

***Microarray analyses:  
From mouse model to predicting human survival***

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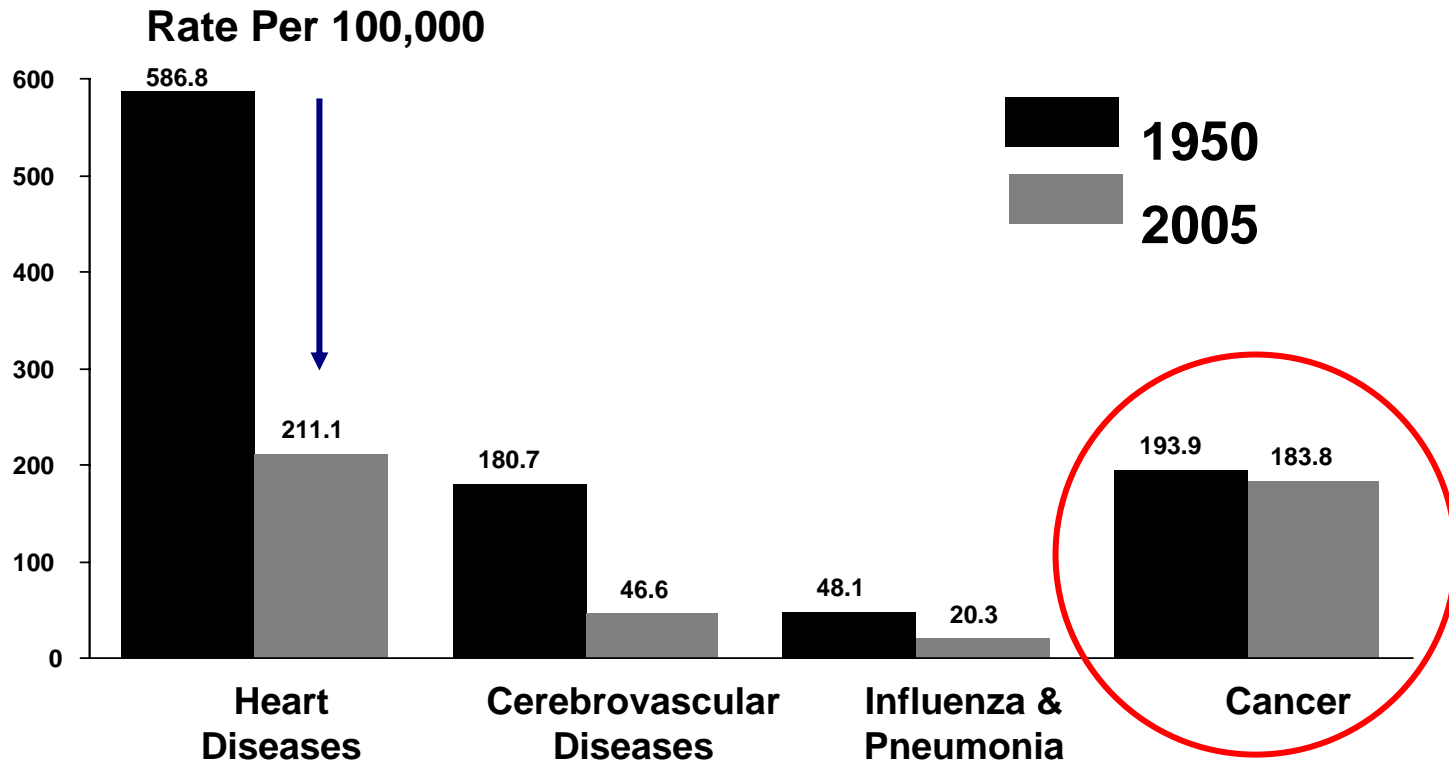
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# Other team members

- Josh Smith
- Bing Zhang
- Pengcheng Lu
- Yu Shyr
- .....

# Change in the US Death Rates\* by Cause, 1950 & 2005



\* Age-adjusted to 2000 US standard population.

Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.

