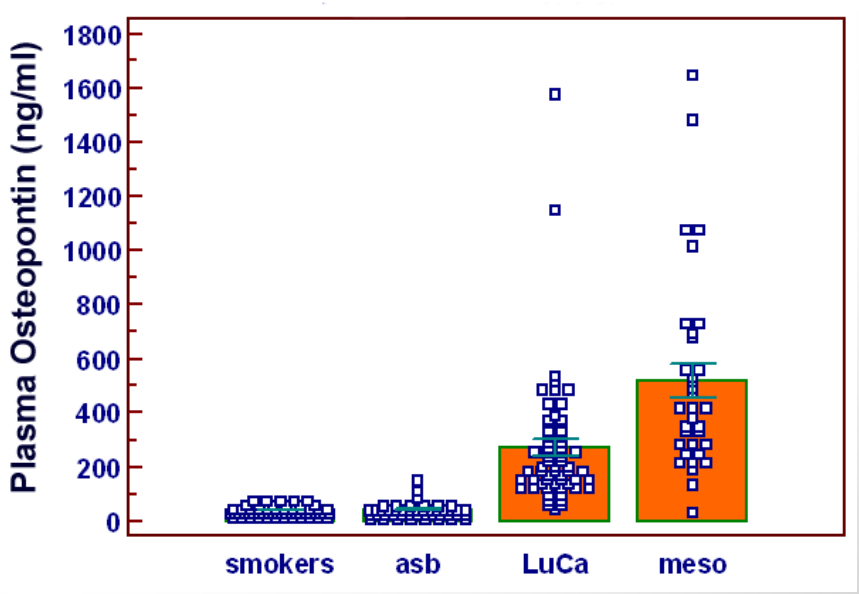
- **Serum worked *but could be erroneous*.**
- **Follow-up series of investigations to**
  - **test plasma osteopontin as a biomarker (34)**
  - **Measure levels in asbestos exposed (45), lung cancer (60), and smokers with dysplasia (56)**

Plasma Osteopontin Levels: Thoracic Malignancies And Controls

	Smokers (n=56)	Asbestos (n=45)	Lung Cancer (n=60)	Meso (n=34)
Mean OPN (ng/ml)	40±2 (10-84)	42±4 (8-148)	271±31 (45-1575)	520±63 (33-1645)

NS

P<0.0001

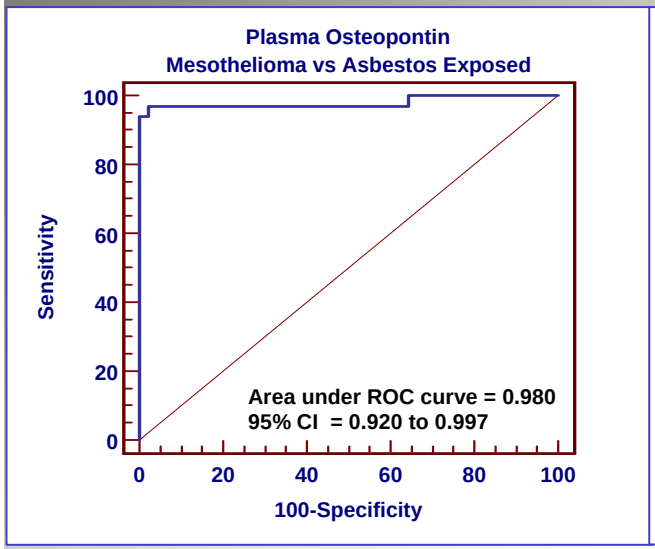


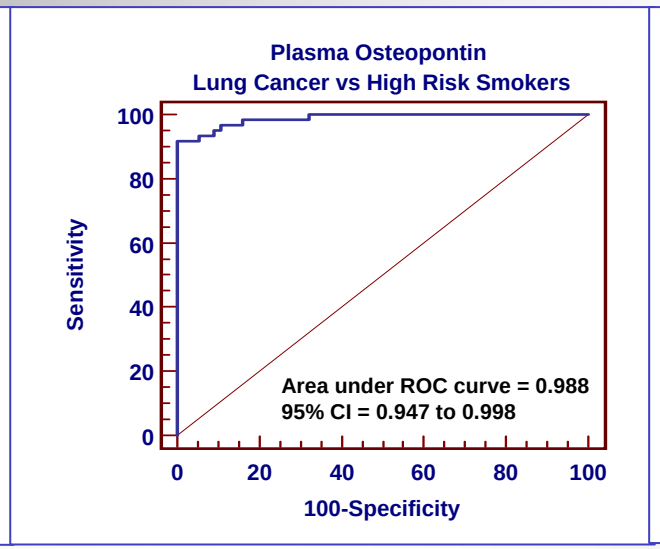
P<0.0001

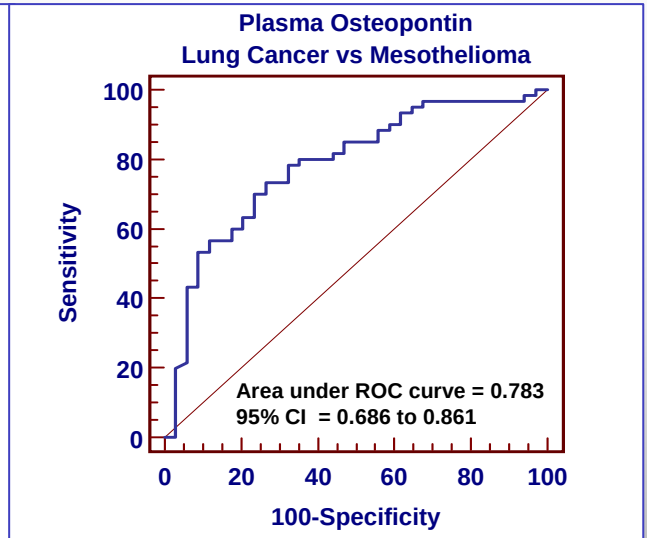
P<0.0001

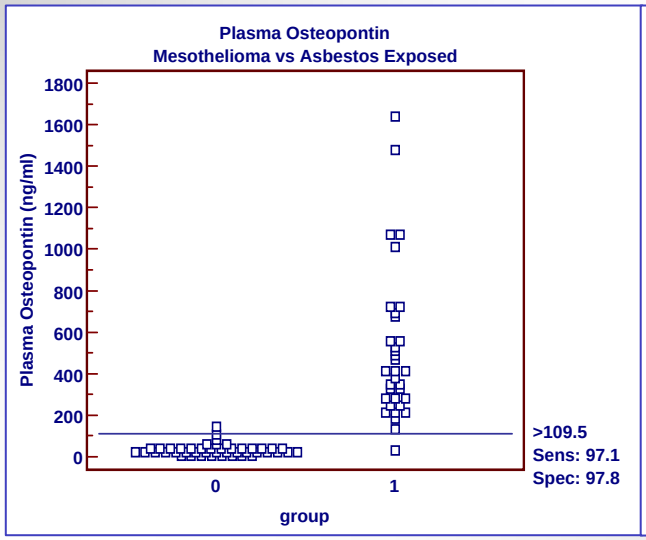
Plasma osteopontin looks as promising as serum osteopontin for MPM

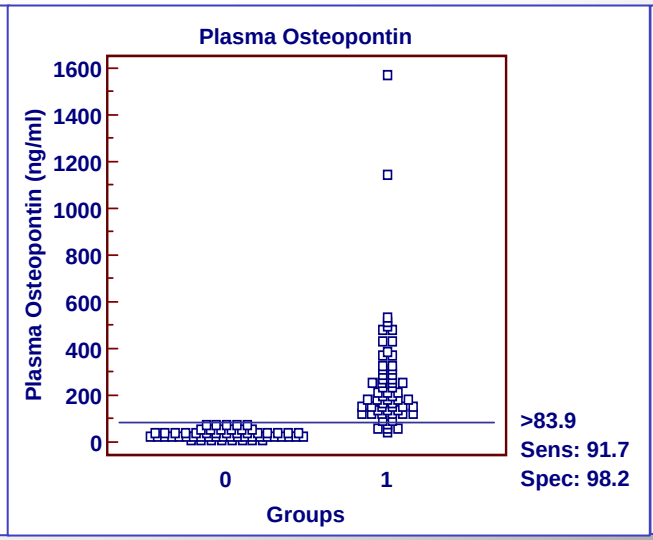
ROC Curves: Tumors vs Controls: Tumors vs Tumors

