Molecular Correlates with IL-2 Response in Renal Cancer

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IL-2 Therapy for RCC-2004

High dose IL-2 remains the preferred therapy for...

- appropriately selected patients
- with access to such treatment

Efforts to improve selection criteria are warranted

Additional Opportunities for Patient Selection: IL-2

- ◆ **Histologic Factors**
  (Upton et al Proc ASCO 2003)

- ◆ **Molecular studies (CAIX Staining)**
  (Bui et al Clin Ca Res 2002; Atkins et al Proc ASCO 2004)

- ◆ **Expression Profiling**

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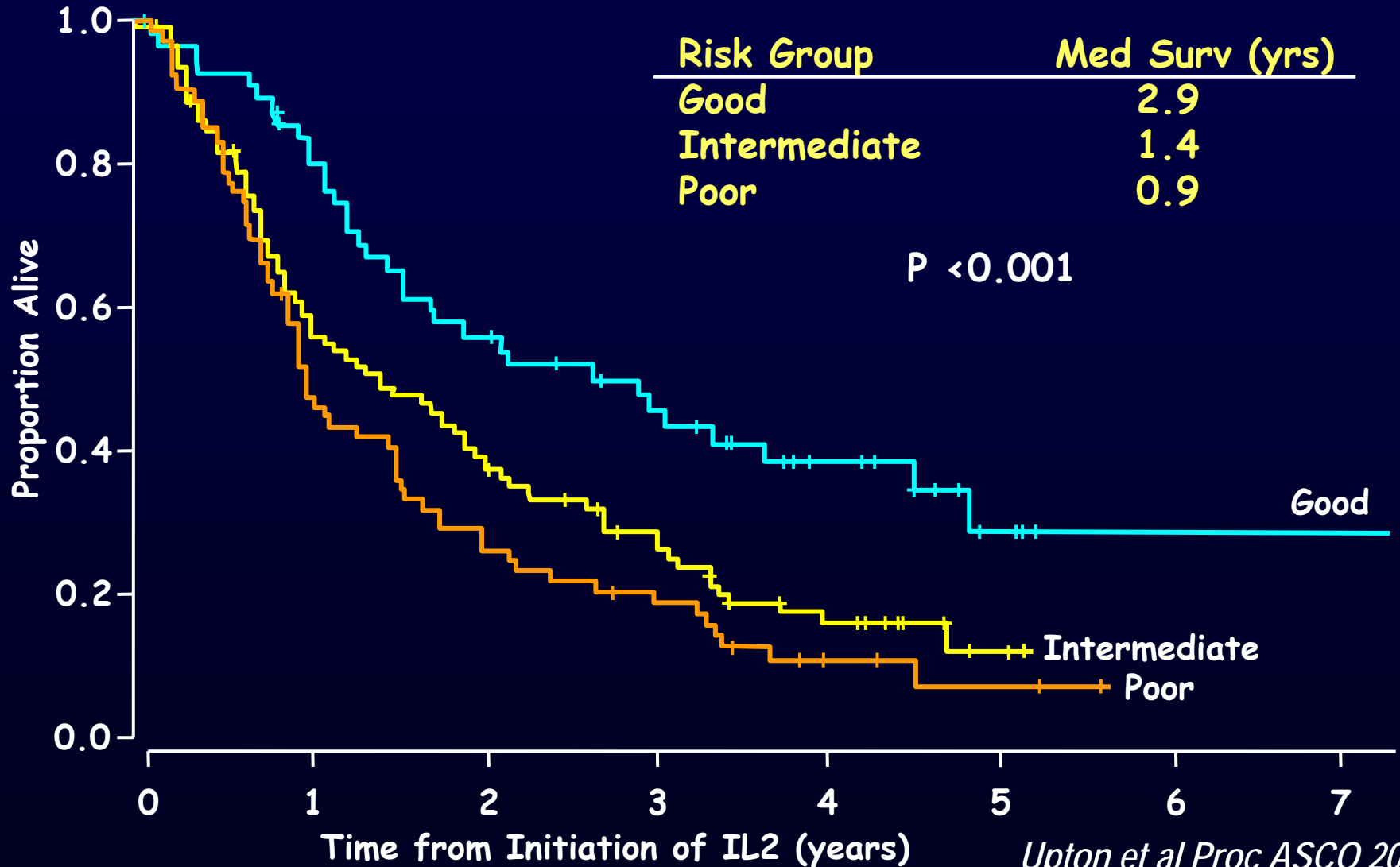
♦ **Expression Profiling**

Pathologic Correlates of Response to IL-2

Non-Clear cell histology associated with poor response

Clear Cell Pathology	Risk Group	Primary N=146 RR (%)	Mets N=66 RR (%)
Alveolar > 50% No papillary No granular	Good	39%	25%
Alveolar < 50% Granular < 50% No papillary	Intermediate	19%	9%
Others	Poor	4%	0%

Upton et al Proc ASCO 2003

Pathologic Correlates of Response to IL-2

Risk Group	Med Surv (yrs)
Good	2.9
Intermediate	1.4
Poor	0.9

P <0.001

Good

Intermediate

Poor

Proportion Alive

Time from Initiation of IL2 (years)

