Gene Expression Profiling of Tumors by Microarray analysis using RNA from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Samples

Kathleen Danenberg, Response Genetics, Inc.
Eric Collisson, UCSF
Margaret Tempero, UCSF

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Forward Looking Statements

This presentation contains forward-looking statements. These statements relate to future events or our future financial performance and involve known and unknown risks, uncertainties and other factors that may cause our or our industry’s actual results, levels of activity, performance or achievements to be materially different from any future results, levels of activity, performance or achievements expressed, implied or inferred by these forward-looking statements. In some cases, you can identify forward-looking statements by terminology such as “may,” “will,” “should,” “could,” “would,” “expects,” “plans,” “intends,” “anticipates,” “believes,” “estimates,” “predicts,” “projects,” “potential” or “continue” or the negative of such terms and other comparable terminology.

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Response Genetics (RGI)

Mission: Personalized Medicine:

Maximizing pharmacogenomic information from clinical trial specimens

Getting The Right Therapy to the Right Patient

---The first time

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"The goal of personalized medicine is to get the best medical outcomes by choosing treatments that work well with a person's genomic profile..”

Felix Frueh, CDER/FDA

Assoc. Director for Genomics

Response Genetics Inc. (RGI)

RGI is a leading service provider of pharmacogenomic data from paraffin tissue for the pharmaceutical industry.

- **Response Genetics Inc., Los Angeles, CA (Established in 1999)**
- **Response Genetics LTD, Edinburgh (Established in 2007)**
    - **42 employees**
    - **Patented and patent pending technologies (11 patents issued)**
    - **Research collaboration with premier academic institutions**
    - **Processing under CLIA in Los Angeles**
    - **GLP labs planned in Asia (Japan, China and India)**
    - **NASDAQ listed as RGDX**

- **Development of Diagnostic Tests and Pharmacogenomic Service Provider to the Pharmaceutical Industry**
  - Extraction of DNA and RNA from Microdissected formalin fixed paraffin embedded (FFPE) samples
    - Validated assays for gene expression/SNPs
    - Global analysis of gene expression from FFPE
    - Analyzed over 30,000 FFPE samples

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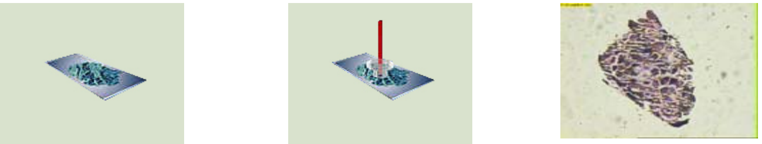
RGI Assays

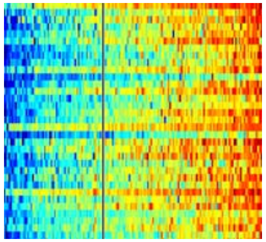


FFPE tumor micro-dissection

RNA/DNA Isolation

RNA

DNA

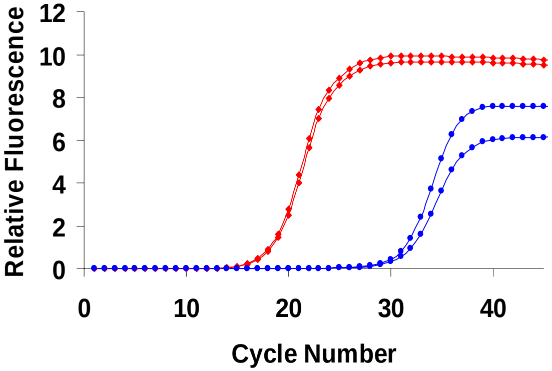


Micro Array
RGI-2

Gene Amplification/ SNP
MIP analysis

RT

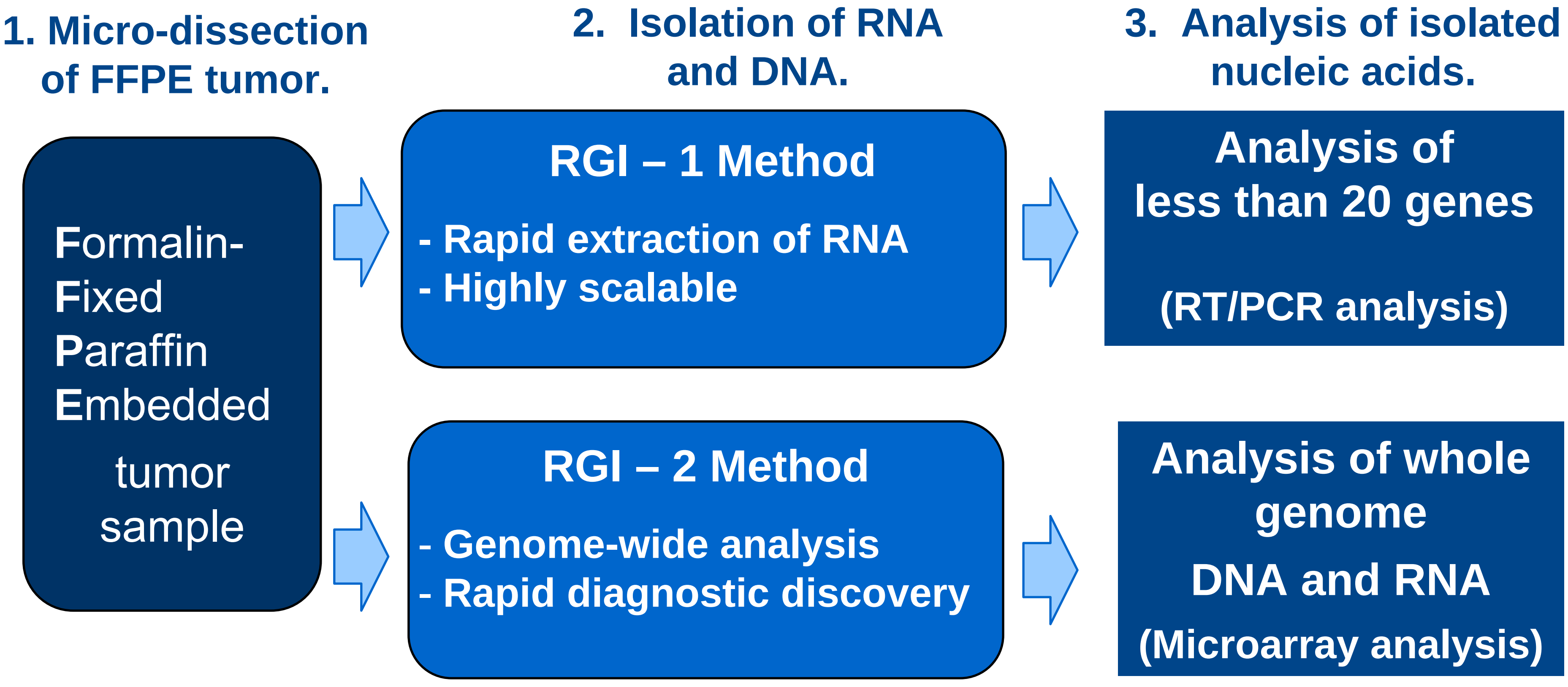
RGI-1



PCR with TaqMan®

Data Analysis

Comprehensive Methodology



1. Micro-dissection of FFPE tumor.

2. Isolation of RNA and DNA.

3. Analysis of isolated nucleic acids.

Formalin-Fixed Paraffin Embedded tumor sample

RGI – 1 Method

- **Rapid extraction of RNA**
- **Highly scalable**

Analysis of less than 20 genes

(RT/PCR analysis)

RGI – 2 Method

- **Genome-wide analysis**
- **Rapid diagnostic discovery**

Analysis of whole genome

DNA and RNA

(Microarray analysis)

Our technology enables rapid development of diagnostic tests.

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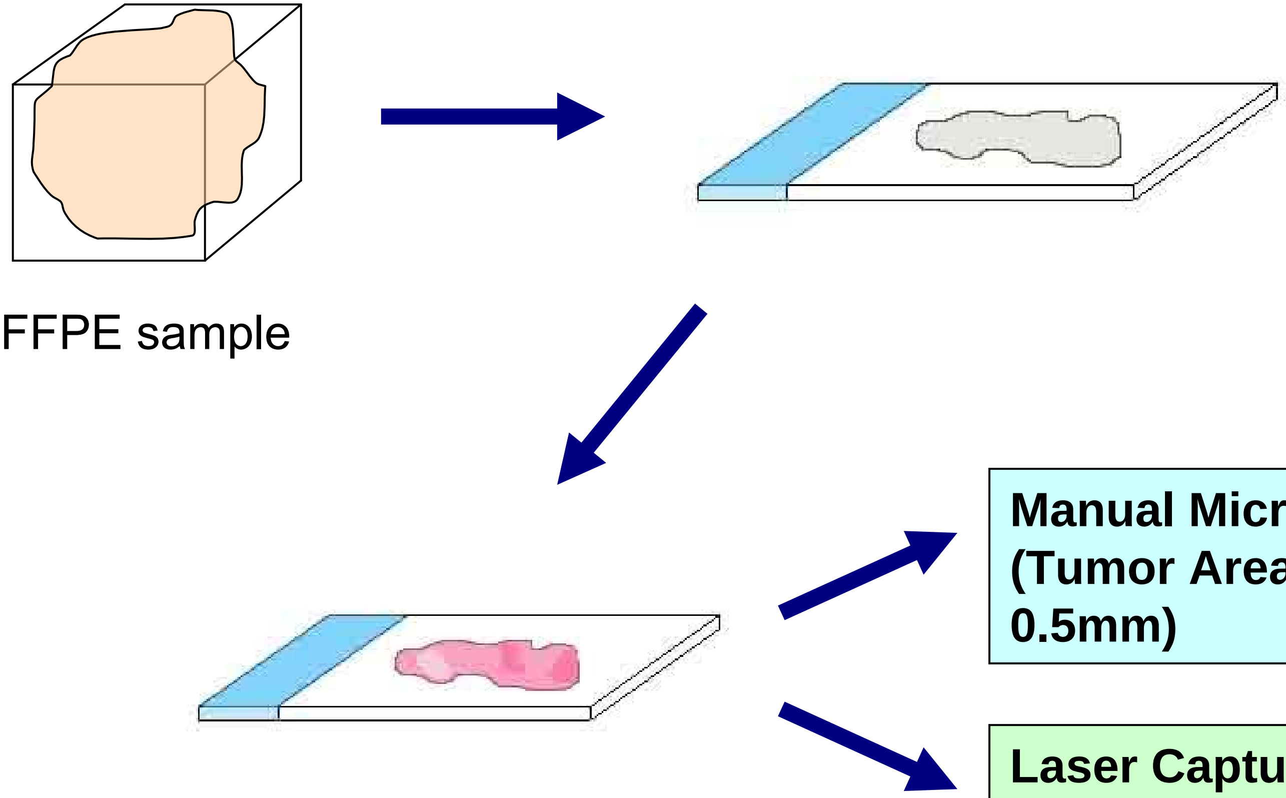
- RGI has technology for isolating DNA and RNA from the same microdissected sample---thereby conserving valuable clinical trial specimens.
- Our method for isolating “long fragment” RNA is also useful for extraction of micro RNAs from FFPE tissue.
- Microdissection employed for analysis of pharmacogenomics from clinical specimens
  - RGI has microdissected every specimen (over 30,000)

Microdissection: Manual or Laser



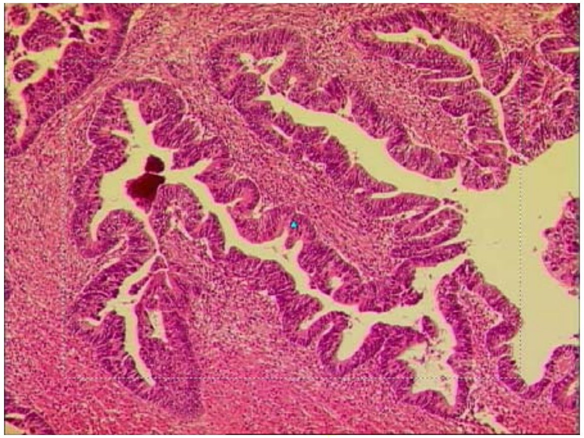
FFPE sample

Nuclear Fast Red (NFR) Staining

Manual Microdissection (Tumor Areas > 0.5mm x 0.5mm)

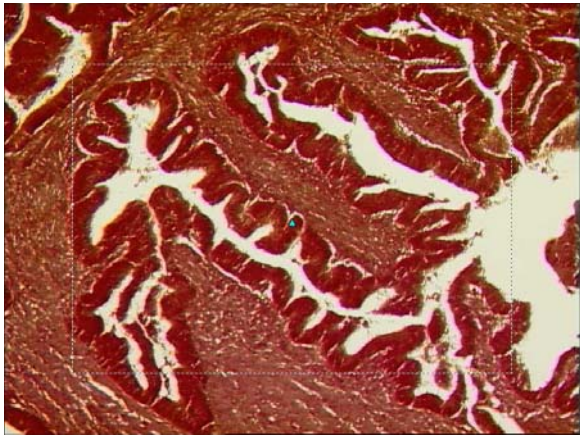
Laser Captured Microdissection (Tumor Areas < 0.5mm x 0.5mm)

Laser Capture Microdissection

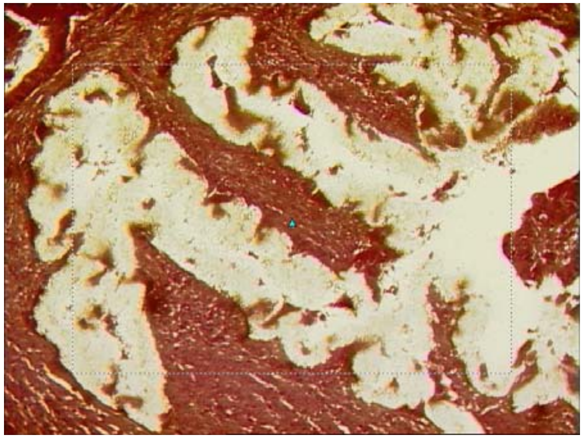


Before dissection

HE staining



After dissection



Nuclear fast red (NFR) staining

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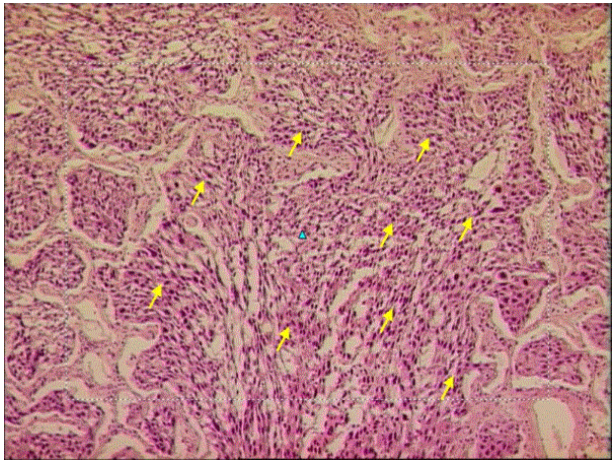
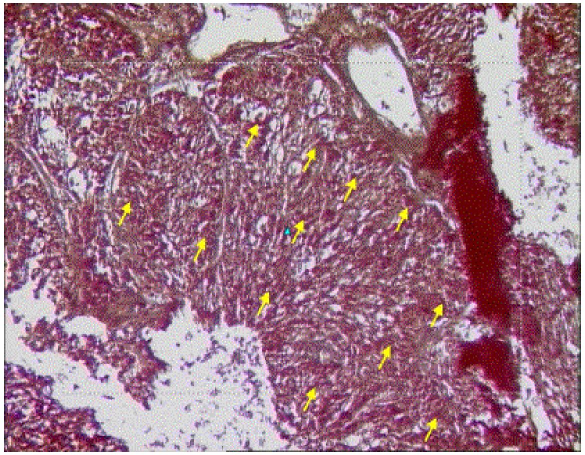
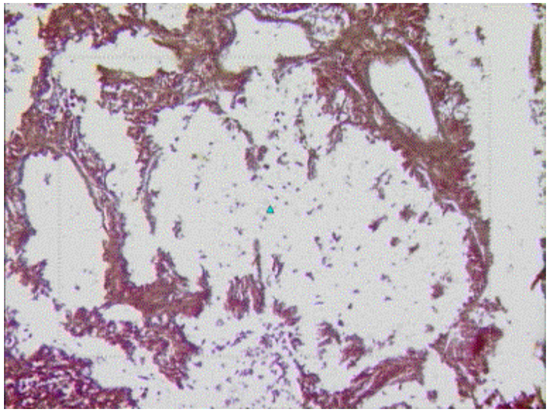
Laser Capture Microdissection



Lung tumor H & E



Before Dissection



After LCM

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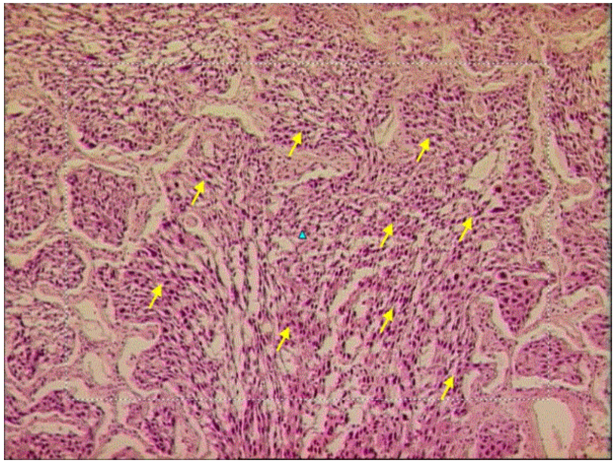
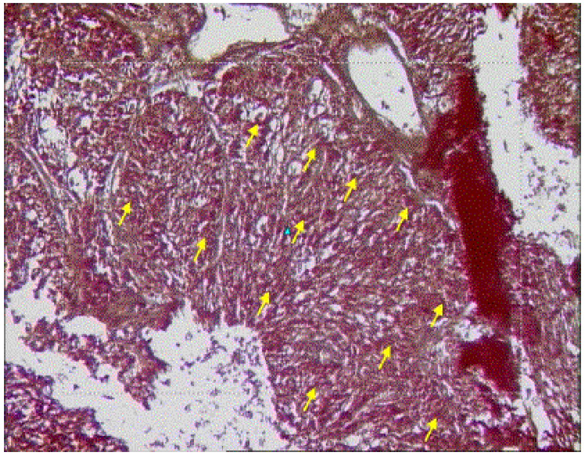
Manual Microdissection