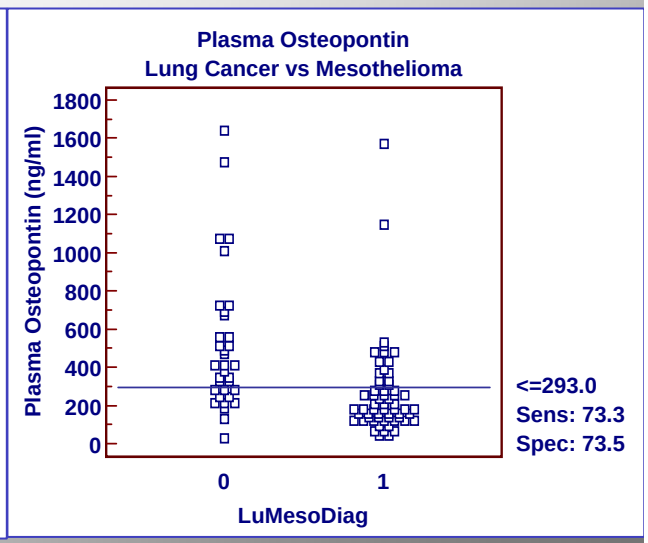








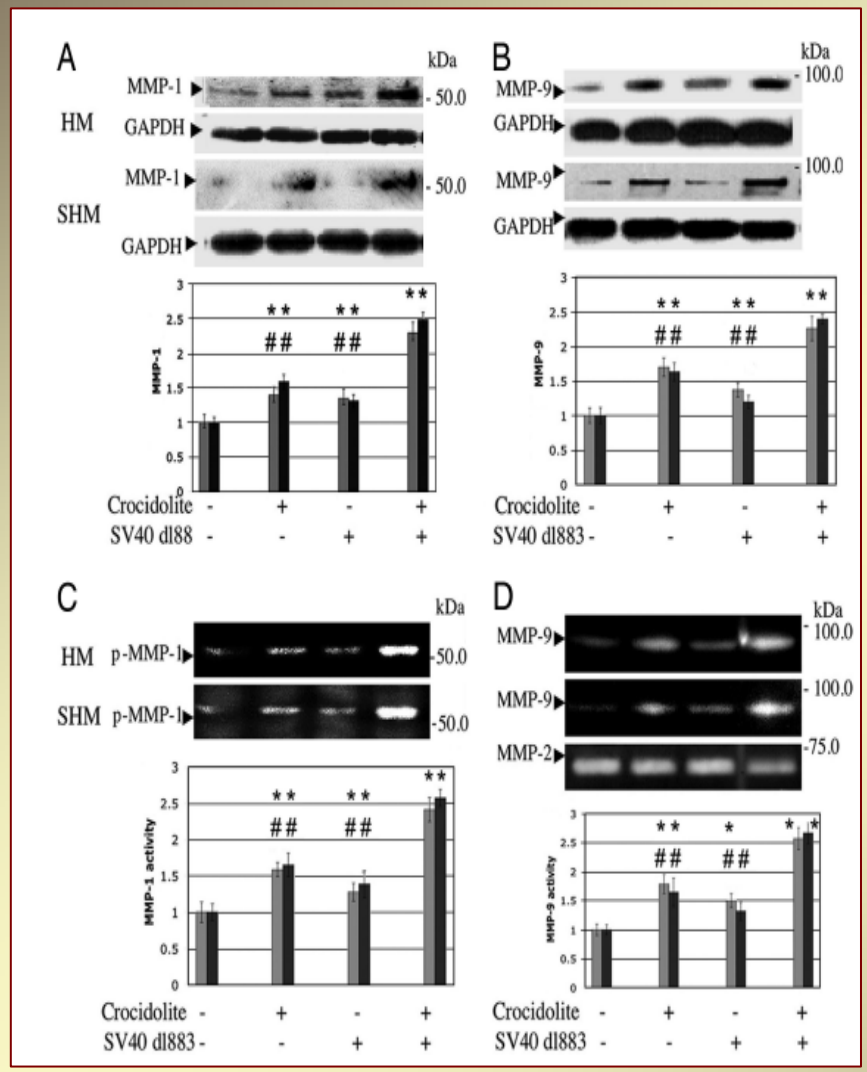


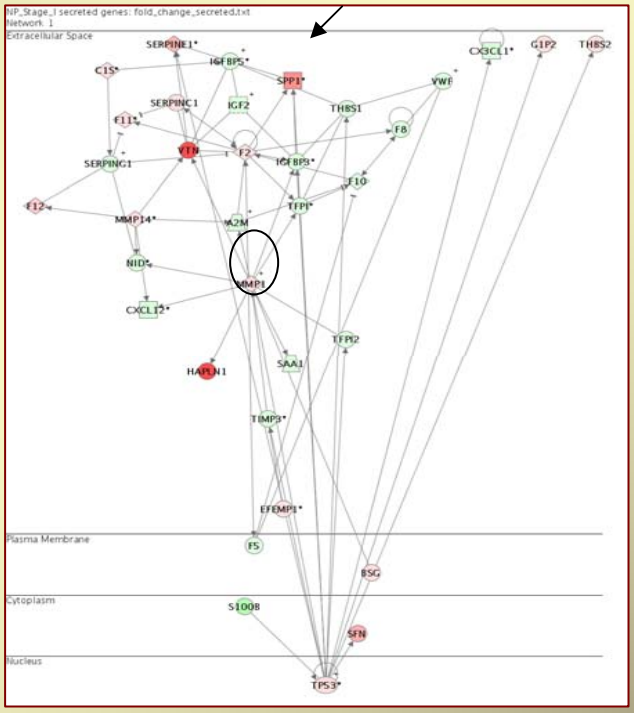


Osteopontin Conclusions

- Both serum and plasma osteopontin are elevated in MPM compared to high risk asbestos controls
- Plasma Osteopontin levels are also elevated in Lung Cancer and could be confused with MPM
  - *Need other markers to distinguish between the two*
- The exact ranges for asbestos exposed cohorts must be studied in greater numbers of patients and in different geographies
  - ***This should be done in the context of an EDRN validation trial as an initial step***

What about other markers?
MMP1 and MMP9





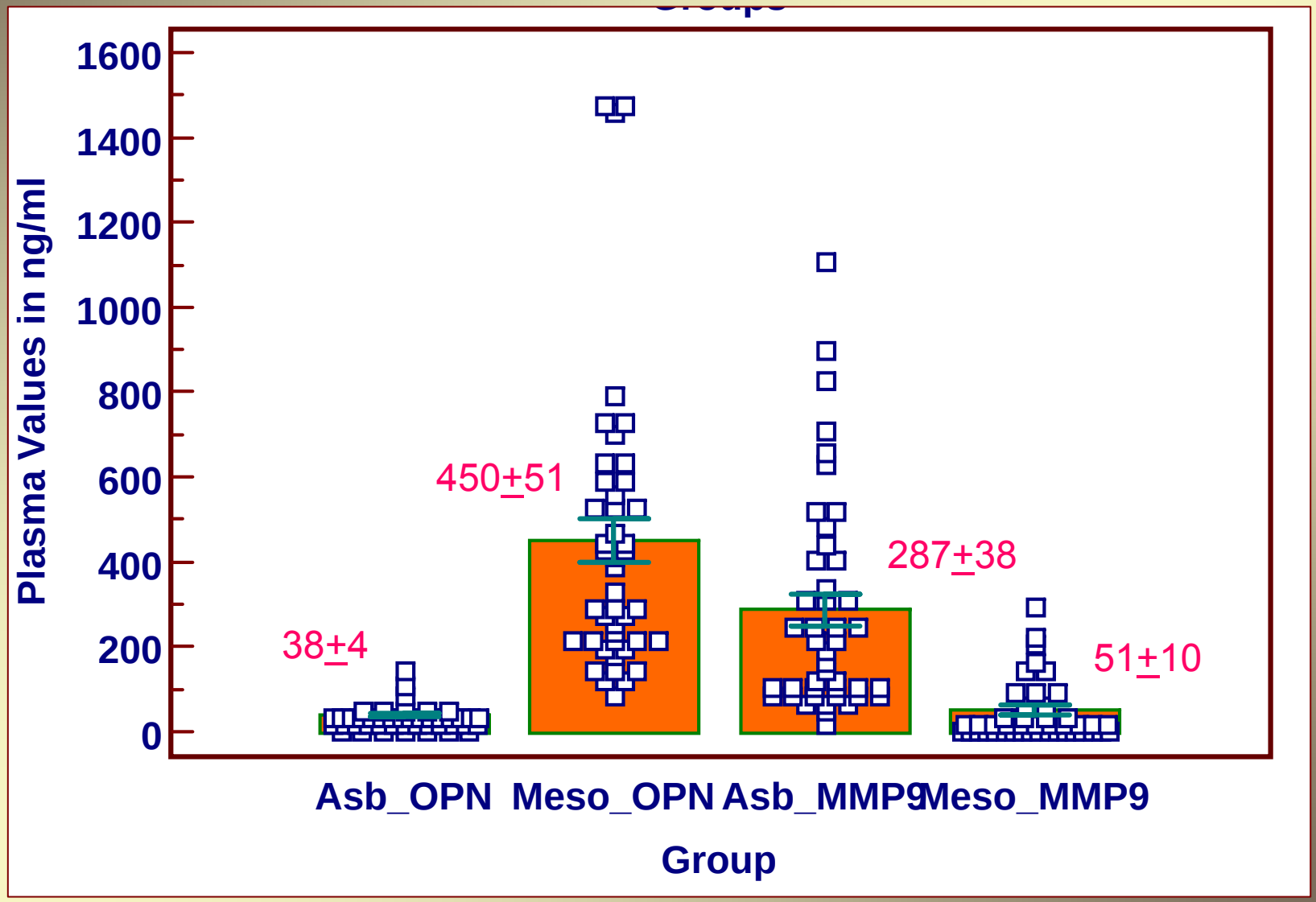
Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters

Barbara Kroczynska*, Rochelle Cutrone*, Maurizio Bocchetta*, Haining Yang*, Amira G. Elmishad*, Pamela Vacek†, Maria Ramos-Nino‡, Brooke T. Mossman‡, Harvey I. Pass§, and Michele Carbone*¶

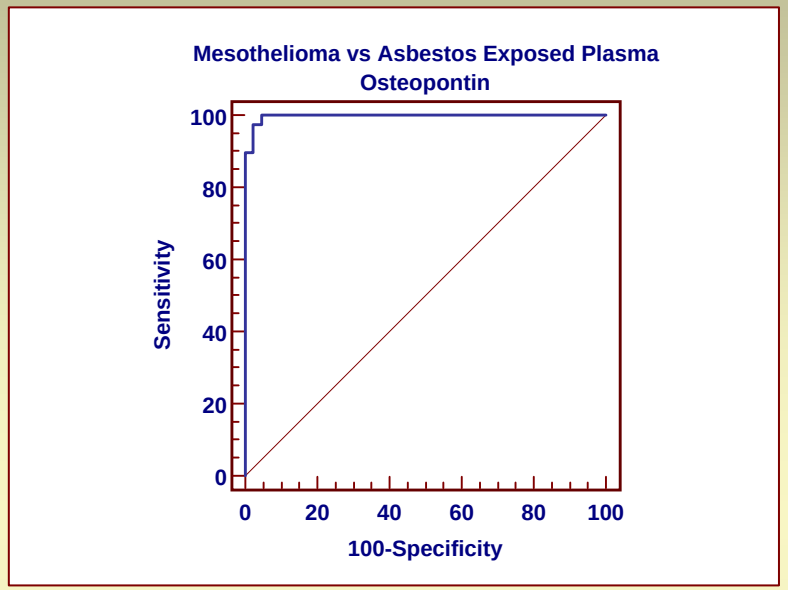
*Thoracic Oncology Program, Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL 60153; Departments of †Medical Biostatistics and ‡Pathology, College of Medicine, University of Vermont, Burlington, VT 05404; and §Department of Thoracic Surgery, New York University, New York, NY 10016

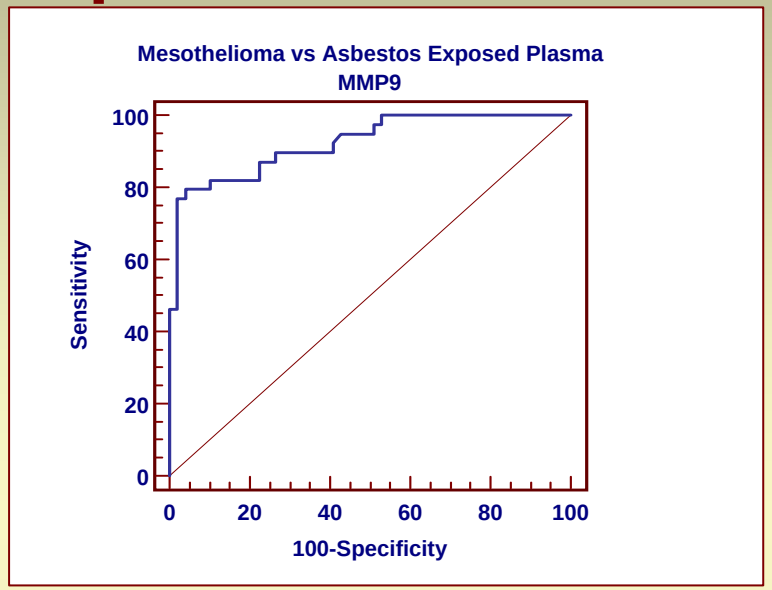
Edited by Baruch S. Blumberg, Fox Chase Cancer Center, Philadelphia, PA, and approved July 21, 2006 (received for review June 5, 2006)

