The Challenges of Tissue Based investigation in Prostate Cancer

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THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER
Making Cancer History®

The Challenge

- Tumor Volume
- Tumor heterogeneity
- Limitation of individual discovery platforms
- Pre -analytic determinates

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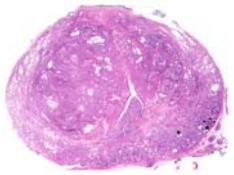
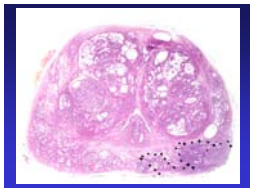
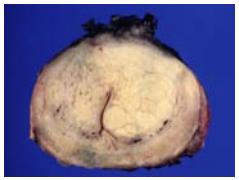
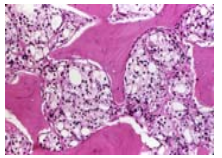
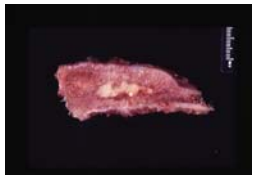
Tissue Resource & Pathology Core











LOCALIZED LOW RISK NEOADJUVANT	LOCALIZED LOW RISK	HIGH RISK NEOADJUVANT	SEROLOGIC RELAPSE	ANDROGEN-DEPENDENT METASTATIC	ANDROGEN INDEPENDENT	BONE METASTASES

Prospective Tissue Procuremen

Retrospective & Prospective Tissue Procurement

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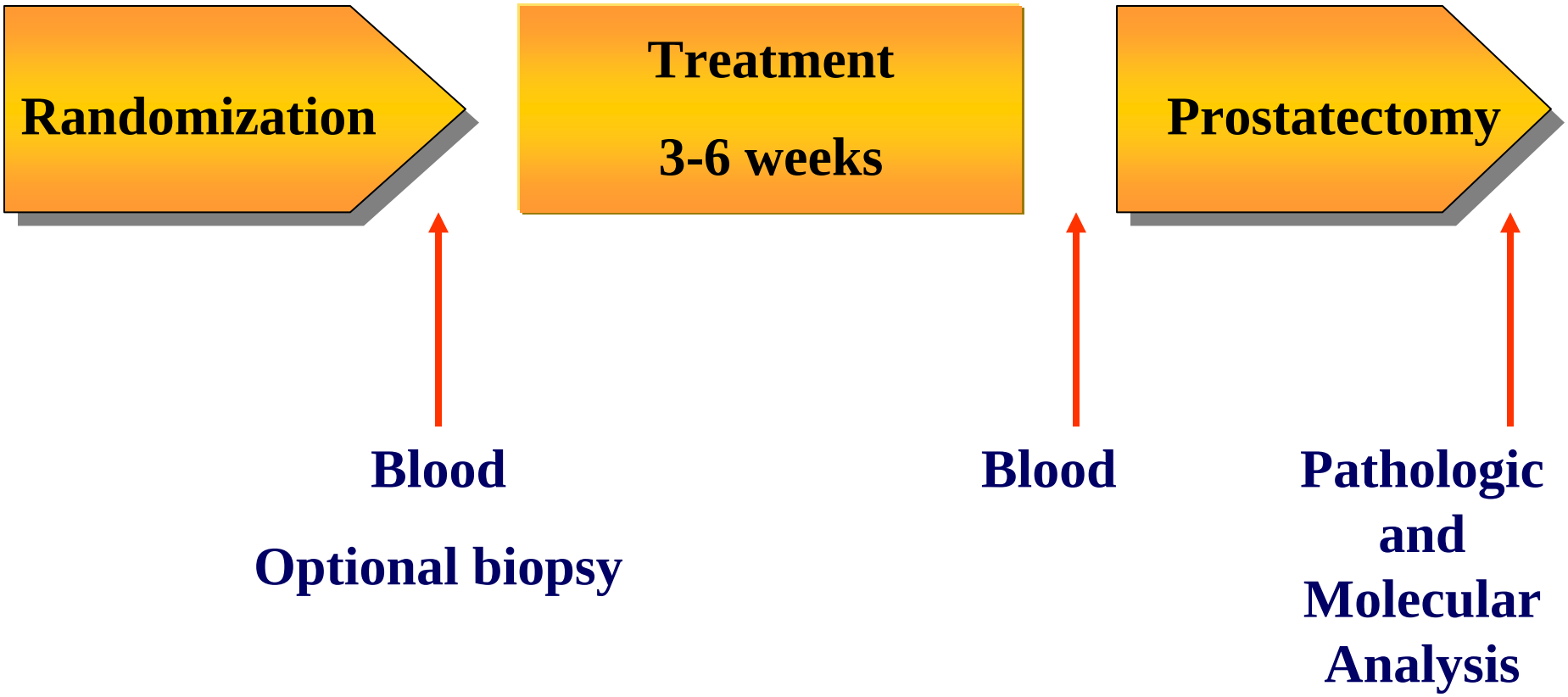
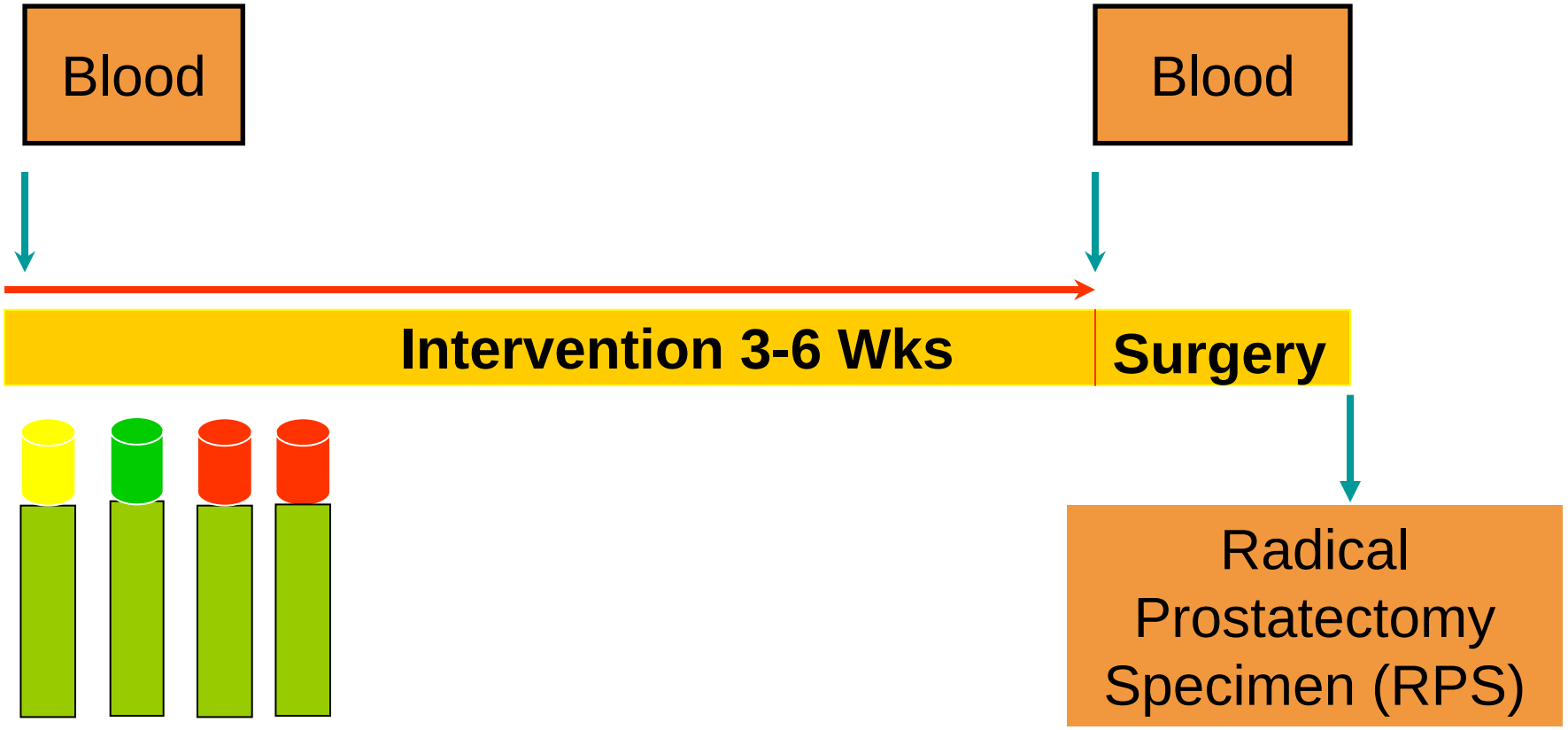
A Preoperative Selenium and Vitamin E Trial in Prostate Cancer

PSA < 10 ng/mL

- **GS </= 7**



Biomarker Analyses



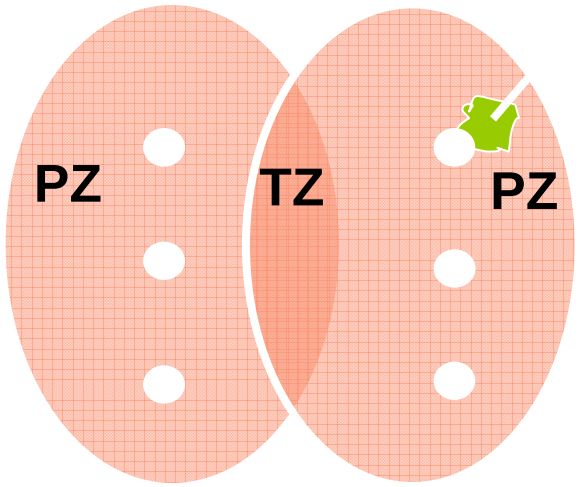
Archived: Serum, Plasma, Lymphocytes

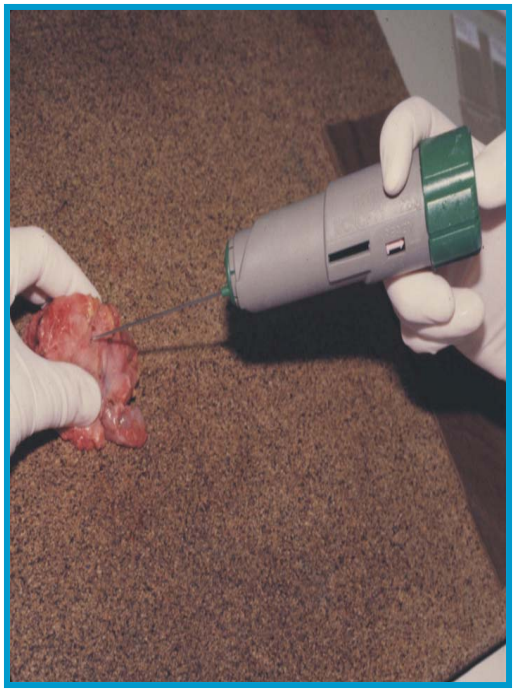
RPS Sextant Biopsy **(X 5)**

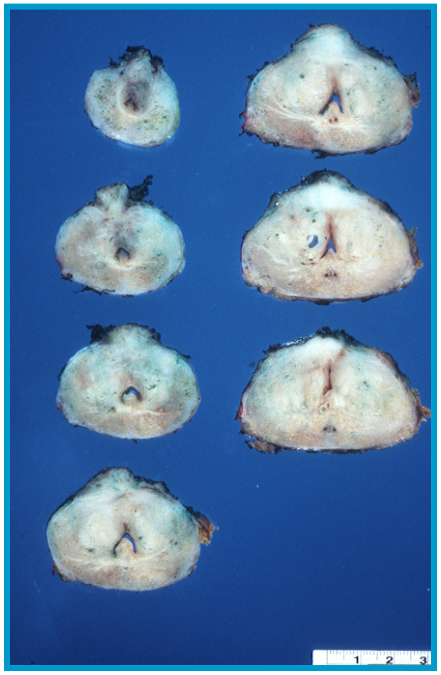
Kim J, 2008

Biomarker Expression: Core Biopsies ⟷ RPS Sections

Diagnostic Biopsies ⟷ **RPS Biopsies** ⟷ **RPS Sections**



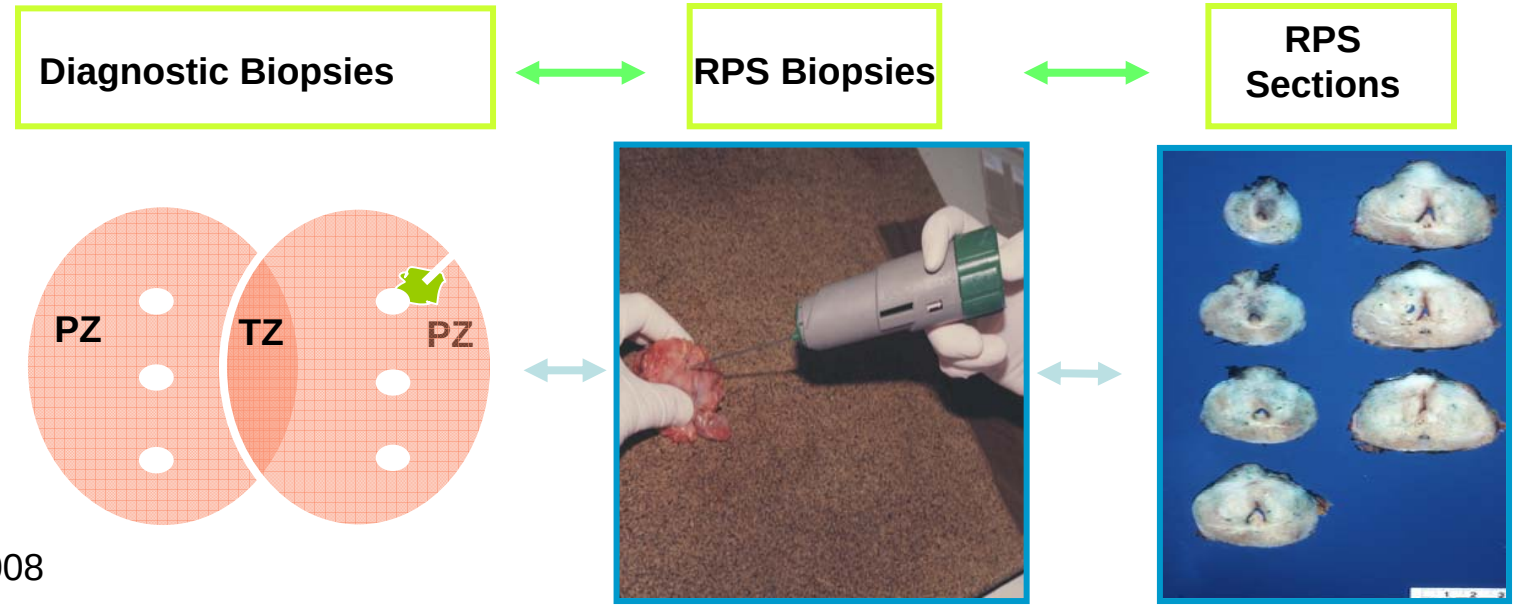




Kim J, 2008

Ex Vivo RPS Biopsy

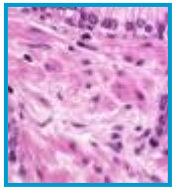
Positive Biopsy	**22/35 RPS (27 cores)**
Tumor focus	
Dominant	**19/27**
Ca #2	**4/27**
Other	**4/27**



Kim J, 2008

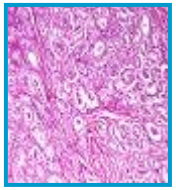
Gene Expression Profiling

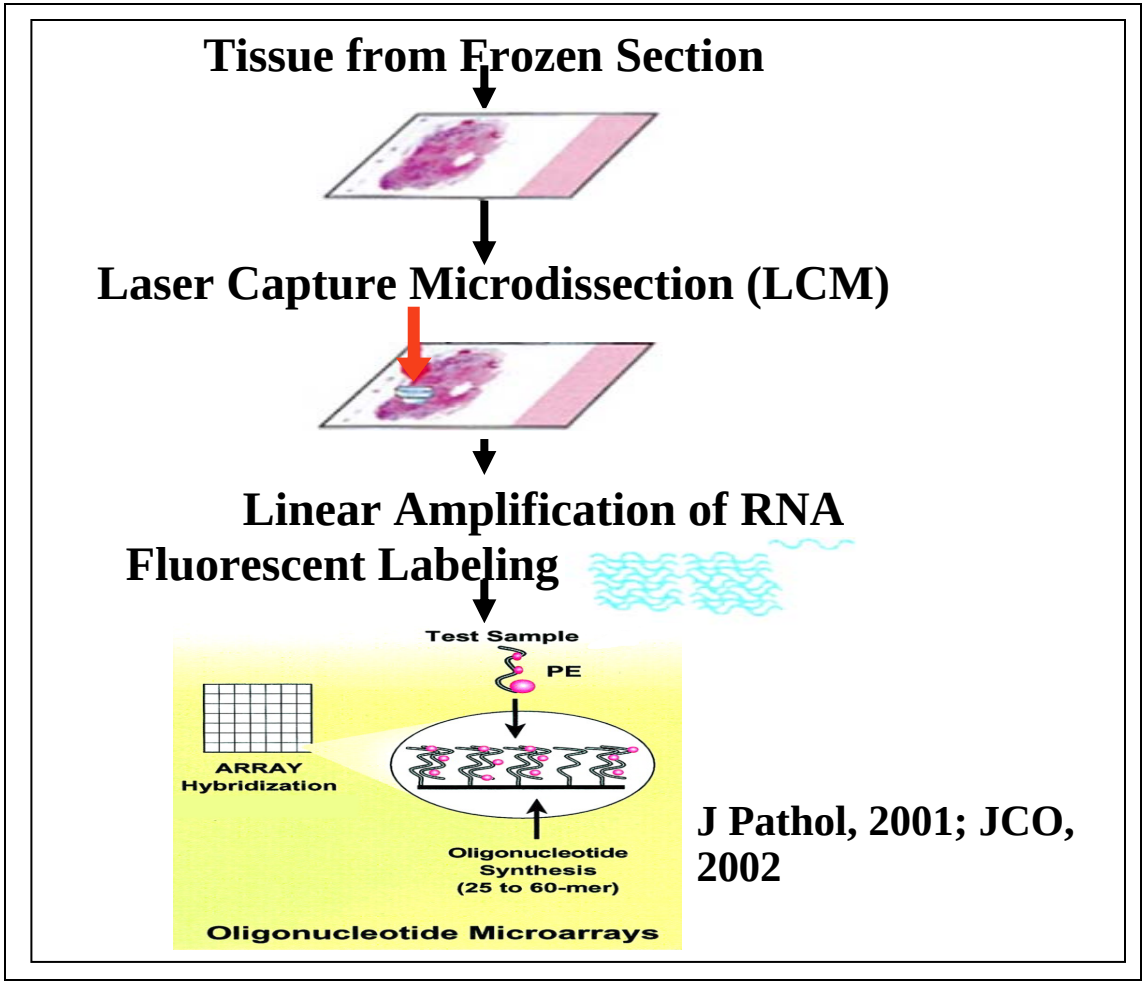
Stromal Cells



Normal Cells

Tumor Cells





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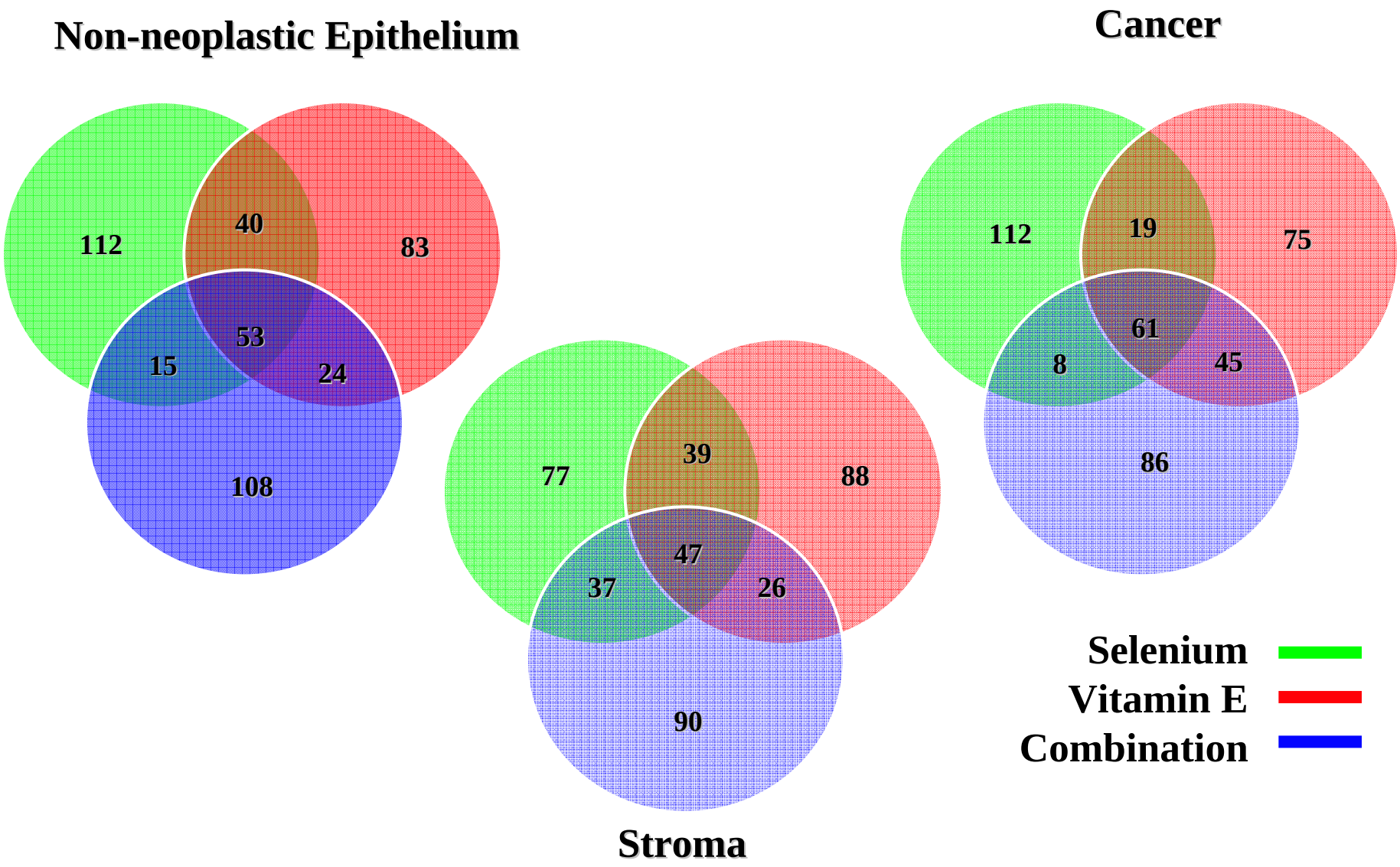
The Prostate is An Organ Composed of different Tissues and Cells

