Characterization of Complete Response to IL-2 Using Gene Expression Analysis and Tissue Array Validation in Metastatic RCCa

Allan J. Pantuck, M.D.
UCLA Department of Urology
Society of Biologic Therapy
San Francisco, CA
November 5, 2004

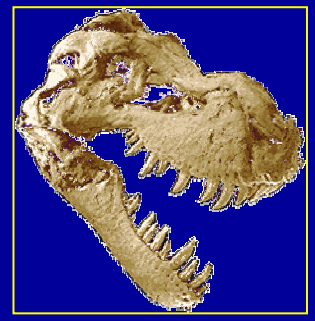
Goals

- Identification of prognostic factors to select patients most likely to respond to IL-2 to maximize therapeutic efficacy and minimize toxicity

- To better understand mechanisms and pathways important for cytokine immune response

- To identify possible new therapeutic targets and design new treatment strategies

Predicting IL-2 Response: Ancient History



Phenotypic Analysis of PBL in Responders and Non Responders

Pt. #	age	Type of Response	Type of TIL	$CD56^+CD3^-$ A	$CD56^+CD3^-$ B	$CD56^-CD3^+$ A	$CD56^-CD3^+$ B	$CD56^+CD3^-$ / $CD56^-CD3^+$ A	$CD56^+CD3^-$ / $CD56^-CD3^+$ B
57	66	CR	(IFN-α/pTIL)	51	1	18	60	2.83	0.02
65	73	CR	(IFN-γ/pTIL)	46	8	17	59	2.71	0.14
87	42	CR	(TNF-α/pTIL)	73	28	10	45	7.30	0.62
63	61	CR	(- /CD8+)	28	9	31	52	0.90	0.17
82	71	CR	(- /CD8+)	41	18	33	58	1.24	0.31
78	58	PR	(TNF-α/pTIL)	15	12	36	39	0.42	0.30
80	45	PR	(- /CD8+)	28	24	48	50	0.58	0.48
90	53	PR	(IFN-α/pTIL)	65	15	25	70	2.60	0.21
	Mean			43*	14	27**	54	2.60***	0.28
	± S.D.			20	9	12	9	2.24	0.19
64	79	NR	(IFN-α/pTIL)	3	29	48	29	0.06	1.00
67	47	NR	(TNF-α/pTIL)	7	9	68	66	0.10	0.14
86	70	NR	(TNF-α/pTIL)	14	18	67	59	0.21	0.31
89	61	NR	(IFN-γ/pTIL)	16	13	58	52	0.28	0.25
96	55	NR	(IL-2/pTIL)	52	20	34	66	1.53	0.30
74	61	NR	(- /CD8+)	33	24	27	34	1.22	0.71
75	57	NR	(- /CD8+)	14	26	58	34	0.24	0.76
81	55	NR	(- /CD8+)	6	10	69	59	0.09	0.17
88	70	NR	(- /CD8+)	13	9	54	58	0.24	0.16
	Mean			18	18	54	51	0.44	0.42
	± S.D.			16	8	15	15	0.54	0.36
Normal value (n=3)				11 ± 3		64 ± 16		0.18 ± 0.2	

* $p < 0.01$ (R vs. NR) and $p < 0.005$ (A vs. B), ** $P < 0.005$ (R vs. NR) and $p < 0.005$ (A vs. B)
*** $P < 0.05$ (R vs. NR) and $P < 0.05$ (A vs. B), R = responder; NR = non-responder;
PR = partial responder

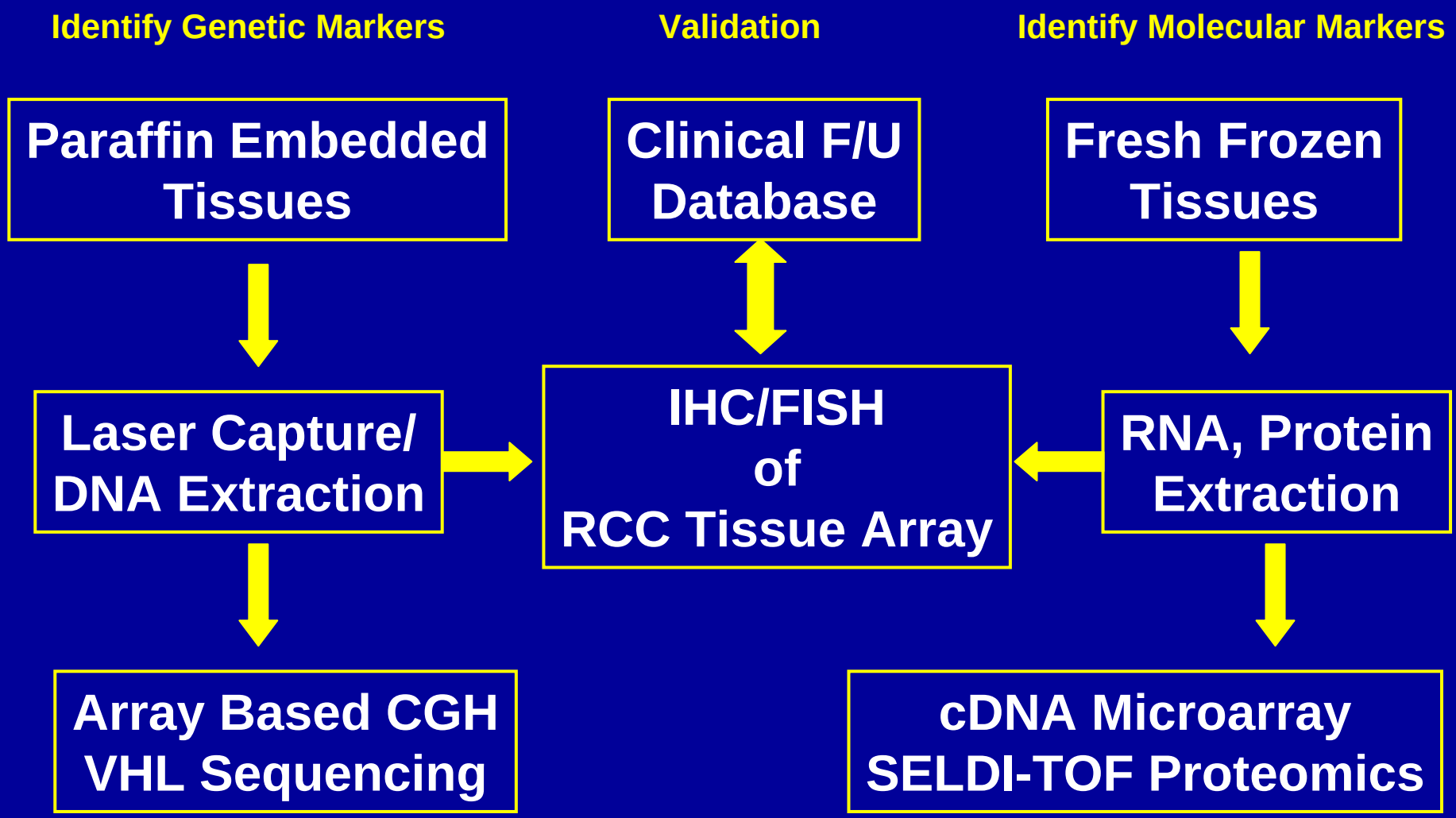
Belldegrun et al JOI, 1996

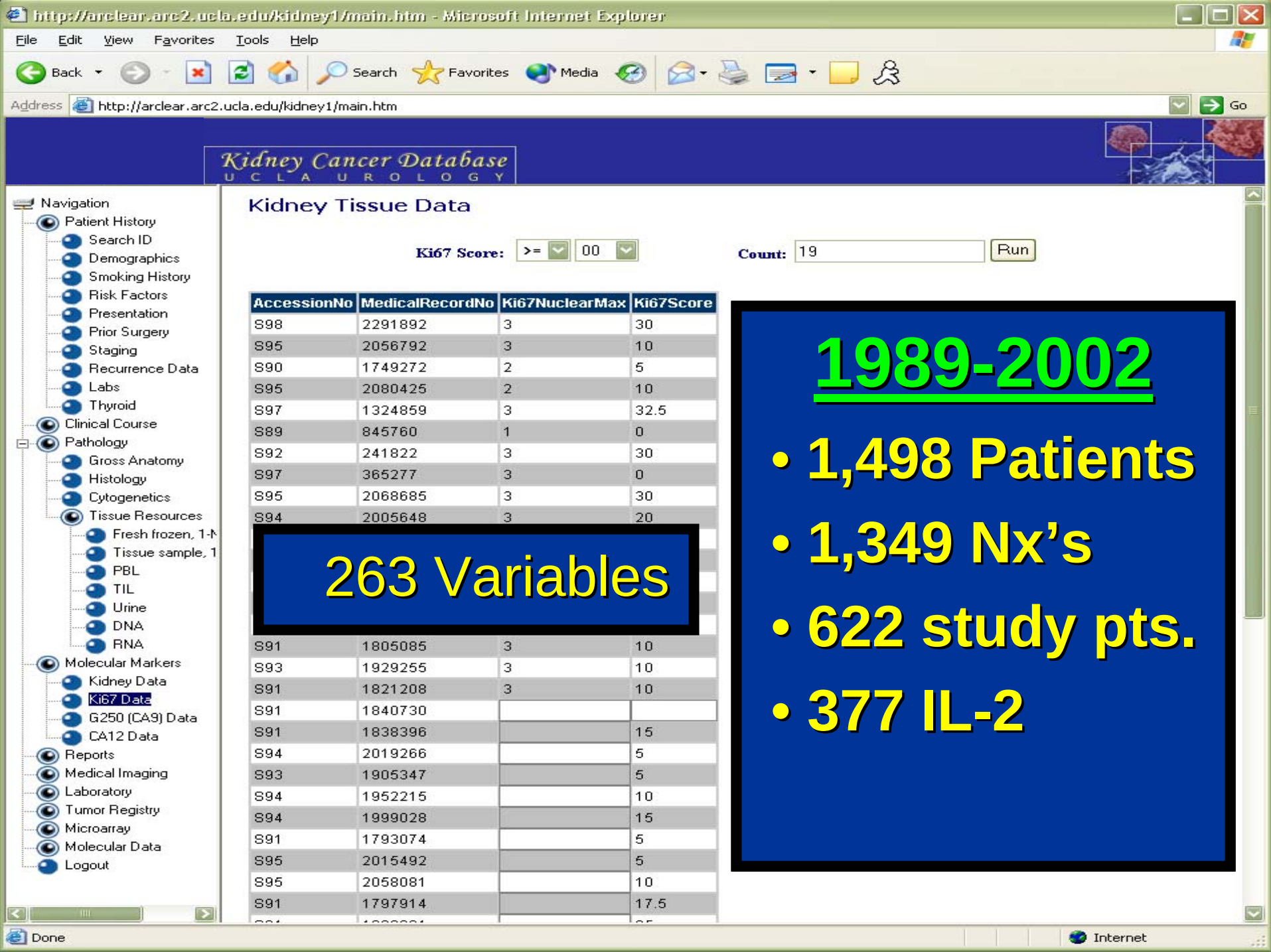
Predicting IL-2 Response
The Middle Ages
Clinical Algorithms