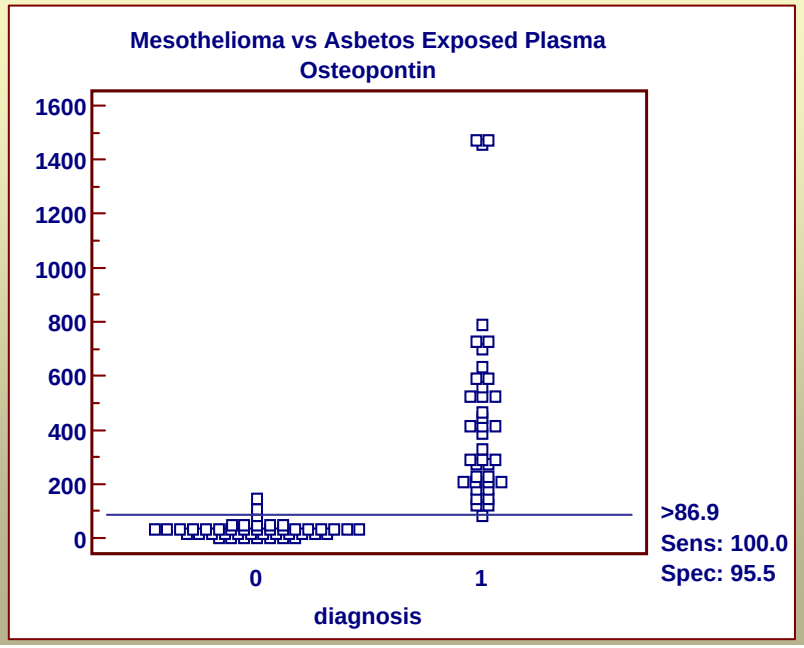
Plasma OPN and MMP9 in mesotheliomas and asbestos exposed cohorts

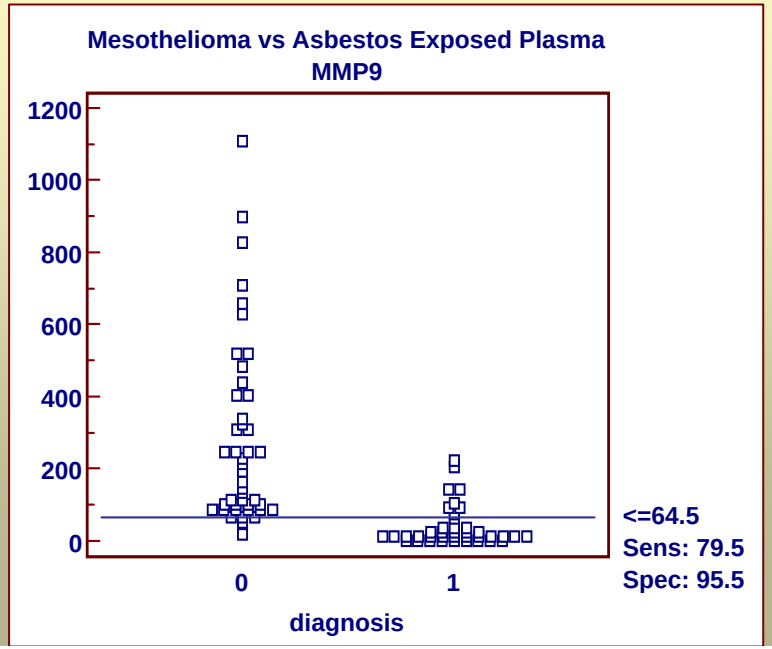


MMP9 and OPN: MPM and Asbestos Exposed Cohorts Matched Plasma Specimens

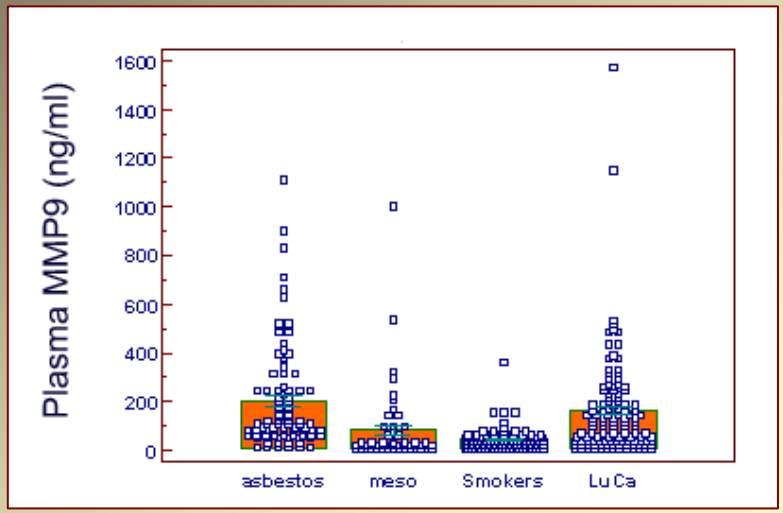


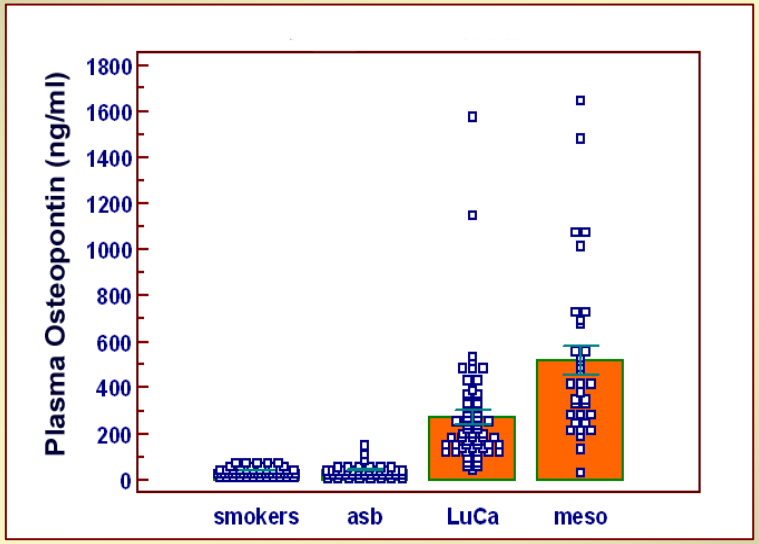






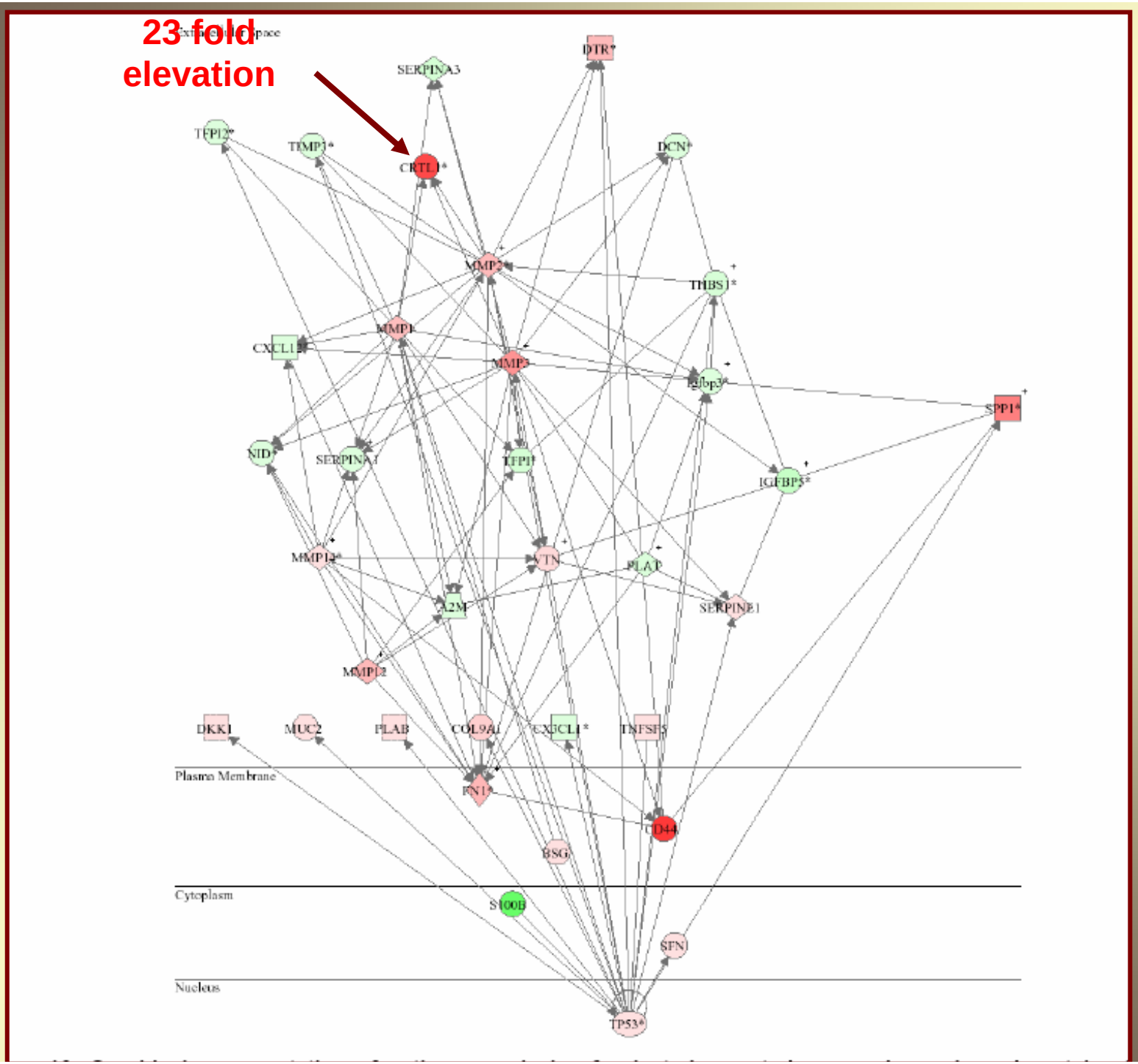
Moreover, MMP9 is elevated in lung cancer



