EARLY DETECTION RESEARCH NETWORK UPDATE:

SCIENTIFIC HIGHLIGHTS

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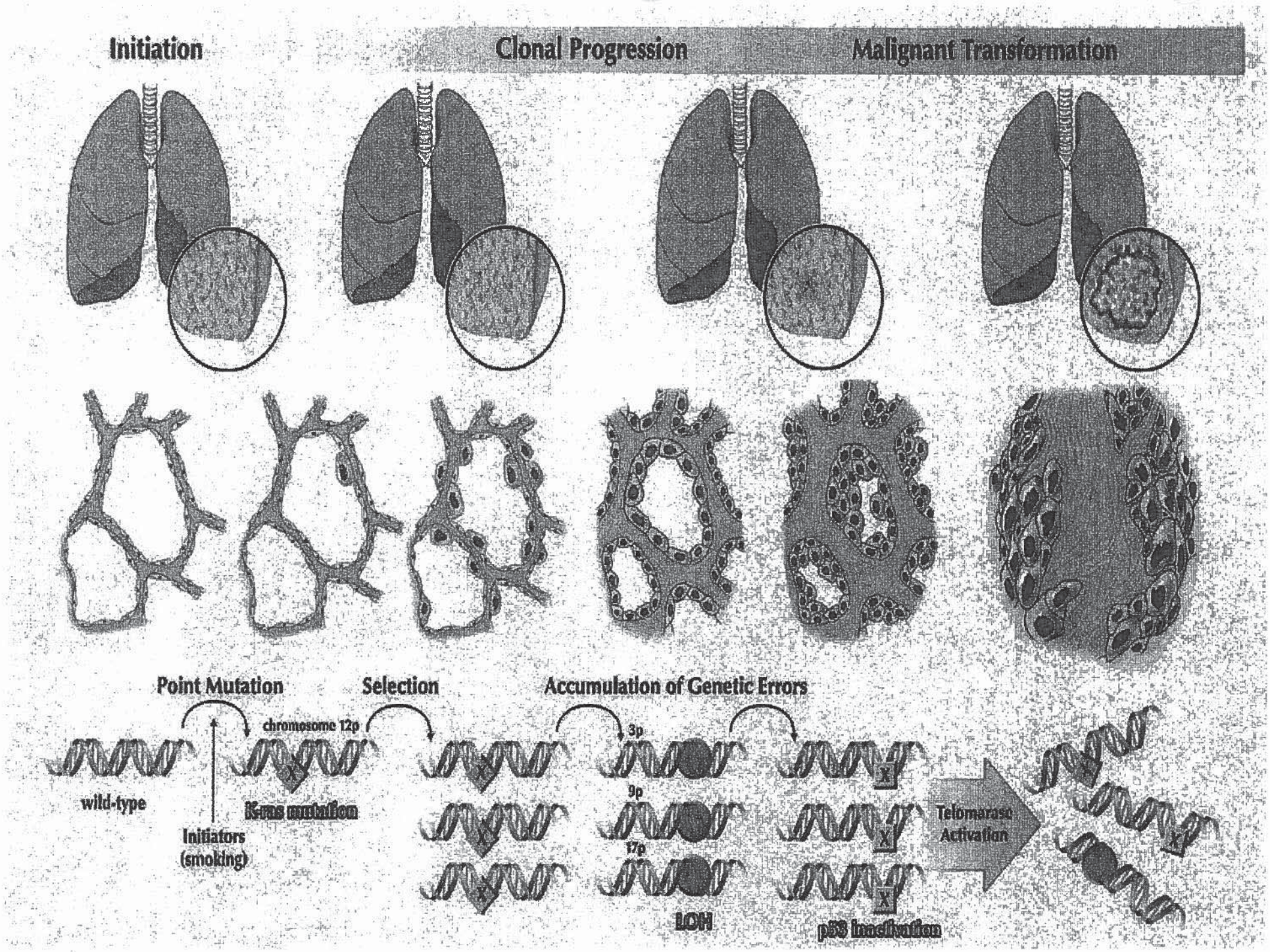
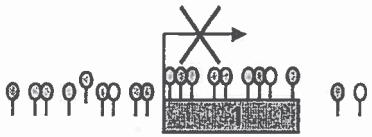
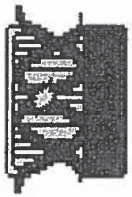

Initiation
Clonal Progression
Malignant Transformation
Point Mutation
Selection
Accumulation of Genetic Errors
chromosome 12p
3p
9p
17p
wild-type
K-ras mutation
Initiators
(smoking)
LOH
p53 inactivation
Telomerase
Activation

Molecular Progression of HNSCC





9p-(p16)

17p-(p53)

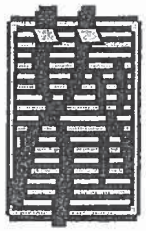
11q-,14q-,8p-

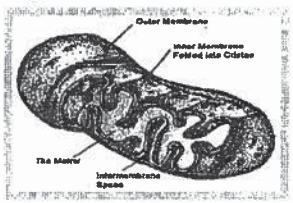
Precursor lesion → Dysplasia → CIS → Invasive Cancer