Assumption: the application of therapy will result in tissue, or cell-type specific alterations in gene expression

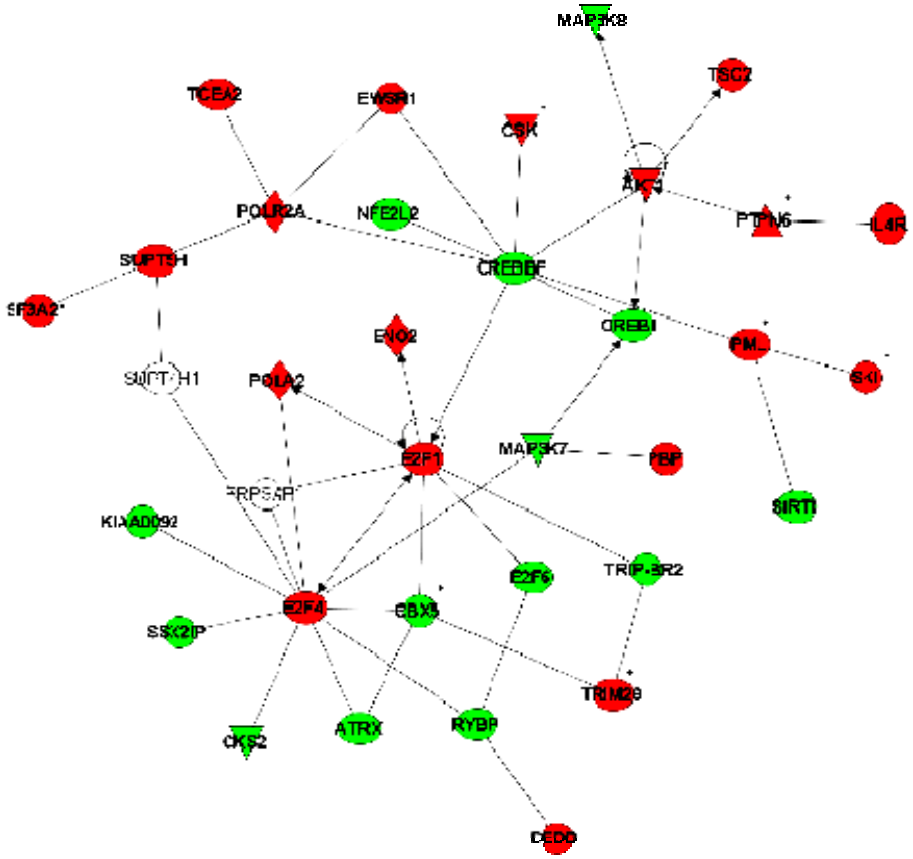
Cell-Type and Treatment Specific Effects in Gene Expression Profile



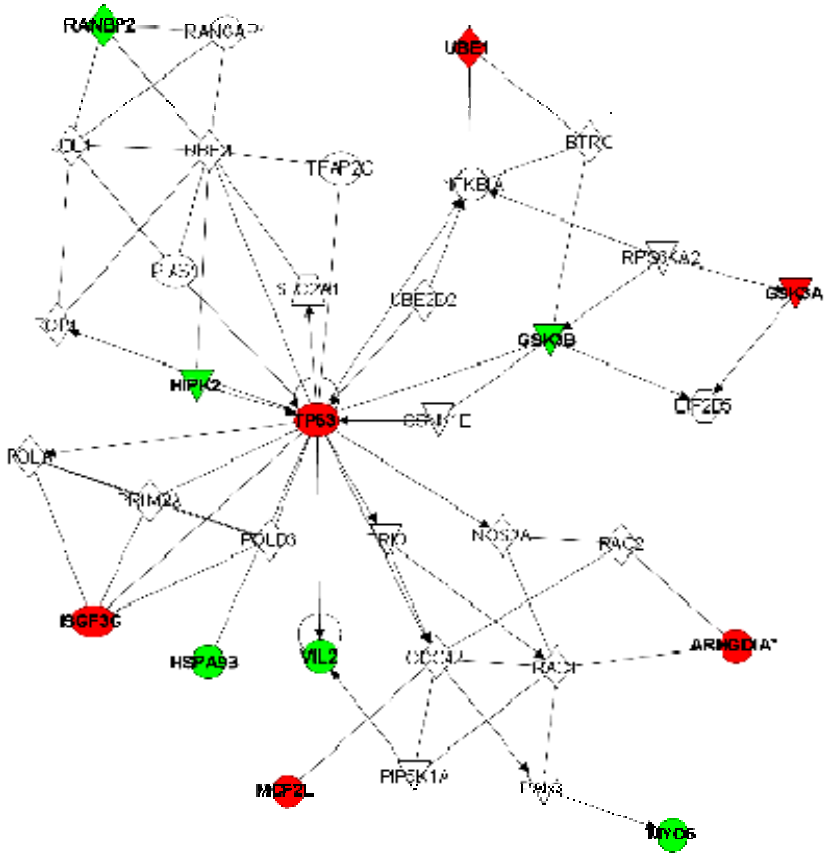
Interacting molecular pathways regulate Prostate Cancer growth

Assumption: signaling networks are modulated in a cell specific manner

Response of Stromal Cells and Cancer Cells Differs: Network Analysis to Identify Hubs

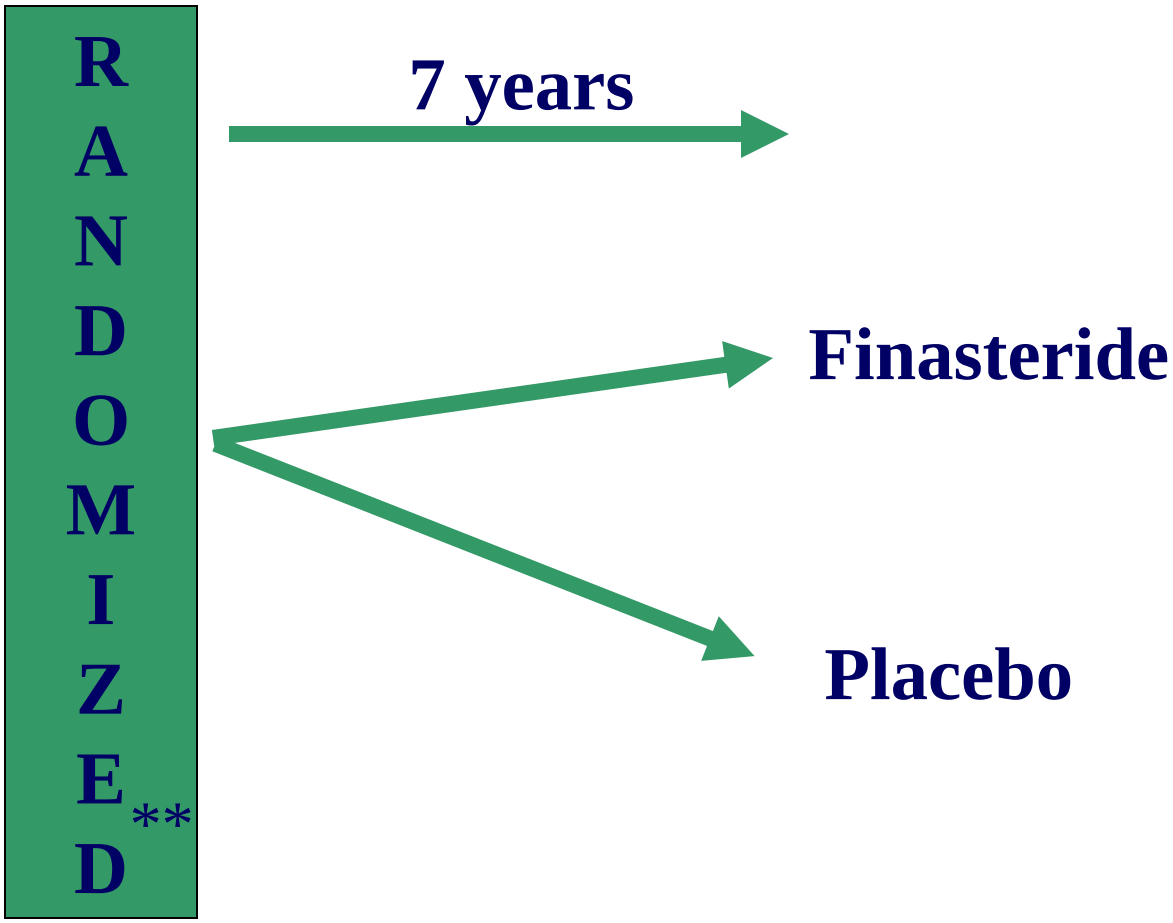


Stromal Cells



Cancer Cells

Prostate Cancer Prevention Trial



** Dynamic allocation

Pathways to Androgen Independence

Androgen-Dependent Prostate Cancer

↓

Hormone Ablation Therapy

↓

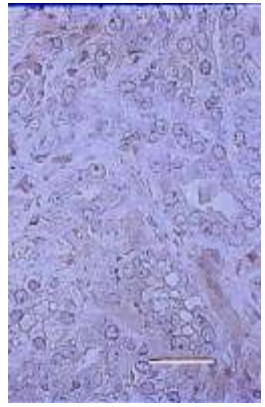
Adaptation

↓

Acquisition of Complimentary Genetic Lesions

↓

Clonal Expansion



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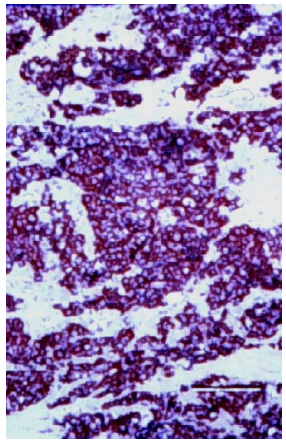

Conclusion

Limitations of small volume prostate cancer can be reduced by using immediate ex-vivo biopsy strategy

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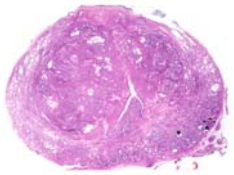

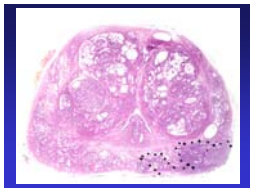

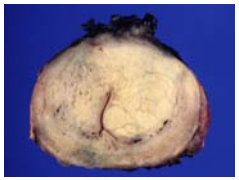

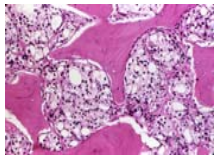

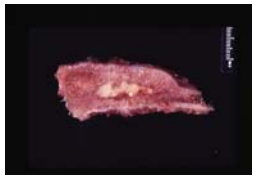

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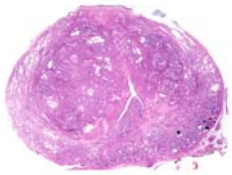

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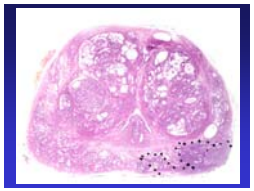

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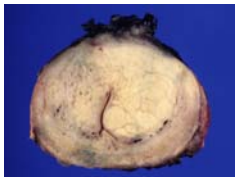
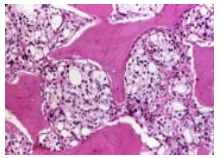
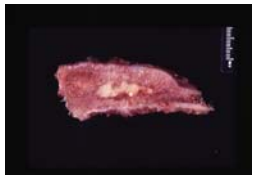


HIGH RISK NEOADJUVANT

SEROLOGIC RELAPSE



ANDROGEN-DEPENDENT METASTATIC



ANDROGEN INDEPENDENT

BONE METASTASES

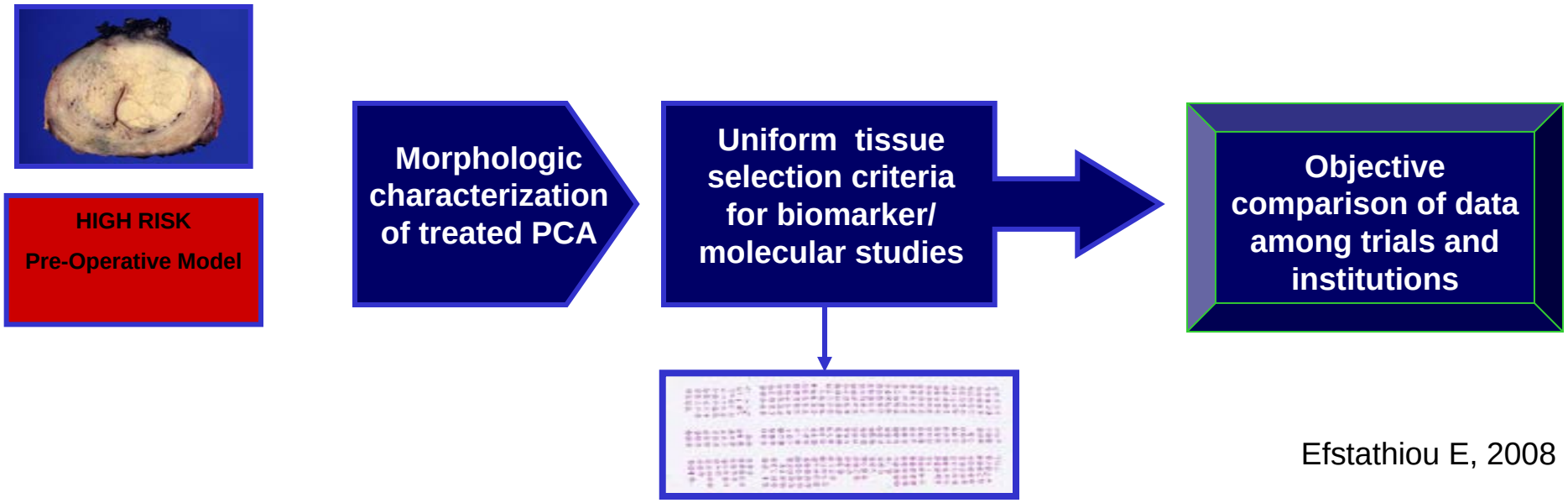
Prospective Tissue Procuremen

Retrospective & Prospective Tissue Procurement

Pre-Operative Model

Standardization of Morphologic Characteristics

- Allows for objective comparison of data
- Leads to uniform tissue selection criteria for biomarker / molecular studies