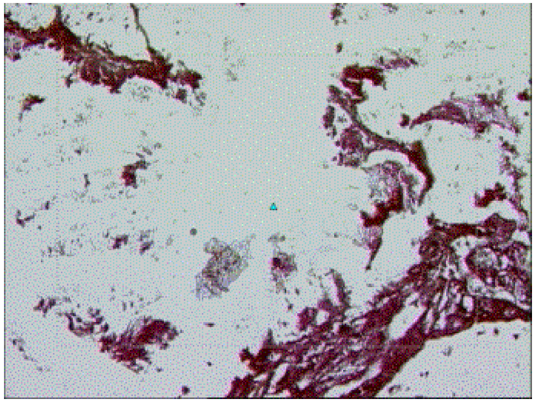


Lung tumor H & E



Before Dissection

NSCLC



After Manual Dissection

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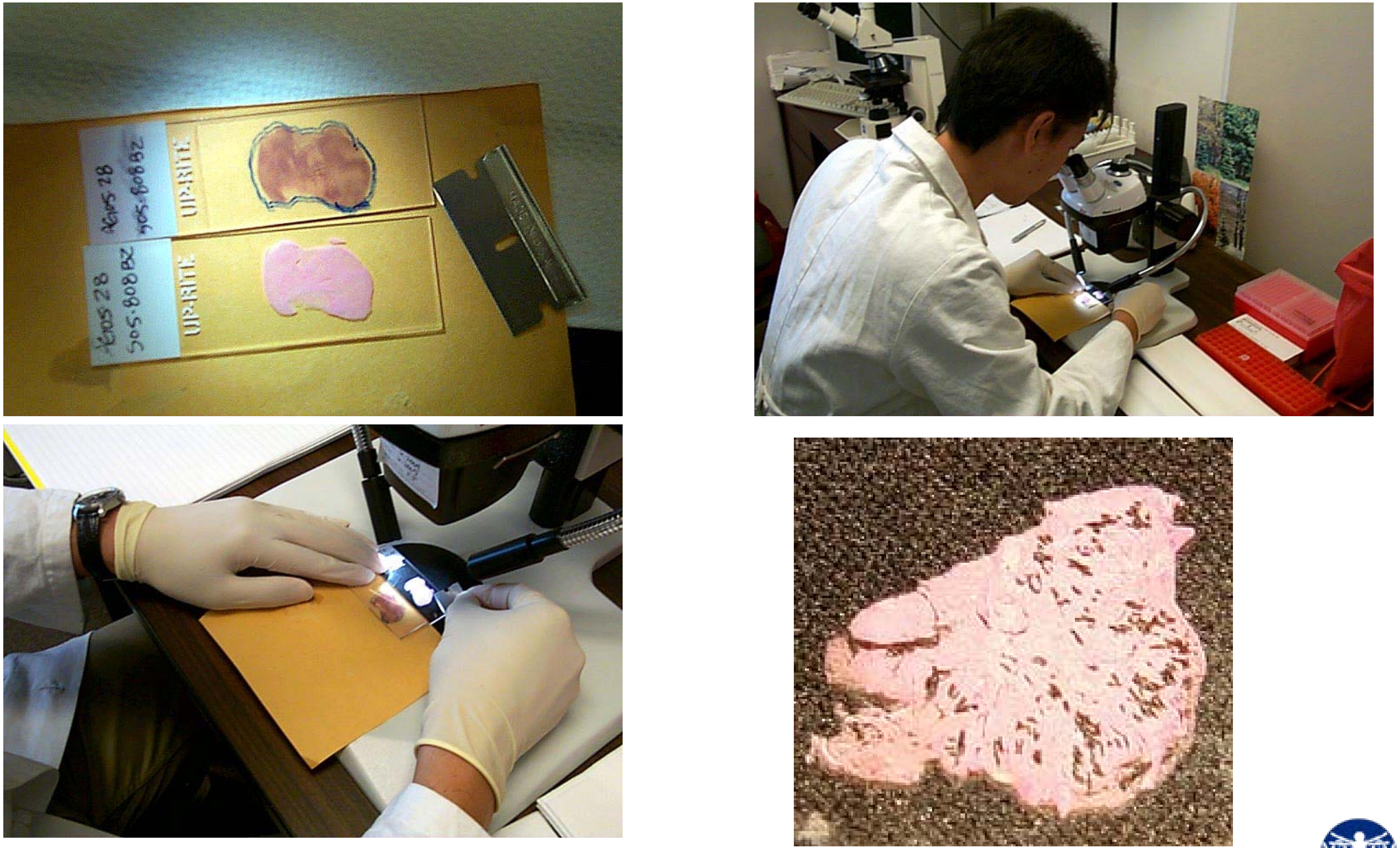
Manual Microdissection



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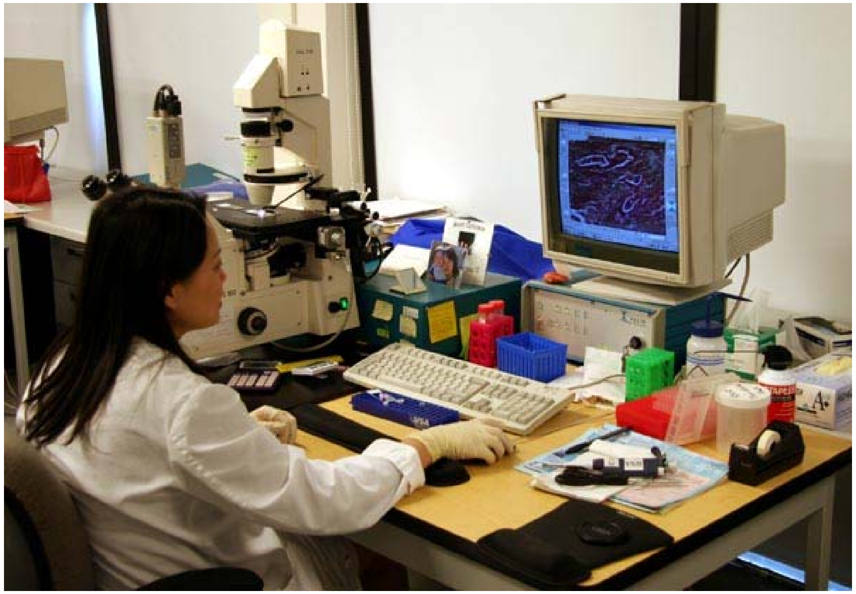
Laser Captured Microdissection
(Required for Approx. 5% of samples)



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Early Stage NSCLC: Current Practice

Stage I
lung cancer patients

Surgery

Post-surgery observation

50% relapse
within 5 years

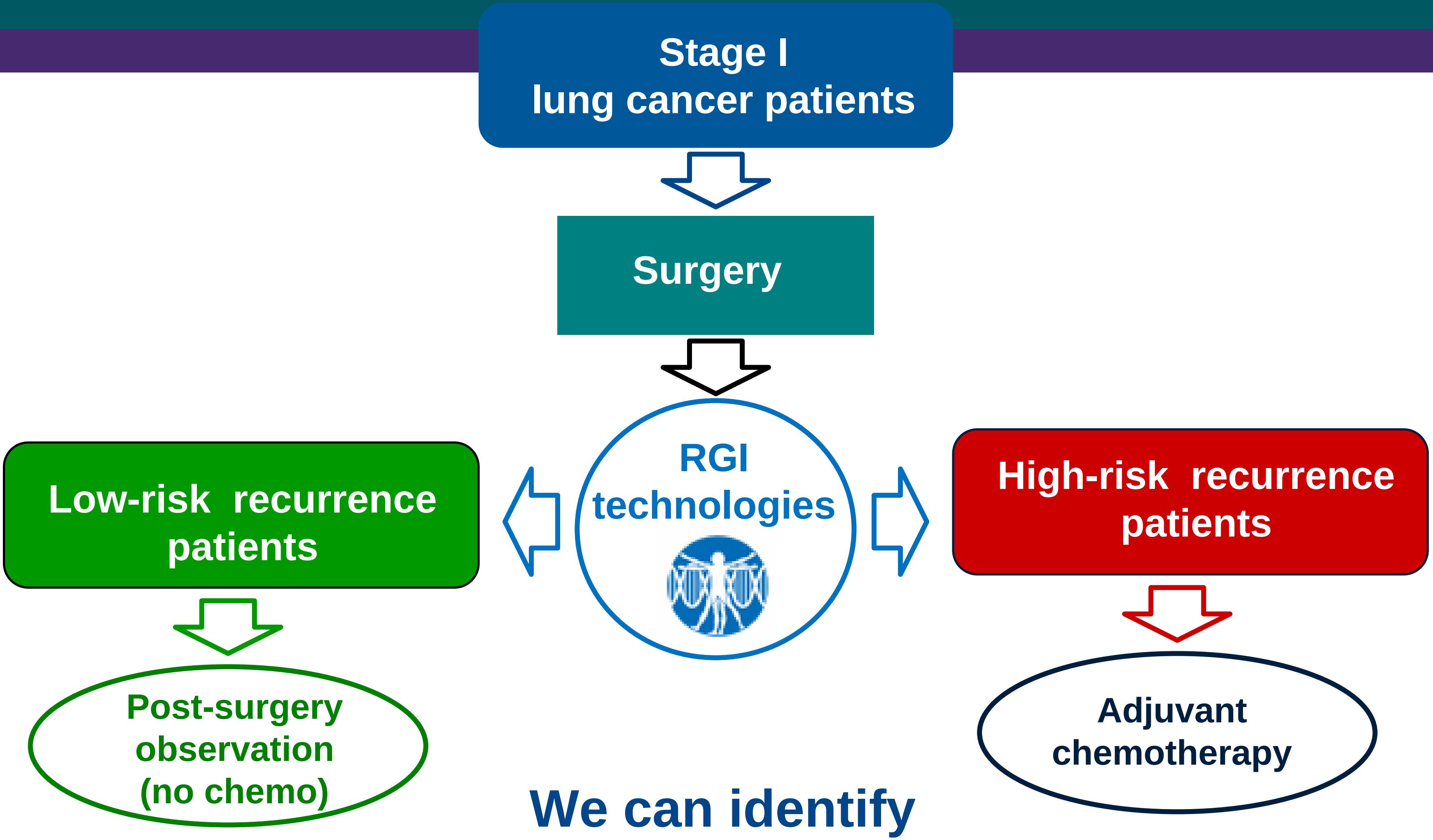
Stage I patients have significant risk of post-surgery relapse.

Stage II, III-A
lung cancer patients

Surgery

Adjuvant chemotherapy

Cancer Diagnostics: Solution



Stage I lung cancer patients

Surgery

RGI technologies

Low-risk recurrence patients

Post-surgery observation (no chemo)

High-risk recurrence patients

Adjuvant chemotherapy

We can identify high-risk Stage I patients in need of adjuvant chemotherapy through pharmacogenomics

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Paraffin Embedded, Formalin Fixed (FFPE) Early Stage NSCLC Patient Specimens Were analyzed by Microarray* for Gene Signatures Related to Differences in

- **Overall and Disease Free Survival after Surgery**
- **Histological Subtypes**
- **Progression Through Various Stages**

*Affymetrix U133 plus 2.0 Chips

ASCO 2006, David Harpole (Duke Univ) and RGI

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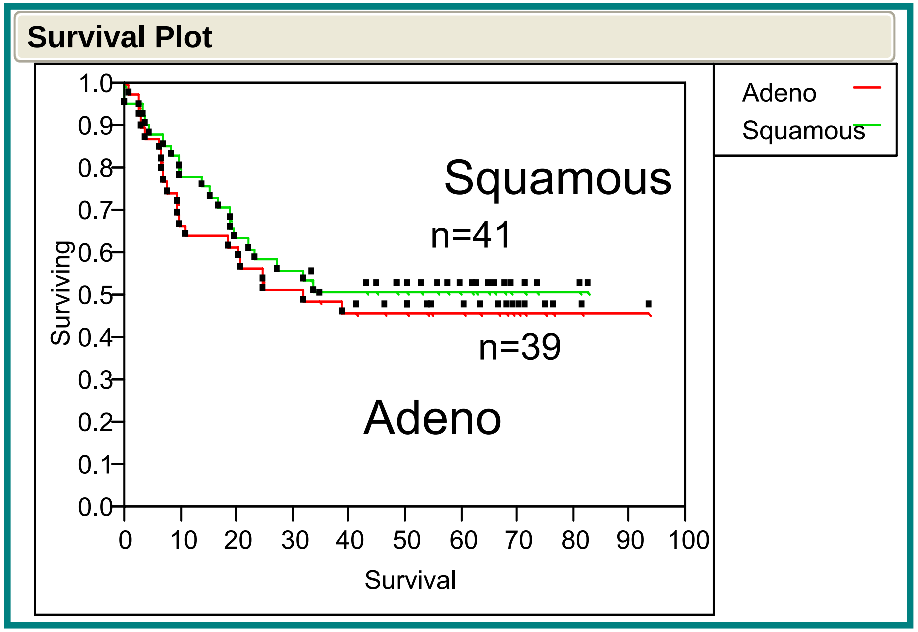
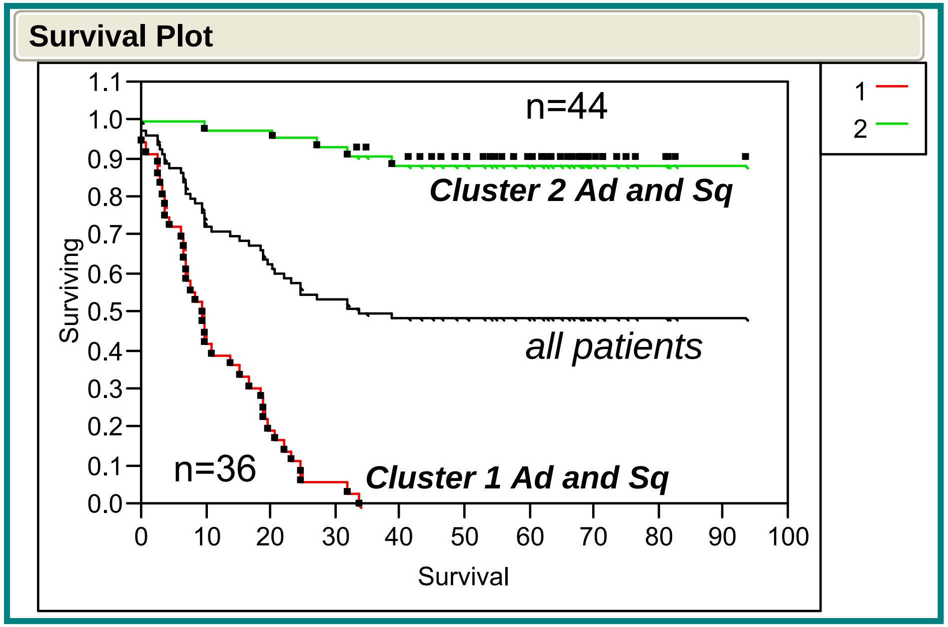
Survival of NSCLC Stage 1 Adeno and Squamous after Surgery using Molecular Staging

Overall Path Stage 1



Molecular Stage Path Stage 1



p<0.0001

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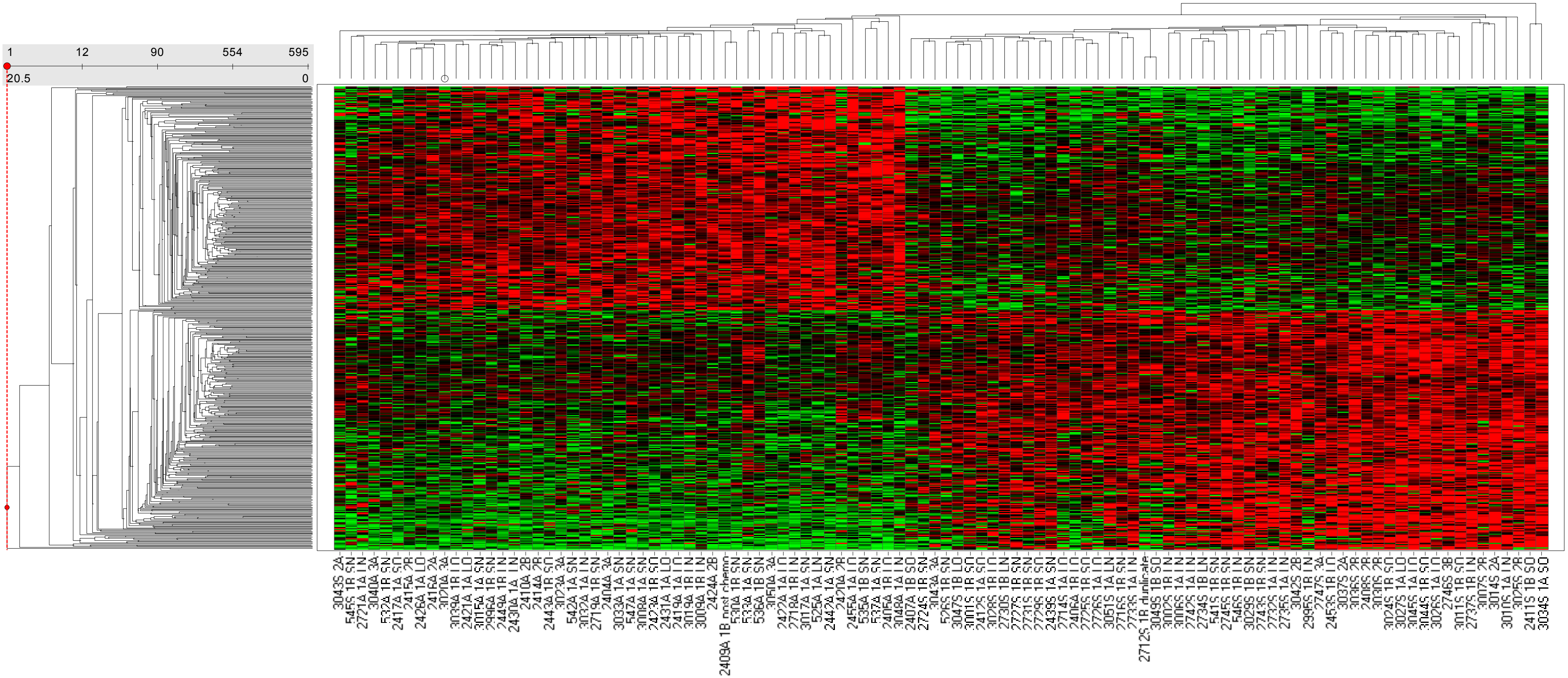
Hierarchical Clustering:
Histology NSCLC Adeno vs. Squamous