Characteristic	Hazard Ratio	p value
Constitutional Sx's	1.9	0.005
N Stage	1.4	0.002
Metastasis location	2.0	<0.001
Sarcomatoid Hist	2.3	0.003
TSH level	1.4	0.038

Leibovich et al
Cancer, 2002

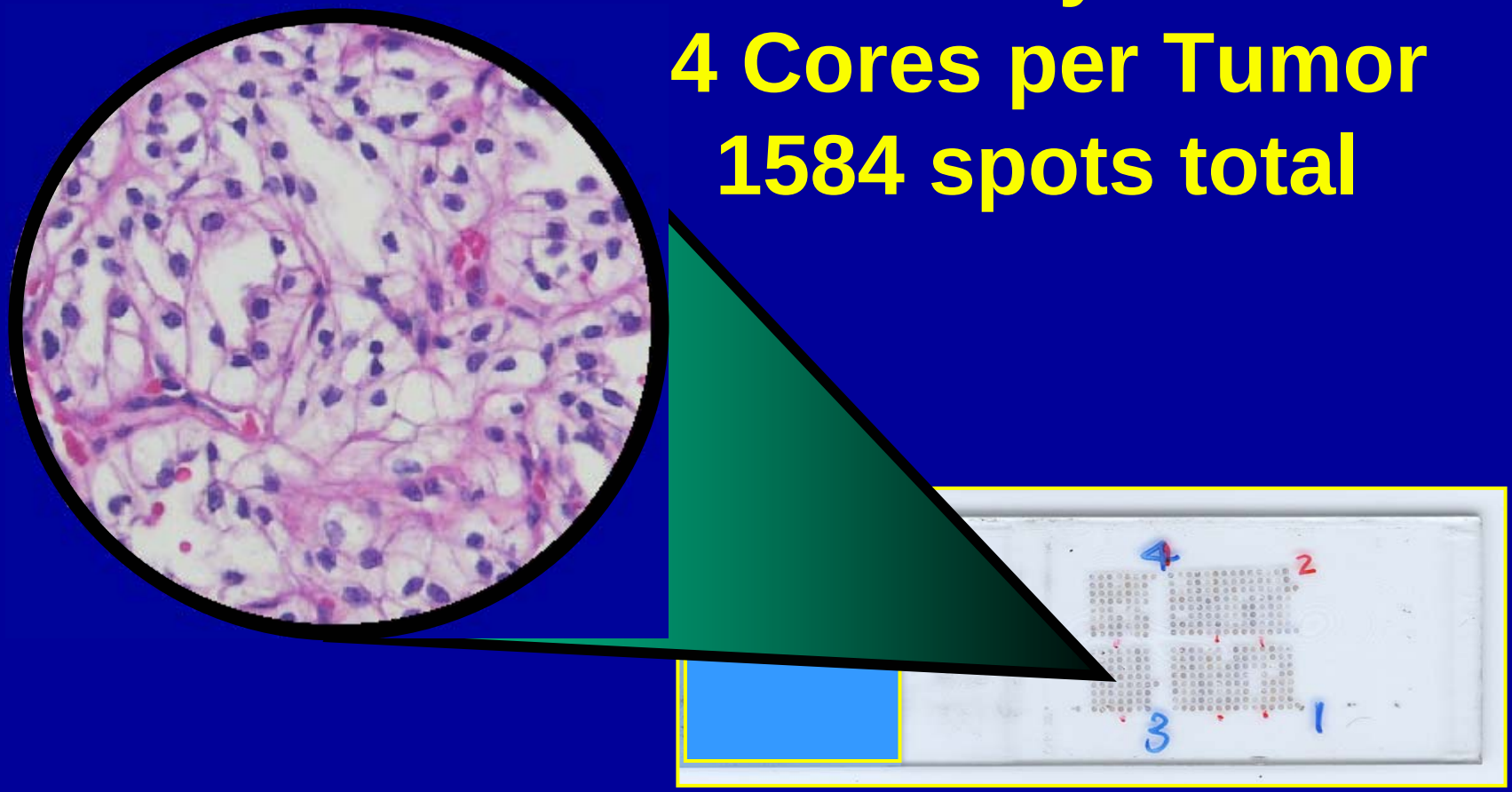
Predicting IL-2 Response
The Modern Era



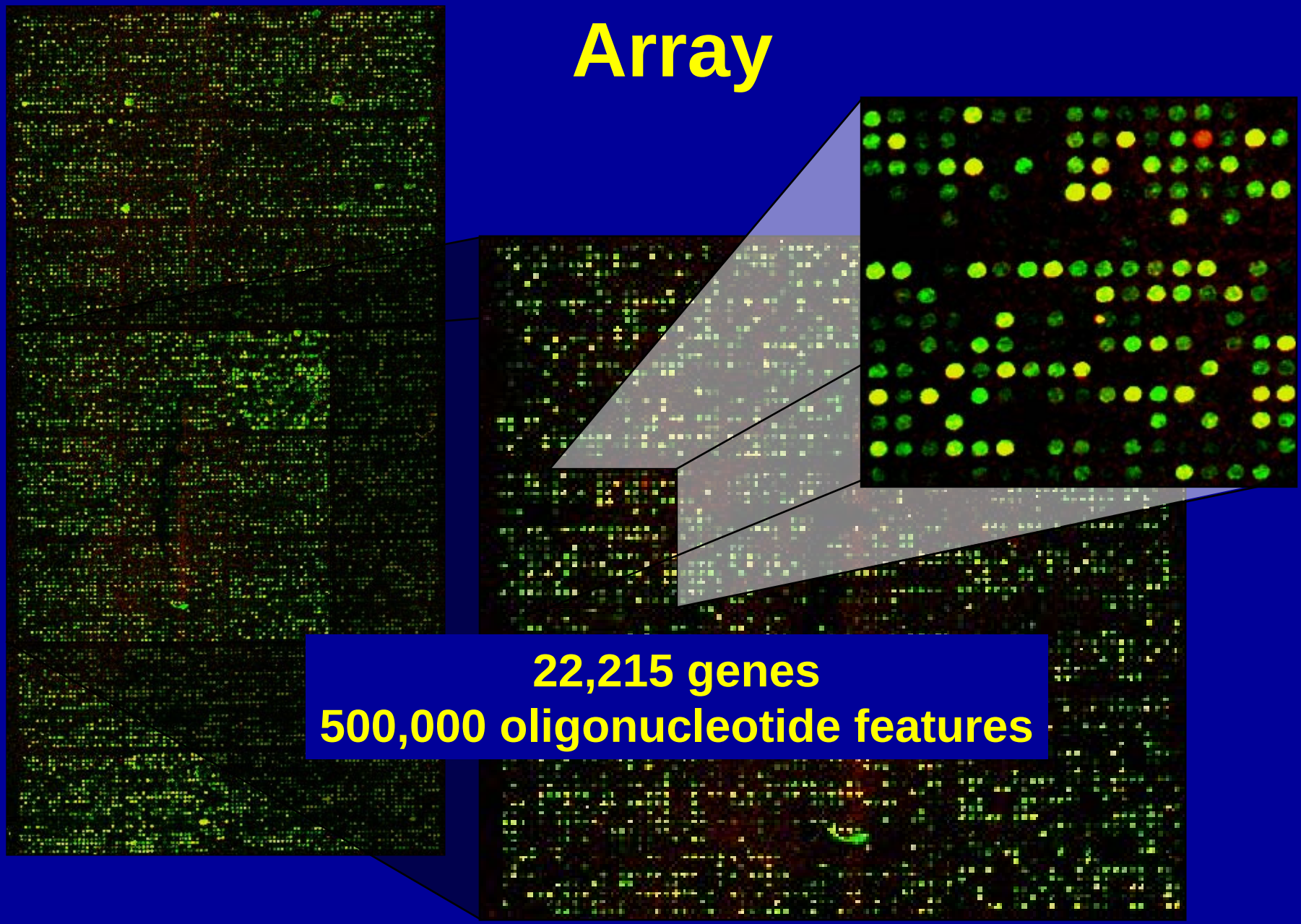

http://arclear.arc2.ucla.edu/kidney1/main.htm - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://arclear.arc2.ucla.edu/kidney1/main.htm
Go
Kidney Cancer Database
UCLA UROLOGY
Navigation
Patient History
Search ID
Demographics
Smoking History
Risk Factors
Presentation
Prior Surgery
Staging
Recurrence Data
Labs
Thyroid
Clinical Course
Pathology
Gross Anatomy
Histology
Cytogenetics
Tissue Resources
Fresh frozen, 1-N
Tissue sample, 1
PBL
TIL
Urine
DNA
RNA
Molecular Markers
Kidney Data
Ki67 Data
G250 (CA9) Data
CA12 Data
Reports
Medical Imaging
Laboratory
Tumor Registry
Microarray
Molecular Data
Logout
Kidney Tissue Data
Ki67 Score: >= 00
Count: 19
Run
AccessionNo MedicalRecordNo Ki67NuclearMax Ki67Score
S98 2291892 3 30
S95 2056792 3 10
S90 1749272 2 5
S95 2080425 2 10
S97 1324859 3 32.5
S89 845760 1 0
S92 241822 3 30
S97 365277 3 0
S95 2068685 3 30
S94 2005648 3 20
263 Variables
S91 1805085 3 10
S93 1929255 3 10
S91 1821208 3 10
S91 1840730
S91 1838396 15
S94 2019266 5
S93 1905347 5
S94 1952215 10
S94 1999028 15
S91 1793074 5
S95 2015492 5
S95 2058081 10
S91 1797914 17.5
1989-2002
• 1,498 Patients
• 1,349 Nx's
• 622 study pts.
• 377 IL-2
Done
Internet