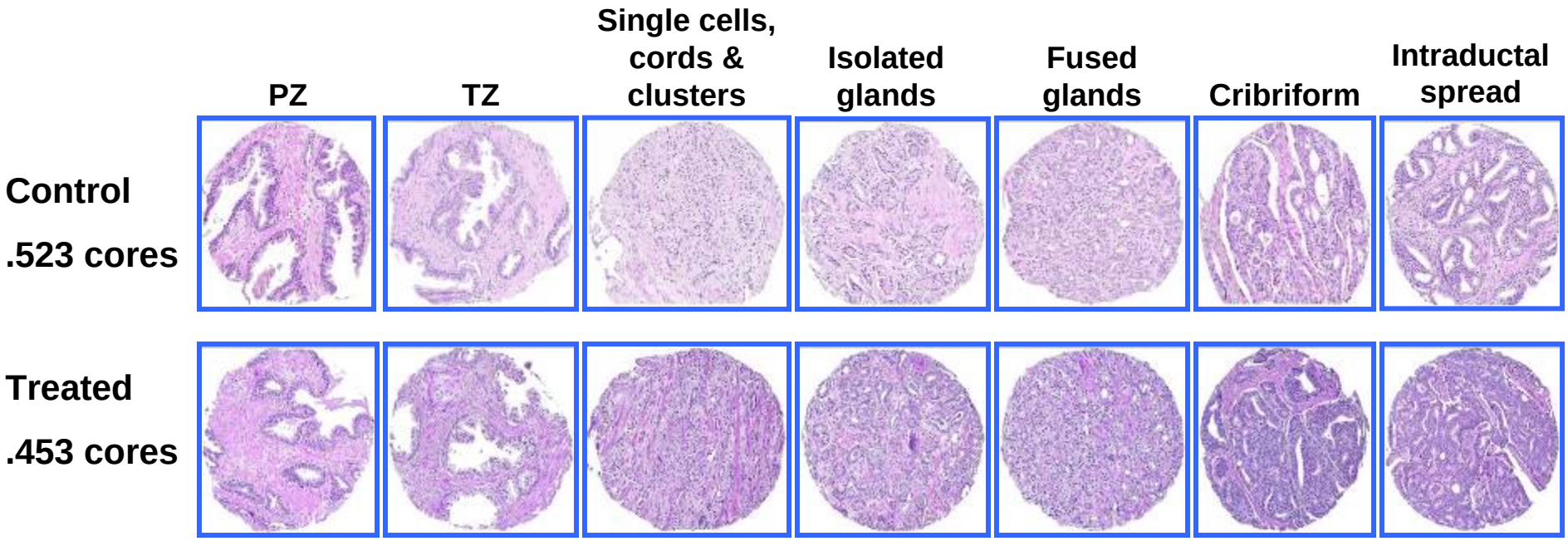


Efstathiou E, 2008

Tissue Microarray Construction

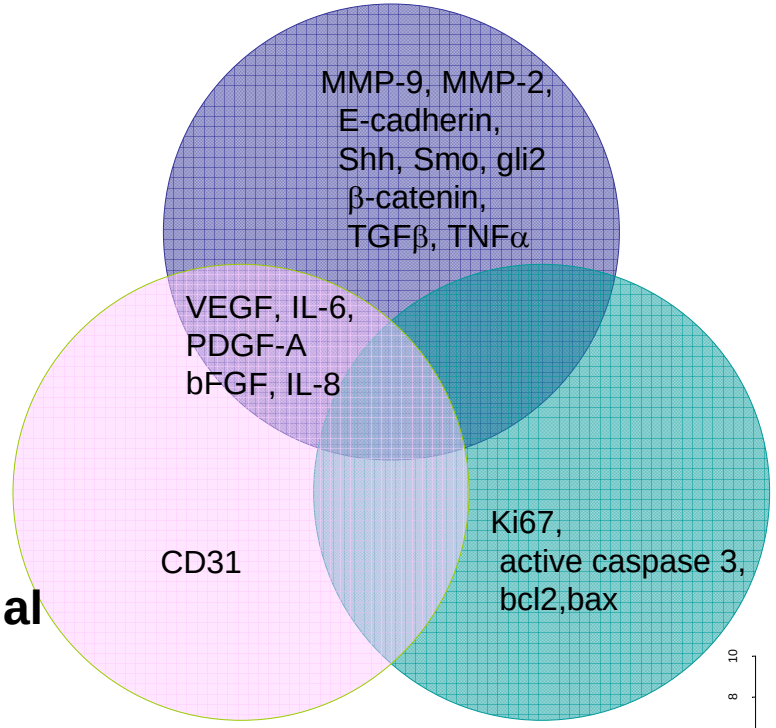
Pre-Operative Model

Thalidomide Trial



Efstathiou E, 2008

Effect on stromal-epithelial interaction

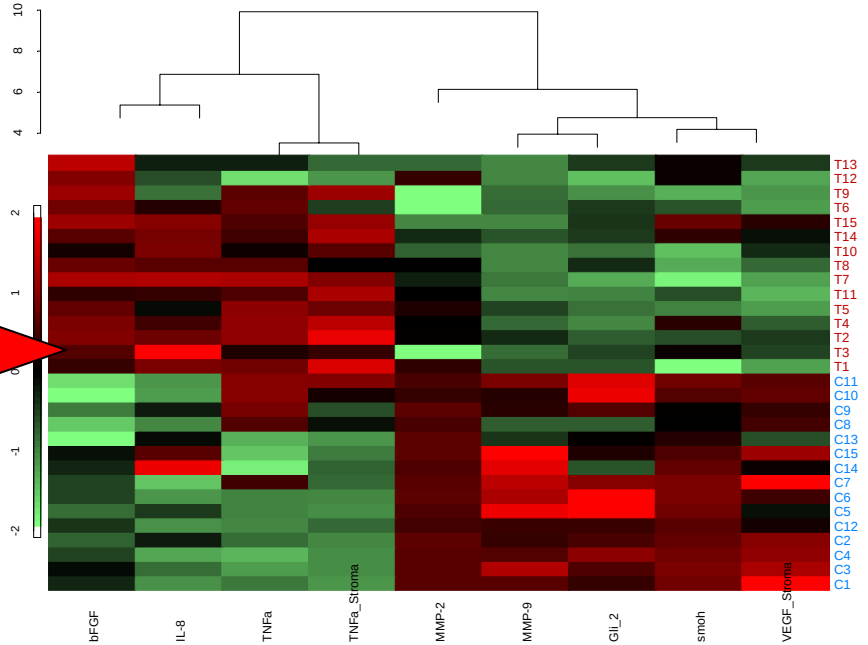


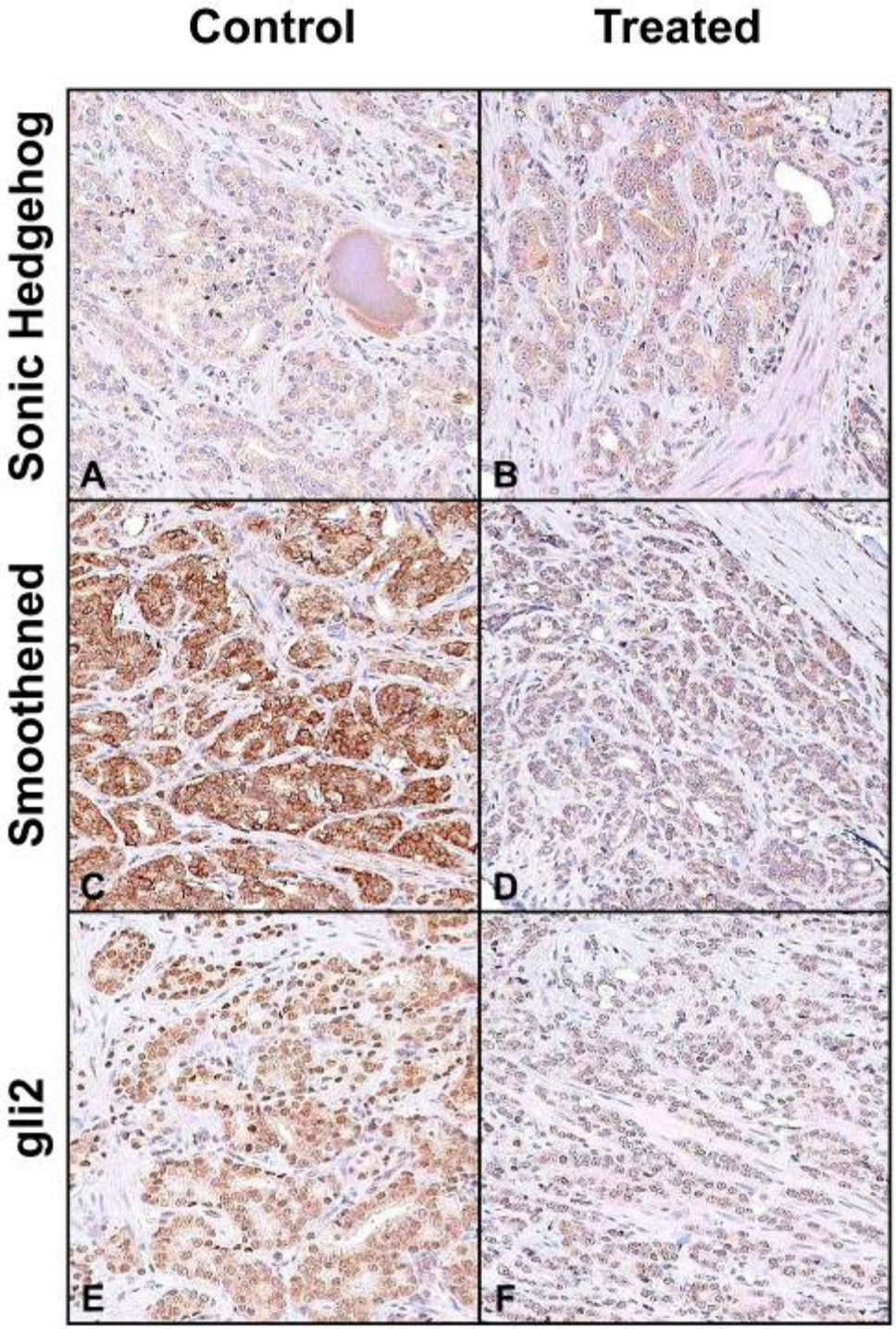
Effect on endothelial cells

25 Markers Interrogated both in epithelium and stroma (when applicable)

Effect on epithelial-cell proliferation and survival

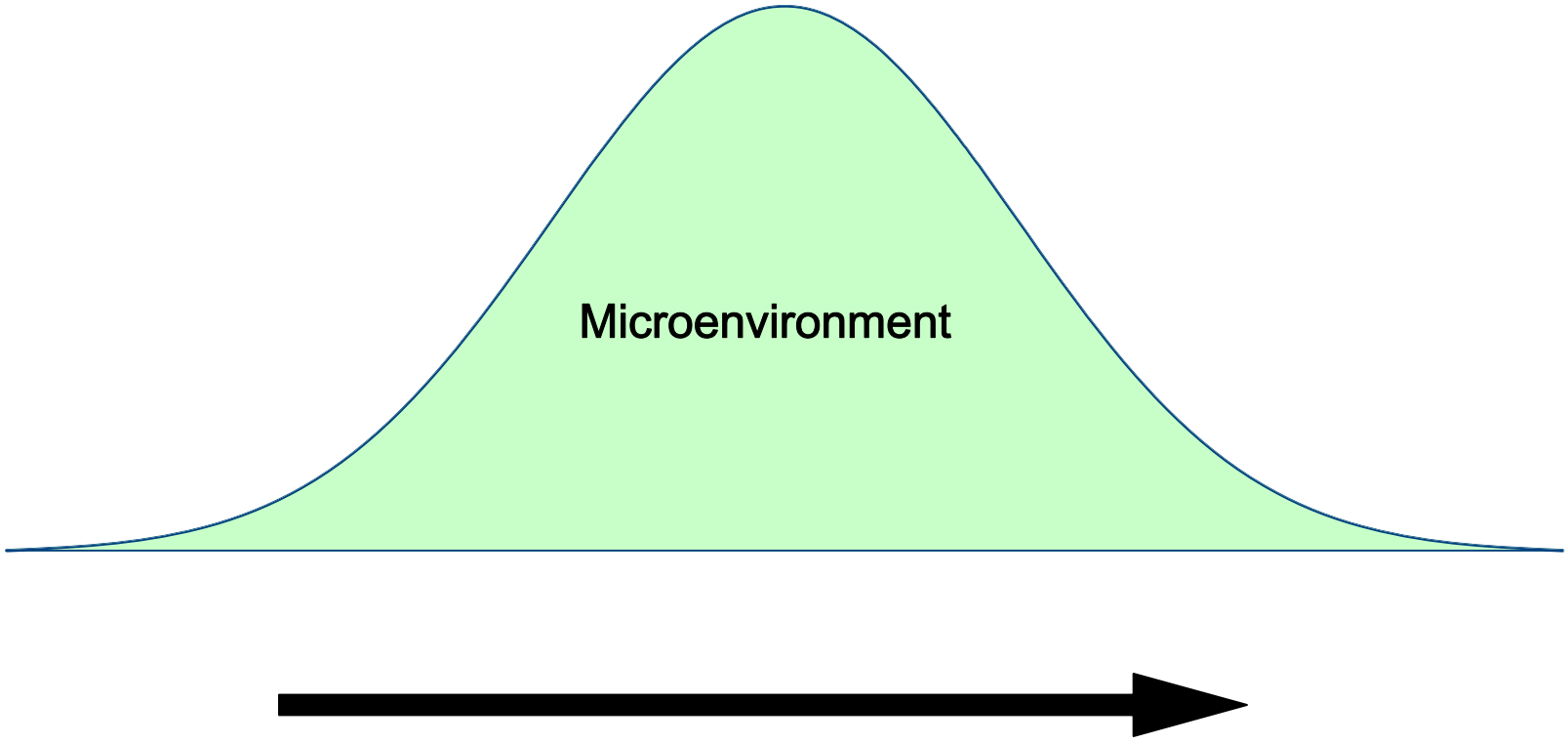
Significant Findings





Efstathiou E, 2007

Serial Modulation
Microenvironment & Neoplastic Epithelium



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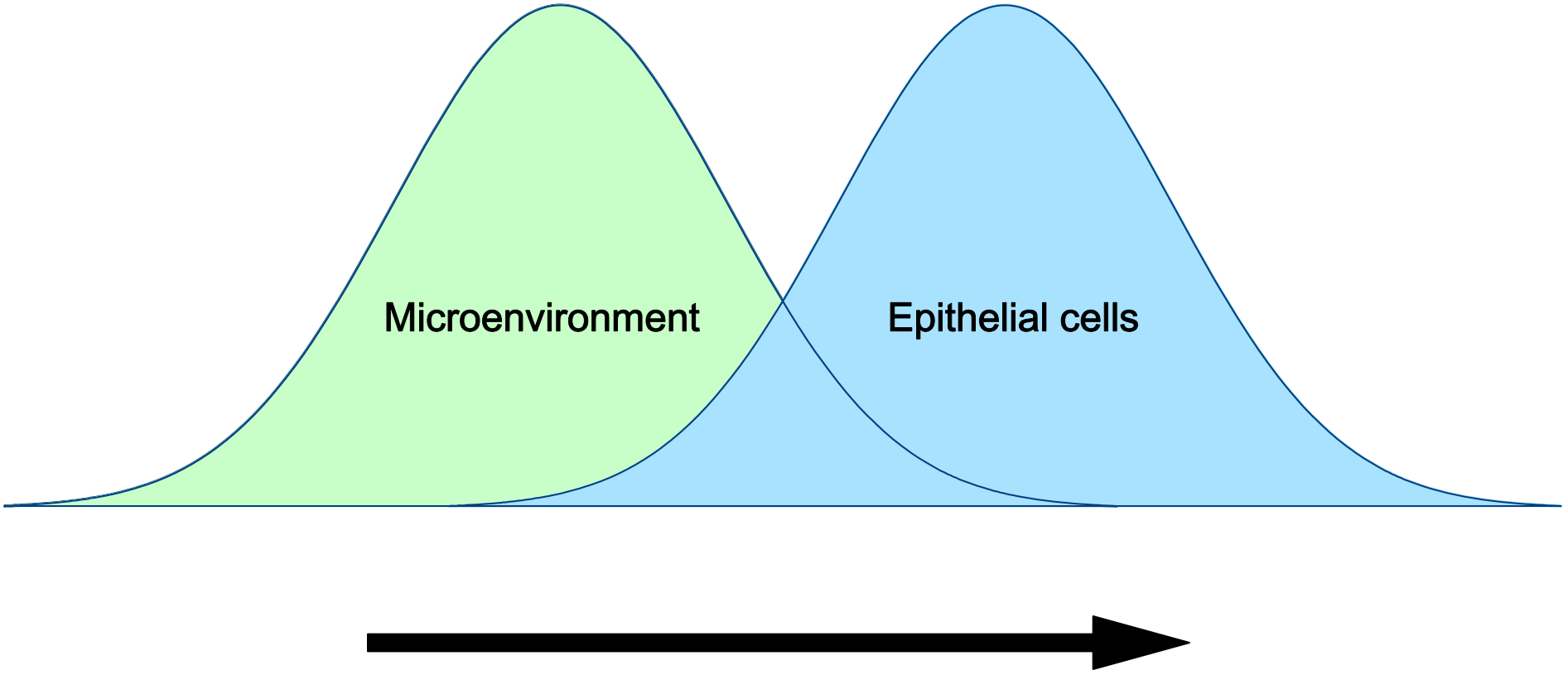

Efstathiou E, 2007

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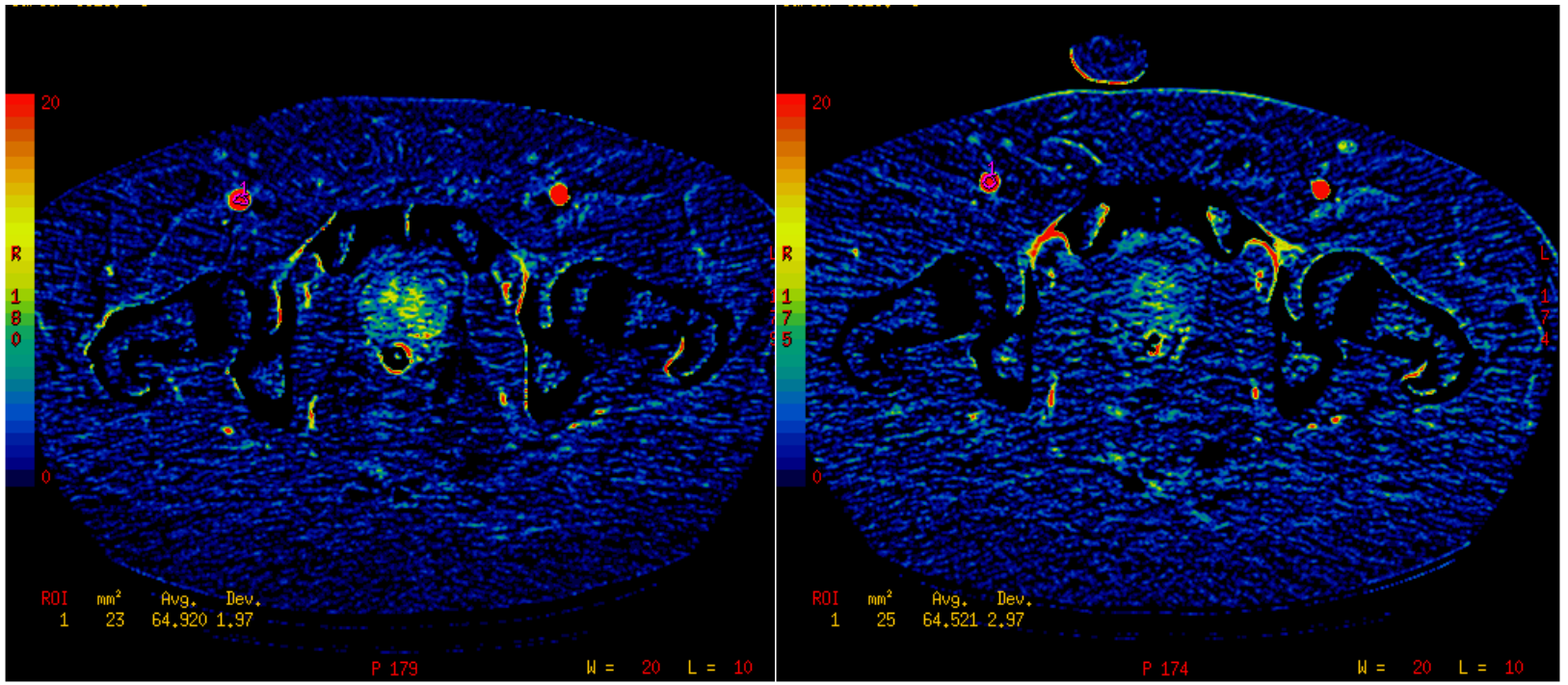
CT Perfusion Study
(confirmation with imaging)



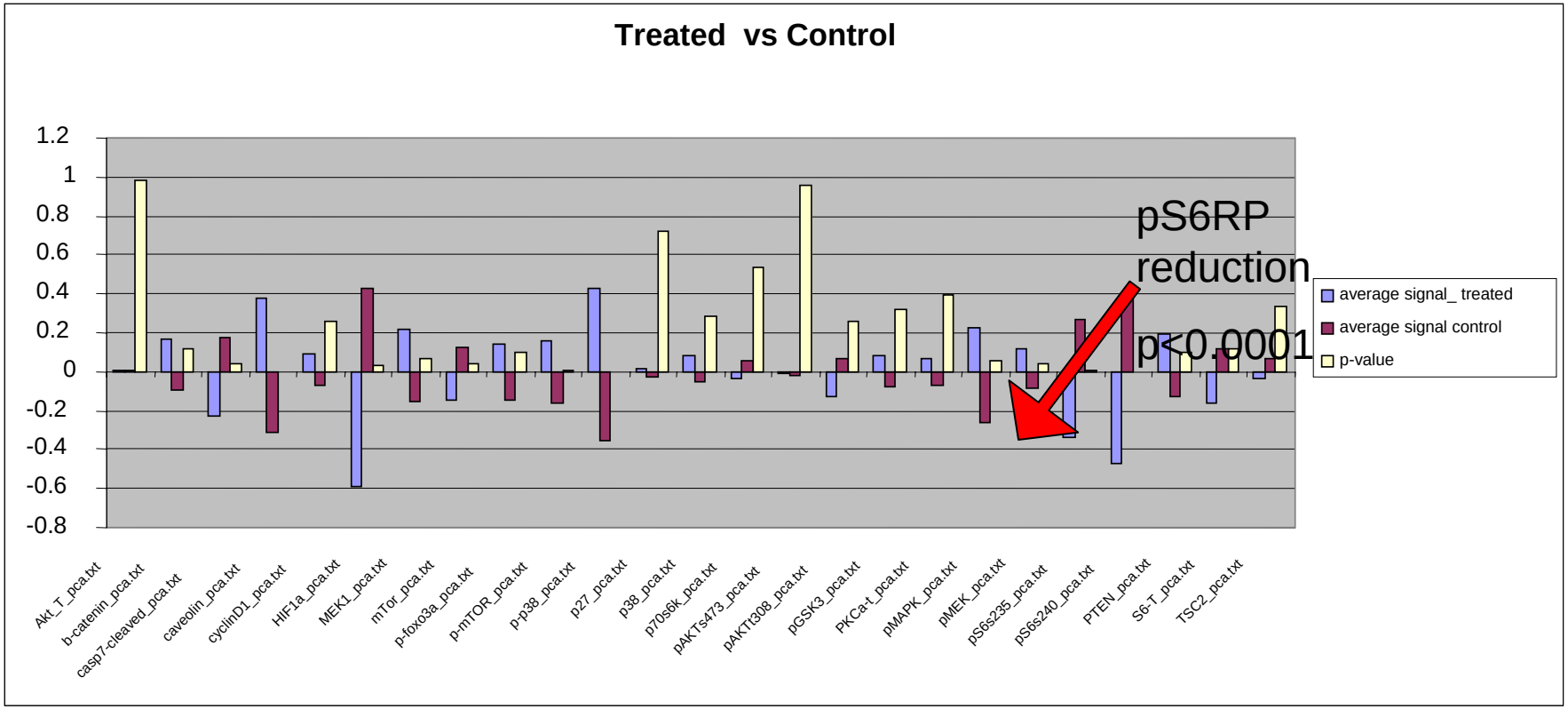
Pretreatment

Posttreatment

Efstathiou E, 2008

Integration of Reverse Phase & Tissue Microarray

(MTOR inhibition Prostate Cancer)