Adeno ***Squamous***



p<0.000005

Response Genetics and David Harpole, Duke

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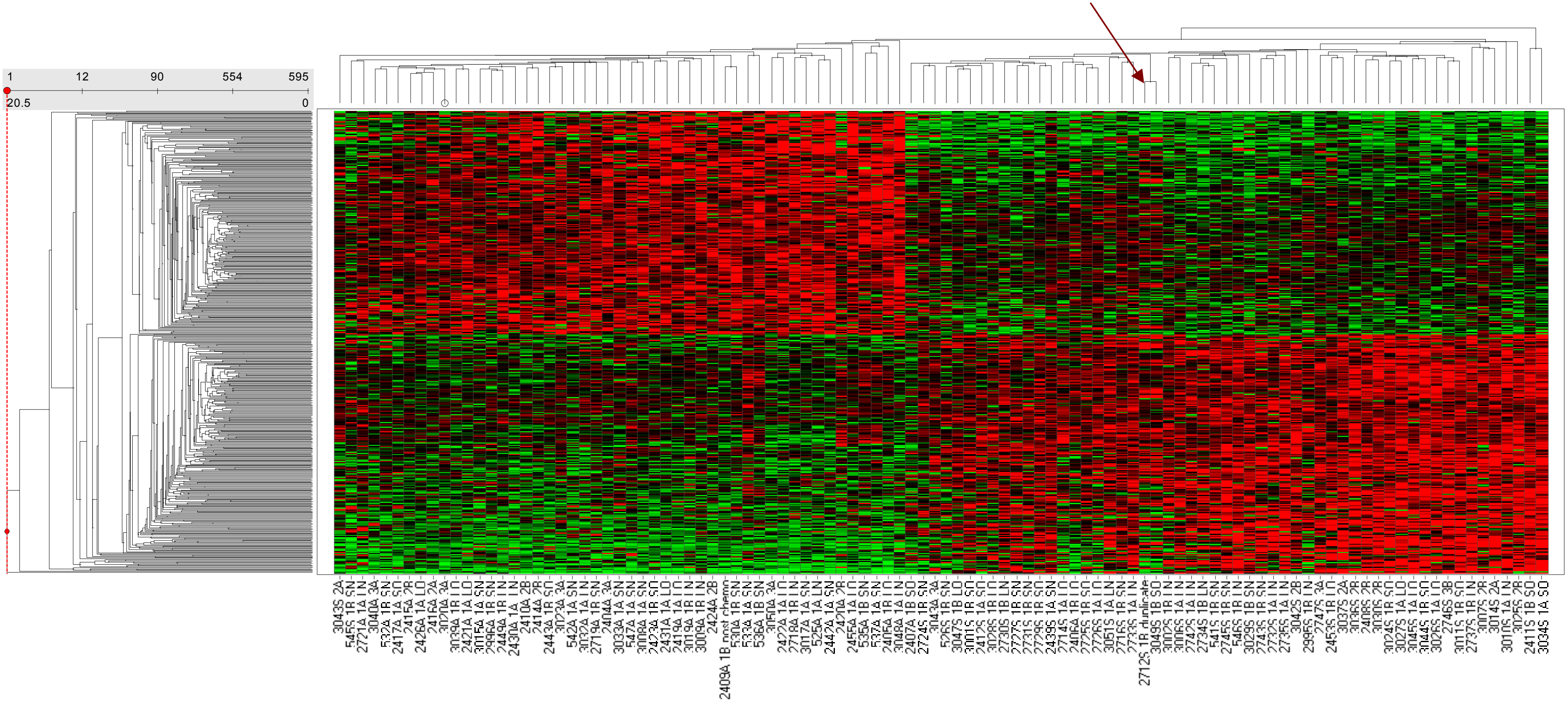
Hierarchical Clustering:
Histology NSCLC Adeno vs. Squamous

Adeno

Same Patient (2 samples)

Squamous



p<0.000005

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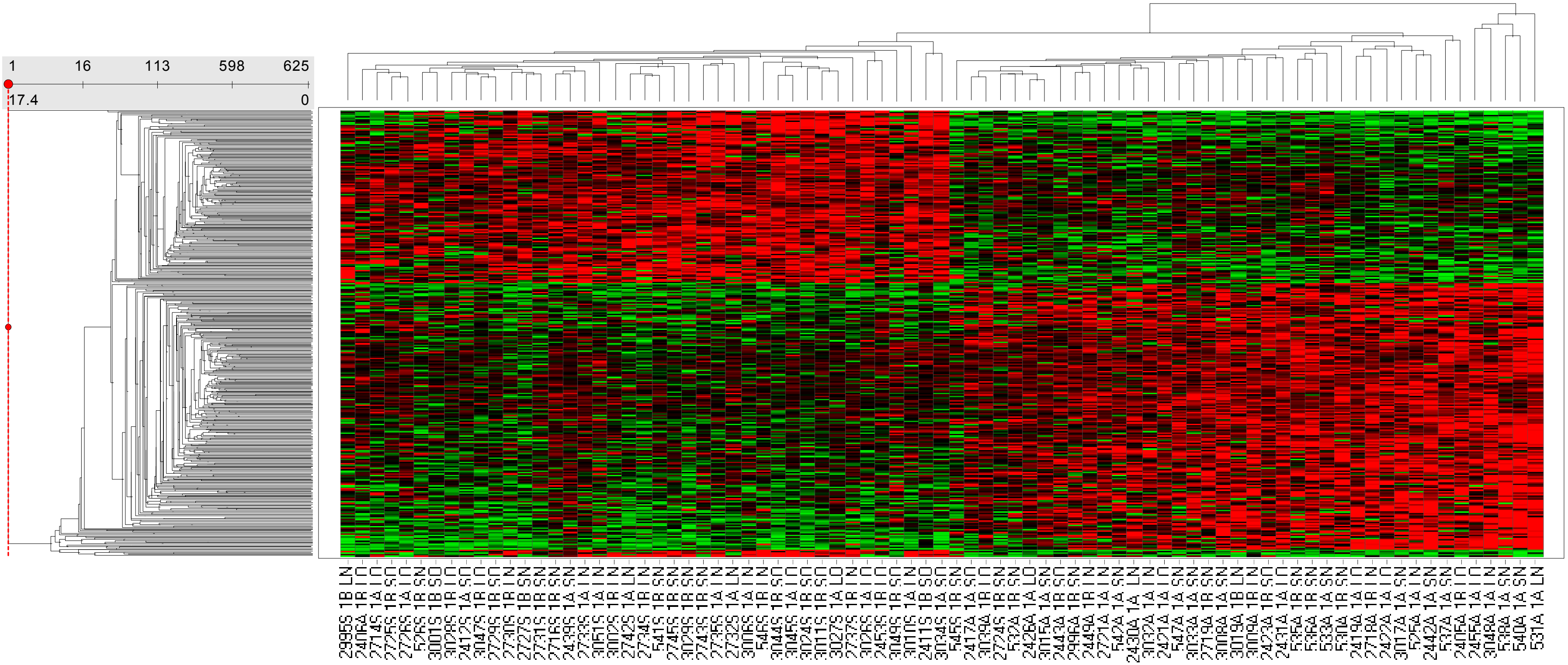
Hierarchical Clustering: Histology NSCLC Adeno vs. Squamous



Colors:

Default

0.0005 2

Order by Hierarchical Clustering (order)

$p<0.00005$

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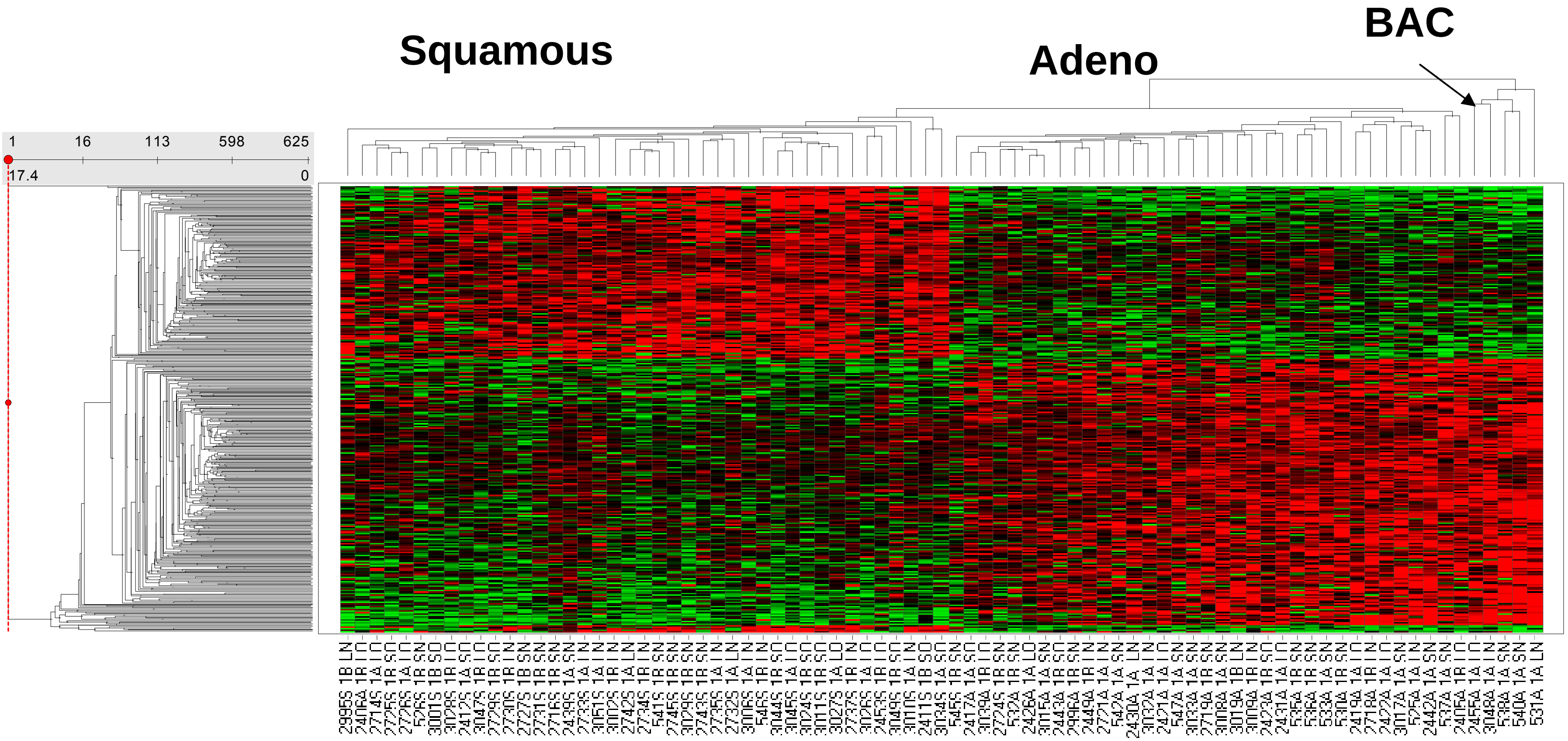
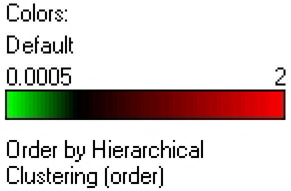
Hierarchical Clustering:
Histology NSCLC Adeno vs. Squamous