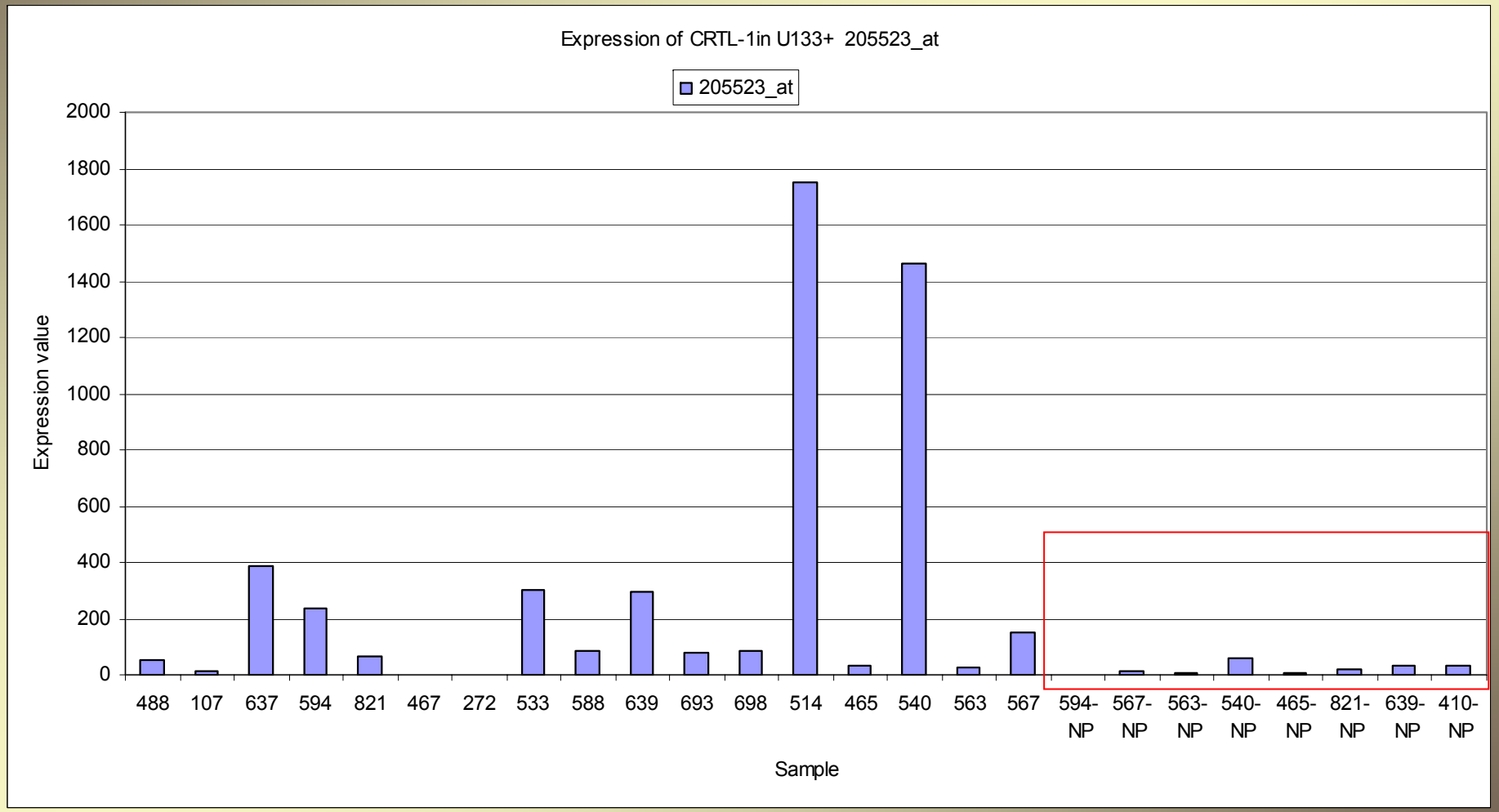


- MMP9 is NOT elevated in MPM
- MMP9 IS elevated in lung cancer
  - **Possible better discrimination between the two by combining with osteopontin?**

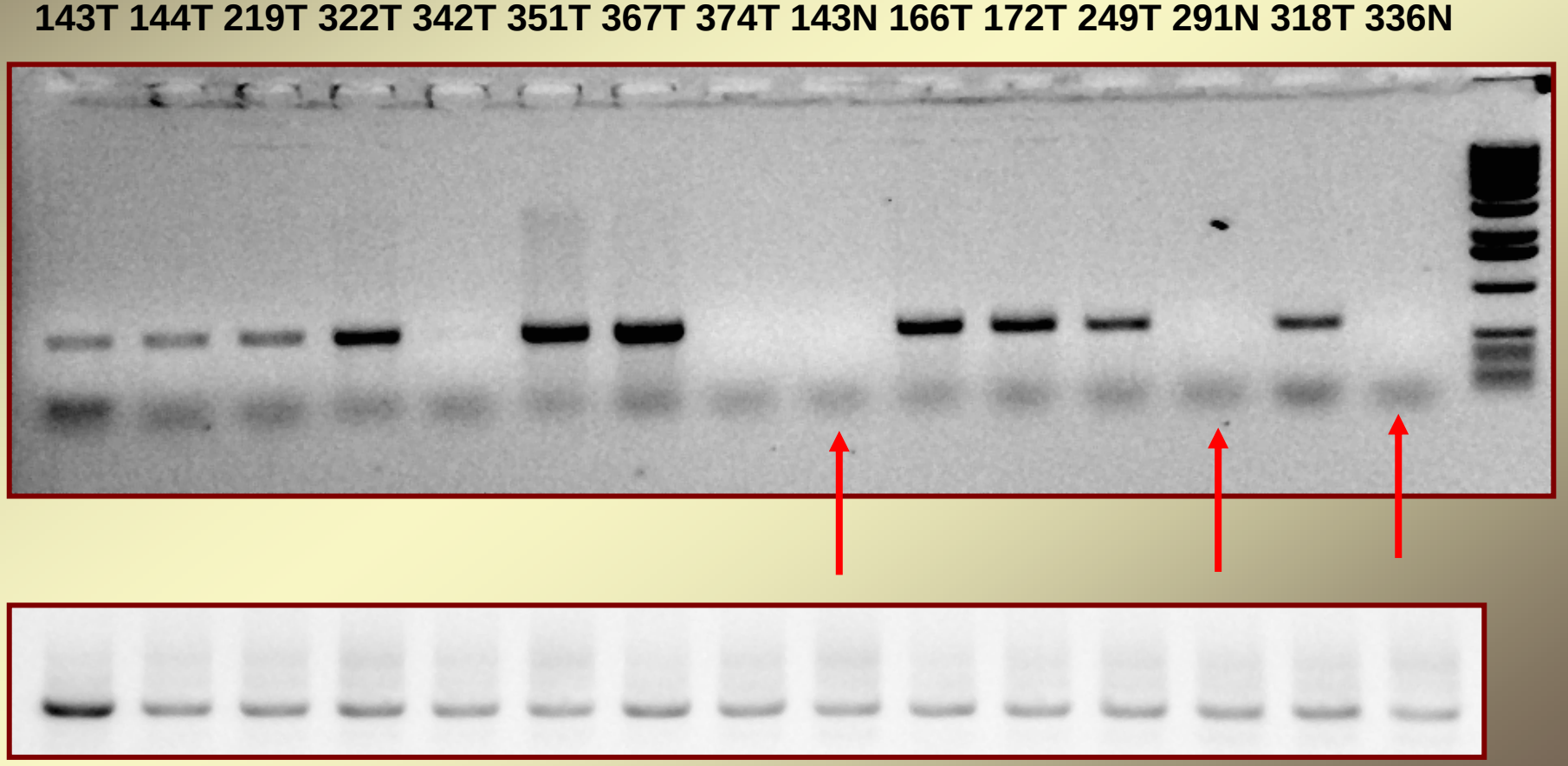
What about other markers?


23-fold elevation
SERPINA3
ITR*
TFPI2*
TIMP1*
CRTL1*
DCN*
MMP2*
THBS1*
MMP1
CXCL12*
MMP3*
Igfbp3*
SPP1*
NID*
SERPINA1
TFPI*
IGFBP5*
MMP14*
VTN*
PLAT*
A2M*
SERPINE1
MMP12*
DKK1
MUC2
PLAB
COL9A1
CXCL1*
TNFSF5
Plasma Membrane
FN1*
CD44
BSG
Cytoplasm
S100B
SFN
Nucleus
TP53*

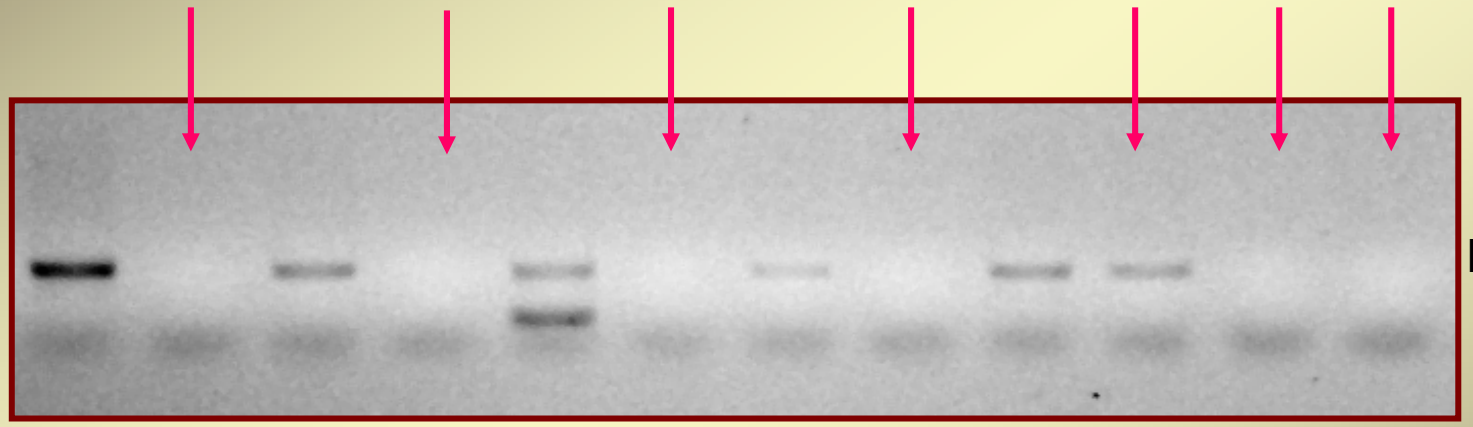
Microarray CRTL1/HAPLN1 expression data in mesothelioma patients



HAPLN1 differential expression in mesothelioma and normal pleura samples (RT-PCR)