Efstathiou & Tsavachidou

Conclusions

- **Multiplatform confirmation can provide confidence in results**
- **Hypothesis testing more reliable than discovery**
- **Fixed genotoxic stress in “pre - operative model” may limit effects of heterogeneity and more efficiently inform**

The Challenge

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Robotic Assisted Prostatectomy

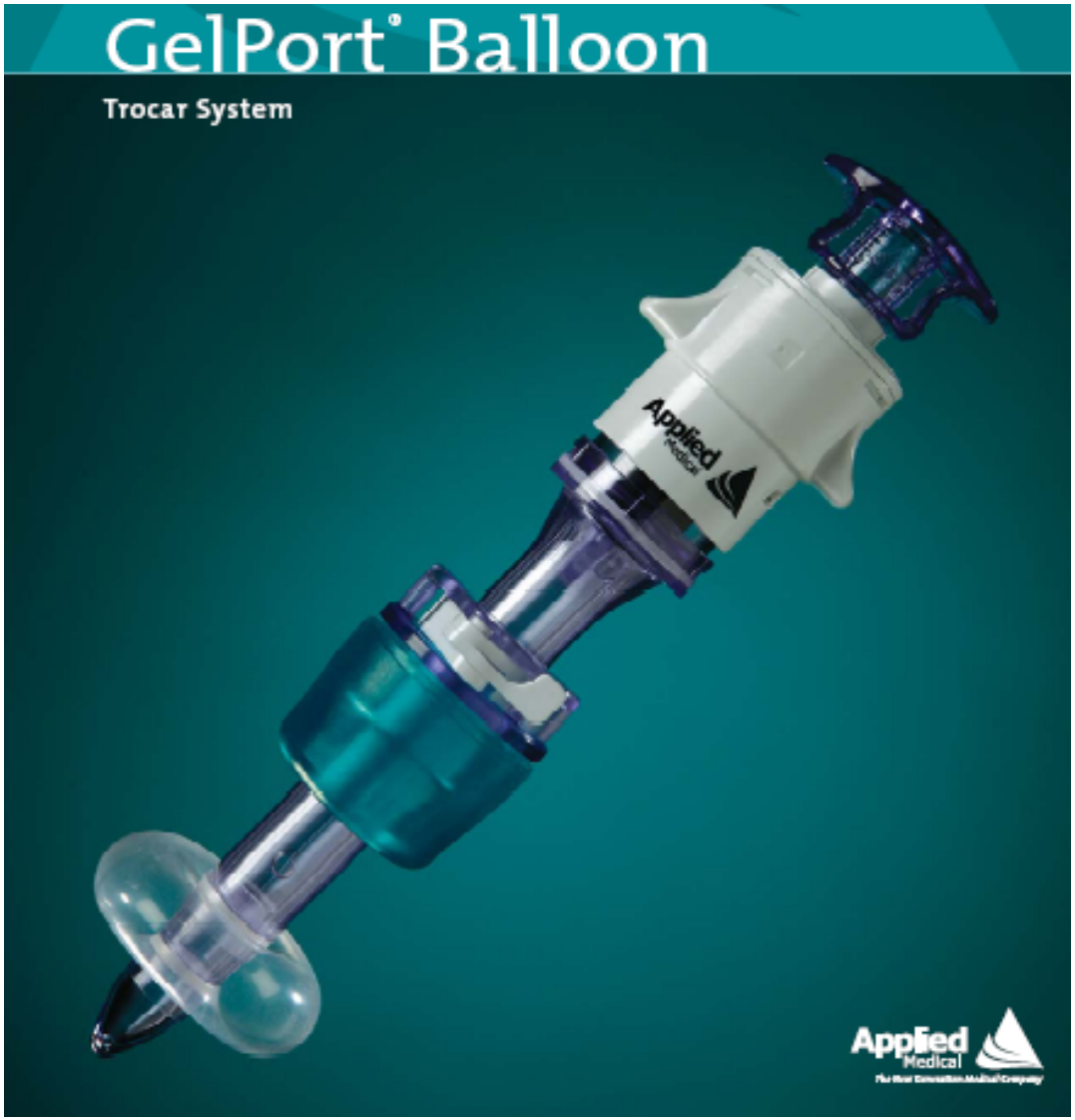
(11 discrete steps)

1.	Dissect SV/Vasa 20	
2.	Drop bladder	8
3.	Endopelvics	10
4.	DVC	10
5.	Anterior bladder 6	
6.	Posterior bladder 10	
7.	Pedicles	40
8.	Urethra	11
9.	PLND	17
10.	Posterior anastomosis	15
11.	Anterior anastomosis	14
	Totals	140

- **Pedicles are divided at the end of step 7,**
- **average warm ischemia time 57 min!**
- **if trainees involved :warm ischemia increase by 60!**

Davis J, 2008

Potential solution: immediate extraction/reconstruction with a balloon port



Davis J, 2008

Immediate Extraction

- **For patients on a study with molecular endpoints**
- **Last pedicle saved to the end, near urethra**
- **Specimen placed in a bag, incision enlarged for removal—to ice**
- **Suture reconstruction to size of port—balloon completes the pneumoperitoneum seal**
- **Surgeon completes the case**
- **Average pedicle division to ice time in the 5-10 minute range**

Davis J, 2008

Conclusions

- **Robotic surgery is here!**
- **Modification needed to meet challenge of delayed “extraction”**
- **Unique opportunity to reduce heterogeneity of surgically induced pre-analytic determinates by codifying surgical behavior**

Acknowledgments

- National Cancer Institute
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- Koch Center for Applied Science of Genitourinary Cancer
- Prostate Cancer Foundation
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- M.D. Anderson Prostate Cancer SPORE

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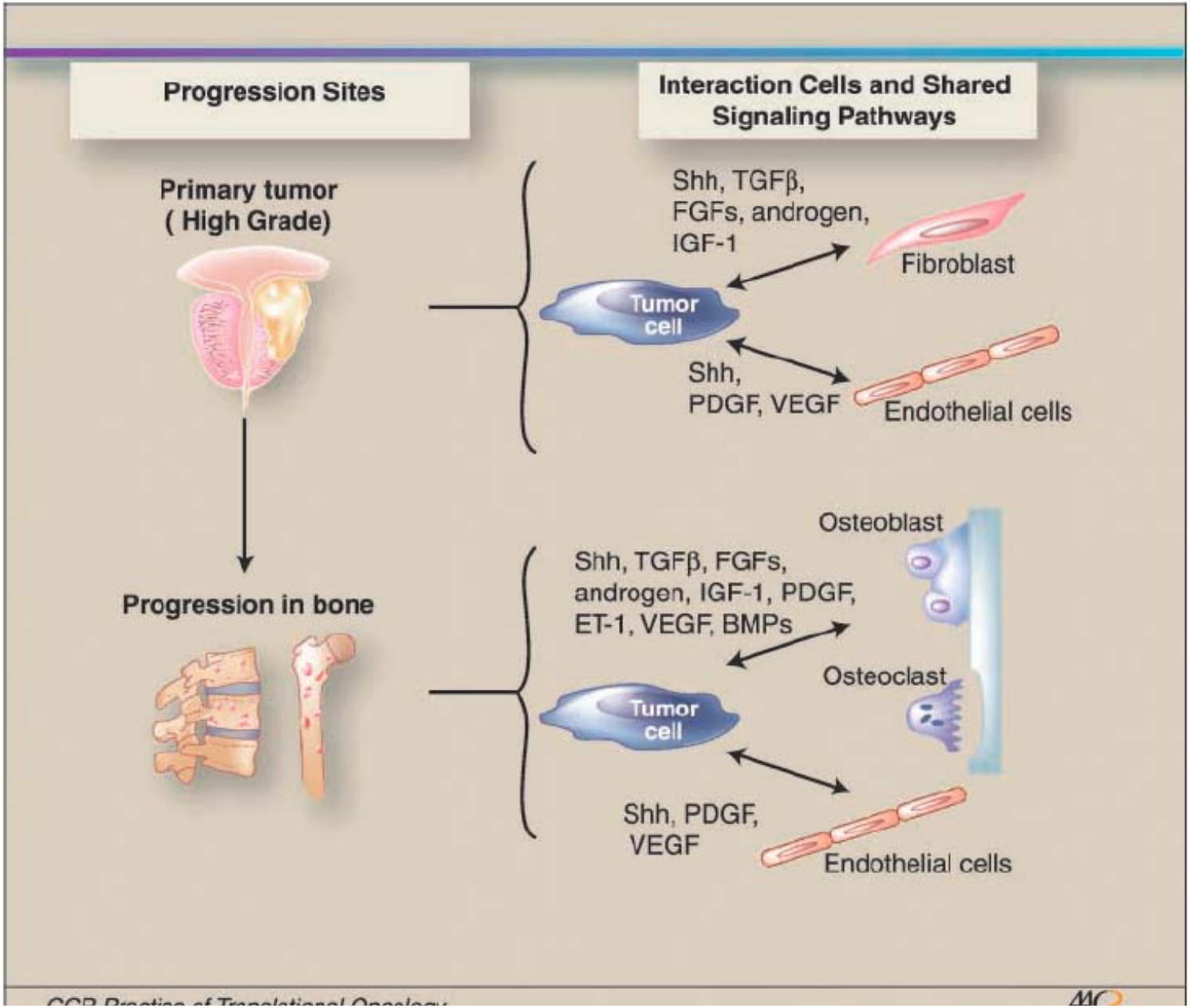
  Nora Navone, MD PhD
- Molecular Pathology:
  Sue Hwa Lin, PhD
- Systems Biology:
  Gordon Mills, MD PhD
  Dimitra Tsavachidou, MD PhD
- Pathology: Patricia Troncoso, MD

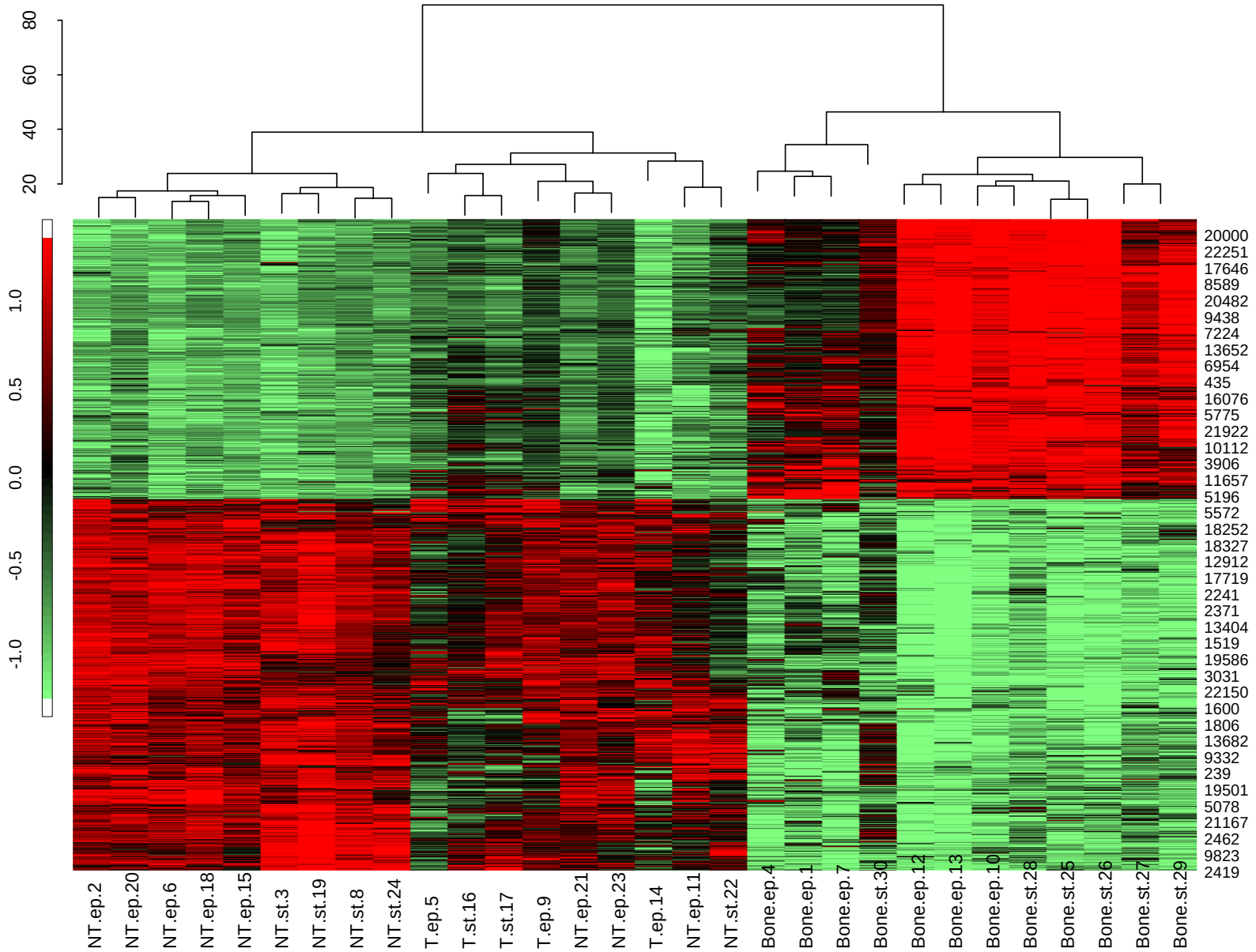
Testing Title Design

Testing text design

Shared Signaling Pathways Implicated in the Tumor Microenvironment of High Risk Prostate Cancer & Bone.