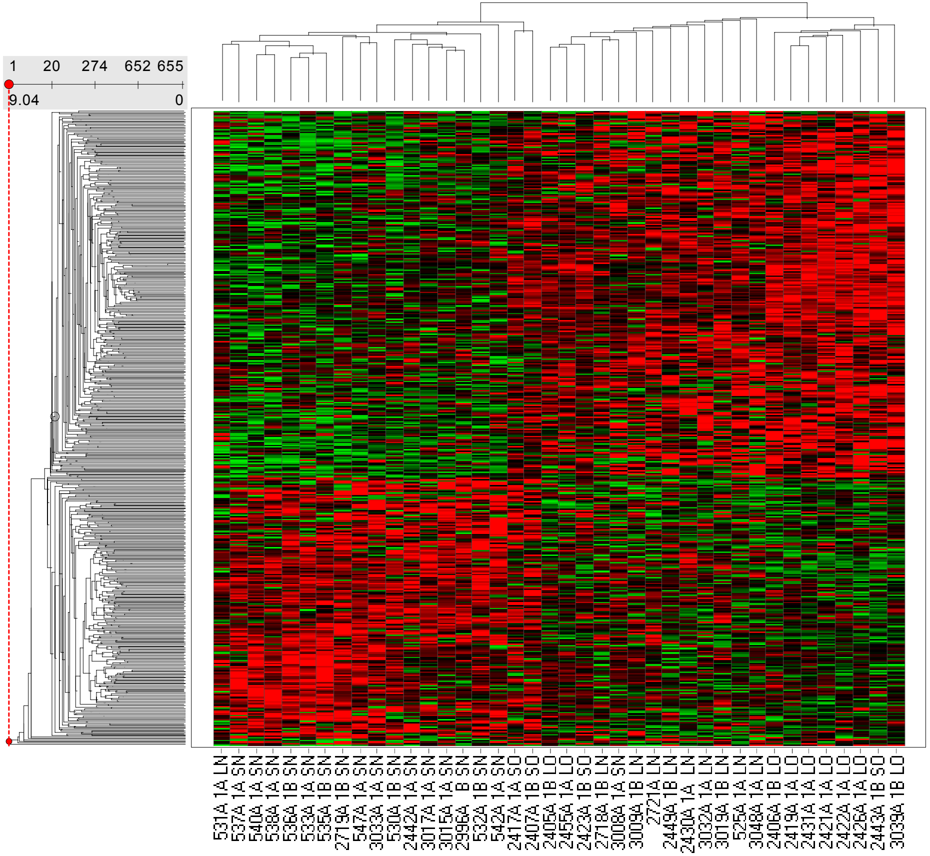


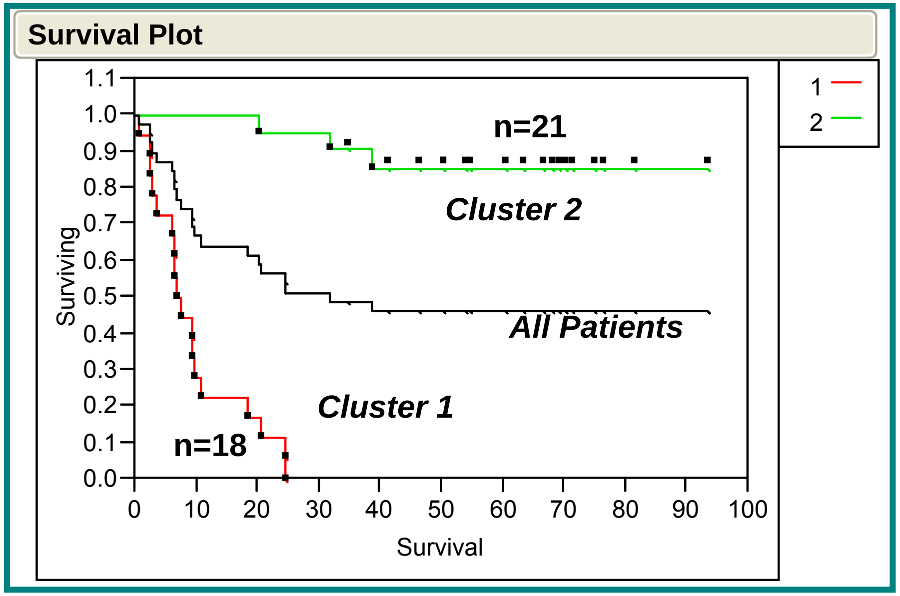
p<0.00005

Survival of NSCLC Patients After Surgery: Adeno Stage 1

Cluster 1
<3 Yr

Cluster 2
>3 Yr





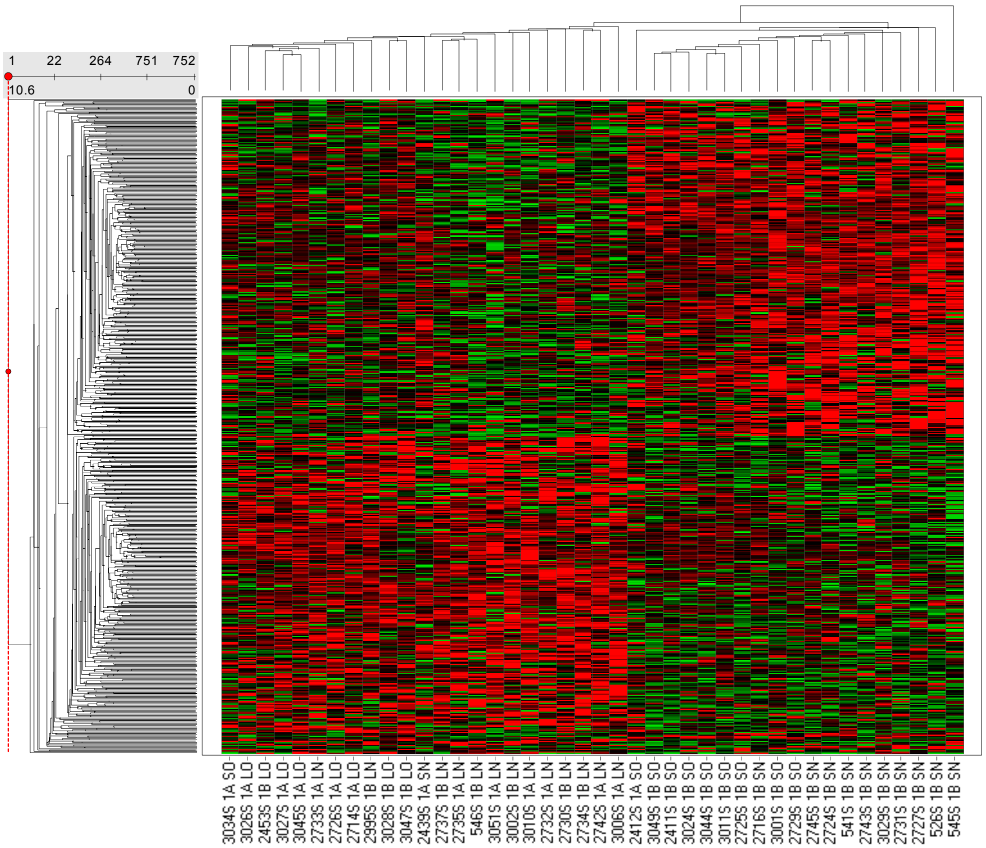
p<0.0001

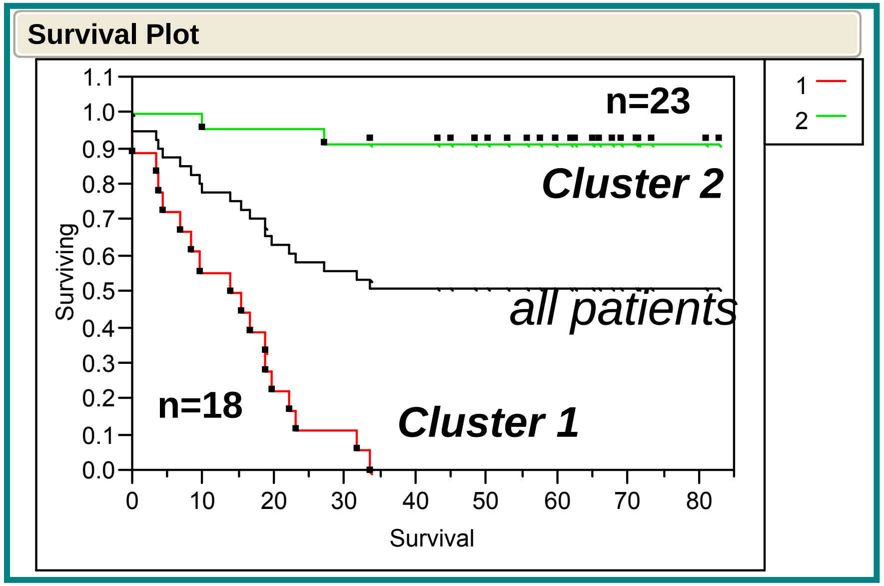
RESPONSE GENETICS

Survival of NSCLC Patients After Surgery: Squamous Stage 1

Cluster 2:
>3 Yr.

Cluster 1:
<3 Yr





p<0.0001

Colors:
Default
0.005 2

Order by Hierarchical
Clustering (order)

p<0.002

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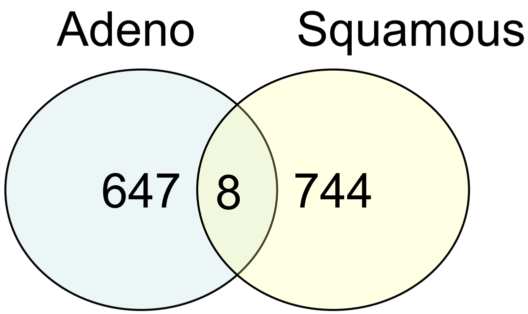
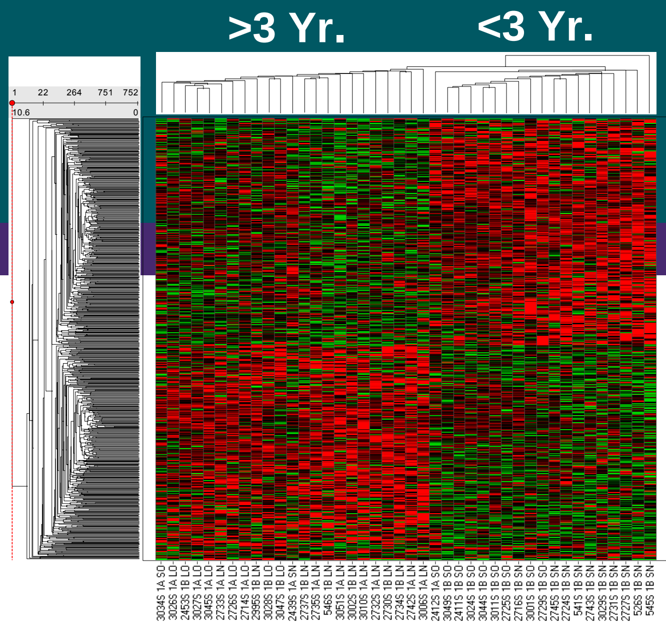
Venn Diagram



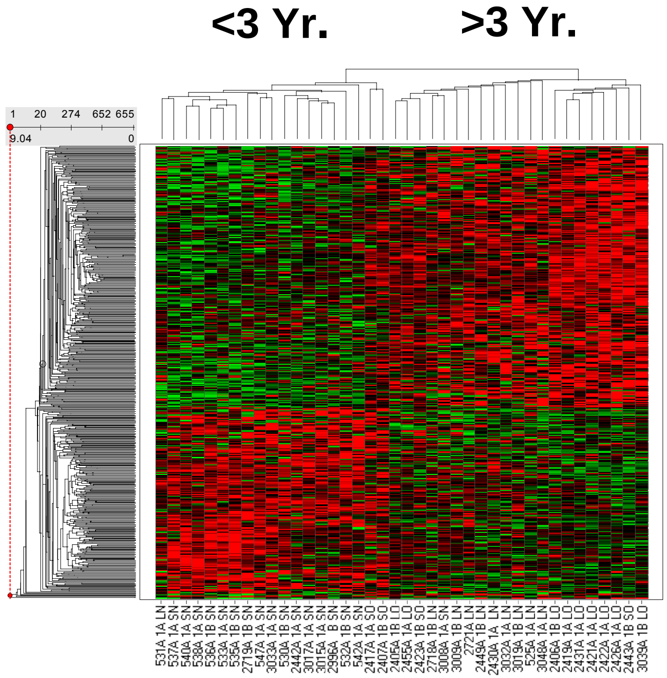
Number of Genes in Union 1399

Number of Genes in Intersection 8

Squamous



Adeno



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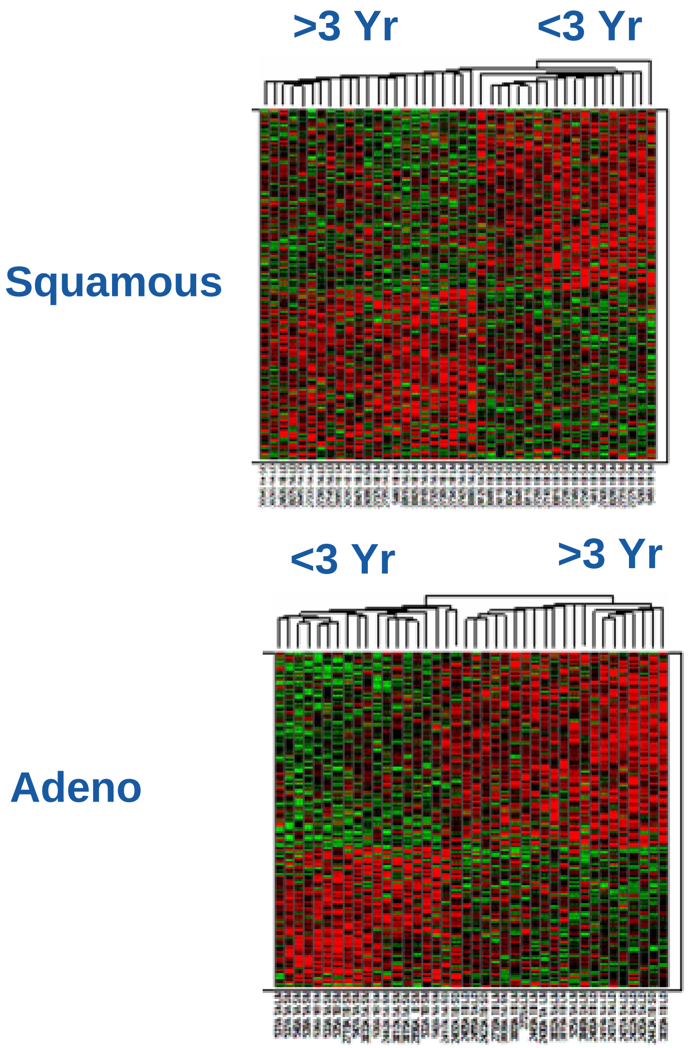
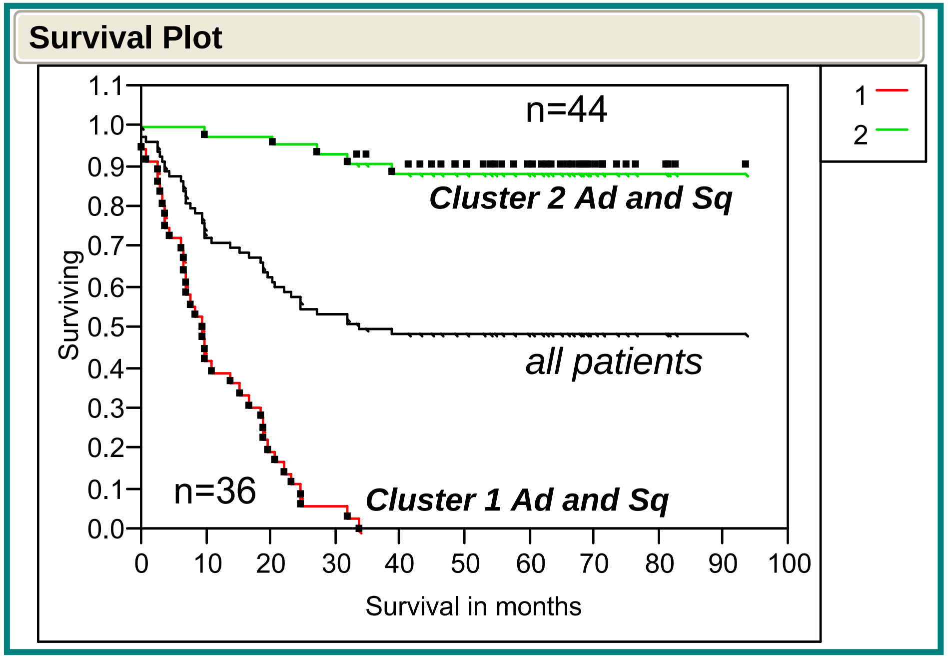
Development of Gene Signature for Risk of Recurrence in early Lung Cancer After Surgery





Data generated from paraffin tissue
$p<0.0001$

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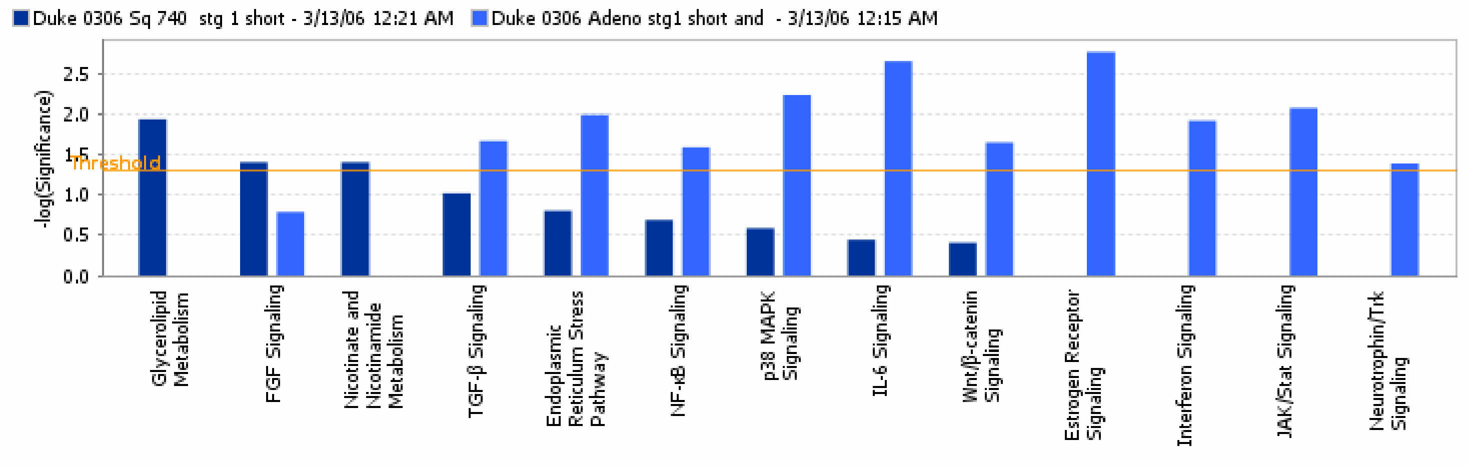
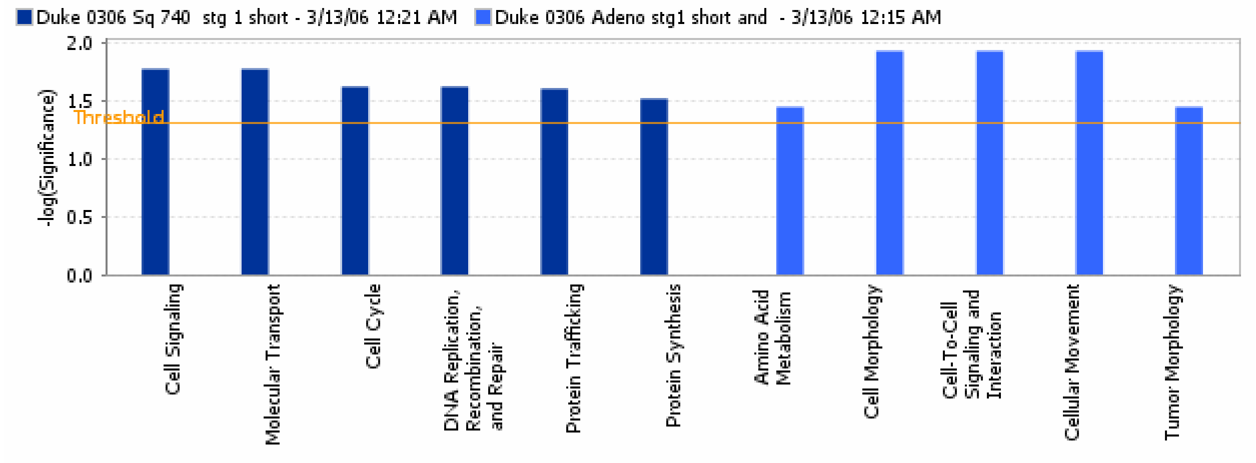
Differential Pathways and Functions Involved in Survival between Adeno and Squamous NSCLC





Response Genetics and David Harpole, Duke

A Pancreatic Cancer Genetic Risk Prediction Model Derived from Paraffin-Embedded Tissue

- **Methods:**
  - **Pathologically confirmed pancreatic ductal adenocarcinoma from FFPE pancreaticoduodenoectomy specimens from a single center collected from 1993-2003**

  - **20 FFPE Specimens: 14 long-term and 6 short-term survivors were analyzed for genes predicting surgical outcome by Affymetrix Gene Expression Profiling (U133 plus 2.0)**

Margaret Tempero, Eric Collisson, UCSF and RGI. ASCO GI 2007

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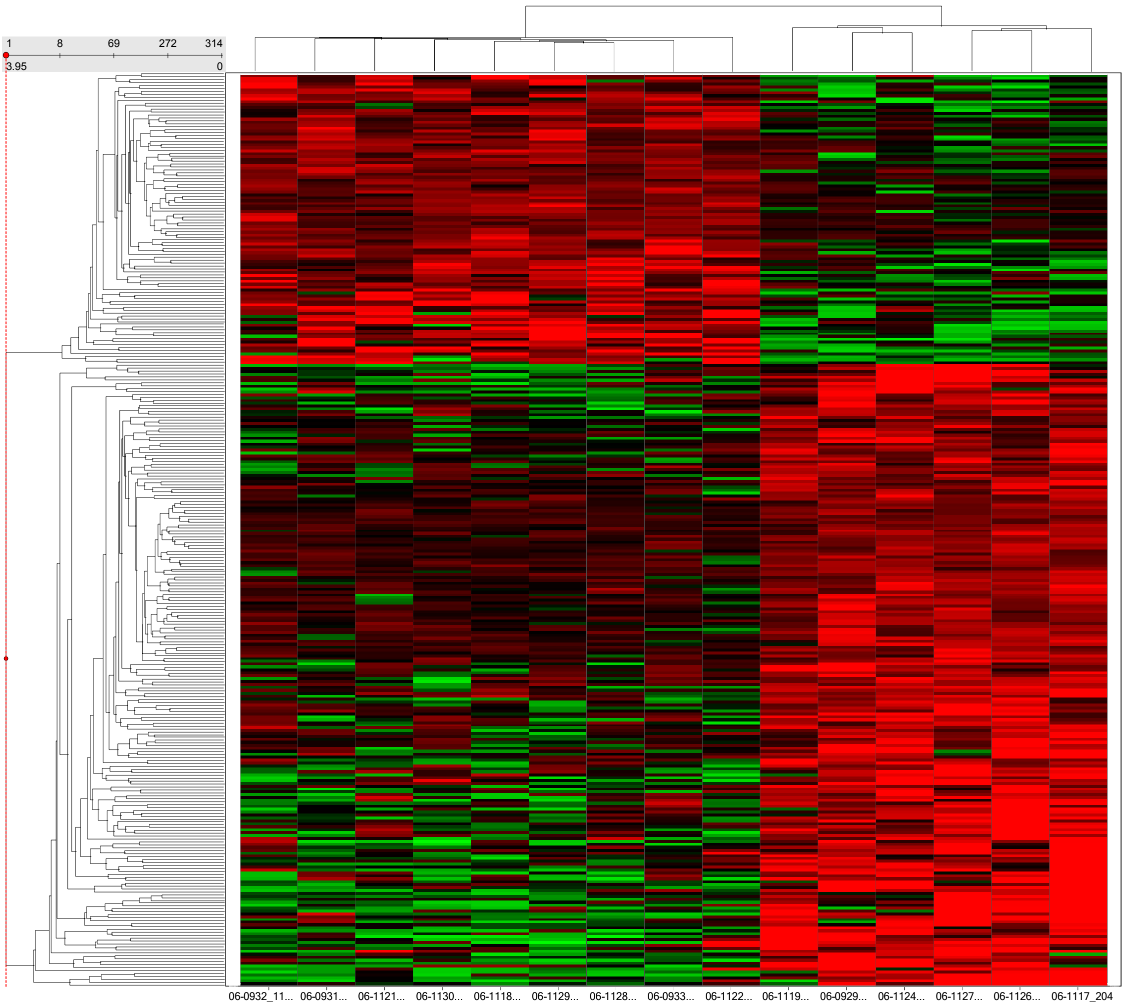
Hierarchical Clustering: Survival after Pancreatic Surgery