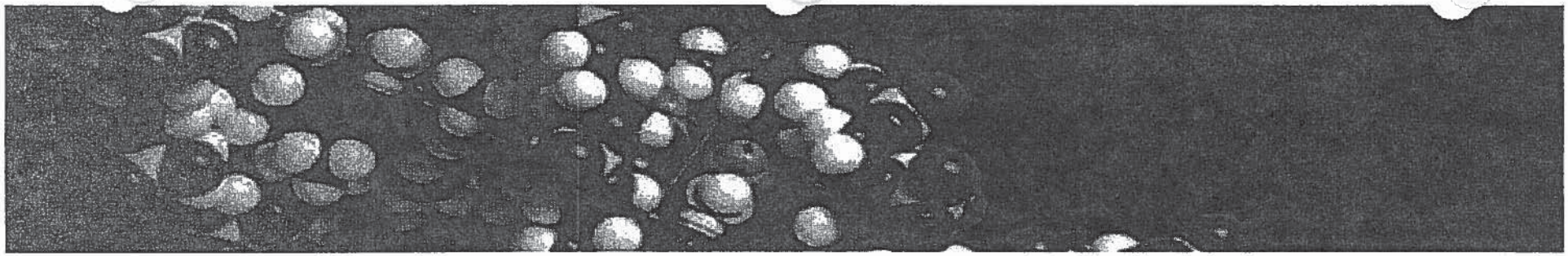
3p-

Cyclin D1 +

4pq-,13q-





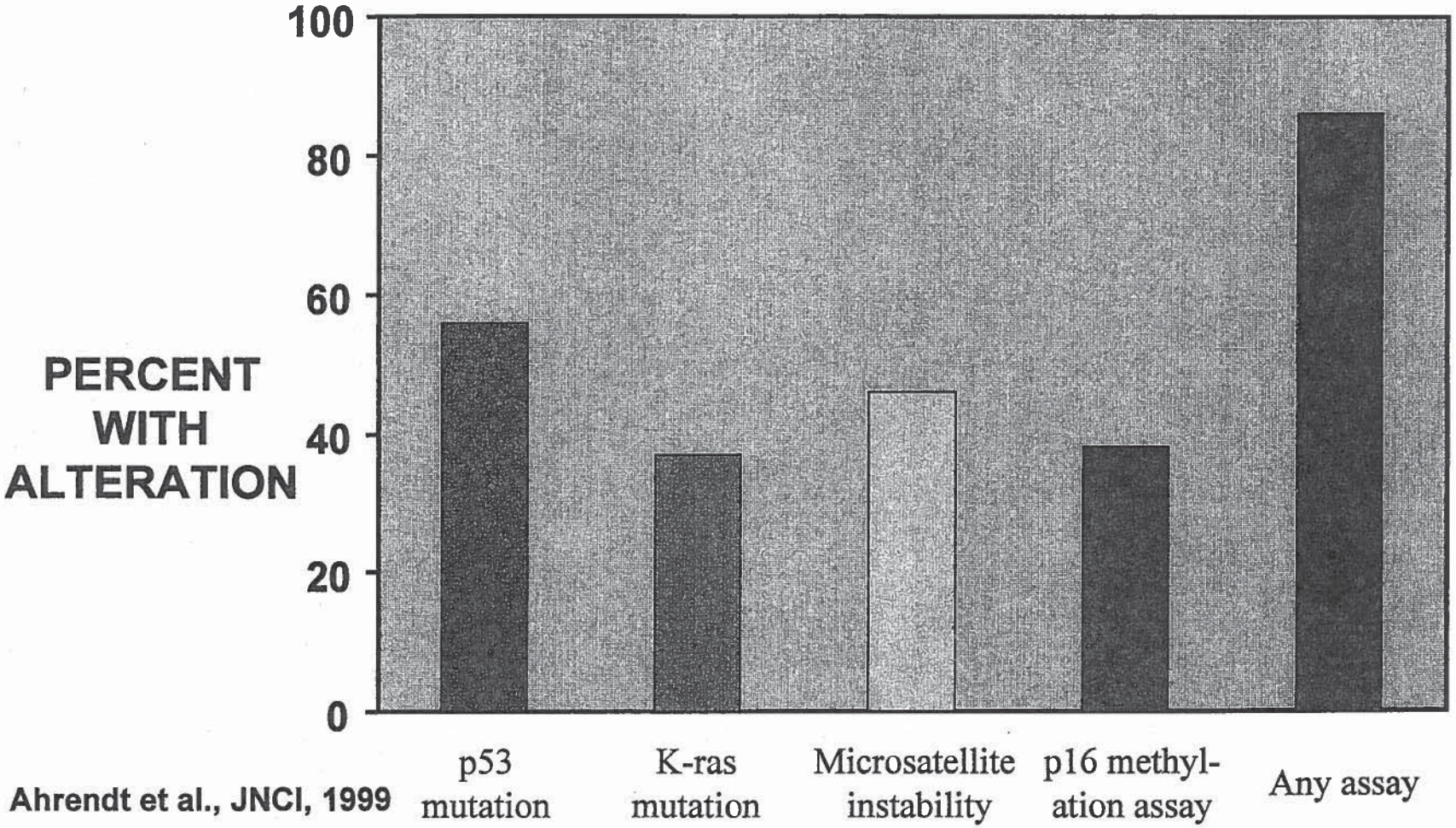


Pipeline for Biomarker Validation

Preclinical Exploratory	*PHASE 1*	*Promising directions identified* ***Many***
Clinical Assay and Validation	*PHASE 2*	*Clinical assay detects established disease* ***Many***
Retrospective Longitudinal	*PHASE 3*	*Biomarker detects preclinical disease and a "screen positive" rule defined* ***Some***
Prospective Screening	*PHASE 4*	*Extent and characteristics of disease detected by the test and the false referral rate are identified* ***Few***
Cancer Control	*PHASE 5*	*Impact of screening on reducing burden of disease on population is quantified*

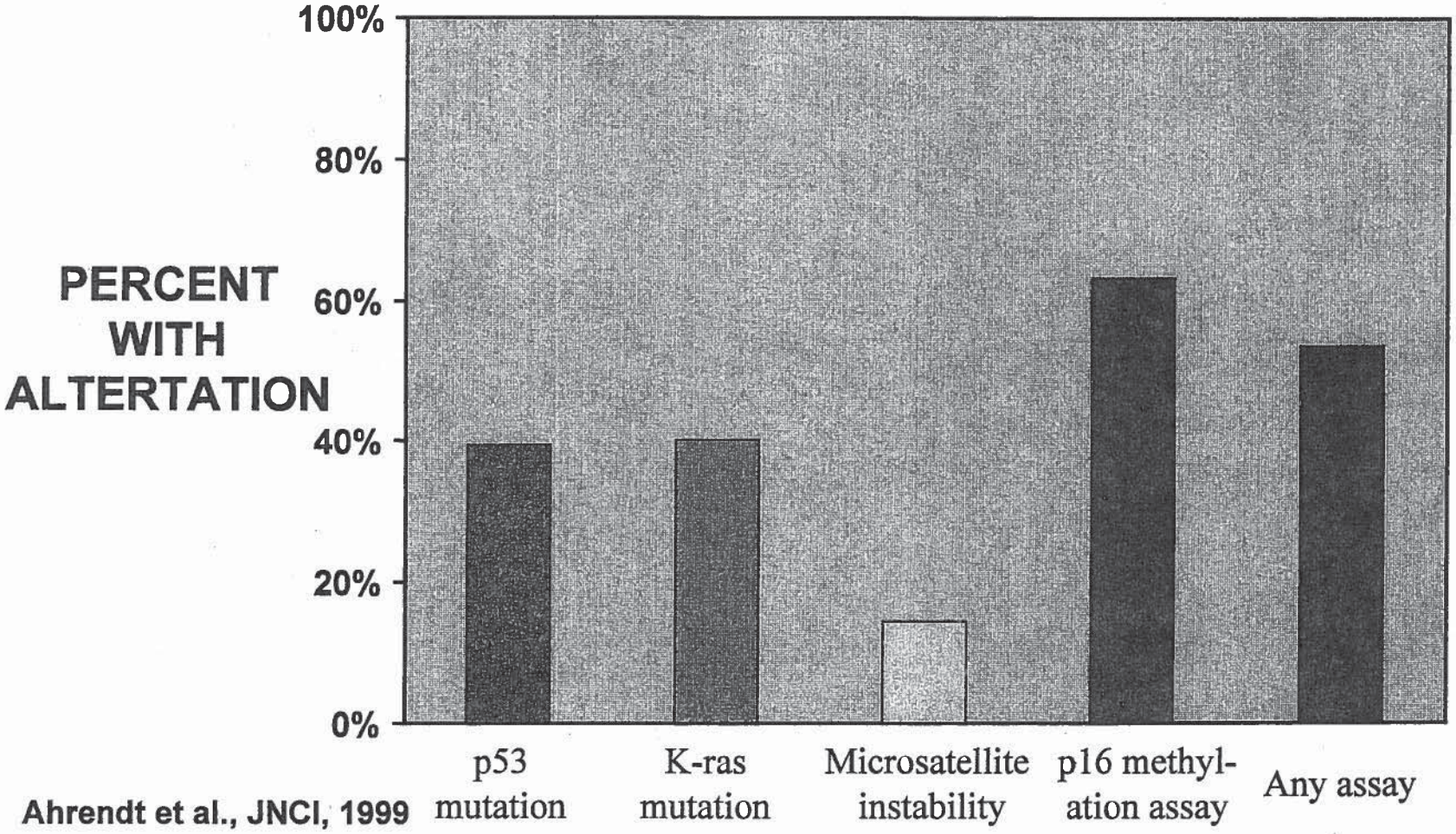
MOLECULAR SCREENING - ALTERATIONS IN TUMOR

50 patients with NSCLC



Ahrendt et al., JNCI, 1999

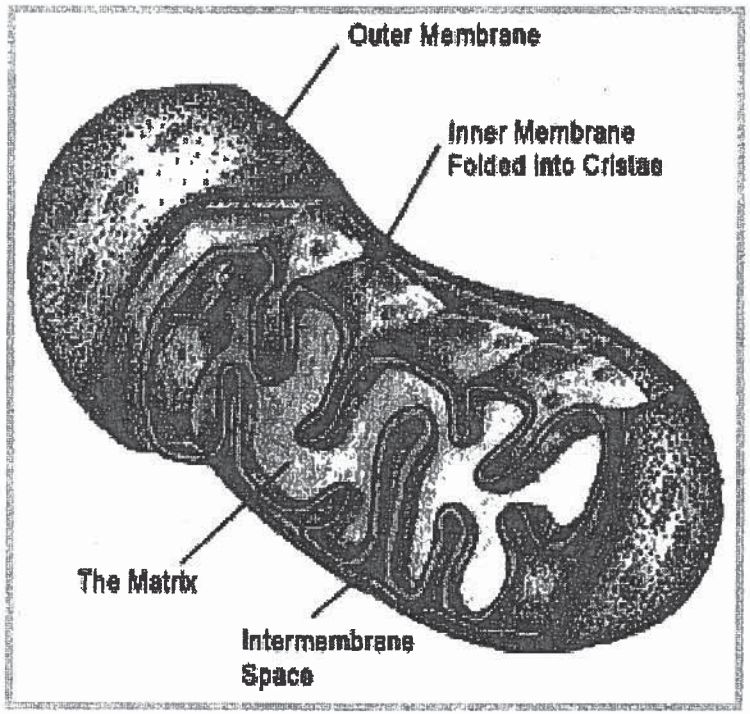
MOLECULAR SCREENING - ALTERATIONS IN BAL



Ahrendt et al., JNCI, 1999

MITOCHONDRIAL-DNA FEATURES

MITOCHONDRIA
ORGANELLA



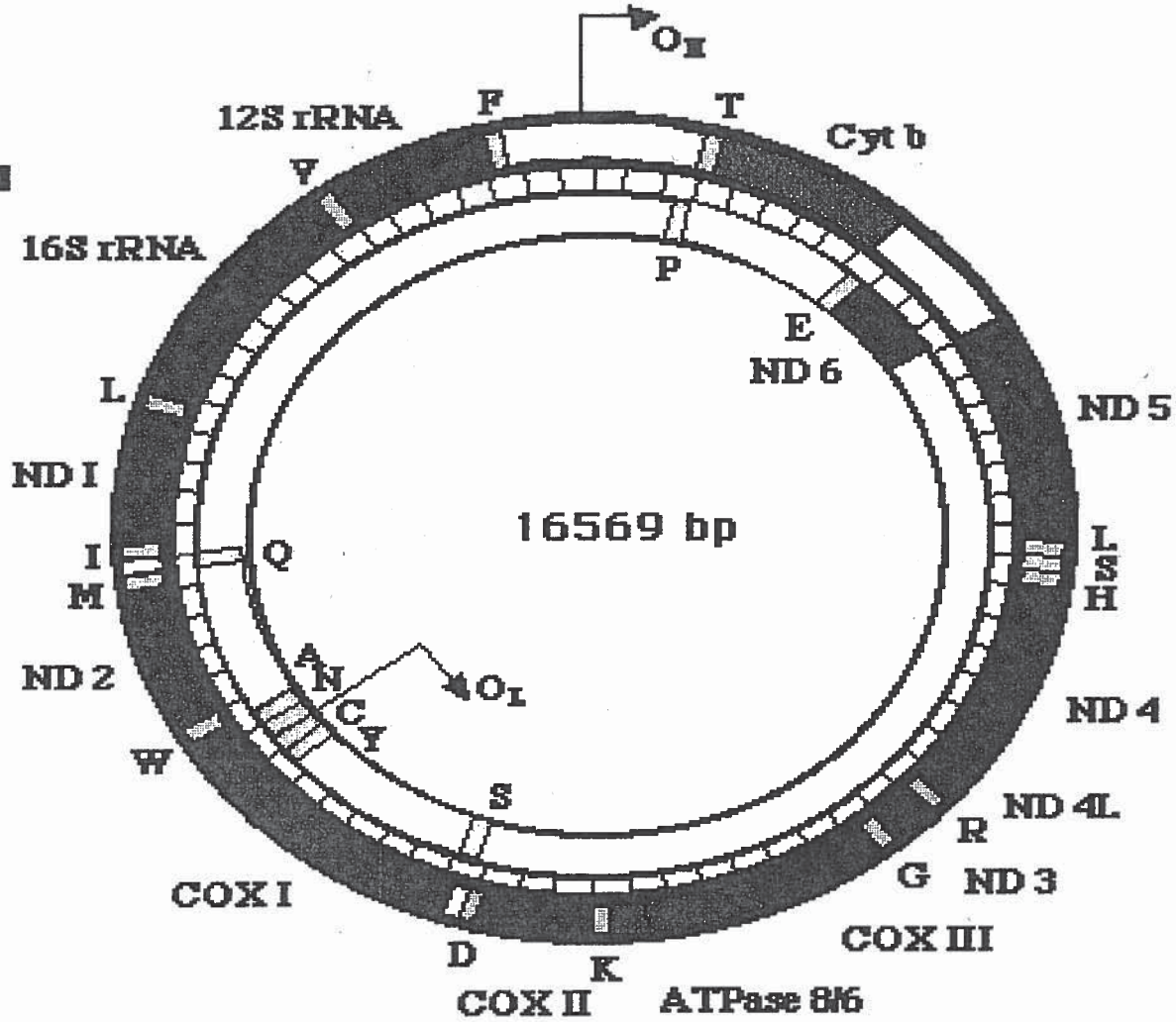
- Human mtDNA is a 16,569-bp closed circular molecule that codes for 13 genes.