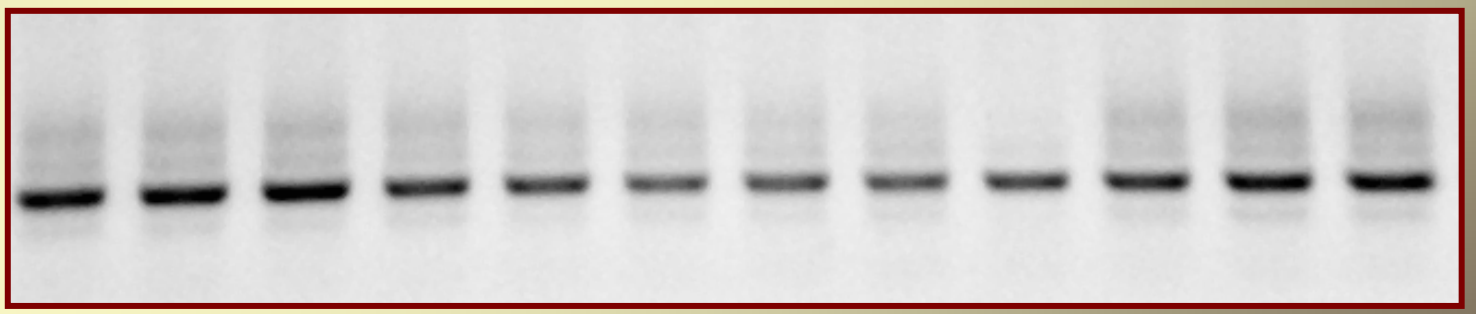


Expression of HAPLN in matched tissues (normal pleura/mesothelioma)



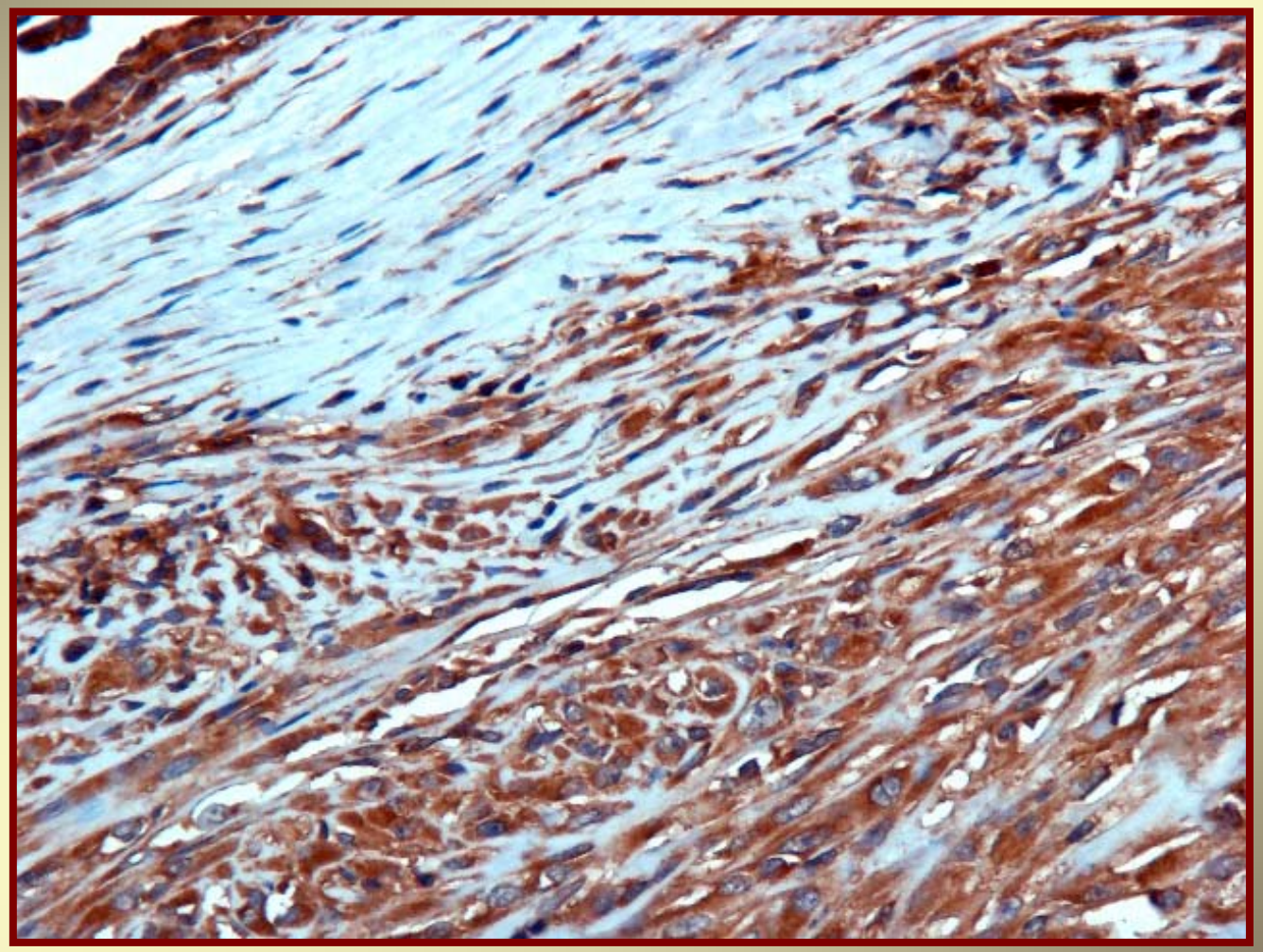
HAPLN1

T	N	T	N	T	N	T	N	T	N	T	N	N	N
978T		997		NYU11		821		930		978		155	128



PPIA

Mesothelioma, HAPLN1 antibodies (Genosis)



Preparations for validation of SMRP, osteopontin

Plans for EDRN Validation

- **Every two week conference calls**
  - Harvey Pass, BDL
  - Mark Thornquist, DMCC
  - Jackie Dahlgreen, DMCC
  - Karl Krueger, NCI
- **Protocol Formulation**
  - Definition of Ranges for Controls
  - ROC vs MPM
  - Retrospective/prospective studies

Validation Trial

- ***Phase I***
  - Identification and assemblage of representative cohorts of individuals
    - with MPM
    - no malignancies but increased risk for MM due to asbestos exposure
    - (optionally) lung malignancies other than MM.

Mt. Sinai Selikoff Foundation

- Nationwide registry of 2900 insulators workers for which data is available up to 1994
  - Approximately 1600 are dead
  - Approximately 120 MPMs developed of which 3/5 were abdominal

Libby Montana

- Vermiculite mining in and near the city of Libby, Montana began in the 1920s and was continued by the W.R. Grace Company from 1963 until 1990. The vermiculite ore mined in Libby was contaminated with ***tremolite asbestos.***
- ***For the 20-year period*** (1979–1998) ***examined, mortality from asbestosis was approximately 40 times higher than the rest of Montana and 60 times higher than the rest of the United States***.
- Pleural abnormalities on chest radiography were seen in 17.8% of participants 6,668 participants 18 years and older and interstitial abnormalities were seen in less than 1% of participants undergoing chest radiography.
- The prevalence of radiographic pleural and interstitial abnormalities was highest in W.R. Grace workers: 51% (186 of 365).
- Of those participants who reported no apparent exposure, 6.7% had pleural abnormalities. Factors most strongly related to having pleural abnormalities were 1) having been a W.R. Grace/ Zonolite worker, 2) having household contact with a W.R. Grace/Zonolite worker, and 3) being a male.

Libby, Montana

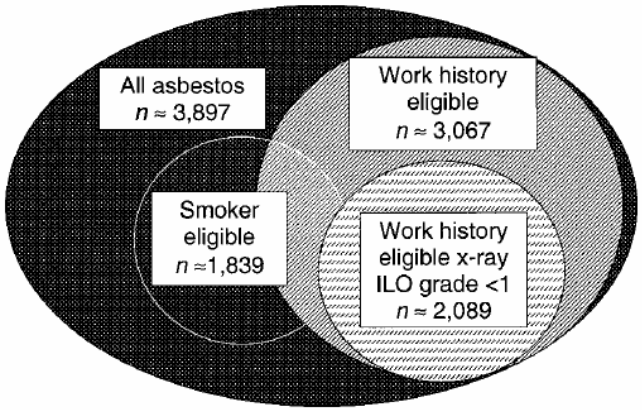
Vermiculite mines

PLCO
(Prostate, Lung, Colon, Ovarian NCI Screening Program)

- 1992-2001 enrollment, Screening until 2007
- CXR vs no CXR
  - Current, former, or never smokers
  - Minimal occupational demographics available
- 21 mesotheliomas were diagnosed

CARET

- CARET
  - multicenter randomized, doubleblinded, placebo-controlled trial examining vitamin A and β-carotene in preventing lung cancer
- Asbestos exposed cohort followed 9-17 years
- CXR, PFTs, sera at baseline



- 47 mesotheliomas developed
  - 38 asbestos arm
  - 9 smoking arm
  - 6 with serum before and after diagnosis
  - 11 with serum less than one year prior to diagnosis

Validation Trial

- ***Phase 2***
  - determine what the characteristics of markers in the screening population, which will include mesothelioma cases and asbestos-exposed controls.

Validation Trial

- ***Phase 2a***
  - the cut point between what the marker says is positive and negative will be established.
    - the distribution of SMRP and Osteopontin in controls will be reviewed for geographic differences and cohort differences (i.e. Libby vs Caret vs Selikoff vs New York Rom Cohort)

Validation Trial

- ***Phase 2b***,
  - current cases will be examined to see what the sensitivity is to draw ROC curves
  - Important to obtain surgical cases in order to draw ROC curves for early (i.e. Stage I) cases

Validation Trial

- ***Phase 2c***
  - "peri-mesothelioma" cases from the CARET and the PLCO trials will be examined for temporally related changes in the markers

Cohort Mobilization

Cohort	MPM Serum	MPM Plasma	Lung Cancer Sera	Lung Cancer Plasma	Asbestos Controls Sera	Asbestos Controls Plasma	Notes
Pass Archives Pre NYU	98	20	Published	Published	Published	Published	1990-2005
NYU Archives	7	7	100	100	0	0	2006-
Rom CVEC	0	0	160	160	300	300	2003-
Sinai Selikoff	56*	0	0	0	1769	0	1981-1982
Libby, Montana	0	0	0	0	300	0	2005-
PLCO	21&	21	0	0	0	0	***Serial draws***, not all with sera at the time of dx
CARET	47&	47	0	0	3,897	3,897	***Serial draws***, not all with sera at the time of dx
Wittenoon, Australia	50	0	0	0	200	0	

*sera not drawn at time of diagnosis

&"peri-mesothelioma bloods"