>645 days **<204 days**



A 314 Gene Profile Predictive of Long and Short Survival after Pancreatic Surgery was Determined Using T-Test analysis and Hierarchical Clustering

p=0.004

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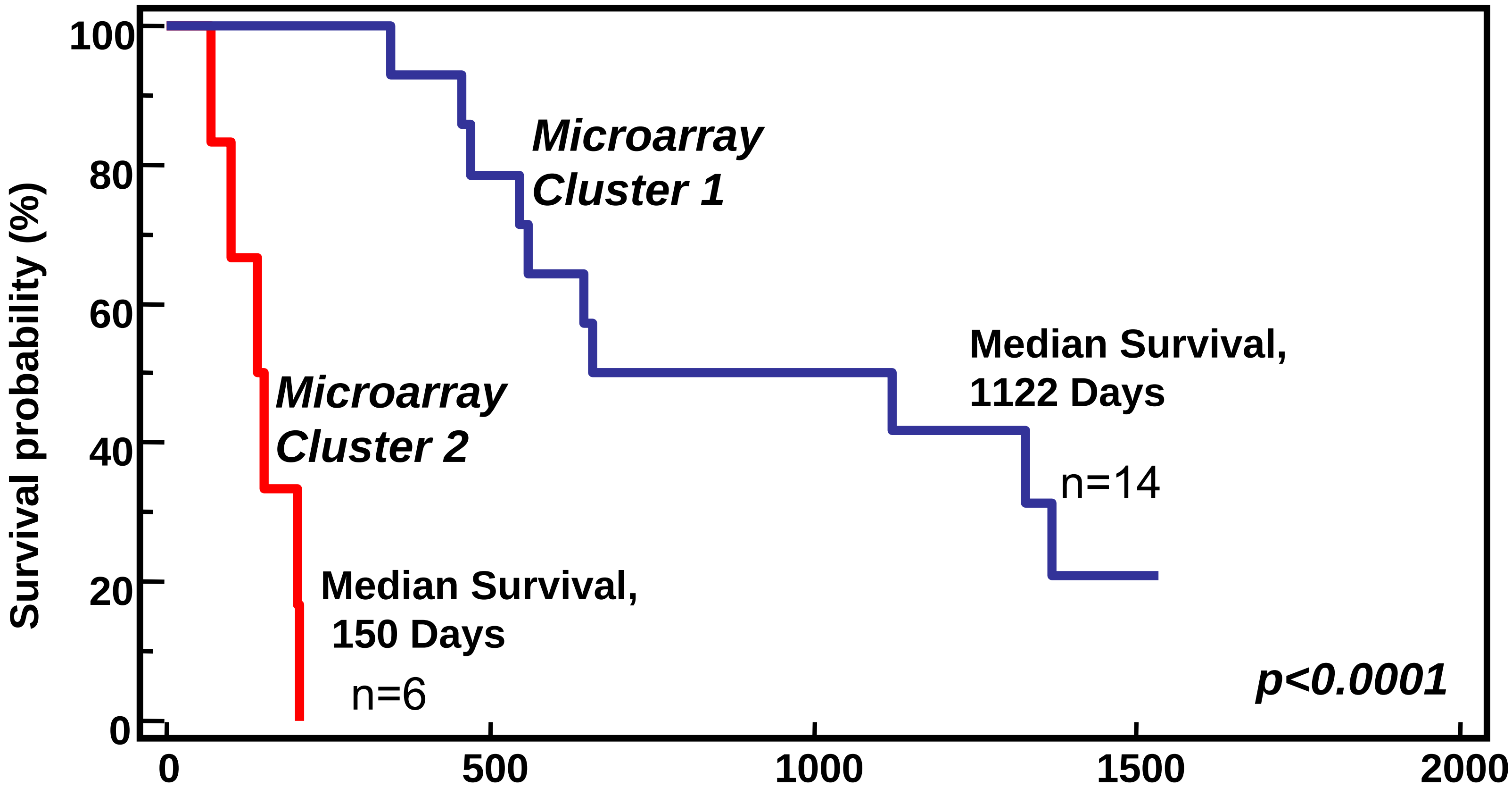
Survival after Pancreatic Cancer Surgery



Hazard Ratio, 7.72

95% CI, 28.49-1801.61

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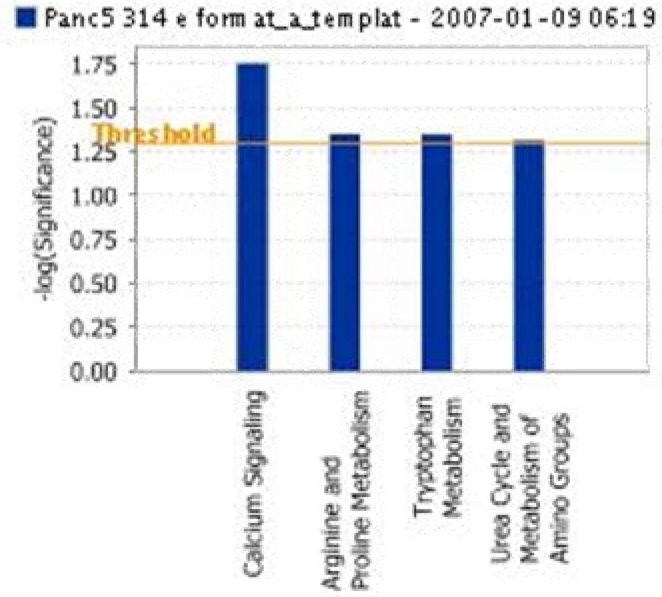
Pathway Analysis of Differentially Expressed Genes between Long and Short Survivors

Calcium Signaling: Most significant pathway associated with outcome after pancreatic surgery



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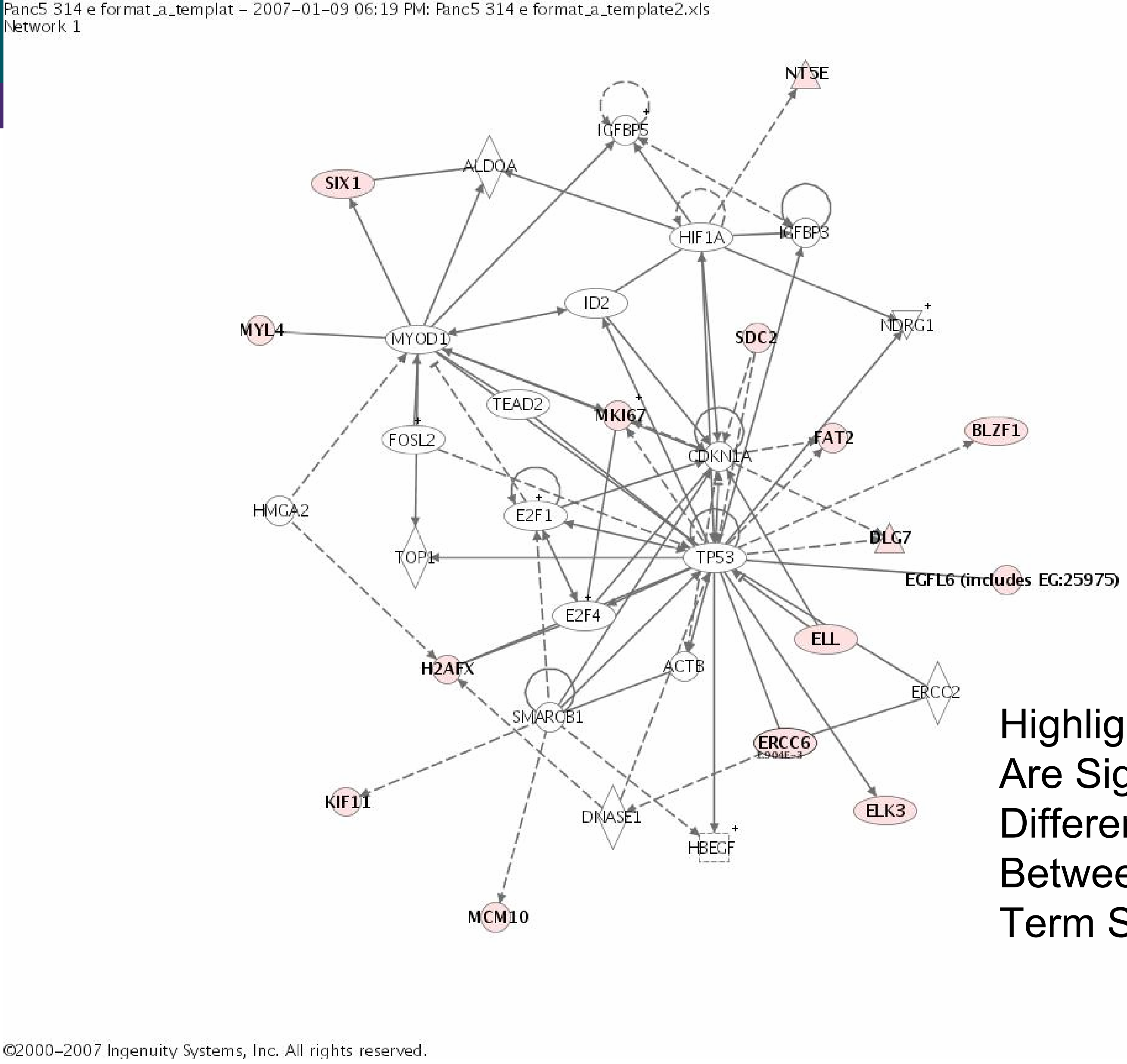
Genes Involved in the Calcium Signaling Pathway

name	synonym	description	affymetrix	p-value	networks	location	family
ATP2A1	ATP2A, Calcium transporting ATPase, SERCA1, Serca1a, SR calcium ATPase	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	230693_at	0.004	5	Cytoplasm	transporter
ATP2C1	1700121J11Rik, ATP2C1A, AW061228, BCPM, D930003G21Rik, HHD, hSPCA1, KIAA1347, MGC58010, MGC93231, PMR1, SPCA, SPCA1	ATPase, Ca++ transporting, type 2C, member 1	209934_s_at	0.003		Cytoplasm	transporter
HDAC8	2610007D20Rik, Hdac8 (predicted), HDACL1, RGD1562895, RPD3	histone deacetylase 8	223909_s_at	0.003	16	Nucleus	transcription regulator
HDAC9	AV022454, D030072B18Rik, DKFZp779K1053, HD7, HD7B, HD9, HDAC, HDAC C, HDAC7, HDAC7B, HDAC9B, HDAC9FL, HDRP, KIAA0744, MITR, mKIAA0744, RGD1563092	histone deacetylase 9	1552760_at	0.001	2	Nucleus	transcription regulator
MYL4	ALC1, AMLC, ATELC, ELC, ELC1a, GT1, MLC1, MLC1A, MLC2A, MYLA, PRO1957	myosin, light polypeptide 4, alkali; atrial, embryonic	210395_x_at	0.003	1	Cytoplasm	other
TPM1	AA986836, Alpha tropomyosin, alpha-TM, C76867, HTM-alpha, Slow-twitch alpha tropomyosin, Tm alpha, Tma2, Tmpa, TMSA, TPM1-alpha, TPM1-kappa, Tropomyosin 5b, Tropomyosin Alpha	tropomyosin 1 (alpha)	238688_at	0.002	4	Cytoplasm	other

Networks involving Genes Predictive of Long and Short Survival After Pancreatic Surgery included:

- **P53/P21**
- **HDAC/SMAD**
- **TNF**
- **MYC**

P53/P21



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Network 1

Highlighted Genes Are Significantly Differentially Expressed Between Long and Short Term Survivors

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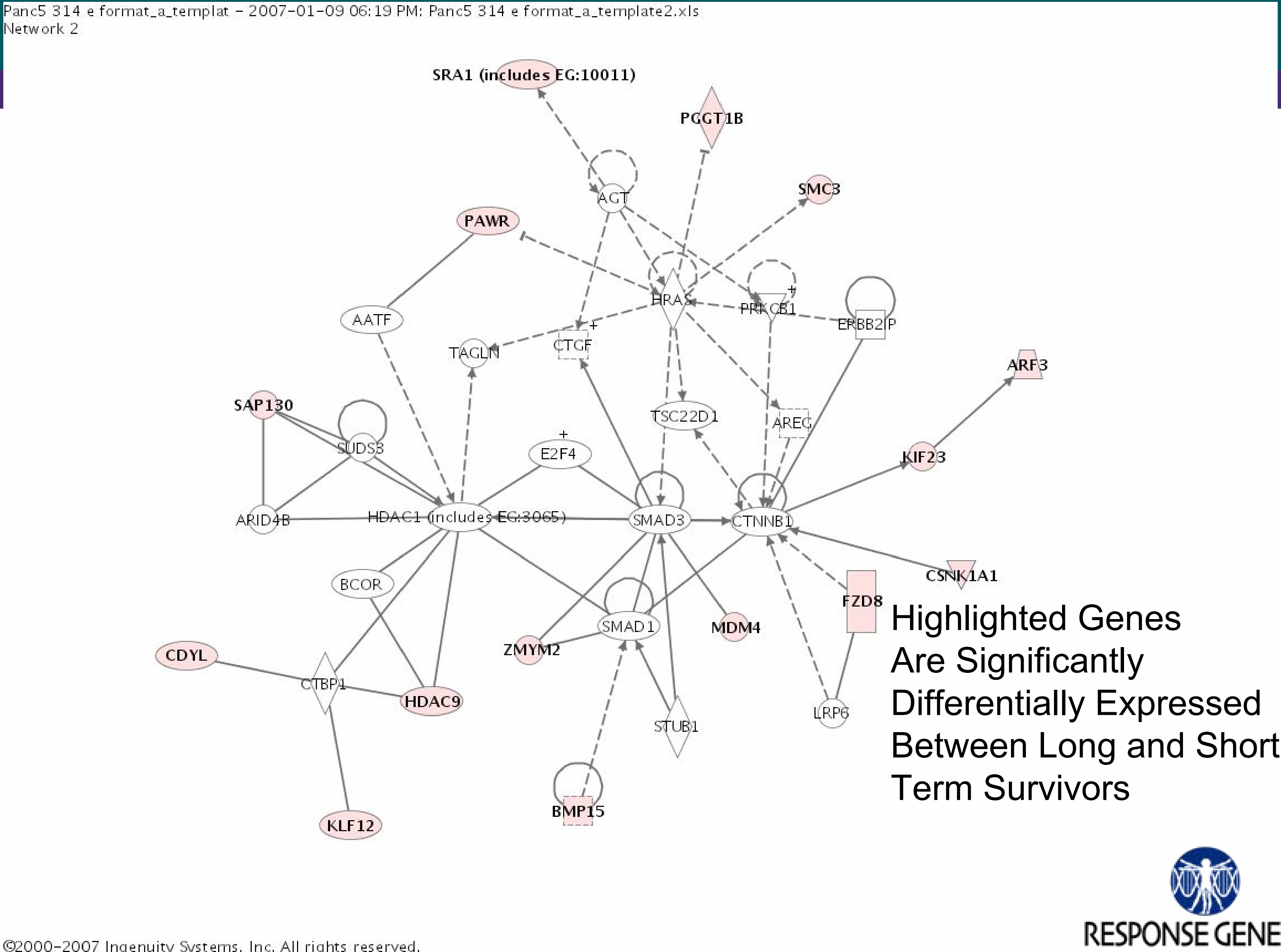
HDAC/SMAD



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Network 2

Highlighted Genes Are Significantly Differentially Expressed Between Long and Short Term Survivors