Upton et al Proc ASCO 2003

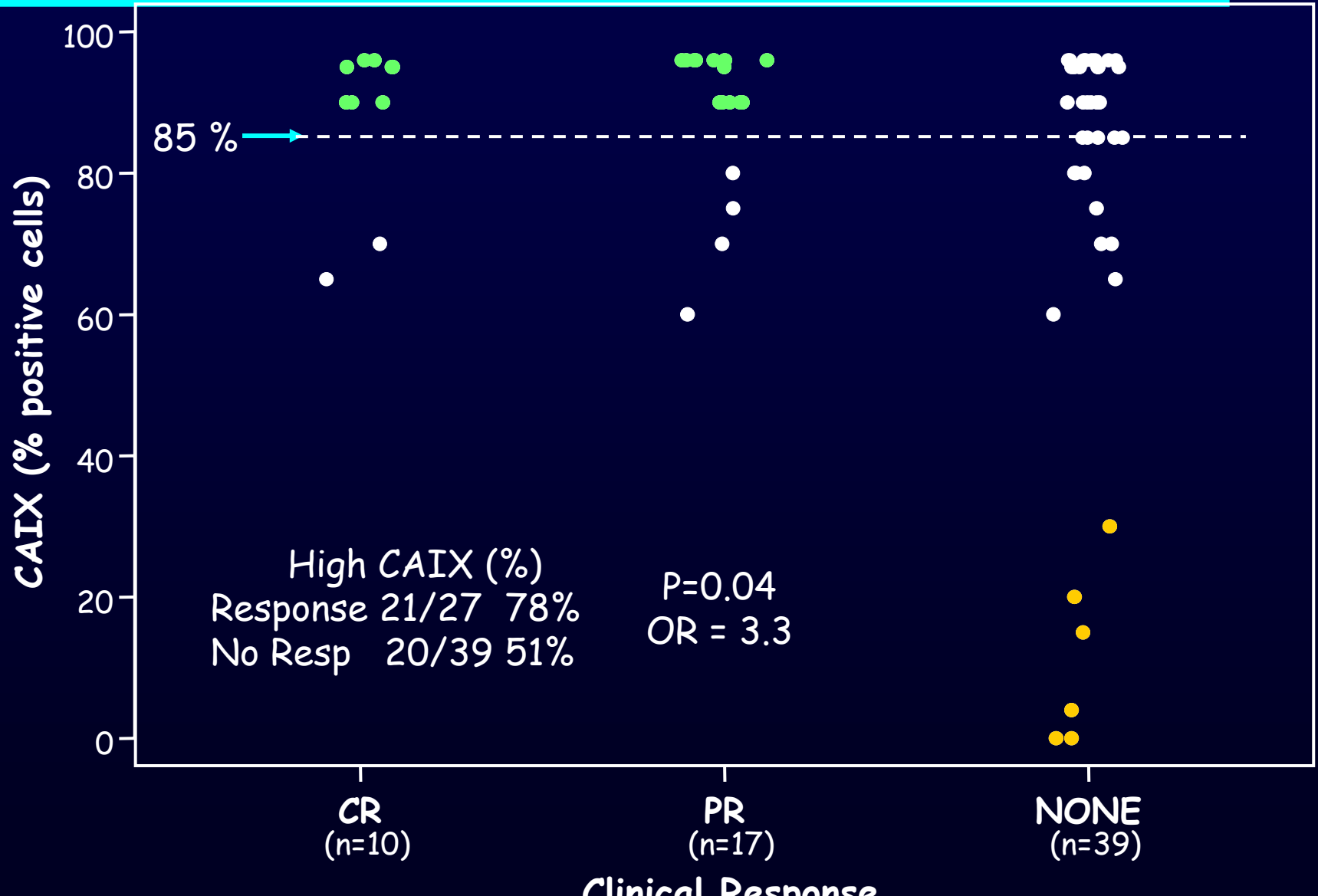# Additional Opportunities for Patient Selection: IL-2

