

# The P38 network and a miR-9 control mechanism as driving GBM disease outcome

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# CAMDA challenge

“gaining better insight from an integration of heterogeneous large-scale data”

“the Glioblastoma multiforme subset of The Cancer Genome Atlas (TCGA)”

# Data

This repository is unusual in that it provides publicly, for several hundred patients, profiles of

- gene transcript expression (435 cancer patients versus 11 controls)
- miRNA expression (426 tumour samples versus 10 controls)
- genomic DNA methylation (256 tumour samples versus a control)
- copy number variation (465 tumour samples versus 430 controls [402 matched normals])

# TCGA



“...a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing”

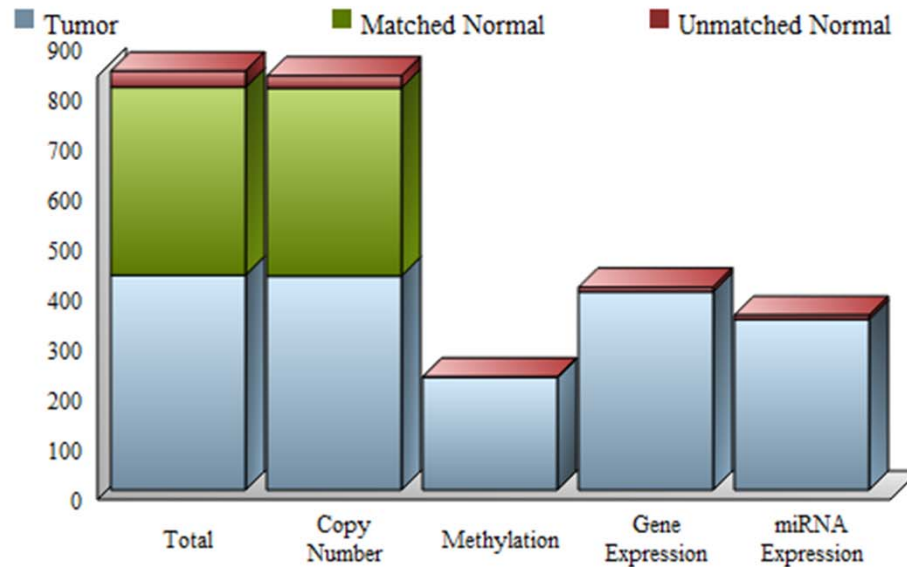
Available Cancer Types	# Patients with Samples	# Downloadable Tumor Samples	Date Last Updated (mm/dd/yy)
Acute Myeloid Leukemia [LAML]	202	200	02/17/11
Bladder Urothelial Carcinoma [BLCA]	38	35	07/07/11
Brain Lower Grade Glioma [LGG]	50	50	07/10/11
Breast invasive carcinoma [BRCA]	801	532	06/30/11
Cervical Squamous Cell Carcinoma [CESC]	42	23	06/30/11
Colon adenocarcinoma [COAD]	380	333	07/07/11
Glioblastoma multiforme [GBM]	597	536	07/07/11
Head and Neck squamous cell carcinoma [HNSC]	93	93	06/06/11
Kidney renal clear cell carcinoma [KIRC]	502	499	07/10/11
Kidney renal papillary cell carcinoma [KIRP]	43	43	07/10/11
Liver hepatocellular carcinoma [LIHC]	45	45	07/07/11
Lung adenocarcinoma [LUAD]	237	170	07/10/11
Lung squamous cell carcinoma [LUSC]	213	184	07/06/11
Ovarian serous cystadenocarcinoma [OV]	594	586	06/24/11
Pancreatic adenocarcinoma [PAAD]	7	0	07/06/11
Prostate adenocarcinoma [PRAD]	83	83	07/07/11
Rectum adenocarcinoma [READ]	153	123	07/07/11
Stomach adenocarcinoma [STAD]	132	109	07/01/11
Thyroid carcinoma [THCA]	86	60	07/08/11
Uterine Corpus Endometrioid Carcinoma [UCEC]	350	270	06/30/11

# CAMDA TCGA data

Target number of Glioblastoma multiforme samples:  
500 (number subject to change)

[Run Query on  
Glioblastoma multiforme](#)

Glioblastoma multiforme [GBM]	Number of Samples				
	Total	Copy Number	Methylation	Gene Expression	miRNA Expression
Tumor	536	534	281	495	426
Matched Normal	469	469	0	0	0
Unmatched Normal	40	30	1	11	10

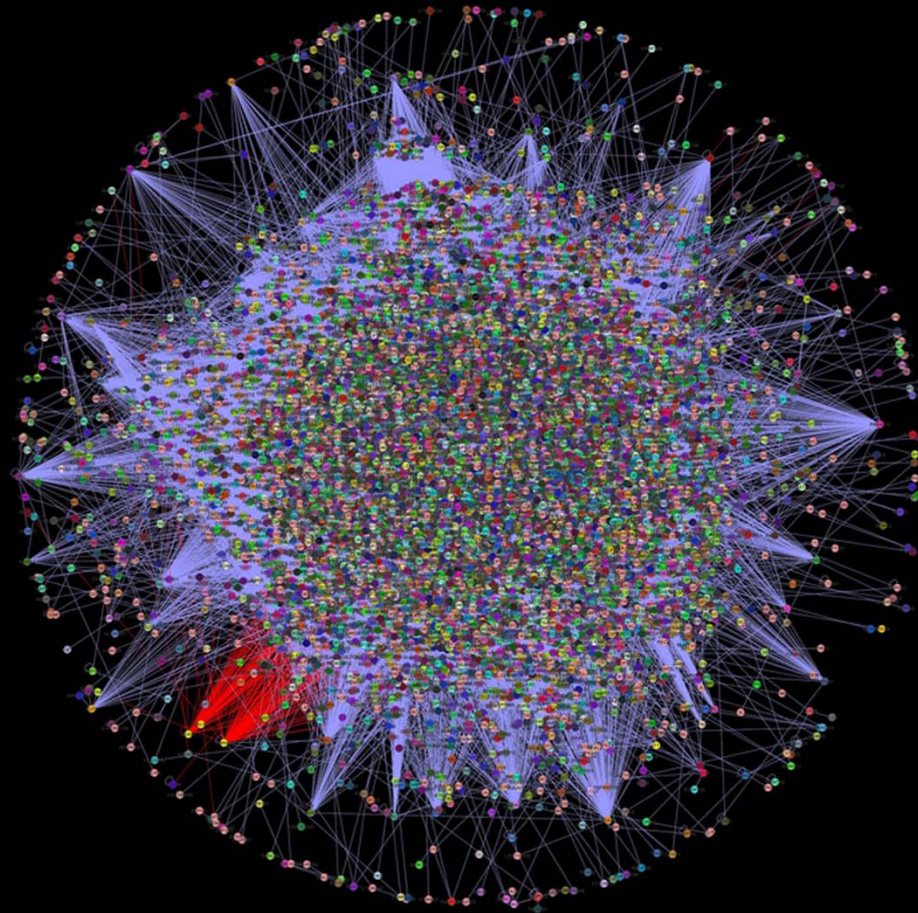


We are interested in a network view of  
the data

We are using the **network** as the **biomarker**

Which network?

# Curated interactome



*Andrew Garrow*



# Parsed Curated interactome

**NATIONAL CANCER INSTITUTE**  
**nature** PathwayInteractionDatabase

**Browse pathways**

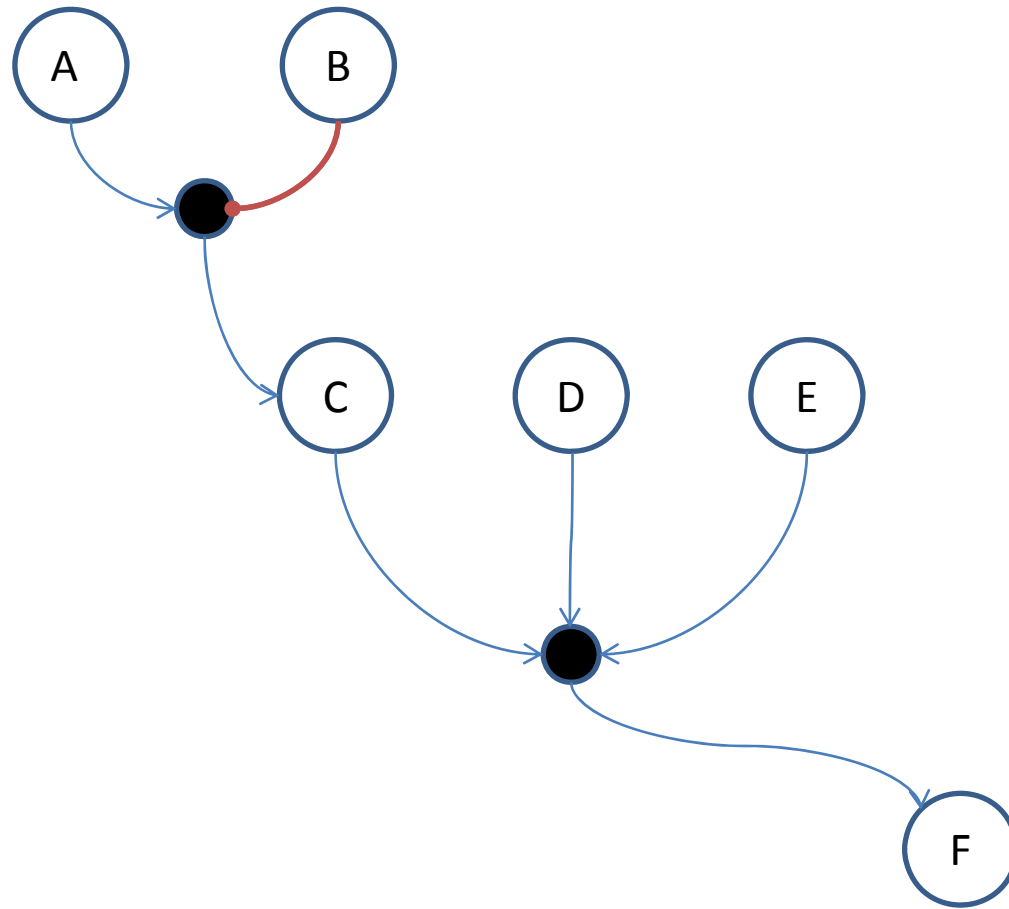
If you don't find your pathway of interest either in the Browse Pathways list or the [Curation Calendar](#), please [Suggest a Pathway](#).