TISSUE ARRAY CONSTRUCTION

396 Kidney Tumors
4 Cores per Tumor
1584 spots total



Affymetrix U133A Gene Chip Array



22,215 genes
500,000 oligonucleotide features

cDNA Microarray Data Anaysis

CEL files were imported into dChip to compute the model based expression index for each gene

To reveal the global pattern of the arrays, we used unsupervised learning analysis, (hierarchical clustering and multi-dimensional scaling plots)

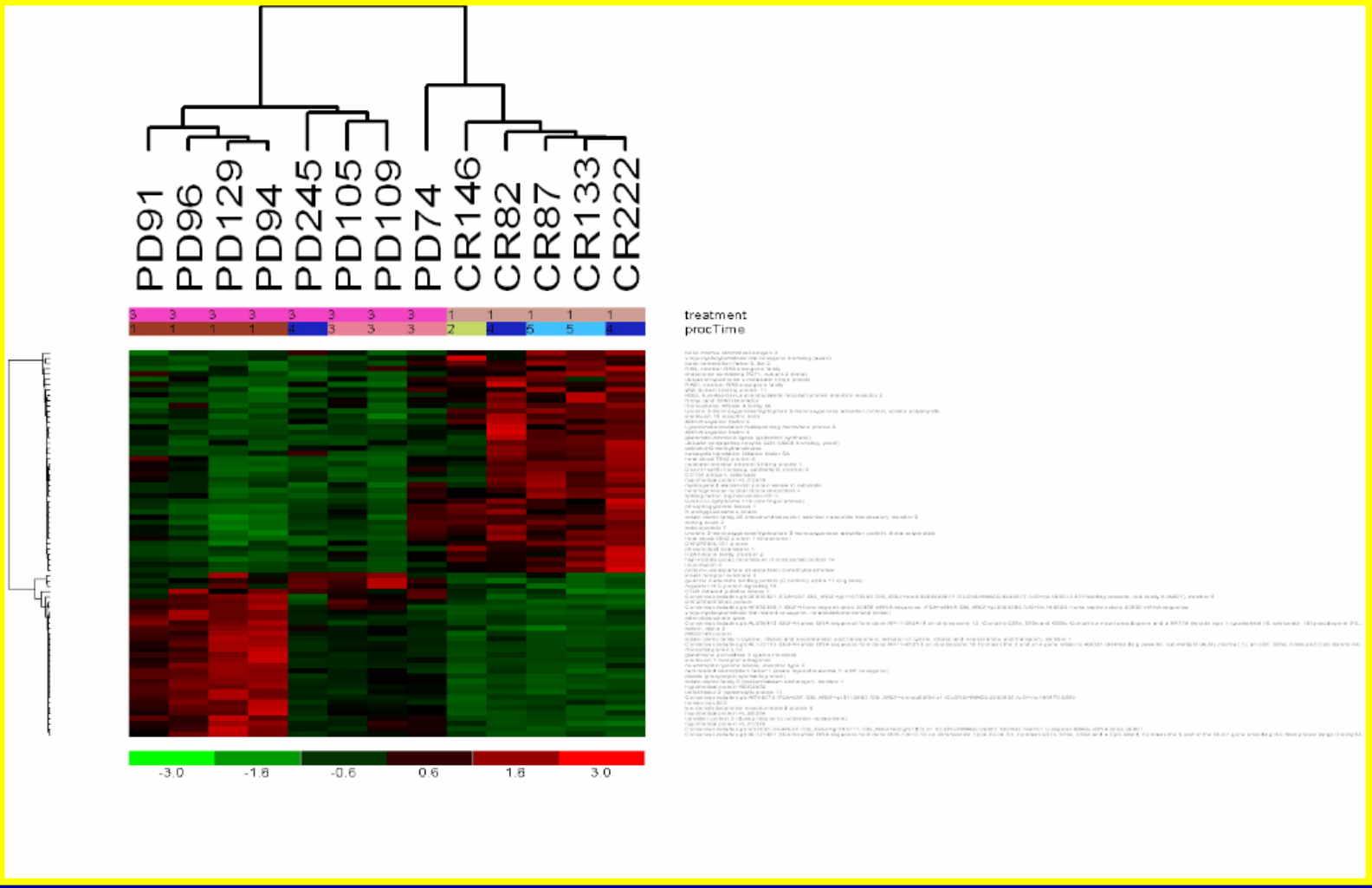
To filter out significant genes, we used the standard two sample t-test in pair-wise comparisons involving different treatment groups. The selection criterion are as follows:

1. the fold change >1.2;
2. |difference|>100; and
3. p-value < 0.05

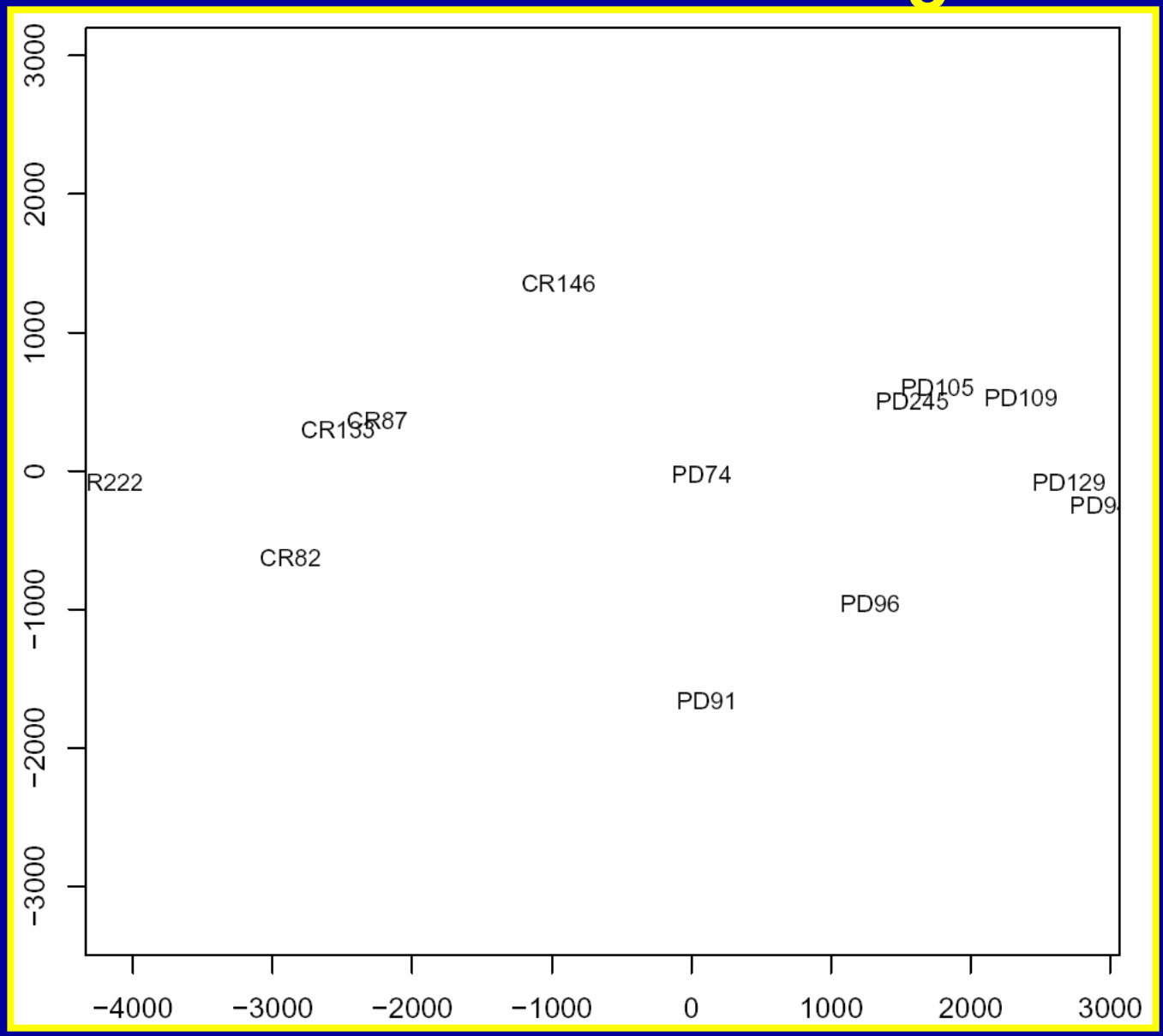
Molecular Analysis of IL-2 Response
Top 566 genes: PD vs CR

probe set
guanine nucleotide binding protein (G protein), alpha 11 (Gq class)
solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocat
tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein
KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2
phosphoglycerate kinase 1
RAN, member RAS oncogene family
heat shock 60kD protein 1 (chaperonin)
H2A histone family, member Z
high-mobility group (nonhistone chromosomal) protein 14
ADP-ribosylation factor 4
heterogeneous nuclear ribonucleoprotein F
bone marrow stromal cell antigen 2
myristoylated alanine-rich protein kinase C substrate
Lysosomal-associated multispanning membrane protein-5
chaperonin containing TCP1, subunit 2 (beta)
ubiquinol-cytochrome c reductase hinge protein
v-myc myelocytomatosis viral oncogene homolog (avian)
phospholipid scramblase 1
glutathione peroxidase 2 (gastrointestinal)
splicing factor, arginine/serine-rich 3
actinin, alpha 2
N-myc (and STAT) interactor
low density lipoprotein receptor-related protein 6
solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, a
regulator of G-protein signalling 12
DKFZP566F0546 protein
transition protein 2 (during histone to protamine replacement)
RAB1, member RAS oncogene family
nuclease sensitive element binding protein 1
CD164 antigen, sialomucin
sorting nexin 3
DnaJ (Hsp40) homolog, subfamily B, member 6
catechol-O-methyltransferase
PTEN

