Proteomic signatures of non-small cell lung cancer

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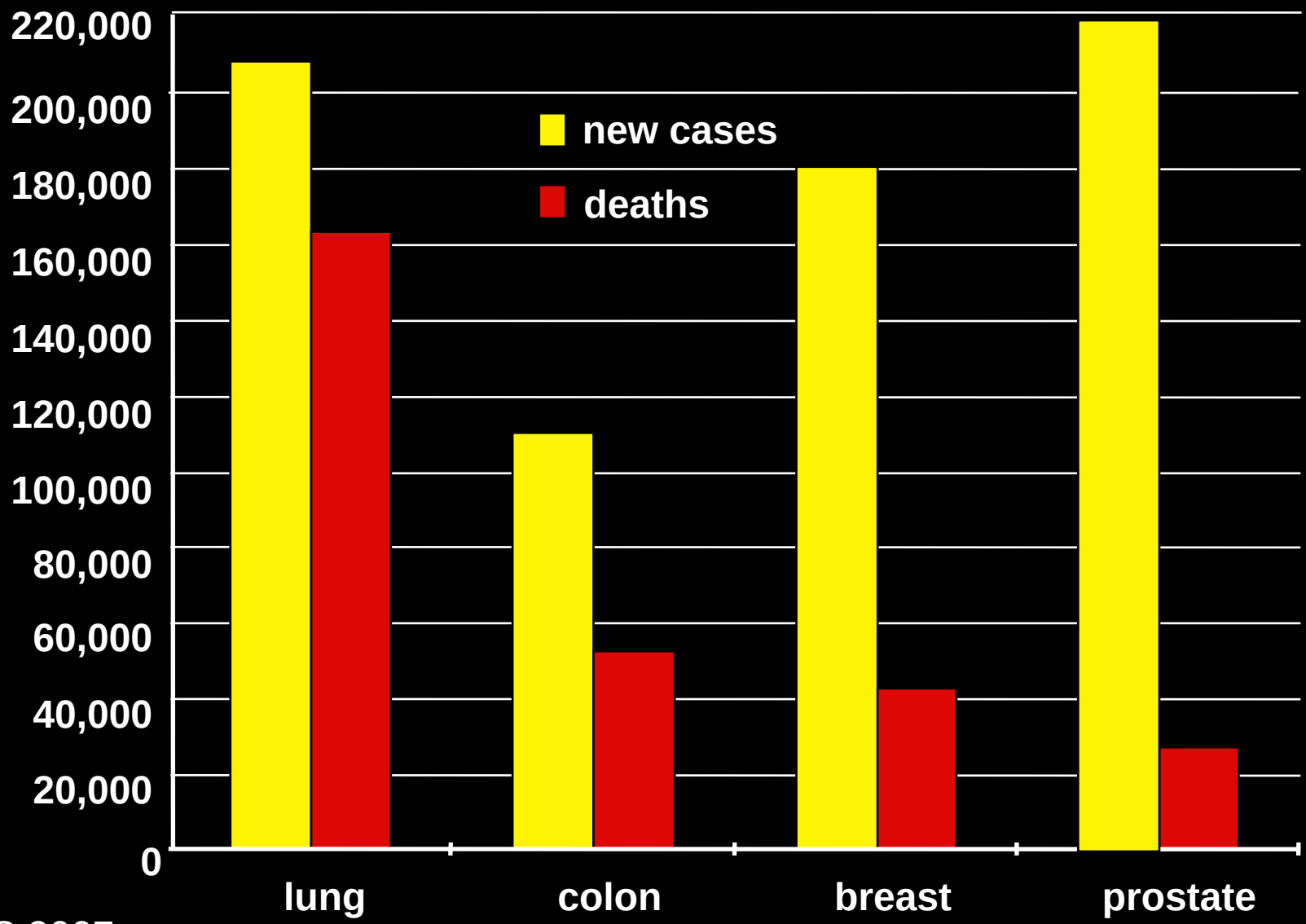
U.S. Cancer Mortality, 2007



ACS 2007

Improving outcomes in NSCLC with better molecular signatures

- NO proven screening strategy
- NO proven biomarkers for
  - Early detection - more curable
  - Diagnosis - avoid futile thoracotomies or missed cures
  - Prediction of response to therapy - individualized therapy
- We have to move beyond light microscopy in clinical decision-making toward use of a “molecular signature”.

Proteomics

- Analyzes patterns of protein expression
- Potential advantages:
  - Most nucleic acid sequences have their effect via translation into proteins
  - Protein expression is often not tightly associated with RNA expression
  - Ability to detect post-translational modifications - proteolytic, phosphorylation, lipidation, ubiquitination, etc.

Challenges

- Orders of magnitude more complex than the genome.
- Huge range of expression levels.
- Highly unstable.
- No amplification technologies.

Points to consider

- Protein patterns should be better biomarkers than RNA/DNA
- Increasing awareness of problems
  - Overfitting, recursive testing of “test sets”, mixing of training and testing sets
  - Systematic bias in analysis or sample collection
  - Mixing of prognostic and predictive endpoints

Early detection - lung cancer diagnosis from peripheral blood

Both tumor-derived proteins and host-reactive proteins potentially important

Blood diagnostics tested in lung cancer

- CYFRA21-1, NSE, CA15-3, CA19-9 and CA125
- VEGF
- Circulating DNA
- IL-6, TNF-alpha, and leptin
- CRP, SAA
- Bcl-2, MIF
- Beta defensins, ADAM8, MMP9
- Many others…

Sensitivity at 95% Specificity

Marker	Sensitivity
CEA	26 - 33%
SCC	39 - 41%
CYFRA 21.1	36 - 81%

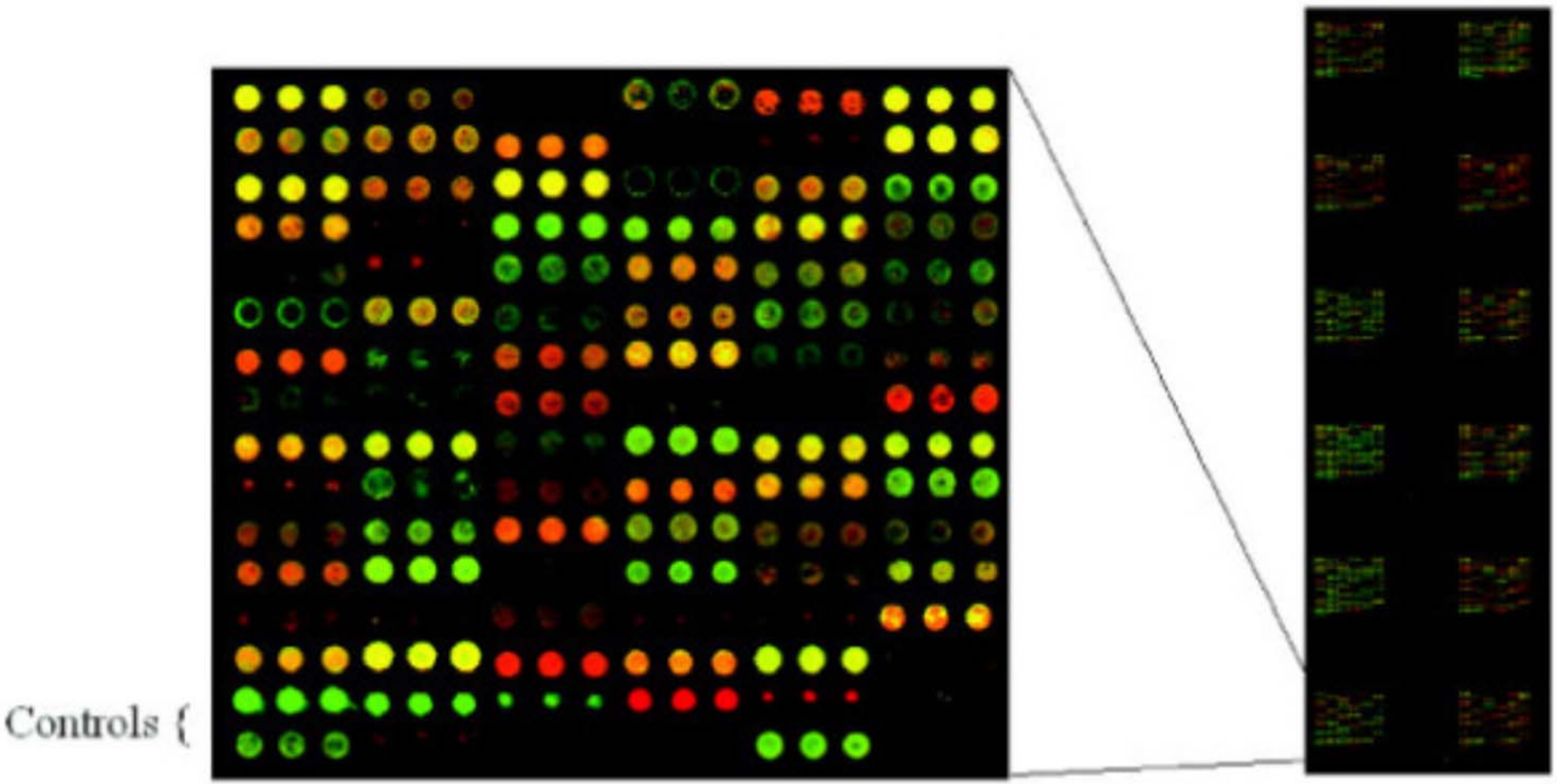
Can combinations of known markers improve the accuracy of the test?

Antibody microarrays,
Luminex analysis

Antibody microarrays

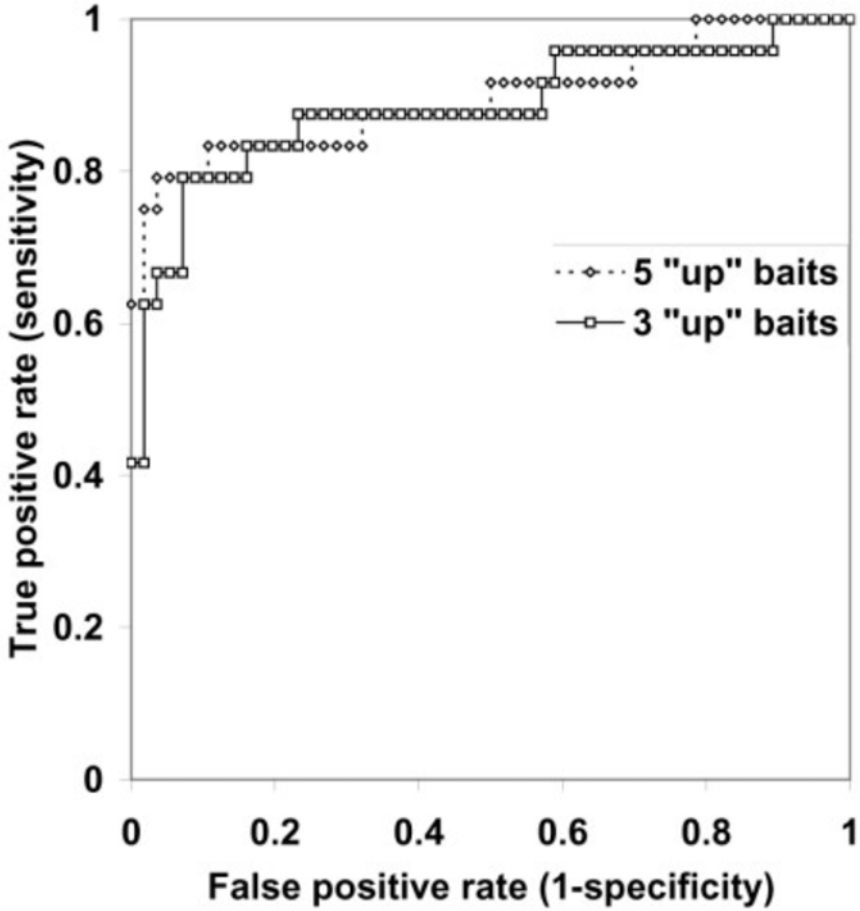
- Gao et al, BMC Cancer. 2005 Aug 23;5:110.
- 84 antibodies, 80 antigens
- 24 lung cancers, 24 healthy and 32 pts with COPD
- CRP (13.3 fold), SAA (2.0 fold), AAT (1.4 fold) and MUC1 (1.4 fold)

Antibody array: evaluating blood for a panel of candidate biomarkers



Gao et al, BMC Cancer. 2005 Aug 23;5:110.