**Jump straight to the search source**

- [NCI-Nature curated pathways](#)
- [BioCarta imported pathways](#)
- [Reactome imported pathways](#)

**NCI-NATURE CURATED**

Pathway	Source
<a href="#">ALK1 pathway</a>	NCI-Nature curated
<a href="#">AP-1 transcription factor network</a>	NCI-Nature curated
<a href="#">ATF-2 transcription factor network</a>	NCI-Nature curated
<a href="#">ATM pathway</a>	NCI-Nature curated
<a href="#">ATR signaling pathway</a>	NCI-Nature curated
<a href="#">Alpha-synuclein signaling</a>	NCI-Nature curated
<a href="#">Alternative NF-kappaB pathway</a>	NCI-Nature curated
<a href="#">Androgen-mediated signaling</a>	NCI-Nature curated
<a href="#">Angiopoietin receptor Tie2-mediated signaling</a>	NCI-Nature curated
<a href="#">Arf1 pathway</a>	NCI-Nature curated
<a href="#">Arf6 signaling events</a>	NCI-Nature curated
<a href="#">Atypical NF-kappaB pathway</a>	NCI-Nature curated
<a href="#">BARO1 signaling events</a>	NCI-Nature curated
<a href="#">BCR signaling pathway</a>	NCI-Nature curated
<a href="#">BMP receptor signaling</a>	NCI-Nature curated



# Actual gene expression abundance experiment

Gene	A	B	C	D	E	F
Expression	✓	-	✓	✓	✓	✓

Sample 1

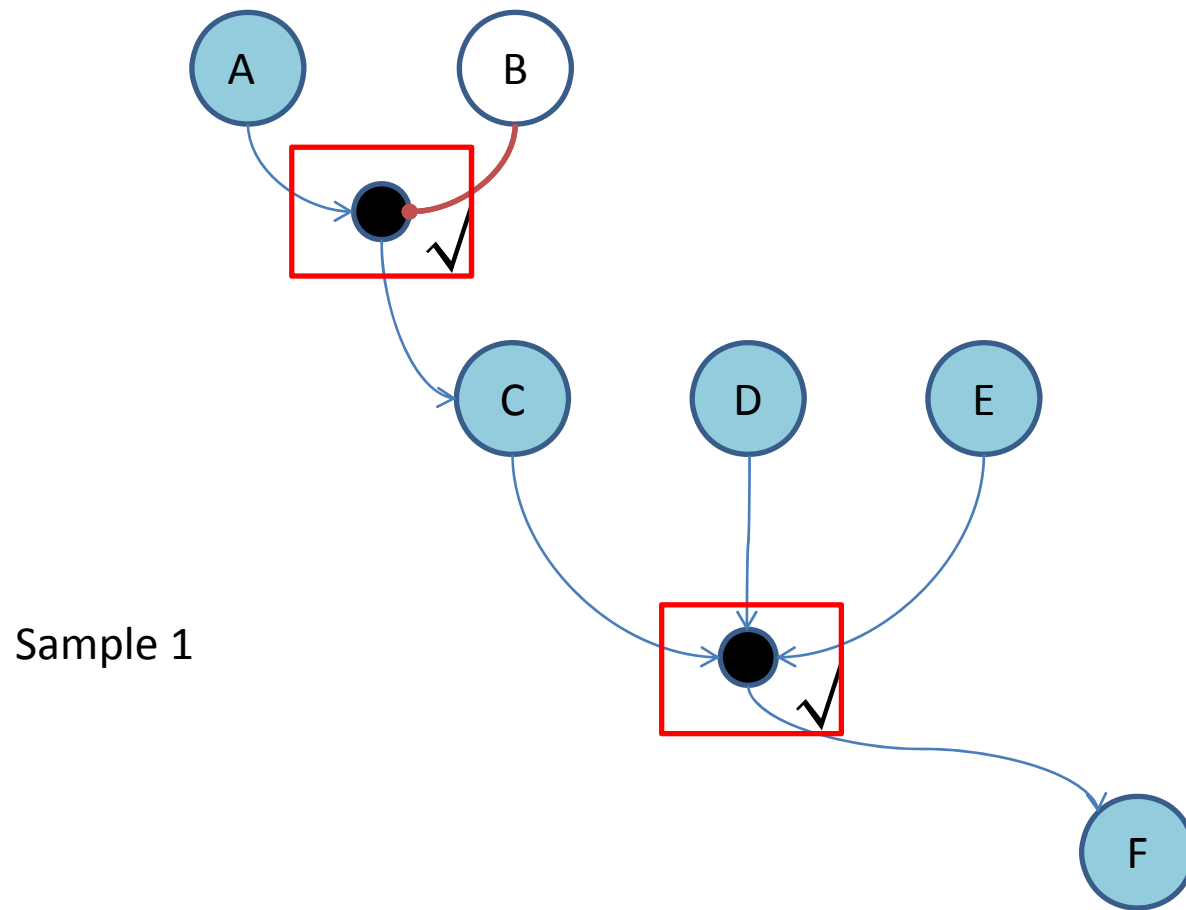
Gene	A	B	C	D	E	F
Expression	✓	✓	✓	-	✓	✓

Sample 2

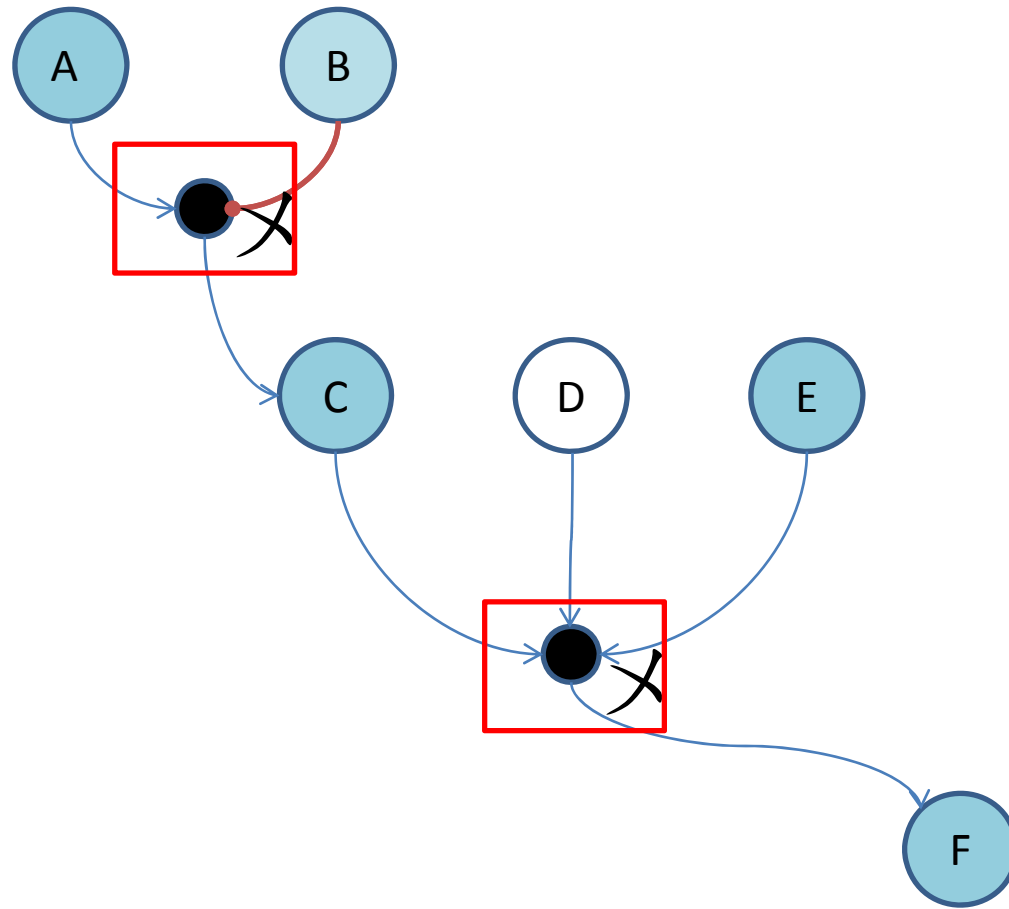
Gene	A	B	C	D	E	F
Expression	✓	-	✓	✓	-	✓

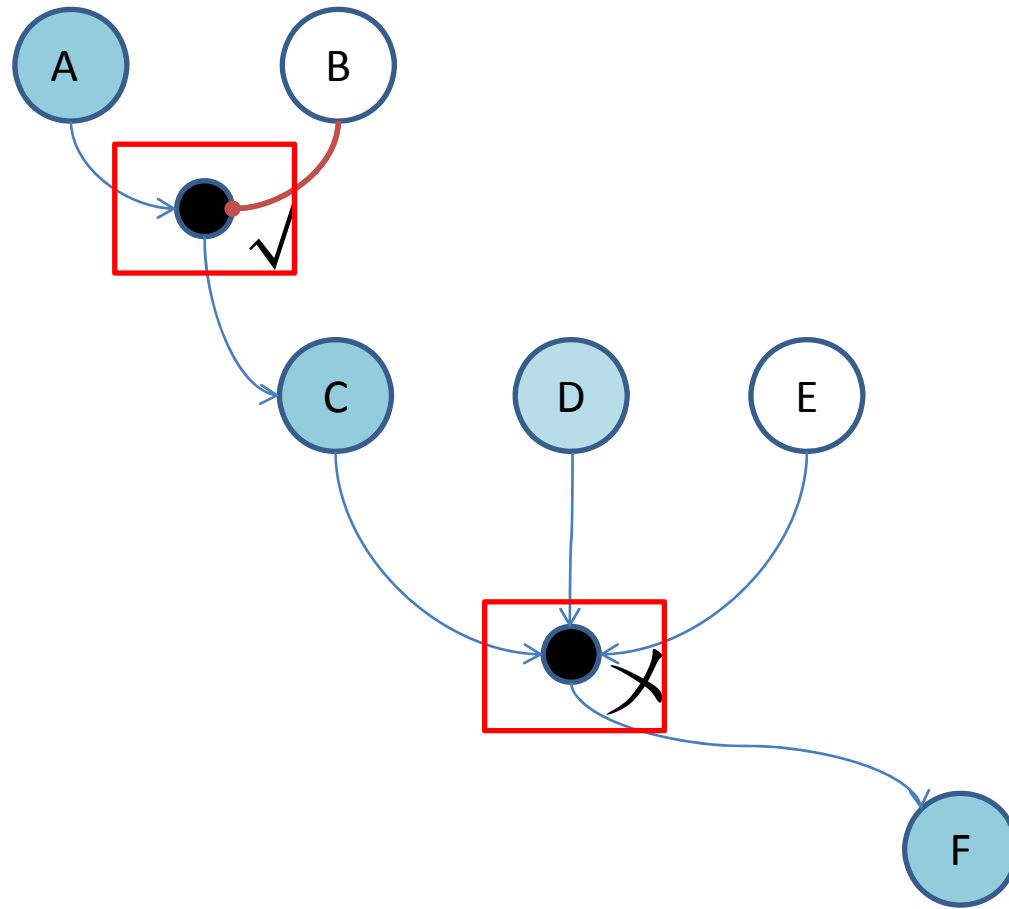
Sample 3

Do the stories match?