-Among these genes are rRNAs, tRNAs and genes essential for the OXPHOS.

-mtDNA is transmitted maternally.

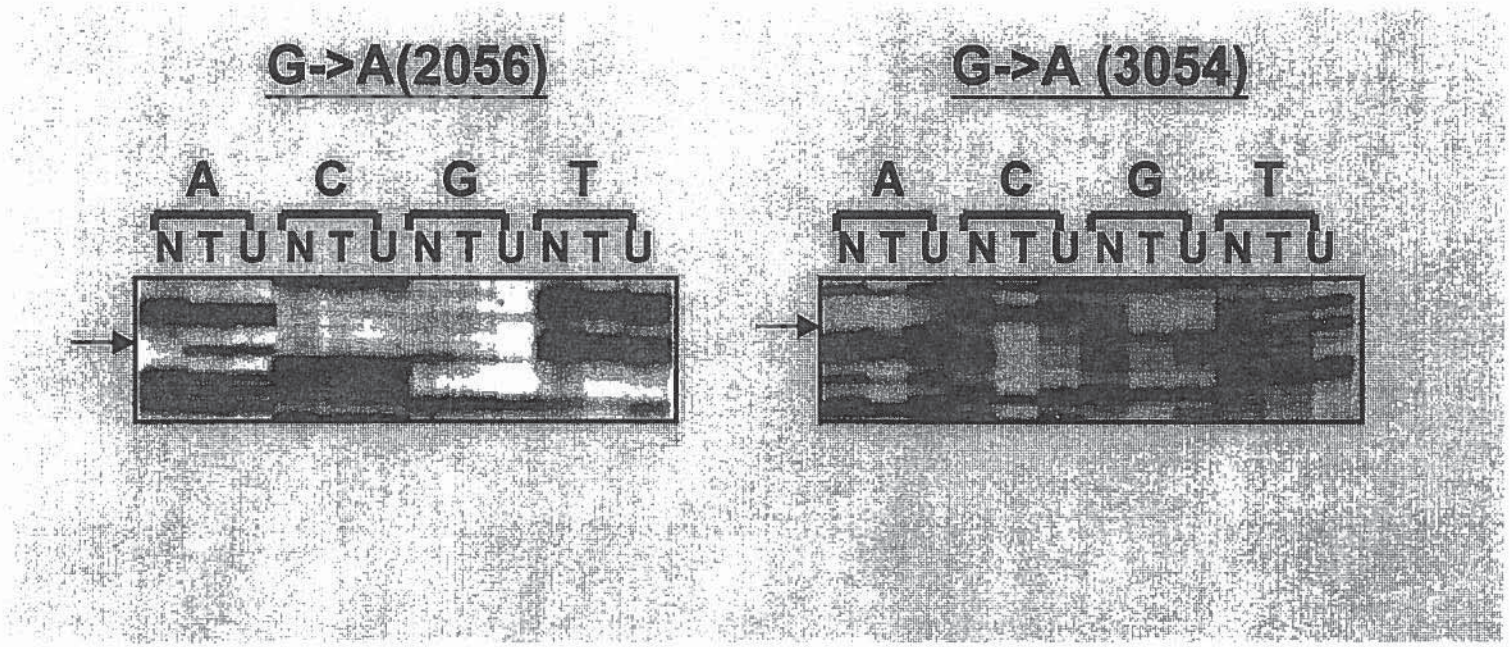
-Mutations in the mtDNA has been associated with degenerative diseases, aging and cancer.