Antibody array ROC results

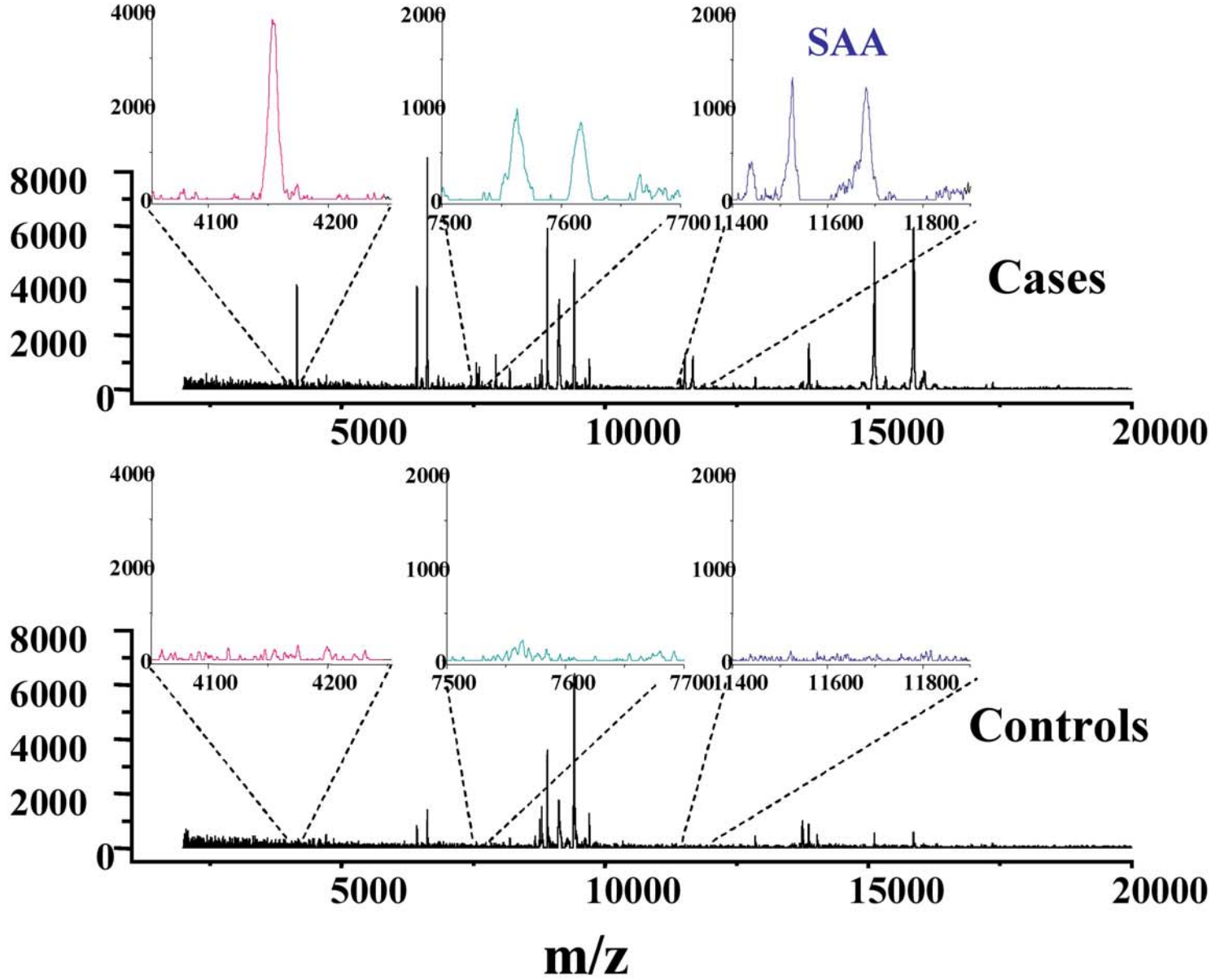


CRP
SAA
AAT
MUC1

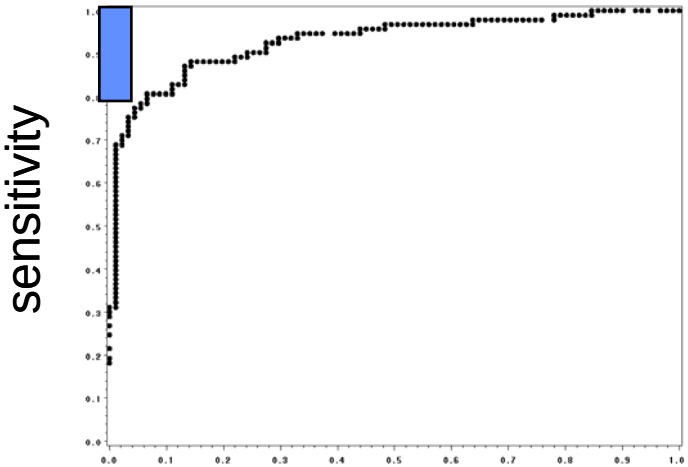
Gao et al, BMC Cancer. 2005 Aug 23;5:110.

Unbiased discovery

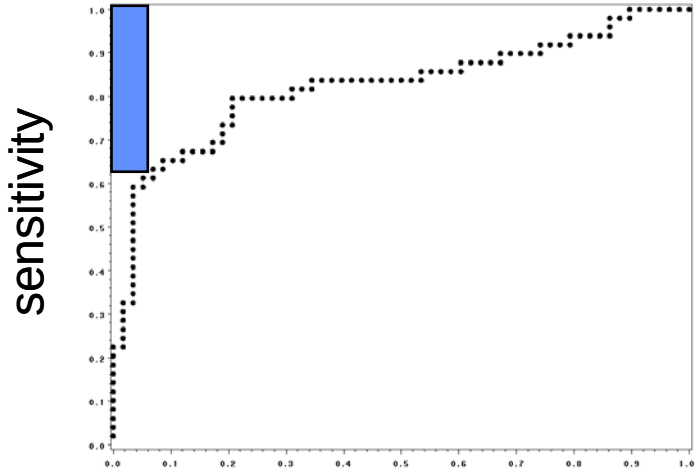
Discriminant features by MALDI



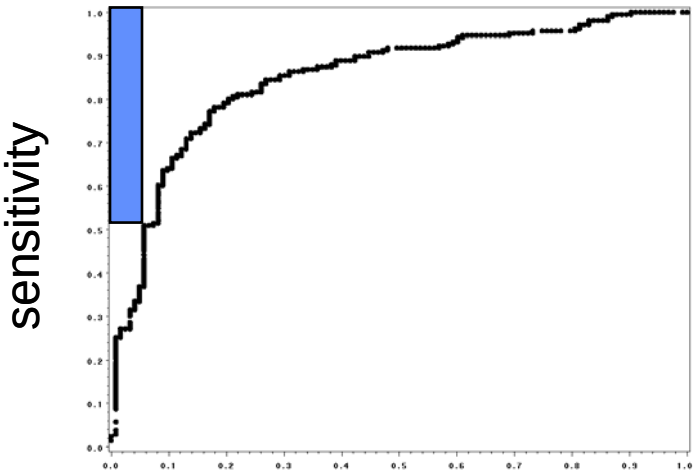
291- Training set



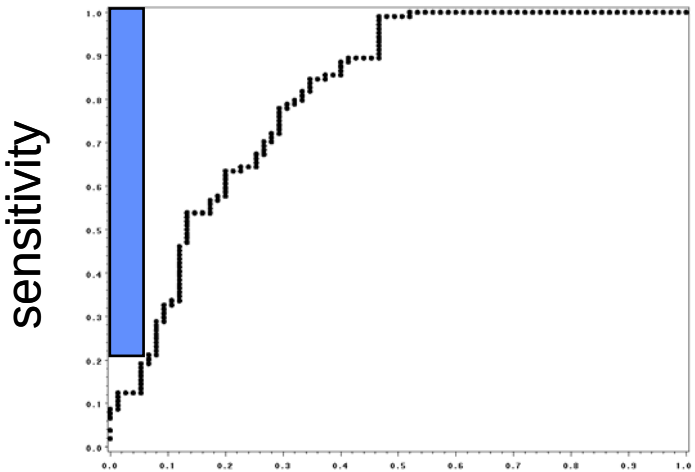
291- Testing Set



508- Training set

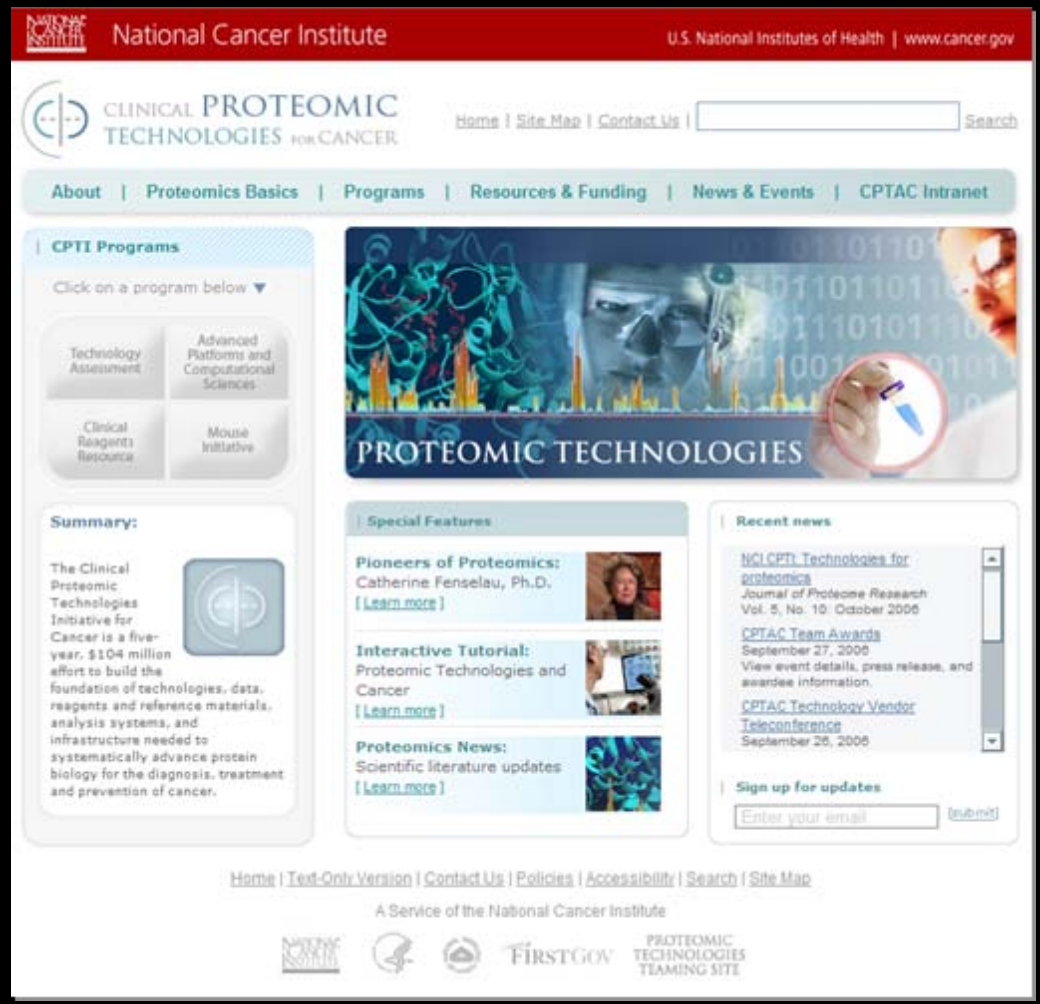


508- Testing Set



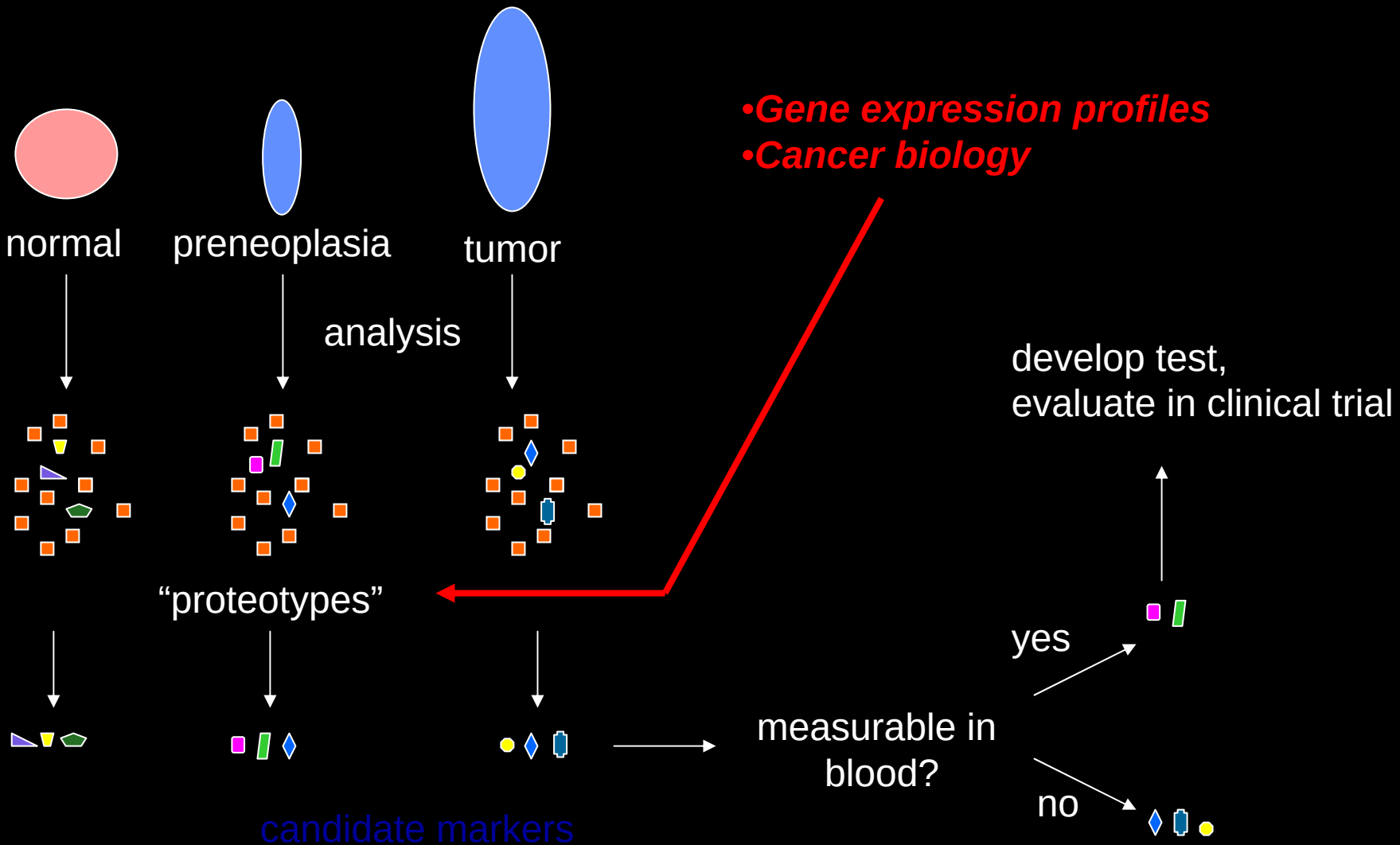
NCI Clinical Proteomic Technologies Initiative for Cancer