Sample 2





Sample 3

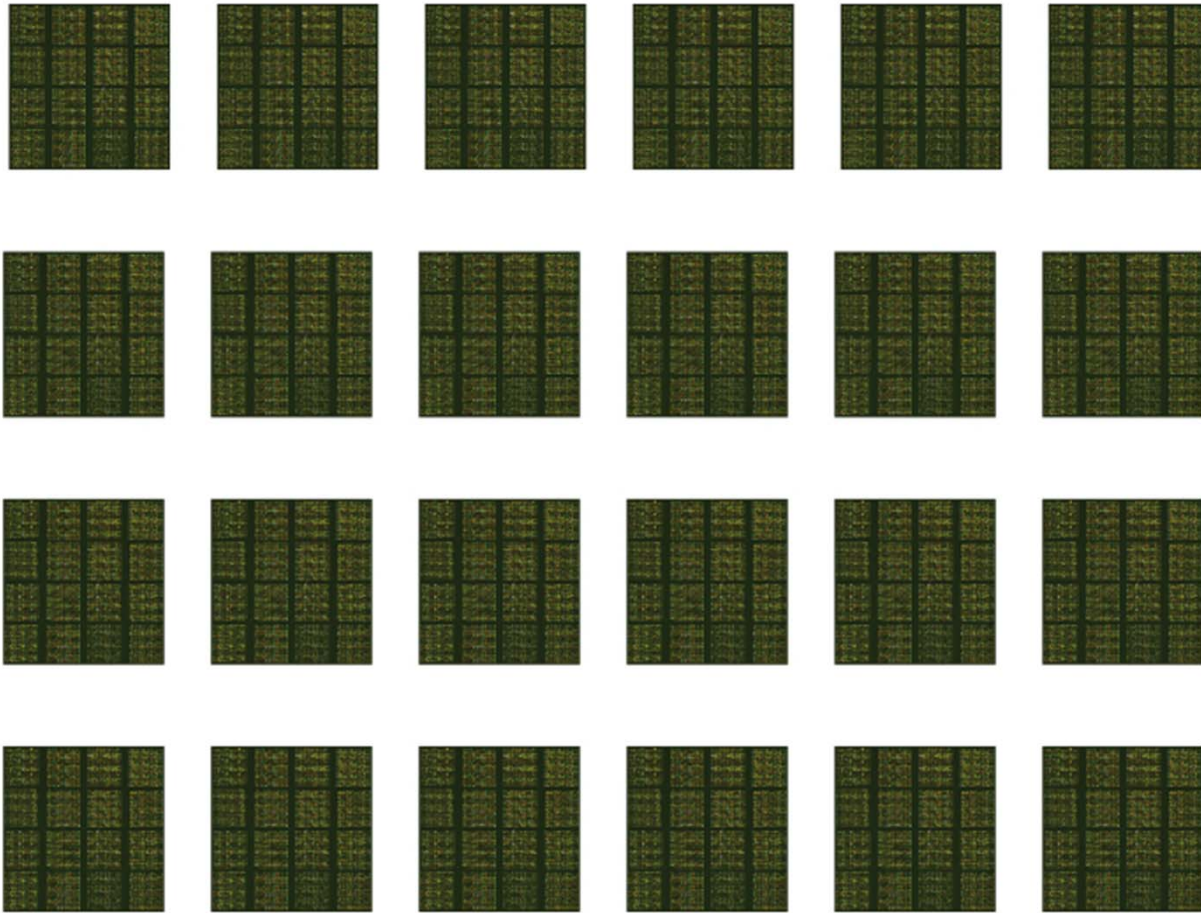
How does this translate to high  
throughput?