Top 1077 genes (bone vs others, FDR=.0001) by mixed model



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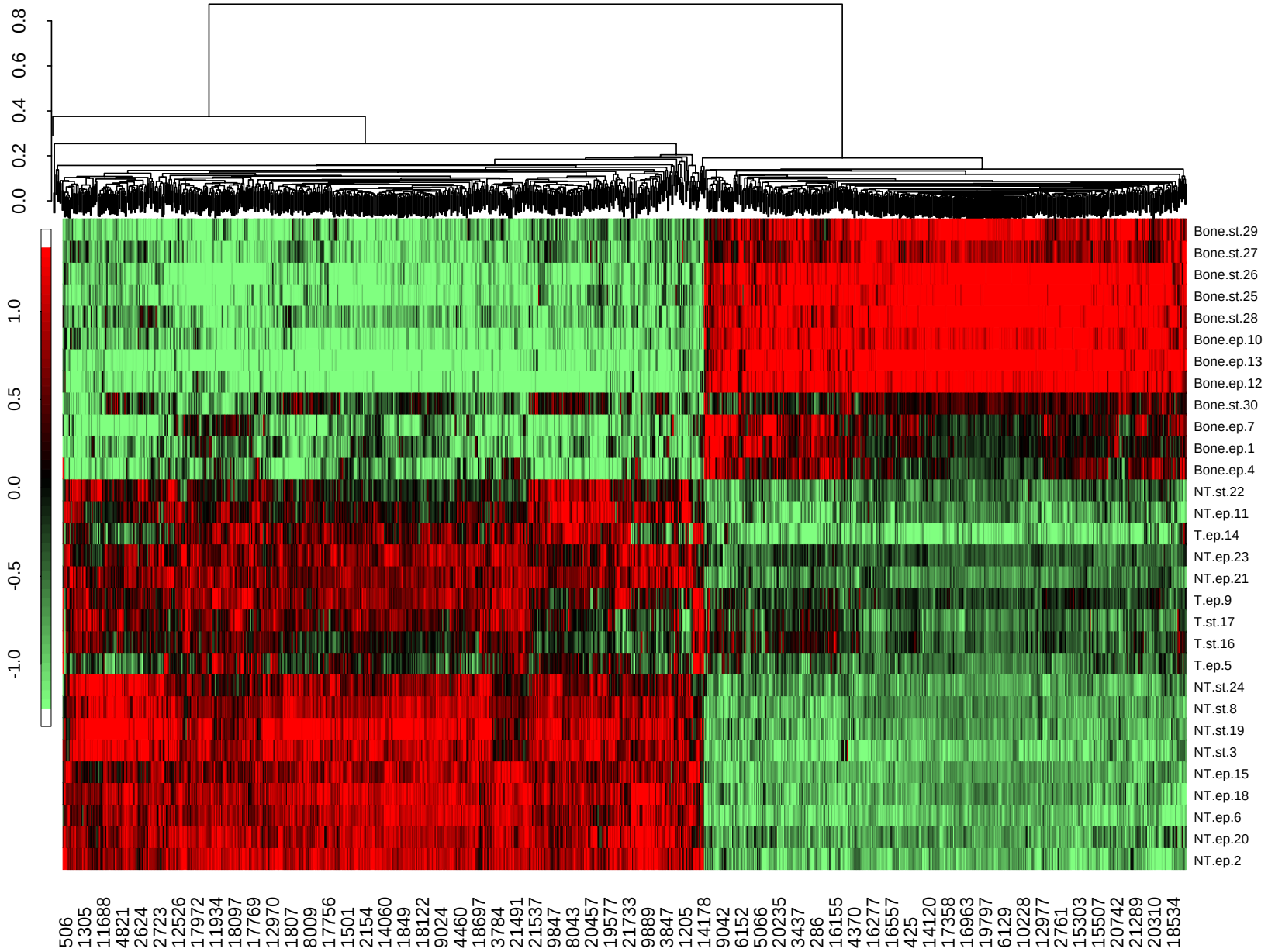
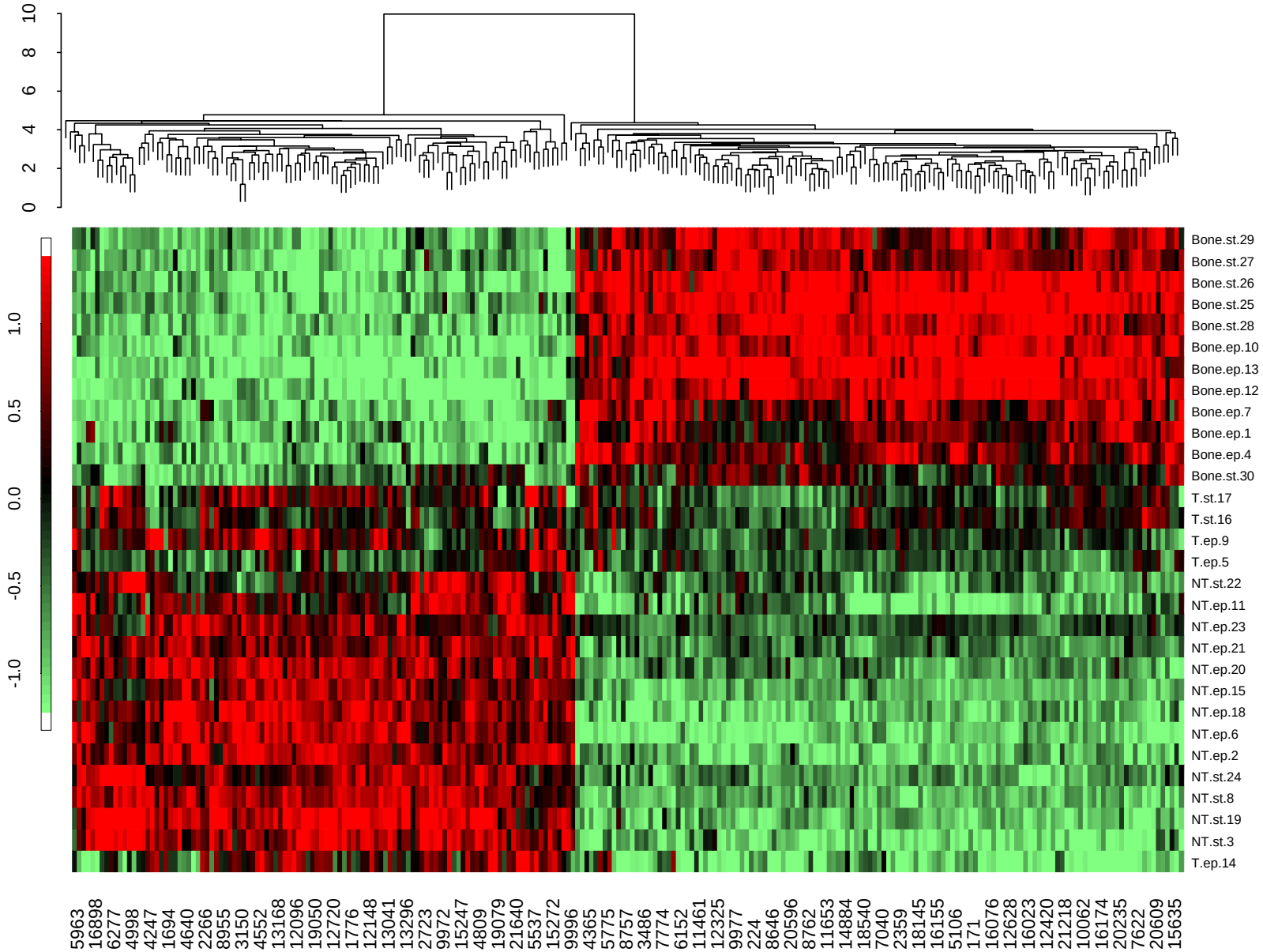
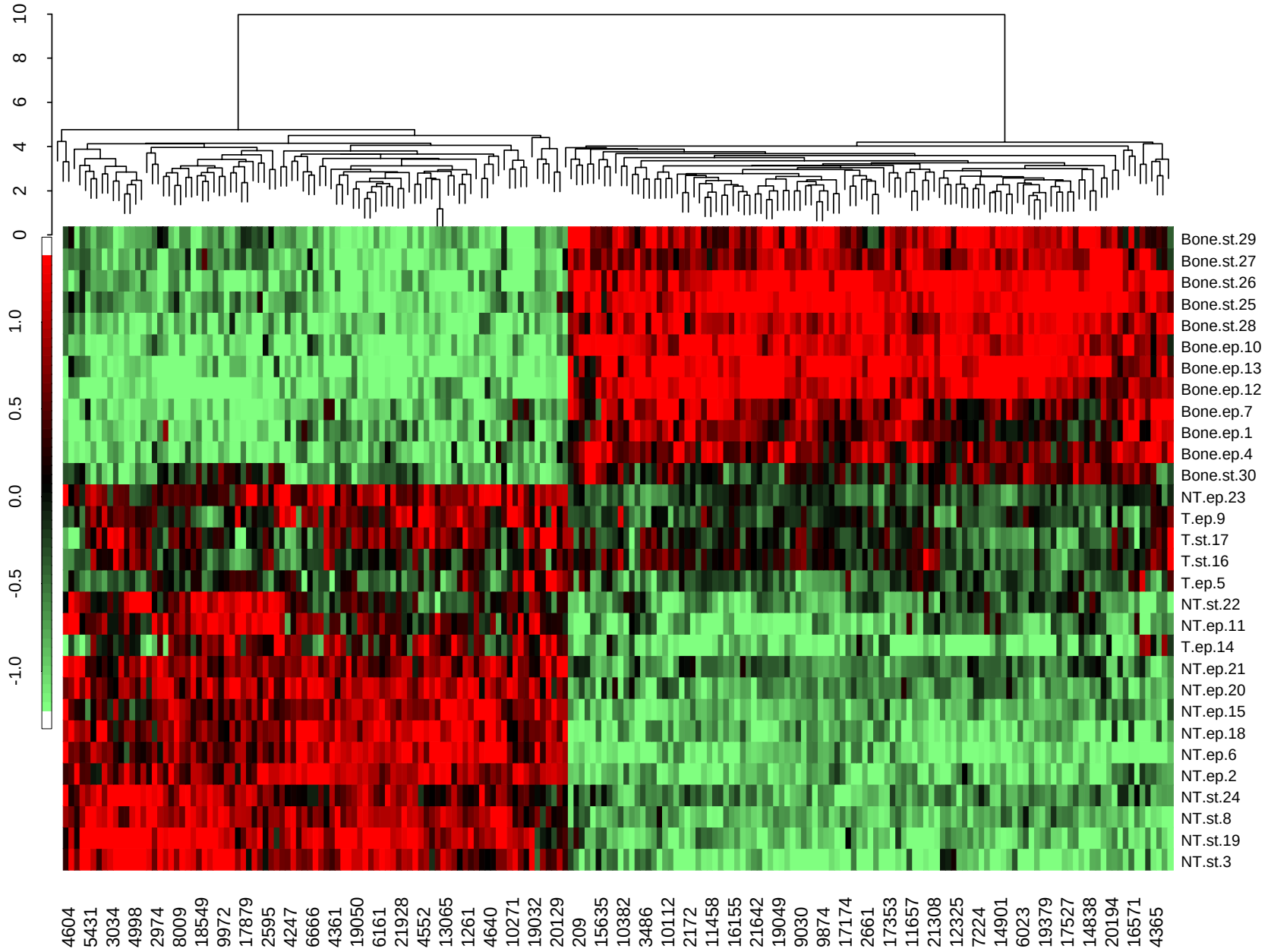


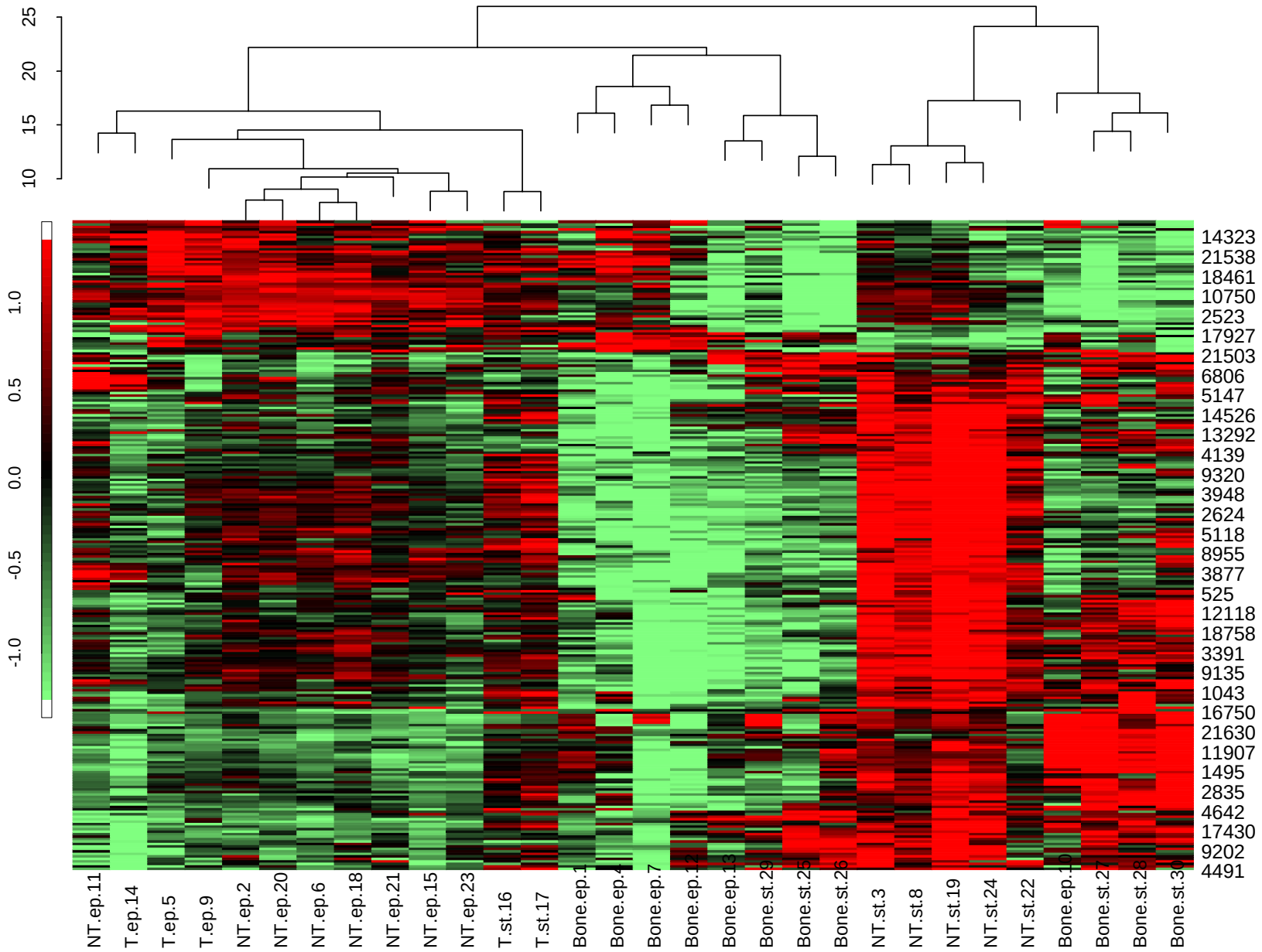
243 genes with smallest SD within bones among 1077 genes

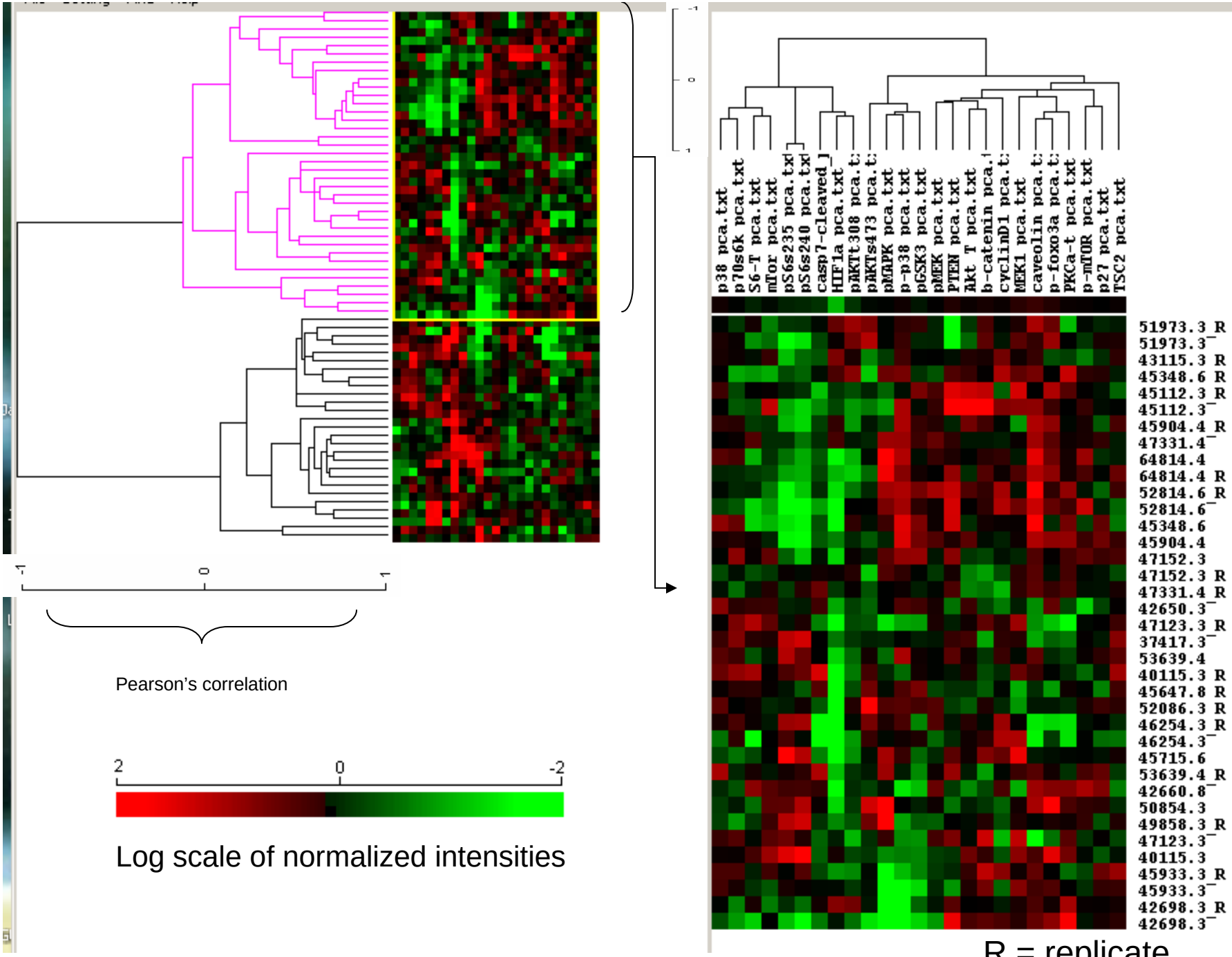


200 genes with smallest SD within bones among 1077 genes



Top 262 genes (stroma vs ep FDR= 0.01) by mixed model




-1
0
1
Pearson's correlation
2
0
-2
Log scale of normalized intensities
p38 pca.txt
p70s6k pca.txt
S6-T pca.txt
mTor pca.txt
pS6s235 pca.txt
pS6s240 pca.txt
casp7-cleaved_1
HIF1a pca.txt
pAKTt308 pca.t:
pAKTs473 pca.t:
pMAPK pca.txt
p-p38 pca.txt
pGSK3 pca.txt
pMEK pca.txt
PTEN pca.txt
Akt T pca.txt
b-catenin pca.t
cyclinD1 pca.t:
MEK1 pca.txt
caveolin pca.t:
p-foxo3a pca.t:
PKCa-t pca.txt
p-mTOR pca.txt
p27 pca.txt
TSC2 pca.txt
51973.3_R
51973.3
43115.3_R
45348.6_R
45112.3_R
45112.3
45904.4_R
47331.4
64814.4
64814.4_R
52814.6_R
52814.6
45348.6
45904.4
47152.3
47152.3_R
47331.4_R
42650.3
47123.3_R
37417.3
53639.4
40115.3_R
45647.8_R
52086.3_R
46254.3_R
46254.3
45715.6
53639.4_R
42660.8
50854.3
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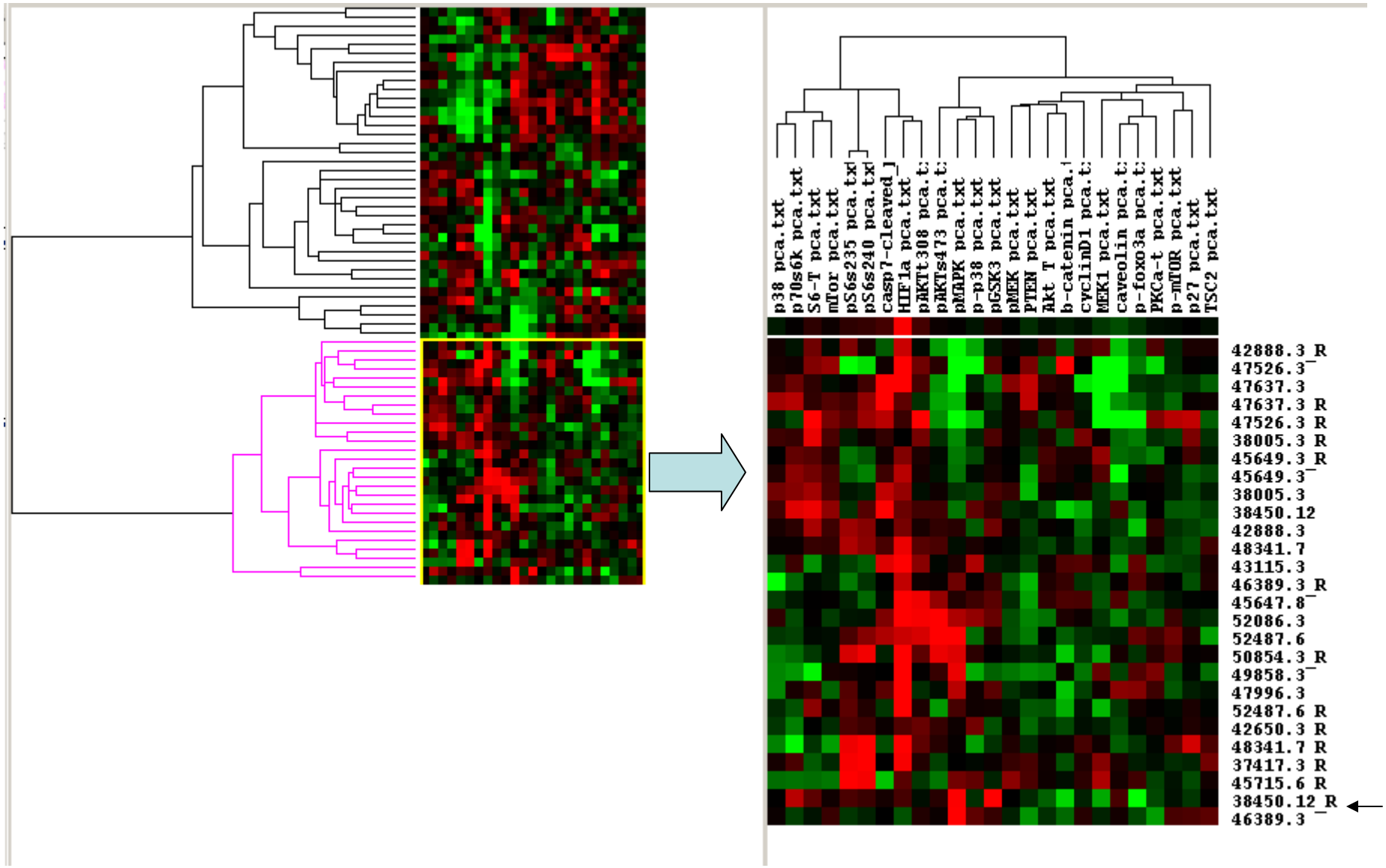
40115.3
45933.3_R
45933.3
42698.3_R
42698.3
_R = replicate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pMEK pca.txt
PTEN pca.txt
Akt T pca.txt
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cyclinD1 pca.t
MEK1 pca.txt
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p27 pca.txt
TSC2 pca.txt
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47526.3
47637.3
47637.3 R
47526.3 R
38005.3 R
45649.3 R
45649.3
38005.3
38450.12
42888.3
48341.7
43115.3
46389.3 R
45647.8
52086.3
52487.6
50854.3 R
49858.3
47996.3
52487.6 R
42650.3 R
48341.7 R
37417.3 R
45715.6 R
38450.12 R
46389.3

The ‘high risk’ preoperative model

The experimental platform to test hypotheses on effects of compounds on the tumor microenvironment after limited exposure in a clinically meaningful context.