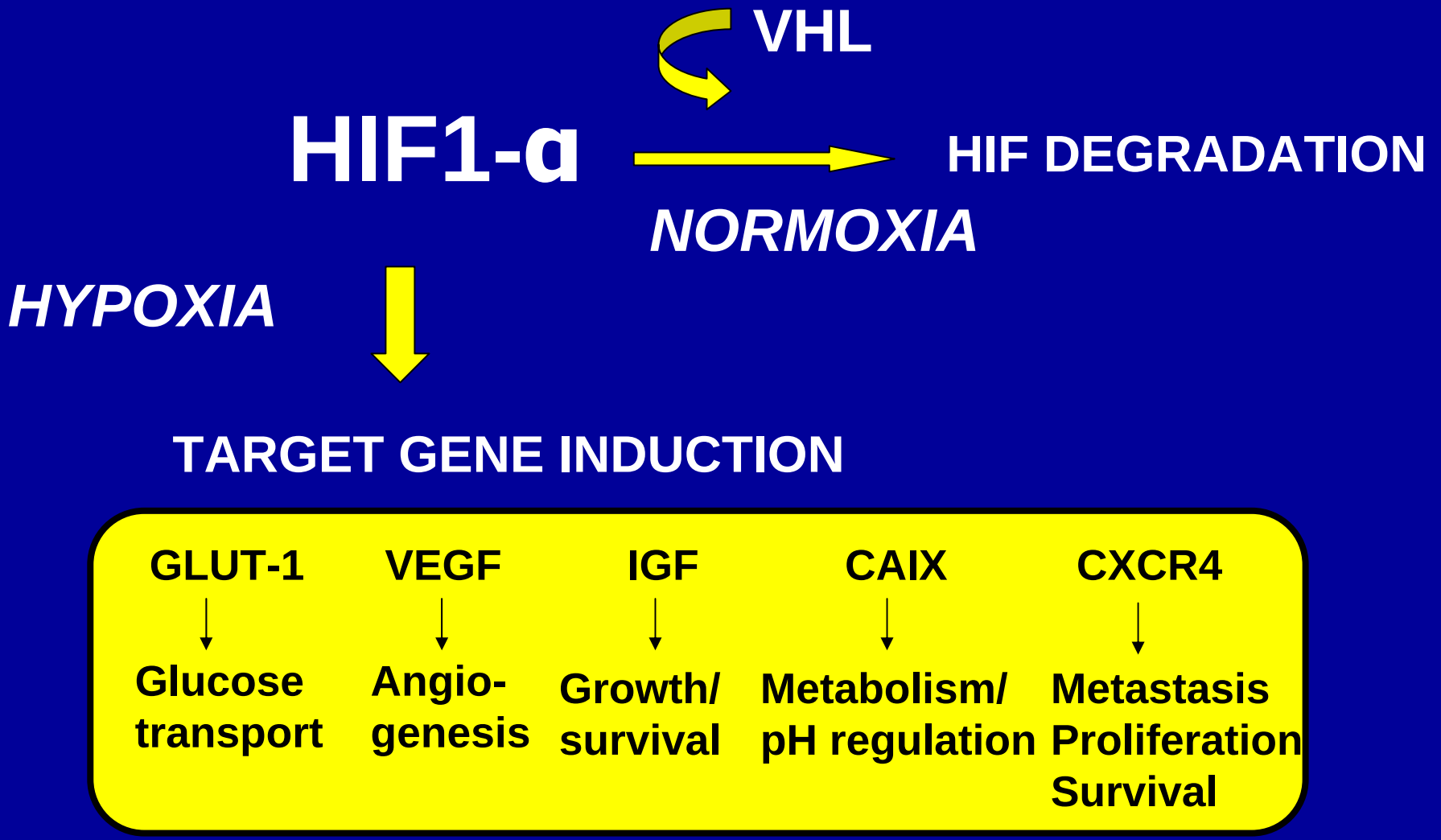
Top 73 Genes PD vs. CR Hierarchical Clustering Dendrogram



Top 73 Genes PD vs. CR
Multi-Dimensional Scaling Plots



VHL, Hypoxia, and RCC Tumorigenesis



Pantuck et al,
Clin Ca Res, 2003

Differentially Expressed Genes of Interest

CR:

Integrin Associated Proteins (CD47, Fibronectin)
Tumor Suppressors (PTEN)
Heat Shock Proteins 60, 70, 90
MCH Class II
Pro-Apoptotic (Caspase I)
MAP kinase
Thyroid Autoantigen
Carbonic Anhydrase IX

PD:

Chemokines (CXCR4)
Regulators of G protein Signaling
Trefoil Factors
Actin Associated Proteins (actinin)
IGF binding proteins

Differentially Expressed Genes of Interest

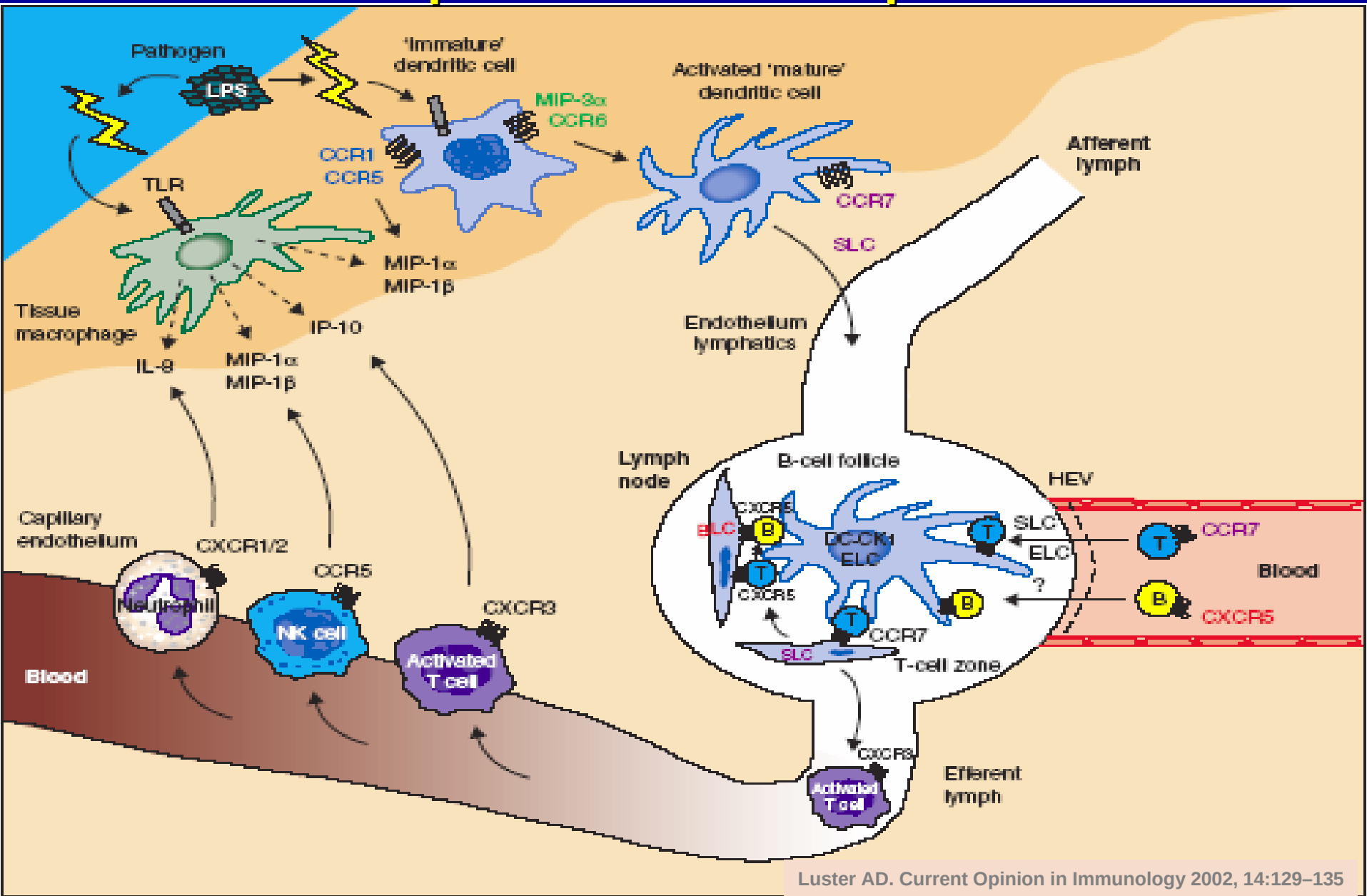
CR:

Integrin Associated Proteins (CD47, Fibronectin)
Tumor Suppressors (PTEN)
Heat Shock Proteins 60, 70, 90
MCH Class II
Pro-Apoptotic (Caspase I)
MAP kinase
Thyroid Autoantigen
Carbonic Anhydrase IX

PD:

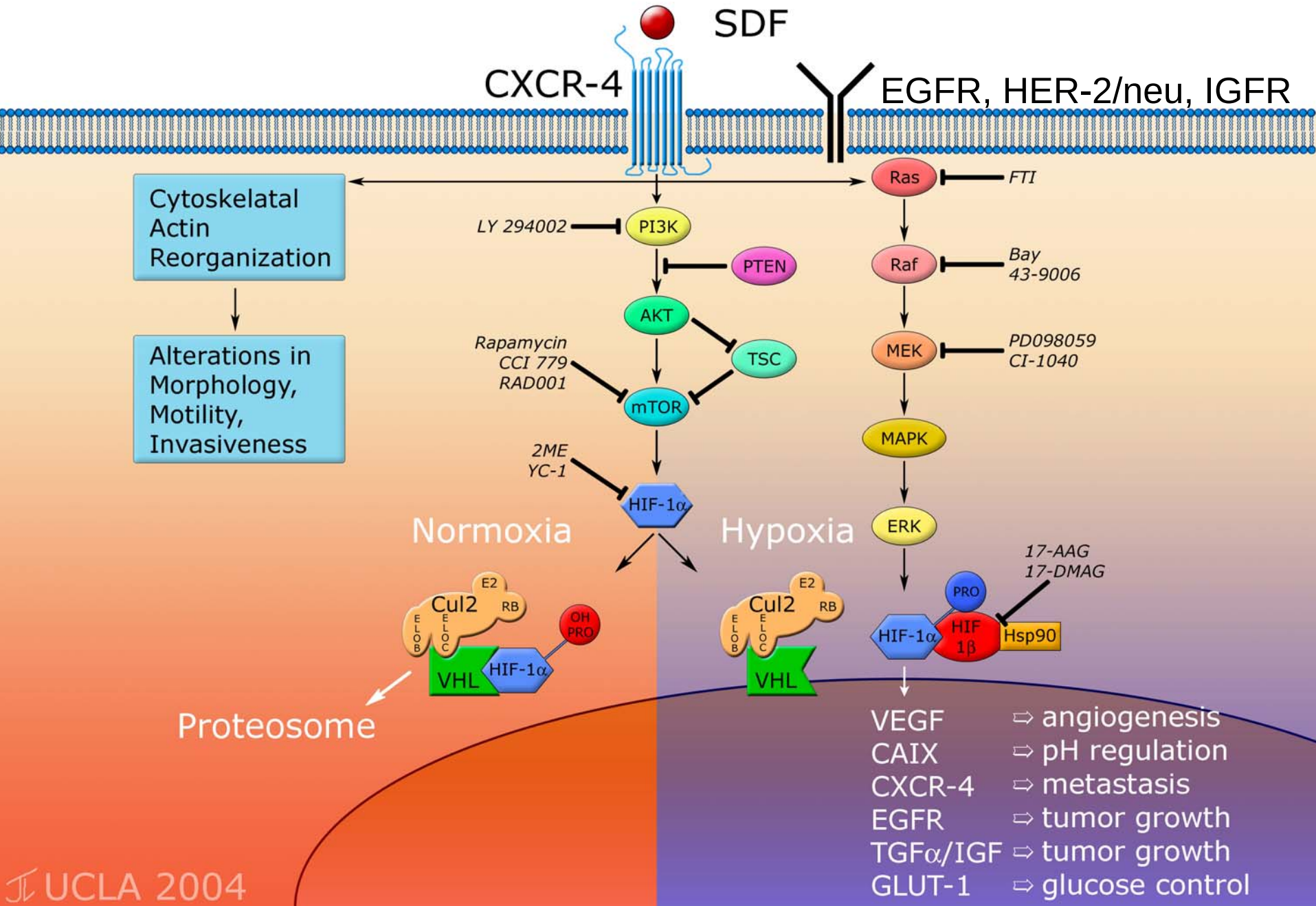
Chemokines (CXCR4)
Regulators of G protein Signaling
Trefoil Factors
Actin Associated Proteins (actinin)
IGF binding proteins

Chemokines Orchestrate Both the Innate and Adaptive Immune Response



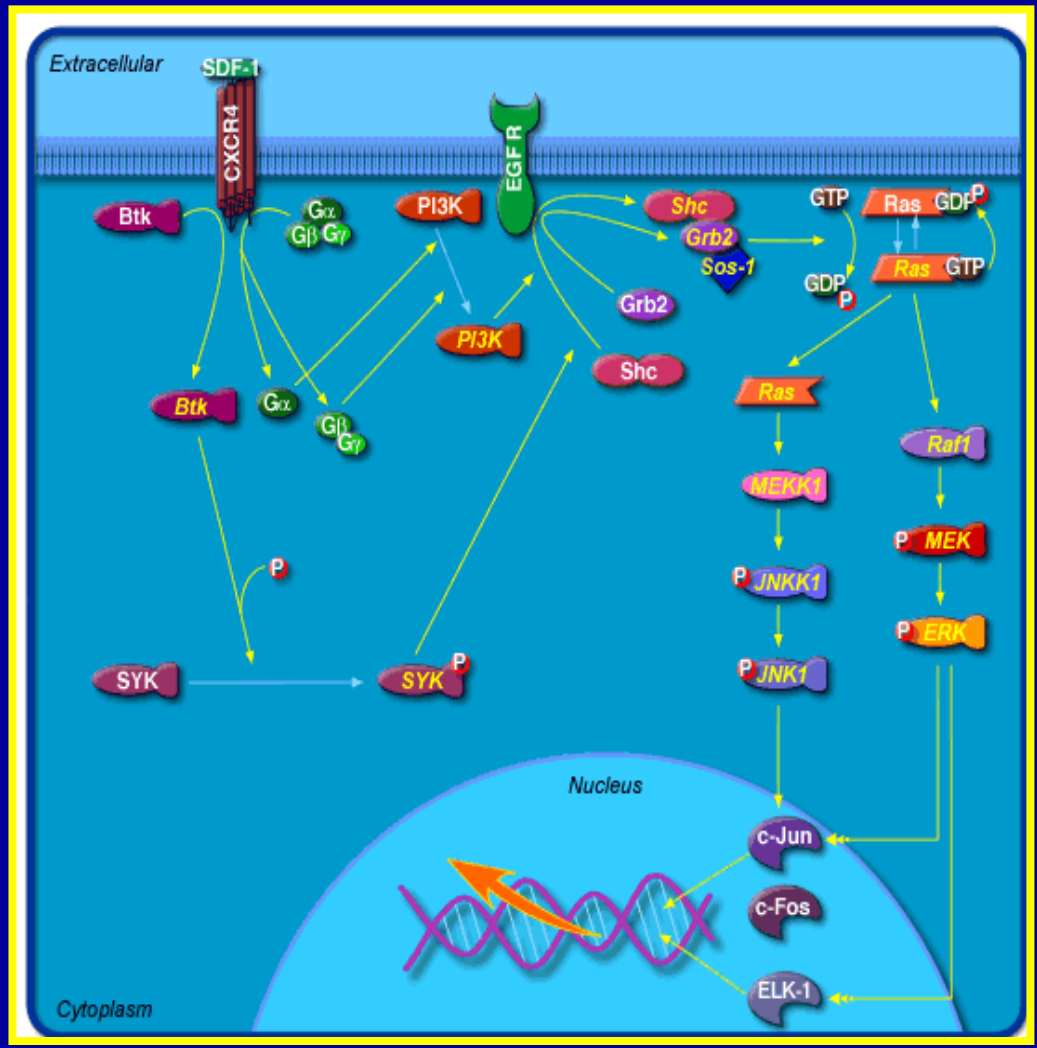
Luster AD. Current Opinion in Immunology 2002, 14:129–135