- 5-year initiative
- Build a foundation of technologies, data, reagents and standards, analysis systems, and infrastructure
- Emphasis on standardization of technology platforms
- Accelerate translation of discovery research and clinical applications

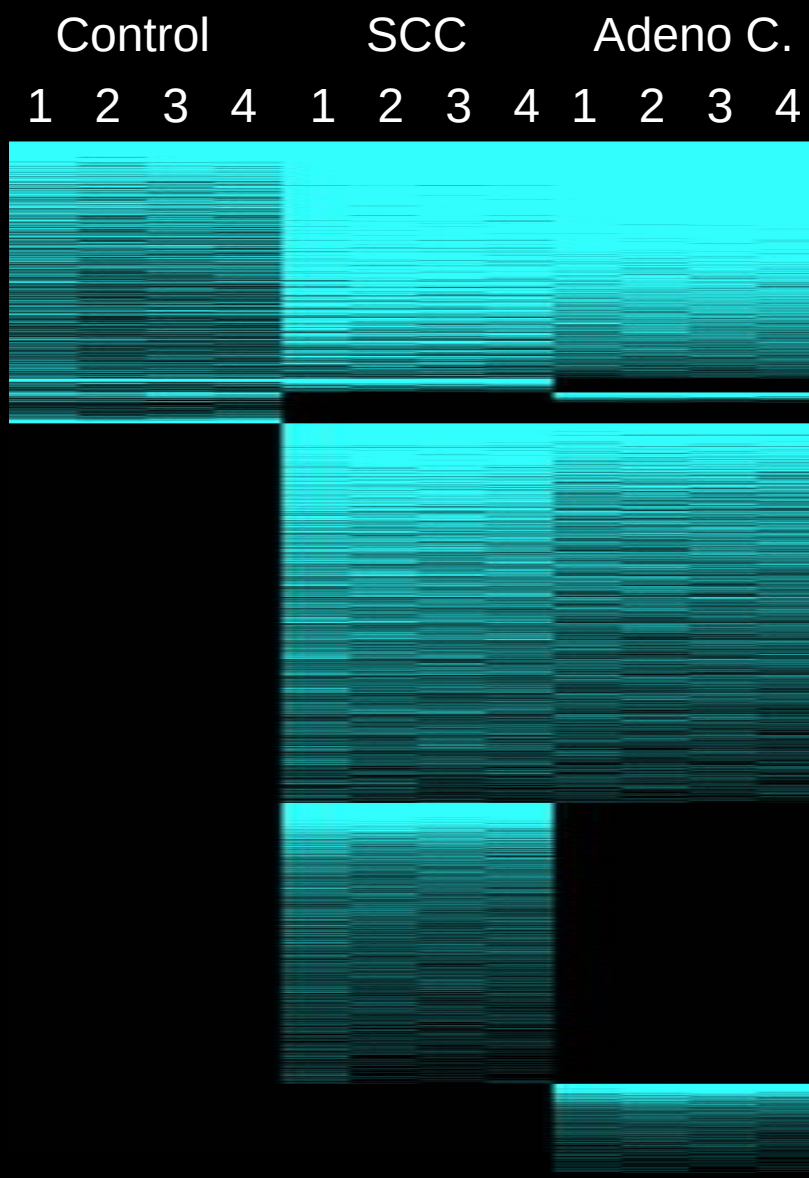


http://proteomics.cancer.gov

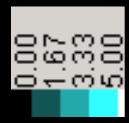
Approach to serum biomarkers by MS



Heatmap view of identified proteins



Total 3273 proteins



All pools:753 proteins

Cancer pools:1204 proteins

SCC pool:891 proteins

Adeno Ca. pools:283 proteins

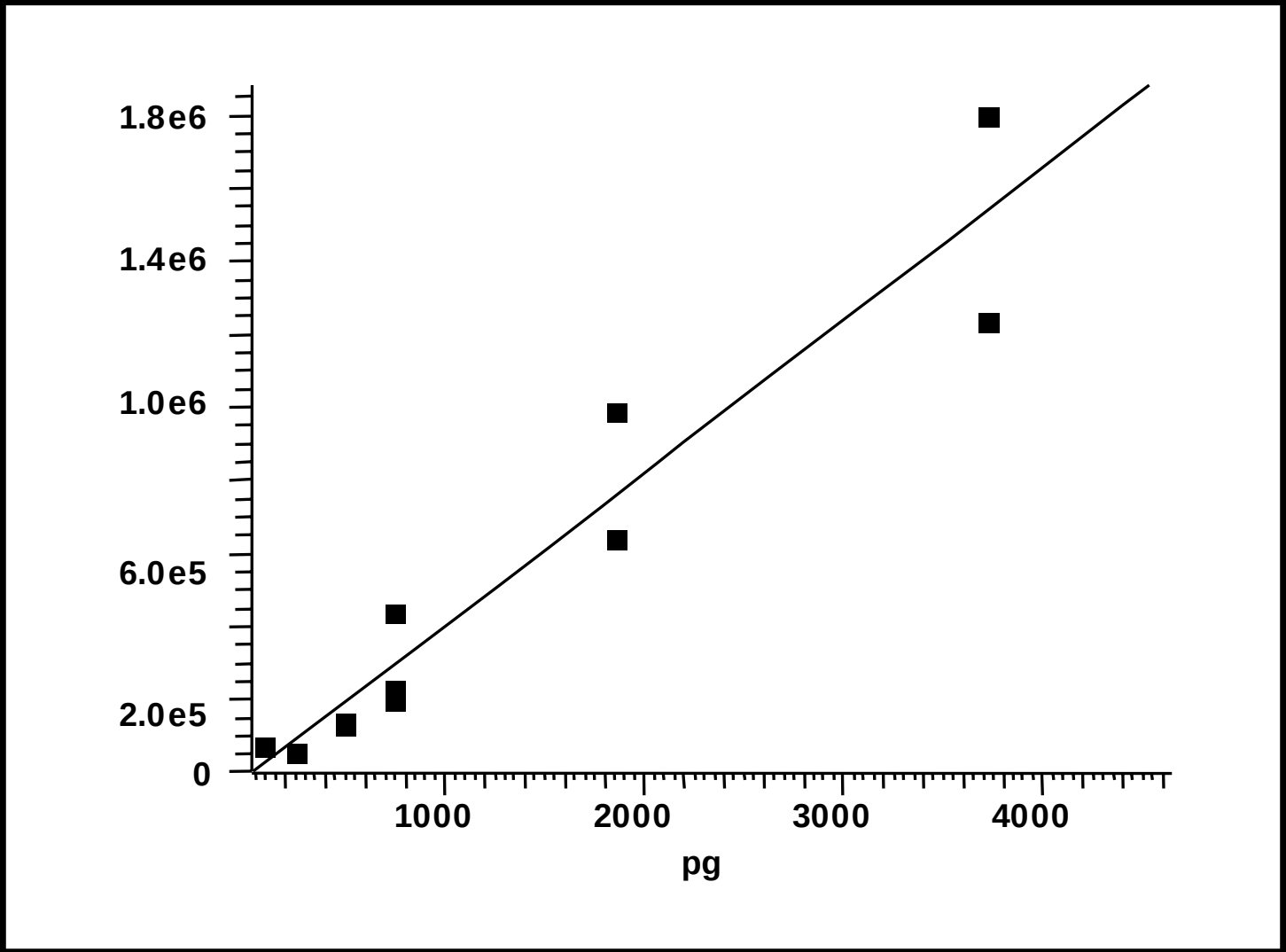
Differential proteins by MS/MS

Protein Name	SUM-N	SUM-T
Major vault protein	0	48
Protein DJ-1	0	48
Splice Isoform Sap-mu-0 of Proacti	0	43
Hepatoma-derived growth factor	0	42
29 kDa protein	0	39
14-3-3 protein theta	0	38
14-3-3 protein beta/alpha	0	38
CDNA FLJ45525 fis, clone BRTHA2	0	36
Galectin-3 binding protein precurs	0	34
Cargo selection protein TIP47	0	33
P63 protein	0	32
60S acidic ribosomal protein P2	0	31
19 kDa protein	0	31
Cathepsin B precursor	0	31
poly(rC)-binding protein 2 isoform	0	29
14-3-3 protein sigma	0	29
Splice Isoform A of Chloride intrac	0	29
14-3-3 protein epsilon	0	28
Chloride intracellular channel prot	0	28
Glucosidase II beta subunit precur	0	28
pyruvate kinase 3 isoform 2	0	28
Lactotransferrin precursor	0	28

Candidate marker identification

		Pooled sample shotgun		
Name		**Control**	**SCC**	**Adeno Ca.**
CD26	IPI00018953	**0**	**4**	**7**
CYFRA 21-1	IPI00479145	**0**	**372**	**330**
NSE	IPI00216171	**0**	**35**	**53**
KL-6/MUC1	IPI00013955	**0**	**0**	**8**
Napsin A	IPI00014055	**0**	**0**	**34**
Plunc	IPI00009856	**0**	**0**	**10**
SCC	IPI00022204	**0**	**9**	**0**
CEA	IPI00654795	**0**	**8**	**13**

Peptide quantitation in serum



Shotgun Conclusions

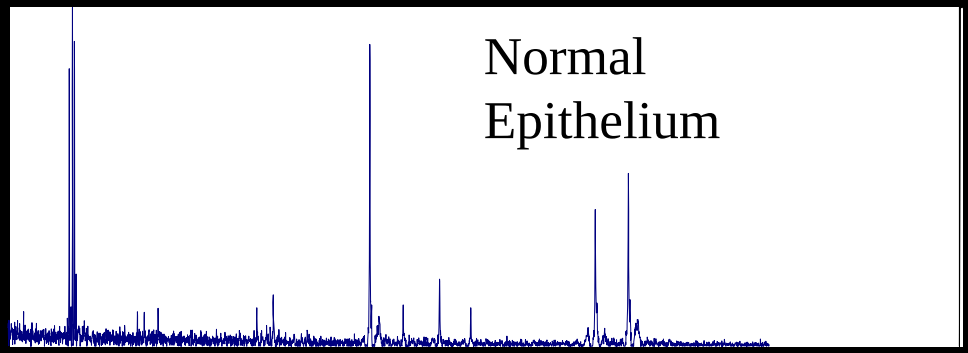
- We identified >5,000 proteins from Normal, Adeno Ca. and SCC
- For tumor marker discovery, shotgun proteomics has promising power to find molecules expressed and post-translationally modified in tumor tissues at the protein level.
- We are in the process of evaluating these markers as preneoplastic markers in tissue and diagnostic markers in peripheral blood.

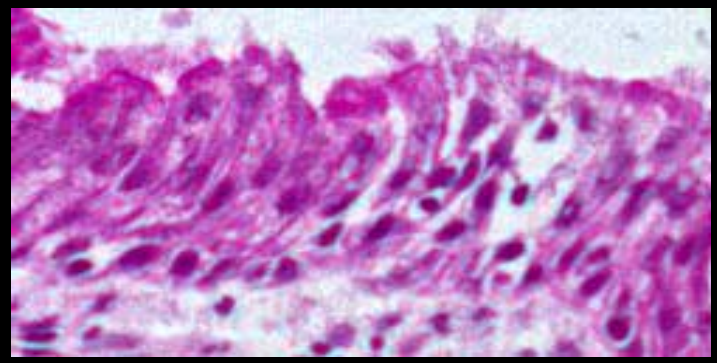
Diagnosis of preneoplastic lesions

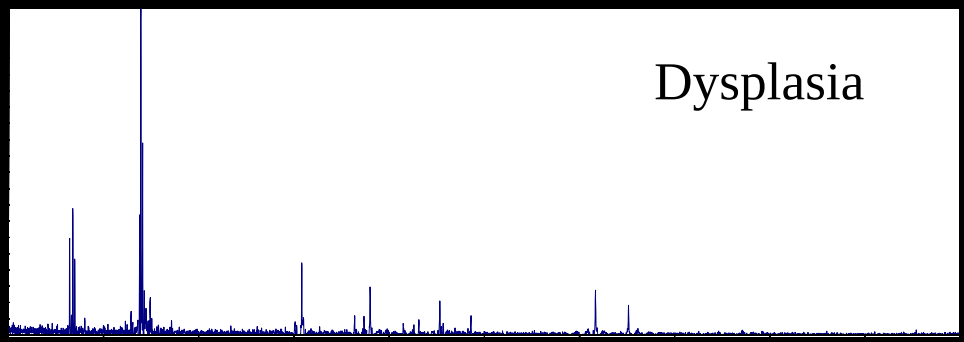
Which will progress to cancer and which will not?