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Methods
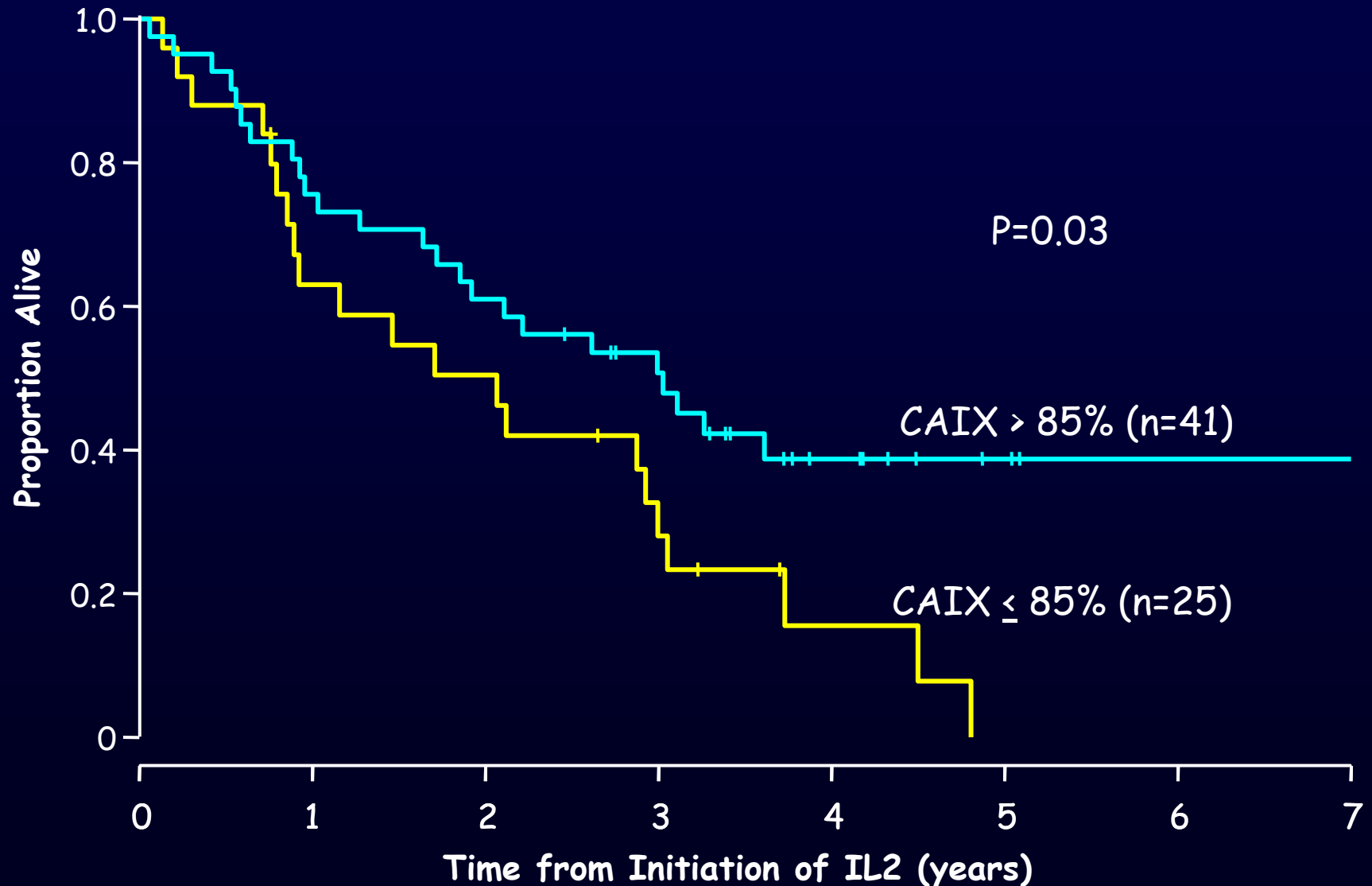
- Collected tissue blocks from patients enrolled in Cytokine Working Group (CWG) IL-2 trials (Upton, ASCO 2003)

- Enriched collection for responding patients- "nested case-control study"

- Selected representative tissue samples from each block

- Stained for CAIX expression using MN-75 Ab from Eric Stanbridge

- Correlated staining results with IL-2 response, survival, IL-2 dose, and pathologic risk group

CAIX Expression and IL-2 Response

High CAIX (%)
Response 21/27 78%
No Resp   20/39 51%

P=0.04
OR = 3.3

CAIX (% positive cells)

85 %

CR (n=10) PR (n=17) NONE (n=39)

Clinical Response

CAIX Expression and IL-2 Response

Proposed New Model

Pathology Risk Group	CAIX Staining	
	Low	High
Good		
Intermed		← Good
Poor	↑	

Poor

M Atkins

Proposed New Model

Refined Pathology Risk Group		Non-Responder (n=39)	Responder (n=27)
Good risk path or intermediate path with high CAIX	Good	18 (46%)	26 (96%)
Poor risk path or intermediate path with low CAIX	Poor	21 (54%)	1 (4%)

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IL-2 Related Survival and Refined Pathology Risk Group

Proportion Alive

Time from Initiation of IL2 (years)