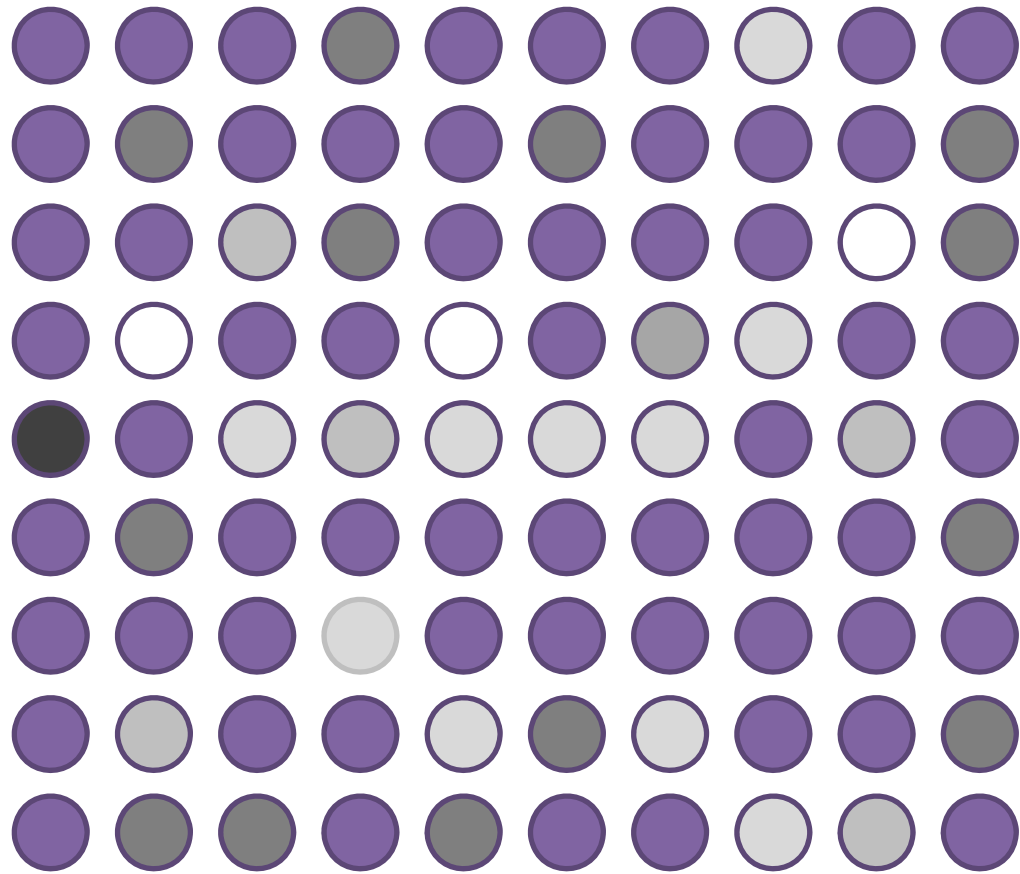
Multiple pathways  
over

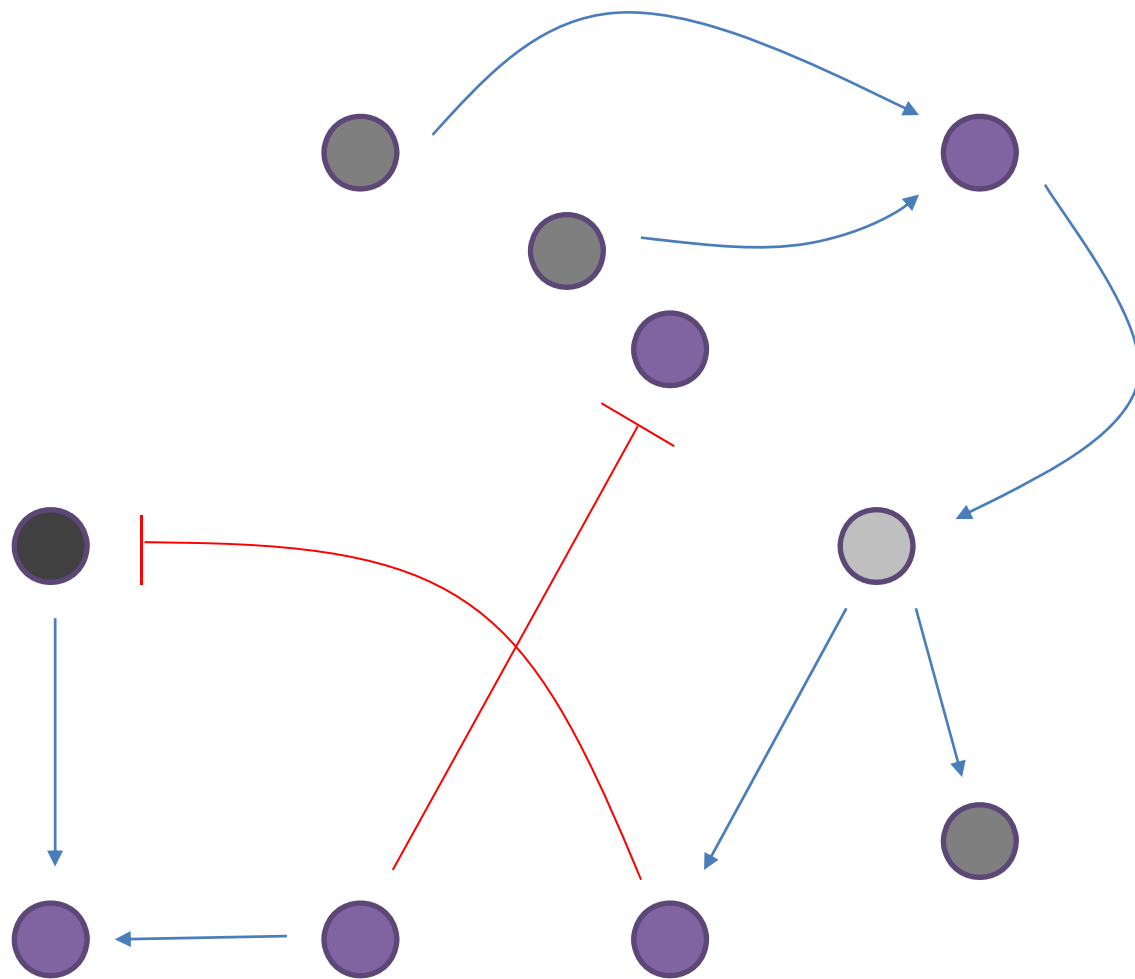
Multiple samples

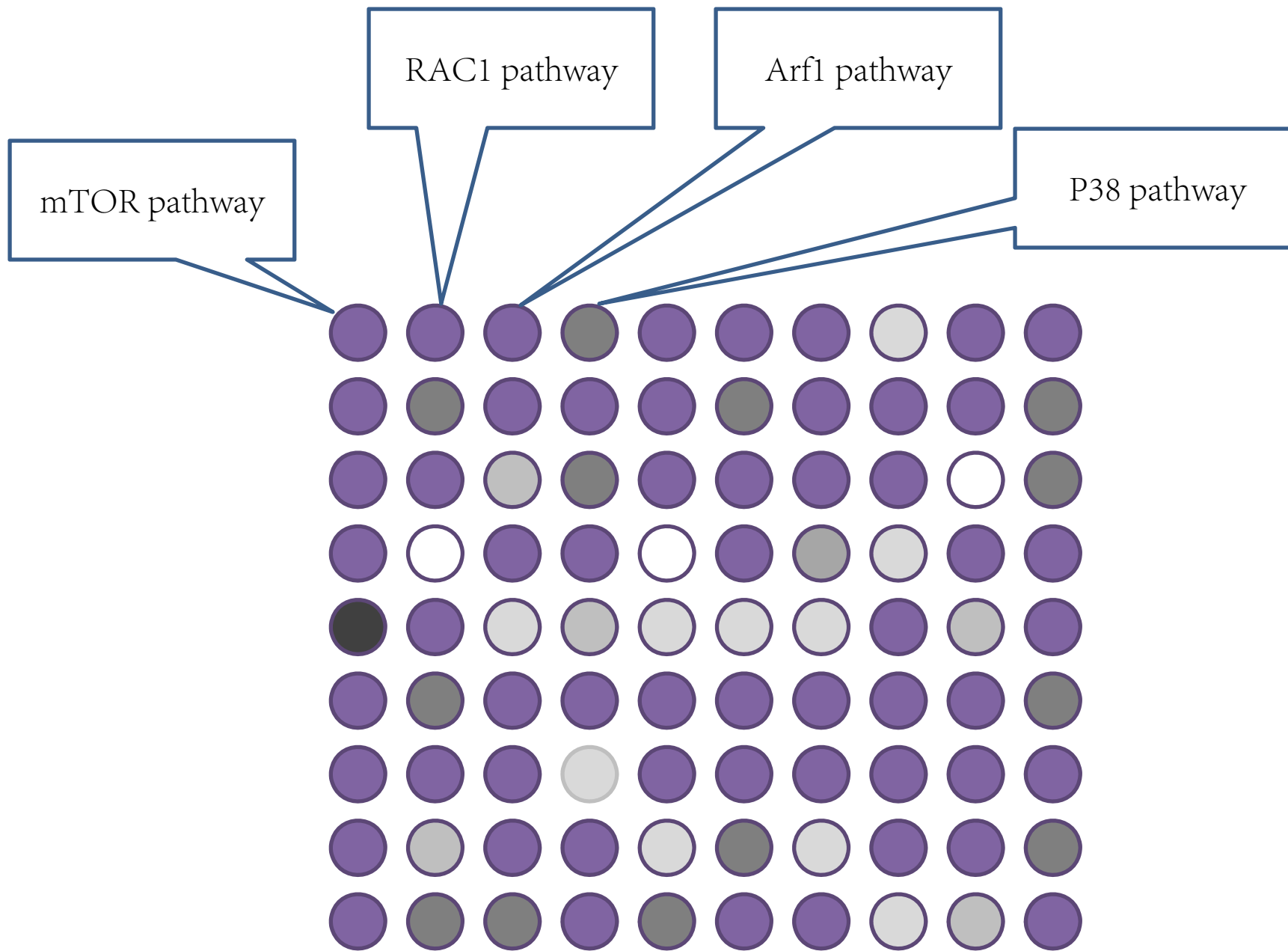


# Microarrays

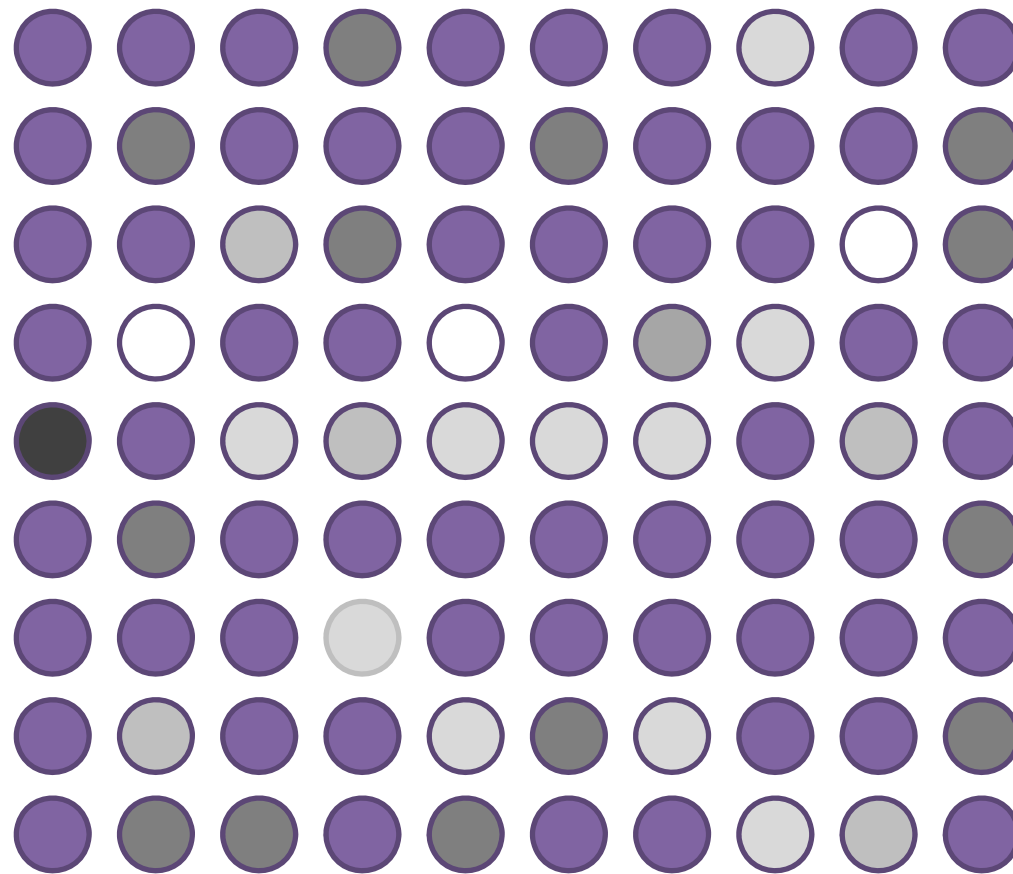








# Generation of global pathway metric



And we are using this global  
pathway metric as a basis to  
biomark samples

# Biomarker definition

The official NIH definition of a biomarker is: “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

# Biomarker

- Biochemical – cholesterol
- Physical - weight
- Physiologic – Blood pressure, heart rate
- Anatomic – ultrasound
- Histologic – tissue under the microscope



What do we biomark?

Risk



Diagnosis



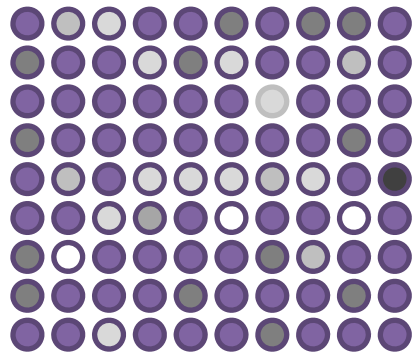
Prognosis



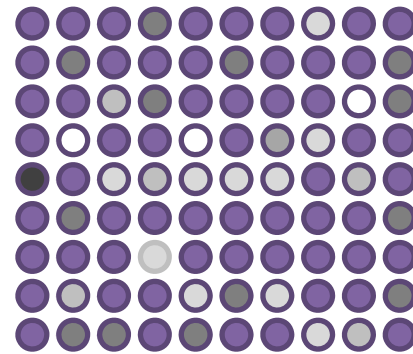
# Phenotype comparison



Tumor

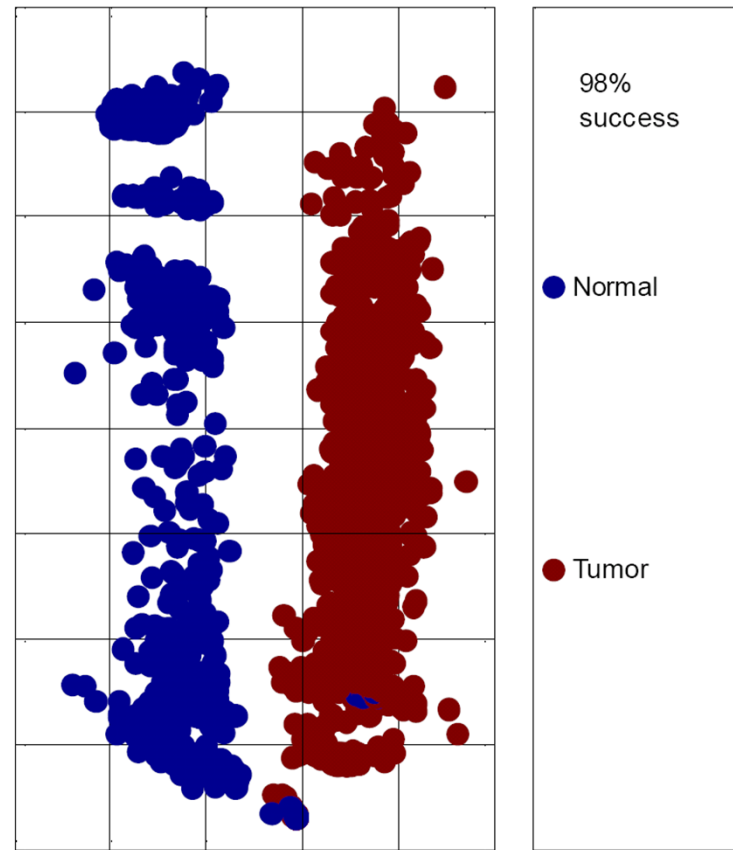


Normal



# Basic Oncogenic Signature

- Recode the data into meaningful units
- Dimensionality reduction
- Intuitive analyses
- Experimental directions