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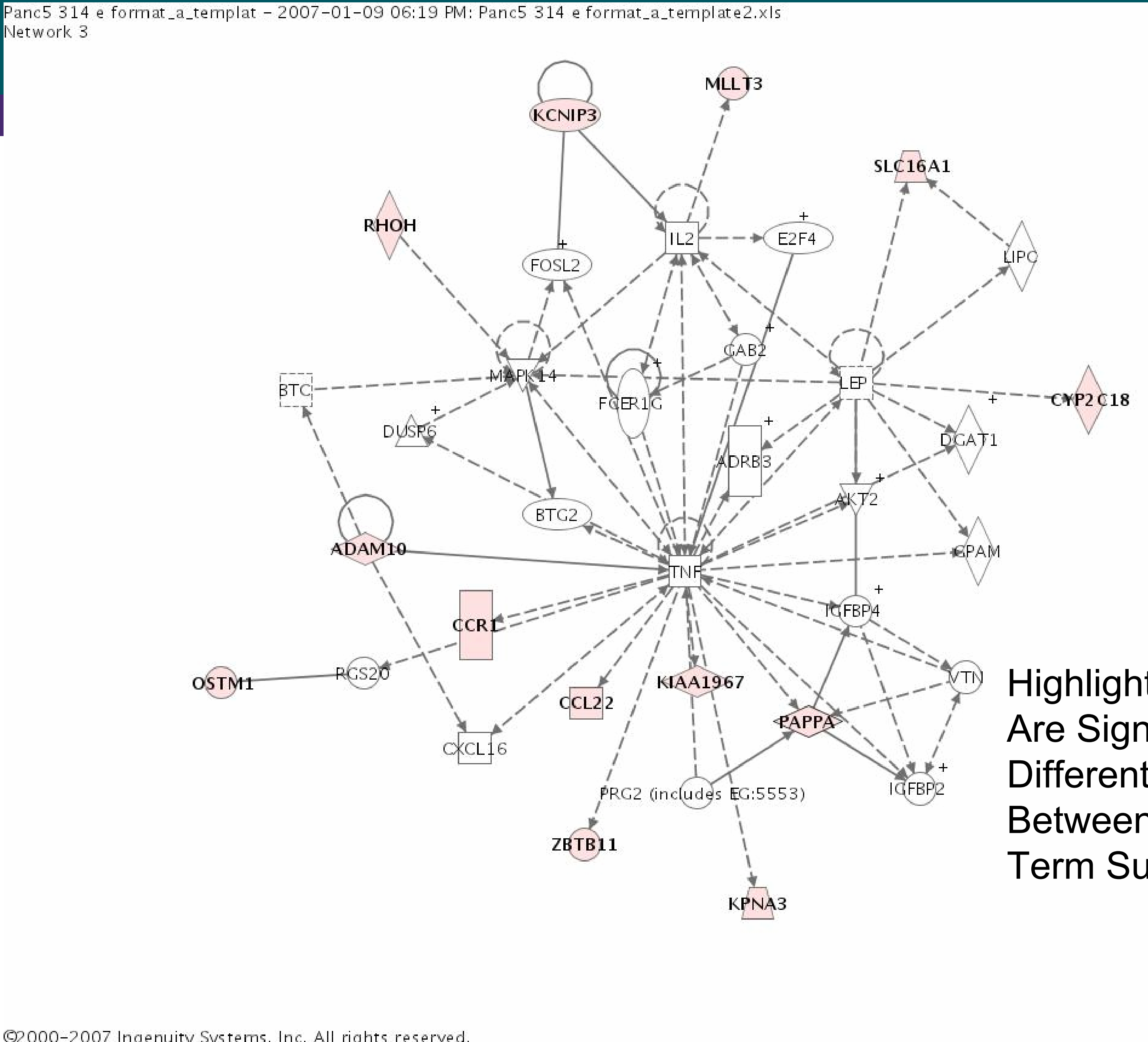
TNF



Panc5 314 e format_a_templat - 2007-01-09 06:19 PM: Panc5 314 e format_a_template2.xls
Network 3

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Highlighted Genes Are Significantly Differentially Expressed Between Long and Short Term Survivors

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MYC

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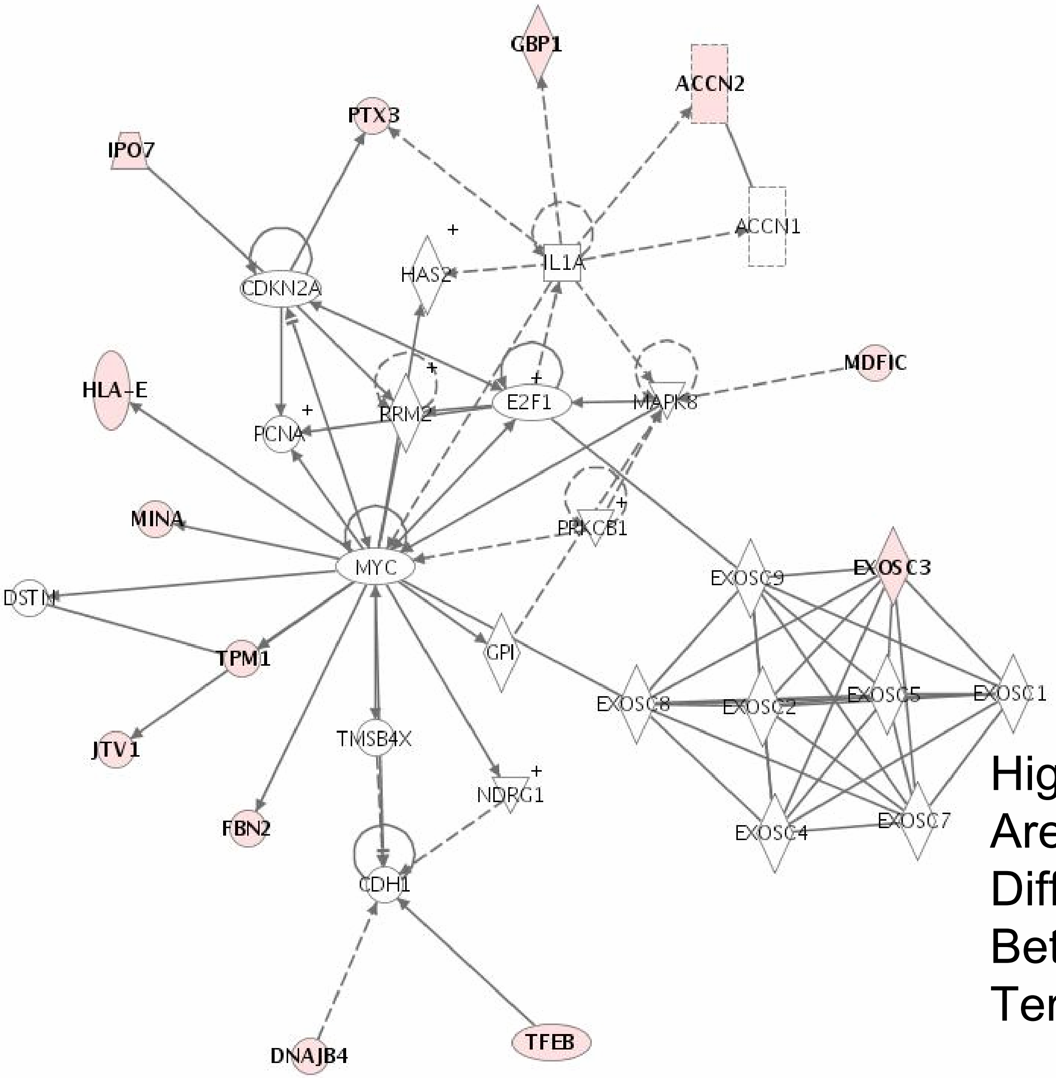
Network 4



Highlighted Genes Are Significantly Differentially Expressed Between Long and Short Term Survivors

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Results

- **Specific Gene Clusters created with supervised analyses based on outcomes identified 314 differentially expressed genes between the long term and short term survivor groups.**
- **Kaplan Meier survival analyses stratifying on clusters revealed significant differences in survival, median survivals of 150 days vs 1122 days, $p<0.001$**
- **Genes differentially regulated were involved in Calcium Signaling. Networks included p53, SMAD, MYC and TNF.**

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Analysis of DNA from Same Pancreatic Cancer Samples using Molecular Inversion Probes (MIPs)

Eric Collisson, UCSF

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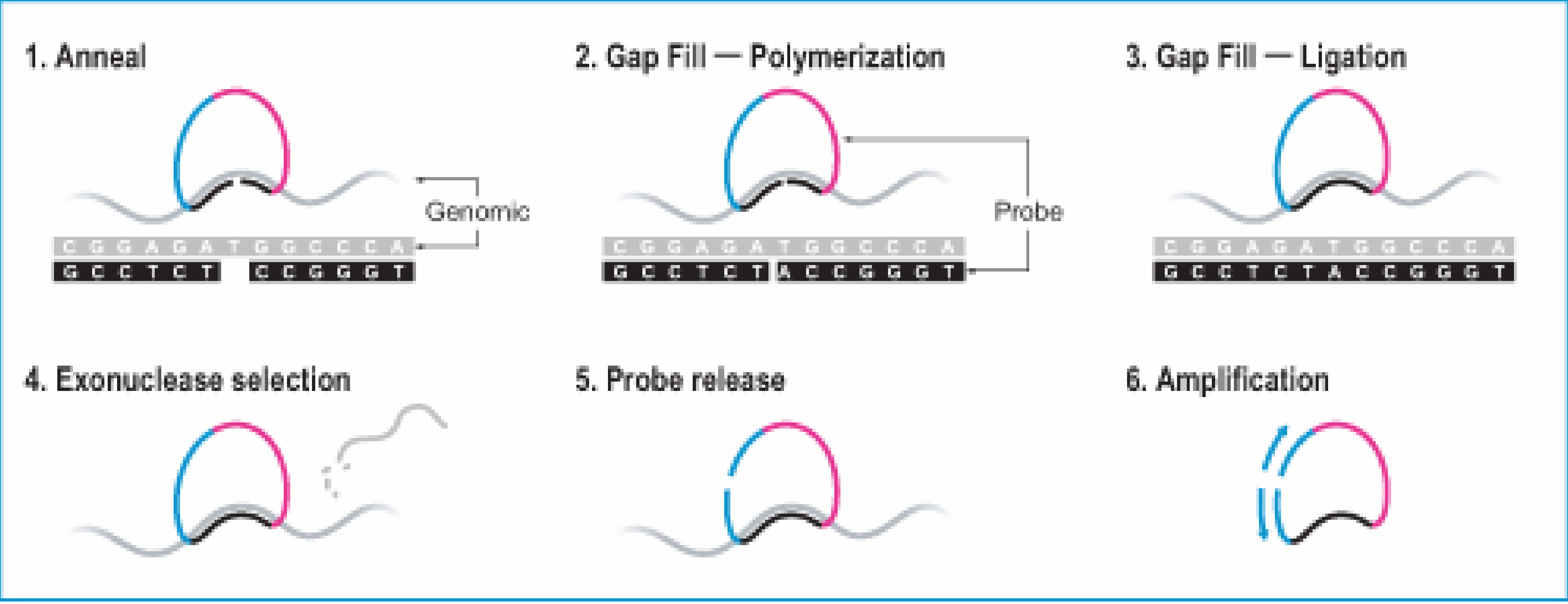
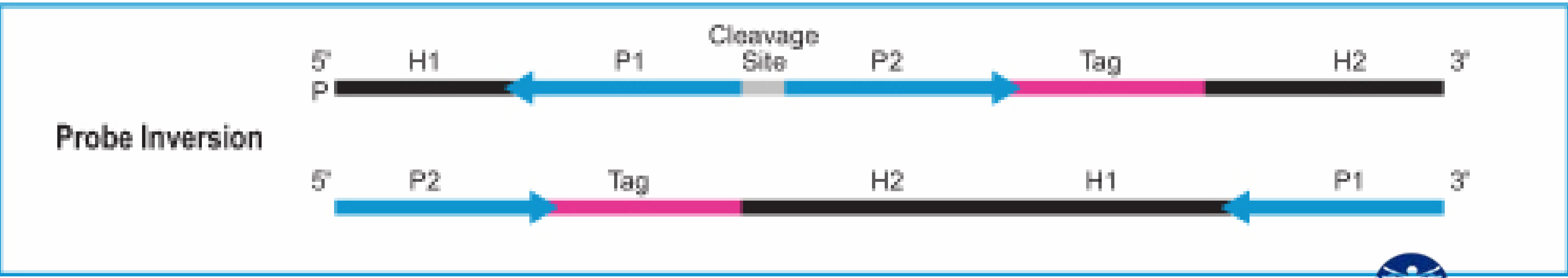
Molecular Inversion Probes





Eric Collisson, UCSF

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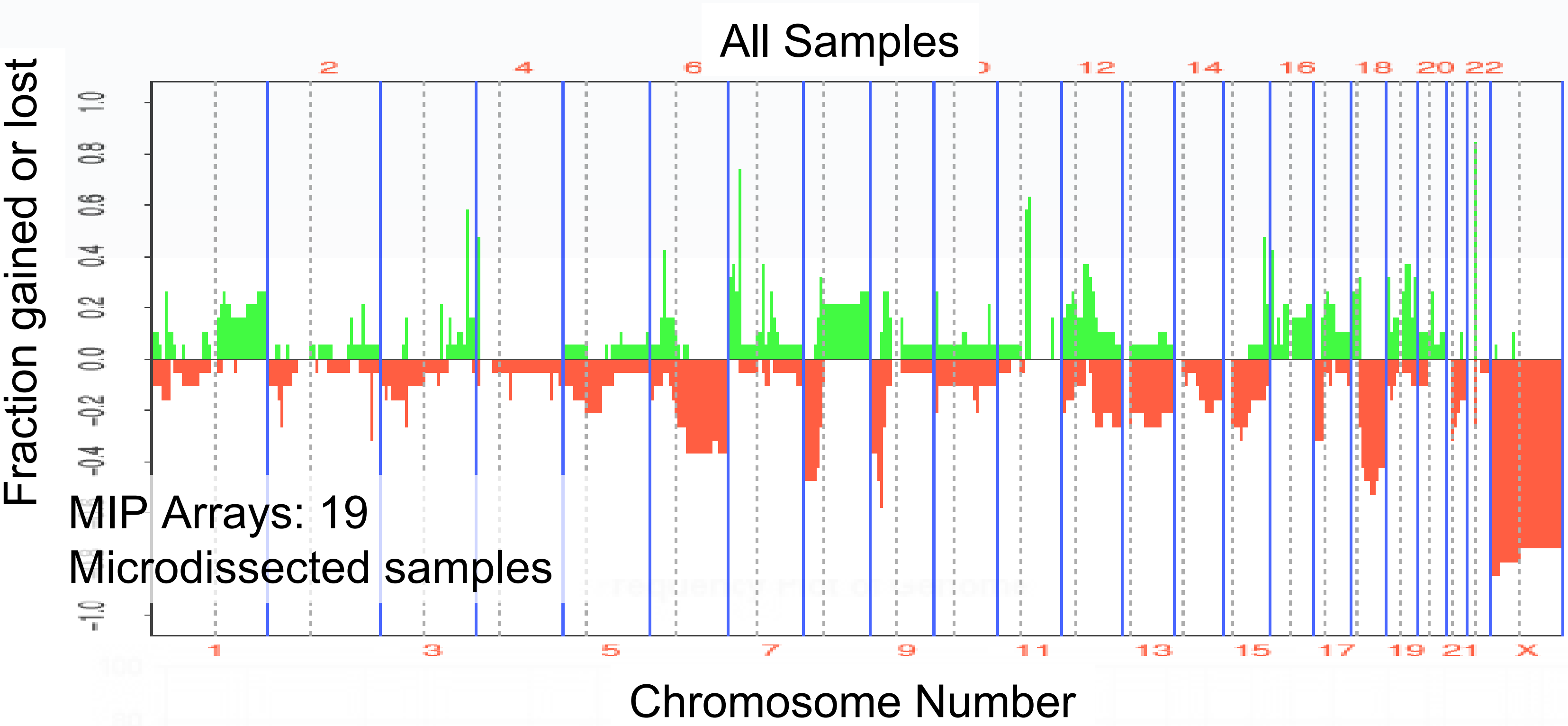
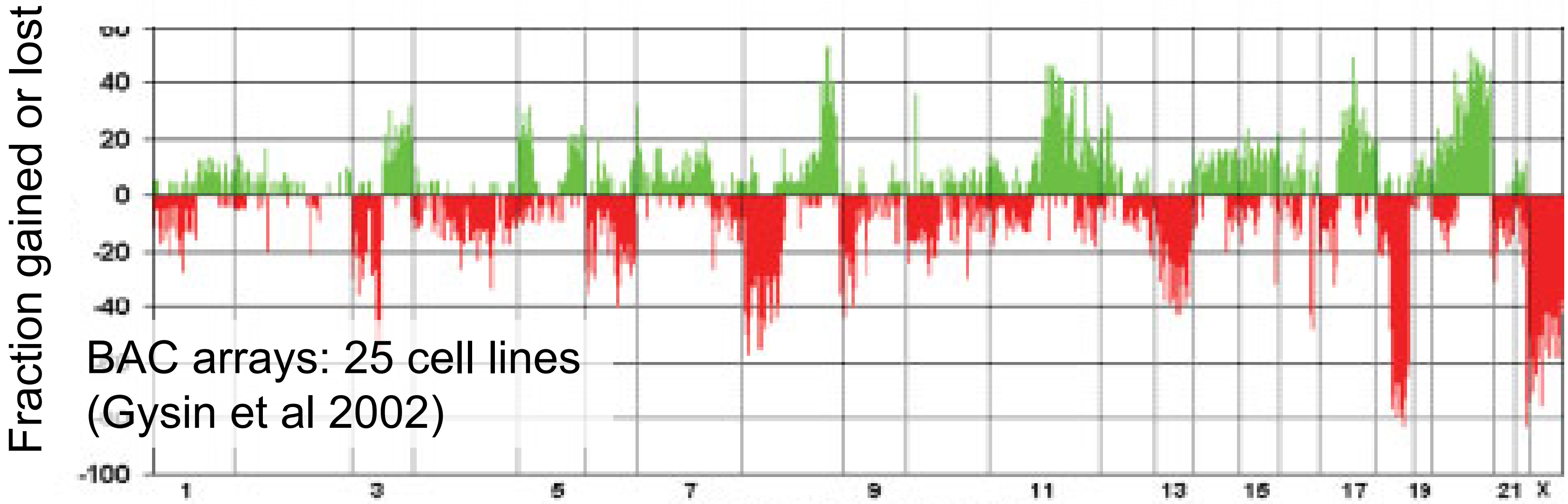
MIPs Advantages