Material /Methods for tissue interrogation

- Materials

RPS of patients treated with the compound of interest and matched controls

(by preop characteristics, ie. clinical stage, biopsy GS, PSA etc)

- Methods

➢ TMAs (extensive representation of heterogeneity, epithelium, stroma, non-tumor, tumor)

➢ RPPAs by isolation of tumor microenvironment components (LCM /UV cut technologies)

(Controls for TMAs and RPPAs of different origin)

Efstathiou E, 2008

Initial Modulation of the Tumor Microenvironment Accounts for Thalidomide Activity in Prostate Cancer

(Efstathiou et al, CCR 2007 ;13(4):1224-31)

- First clinical evidence to support the hypothesis that the reported thalidomide clinical efficacy is attributable to early modulation of the tumor microenvironment

✓ Antiangiogenic effect
  - CD31 reduction
  - VEGF,IL6 reduction

✓ Unlinking of broader stromal-epithelial interactions
  - Attenuation of hedgehog signaling
  - MMP9+MMP2/ E cadherin changed to favor e-cadherin

Coordinated Modulation of Sonic Hedgehog and Androgen Signaling in the Prostate Cancer Microenvironment by Chemo-Hormonal Therapy.

(ASCO, Chicago 2007 abstr 5066 , Prostate Poster discussion)

- **Results:**

Following androgen ablation (alone or in combination with chemotherapy) there is an increase in hedgehog signaling activity in the residual tumor epithelium and stroma

Active hedgehog signaling was parallel in the tumor epithelium and adjacent stroma

- **Conclusion**

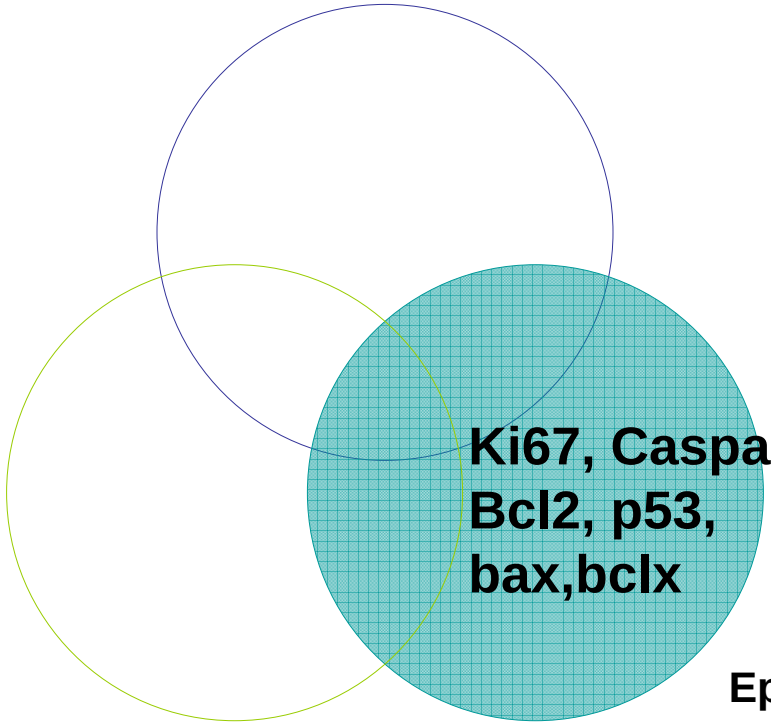
Sonic Hedgehog and androgen pathways are modulated in a manner that suggests they behave in a compensatory fashion, and are determinants of therapy response.

Efstathiou E, 2008

Mixed Model effects comparison	**Mean control (sd)**	**Mean AA (sd)**	**Mean CH (sd)**	**P value Control vs AA**	**P value Control vs CH**
Gli2 Epithelium	**39.6 (20.7)**	**79.6 (17.2)**	**85.5 (11.9)**	**<0.0001**	**<0.0001**
Gli2 Stroma	**22.9 (15.2)**	**43.3 (17.9)**	**54.7 (15.4)**	**<0.0001**	**<0.0001**
Smoothened Epithelium	**68.9 (18.2)**	**81.1 (14.2)**	**83.5 (8.9)**	**0.0047**	**0.0008**
Smoothened Stroma	**16.1 (11.2)**	**21.6 (10.7)**	**28.2 (13.9)**	**0.118**	**0.0011**
Shh Epithelium	**49.9 (24.8)**	**61.3 (19.2)**	**62.6 (22.4)**	**0.085**	**0.05**
Shh Stroma	**4.6 (6.5)**	**12.8 (13.5)**	**19.4 (13.9)**	**0.0203**	**0.0001**

Pearson's correlation	**Gli2. Epithelium**	**Gli2 Stroma**	**Smoothened Epithelium**	**Smoothened Stroma**	**Shh Epithelium**	**Shh Stroma**
Gli2. Epithelium	1	**0.789**	0.516	0.359	0.349	0.545
Gli2 Stroma	**0.789**	1	0.443	0.601	0.305	0.731
Smoothened Epithelium	0.516	0.443	1	0.449	0.496	0.359
Smoothened Stroma	0.359	0.601	0.449	1	0.172	0.552
Shh Epithelium	0.349	0.305	0.496	0.172	1	0.333
Shh Stroma	0.545	0.731	0.352	0.552	0.333	1

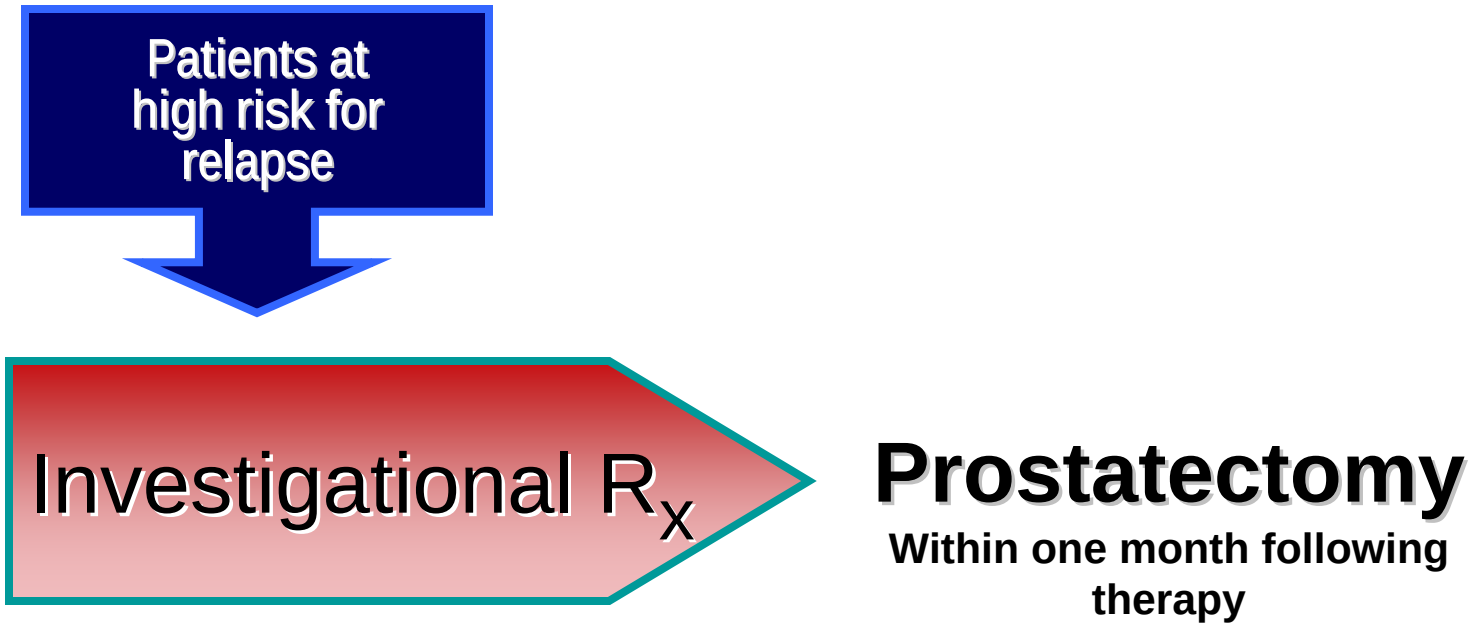
Tumor Microenvironment



Effect on
Epithelial proliferation
survival

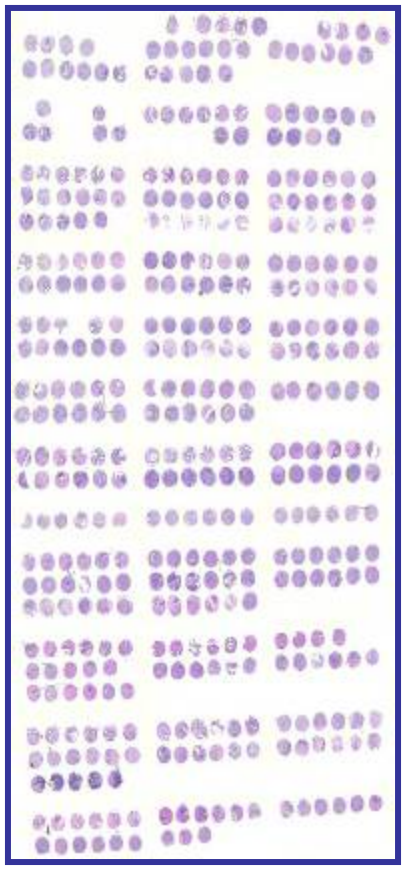
Efstathiou E, 2008

Treatment Strategy



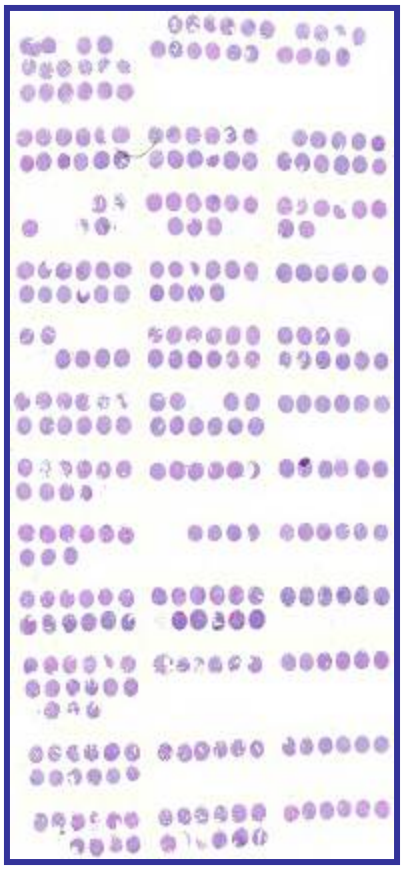
Efstathiou E, 2008

Thalidomide Trial



Control (15 RPS)

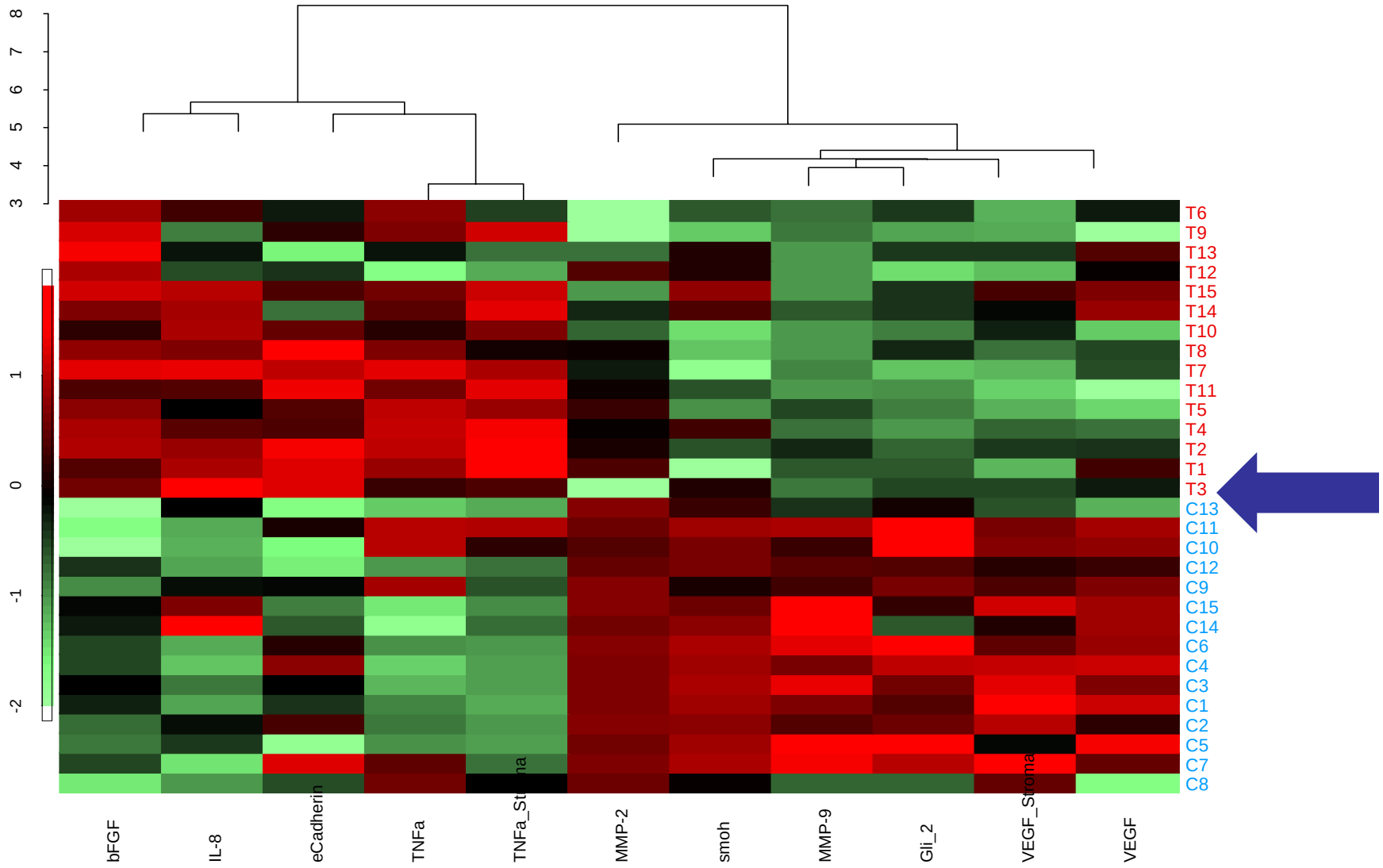
.523 cores



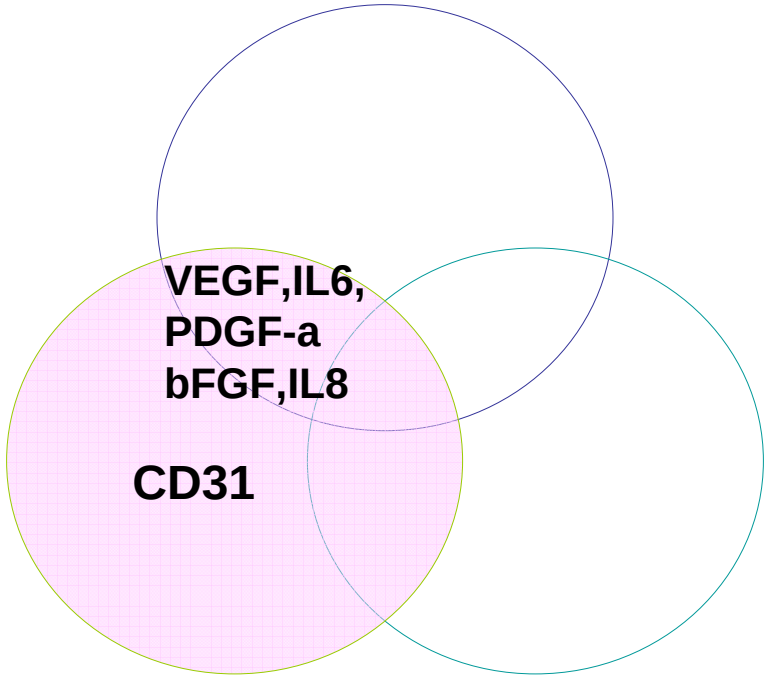
Treated (15 RPS)