Structure of the mitochondrial DNA



ATPase 8/6 | Complex 1 | tRNAs
Cytochrome b | Complex 4 | rRNAs

Detection of Cancer-derived mtDNA in Bodily Fluids

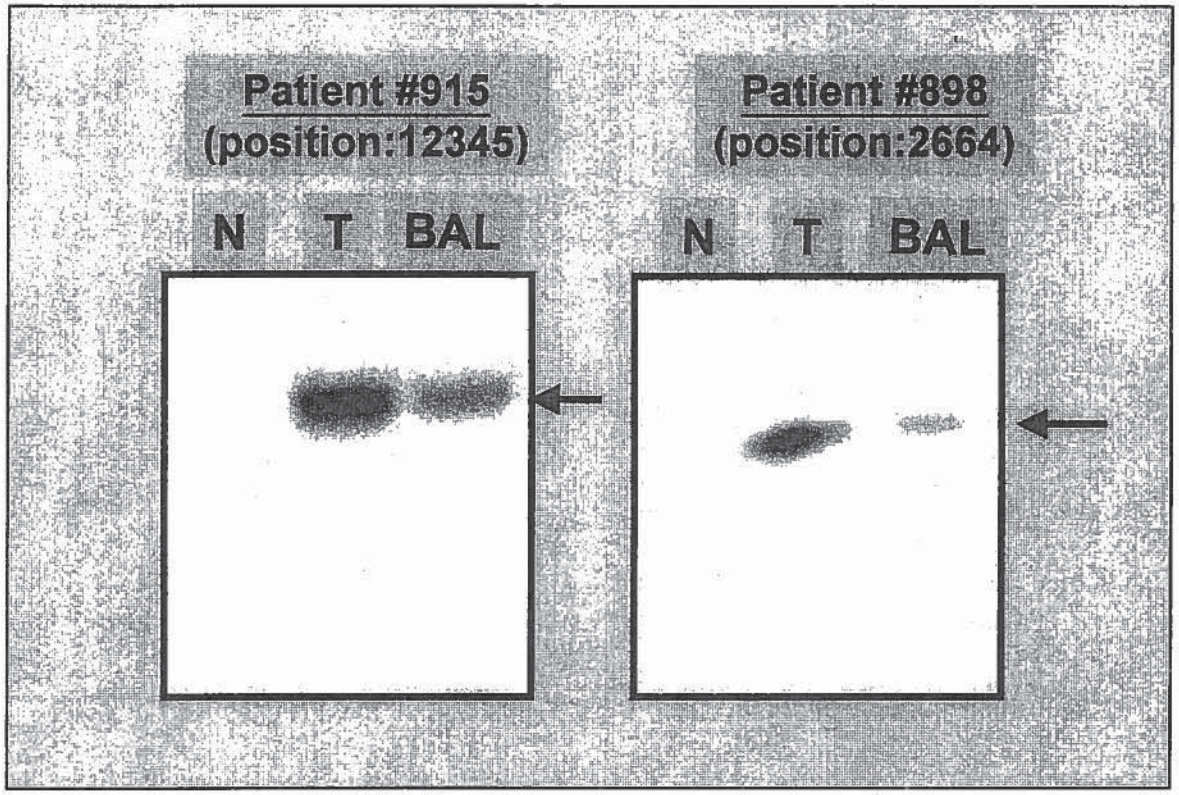


Somatic Mutations of mtDNA in Lung Cancer

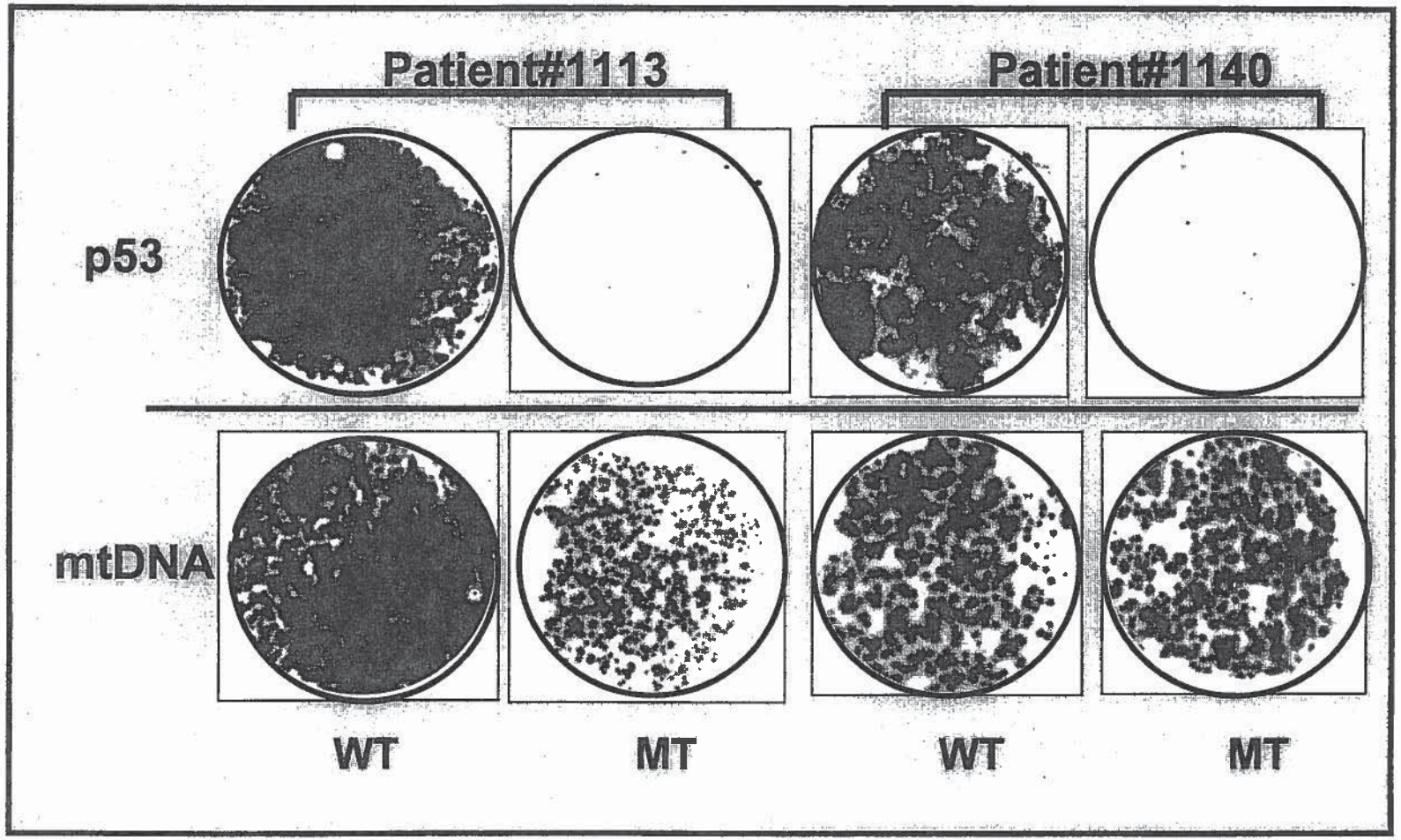
Lung Cancer (6/15, 40%)

1174	150	C->T	N/A	D-loop
1174	195	T->C	N/A	D-loop
902	302	Del C	N/A	D-loop
898	2664	T->C	N/A	16sRNA
915	5521	G-A	N/A	tRNATrp
915	12345	G->A	N/A	tRNALeu
915	16183	C->A	N/A	D-loop
915	16187	C->T	N/A	D-loop
1113	16519	T->C	N/A	D-loop
1140	16380	G->A	N/A	D-loop

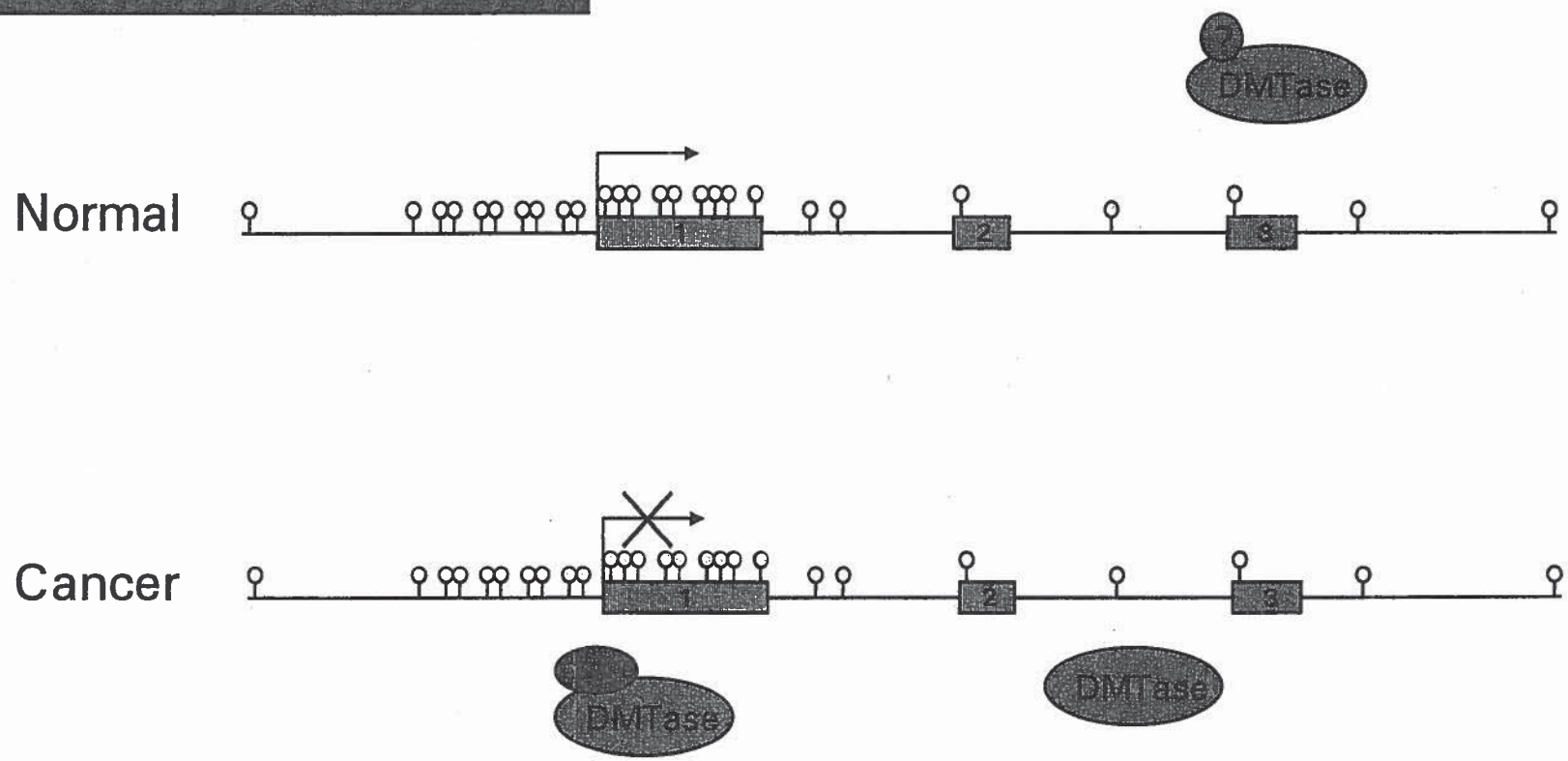
Detection of Tumor-derived mtDNA from BAL



Enrichment of mtDNA in BAL



Methylation changes in Neoplasia



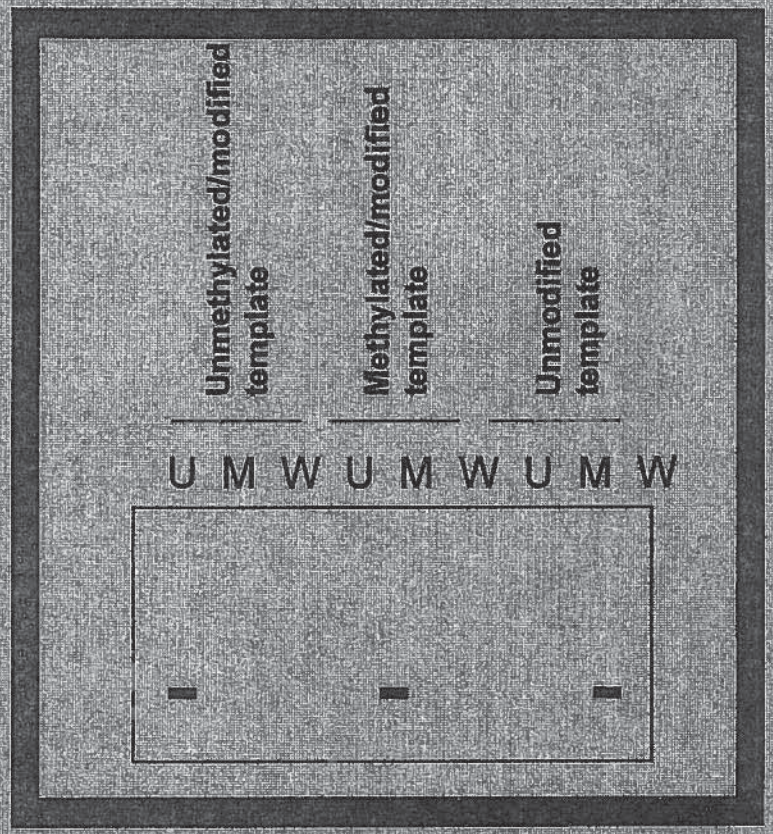
Fundamental Pathways affected by DNA Methylation in Cancer