2005 Mortality Data: US Mortality Data 2005, NCHS, Centers for Disease Control and Prevention, 2008.

# The problem

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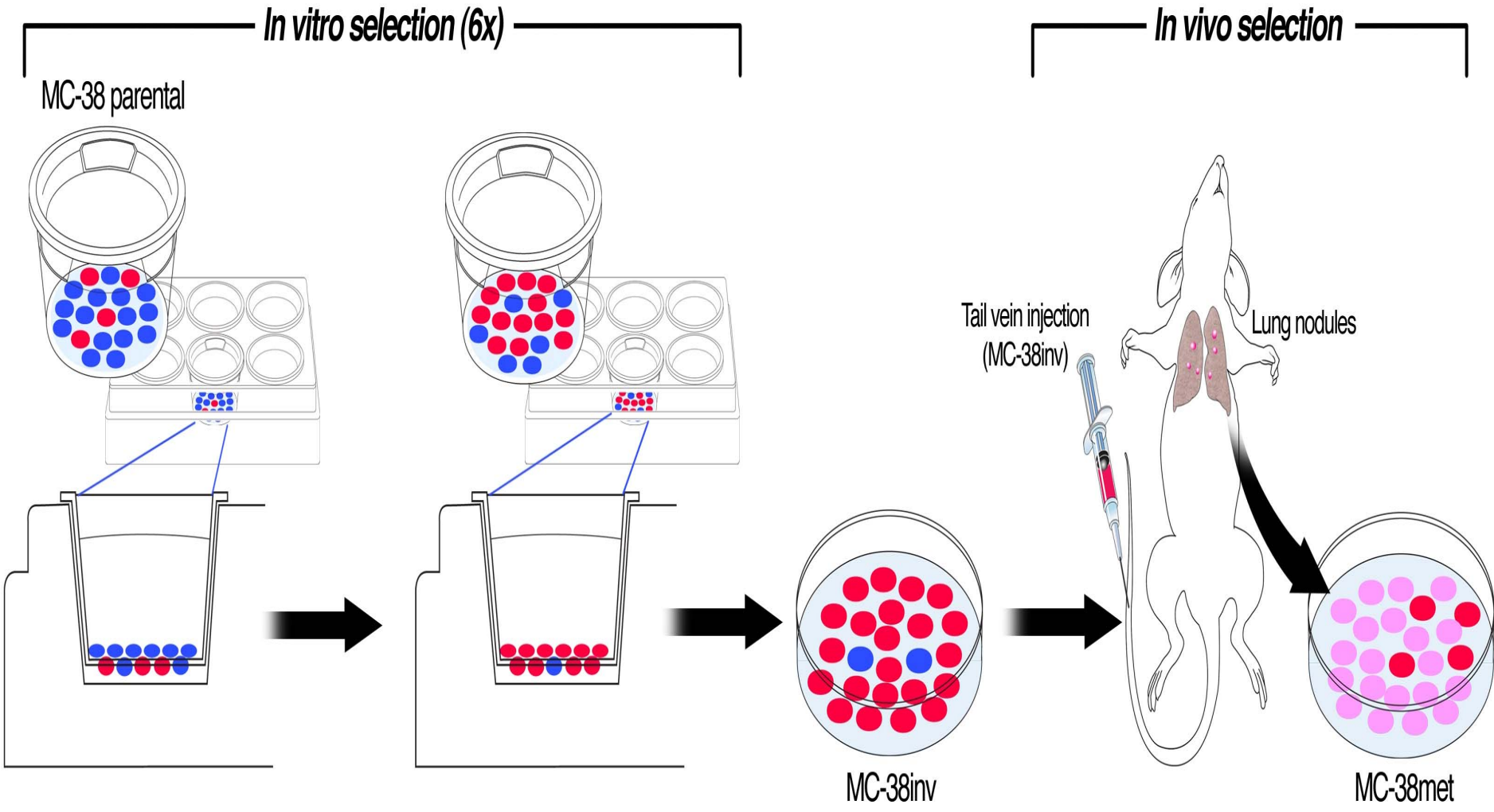
- Colon cancer was estimated to be the **2<sup>nd</sup> leading cause** of cancer-related death in the US for 2008.
- Although a number of groups have identified prognostic gene signatures in colon cancer, few have been based upon the biology of **metastasis**.

# Mouse model

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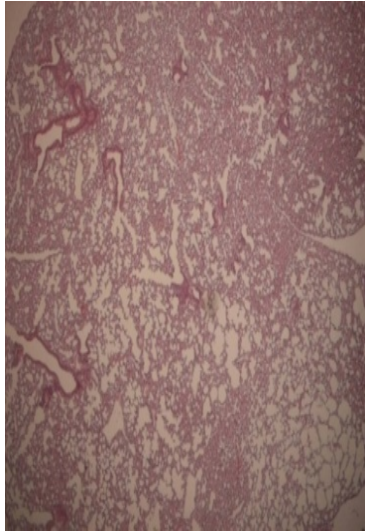
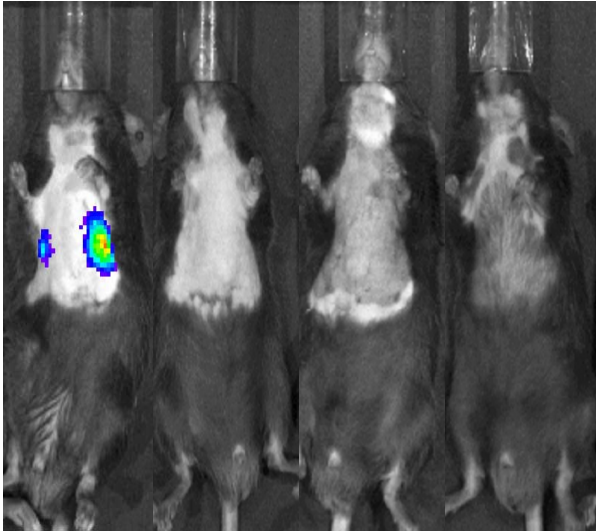
- MC-38 cells were transfected with **firefly luciferase gene** and selected in 0.5mg/mL G418.
- To enrich for invasive MC-38 cells,  $7.5 \times 10^5$  cells were seeded onto 6-well, 8.0  $\mu$ M pore transwell polycarbonate membrane inserts coated with 2.5 mg/mL **matrigel** and incubated with **serum-free** DMEM in the upper chamber and complete DMEM in the bottom well.
- Invading cells were collected after six serial passages through matrigel-coated Boyden chambers.
- The selected invasive cells and the parental luciferase-expressing MC-38 cells were **injected into the tail vein** and the development of lung metastases was assessed. Development of metastases was determined by bioluminescence imaging.
- The MC-38met cells were then derived by culture of tumor cells from a **metastatic lung** tumor.

# Murine model of metastasis

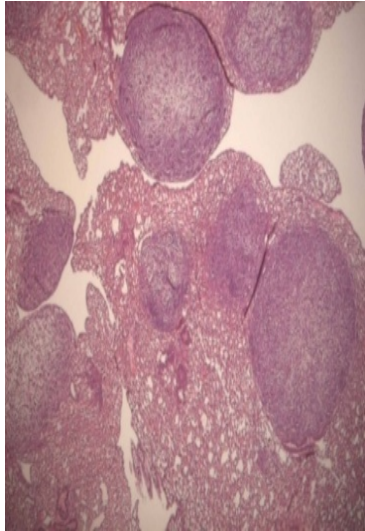
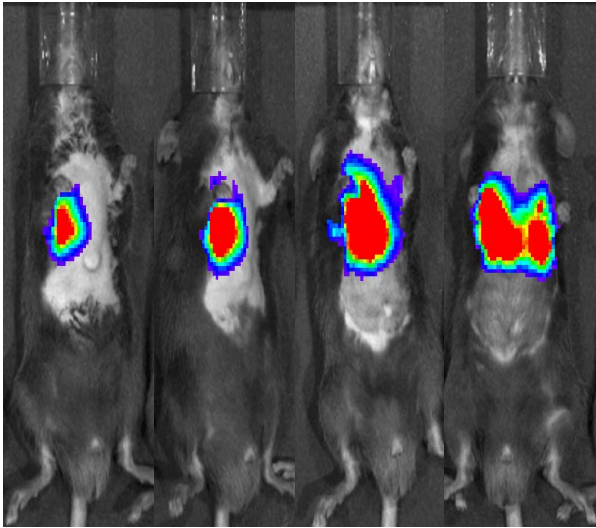