.453 cores

Differentially Expressed Markers



Tumor Microenvironment

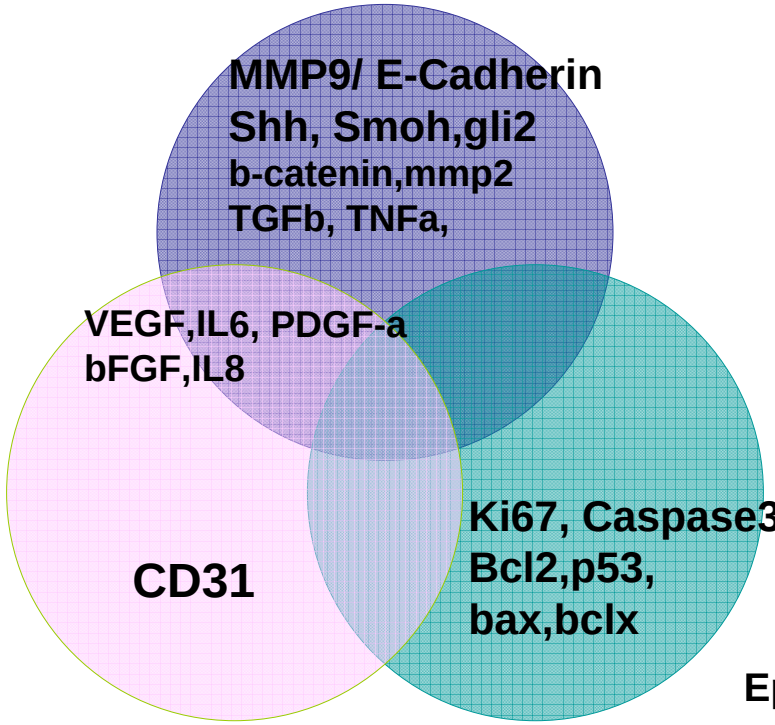


Effect on Endothelial cells

Efstathiou E, 2008

Tumor Microenvironment

Effect on S-E interaction

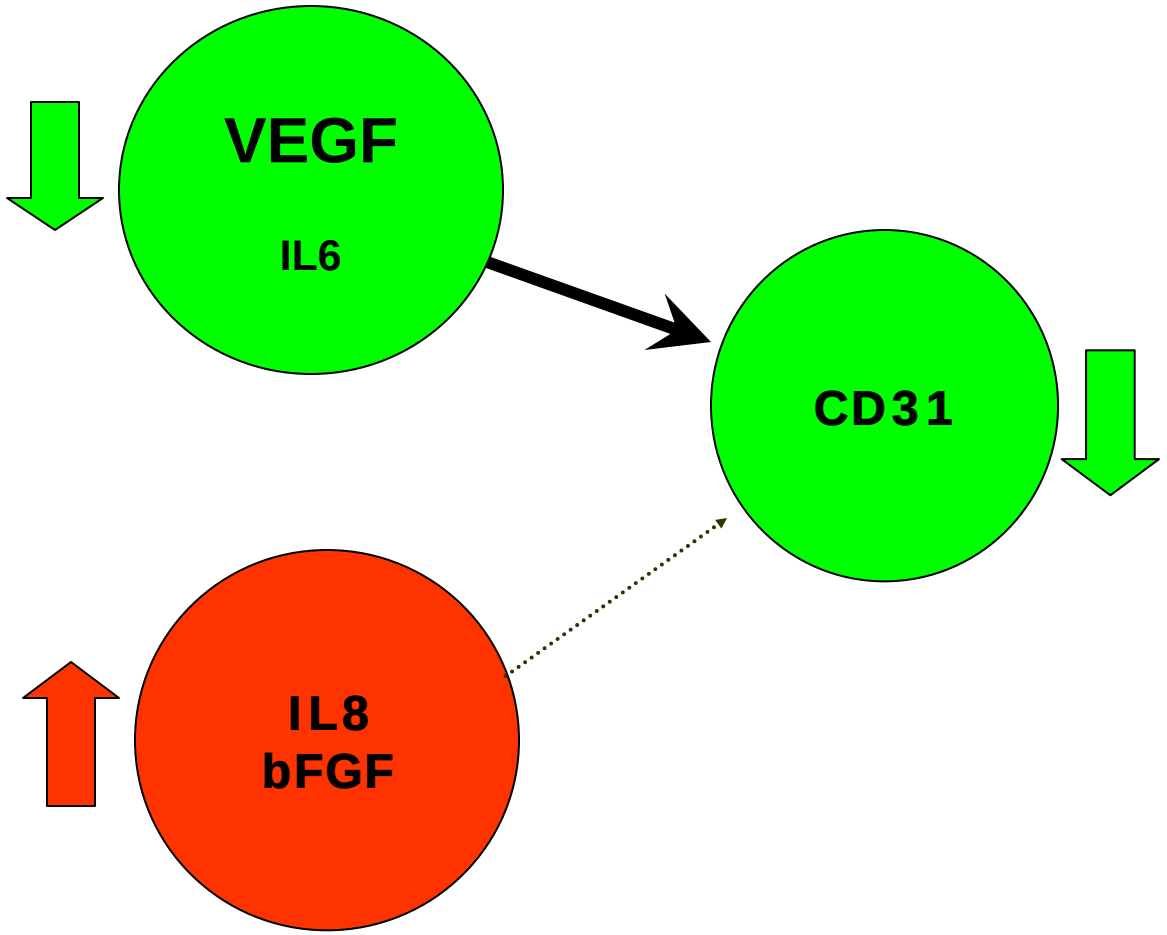


Effect on Endothelial cells

Effect on Epithelial proliferation survival

Comparison of marker grouping in treated and controls

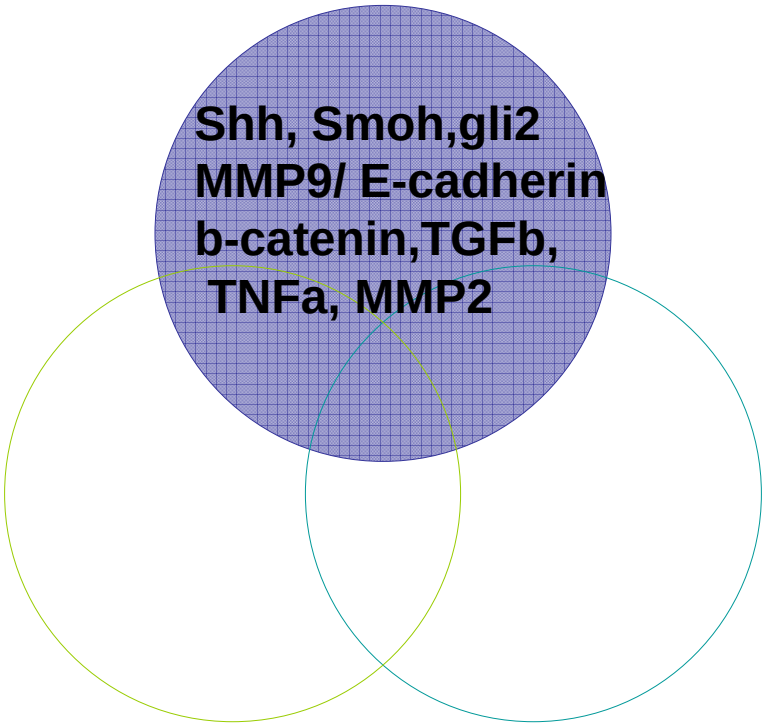
Markers of Angiogenesis



Efstathiou E, 2008

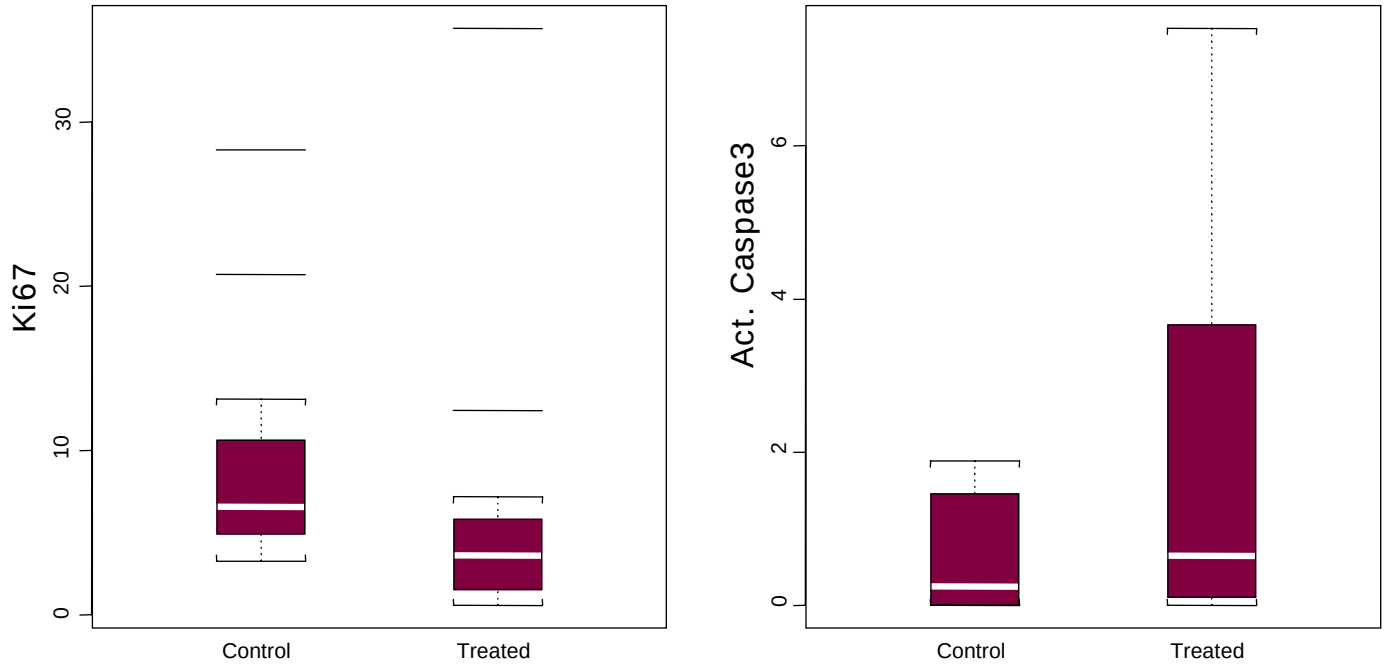
Tumor Microenvironment

Effect on S-E interaction



Efstathiou E, 2008

Tumor Proliferation and Apoptosis treated vs control



Efstathiou E, 2008

Conclusions

- **Markers of shh signaling and vasculogenesis are reduced in treated as compared to control specimens**
- **MMP-9/E-Cadherin ratio favors E-cadherin in treated specimens**
- **No significant change was seen in epithelial markers (Proliferation & Apoptosis)**

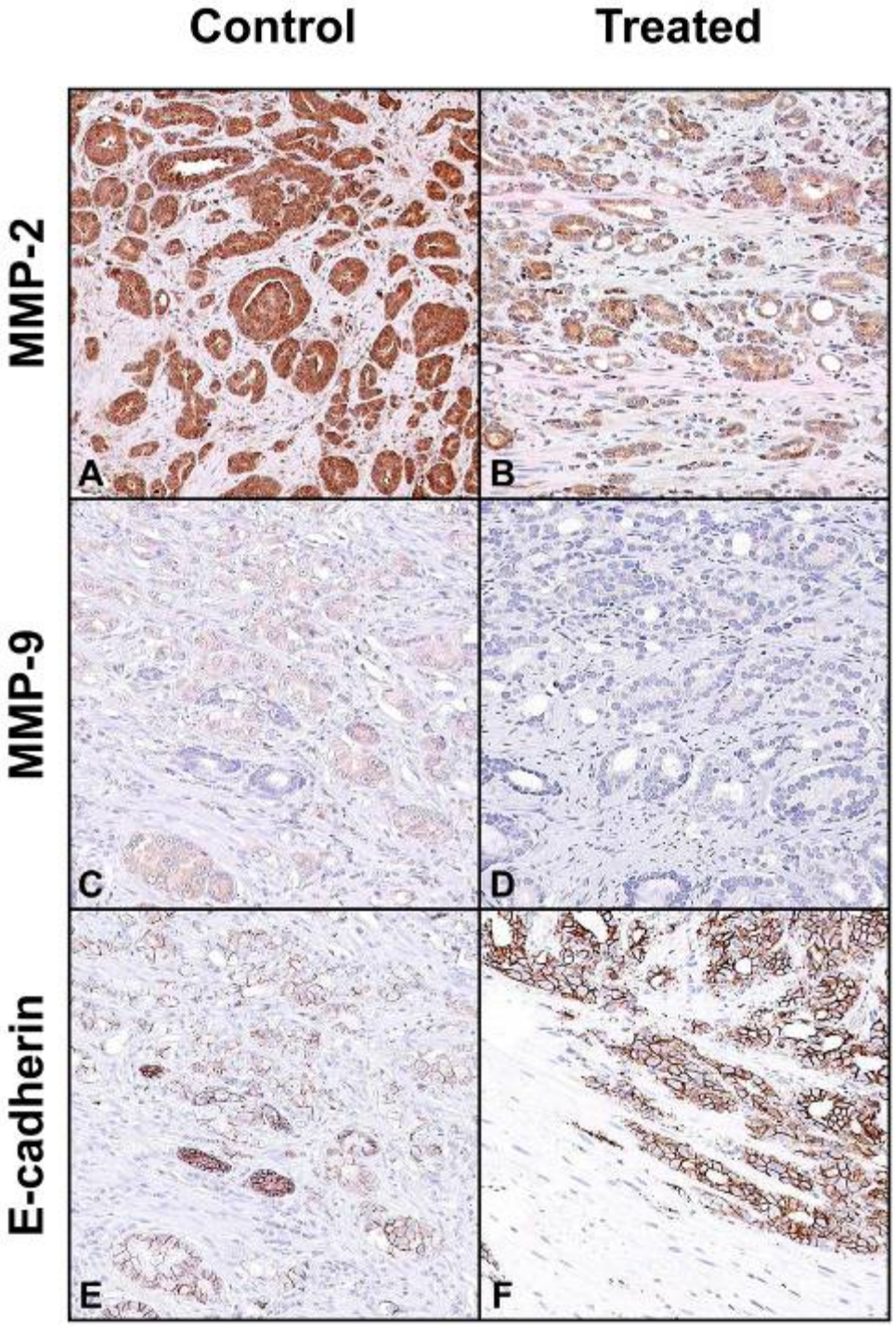
Efstathiou E, 2008

S-E Interaction

	Control Mean (s.d)	**Treated** Mean (s.d)	p value (t-test)	p value (mixed model)
Gli2	2.11 (.5)	1.20 (.3)	**<.0001**	**<.0001**
Smoh	2.84 (.19)	2.33 (.46)	**.0005**	**.0005**
Shh	2.1 (.53)	2.3 (.54)	.7702	.7782
MMP9	1.86 (.74)	.208 (.2)	**<.0001**	**<.0001**
E-Cadherin	2.6 (.15)	2.85 (.18)	**.002**	**.004**

P ≤ .003 for significance

Efstathiou E, 2008



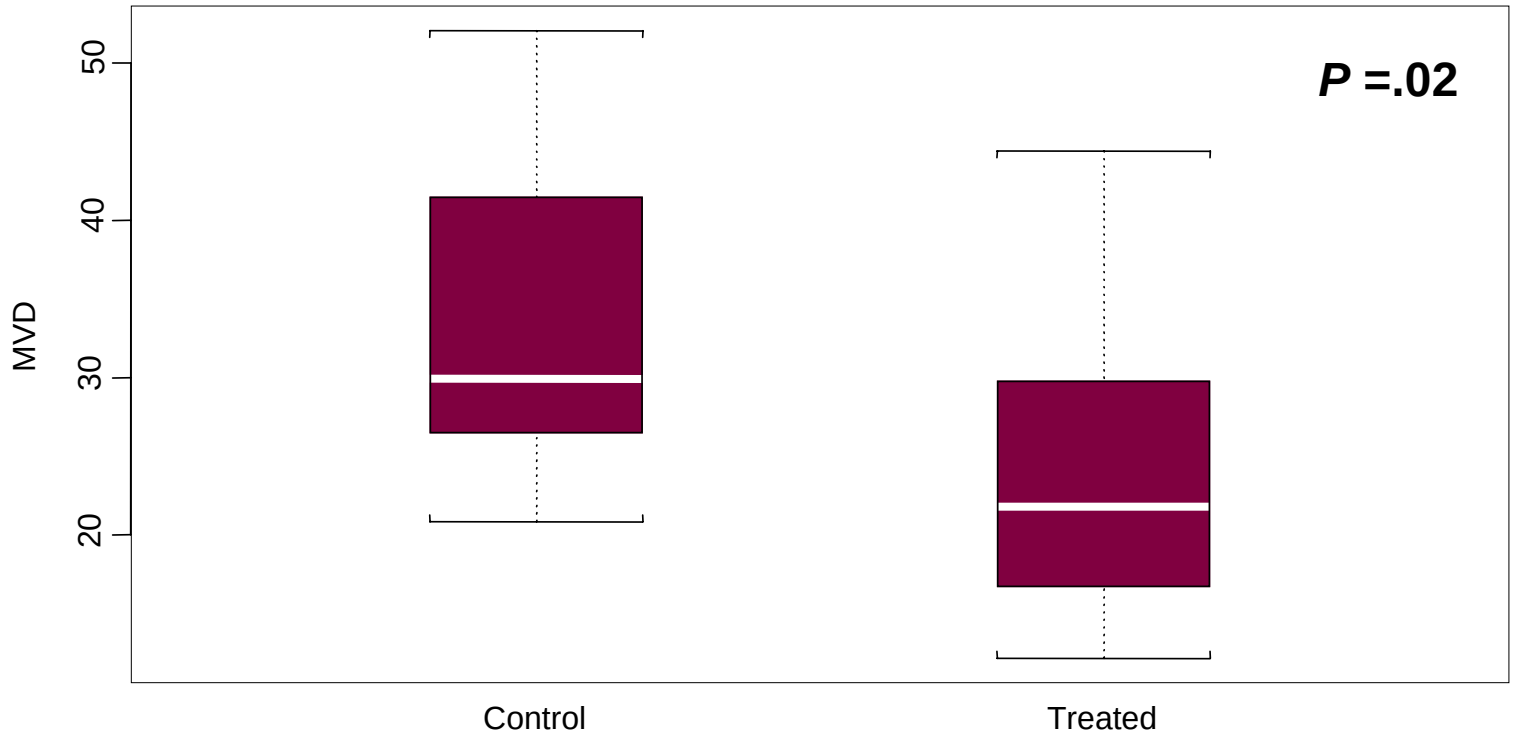
Efstathiou E, 2008

Markers of Angiogenesis

	Control Mean (s.d)	**Treated** Mean (s.d)	p value (t-test)	p value (mixed model)
VEGF	**2.24 (.58)**	**1.63 (.56)**	**.007**	**.004**
VEGF stroma	**.76 (.34)**	**.34 (.18)**	**<.0001**	**<.0001**
IL6	1.68 (.5)	1.23 (.62)	.04	.04
IL6 stroma	1.53 (.4)	1.41 (.49)	.46	.27
PDGF-a	2.7 (.23)	2.59 (.41)	.31	.24
IL8	**.49 (.50)**	**1.26 (.62)**	**.0009**	**.0008**
bFGF	**1.55 (.61)**	**2.55 (.33)**	**<.0001**	**<.0001**