- Altered cell cycle control
  - *Rb, p16, p15, p14*
- DNA damage repair
  - *MLH1, O^6MGMT, GSTp, BRCA1*
- Controlling/limiting apoptosis
  - DAP-kinase
- Invasion/ tumor architecture
  - *E-cadherin, VHL, APC*
- Growth factor response
  - *ER, RARβ,*

Methylation Specific PCR

CCGCATCCGCG $\xrightarrow{NaHSO_3}$ UUGUATUUGUG $\xrightarrow{PCR}$

$C^mCGCATC^mCG^mCG$ $U^mCGUATU^mCG^mCG$



Unmethylated Specific Primer
Methylated Specific Primer
Mismatches in Red

TTGTATTTGT
UUGUATUUGUG Unmethylated/modified template
TCGTATTCGC

TTGTATTTGT
UCGUATUCGCG Methylated/modified template
TCGTATTCGC

TTGTATTTGT
CCGCATCCGCG Unmodified template
TCGTATTCGC

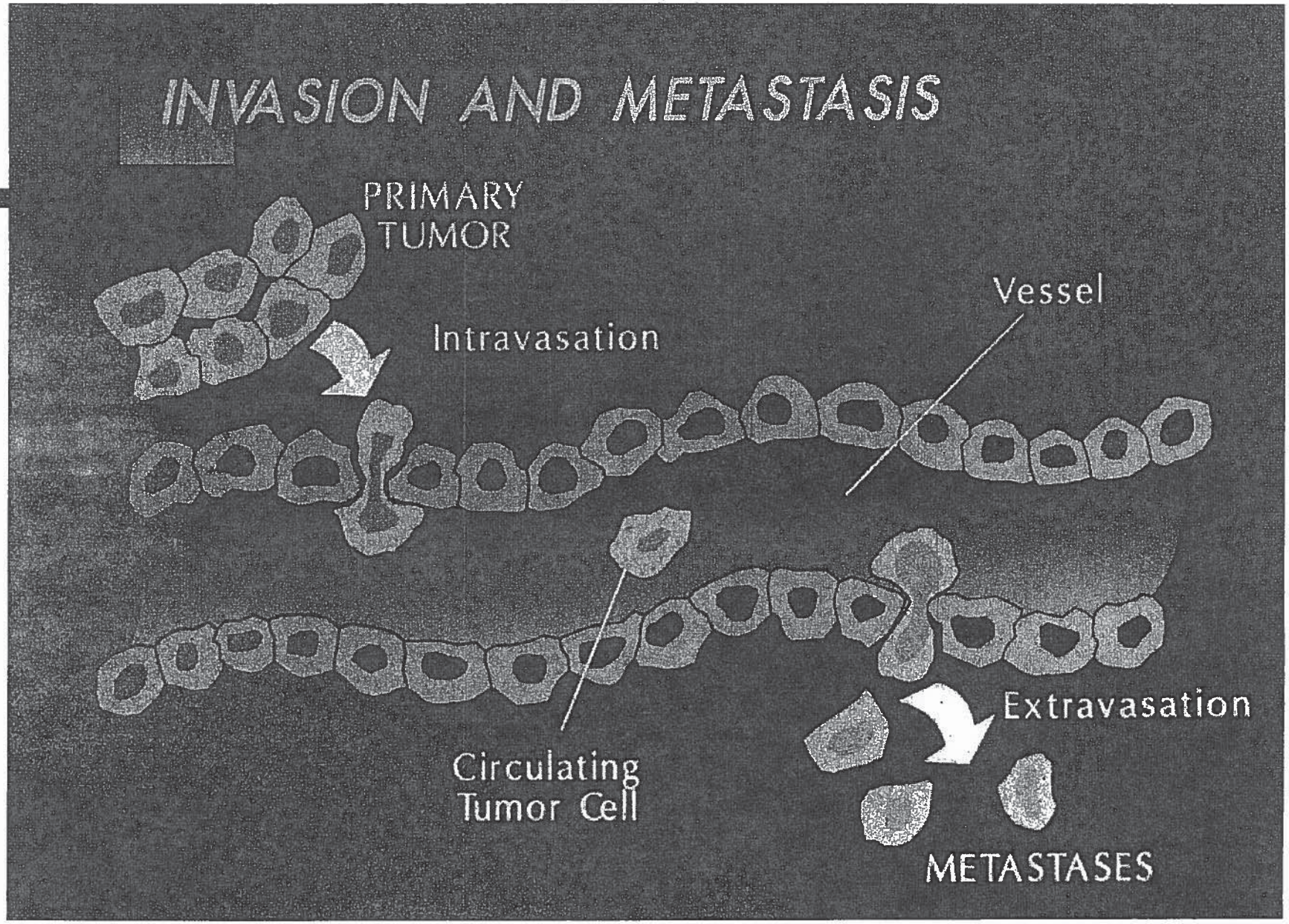
Promoter Hypermethylation in Tumors and Saliva of Head and Neck Cancer Patients.

- 30patients with HNSC
- 15 smoking controls
- 15 nonsmoking controls
- tested 3 genes
  - DAP-K
  - MGMT
  - p16

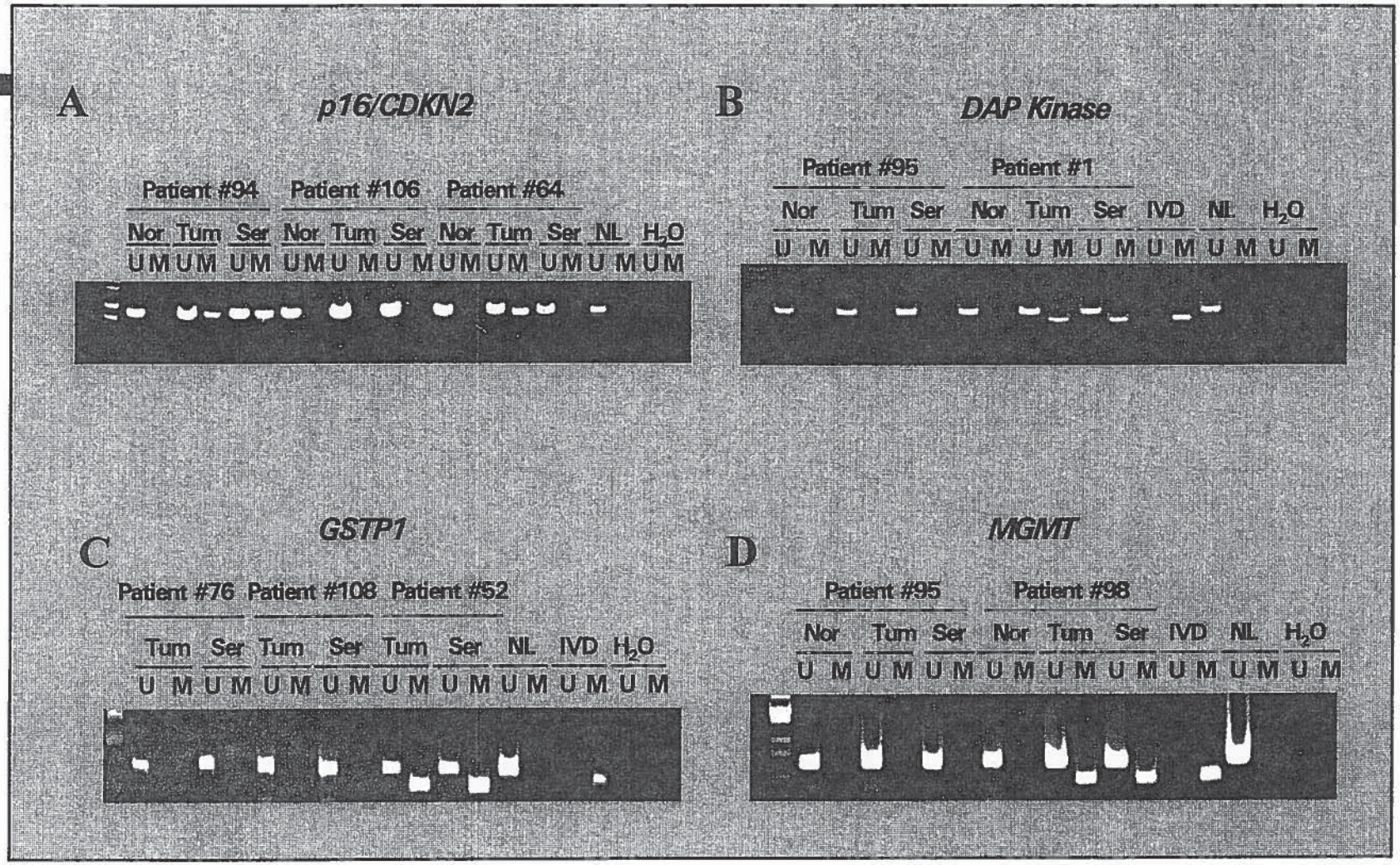
Patient no.	*p16*	*DAP*	*MGMT*	Site	Stage (TNM)
1506	**M/U**	U/U	**M/U**	OP	T4N1M0
1493	U/U	U/U	U/U	OP	rT3N0M0
1507	U/U	**M/U**	**M/U**	LA	T3N1M0
1572	U/U	U/U	U/U	OP	T1N0M0
[redacted]	[redacted]	**M/U**	U/U	OP	T2N2aM0
1584	U/U	U/U	**M/U**	OP	T3N2M0
1627	U/U	U/U	U/U	NP	T4N2Mx
1672	**M/M**	**M/M**	U/U	OP	T3N1M0
1624	U/U	U/U	U/U	OP	T4N3Mx
1620	**M/U**	U/U	U/U	OP	T2N2aM0
1621	U/U	U/U	U/U	LA	T1N0M0
1605	U/U	U/U	U/U	UNK	TxN1M0
1606	U/U	U/U	U/U	OP	T1N1M0
1708	U/U	U/U	U/U	OP	T2N2M0
1691	U/U	U/U	U/U	OC	T4N0M0
1684	**M/M**	U/U	U/U	OP	T1N2AM0
1683	U/U	**M/U**	**M/U**	OP	T1N2bM0
1680	**M/M**	**M/M**	**M/M**	OC	rT4N0
1847	**M/M**	**M/M**	**M/M**	OC	T1N0M0
1850	**M/M**	**M/M**	U/U	OC	T1N0M0
1853	**M/M**	**M/M**	**M/M**	OC	T1N0M0
1855	**M/M**	**M/M**	**M/M**	OC	T1N0M0
1857	U/U	U/U	U/U	HP	T2N0M0
1911	**M/M**	U/U	U/U	OC	T1N2M0
1933	U/U	U/U	U/U	OC	T2N0M0
1858	**M/M**	U/U	U/U	OC	T4N2cM0
1810	**M/M**	**M/U**	U/U	LA	T3N1M0
1565	U/U	U/U	U/U	LA	T4N0M0
1628	**M/M**	U/U	U/U	OC	T1N0M0
1677	U/U	U/U	U/U	OP	T2N1M0