***In vivo* monitoring and *ex vivo* proof of metastases**

**MC-38 parental**



**MC-38inv**



**Days post-injection**

**1      7      14      21**

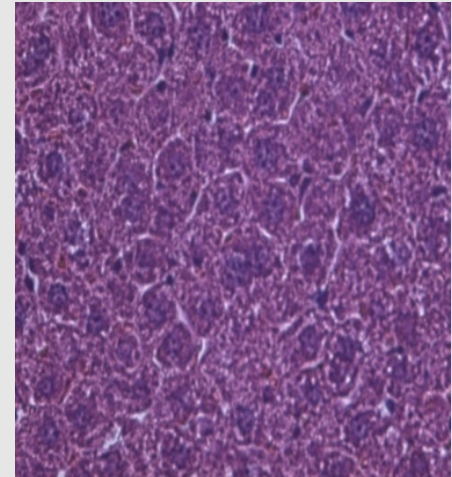
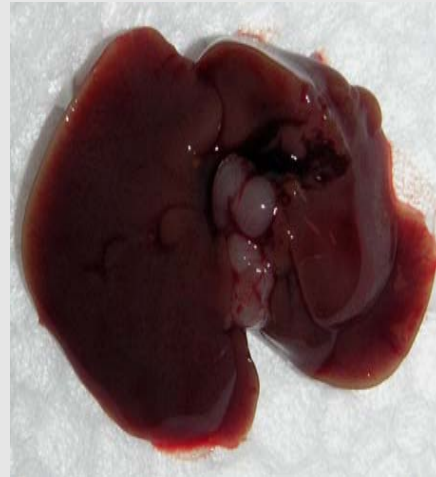
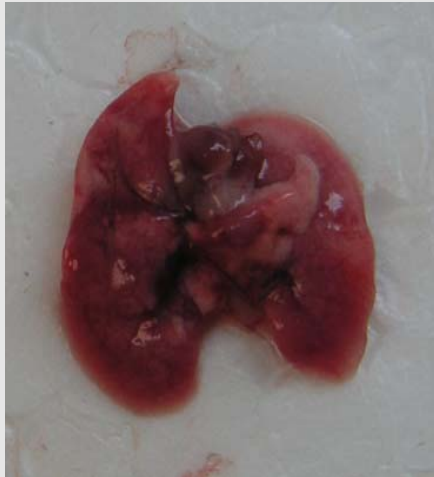
**21      21**

# ***MC-38met cells are highly metastatic in vivo***

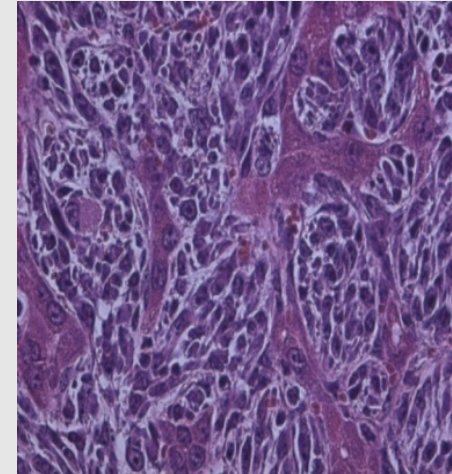
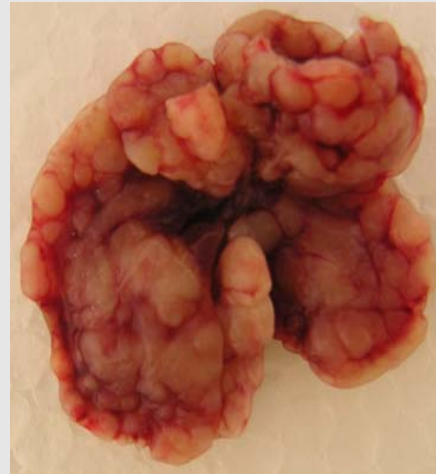
**Tail-vein injection**

**Splenic injection**

**MC-38  
parental**



**MC-38met**



Lung

Liver

# Development of the recurrence signature - Mouse to Man

MC-38 parental vs. MC-38met

Differential gene expression analysis by microarray



300 Differentially expressed genes with human orthologues  
“**metastatic gene signature**”



*PARTIAL TRAINING Set (Vanderbilt Medical Center):*

Refinement of 300 homologous genes with high-risk patients

Refinement: Concordance analysis of MC-38met and VMC high-risk patients



“**34-gene recurrence signature**”



*Get model from FULL 55 patient TRAINING Set*  
(Vanderbilt Medical Center)



TESTING Set (Moffitt Cancer Center): 177 colon cancer patients

## Signature from mouse

MC-38 parental vs. MC-38met  
Differential gene expression analysis by microarray



300 Differentially expressed genes with human orthologues  
“**metastatic gene signature**”

Differentially expressed genes were determined using the limma package in Bioconductor based upon 3 criteria:

- (1) **Fold change** >2;
- (2) False discovery rate (**FDR**) based on the moderated t-test followed by Benjamini and Hochberg's multiple-test adjustment <0.01;
- (3) **Log odds** of differential expression (B-statistic) > 1.