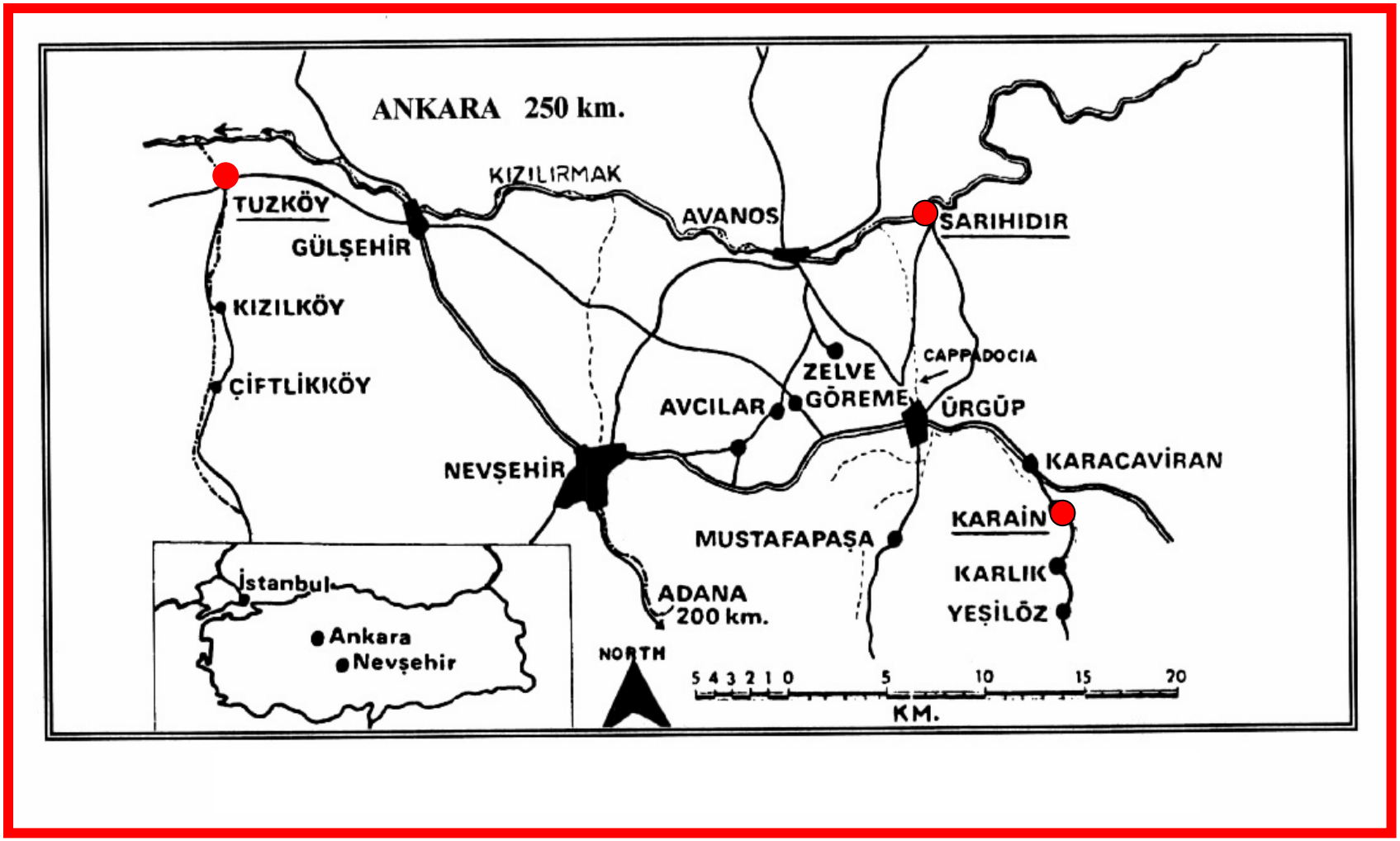
Prospective Validation

- **Cappadochia**
- **New York Asbestos Screening Protocol**
  - Philanthropy
  - Combined with Low Dose Helical CT
  - Defined exposure and age for enrollment
  - Combine with action taken on marker elevation at prevalence scan or rising marker at 6 month intervals

SMRP and Osteopontin

- **Cappadocia**
  - Very important PROSPECTIVE opportunity
- **Collaboration with Michele Carbone MD, PhD, University of Hawaii**
  - Mesothelioma Pathogenesis PO1
    - No funds for biomarker development

• **In the Cappadocian region of Central Anatolia, three villages, Karain, Tuzköy, and Sarihidir, with environmental exposure to erionite are known as "Erionite villages"**



• **Map of Cappadocia region showing the Erionite villages of Karain, Tuzköy and Sarihidir.**

MPM in Cappadocia- Mortality Studies

TABLE 1. Mortality Data of Karain Village between January 1970 and July 1994.

	Cases		Age		M/F
	No.	%	Mean	Range	Ratio
Total deaths	305	100	54.1	1–90	160/145
Deaths due to malignancies	177	58	51.1	18–89	89/88
MPM[a]	150	49.2	50.6	27–89	76/74
MPEM[b]	7	2.3	60.8	48–76	3/4
Gastroesophageal	6	1.9	54.3	46–61	3/3
Lung	4	1.3	43.0	40–46	2/2
Leukemia	3	1.0	30.3	18–53	2/1
Intraabdominal	1	0.3	56		0/1
Head and neck	1	0.3	48		1/0
Skin	1	0.3	77		1/0
Prostate	1	0.3	65		1/–
Endometrium	1	0.3	62		–/1
Ovary	1	0.3	59		–/1
Unknown primary	1	0.3	59		0/1
Other causes of death	128	42	58.8	1–90	71/57

[a] Malignant pleural mesothelioma.
[b] Malignant peritoneal mesothelioma.

TABLE 2. Mortality Data of Tuzköy Village between January 1980 and July 1994.

	Cases		Age		M/F
	No.	%	Mean	Range	Ratio
Total deaths	432	100	52.4	1–90	235/197
Deaths due to malignancies	225	52.1	50.8	15–75	118/107
MPM[a]	105	24.3	49.2	26–75	54/51
MPEM[b]	60	13.9	54.0	30–75	22/38
Intraabdominal	29	6.7	52.5	15–75	19/10
Lung	6	1.4	51.0	38–61	4/2
Gastroesophageal	4	0.9	54.0	35–65	3/1
Leukemia	4	0.9	29.5	18–41	3/1
Colorectal	3	0.7	57.3	41–67	2/1
Mesenchymal	3	0.7	40.7	31–60	3/0
Brain	3	0.7	54.3	36–70	3/0
Skin	2	0.5	55	40–70	2/0
Breast	2	0.5	52	49–55	0/2
Lymphoma	1	0.5	38		1/0
Head and neck	1	0.2	40		1/0
Thyroid	1	0.5	73		0/1
Other causes of death	207	47.9	48.3	1–90	117/90

[a] Malignant pleural mesothelioma.
[b] Malignant peritoneal mesothelioma.

- **Up to 52% of deaths in Karain between 1970 and 1994, and 38% of deaths in Tuzköy between 1980 and 1994 were due to malignant pleural or peritoneal mesothelioma. Periotoneal mesotheliomas were more prevalent in Tuzköy (1).**
- **Besides mesothelioma the incidence of non-mesoteliomal malignancies were found high in erionite villages.**
- **Cancer rates in these villages is about 1000 times more than the normal rate.**

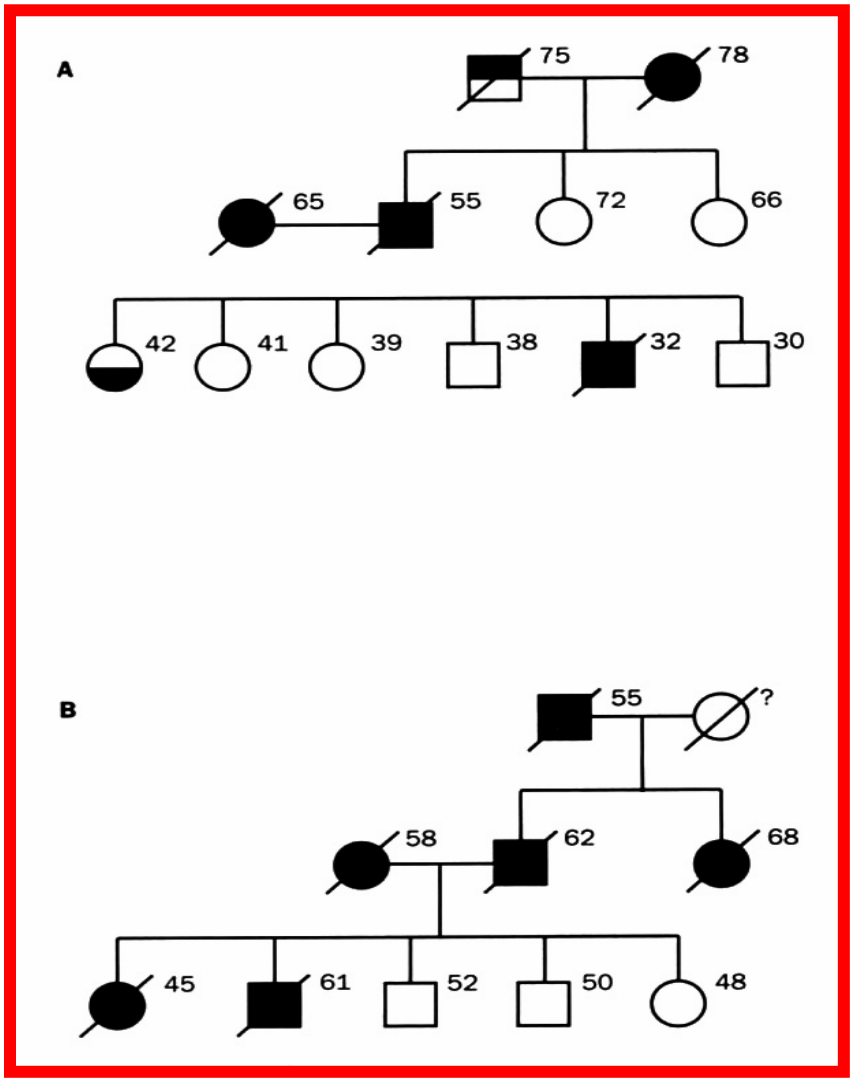
1. Baris B, et al. J Environ Pathol Toxicol Oncol 1996; 15: 183-189.