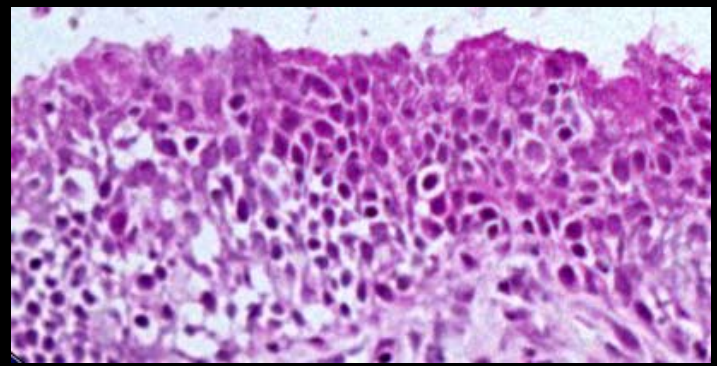
Biomarkers needed to assess efficacy in chemoprevention trials

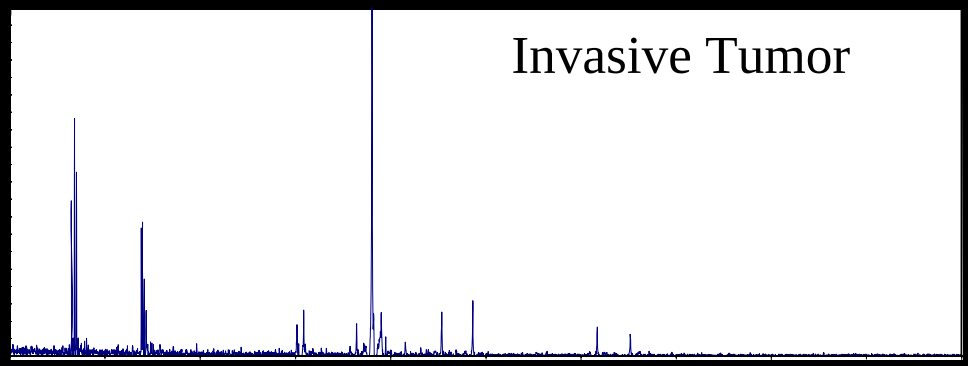
Spectra obtained from different regions of lung squamous cell carcinoma

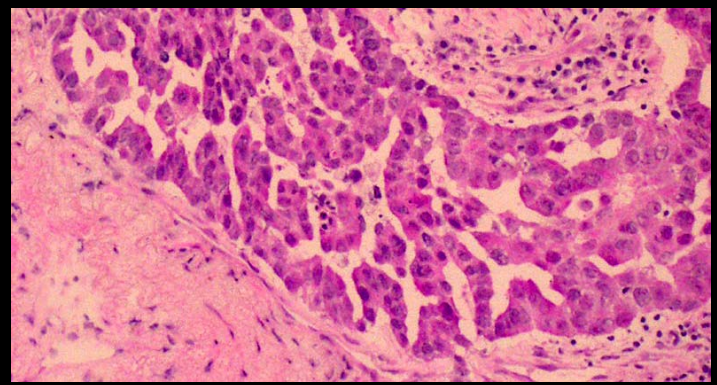


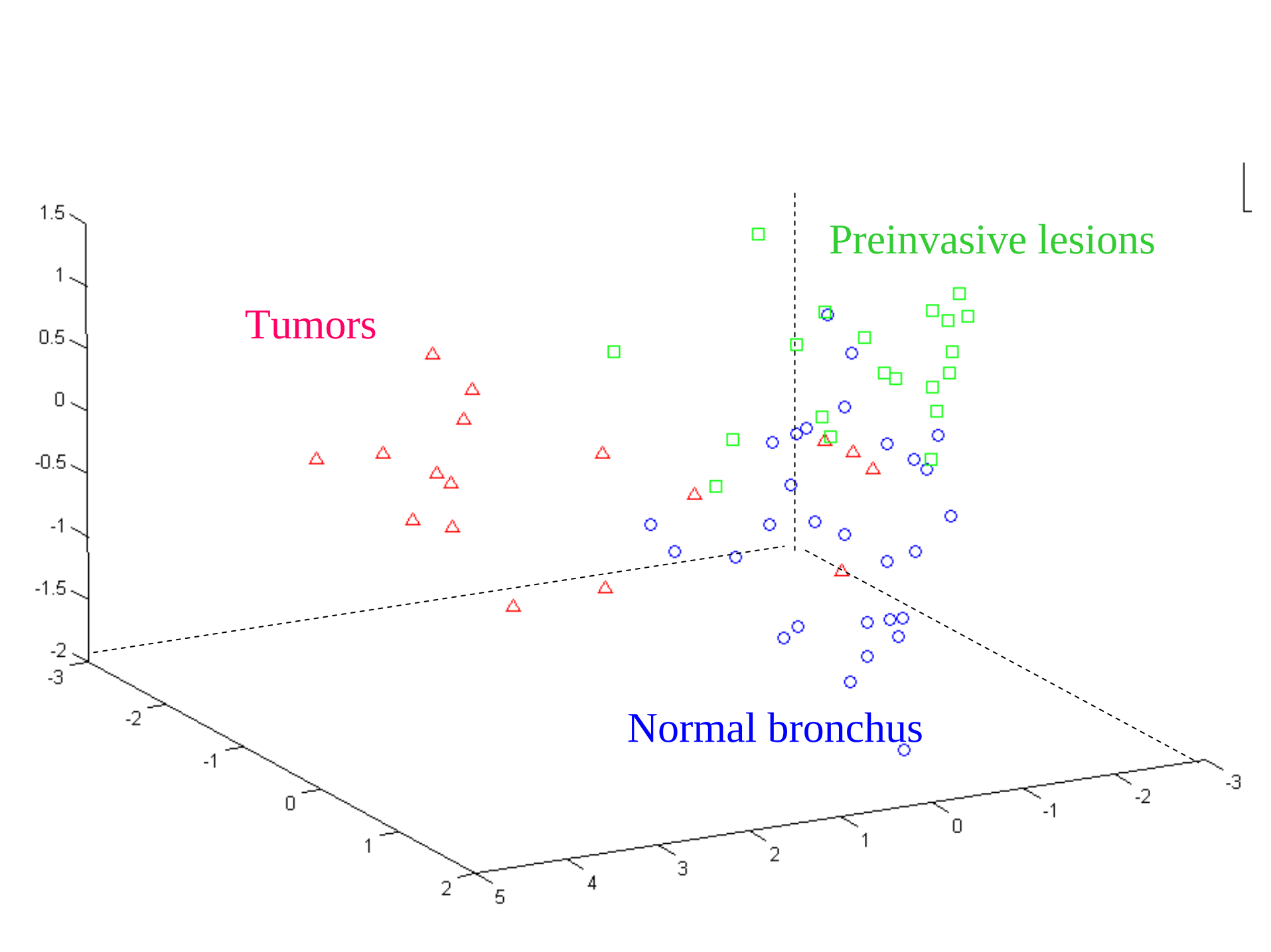











Tumors
Preinvasive lesions
Normal bronchus
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-3
-2
-1
0
1
2
5
4
3
2
1
0
-1
-2
-3

Predictive signatures

Lung Cancer Cell Line Sensitivities

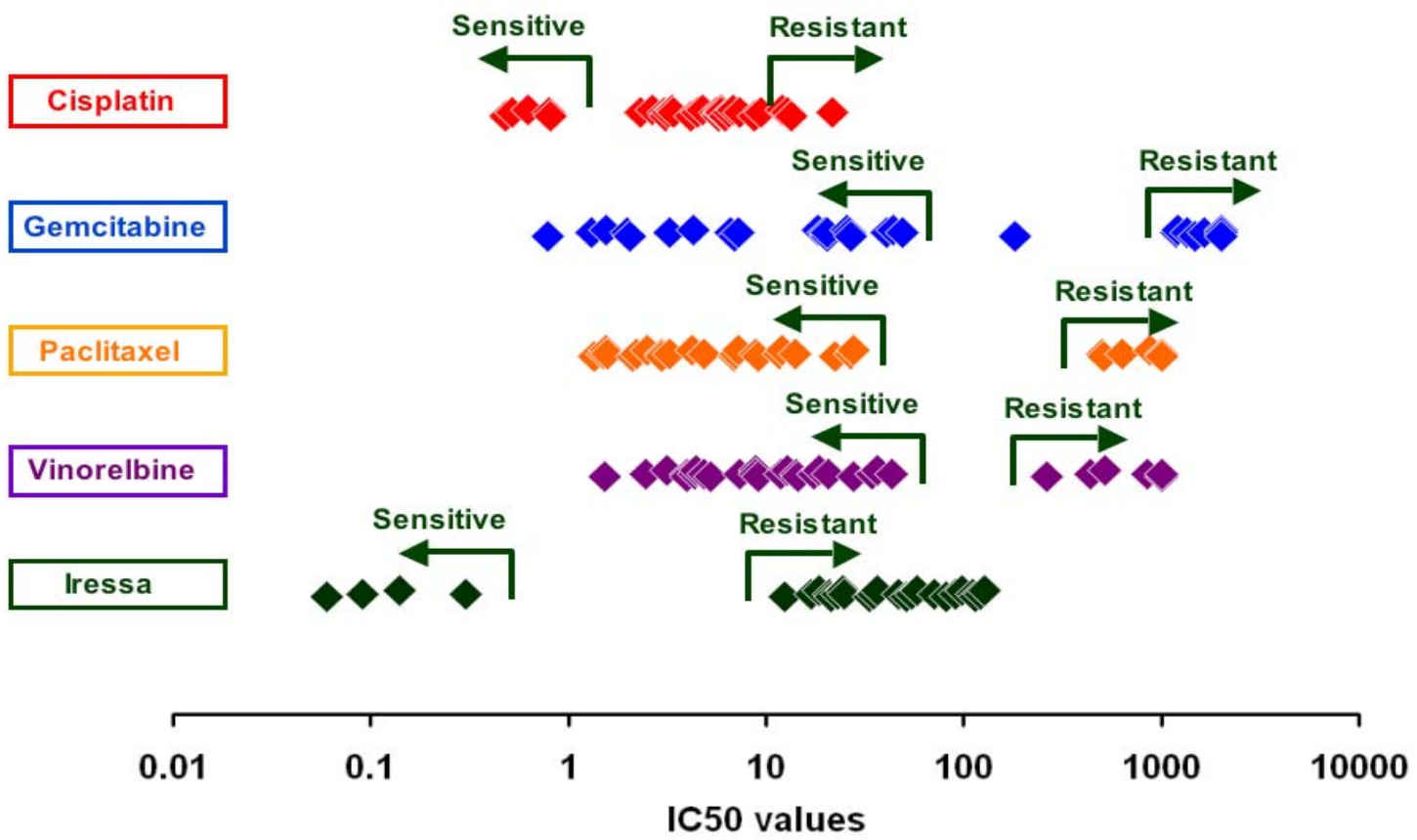
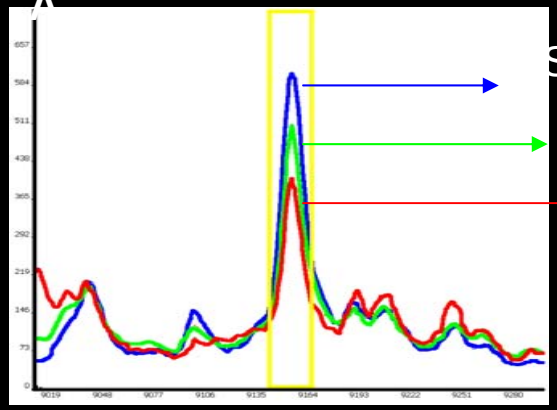


Figure 2 IC_{50} values for each drug show a range of *in vitro* sensitivity across the different cell lines. For most drugs, tumor cell lines fall into distinct sensitivity and resistant phenotypes.

Prediction of response
Discriminant signals

Figure 3

A

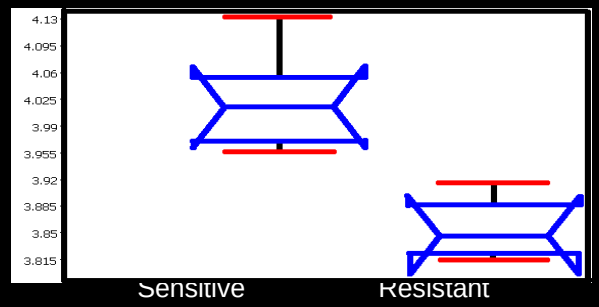


Sensitive

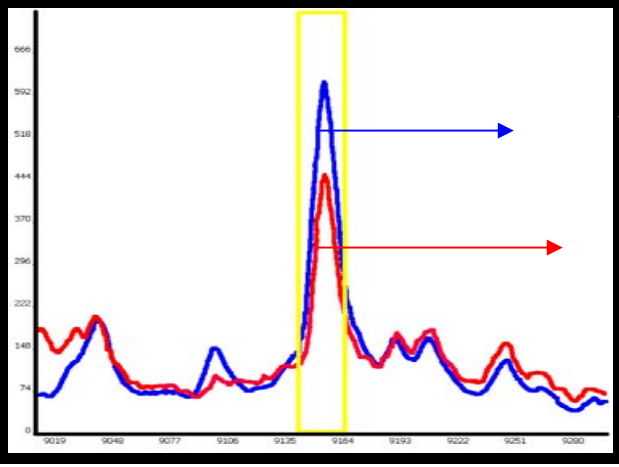
Intermediate

Resistant

Vinorelbine: m/z 9154



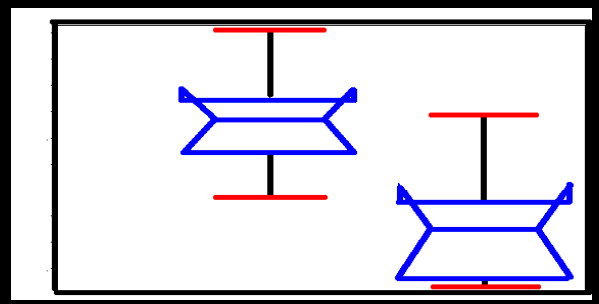
B

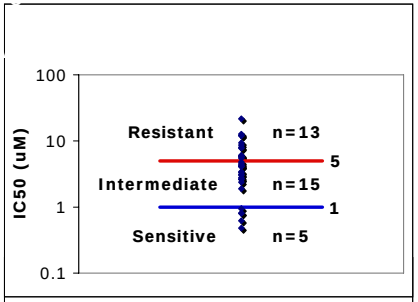


Sensitive

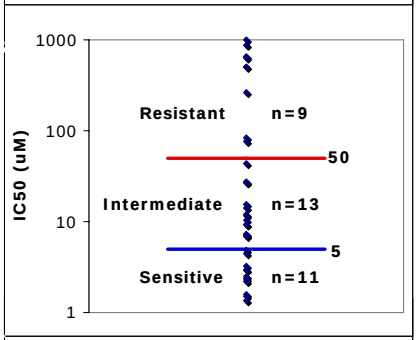
Resistant

Paclitaxel: m/z 9154

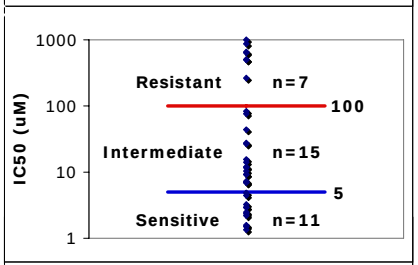




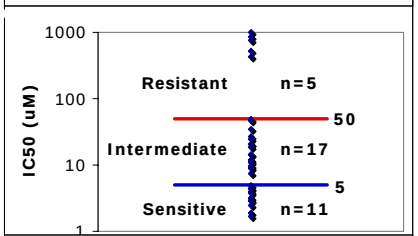
Group 1		Group 2	Overall	Group 1	Group 2
Sens.	vs	Inter. + Res.	**0.76**	0.80	0.75
Sens. + Inter	vs	Res.	**0.79**	0.80	0.77
Sens.	vs	Res.	**0.89**	1.00	0.85