## Signature – from Mouse to Man

300 Differentially expressed genes with human orthologues  
“**metastatic gene signature**”



**PARTIAL TRAINING Set (Vanderbilt Medical Center):**

Refinement of 300 homologous genes with high-risk patients

Refinement: Concordance analysis of MC-38met and VMC high-risk patients



“**34-gene recurrence signature**”

Directional concordance between MC-38met cells and 19 patients from VMC with high-risk of recurrence or cancer-related death (17-stage IV patients and 2-stage III patients) was determined using a cut-off of  $p = 0.08353 \leq 0.10$  (binom.test , at least 13 out of 19 patients have same direction changing as in MC-38 met cells) to refine the metastasis-high-risk signature.

## Predicting human survival

“34-gene recurrence signature”



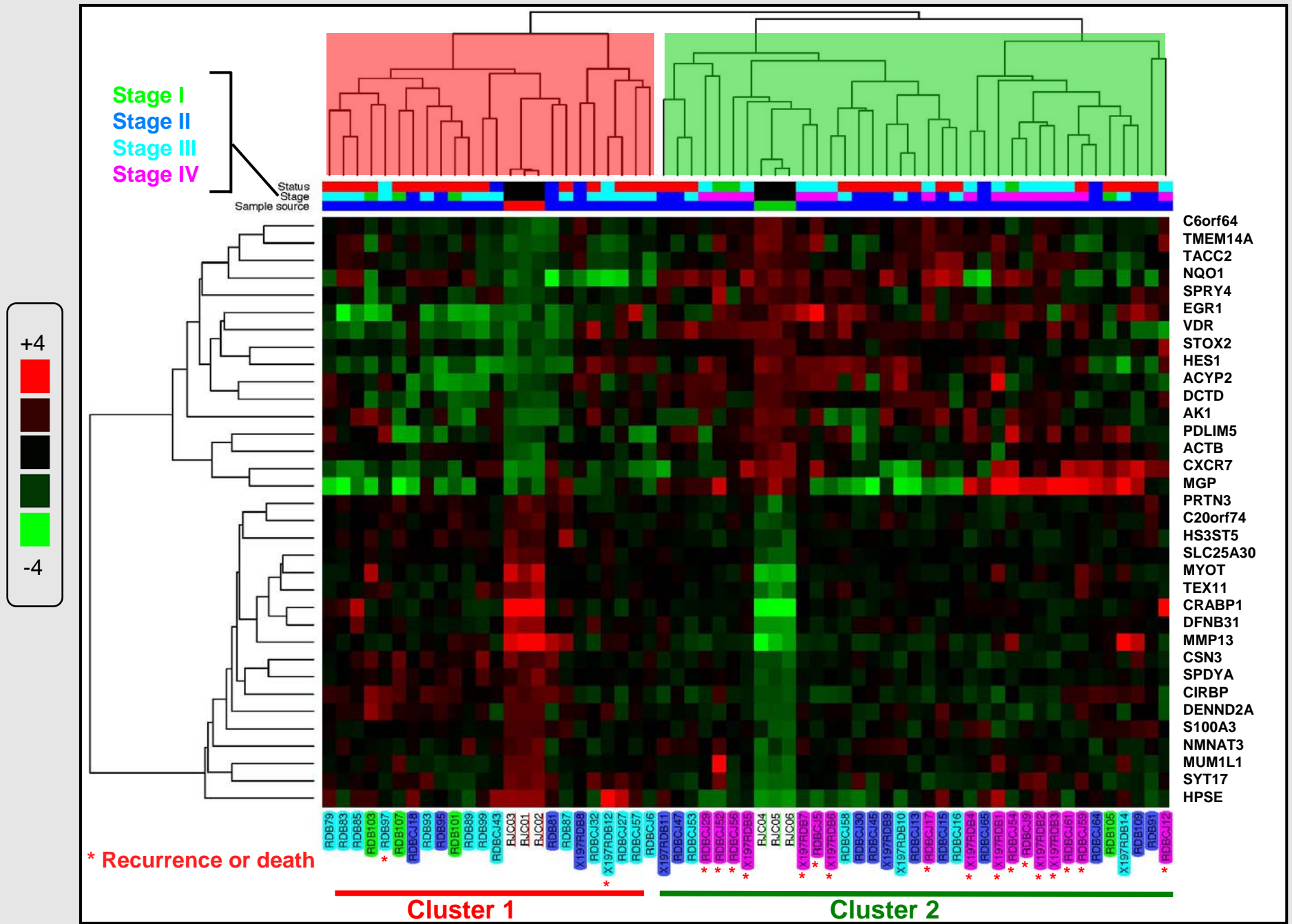
Get model from FULL 55 patient TRAINING Set  
(Vanderbilt Medical Center)



TESTING Set (Moffitt Cancer Center): 177 colon cancer patients

Based on full training dataset, run Cox for each Affymetrix probe (34 genes -> 60 Affymetrix probes), get beta and Wald test stat, use these sign of beta and Wald, and expression testing dataset to calculate compound score for each patient in the test dataset, then use compound scores to predict human survival.

# Functional genomic clustering analysis (VMC 55)



# Characterization of the 34-gene recurrence signature

## Up-regulated Genes

Gene symbol	Fold change	Processes/Networks
<b>CXCR7</b>	<b>3.9</b>	<b>Cancer, survival/growth and chemotaxis</b>
AK1	2.8	Nucleotide binding
ACTB	2.8	Cancer, cell morphology and motility, growth, polarization and adhesion
MGP	2.8	Cell-cell Signaling, branching, migration
<b>HES1</b>	<b>2.8</b>	<b>Cancer, endocrine function, cell death</b>
TMEM14A	2.6	Cell proliferation (target of CREB)
<b>EGR1</b>	<b>2.5</b>	<b>Cancer, endocrine function, cell death</b>
VDR	2.4	Cancer, endocrine function, cell death
C6orf64	2.4	Membrane dynamics
NQO1	2.4	Cancer, cell death
STOX2	2.3	Putative stem cell marker
ACPY2	2.2	Mutated in aromatic rice
SPRY4	2.1	Cancer; cell migration, proliferation, differentiation
DCTD	2.1	Nucleotide biosynthesis
TACC2	2.1	Cancer; biogenesis, morphology, proliferation
PDLIM5	2.0	Cancer, actin binding