- **Small starting amounts of DNA (70ng in this cohort)**
- **DNA length need be on average no longer than 200 bp**
- **Cancer centric and whole genome platforms**
- **LOH and amplification detection as well as genotyping**



MIP Arrays: 19
Microdissected samples



BAC arrays: 25 cell lines
(Gysin et al 2002)

Eric Collisson, UCSF

Chromosome Number

Conclusions

- **We have demonstrated the feasibility of creating a preliminary genetic risk prediction model using FFPE pancreatic tissue.**
  - **This model will be further validated.**
  - **Patients at high risk of poor survival may consider definitive chemotherapy rather than surgery.**

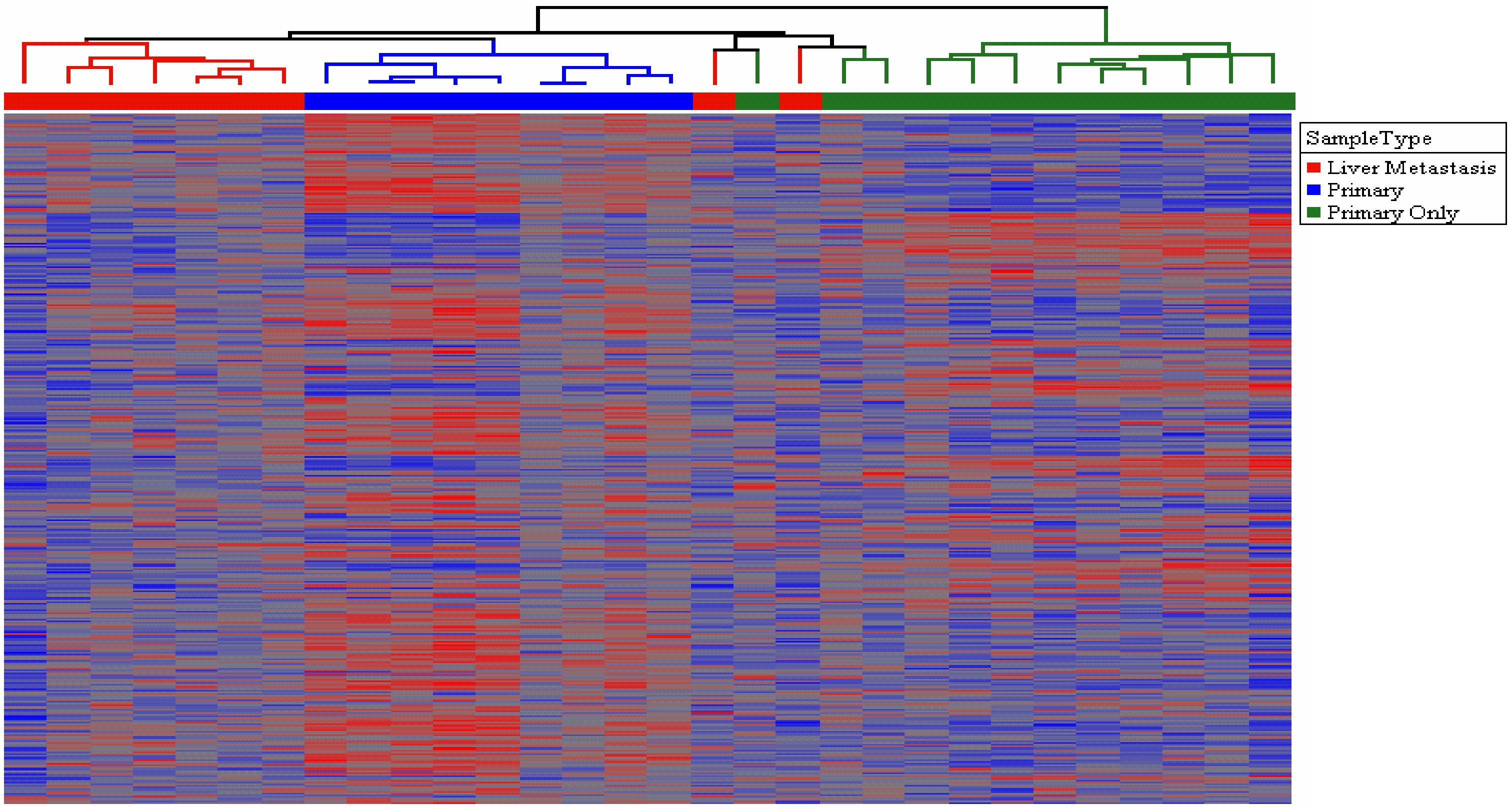
Margaret Tempero, Eric Collison, UCSF

RESPONSE GENETICS™

Molecular Staging in Colorectal Cancer

Liver Mets and matching CRC Metastatic Primary

Non-Metastatic CRC Primary



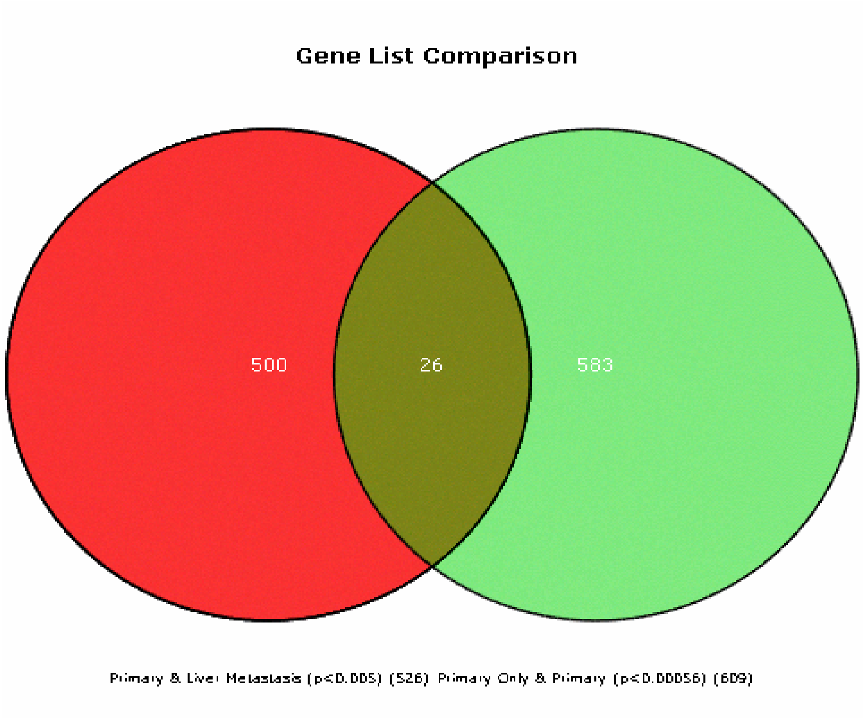
Collaboration with Roche Diagnostics
Shimizu, Lenz, Danenberg et al, ASCO 2006

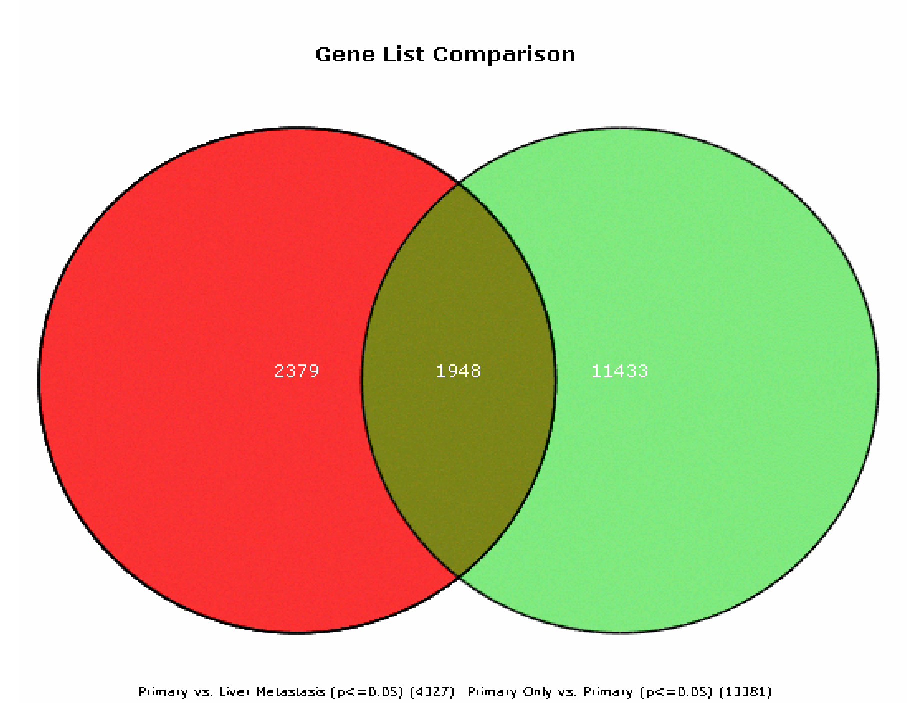
RESPONSE GENETICS™

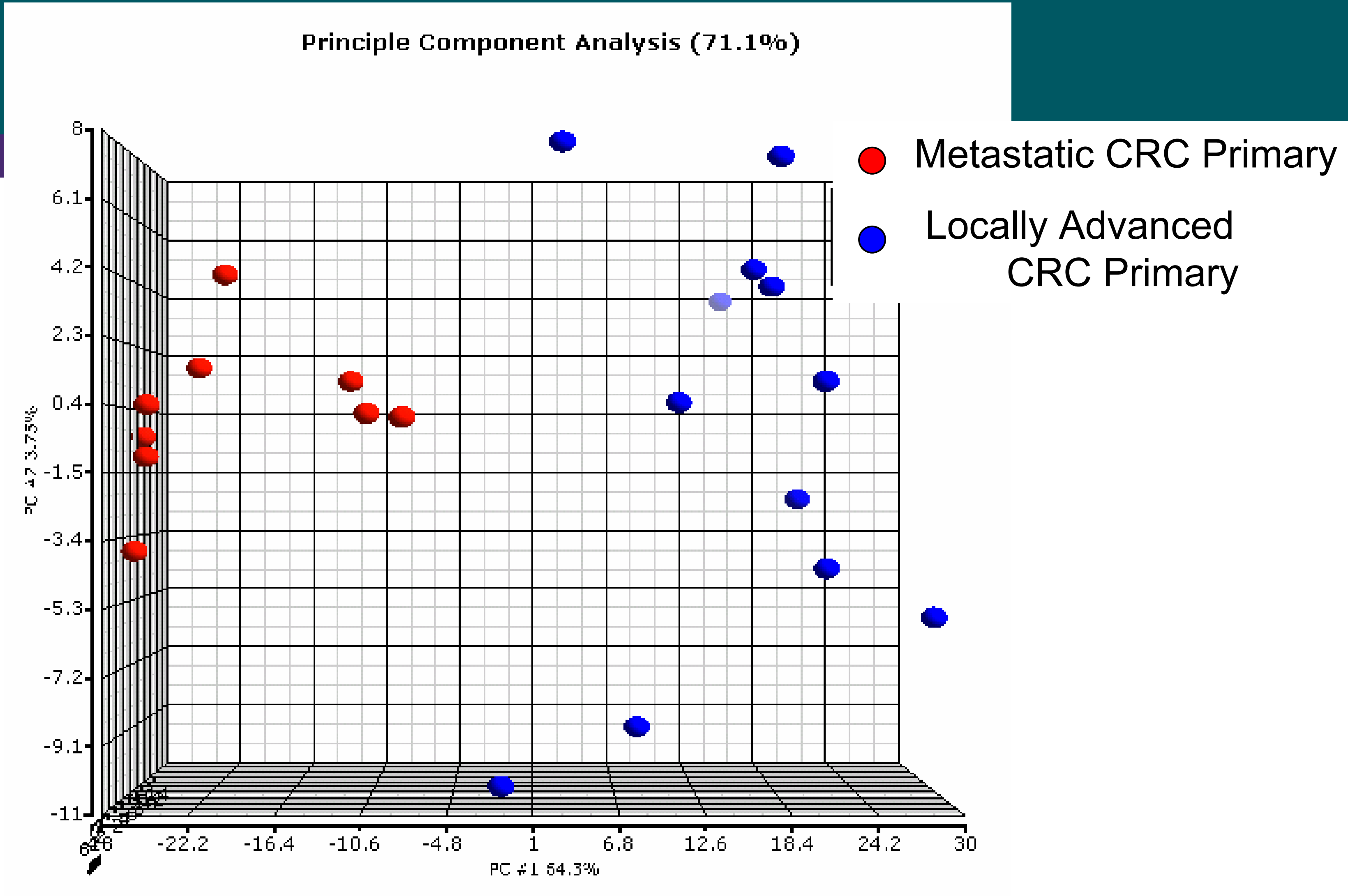
T-test with unequal variance assumption to compare Primary only (N=12) versus Primary with liver metastasis (N=9); 609 differentially expressed probe sets with FDR=0.05 and 13381 with significance p value ≤ 0.05.

Paired T-test to compare Liver Metastasis (N=9) versus Primary (N=9); 2 differentially expressed probe sets with FDR=0.05, 526 with significance p value ≤ 0.005 and 4327 with significance p value ≤ 0.05.

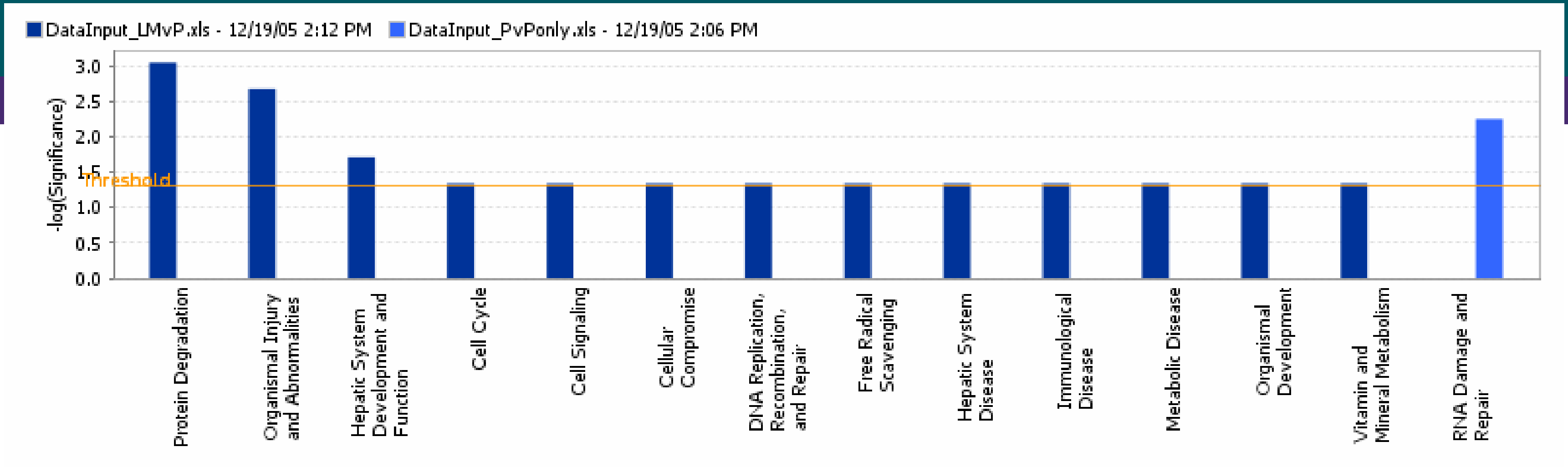
How different are the gene lists between two comparisons?