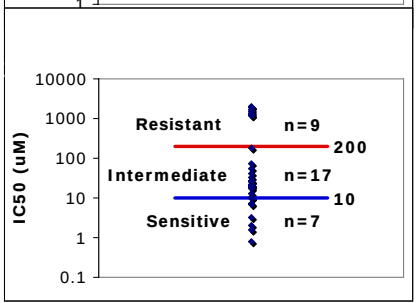
Group 1		Group 2	Overall	Group 1	Group 2
Sens.	vs	Inter. + Res.	**0.82**	0.91	0.77
Sens. + Inter	vs	Res.	**0.91**	0.92	0.89
Sens.	vs	Res.	**0.85**	0.91	0.78



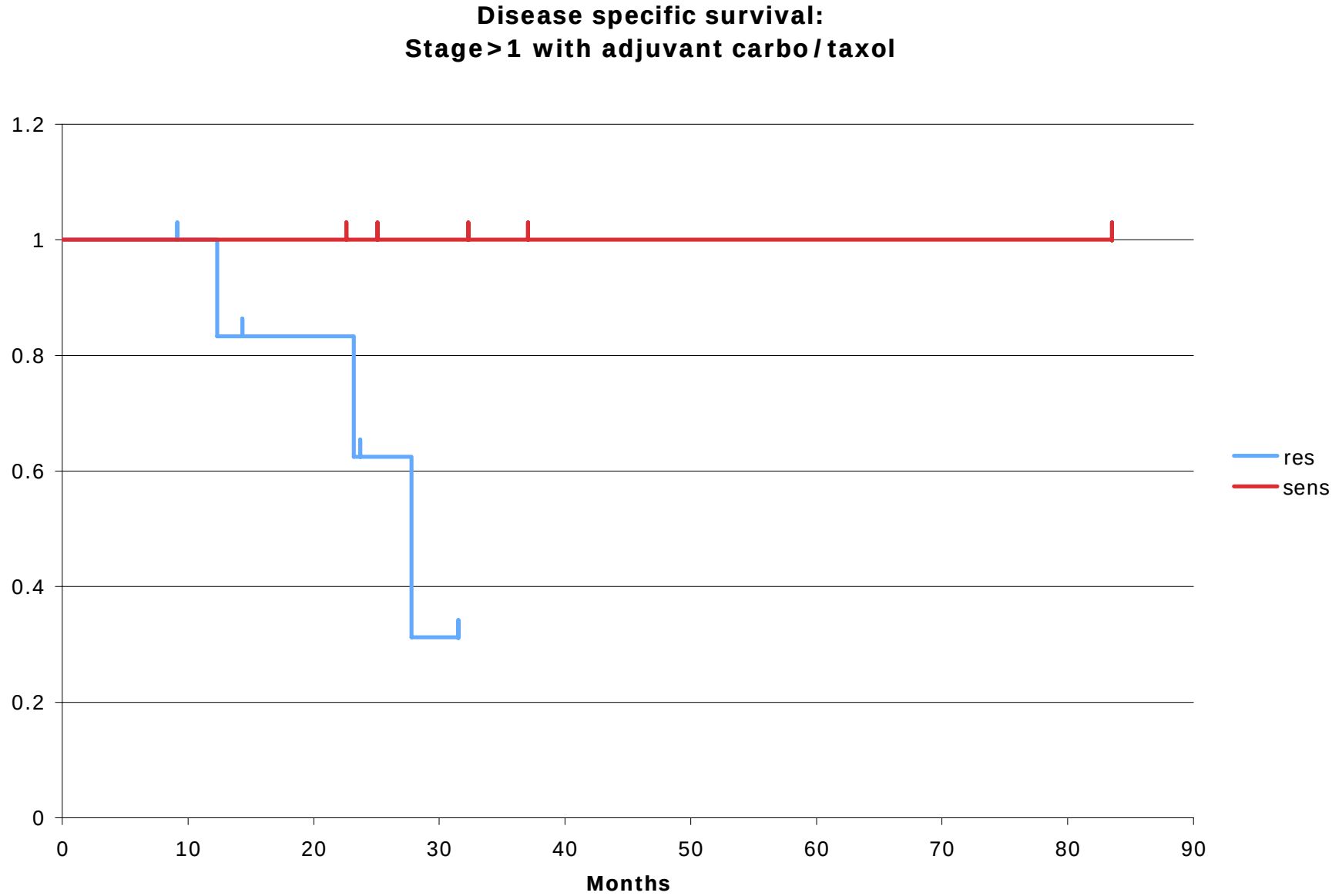
Group 1		Group 2	Overall	Group 1	Group 2
Sens.	vs	Inter. + Res.	**0.82**	0.91	0.77
Sens. + Inter	vs	Res.	**1.00**	1.00	1.00
Sens.	vs	Res.	**0.94**	0.91	1.00

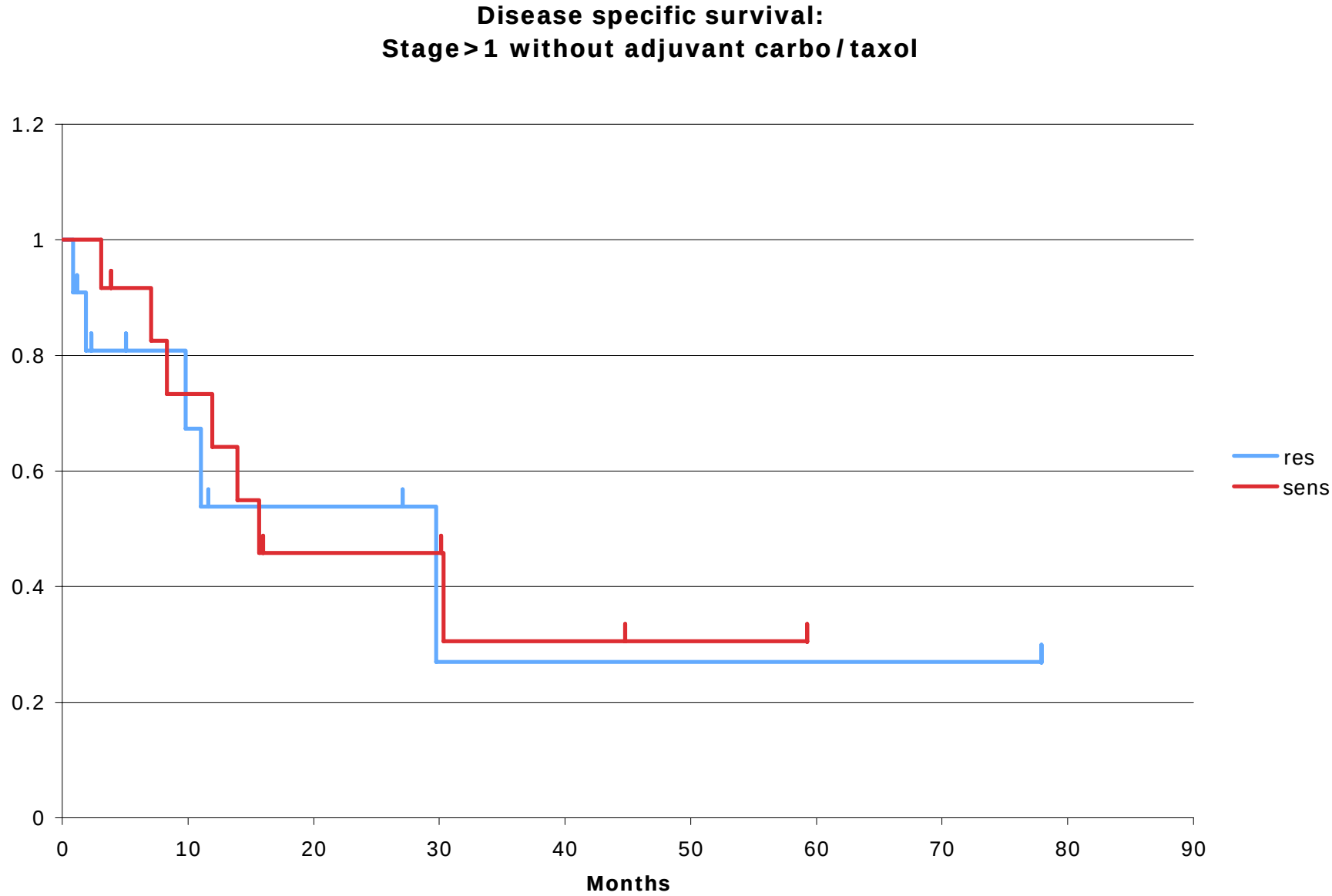


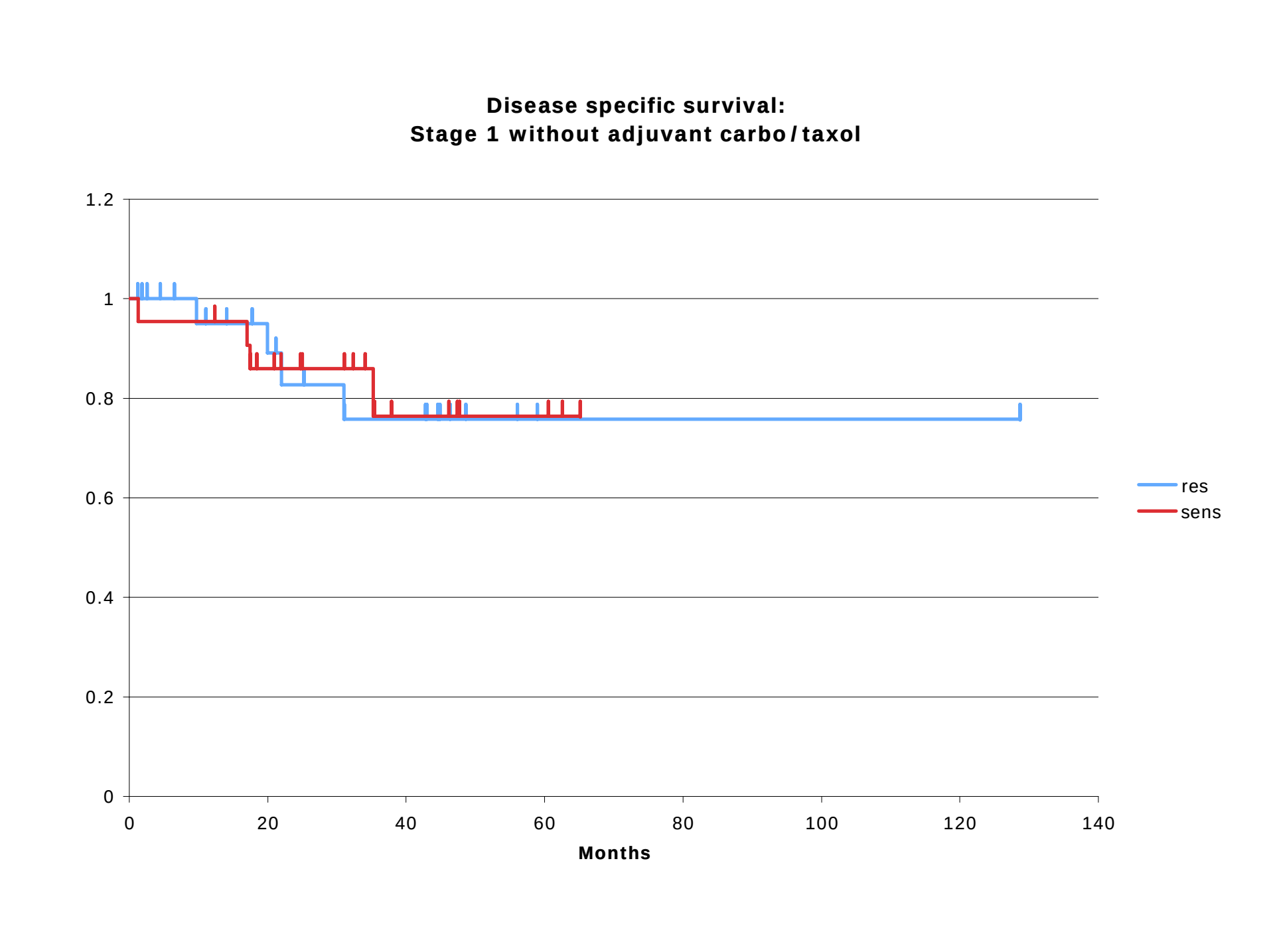
Group 1		Group 2	Overall	Group 1	Group 2
Sens.	vs	Inter. + Res.	**0.67**	0.64	0.68
Sens. + Inter	vs	Res.	**0.94**	0.93	1.00
Sens.	vs	Res.	**1.00**	1.00	1.00