MPM in Cappadocia- Genetic studies: Genetic mapping study(1)

- **Analysis of a six-generation extended pedigree of 526 individuals showed that predisposition to induced MM was genetically transmitted.**

- **It was suggested that vertical transmission of MM occurs probably in an autosomal dominant way**

- **Studies are in progress to identify the gene(s), which increase(s) the susceptibility to erionite and asbestos.**

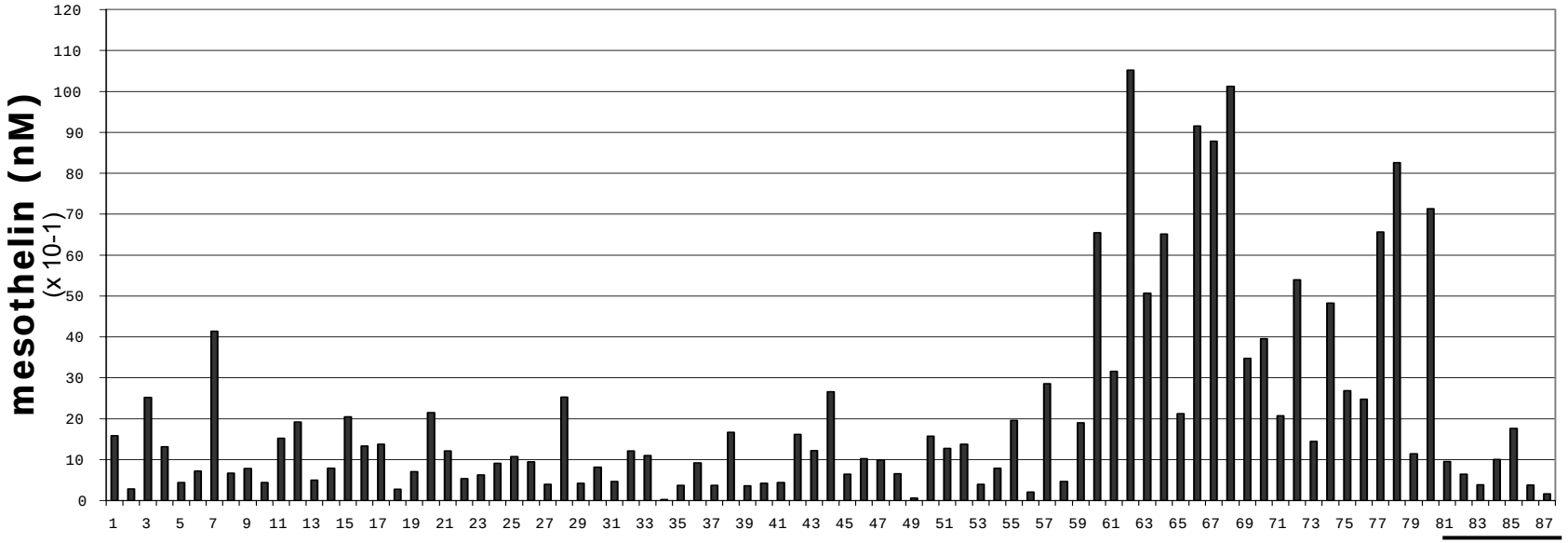


Hammady I-Roushdy, et al.Lancet 2001; 357:444-445.

Cappadocian Studies
March and June 2006

- ***Blood cannot be removed from Turkey***
- Received permission to visit the villages and draw blood
- Laboratory space used at University of Ankara for ELISA reading
- Carbone took SMRP kits from FDI and osteopontin kits from IBL to Ankara

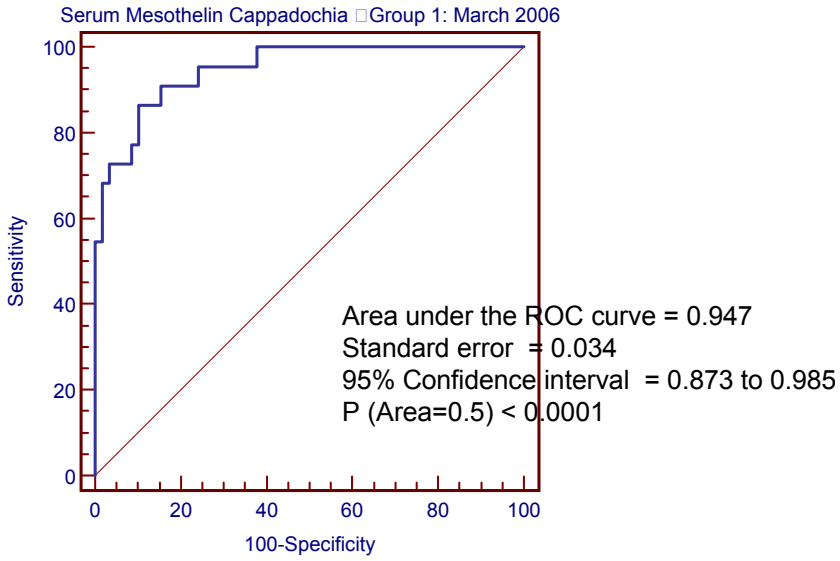
ELISA (March 2006)

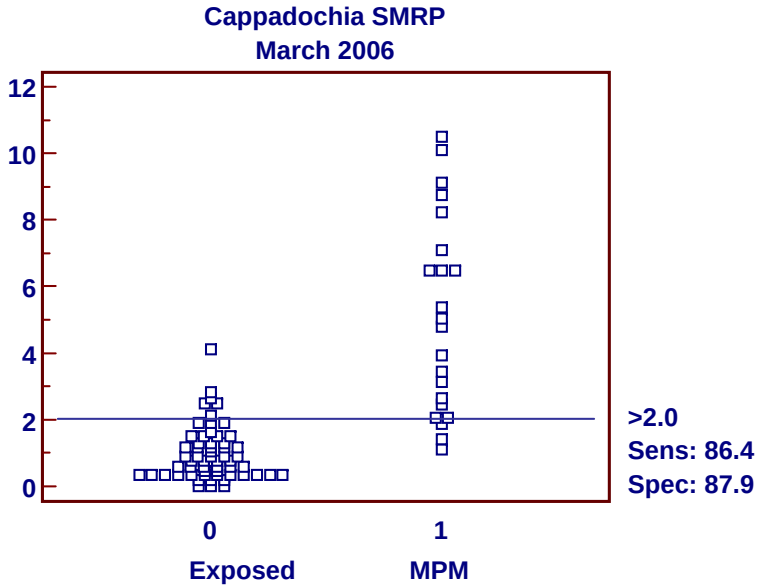


Individuals from erionite villages from Turkey (1-58)

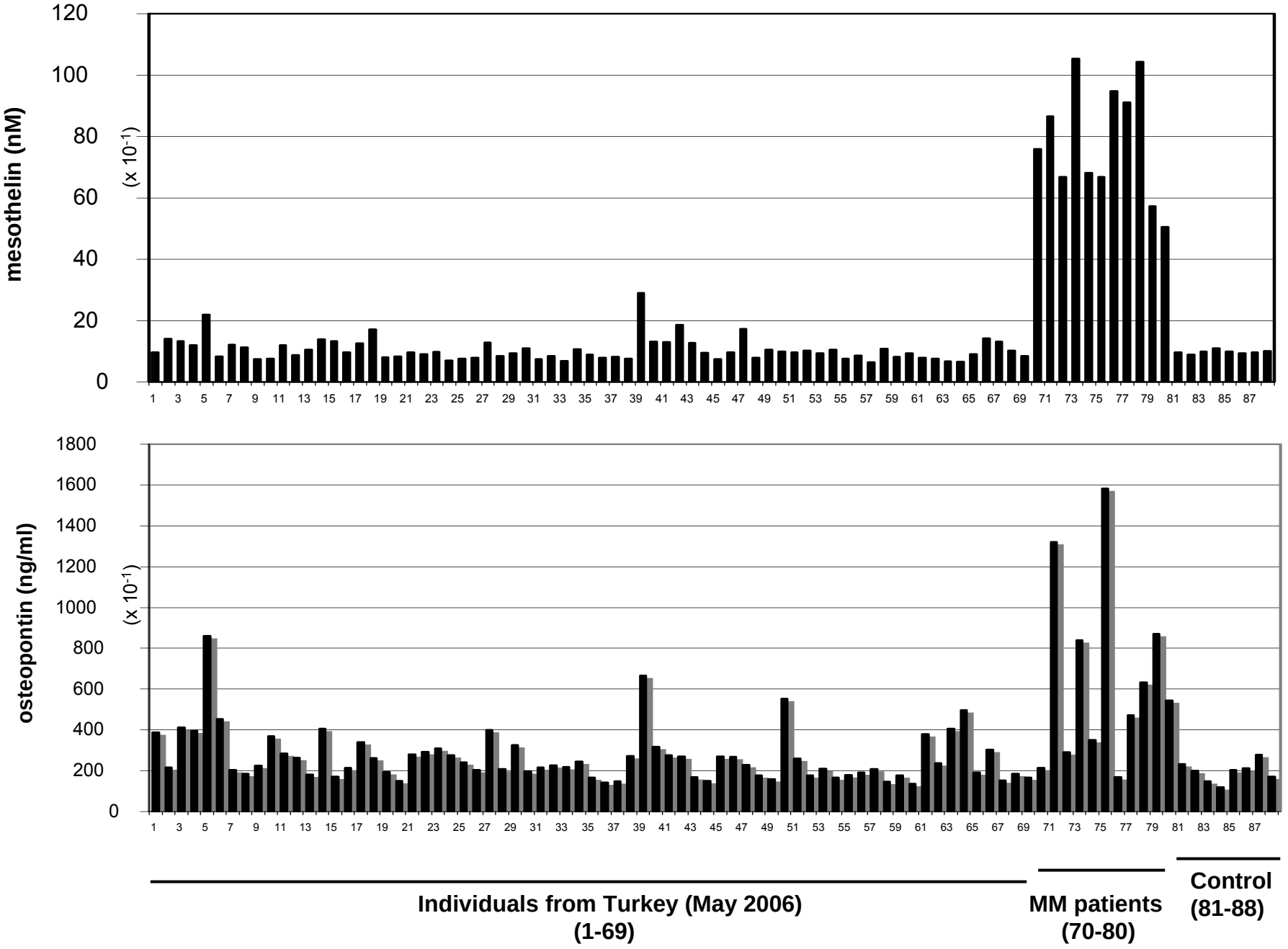
MM (59-80)

control (81-87)

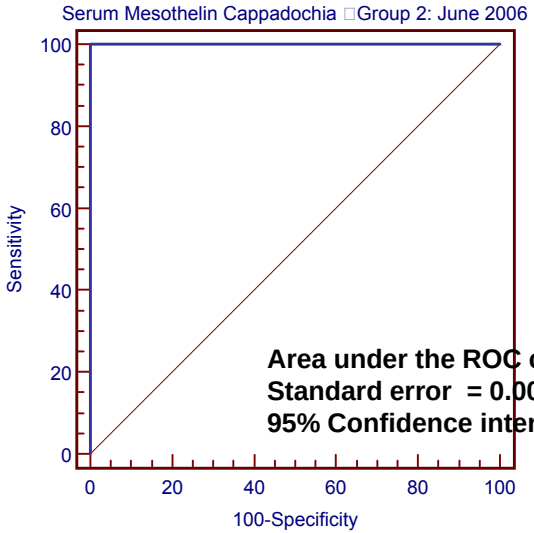




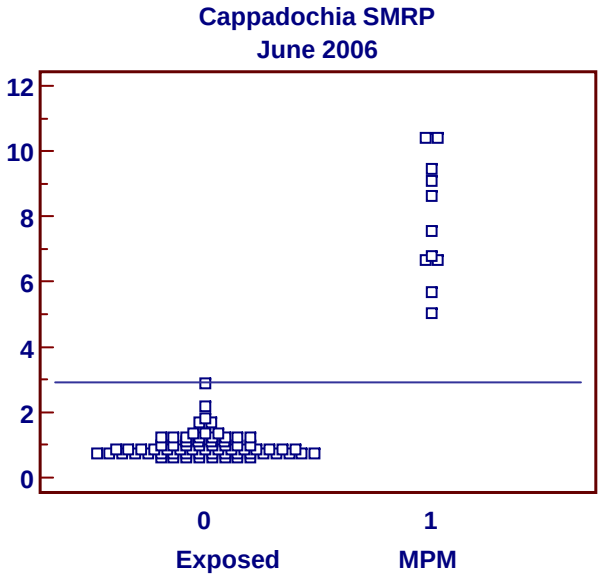
ELISA (June 2006)