CXCR-4 Pathways and RCC

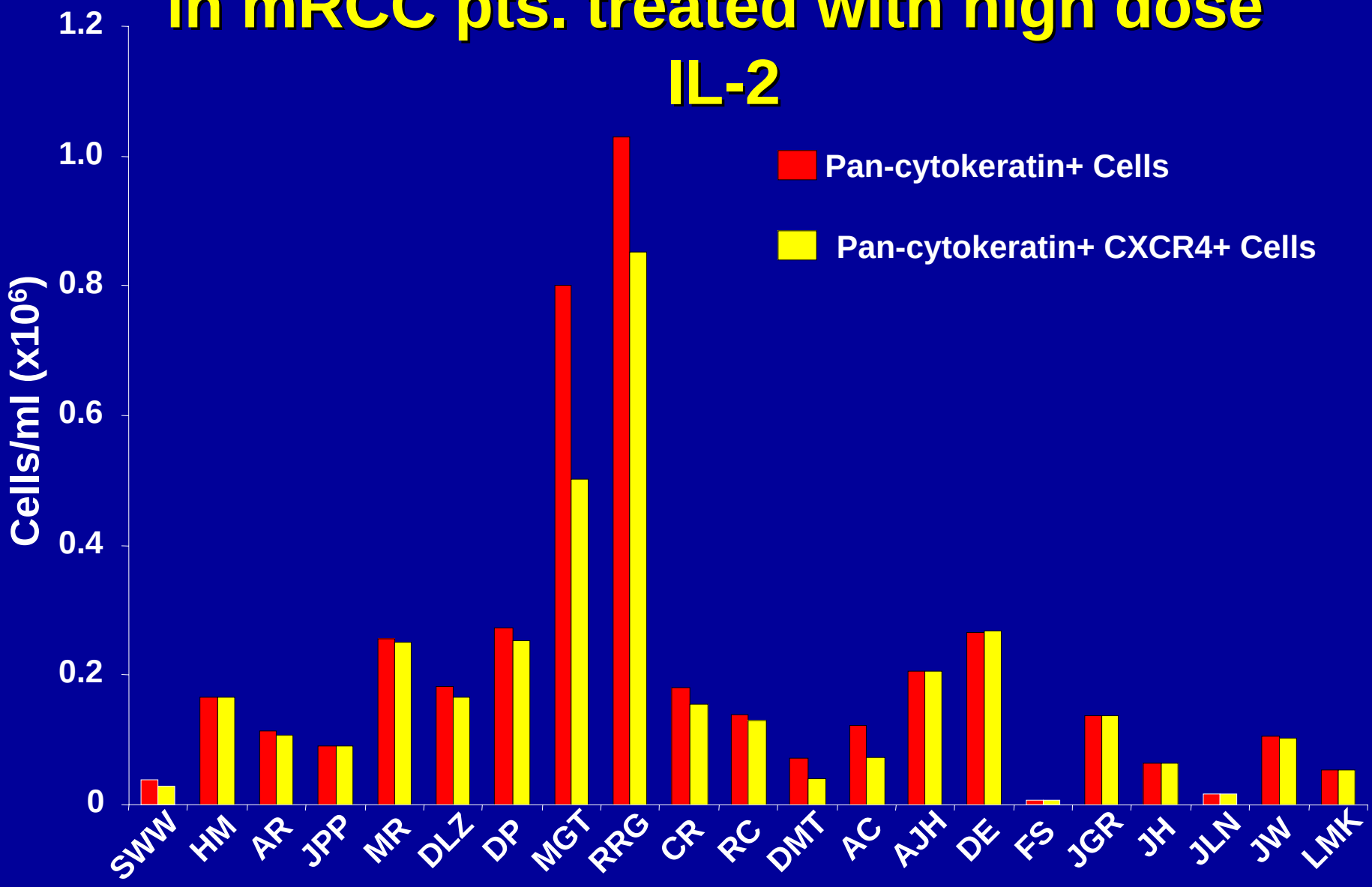


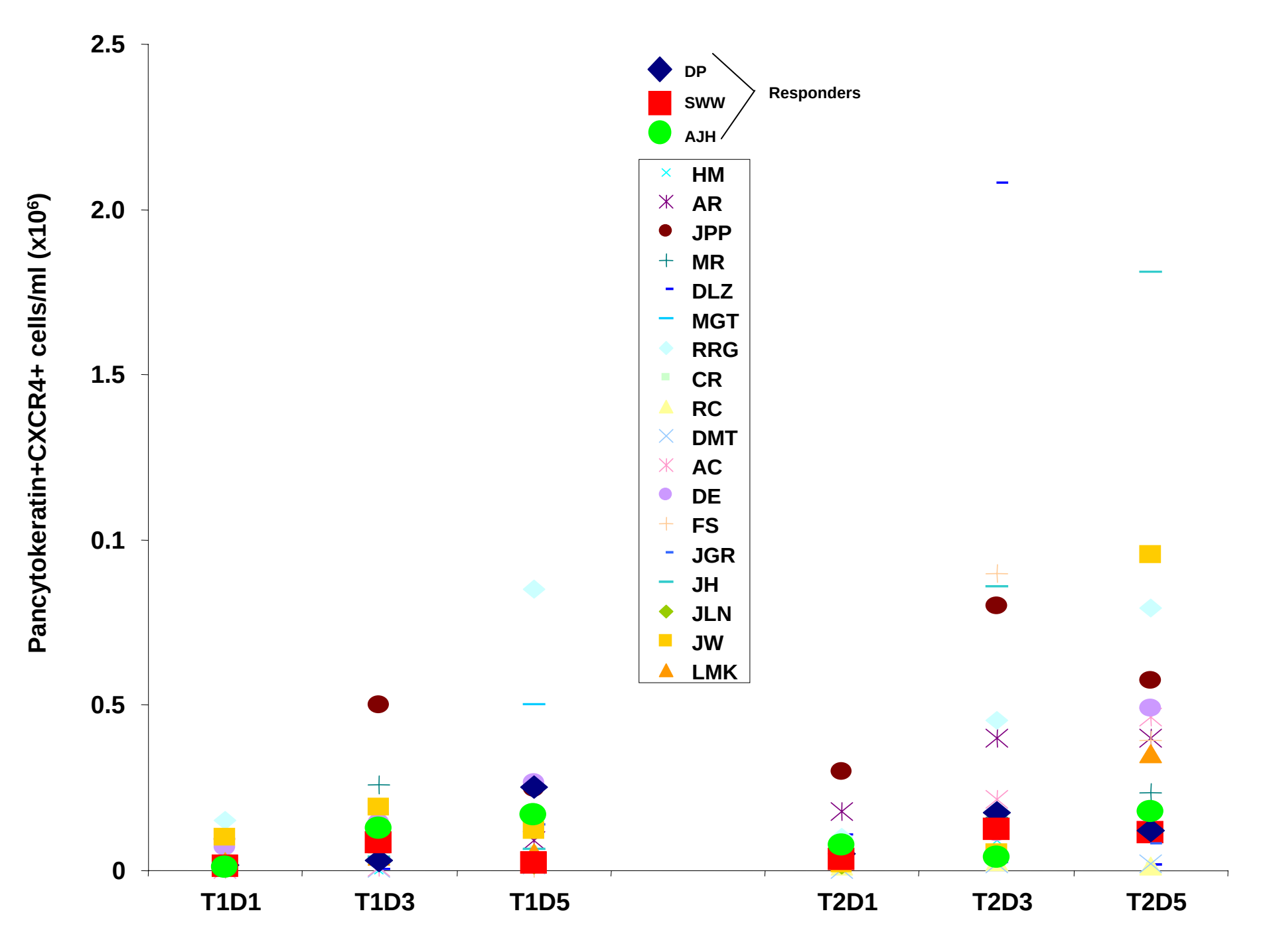
Increased CXCR4: A Marker of IL-2 Non-Responsiveness?

- Receptor for CXCL12/SDF-1
- Regulation of: metastasis proliferation survival



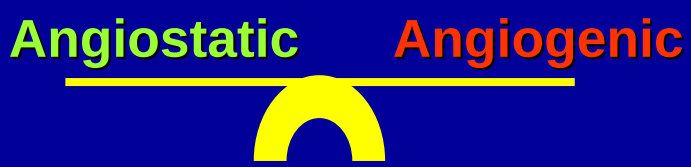
Cytokeratin + CXCR4+ circulating cells in mRCC pts. treated with high dose IL-2




Pancytokeratin+CXCR4+ cells/ml (x10⁶)
2.5
2.0
1.5
0.1
0.5
0
T1D1
T1D3
T1D5
T2D1
T2D3
T2D5
DP
SWW
AJH
Responders
HM
AR
JPP
MR
DLZ
MGT
RRG
CR
RC
DMT
AC
DE
FS
JGR
JH
JLN
JW
LMK

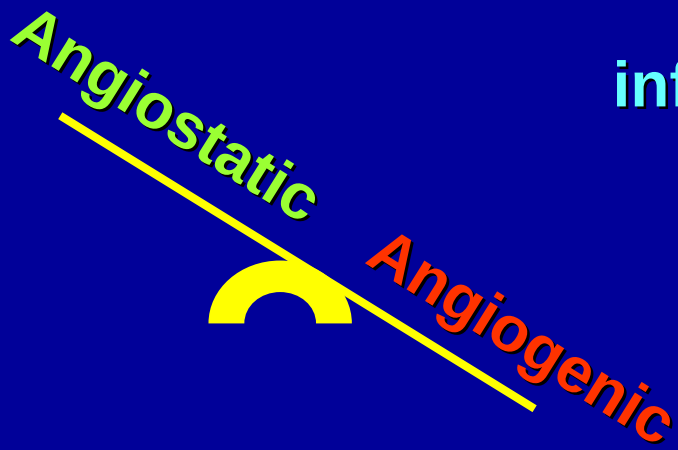
Angiogenesis

Net neovascularization is determined by the balance of angiogenic and angiostatic factors within the local microenvironment



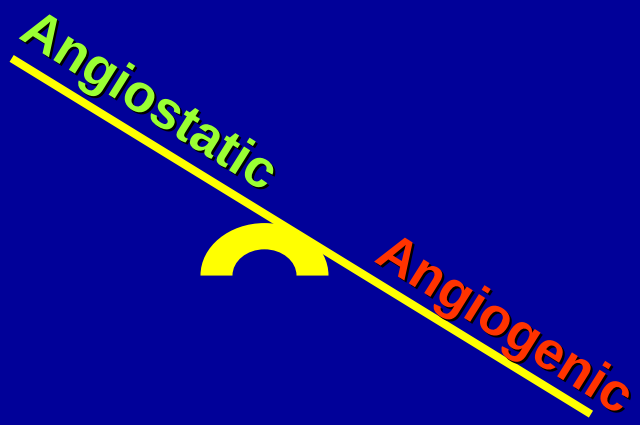
Homeostasis

Wound repair
Chronic inflammation



Tumor growth

Angiogenic Factors and Immunotherapy Response



CR	CXCL9 CXCL10 CXCL11	CXCL12 VEGF
PD	----------	CXCL1 CXCL2 CXCL3 VEGF CXCR4 VEGF B

Carbonic Anhydrase IX

Clinically, high levels (>85%) associated with improved immunotherapy response (UCLA, CWG)