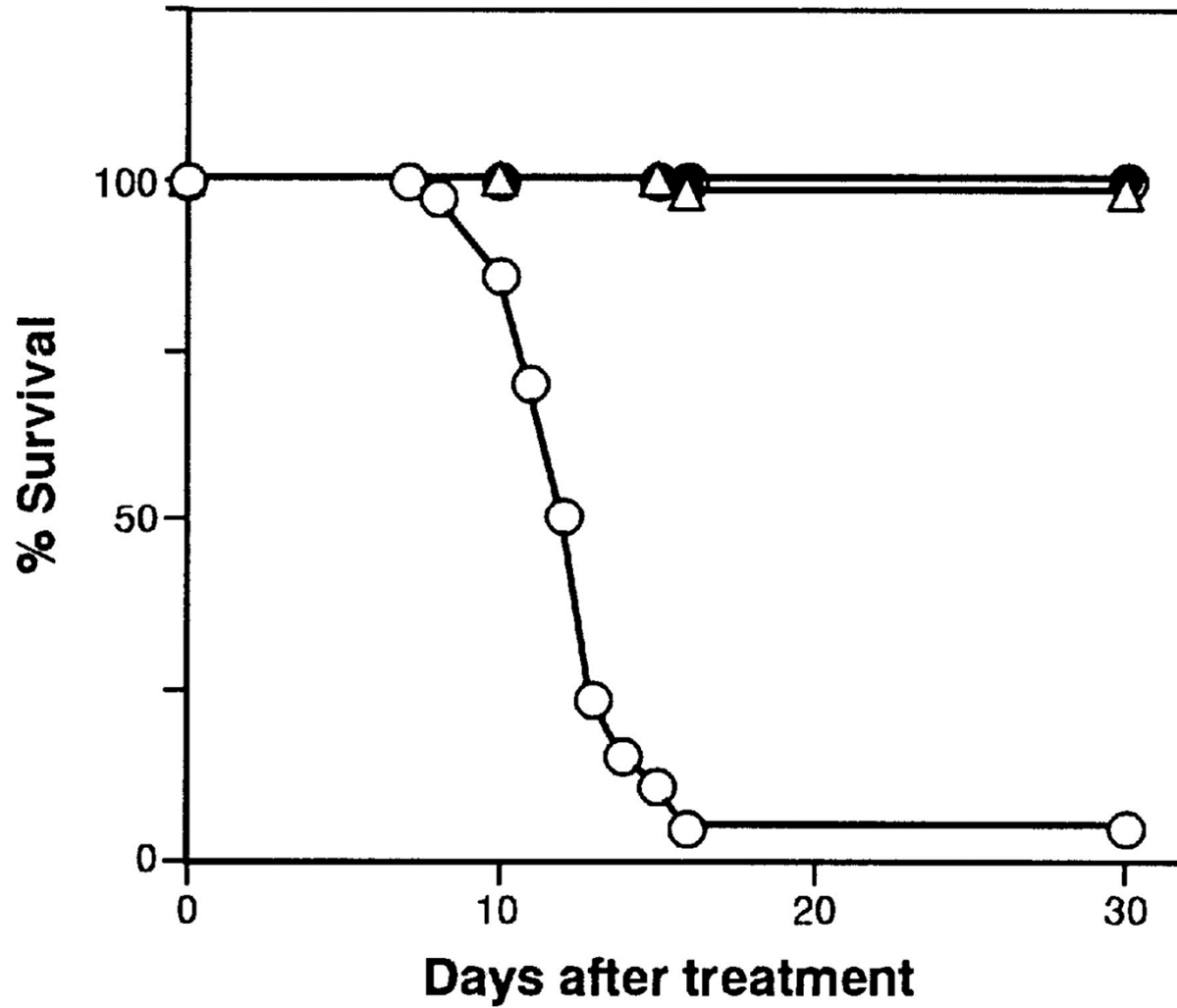


# Phenotypes

- Tumor/normal
- Genomic mutations
- Stage
- Smoker/non

Prognosis

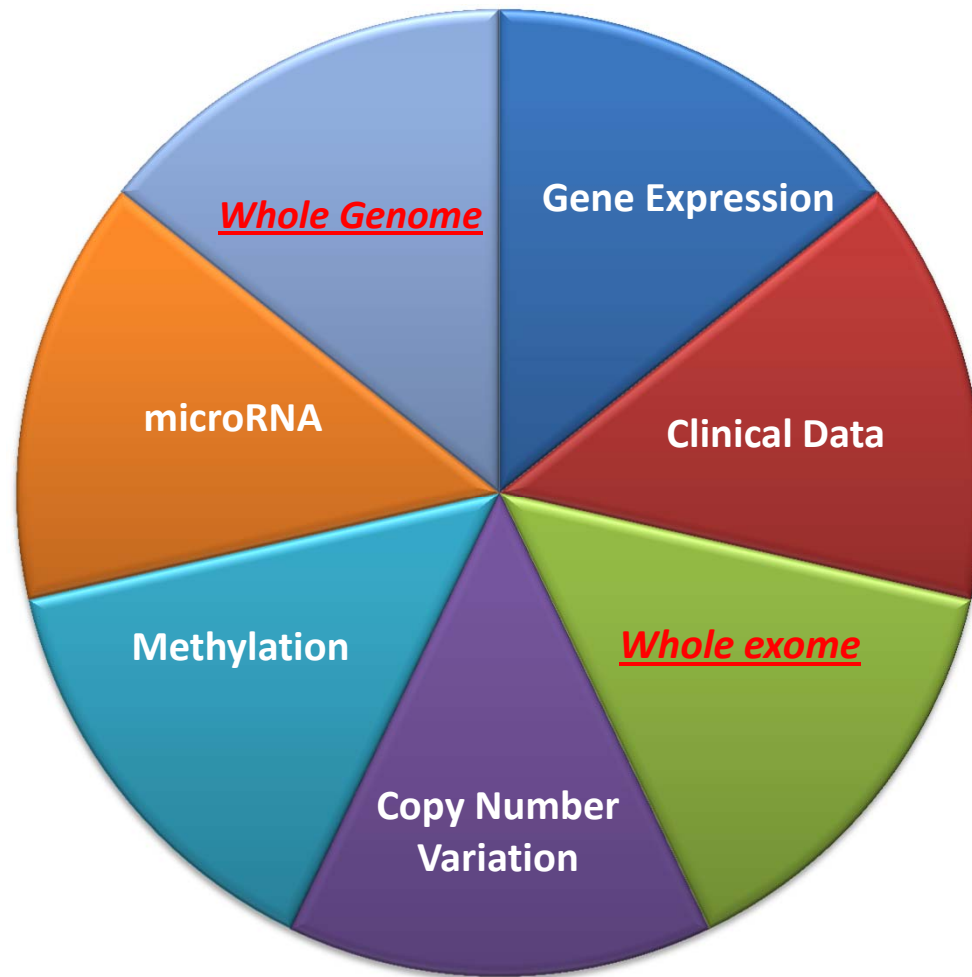
Survival curves of mice after MNU administration.