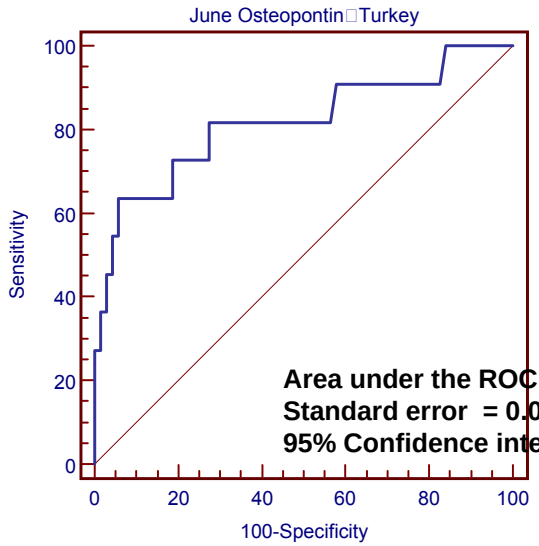
ROC Analysis
Cappadocia June 2006



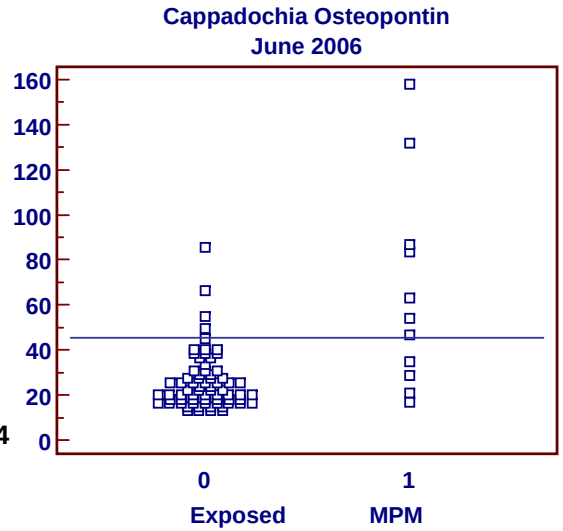
Area under the ROC curve = 1.000
Standard error = 0.000
95% Confidence interval = 0.954 to 1.000



>2.9
Sens: 100.0
Spec: 98.6

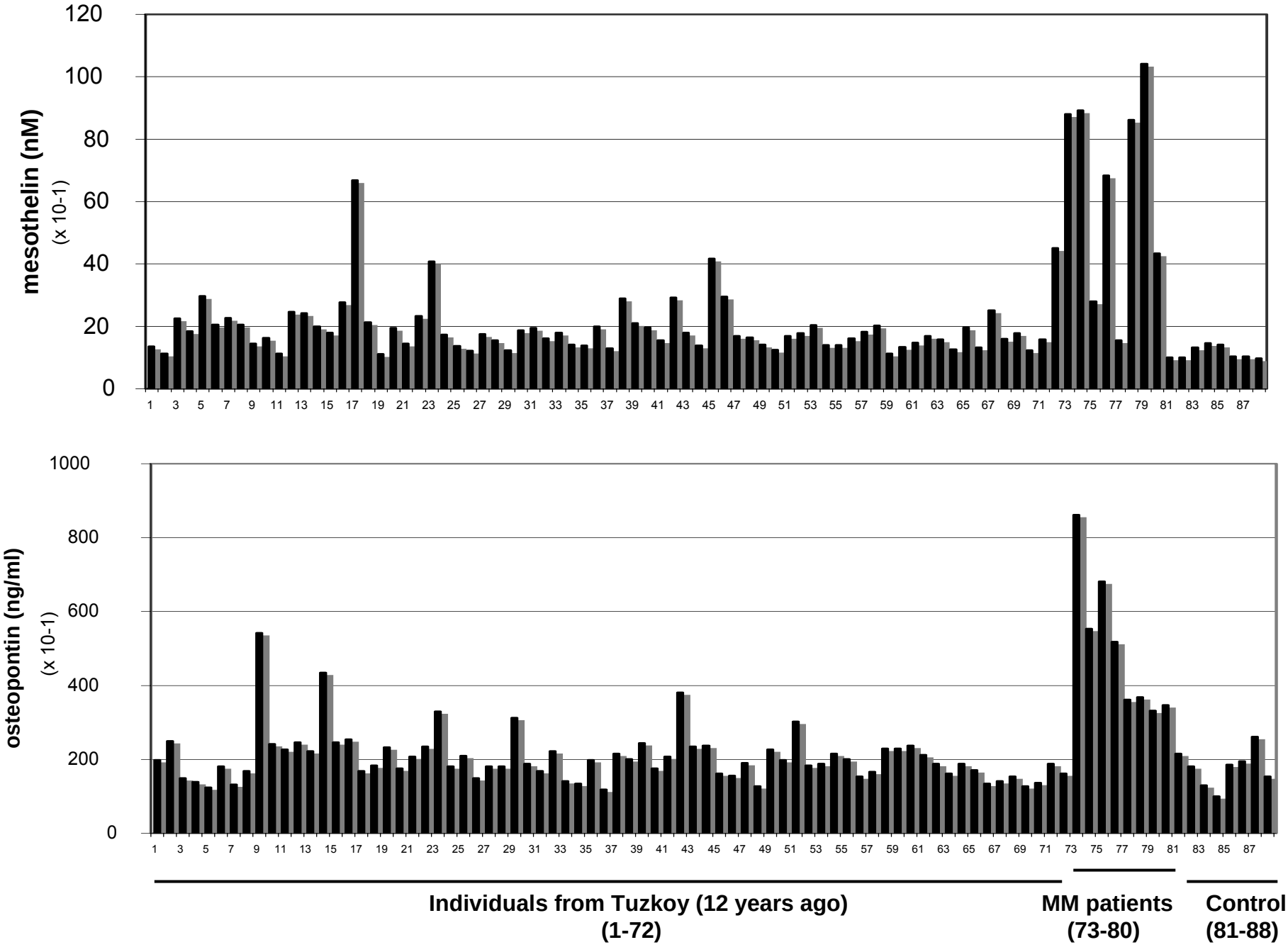


Area under the ROC curve = 0.817
Standard error = 0.081
95% Confidence interval = 0.715 to 0.894

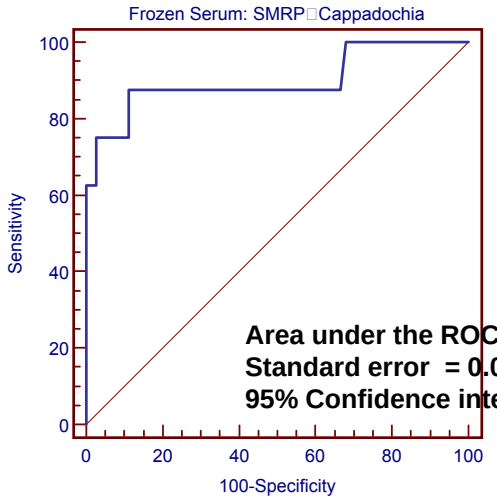


>45.5
Sens: 63.6
Spec: 94.2

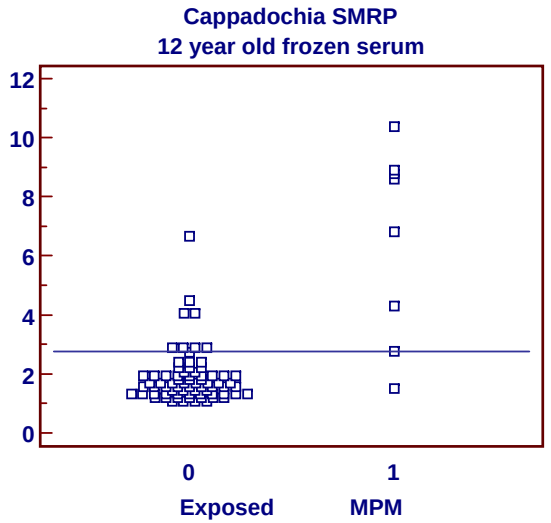
ELISA (06/13/2006)



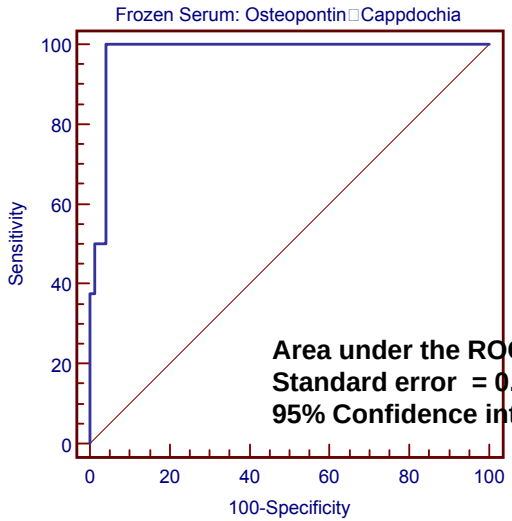
ROC Analysis
Cappadocia Frozen Serum



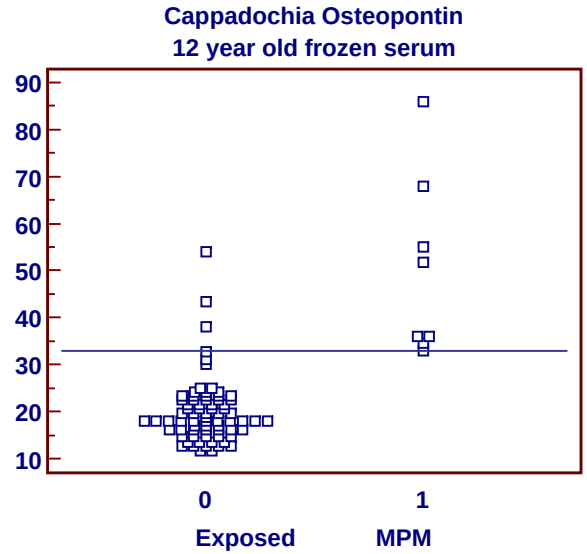
Area under the ROC curve = 0.898
Standard error = 0.075
95% Confidence interval = 0.810 to 0.955



>2.8
Sens: 87.5
Spec: 88.9



Area under the ROC curve = 0.977
Standard error = 0.037
95% Confidence interval = 0.916 to 0.997



>33.0
Sens: 100.0
Spec: 95.8

Partnerships for Pursuing Marker for Screening Indications

- Fujirebio Diagnostics
  - Industrial Partner in EDRN U01
  - Would pursue licensing of patent for osteopontin in asbestos related disease screening
    - Pass/Wali patent application through Wayne State University