CAIX Gene Expression

CR: 1669 **PD:** 941 **PD+ Lymph Nodes:** 611

Tissue Array Validation: Analysis of Genes and IL-2 Response

Carbonic Anhydrases IX and XII

EpCAM

Gelsolin

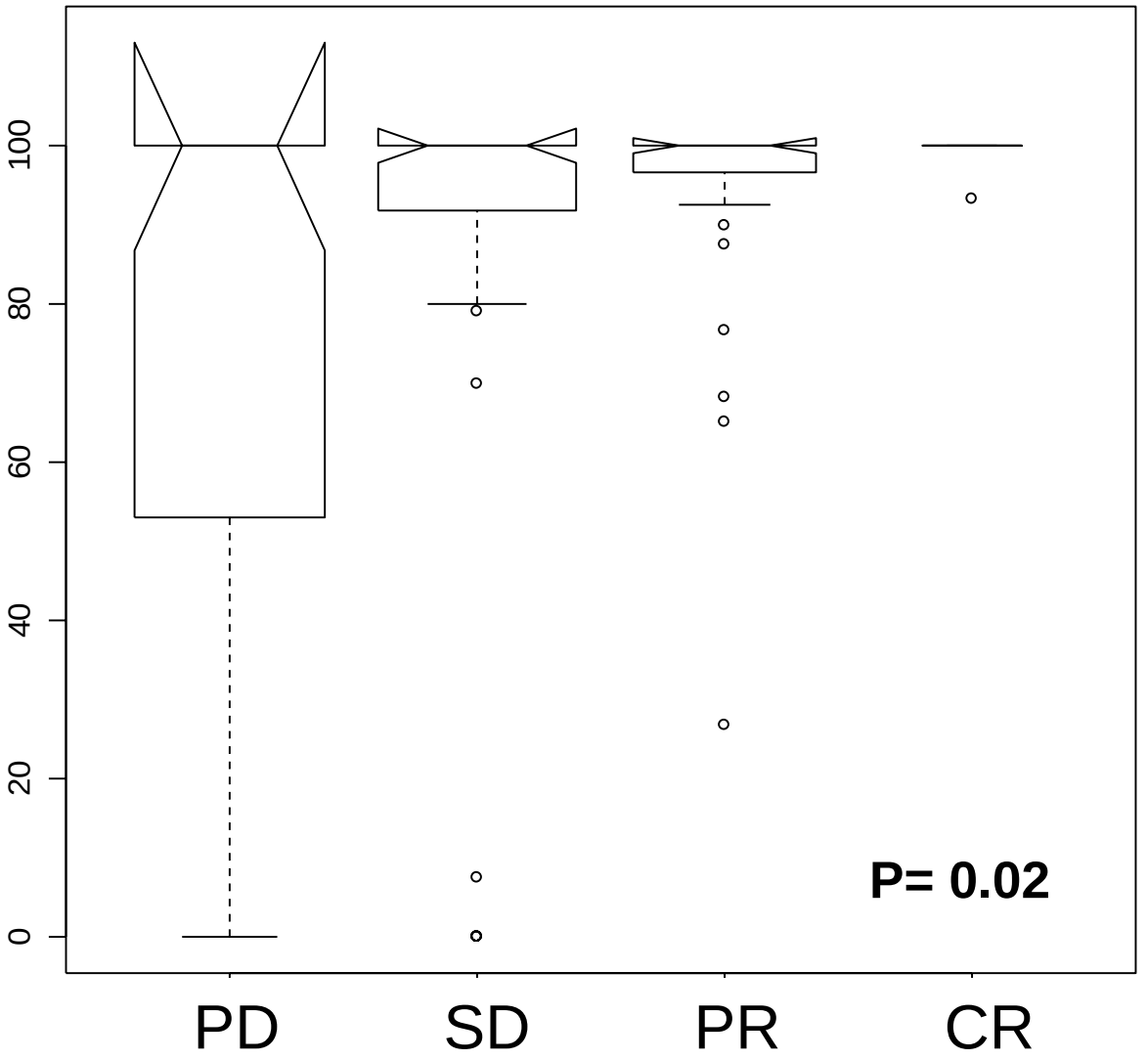
p53

Ki67

PTEN

Vimentin

CAIX by Ordinal IMT Response



Tissue Array Analysis of Genes and IL-2 Response

Univariate Predictors

CAIX > 85%	p = 0.027
EpCAM > 5%	p = 0.037
P53	p = 0.623
PTEN	p = 0.012
Vimentin	p = 0.301
Gelsolin	p = 0.794

Multivariate Predictors

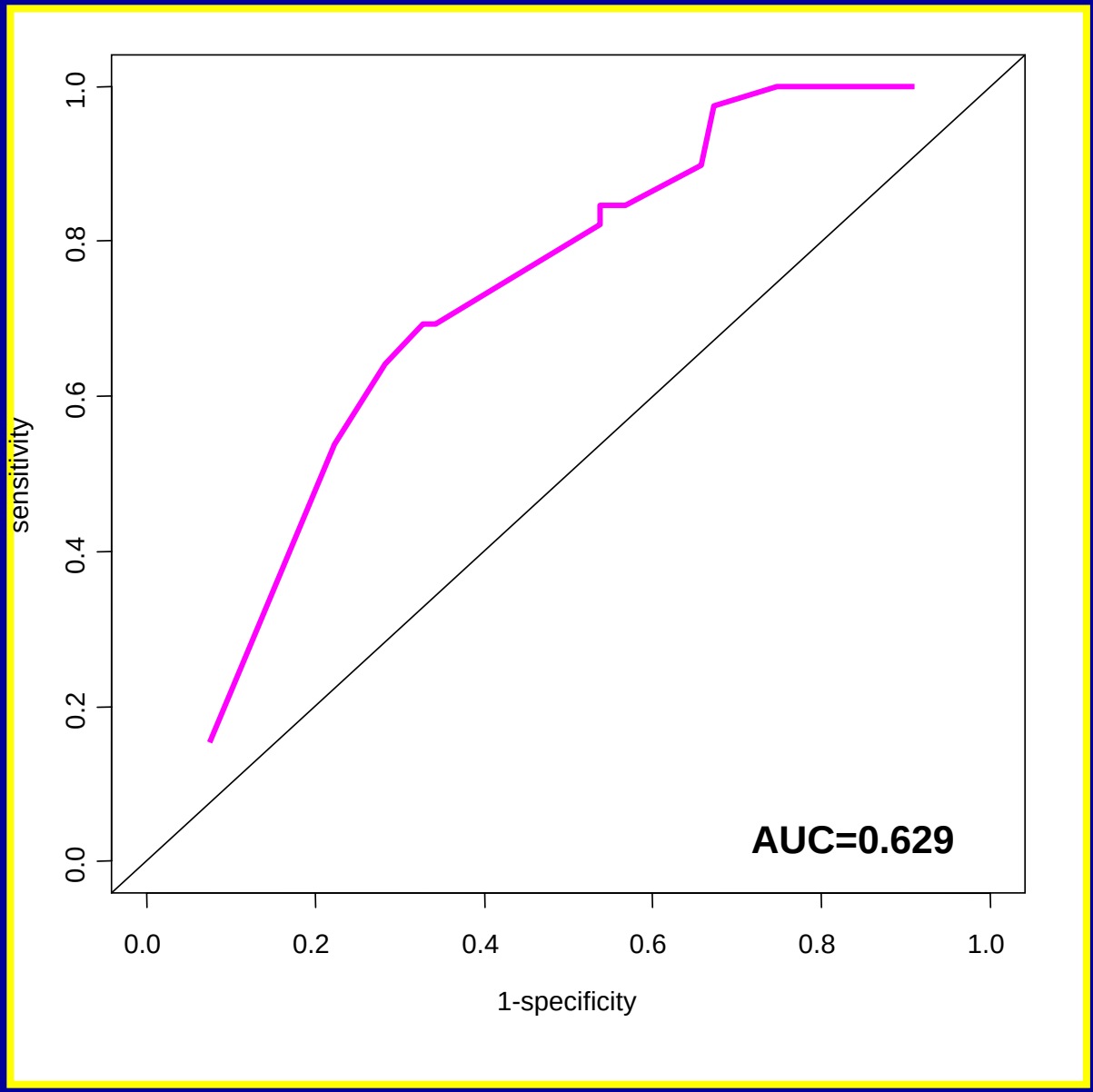
	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.0544731	0.2146256	0.254	0.8002
CA9MemPos.mn	0.0037908	0.0015443	2.455	0.0158
EpDctPos.md	-0.0021168	0.0020954	-1.010	0.3149
p53Pos.md	-0.0009112	0.0049747	-0.183	0.8550
pTENMax.md	0.1316415	0.0680478	1.935	0.0519
VimMax.max	-0.0963226	0.0626230	-1.538	0.1272
GeMax.mn	0.0045761	0.0742013	0.062	0.9509

Tissue Array Analysis of Genes, Clinical Variables, and IL-2 Response

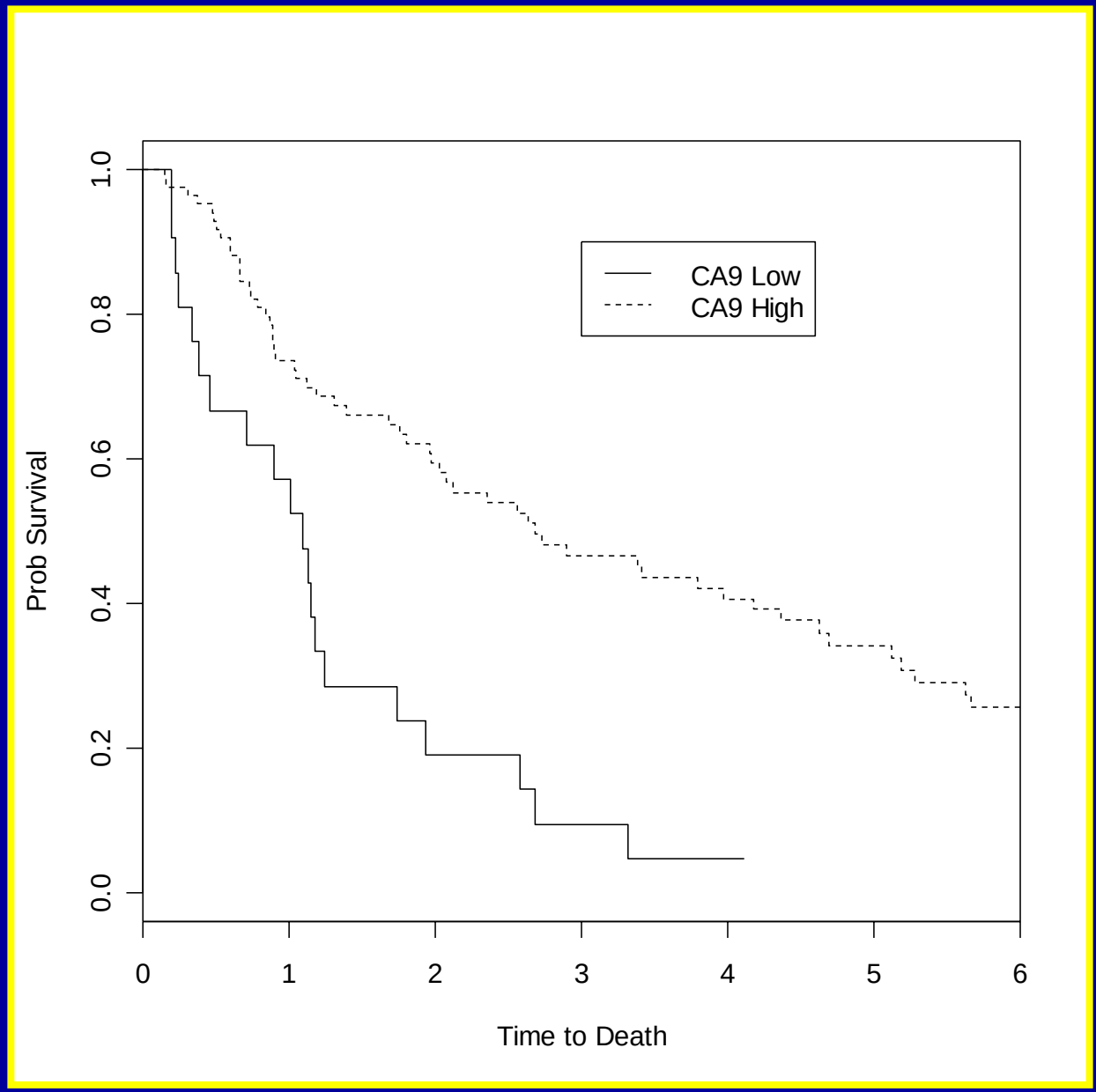
Multivariable Analysis

	Estimate	Std. Error	t value	Pr(>|t|)	
CA9MemPos.mn	0.005649	0.001695	3.333	0.00146	**
EpDctPos.md	-0.003337	0.002053	-1.625	0.10931	
p53Pos.md	-0.002777	0.004600	-0.604	0.54833	
pTENMax.md	0.140579	0.074246	1.893	0.06305	
VimMax.max	-0.119671	0.066276	-1.806	0.07591	
GeMax.mn	0.034811	0.083754	0.416	0.67914	
Male	0.301309	0.115154	2.617	0.01118	*
ECOG	-0.244610	0.099794	-2.451	0.01712	*
nstage	-0.061457	0.067538	-0.910	0.36642	