Kawate H et al. Carcinogenesis 2000;21:301-305

Prognosis is practically the only  
phenotype we worked with in  
this data set

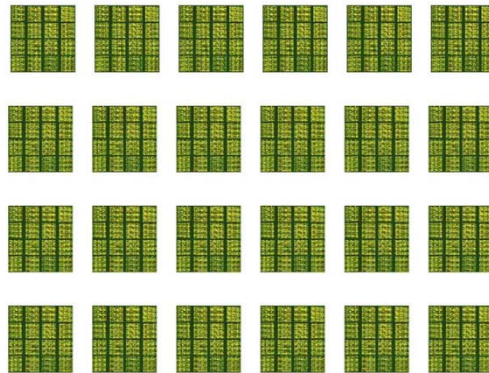
# Back to TCGA GBM data



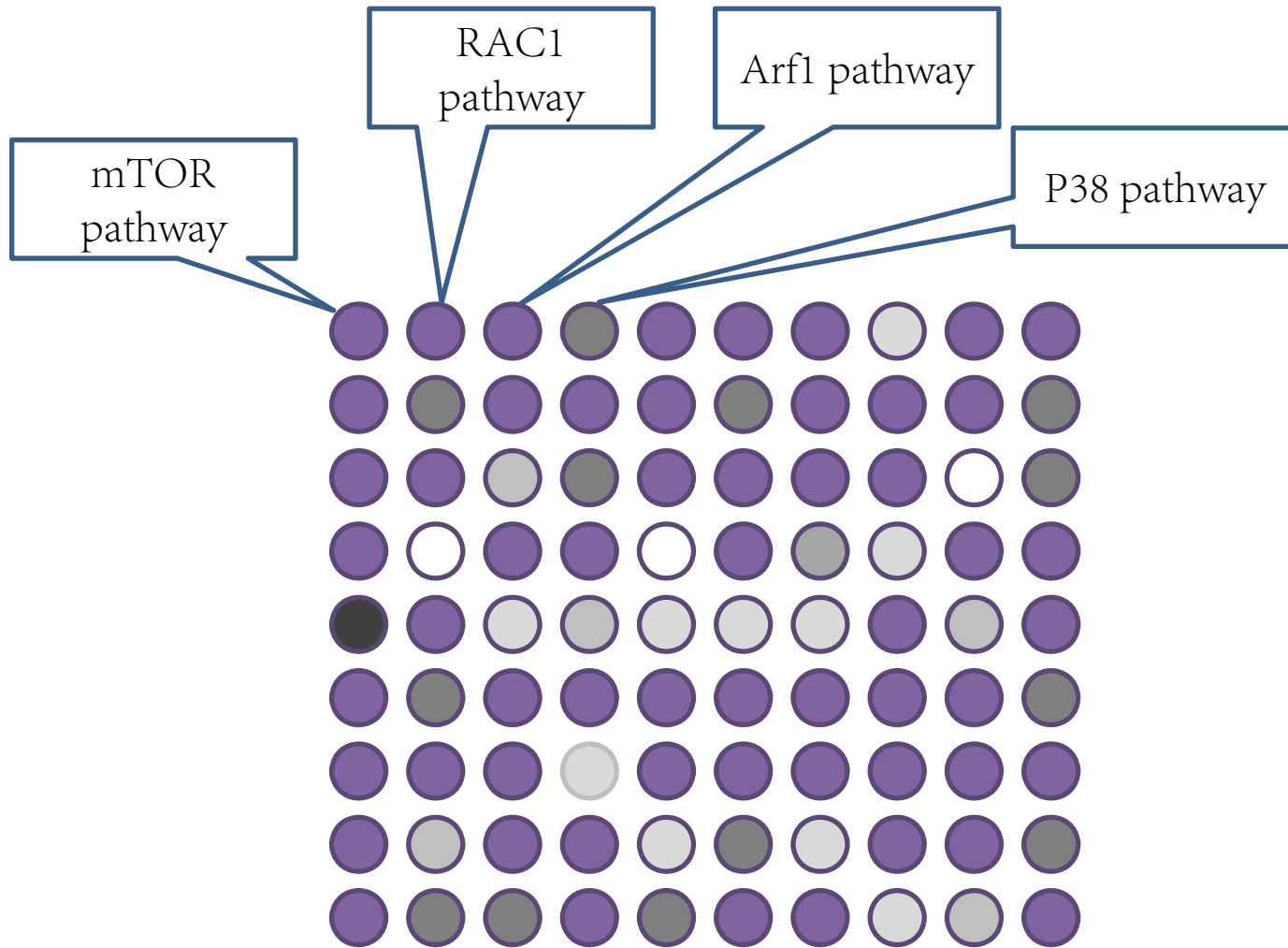


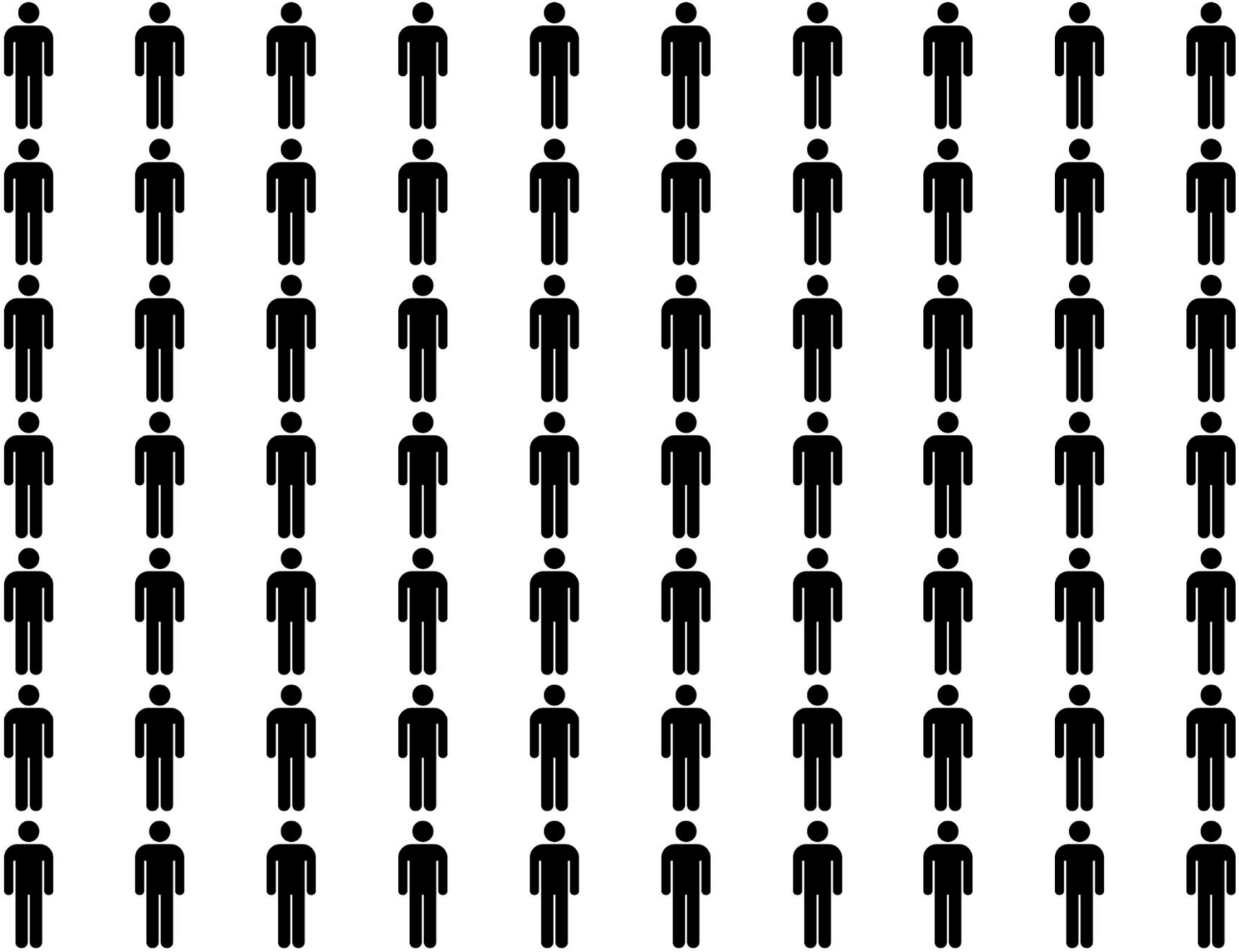
# Back to TCGA GBM data

## Gene Expression

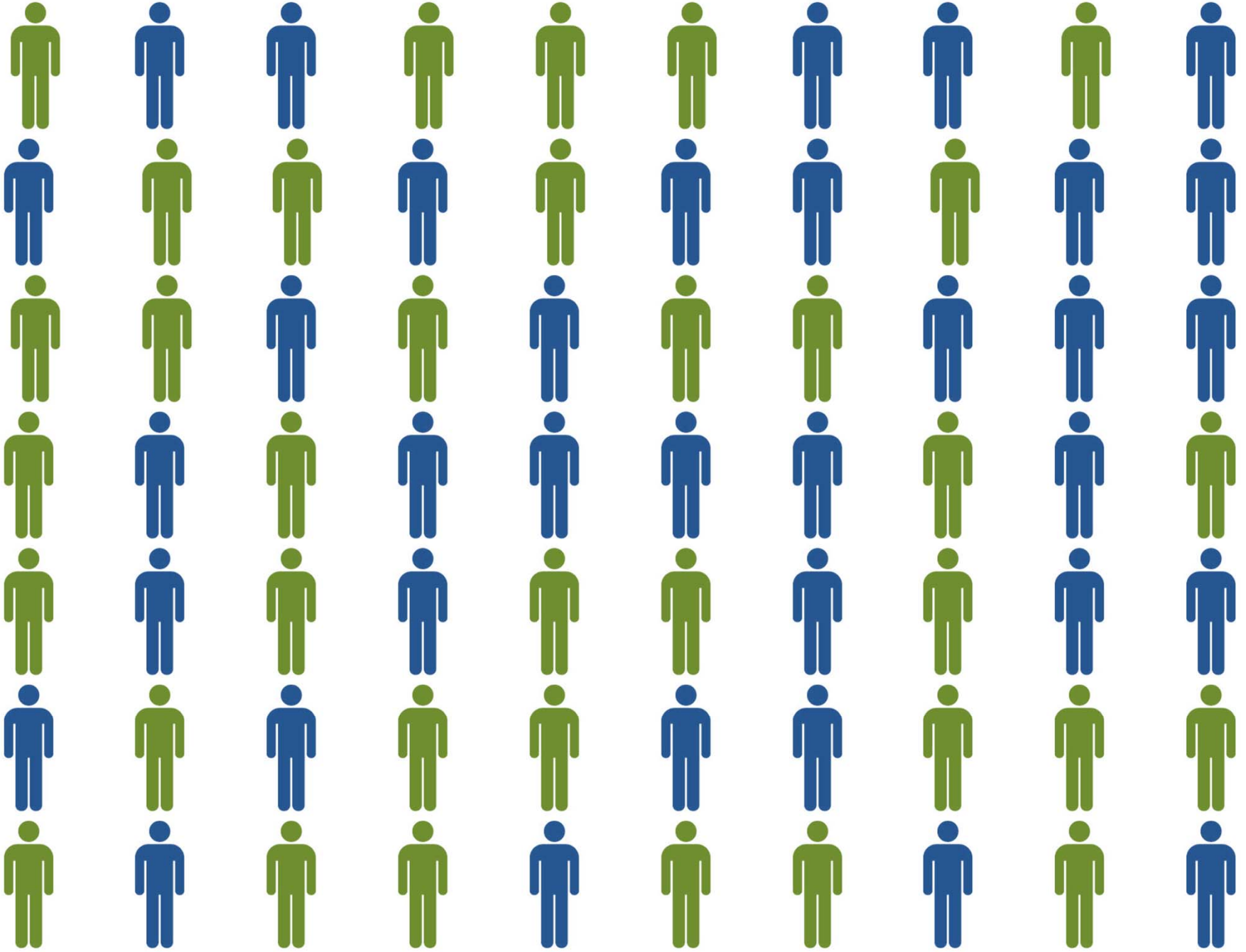


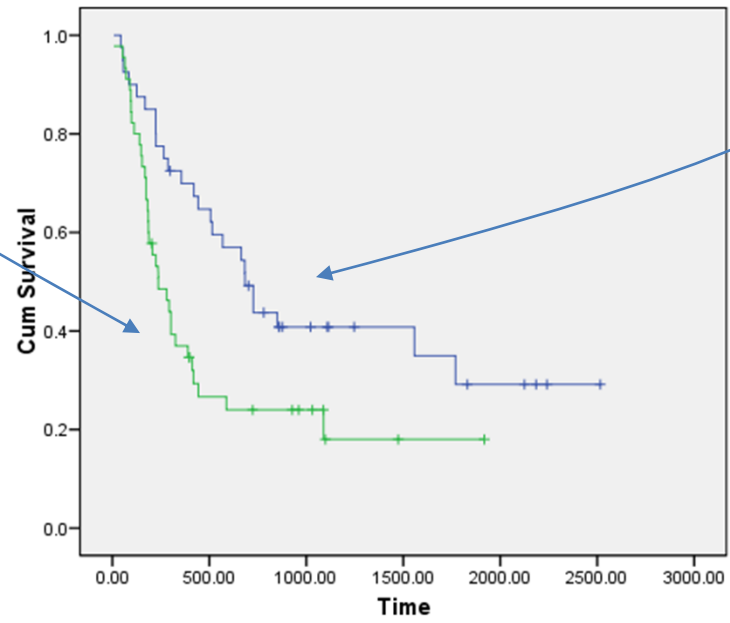
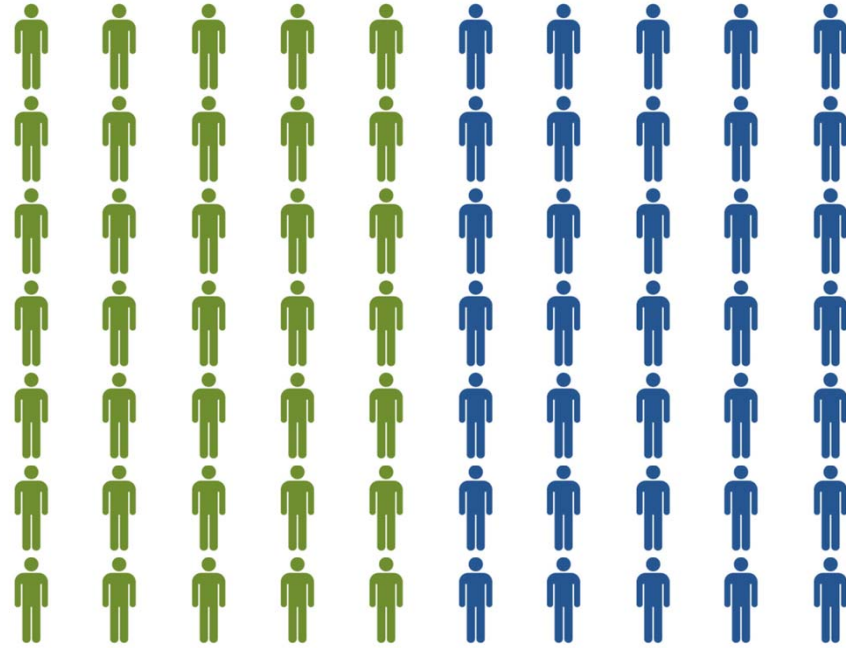