P<0.01

Good

Poor

Additional Opportunities for Patient Selection: IL-2

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♦ **Expression Profiling**

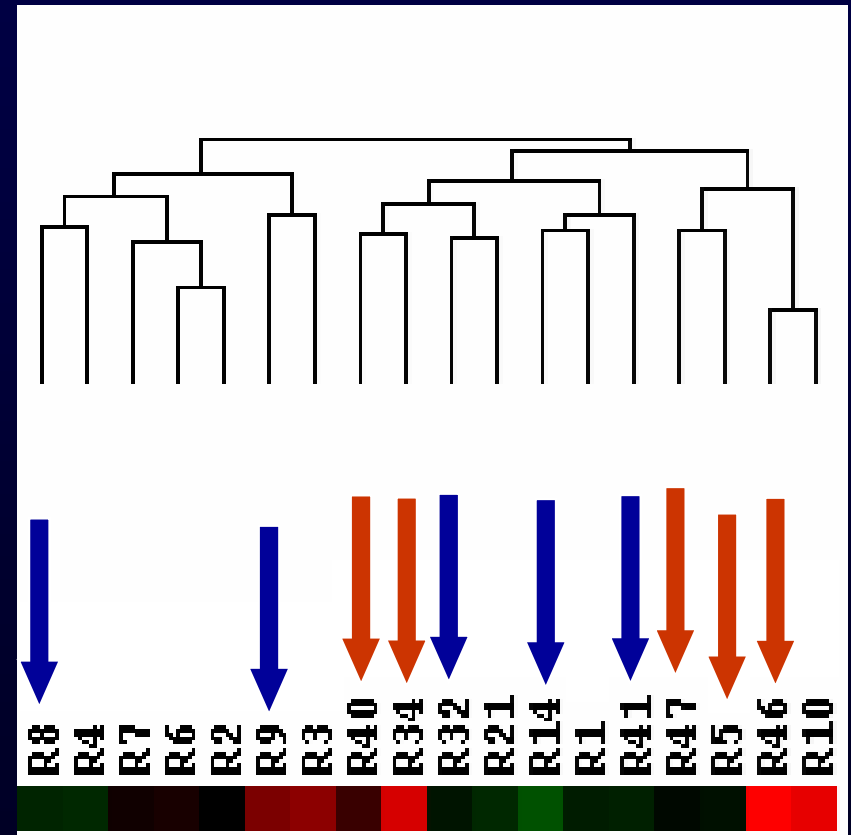# Prediction of Response to IL-2

Expression Analysis

RCC Sample Flow

1) Obtained frozen samples from Renal SPORE Path Core
2) Cut frozen sections, reviewed with pathologist
3) Included samples with RCC without significant necrosis
4) Isolated RNA
5) Created Target
6) Applied to microarrays U133A
7) Performed Q/A to omit poor scans
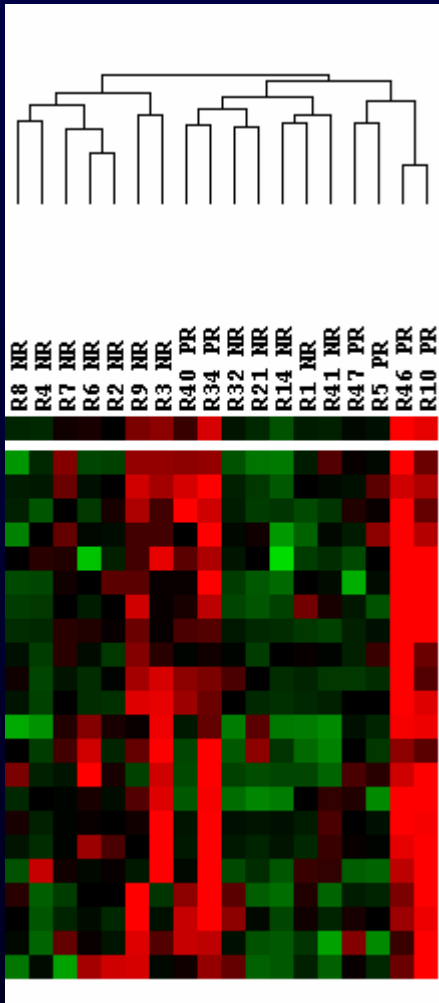
Prediction of Response to IL-2