Gene aberrantly methylated	Tumor postive, n.o/total no. (%)	Saliva positive, n.o/total no.(%)
p16	14/30 (47)	11/14 (79)
Dap-kinase	10/30 (33)	6/10 (60)
MGMT	7/30 (23)	4/7 (50)
Any	17/30 (56)	11/17 (65)

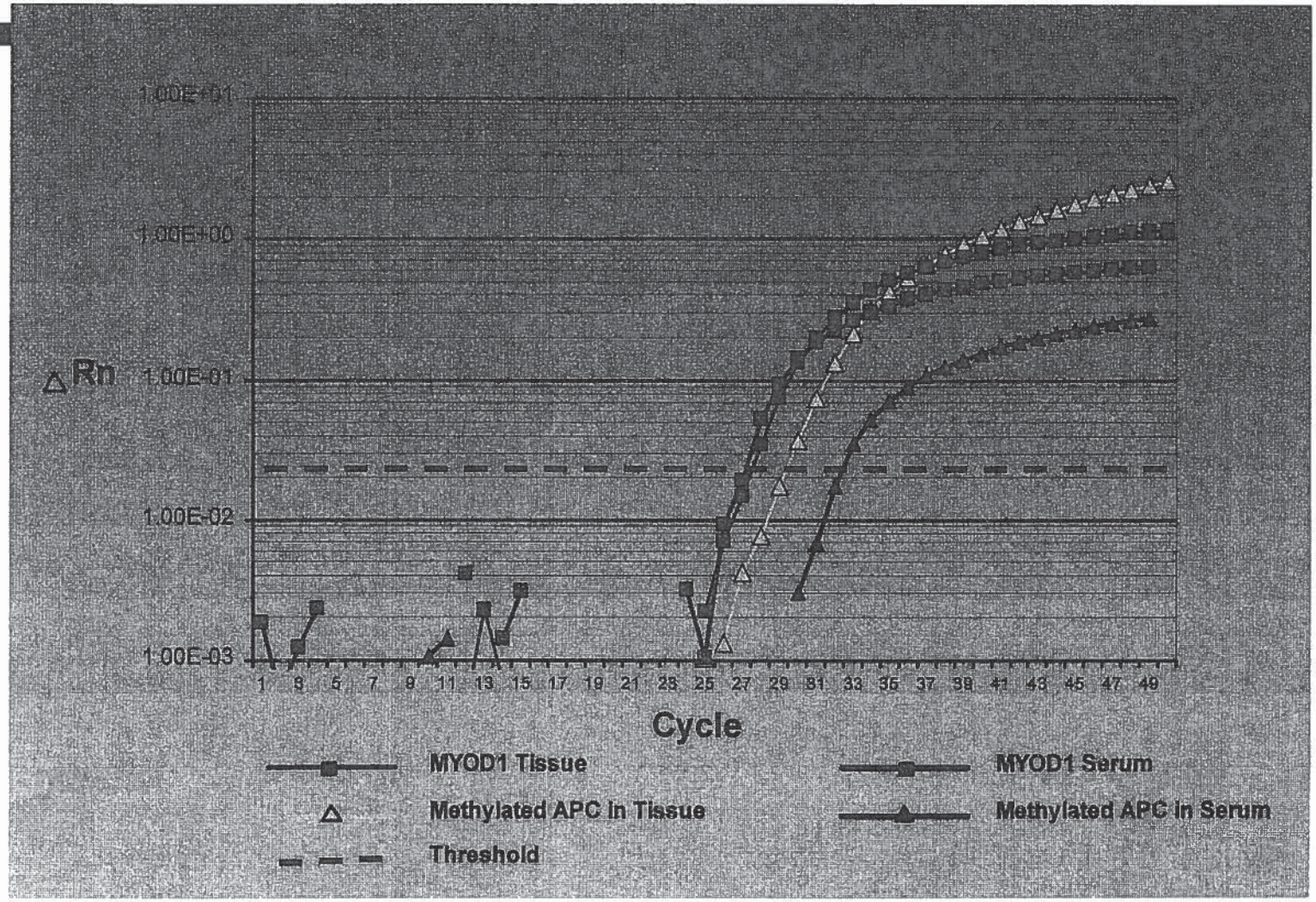
Controls
1/15 smokers positive
0/15 nonsmokers positive


INVASION AND METASTASIS
PRIMARY TUMOR
Intravasation
Vessel
Circulating Tumor Cell
Extravasation
METASTASES

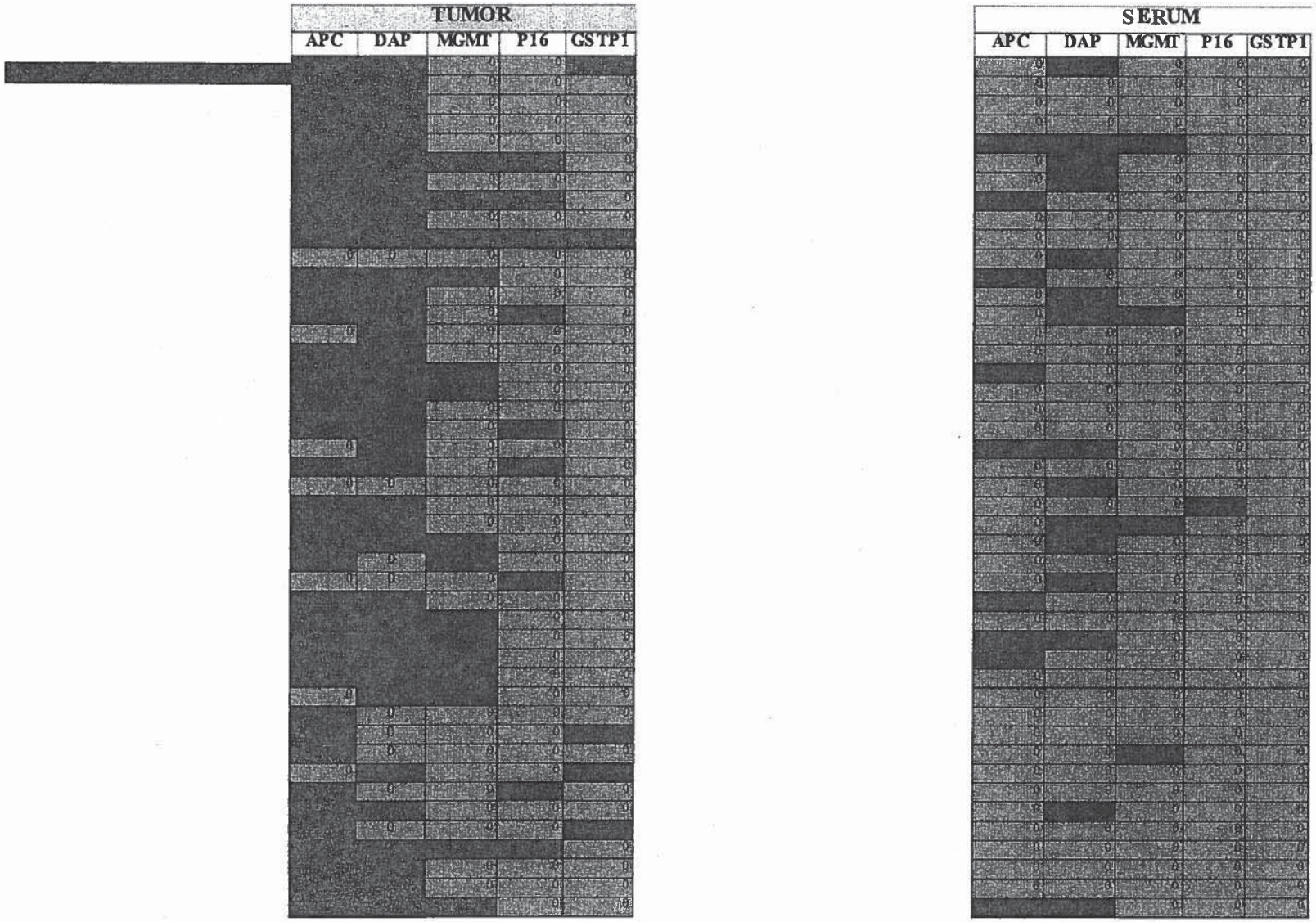
Detection of aberrant promoter methylation in tumor and serum from resectable NSCLCa patients