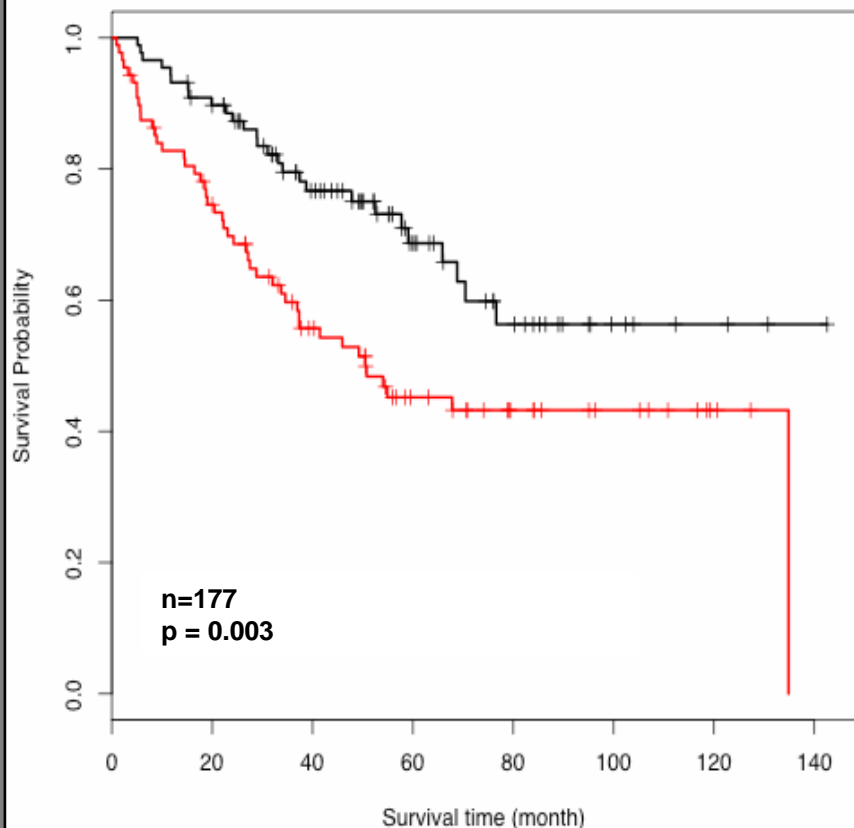
## Down-regulated genes

Gene symbol	Fold change	Processes/Networks
<b>CRABP1</b>	<b>-21.0</b>	<b>Cancer, CpG Island Methylation</b>
<b>MMP13</b>	<b>-17.3</b>	<b>Cell-cell signaling, immune response</b>
MYOT	-7.1	Cancer, actin filaments and stress fibers
DFNB31	-5.1	Cell-cell Signaling
HPSE	-4.7	Cell-cell signaling, immune response
TEX11	-3.7	Cell cycle/division
SYT17	-3.1	Membrane protein
MUM1L1	-2.8	Unknown
SLC25A30	-2.6	Oxidative stress
CSN3	-2.5	Cell cycle, membrane dynamics
NMNAT3	-2.4	Nucleotide biosynthesis
DENND2A	-2.4	Unknown
CIRBP	-2.3	Cancer, nucleotide binding
SPDYA	-2.3	Putative cell cycle
S100A3	-2.2	Cancer
PRTN3	-2.1	Cell-cell signaling, immune response
C20orf74	-2.1	GTPase regulation
HS3ST5	-2.0	Putative epigenetic regulation

<b>Study Demographics</b>	<b><i>VMC-training</i></b>	<b><i>MCC-testing</i></b>
<b><i>Sample size</i></b>	<b>55</b>	<b>177</b>
<b><i>Mean Age (s.d.)</i></b>	<b>62.3 (14.1)</b>	<b>65.5 (13.1)</b>
<b><i>Sex (%male)</i></b>	<b>30 (54.5%)</b>	<b>96 (54.2%)</b>
<b><i>Stage I</i></b>	<b>4 (7.3%)</b>	<b>24 (13.6%)</b>
<b><i>Stage II</i></b>	<b>15 (27.3%)</b>	<b>57 (32.2%)</b>
<b><i>Stage III</i></b>	<b>19 (34.5%)</b>	<b>57 (32.2%)</b>
<b><i>Stage IV</i></b>	<b>17 (30.9%)</b>	<b>39 (22%)</b>
<b><i>Median Follow-up in Months (Min/Max)</i></b>	<b>50.2 (0.4 - 111.3)</b>	<b>48.1 (0.92 - 142.6)</b>
<b><i>Number of deaths</i></b>	<b>20 (36.3%)</b>	<b>73 (41.2%)</b>
<b><i>Caucasian (%)</i></b>	<b>50 (90.9%)</b>	<b>151 (85.3%)</b>
<b><i>Black (%)</i></b>	<b>4 (7.3%)</b>	<b>9 (5.1%)</b>
<b><i>Other (%)</i></b>	<b>1 (1.8%)</b>	<b>17 (9.6%)</b>