Sample ID
R1
R2
R3
R4
R6
R7
R8
R9
R14
R21
R25
R32
R41
R10
R5
R23
R34
R40
R46
R47

Unsupervised Analysis

No dominating gene expression pattern for response to IL-2

Prediction of Response to IL-2

NAME
chemokine (C-X-C motif), receptor 4 (fusin)
chemokine receptor CXCR4
leukocyte surface protein (CD31)
STAT6
 integrin-linked kinase (ILK)
 ras homolog gene family, member B
 glutamyl aminopeptidase (aminopeptidase A) (ENPEP)
 fenestrated-endothelial linked structure protein (FELS)
vascular endothelial growth factor
enolase like 1 (ENO1L1)
cysteine-rich protein 2 (hCRP2)
 aquaporin 1 (channel-forming integral protein, 28kD)
alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidas
prominin (mouse)-like 1
carbonic anhydrase IX (CA9)
TSC501
 N-acetyltransferase Camello 2 (CML2)
 bcl-1
 CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.
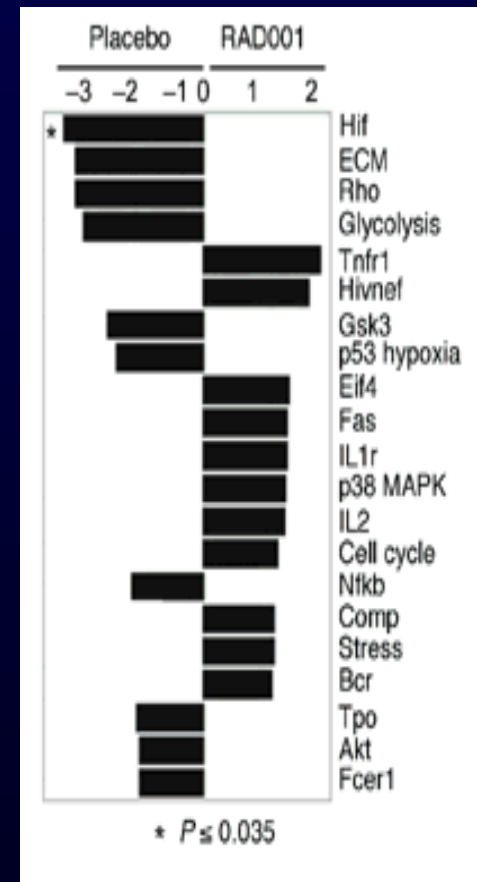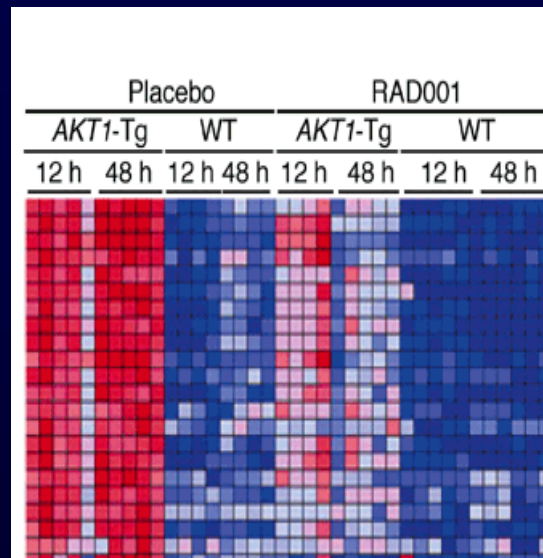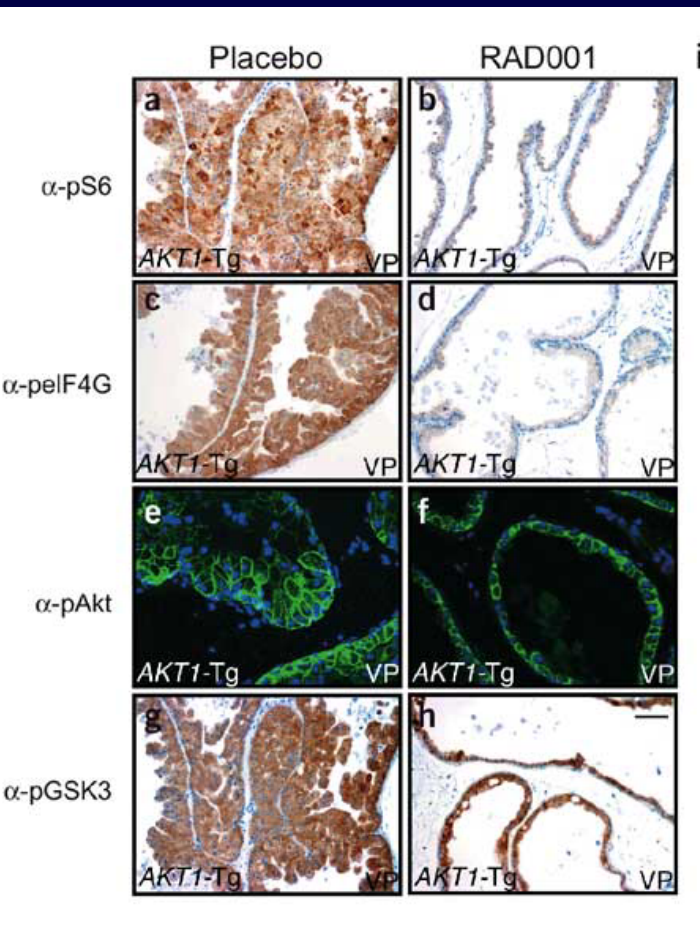 CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.
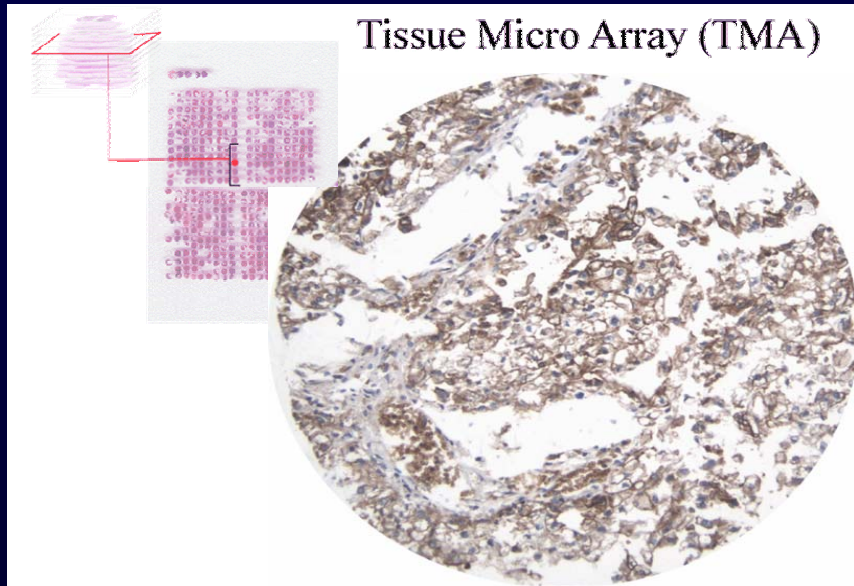 syndecan 1 (SDC1)
 CD59 antigen p18-20
EST
GABAnoradrenaline transporter