Real-Time Amplification Plot: Tissue/Serum



Comparison real-time MSP Tumor vs. Serum *n* = 55



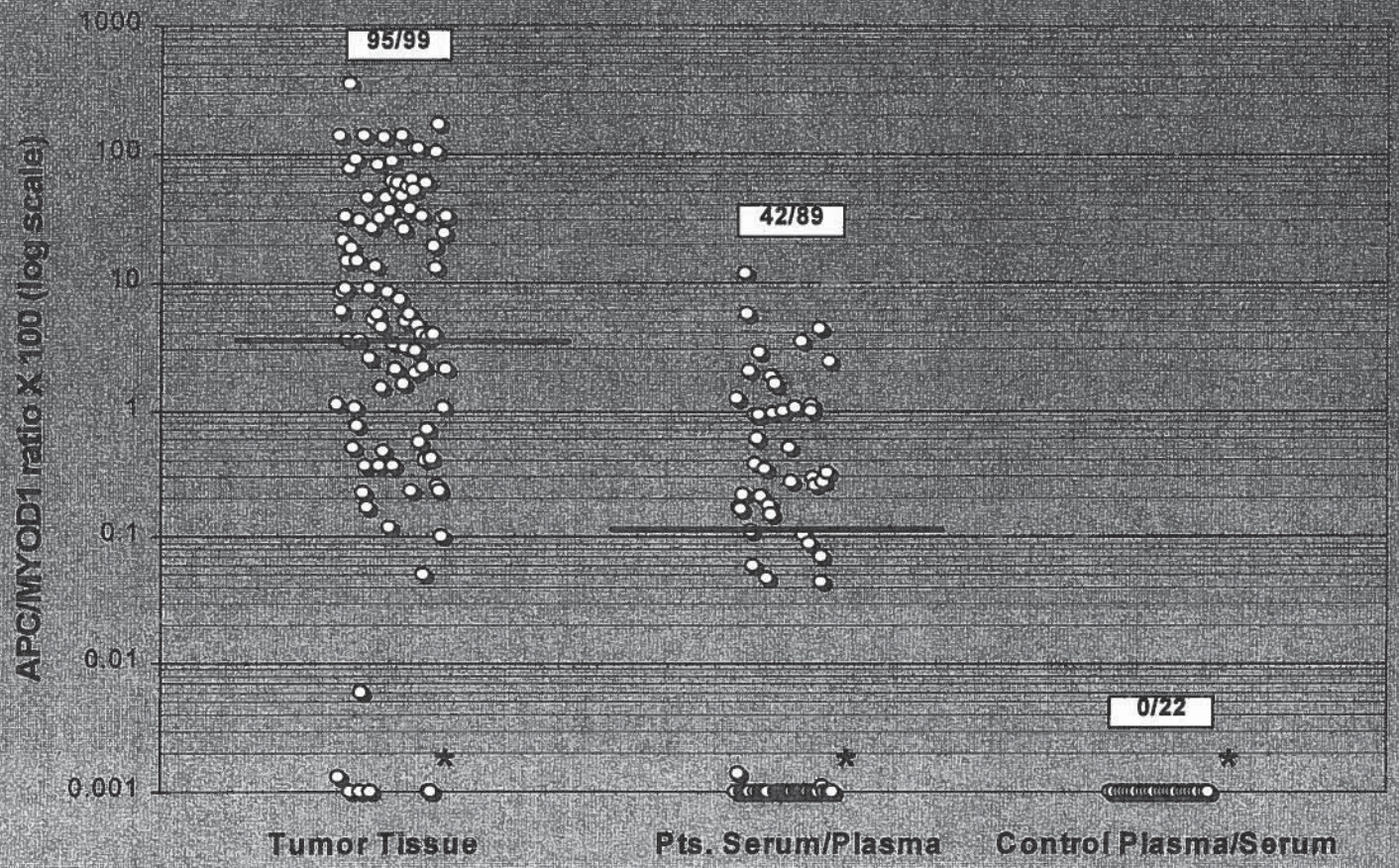
99 NSCLC tissue Samples:

- 95 (96%) Samples Positive
- Significance between APC-Hypermethylation Level and survival

89 Serum and/or Plasma Samples:

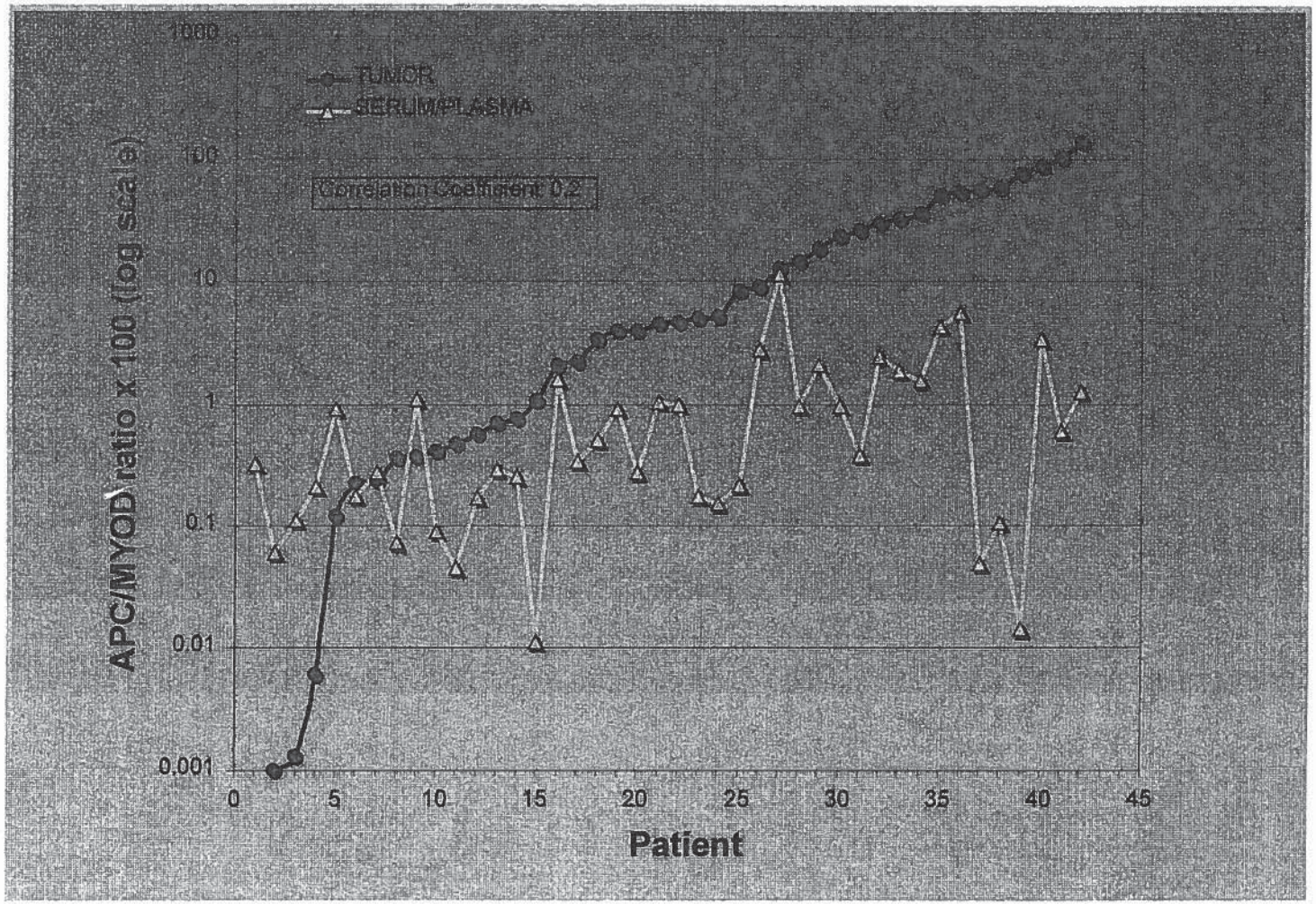
- 42 (47%) Samples Positive
- No correlationbetween APC-Hypermethylation Level in serum and Clinical data

Methylation Levels in Tissue and Serum/Plasma



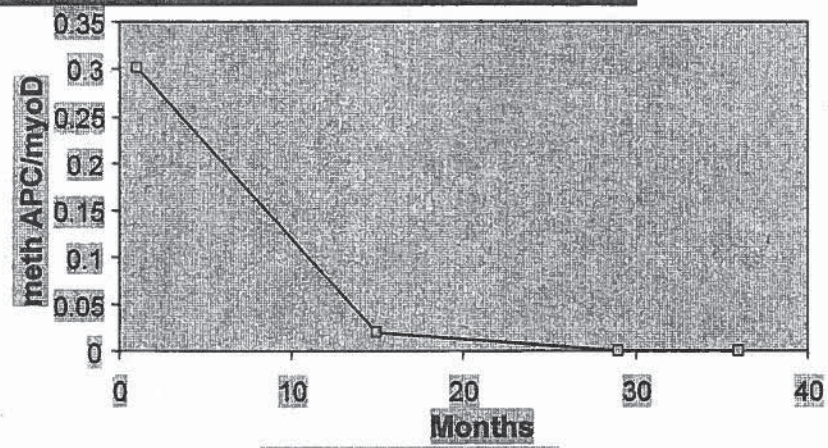
Solid bars indicate the median within a group of patients. Asterisks indicate the samples with 0-values which can not be plotted correctly on a log scale.