# Pathway representations



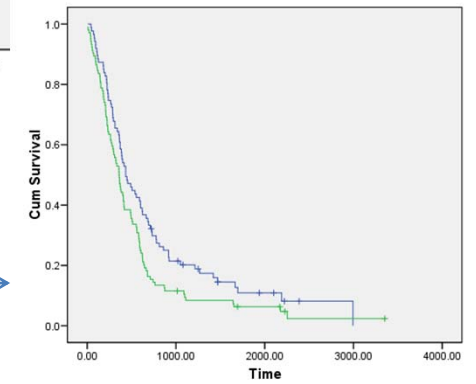
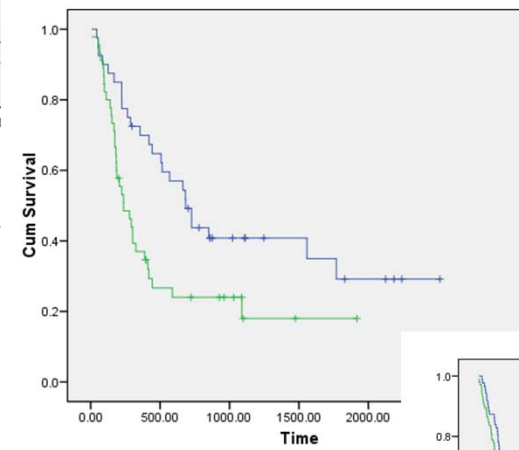
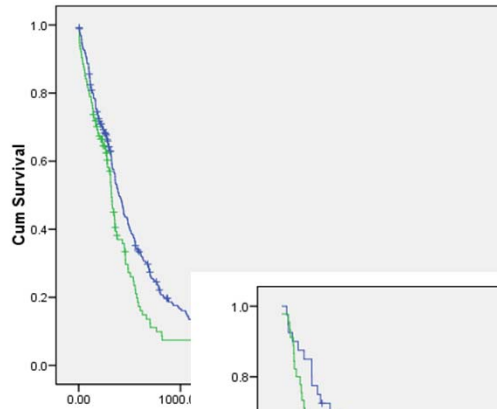






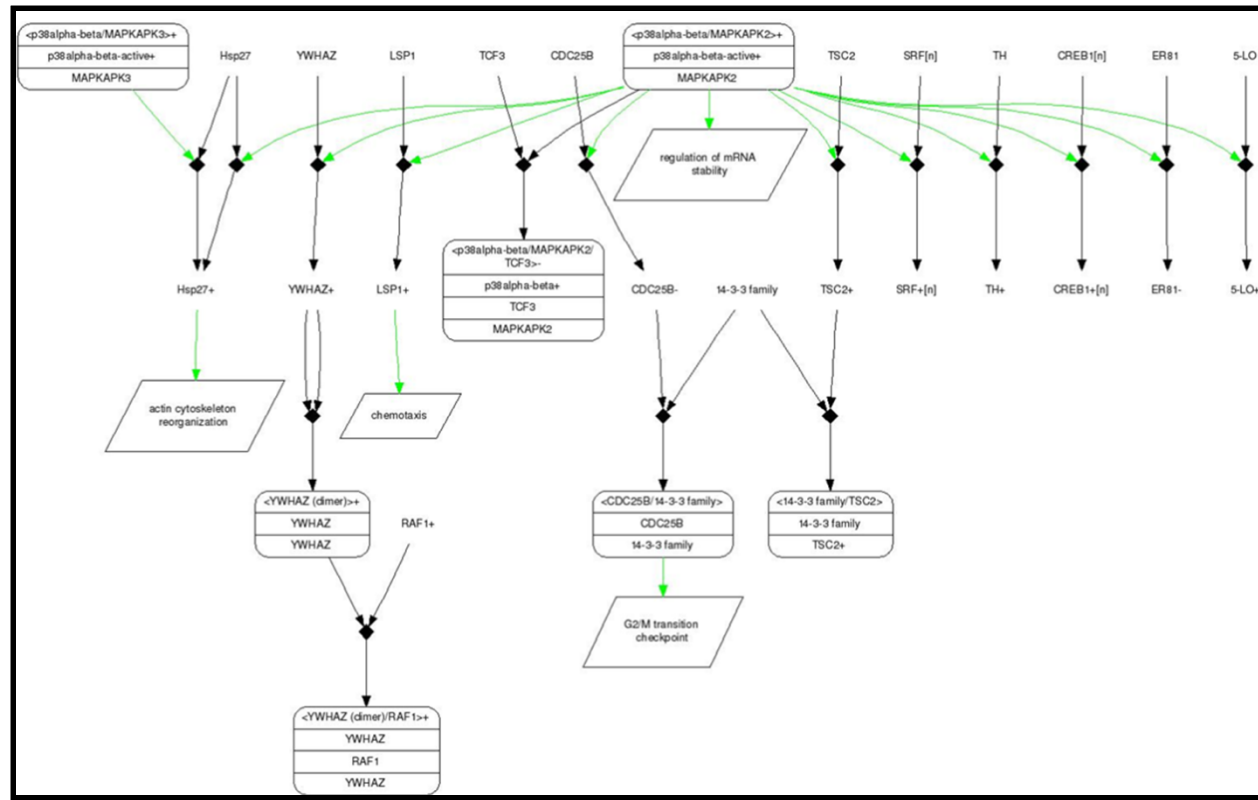


- Pathway 1
- Pathway 2
- Pathway 3
- Pathway 4
- Pathway 5
- Pathway 6
- ...
- Pathway 579



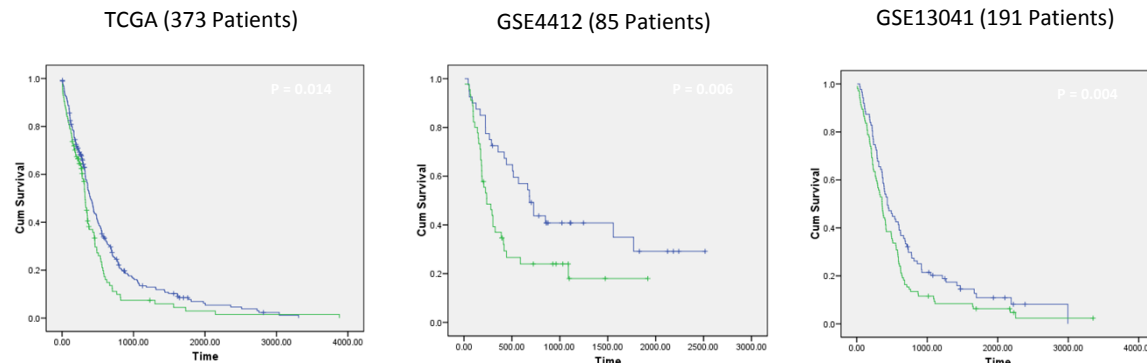
# The p38 pathway is most significant

“p38 signaling mediated by mapkap kinases”