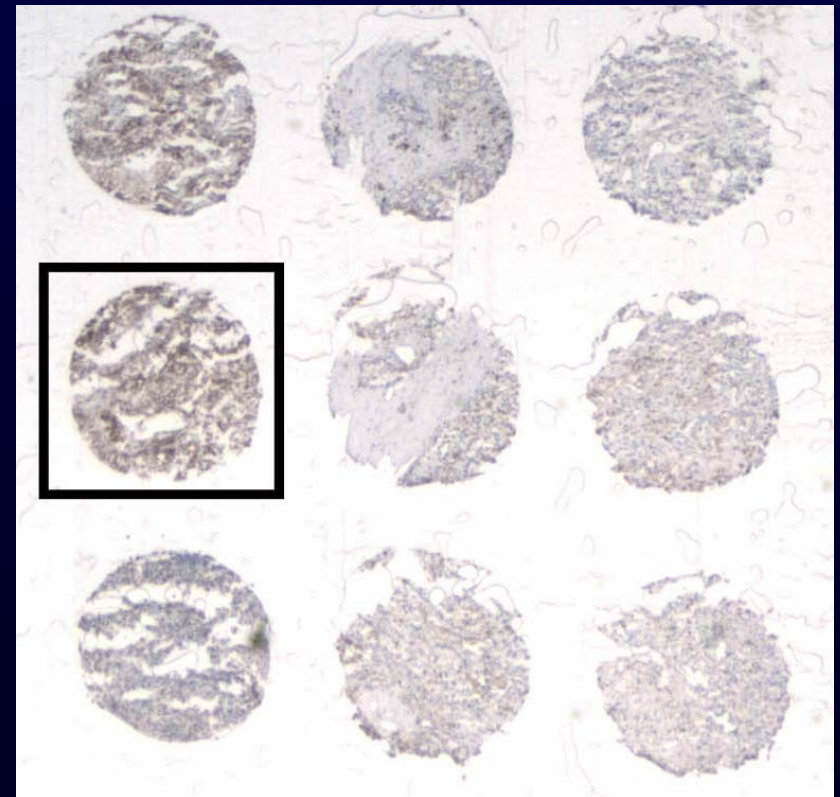# AKT and mTOR inhibition (Prostate)

Glut 1 Staining and RCC

Tissue Micro Array (TMA)

Pathology Data: (N=66)

Non-Clear Cell	8
Clear Cell	58
Alveolar	56
Granular	33
Papillary	4

Pathology Risk Group (Upton):

good	24	(36%)
intermediate	31	(47%)
poor	11	(17%)

Glut 1 Staining and RCC

Most RCC positive for GLUT 1

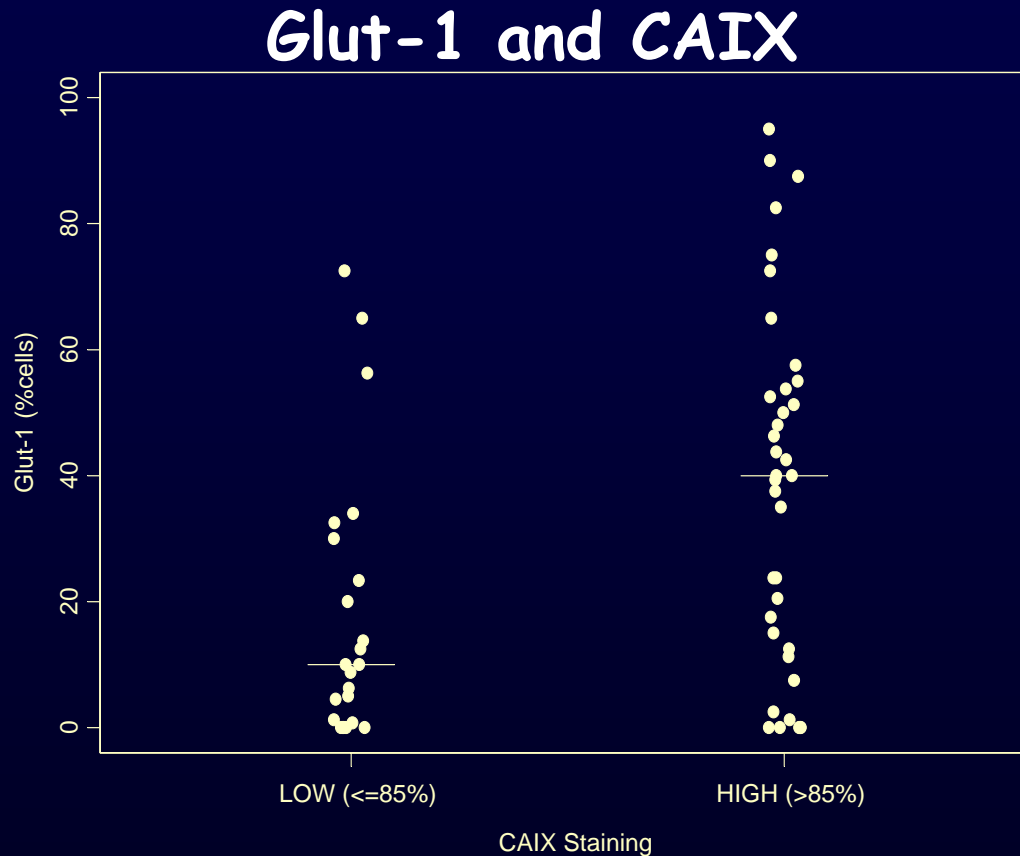   83.1% (51/61) with staining $\geq$ 1+

Heterogeneous staining observed within tumors

    Mean percentage of positive cells 30% (+/-27.6)