# Overall and disease-specific survival in the Moffitt test set

## All Stages: Overall survival

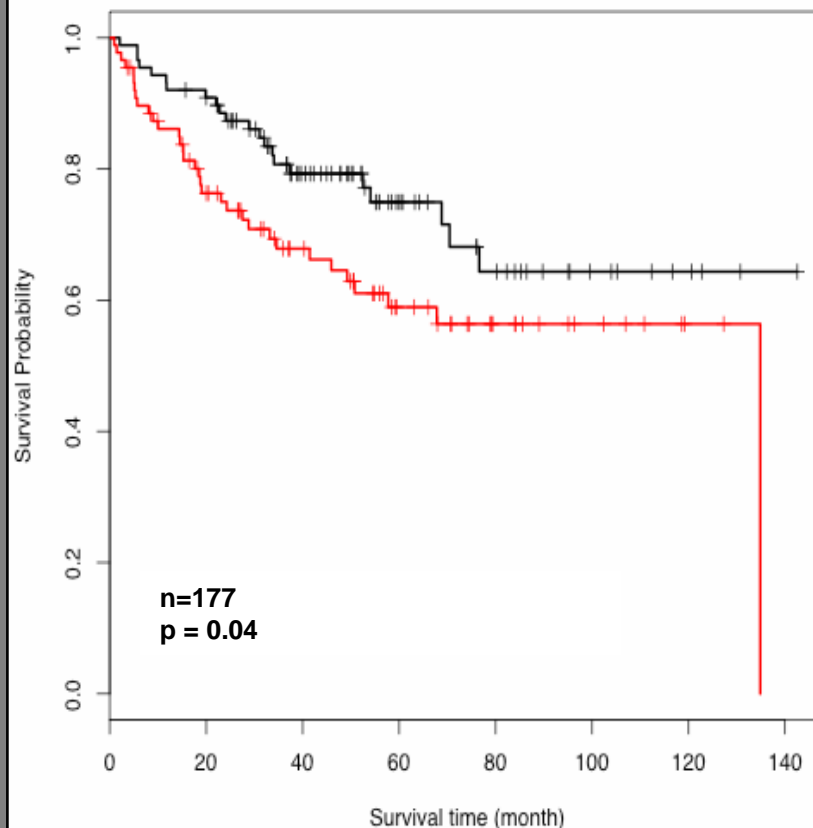


**Recurrence score comparison:**

**Low score (n=88) = 27 deaths**

**High score (n=89) = 46 deaths**

## All Stages: Disease-specific survival



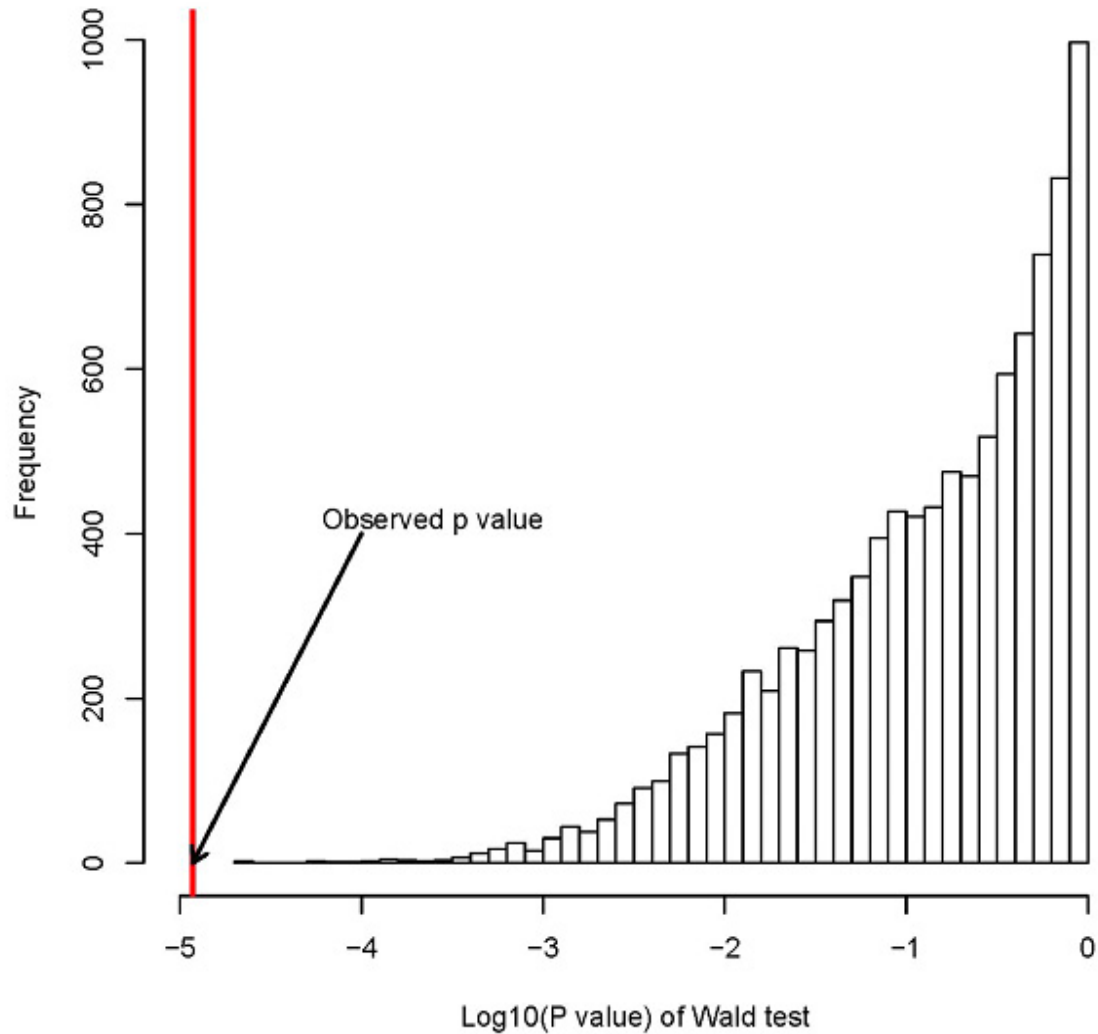