Receiver Operating Curve: Clinical + Molecular Prediction Model

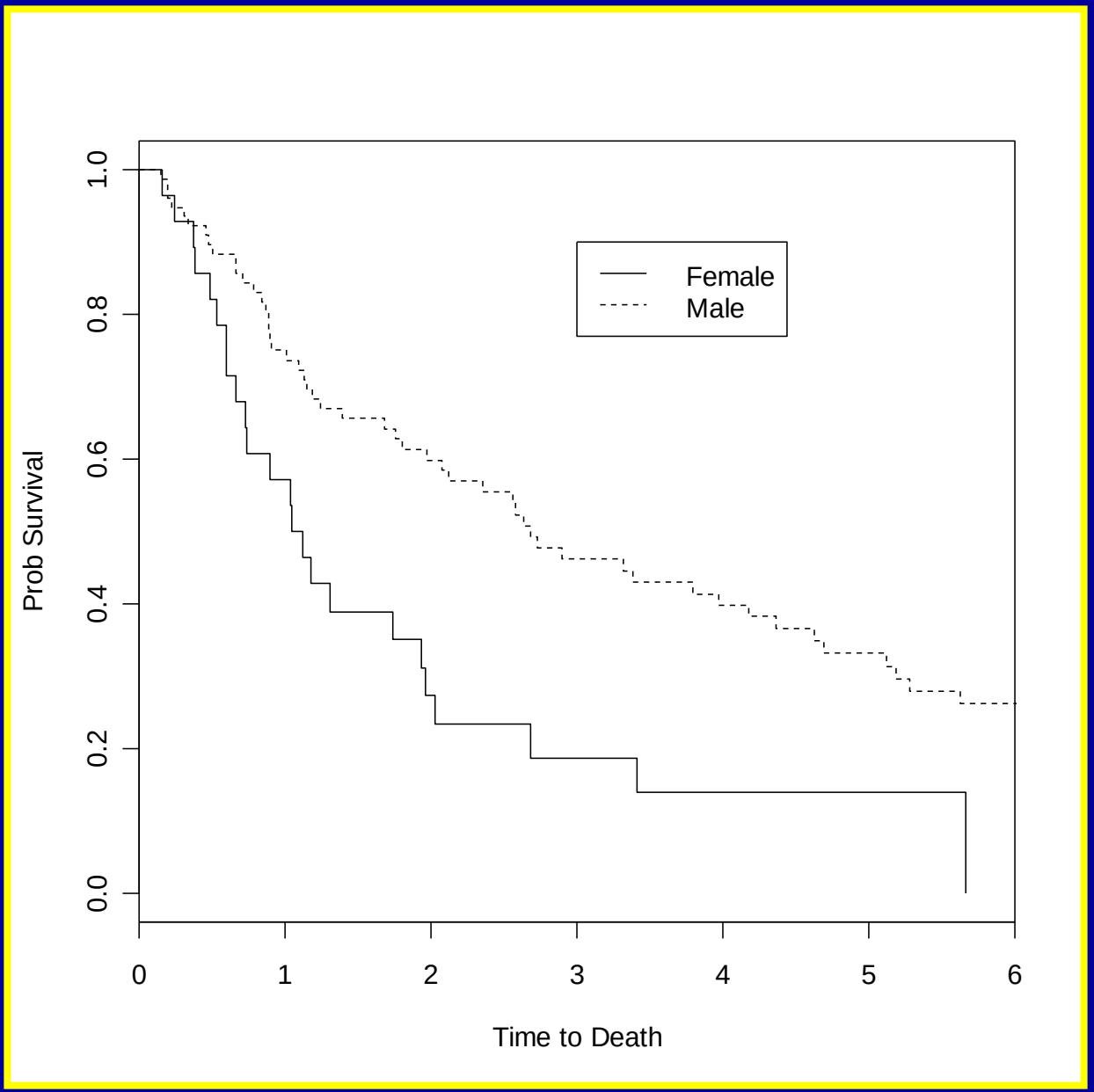


CAIX Expression and Survival in mRCC Treated by IL-2



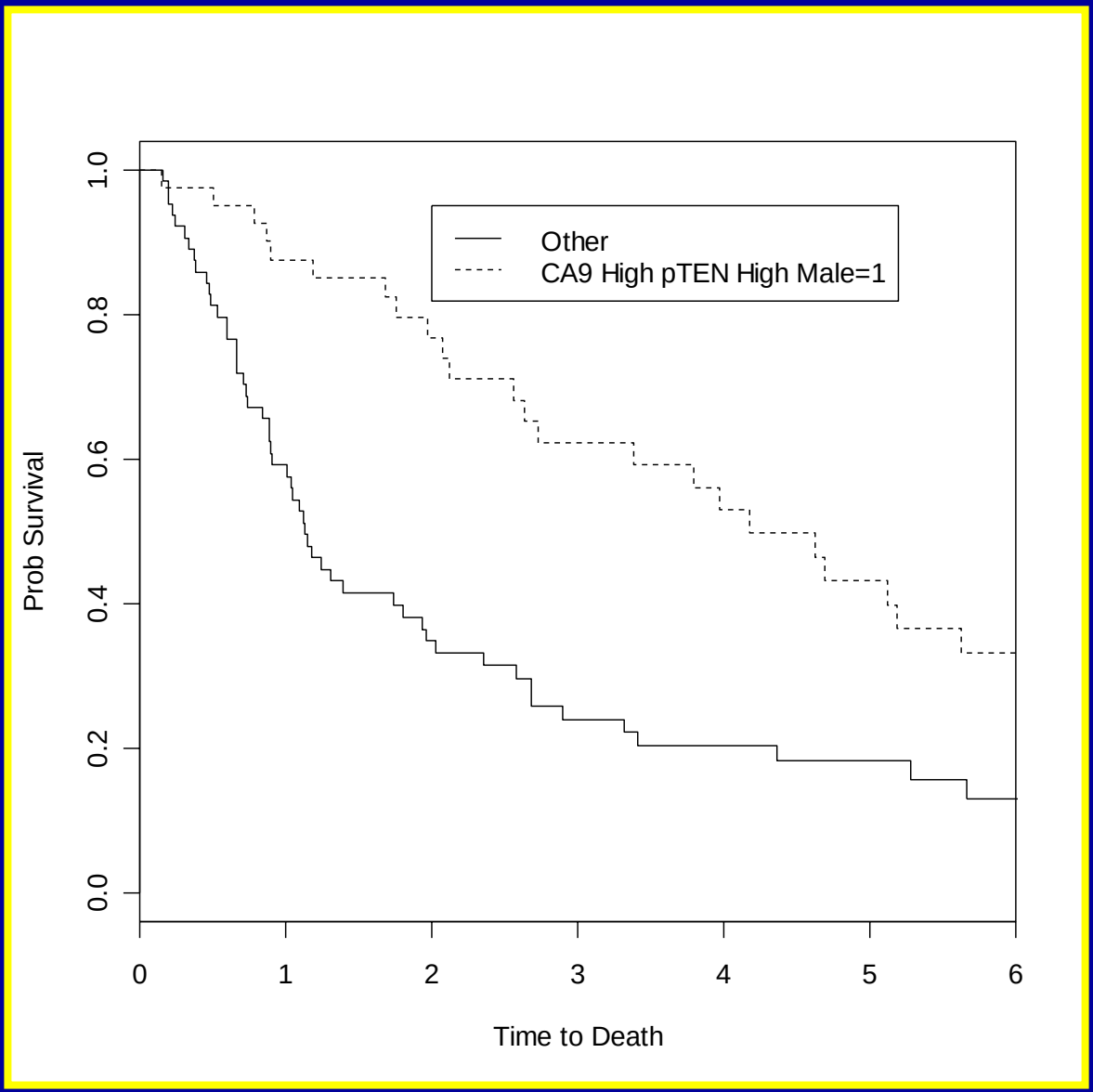
p= 2.4e-05

Gender and Survival in mRCC Treated by IL-2



p= 0.00159

CAIX, PTEN, Gender and Survival in mRCC Treated by IL-2



p= 0.000245

Conclusions

- Archived tumor tissue combined with a strong clinical data base: a powerful combination
- Proof of principle: gene expression analysis can identify relevant gene differences that translate into meaningful variables in an independent tissue array validation
- CAIX, PTEN, CXCR4, and other genes may play important roles in dictating IL-2 treatment response in RCC

Acknowledgements

Arie Belldegrun, MD
David Seligson, MD
Robert Strieter, MD
Karen Riedl, MD
Robert Figlin, MD

UCLA Biomathematics
Robert Elashoff, PhD
Zixing Fang, PhD
Steven Horvath, PhD
Xueli Liu, PhD

Funding: NIH K23CA095151-01