? Correlation with CAIX protein expression

? Correlation with IL2 outcome data

Relationship between CAIX and Glut-1

High CAIX expression appears to correlate
with high Glut-1 expression

Relationship between Glut-1 and IL-2 therapy

1. Glut-1 is NOT associated with response to IL-2 therapy

Glut-1

2. Glut-1 is associated with better survival following IL-2 therapy

Prediction of Response to IL-2

Expression Analysis

Supervised Analysis

10 patients received HDIL-2

♦ **Patient Characteristics-**
- 8 male/ 2 female
- MSKCI criteria: 2 good, 6 intermediate, 2 poor
- Response: 5 PR / 5 PD

♦ Specimens
- All clear cell, 1 with papillary features
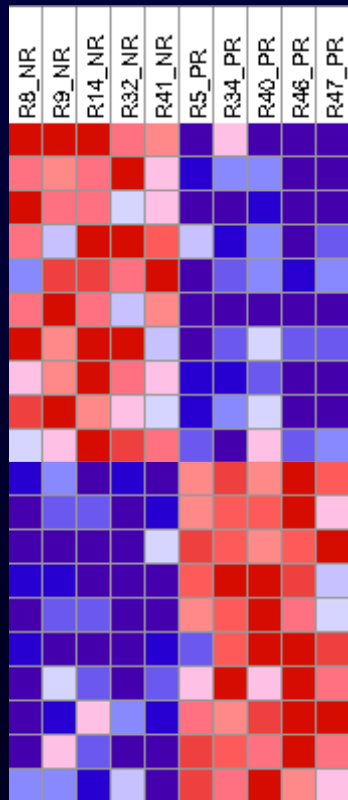- 8 with high CAIX protein expression

Sample ID
R1
R2
R3
R4
R6
R7
R8 ←
R9 ←
R14 ←
R21
R25
R32 ←
R41 ←
R10
R5 ←
R23
R34 ←
R40 ←
R46 ←
R47 ←

Prediction of Response to IL-2

Non-Responders

Responders

Supervised Analysis

Column labels: R8_NR, R9_NR, R14_NR, R32_NR, R41_NR, R5_PR, R34_PR, R40_PR, R46_PR, R47_PR

Top cluster gene list:
- variable charge, Y chromosome
- superoxide dismutase 2, mitochondrial
- hypothetical protein FLJ10815
- hepcidin antimicrobial peptide
- golgin-67
- EST
- interleukin enhancer binding factor 1
- Superoxide dismutase 2, mitochondrial
- Cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 9
- hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)

MnSOD
IL1RA

Bottom cluster gene list:
- neural proliferation, differentiation and control, 1
- tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)
- acetylserotonin O-methyltransferase-like
- MCM7 minichromosome maintenance deficient 7 (S. cerevisiae)
- hypothetical protein FLJ22690
- tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)
- metastasis-associated 1-like 1
- KIAA0323 protein
- EST
- phosphatidic acid phosphatase type 2A

TIMP3

Prediction of Response to IL-2

Supervised Analysis

- 206 genes with expression > 2 fold higher in responders
  - TIMP3 (inhibits MM3- tumor less aggressive)
  - CD 9 (associated with immune responsiveness)
- 197 genes > 2 fold higher in non-responders
  - MnSOD
  - IL-1 RA
  - Both induced by inflammatory cytokines
  - MnSOD increases resistance to TNF mediated apoptosis
- CAIX expression
  - increased 1.8 fold in tumors from responding patients
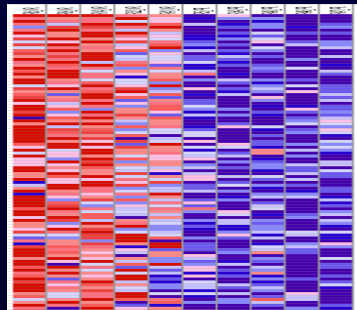 - Clustered with expression of HIF1 target genes

Prediction of Response to IL-2

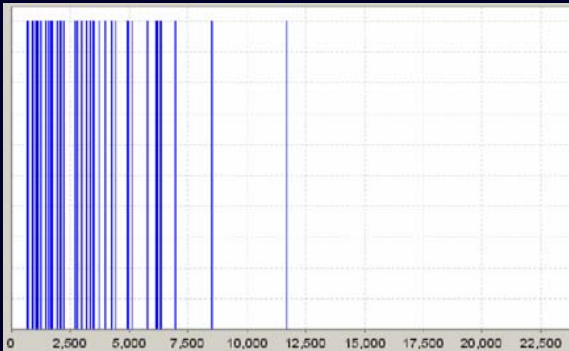## Gene Set Enrichment Analysis (GSEA)