**Recurrence score comparison:**

**Low score (n=88) = 22 deaths**

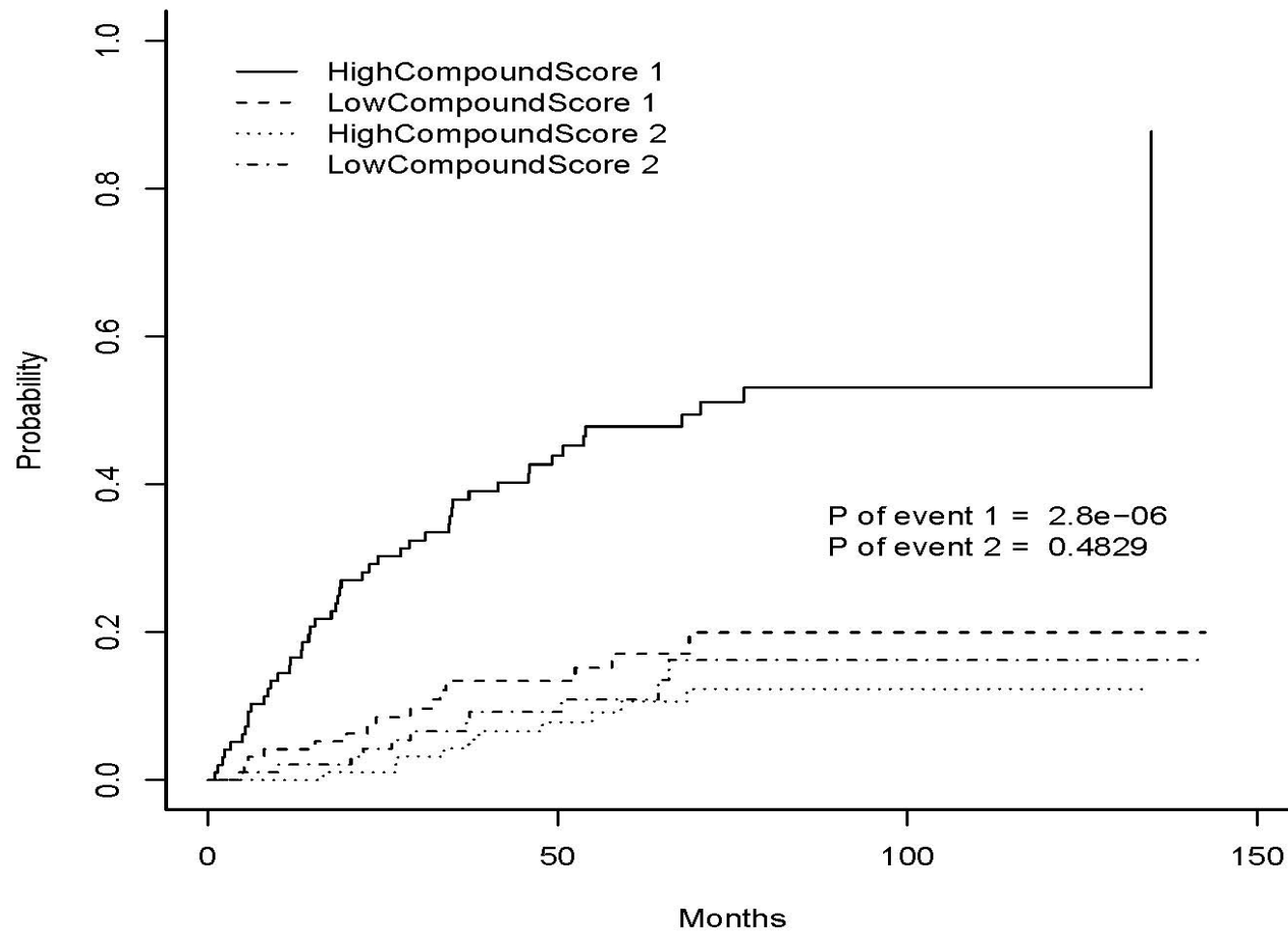
**High score (n=89) = 33 deaths**

# Permutation test

Distribution of 10,000 permutation Wald tests of MCC data with 34-gene poor-prognosis score



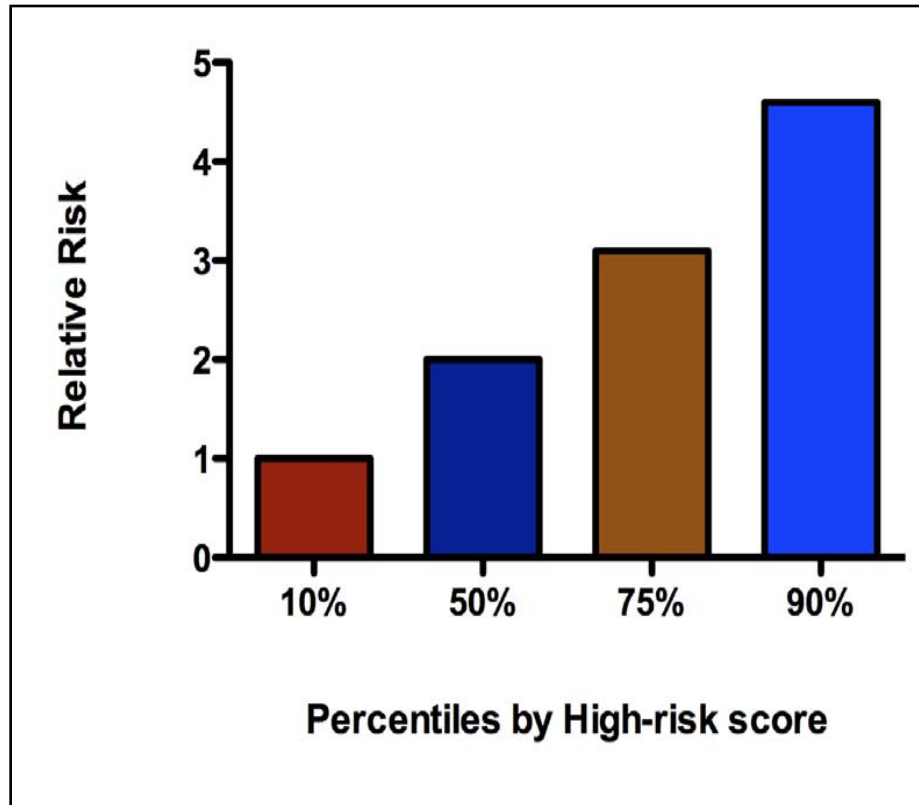
# Competing Risk: death as event 1 and cancer as event 2