Group 1		Group 2	Overall	Group 1	Group 2
Sens.	vs	Inter. + Res.	**0.85**	0.86	0.85
Sens. + Inter	vs	Res.	**0.82**	0.83	0.78
Sens.	vs	Res.	**0.88**	1.00	0.78


Disease specific survival:
Stage>1 with adjuvant carbo/taxol
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
80
90
Months
res
sens

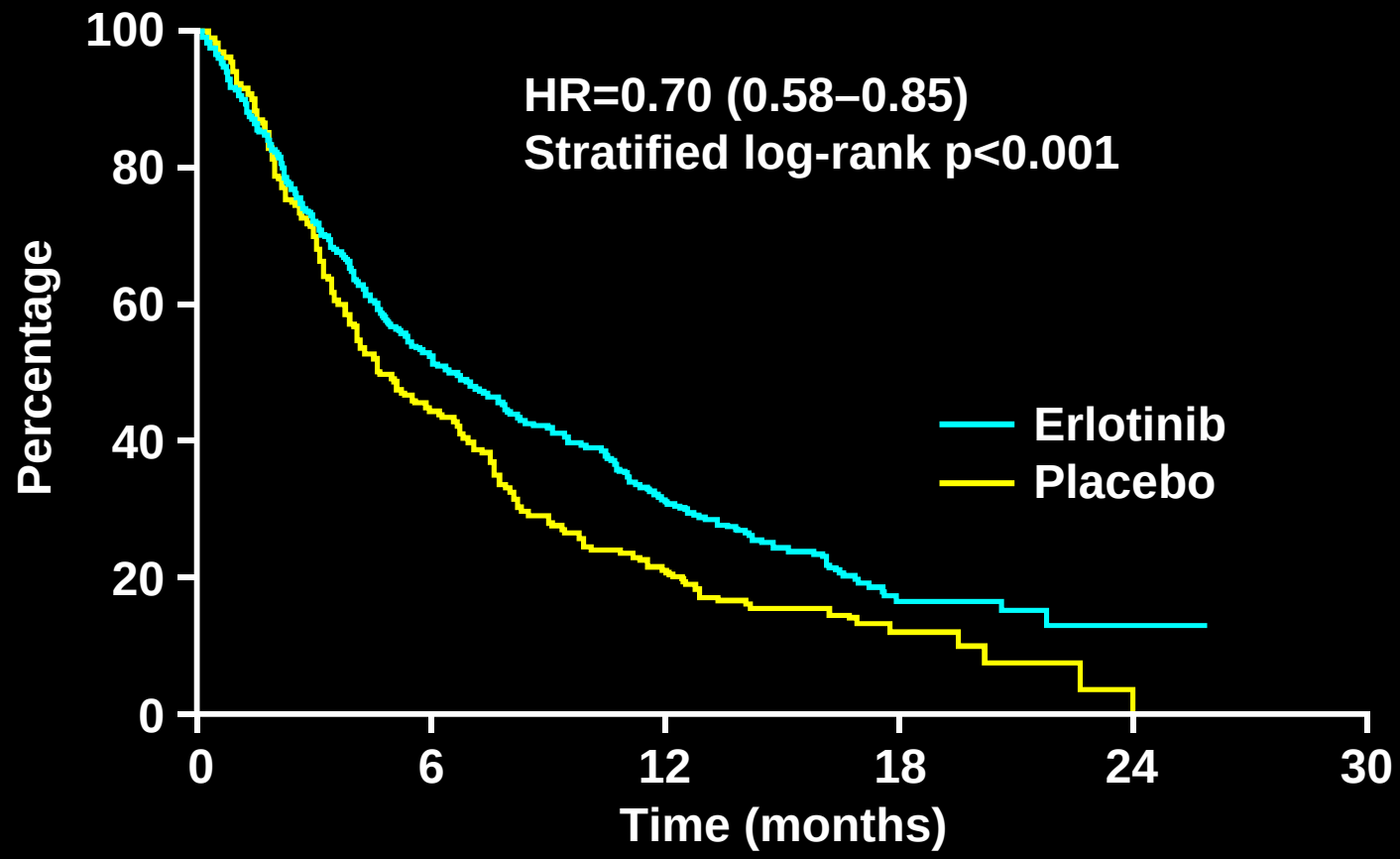

Disease specific survival:
Stage>1 without adjuvant carbo/taxol
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
80
90
Months
res
sens


Disease specific survival:
Stage 1 without adjuvant carbo/taxol
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
Months
res
sens

EGFr TKI

Mutations do not identify all patients with clinical benefit (especially in the west…)

BR.21: overall survival



At risk						
Erlotinib	488	255	145	23	4	0
Placebo	243	107	50	9	0	0

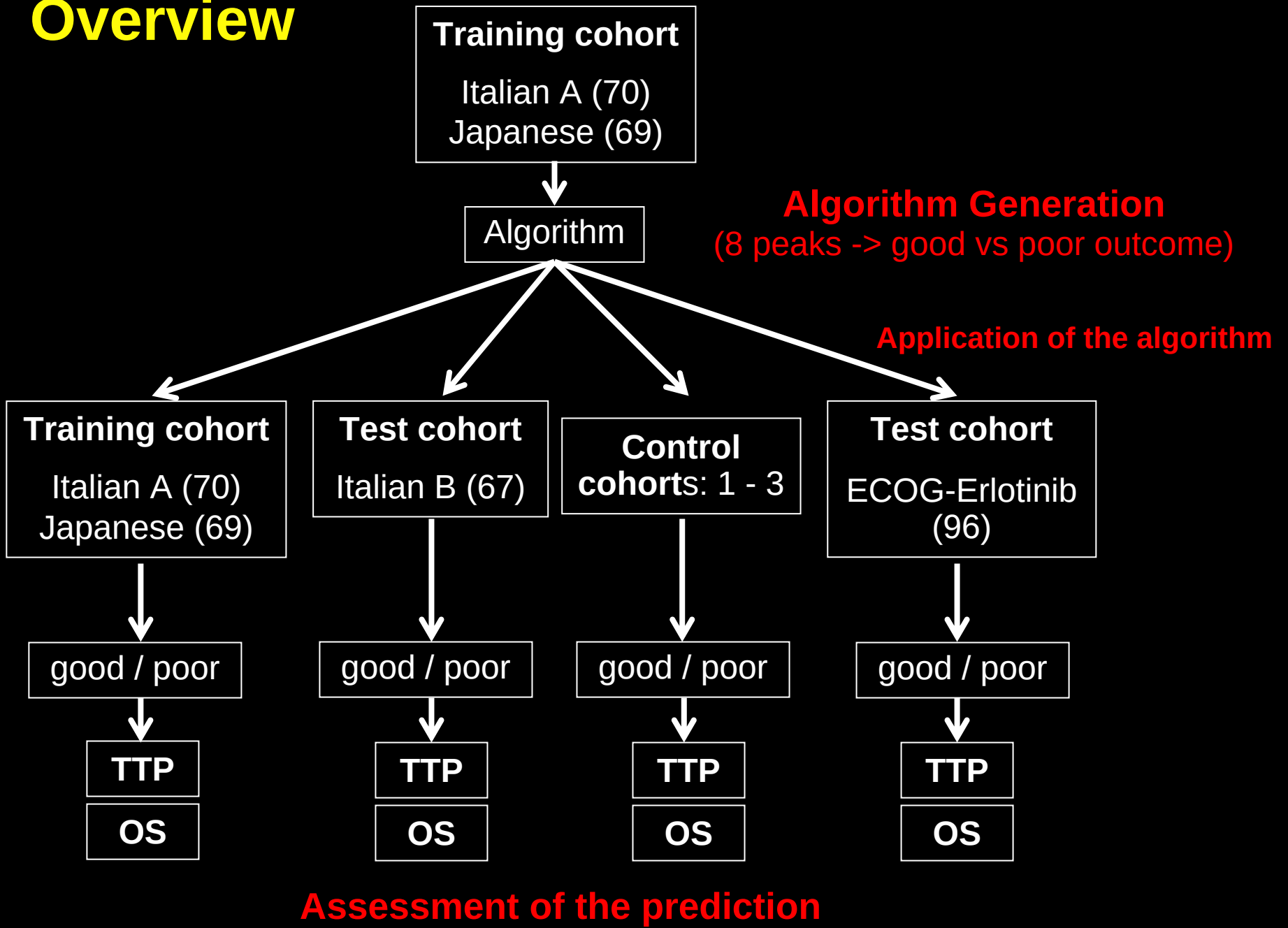
Shepherd F, et al. N Engl J Med 2005.

INTEREST

- Erlotinib v. Docetaxel
- No survival difference by:
  - EGFR mutations
    - Patients with EGFR mutant tumors did better in both arms
  - Ras mutations
    - Patients with ras mutant tumors did worse in both arms
  - FISH, Smoking status - no difference

Predicting response from pre-treatment sera

Overview

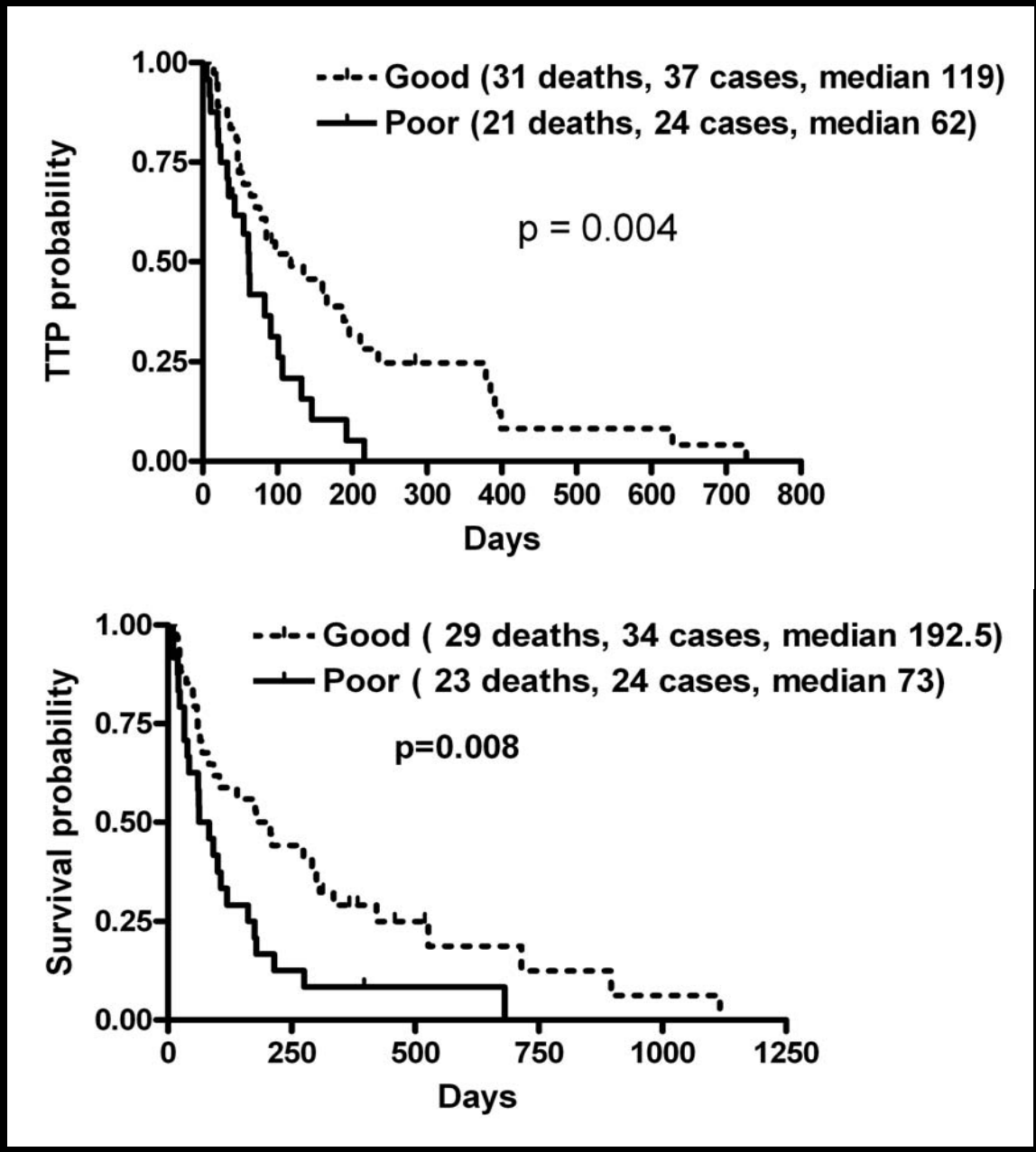

Training cohort
Italian A (70)
Japanese (69)
Algorithm
Algorithm Generation
(8 peaks -> good vs poor outcome)
Application of the algorithm
Training cohort
Italian A (70)
Japanese (69)
Test cohort
Italian B (67)
Control cohorts: 1 - 3
Test cohort
ECOG-Erlotinib (96)
good / poor
good / poor
good / poor
good / poor
TTP
OS
TTP
OS
TTP
OS
TTP
OS
Assessment of the prediction