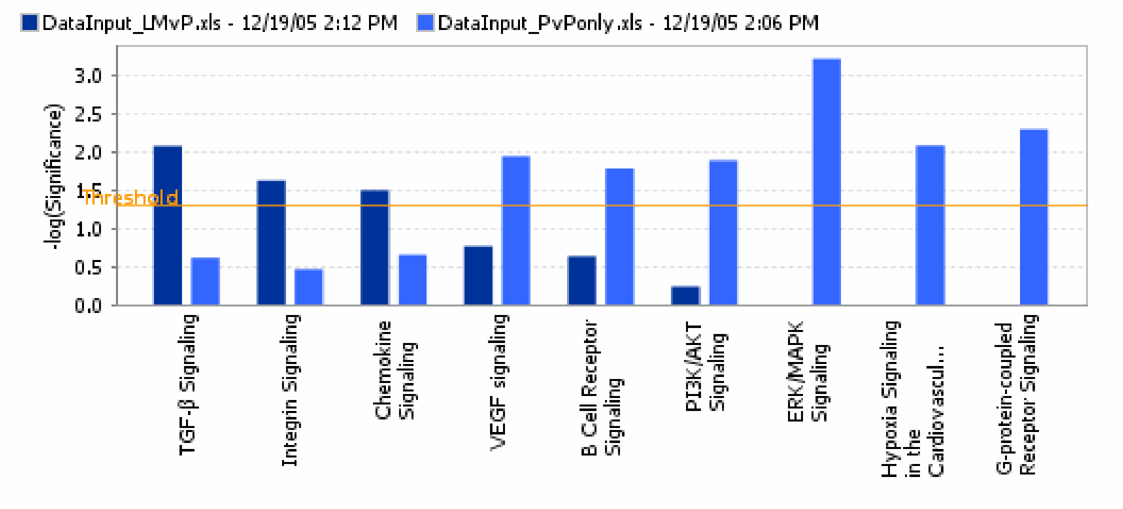






PCA with 609 probe sets (T-test with unequal variance assumption to compare Primary only versus Primary with liver metastasis, FDR=0.05)



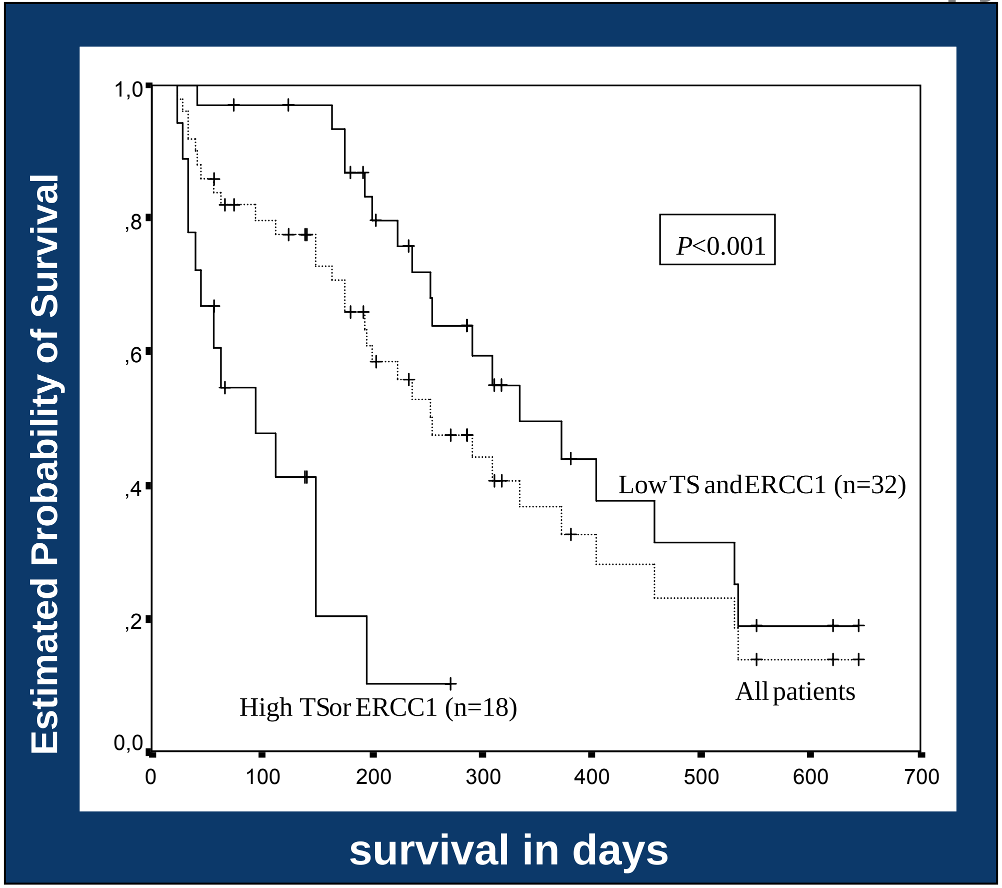


Differential mapping on functions and canonical pathways from IPA analysis (272 and 210 network genes from the gene lists of PvPonly and LMvP comparisons).

RESPONSE GENETICS

Prediction of FolFOX Treatment Outcome in CRC by RT-PCR

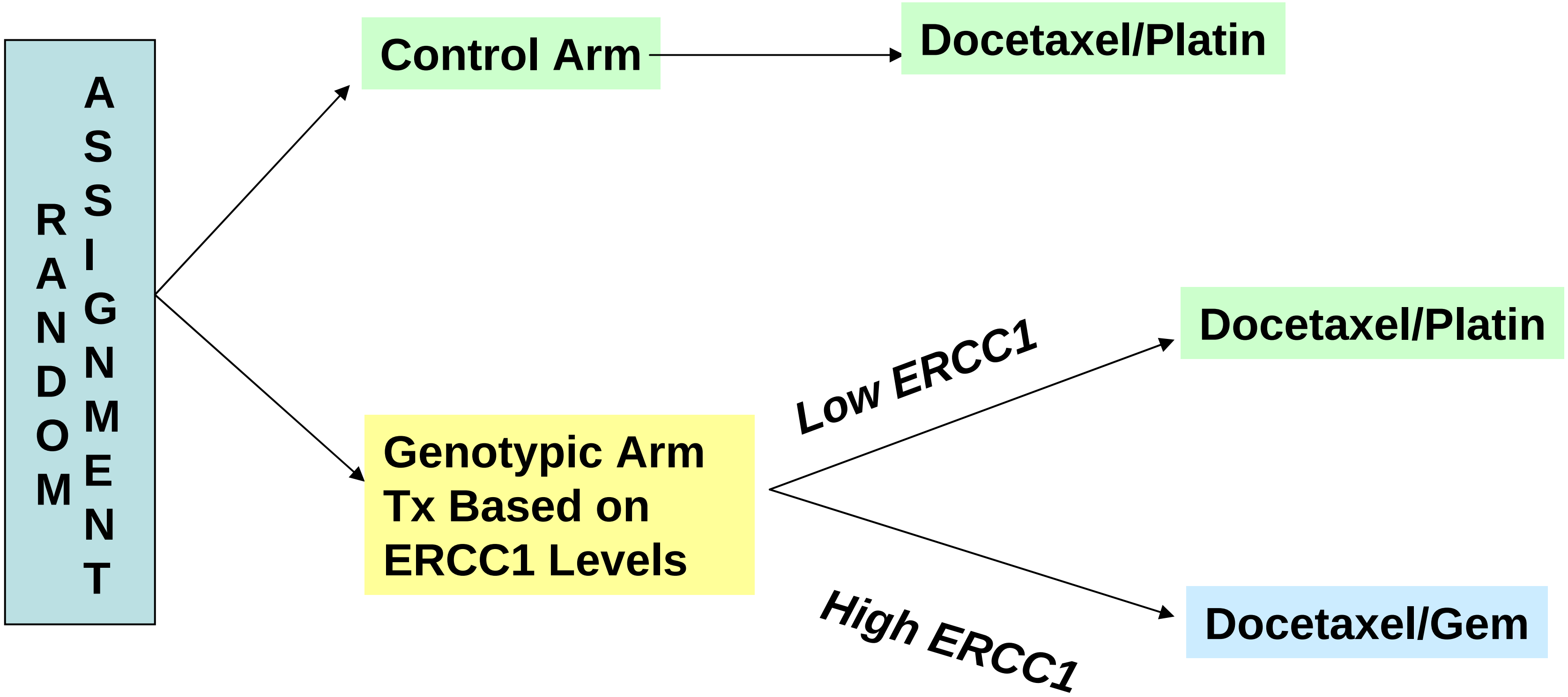
TS and ERCC1 gene expression and survival in CRC patients treated with second line 5-FU/Oxali Therapy



Shirota et al, CCR, 2001

Customizing Cisplatin Based on Quantitative ERCC1 mRNA Expression:

A Phase III Trial in Non Small Cell Lung Cancer



RANDOM ASSIGNMENT

Control Arm → **Docetaxel/Platin**

Genotypic Arm Tx Based on ERCC1 Levels

Low ERCC1 → **Docetaxel/Platin**

High ERCC1 → **Docetaxel/Gem**

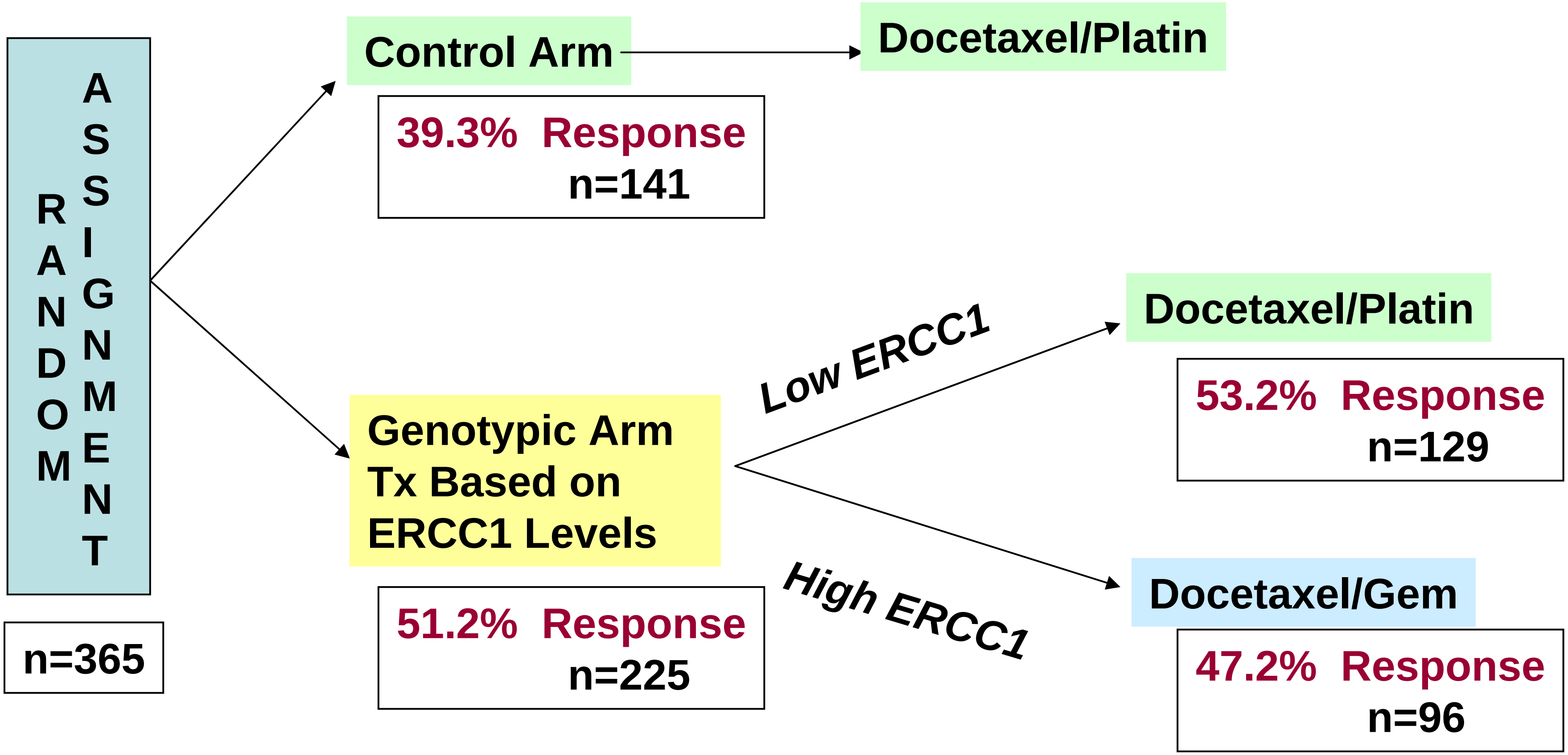
Pre-treatment FFPE biopsies were analyzed by Response Genetics and therapy was directed by level of mRNA ERCC1

Cobo etal, JCO Jul 1 2007: 2747–2754.

RESPONSE GENETICS™

Customizing Cisplatin Based on Quantitative ERCC1 mRNA Expression:
A Phase III Trial in Non Small Cell Lung Cancer

Results:



RANDOM ASSIGNMENT

n=365

Control Arm

39.3% Response
n=141

Docetaxel/Platin

Genotypic Arm
Tx Based on
ERCC1 Levels

51.2% Response
n=225

Low ERCC1

Docetaxel/Platin

53.2% Response
n=129

High ERCC1

Docetaxel/Gem

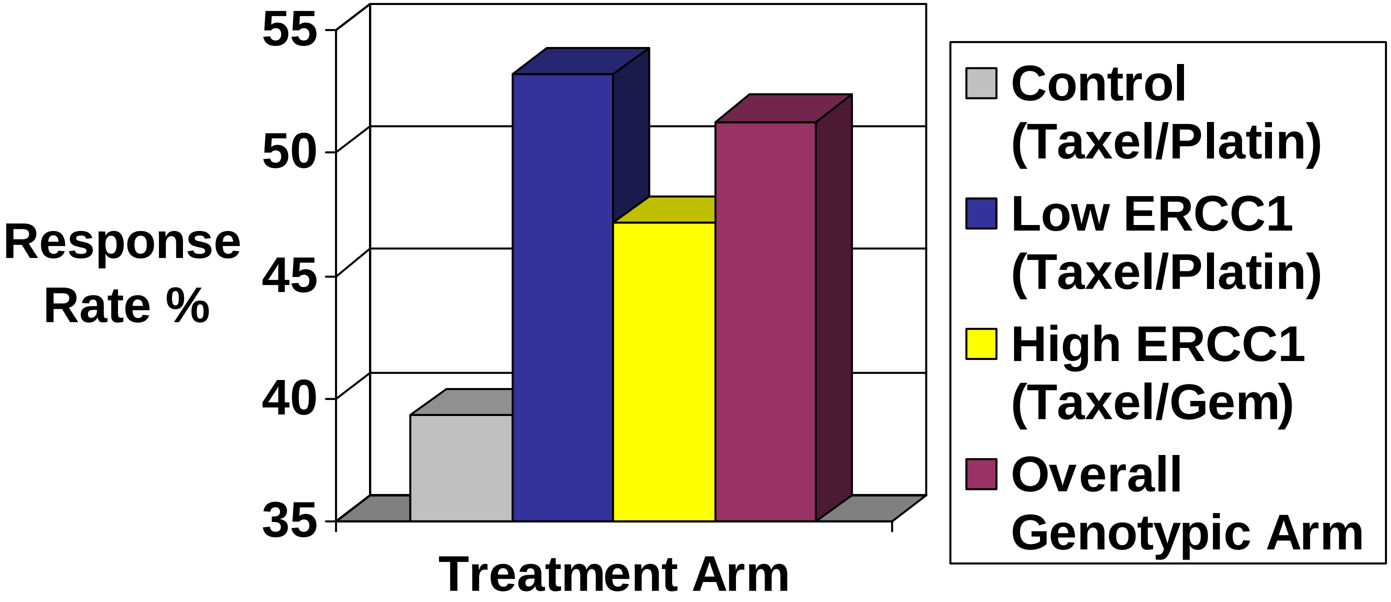
47.2% Response
n=96

***Cobo etal, JCO* Jul 1 2007: 2747–2754.**

RESPONSE GENETICS™

Customizing Cisplatin Based on Quantitative ERCC1 mRNA Expression:
A Phase III Trial in Non Small Cell Lung Cancer

Results: Patient Selection for Therapy Significantly Increased Response Rate



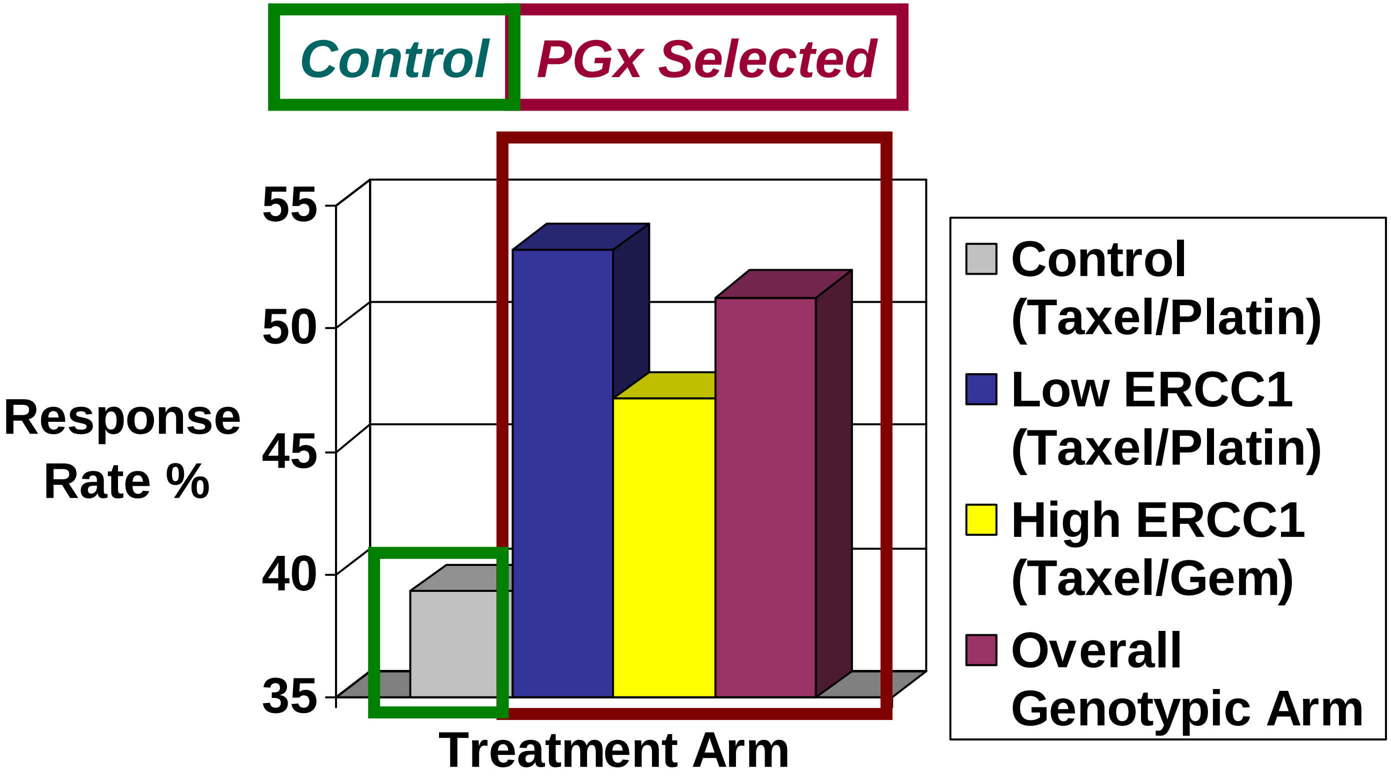
p<0.02
Genotypic vs Control

RESPONSE GENETICS™

Customizing Cisplatin Based on Quantitative ERCC1 mRNA Expression:

A Phase III Trial in Non Small Cell Lung Cancer

Results: Patient Selection for Therapy Significantly Increased Response Rate



$p<0.02$
Genotypic vs Control

Conclusion

- **These results show that it is possible to obtain meaningful gene expression and biological pathway data from microarrays using RNA extracted from FFPE samples.**
- **We intend to use our technologies in an effort to develop “gene signatures” as diagnostics that predict patient outcome to various therapies.**