Correlation between Methylation levels in Tissue and Matching Serum/Plasma

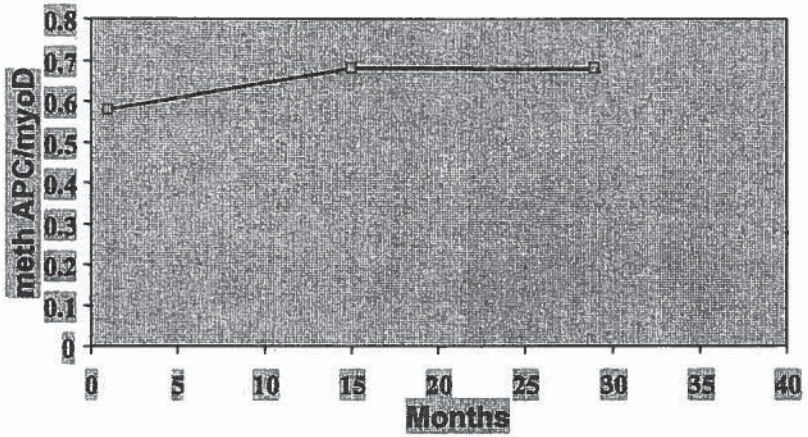


Serum meth APC during treatment in four cases

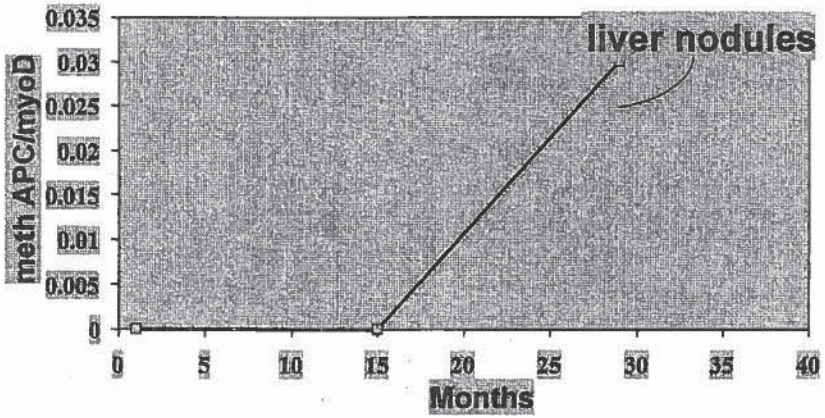
Response



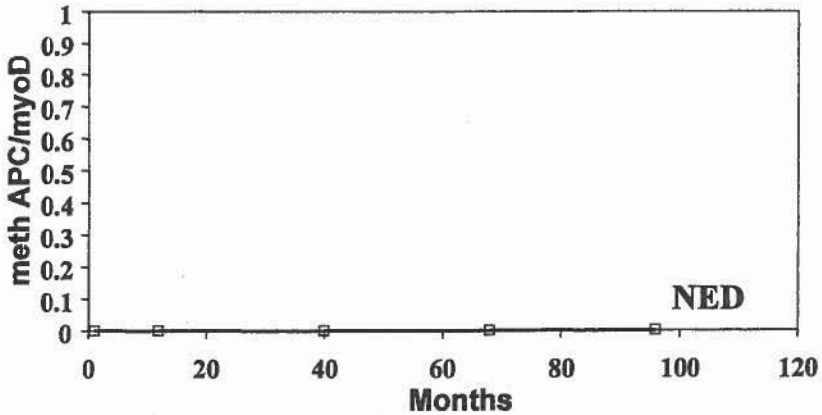
No Change

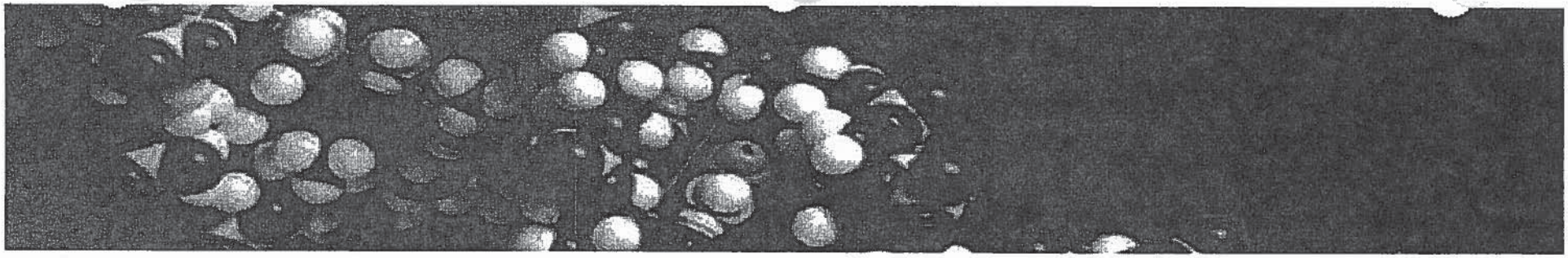


Progression



No Evidence of Disease





- **A panel of methylation markers that theoretically detects 100% of lung cancers has been developed.**

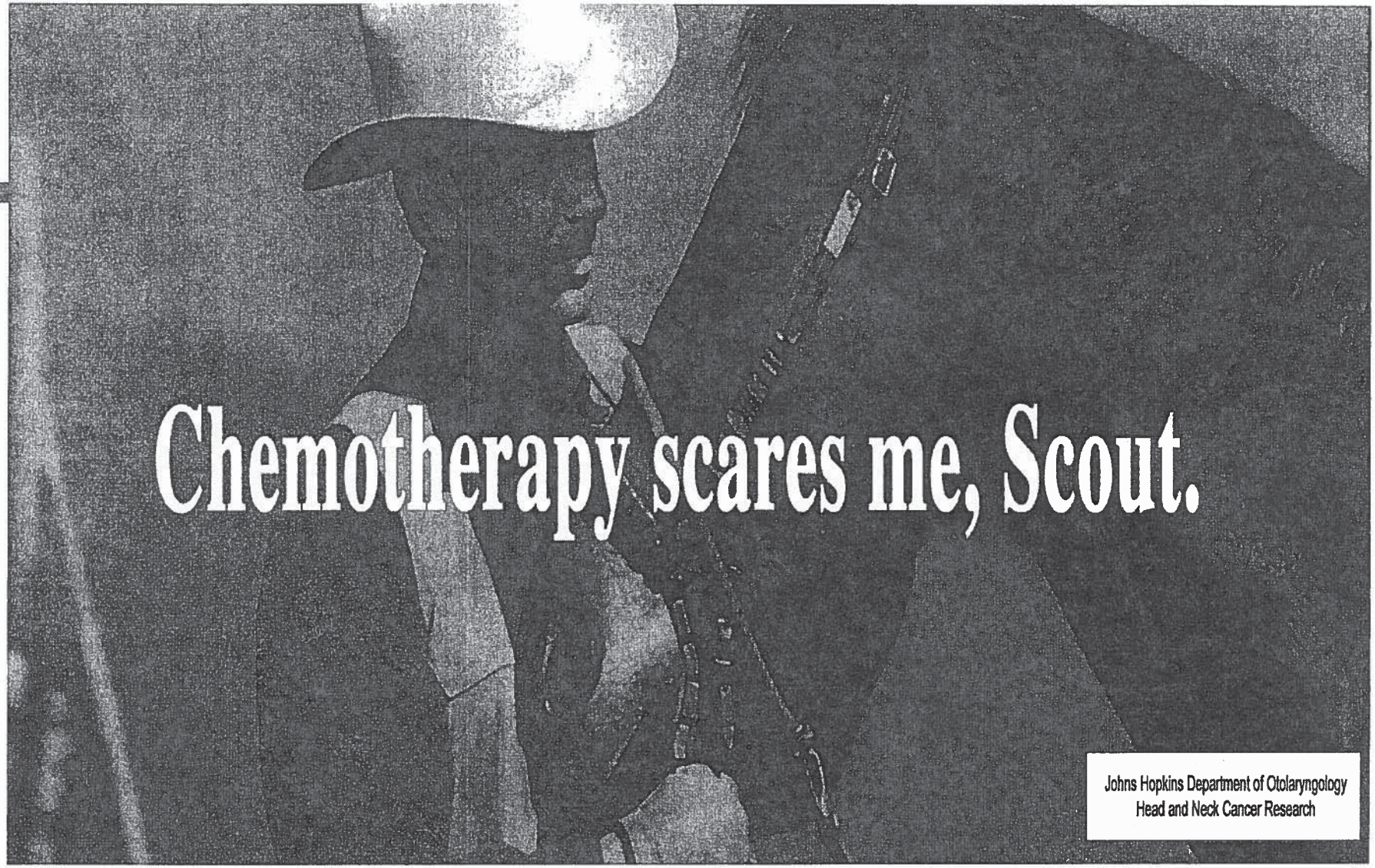
- **Retrospective analysis of longitudinal samples initiated (CARET) (LCWG)**

- **Prospective trial on high risk smokers (piggyback)**

Promoter hypermethylation working group established to cross-check results from four labs, using same set of primers and coded samples

Working Group Members

Melvin Tockman
Adi Gazdar
Wilbur Franklin
David Sidransky
Jim Herman


Chemotherapy scares me, Scout.
Johns Hopkins Department of Otolaryngology
Head and Neck Cancer Research