Concordance of prediction results between Vanderbilt and Colorado

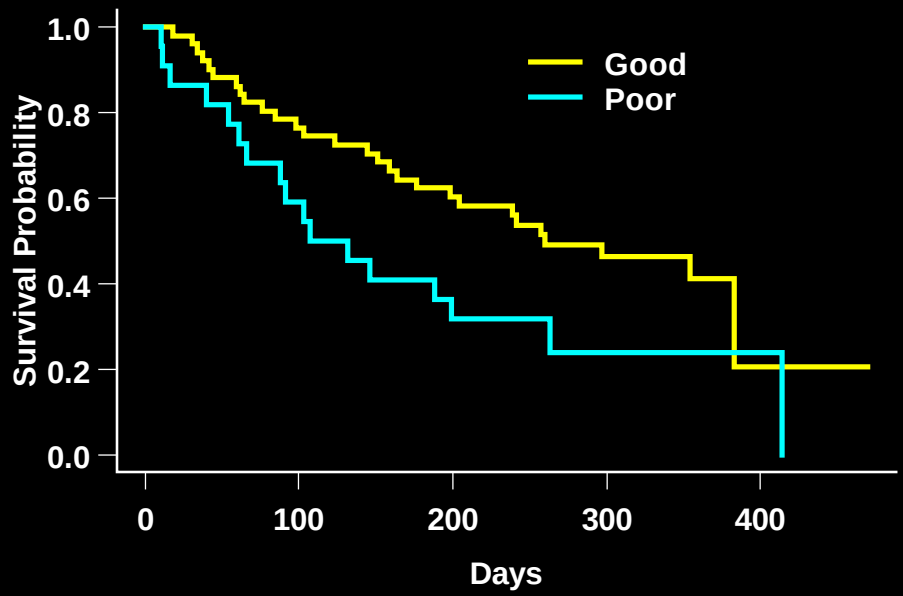
97.1 %

		UCCC		
		Good	Poor	Undefined
VU	Good	139	1	0
	Poor	2	59	0
	Undefined	3	0	2

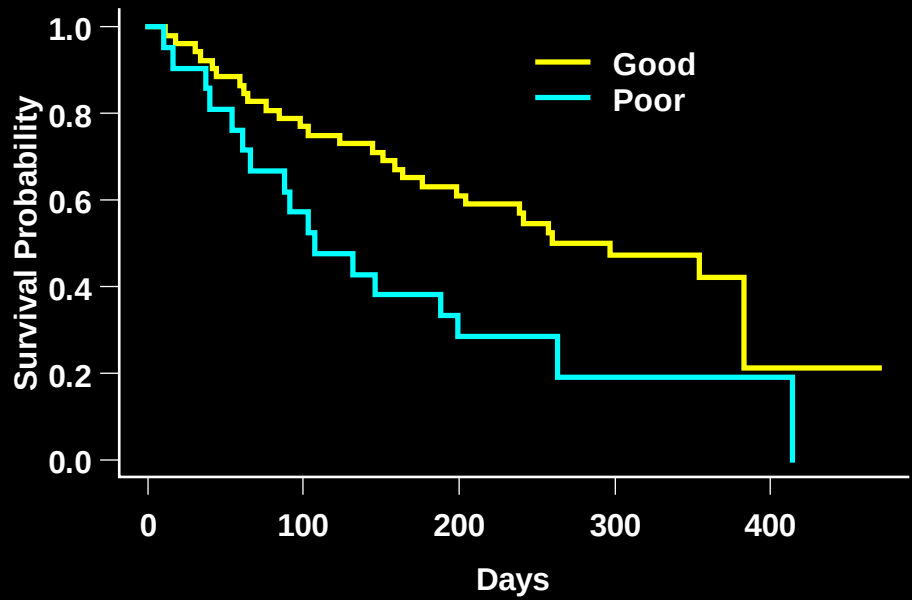
Test cohort (Italian B)



Prediction using serum or plasma (n=73)



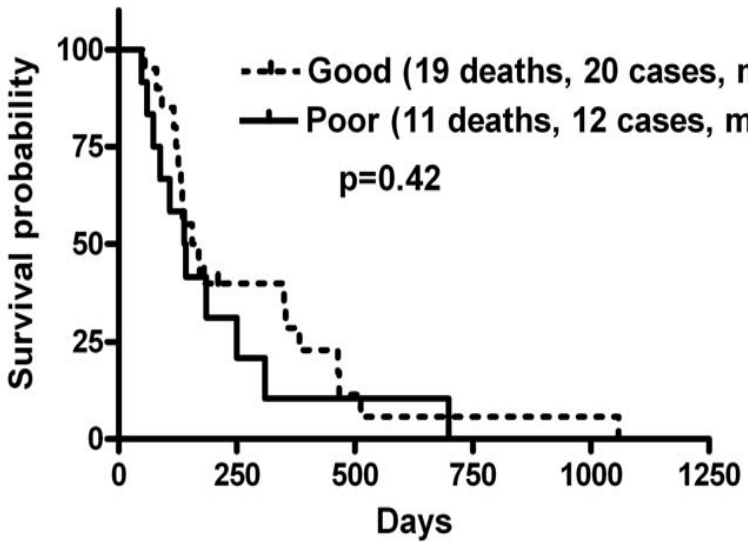
Serum



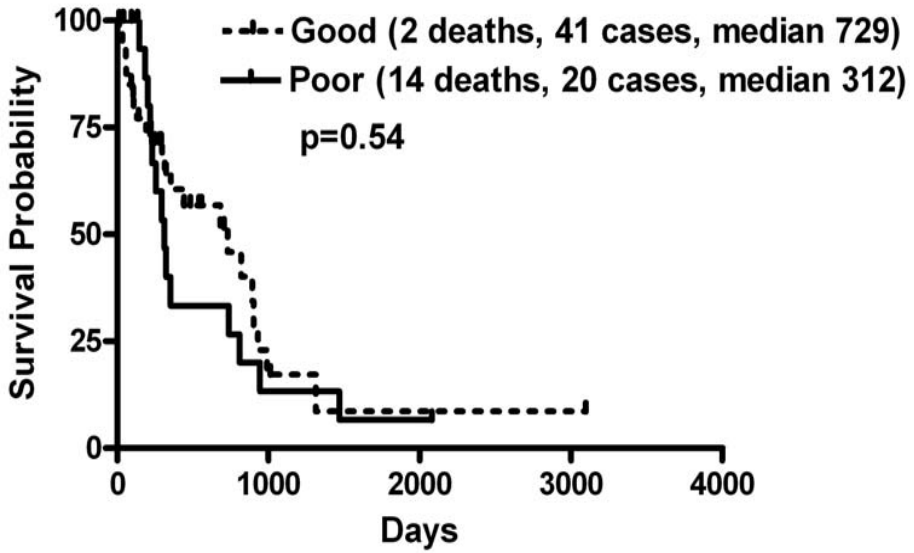
Plasma

Control test cohorts - chemo, no TKIs

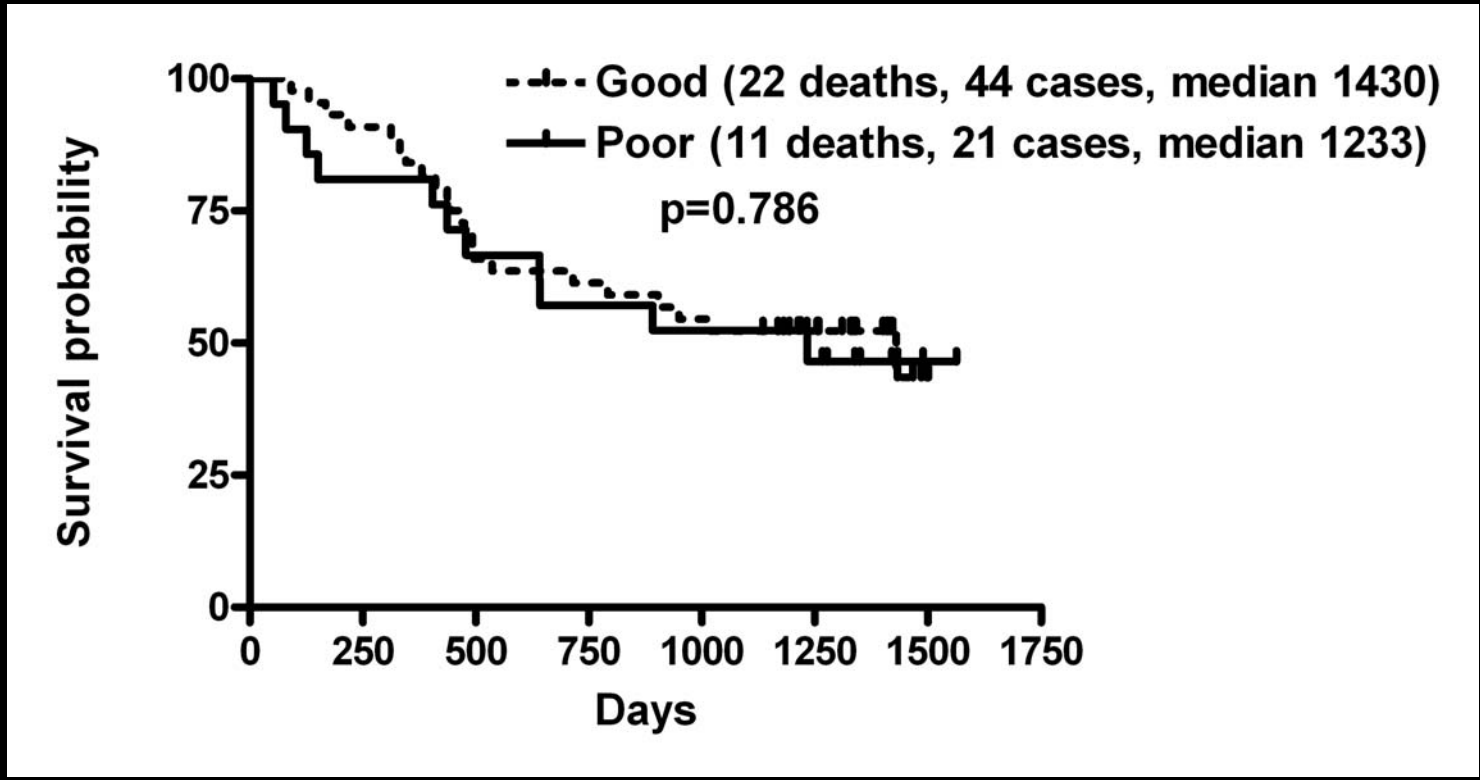
NSCLC patients from Italy (n = 32)



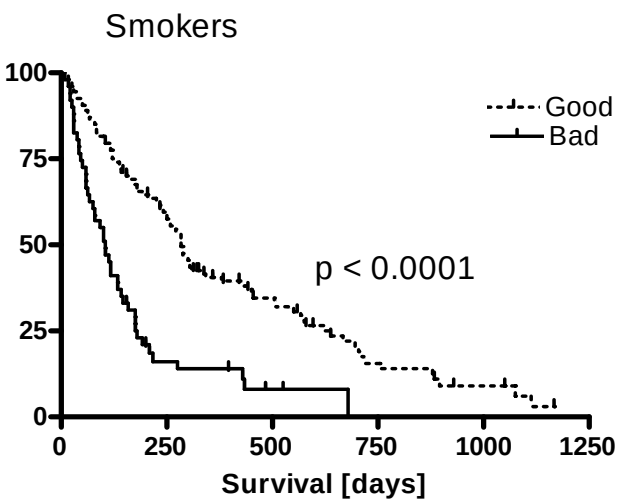
NSCLC patients from Tennessee (n = 61, stage IIIB or IV)

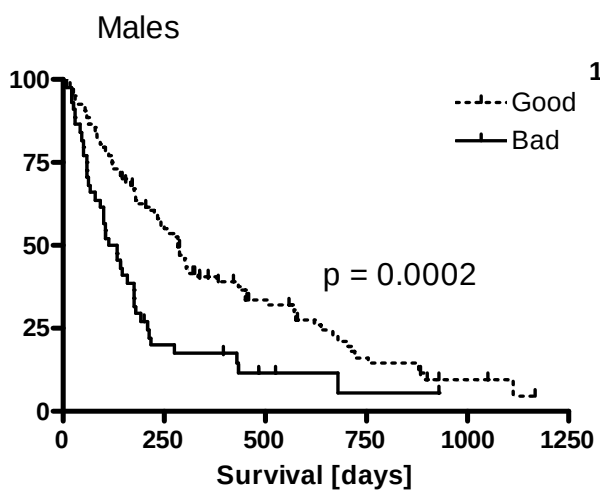


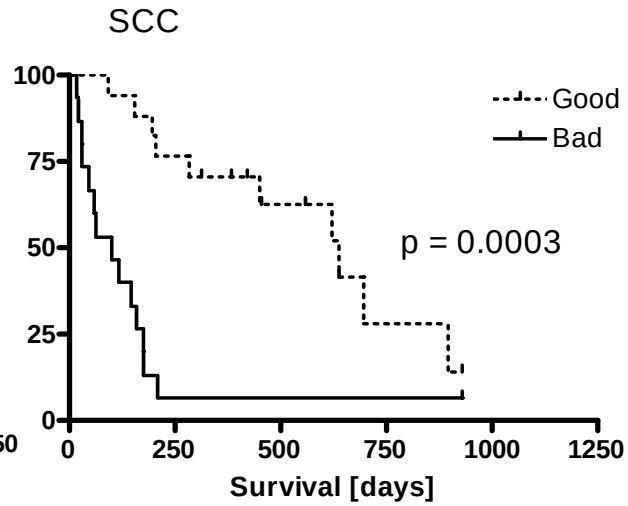
Test cohort 3: NSCLC s/p Surgery only (Poland)