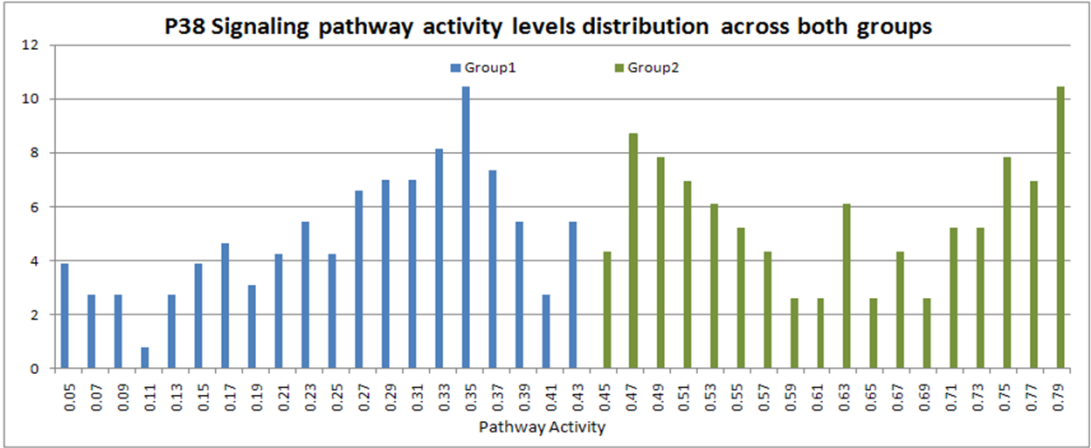




# Across three different datasets



Supporting previous research shows: Nomura N et al. Phorbol 12-myristate 13-acetate (PMA)-induced migration of glioblastoma cells is mediated via p38MAPK/Hsp27 pathway. *Biochem Pharmacol* 2007, 74(5):690-701.



# Copy numbers

## Amplified genes

Gene Symbol	Tumor	Normal
HSP27	21%	2%
CREB1	27%	16%
TCF3	14%	2%
ER81	45%	6%
CDC25B	36%	20%

## Deleted genes

Gene Symbol	Tumor	Normal
MAPKAPK3	20%	11%
LSP1	31%	25%
TH	37%	14%
YWHAZ	63%	27%
ALOX5	68%	7%
RAF1	13%	9%

11 of 13 pathway genes with relevant CNV

# Methylation

- 4 of the pathway genes methylated across all samples

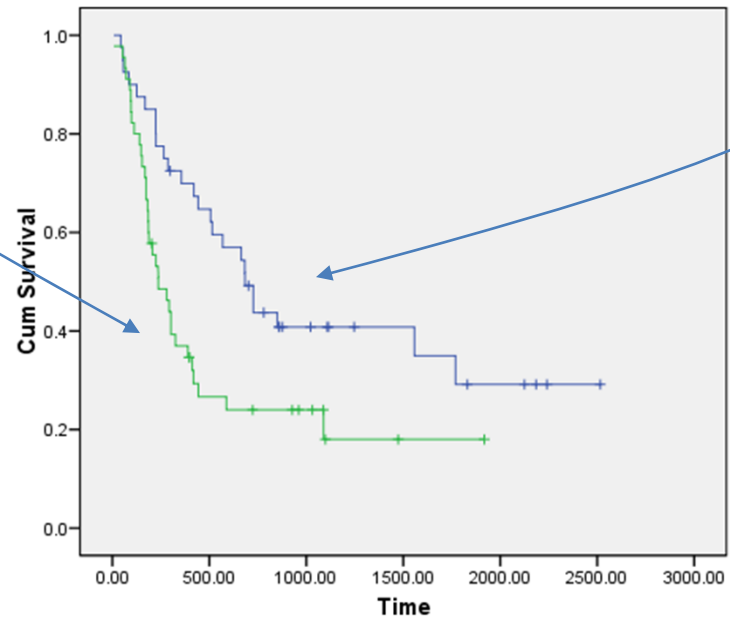
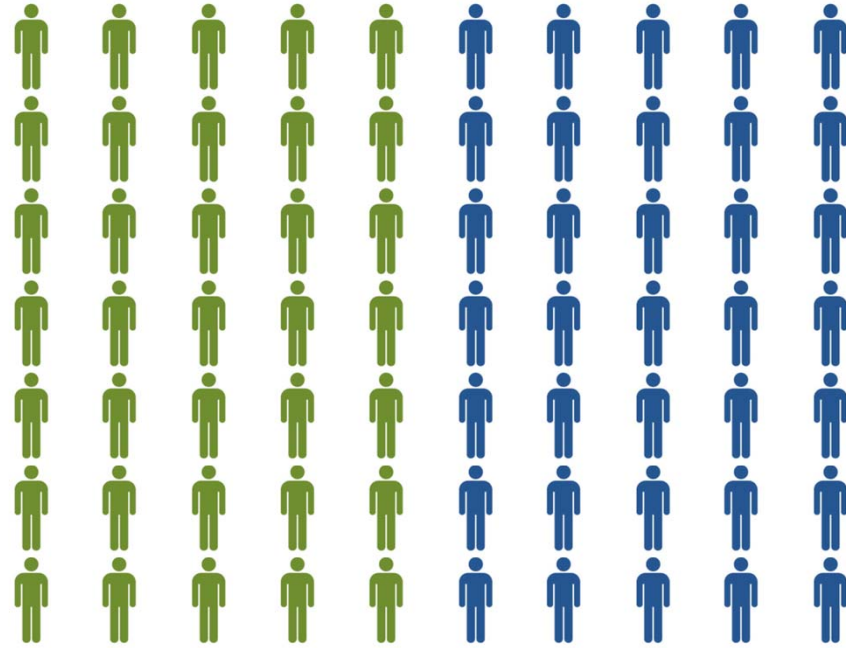
# Micro RNA

Pathway control mechanisms?

We found that 7 out of the 15 genes in the pathway have a possible binding site to miR-9.

# miR-9 :: p-38 pathway

	Number of patients	R <sup>2</sup> Correlation Value	P-value
Group1	241	-0.64	0
Group2	130	0.012	0.8876



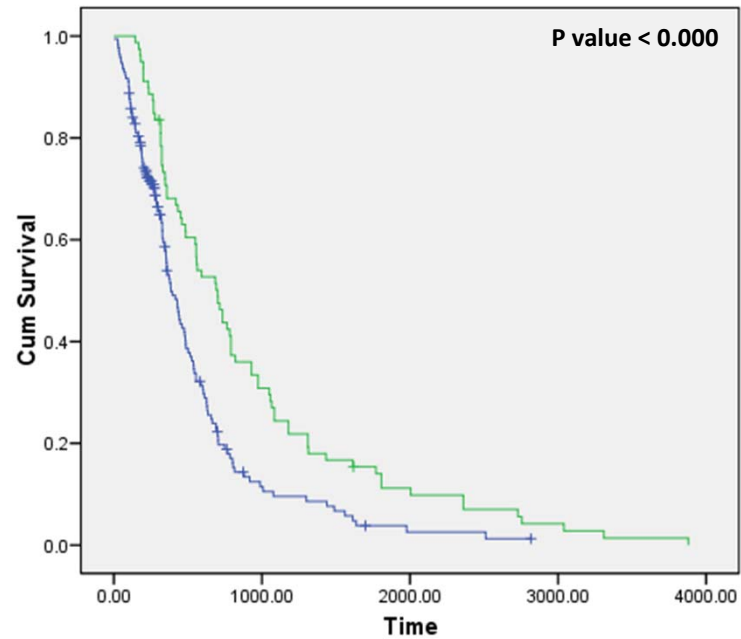
# Drug response

- patients are treated using a wide spectrum of 69 different drugs
- Classified drugs into two groups:
  - drugs that target genes in the p38 pathway
  - And
  - drugs that do not target genes in the pathway



Out of the 69 drugs given to the patients 6 drugs target genes that takes part of the p38 network

Drug Name	Target	Pathway
Accutane	RARA	map kinase inactivation of smrt co-repressor
CCNU	STMN4	Signaling mediated by p38-gamma and p38-delta pathway
Celebrex	COX2	Signaling mediated by p38-alpha and p38-beta pathway
Cis Retinoic Acid	RARA	map kinase inactivation of smrt co-repressor
Sorafenib	RAF1	p38 signaling mediated by MAPKAP kinases
Tamoxifen	ESR1	Signaling mediated by p38-alpha and p38-beta pathway



#### Group1

- Low survival
- 169 patients
- Average overall survival time – 433 days
- Median survival time – 310 days
- All patients did not received p38 targeted drugs

#### Group2

- High survival
- 63 patients
- Average overall survival – 896 days
- Median survival time – 691 days
- All patients received p38 targeted drugs

# Recap

- Pathway behavior over population, using prognosis as phenotype, surfaced the **p38 network**
- The p38 network is **targeted by copy number** variations
- The network as biomarker was proven **robust** in two additional datasets
- (negative) **correlation** between **miR-9** expression levels and the pathway behavior suggested miR-9 as a control mechanism over the pathway
- **miR-9 Binding sites** in a subset of genes in the pathway support the hypothesis
- **Drug** treatment directed towards **gene members** of the p38 network affiliates patients with better prognosis
- Further work can now be done on **sequencing** data
- **Experimentation**

# Acknowledgements

Lab Manager



Helit  
Cohen

## Students

Rotem  
Ben-Hamo



Chen  
Rubinstein



Miri  
Gordin



Jennifer  
Benichou



Moriah  
Cohen



Shai  
Fleger



Ilana  
Brotman



Shai  
Shilo



## NCI collaborators

- Kenneth Buetow
- Carl Schaefer
- Sharon Greenblum

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## HUJI

Liran Carmel

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## Harvard

Francisco Quintana