1) Choose Dataset

IL-2 Responsive v. Non-Responsive

2) Set Class Distinction

3) Determine position of genes in Gene Sets

(Gene Ontology Sets)

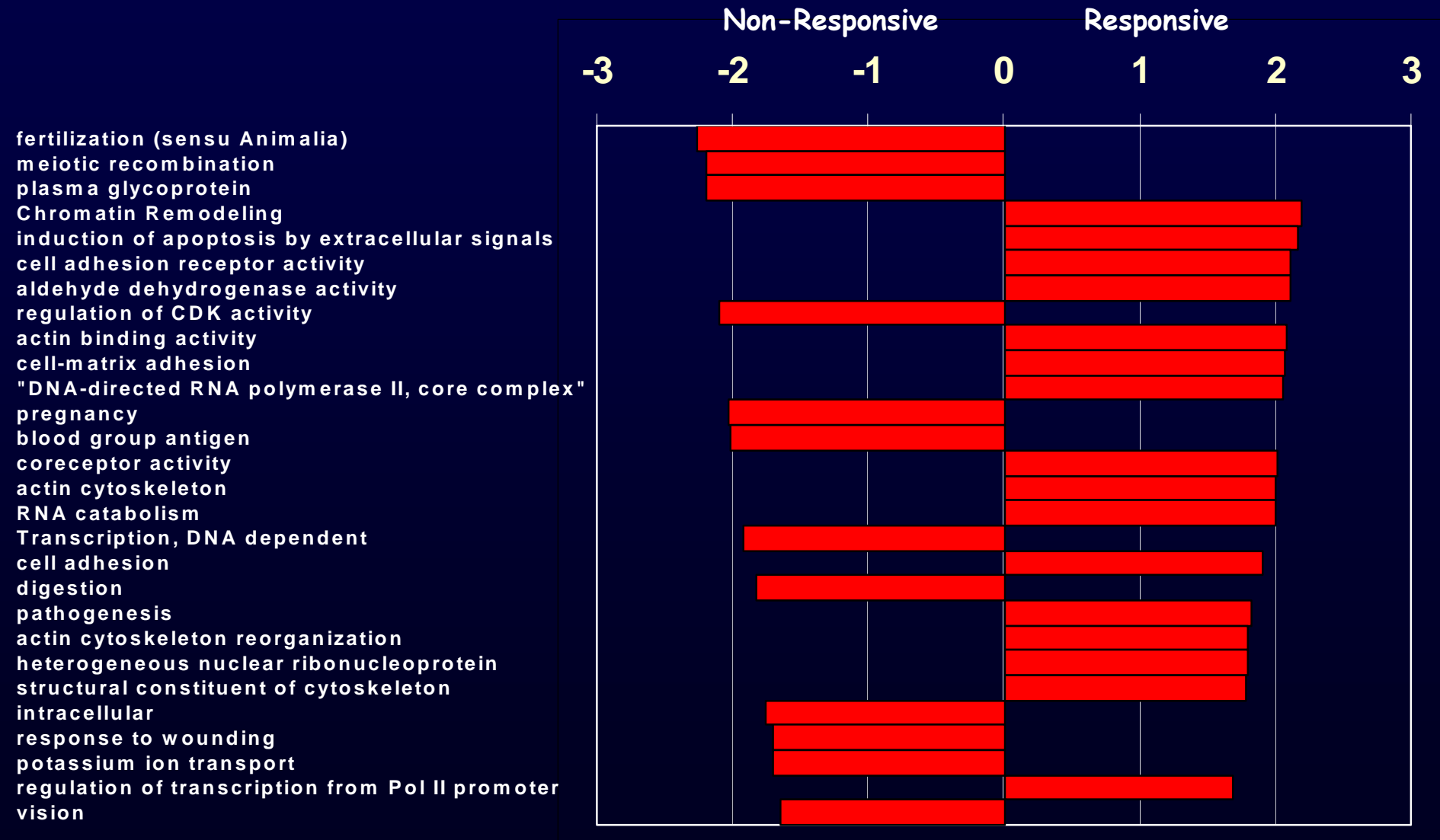**4) Determine "running score" of gene set**

5) Compare with permuted data

p < 0.01

Mootha et al (2003), Subramanian et al (Submitted)

Prediction of Response to IL-2

Prediction of Response to IL-2

Responsive

Chromatin Remodeling
induction of apoptosis by extracellular signals
cell adhesion receptor activity
aldehyde dehydrogenase activity

Prediction of Response to IL-2

Conclusions

- **Pathological and Molecular features of RCC can help anticipate an individual's response to IL-2 therapy**

- **There may be value in combining Pathological risk categories and CAIX staining in a response model**

- **CAIX RNA expression correlates with other HIF targets at RNA and Protein level**

- **Glut-1 expression does not correlate with response to IL-2: more to CAIX correlated response than HIF**

- **Preliminary supervised analyses suggest there are additional expression correlates with IL-2 response**

Acknowledgements

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