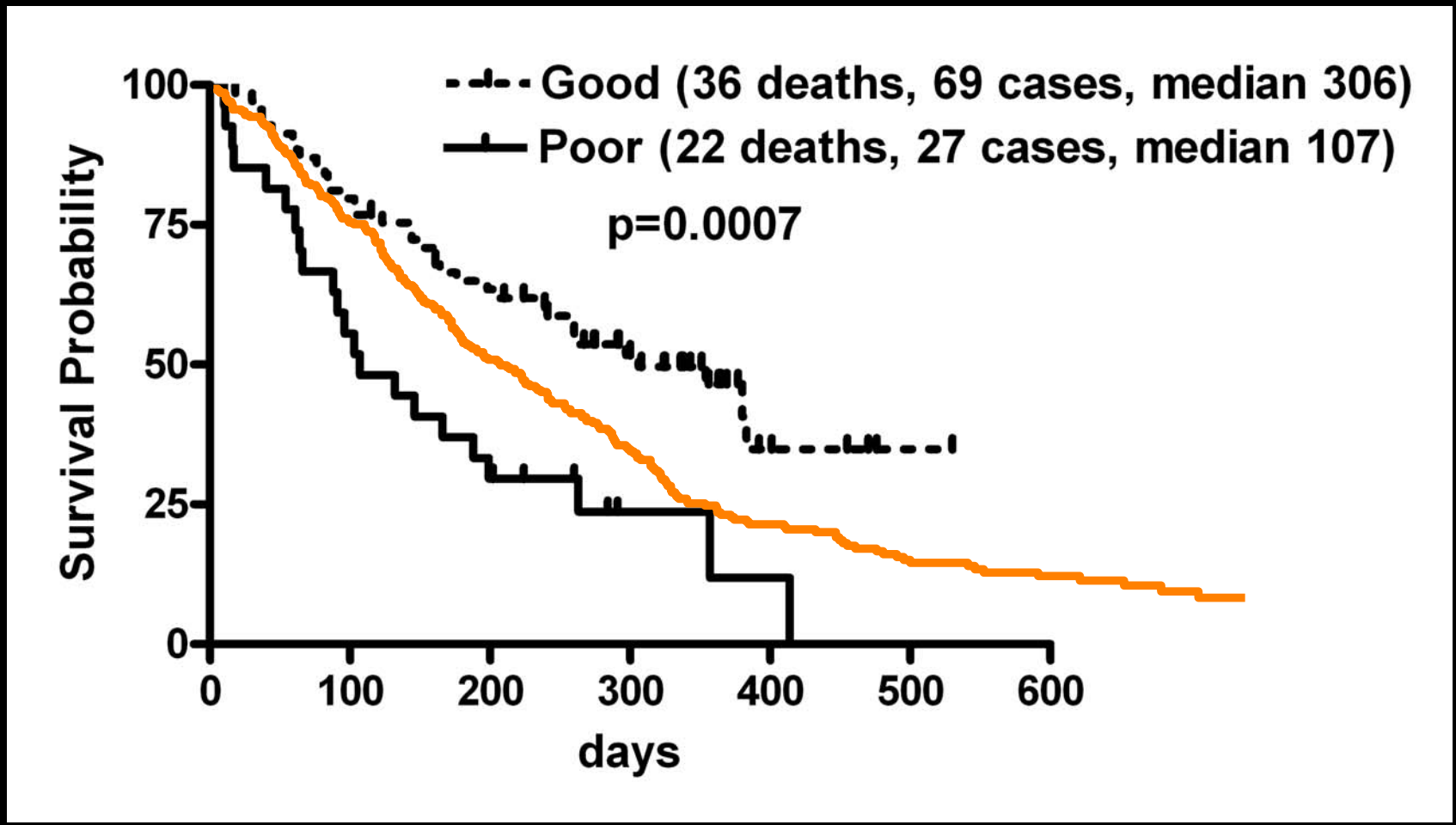
Benefit in subsets



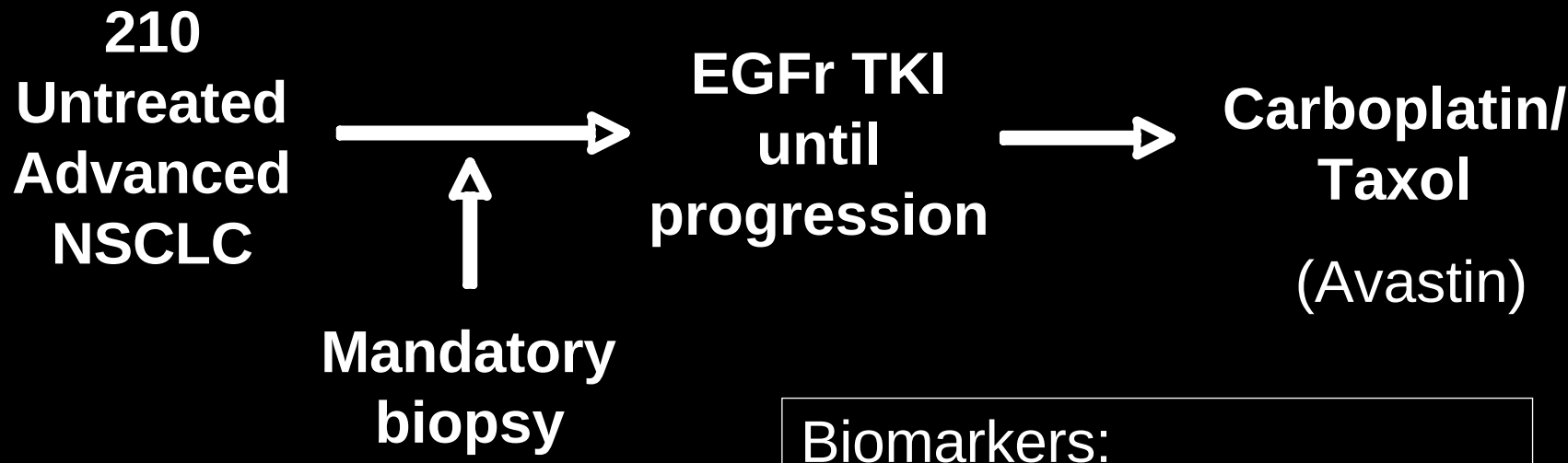




ECOG validation cohort: Advanced NSCLC, first line erlotinib



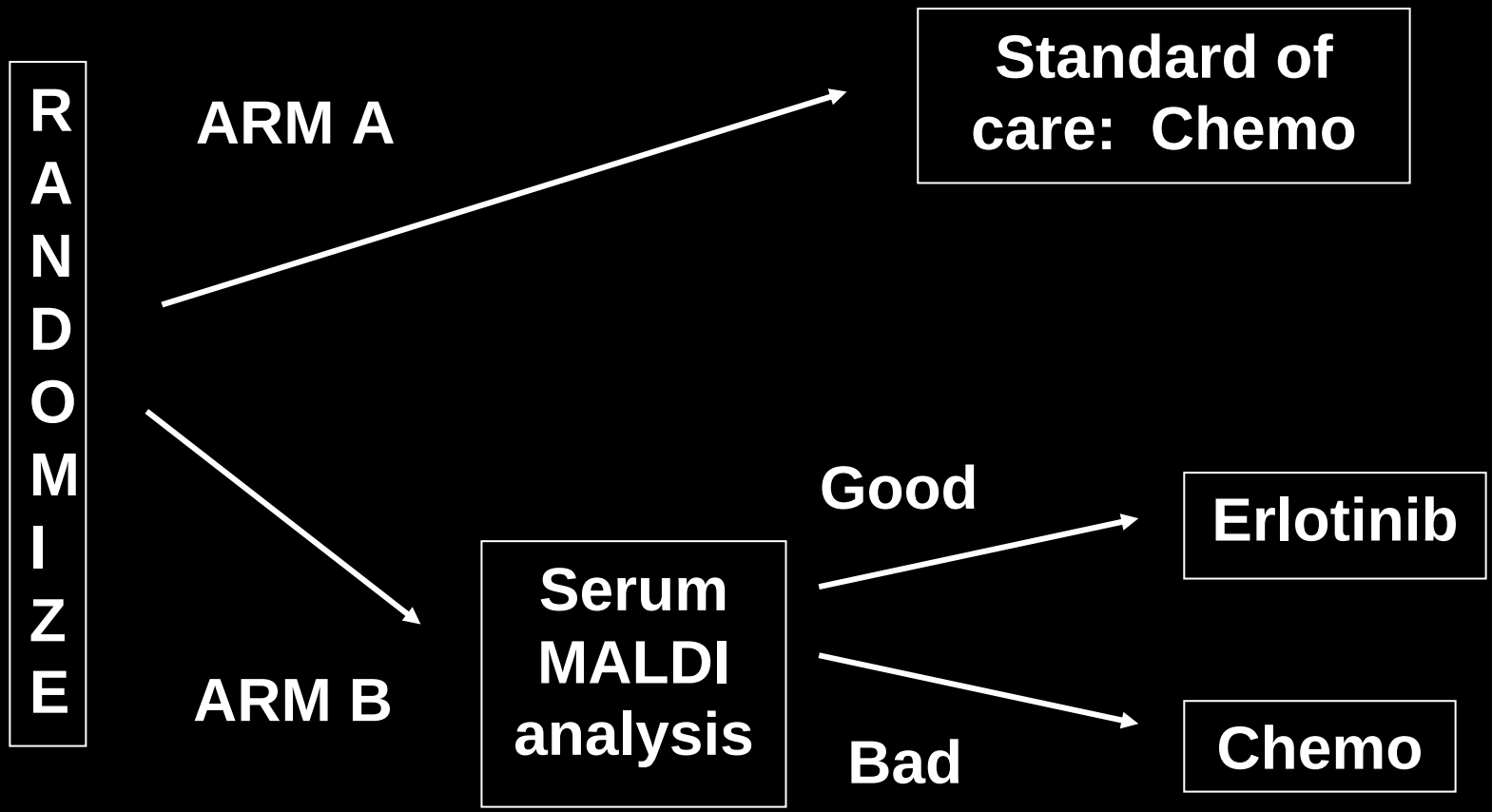
Lung SPECs Trial of erlotinib



Biomarkers:

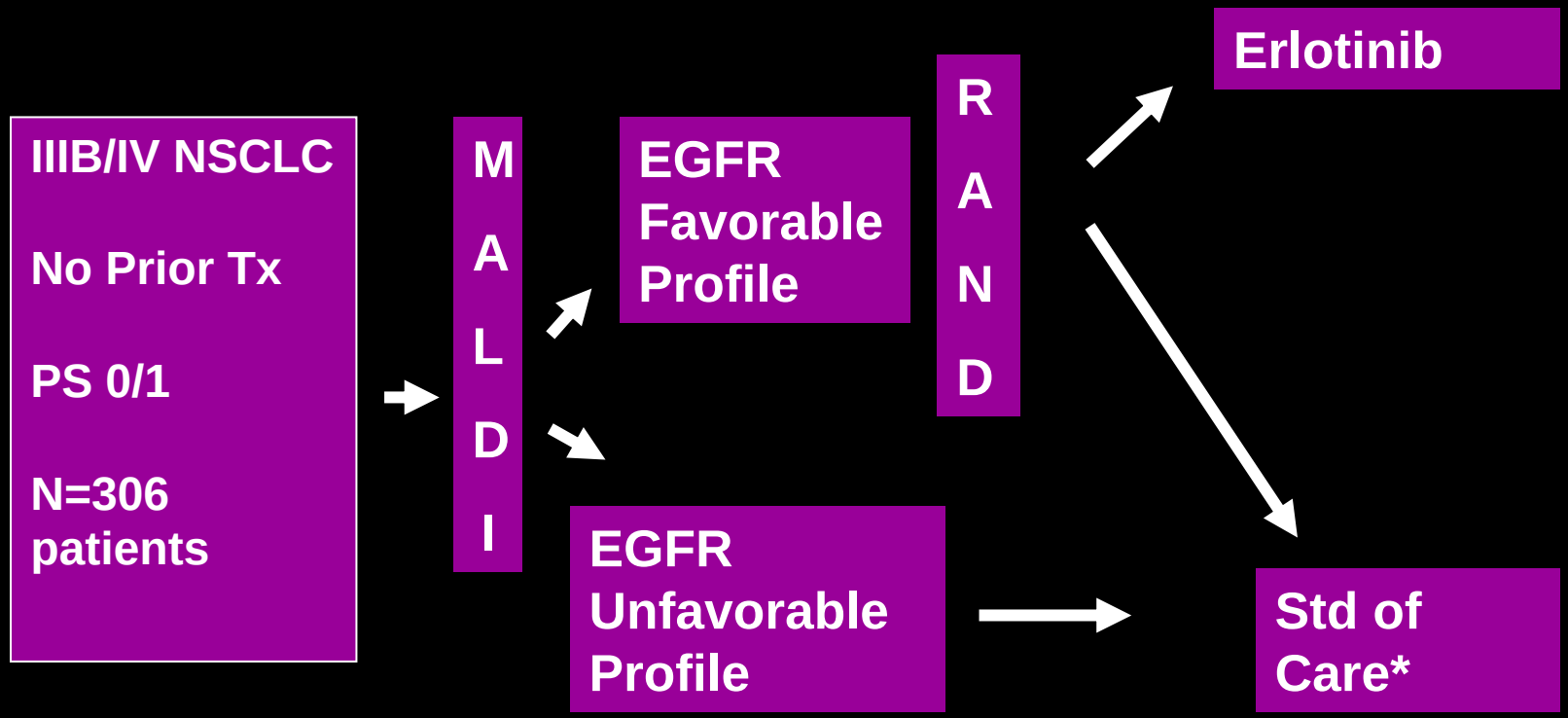
- EGFr mutations
- ras/other mutations
- Proteomics
- FISH
- IHC
- Affy arrays

Potential Trial Schema



Endpoint: survival arm A vs. arm B

ECOG 1507: Study Schema



Std of Care:
Carbo/Pac, Carbo/Doc or Carbo/Gem
Bevacizumab will be given to eligible patients

*** Investigator will be blinded to MALDI status**

Conclusions

- Protein signatures may aid in early detection, prognostication and prediction of lung cancer
- MALDI MS analysis of tumor FNAs may able to predict OS and TTP after treatment with chemotherapy.
- MALDI-TOF MS of pre-treatment peripheral blood may assist in the selection of NSCLC patients who will show improved survival after treatment with EGFR TKIs.
- These are being prospectively tested in the clinic

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