***The 34-gene score is an independent predictor  
of recurrence risk***

	<b>Adjusted Hazard Ratio</b>	<b>p-value</b>	<b>Lower 95% CI</b>	<b>Upper 95% CI</b>
<b>Recurrence score</b>	1.016	<0.001	1.008	1.025
<b>Gender</b>	1.011	0.98	0.481	2.124
<b>Stage</b>	2.119	0.002	1.312	3.424
<b>Age</b>	1.001	0.93	0.974	1.029
<b>Grade</b>	1.446	0.32	0.701	2.985

# Relative risk of cancer-related death by percentile score



# Summary of Results

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- A 34-gene signature was identified using a biological model of metastasis
- The signature was an independent predictor of survival and recurrence in multivariate models

# Conclusions

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- A biologically-based mouse model identified a stage-independent gene expression signature predictive of poor prognosis in patients with colon cancer.

# Publication

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- The current research is accepted by *Gastroenterology*.
- *Gastroenterology* is ranked 1st of 55 journals in the Gastroenterology and Hepatology category on the 2008 Journal Citation Reports®, published by Thomson Reuters, and has an Impact Factor of 12.591.

# Acknowledgements

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- Christian Kis
- John Neff
- Nicole Al-Greene

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- Ramona Deal
- Jeff Franklin

## • Goldenring lab:

- Joseph Roland

## Biostatistics:

- Yu Shyr
- Pengcheng Lu

## Bioinformatics:

- Bing Zhang

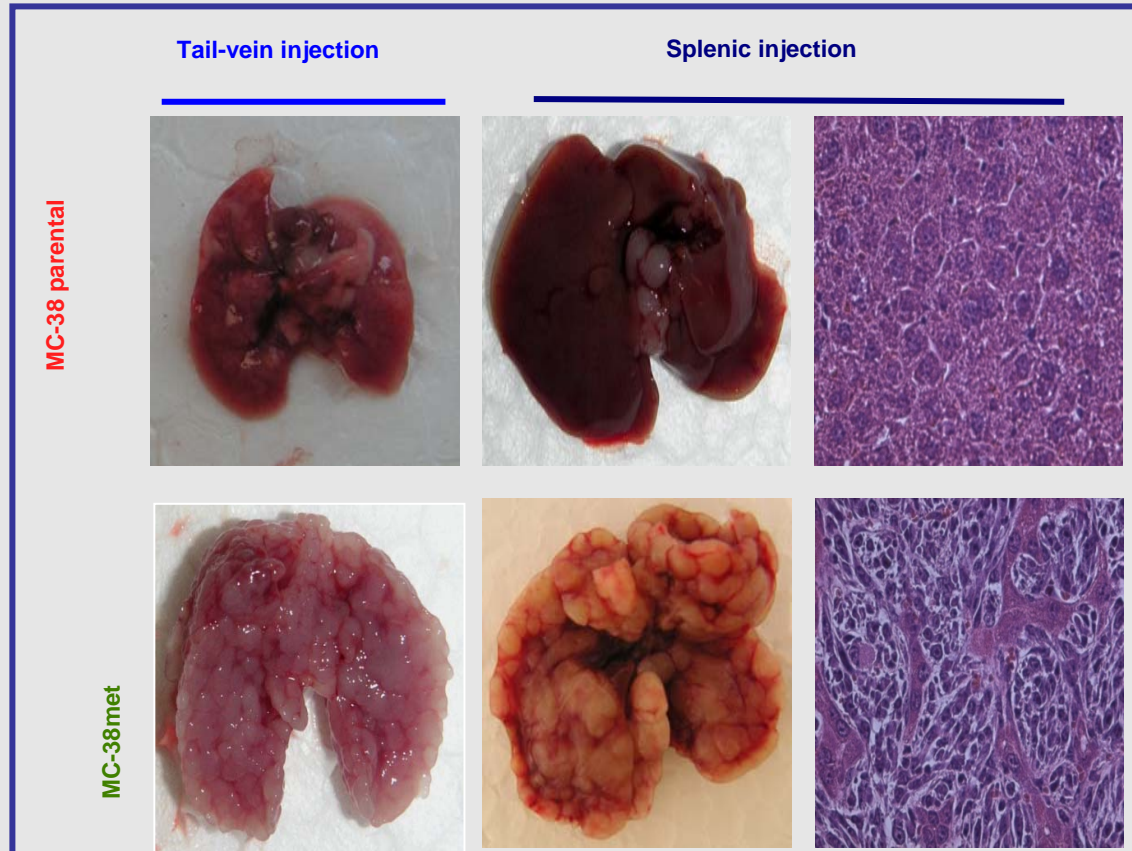
## Outside Collaborators:

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- Steven Eschrich, MCC
- Kay Washington

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  - Dr. Dermody
- SUS Ethicon Scholarship

# Thank you. Questions?



“Few diseases have the power of inspiring fear to the same degree as cancer. . . . It is therefore natural that we should strive to throw light upon its nature; but the road to this discovery is both long and difficult”  
Professor W. Wernstedt, Dean of the Royal Caroline Institute

- 10 December 1927 prior to the presentation of the 1926 Nobel Prize in Physiology or Medicine to Johannes Fibiger

# Last workshop in 2009:

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November 20: Fei Ye, PhD

Statistical practice in high-throughput siRNA/shRNA screens to identify genes mediating sensitivity to chemotherapeutic drugs