$P \leq .003$ for significance

Phenotypic Effect
Modulation of Microvessel Density

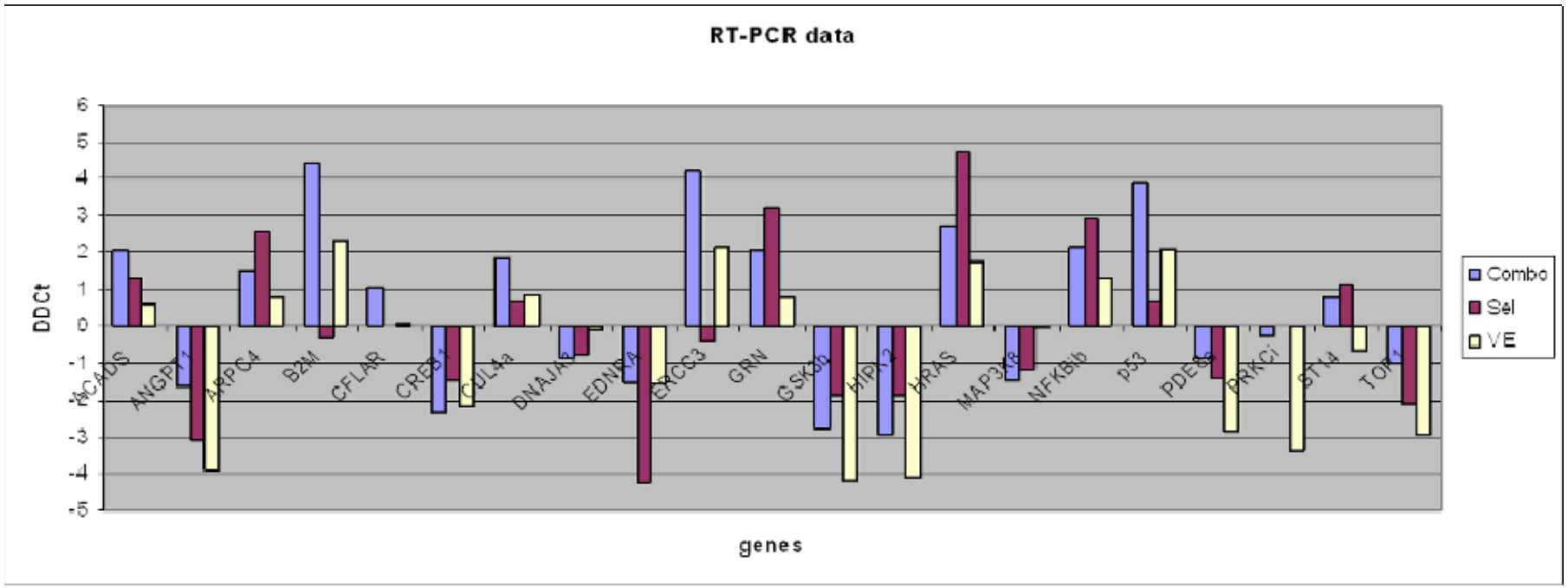


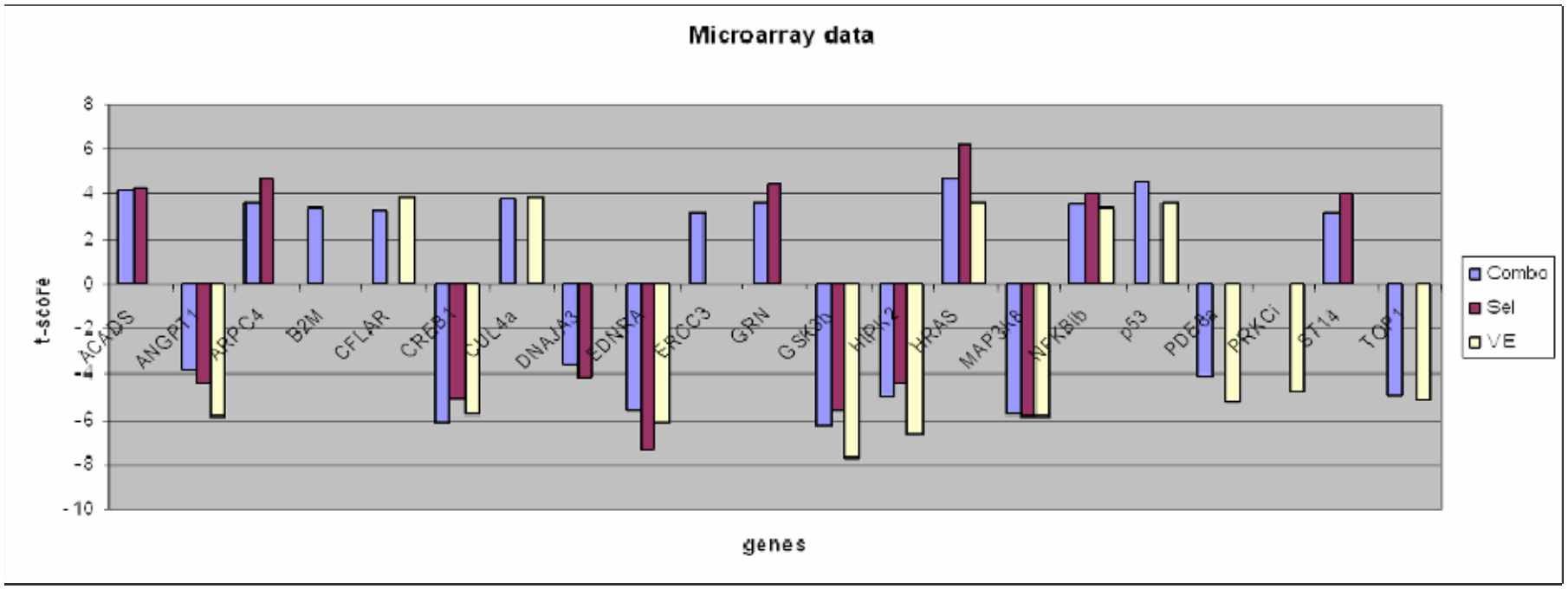
Efstathiou E, 2008

Hypothesis

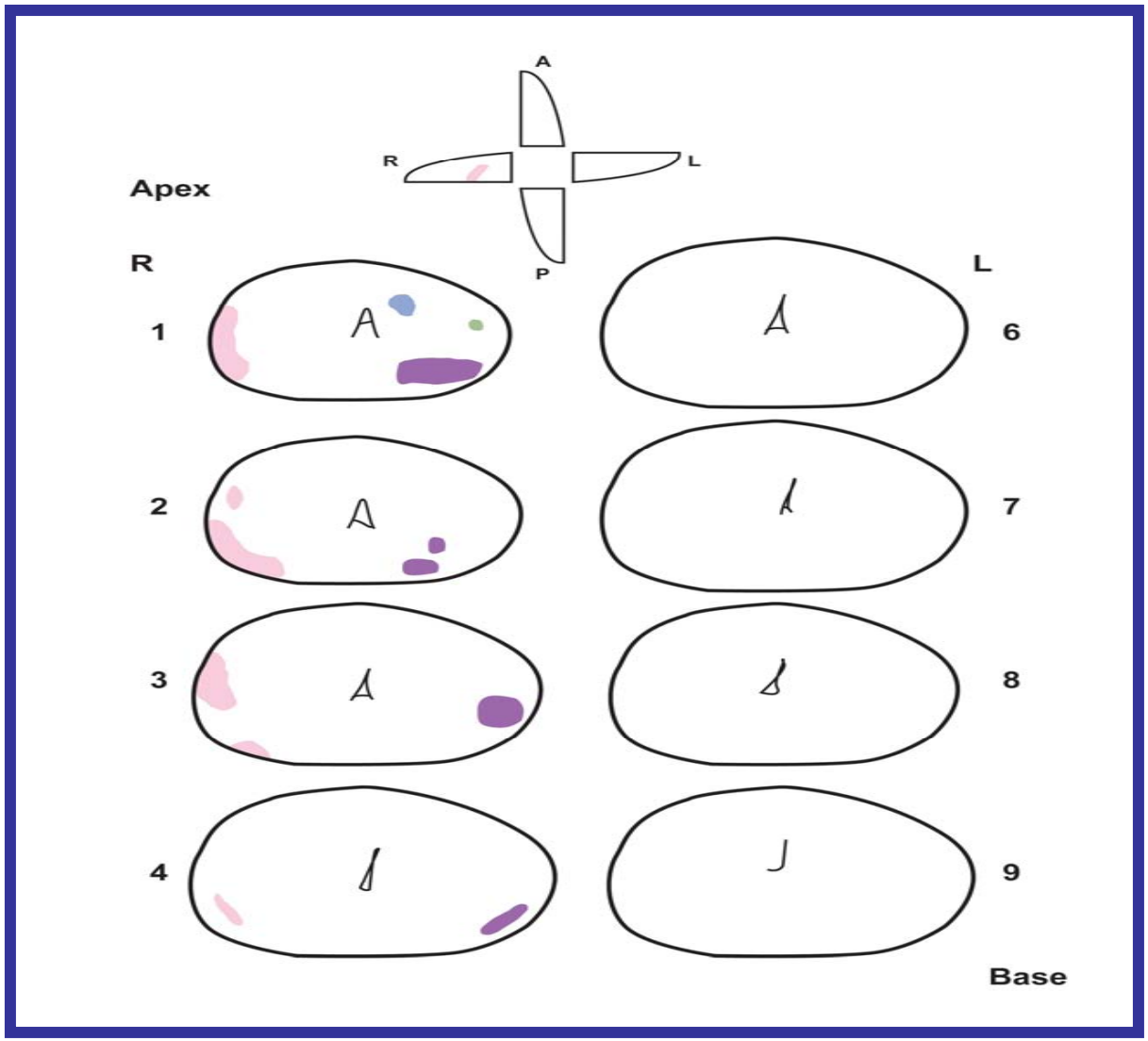
The biologic activity of finasteride may promote identification of molecular events that precede morphologic changes.

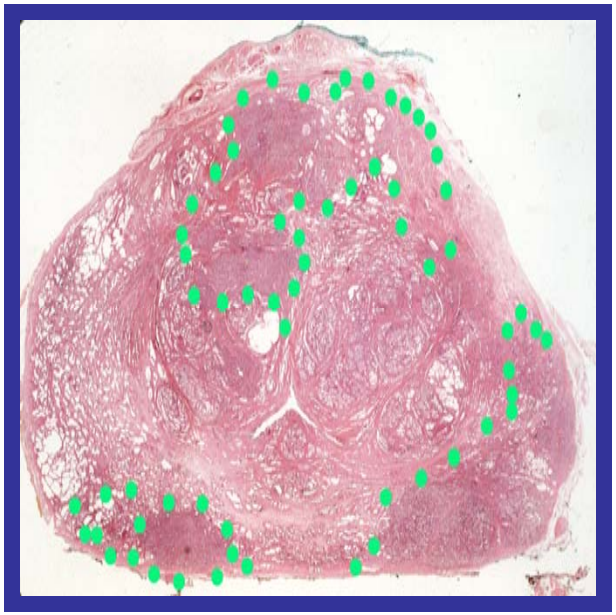
Kim J, 2008


RT-PCR data
DDCt
genes
Combo
Sel
VE


Microarray data
t-score
genes
Combo
Sel
VE

Secondary End Point

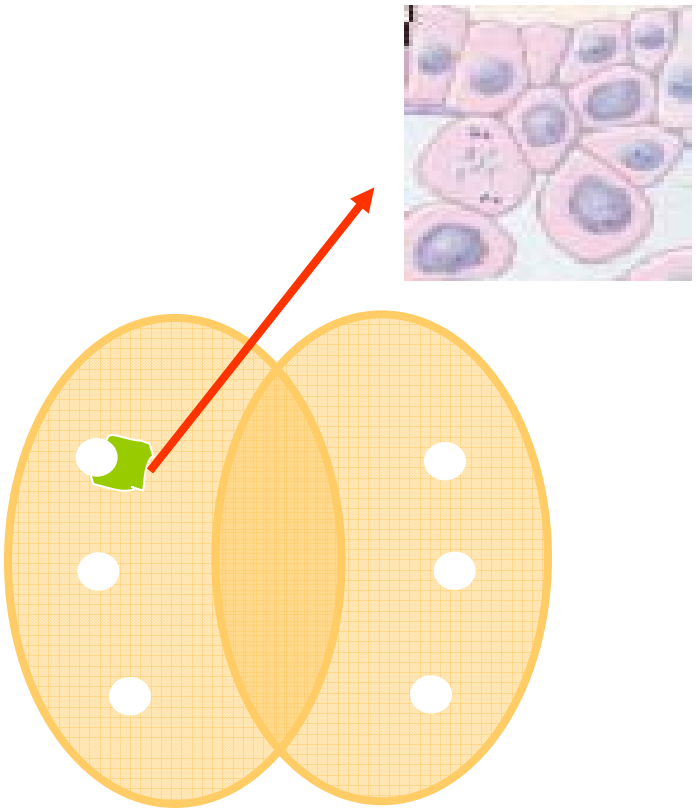




All Tumor Foci in Peripheral Zone

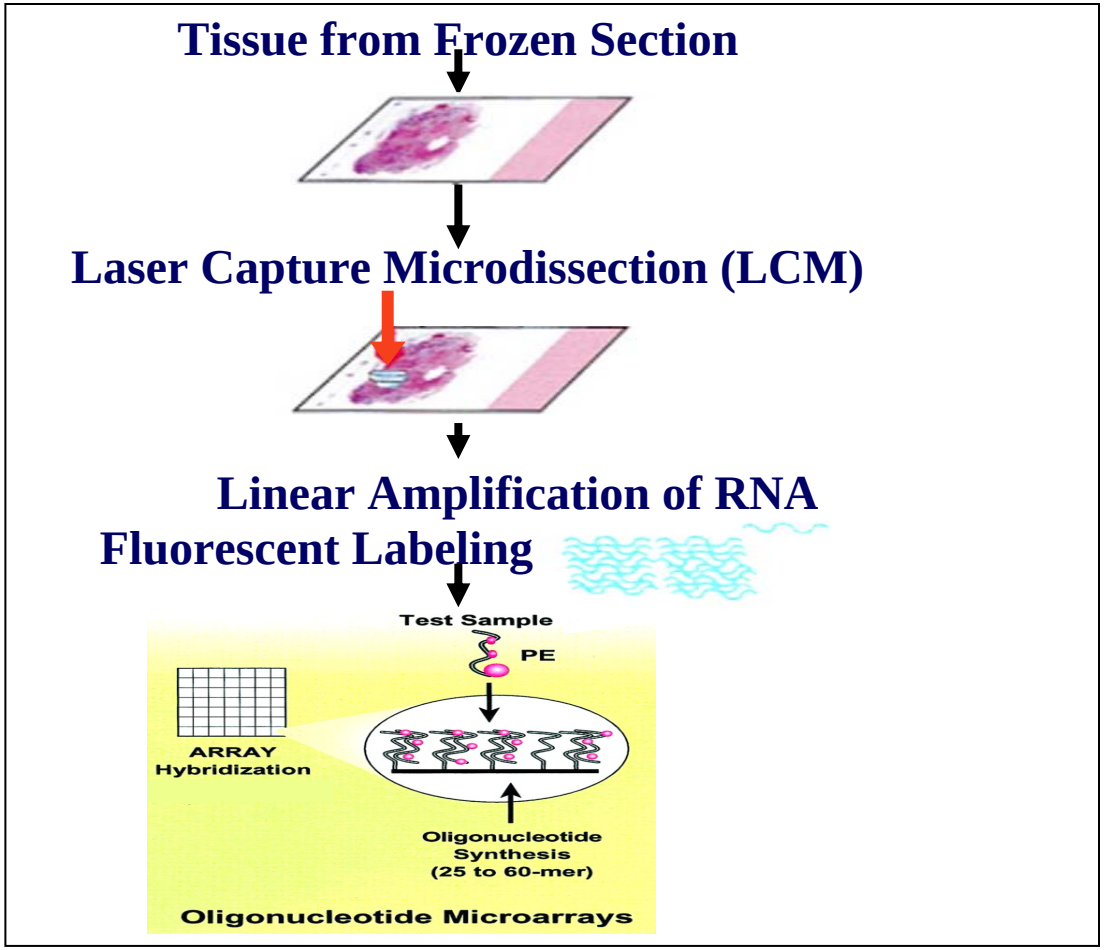
Kim J, 2008

Laser Capture Microdissection Oligonucleotide Microarrays



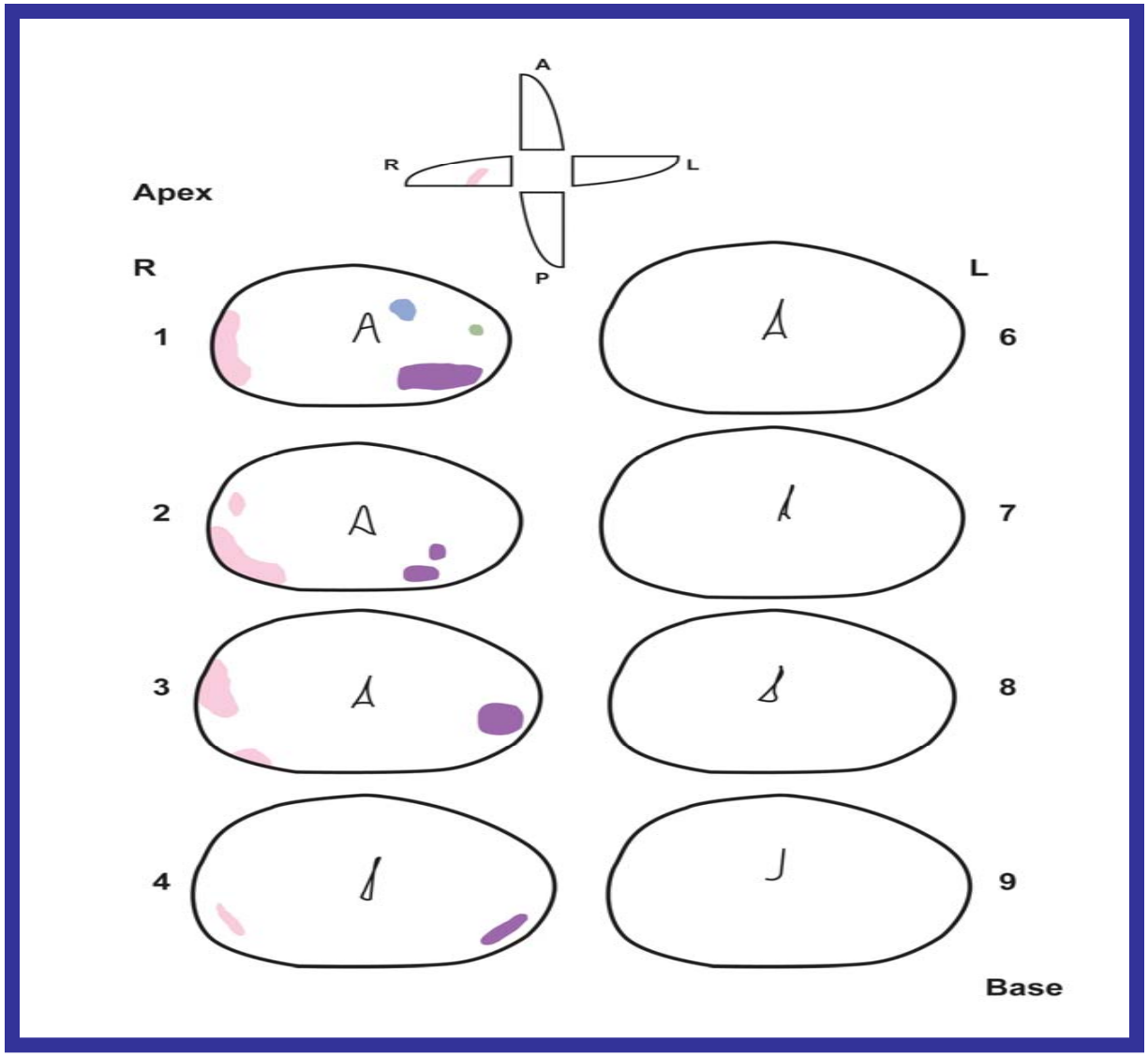
RPS

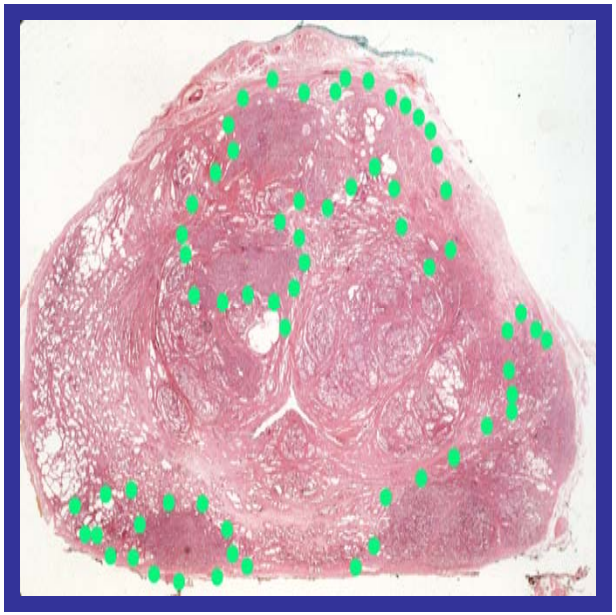
Biopsies



Pathol, 2001; JCO, 2002

Primary End Point

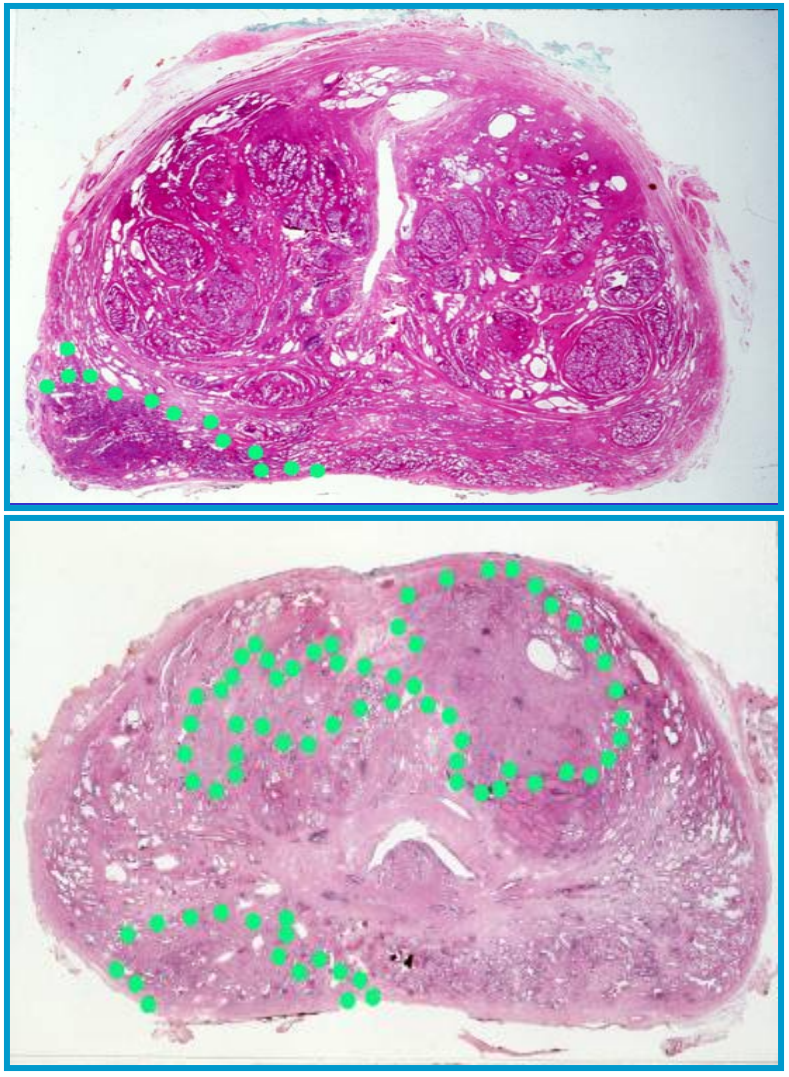




Dominant Tumor Focus in Peripheral Zone

Kim J, 2008

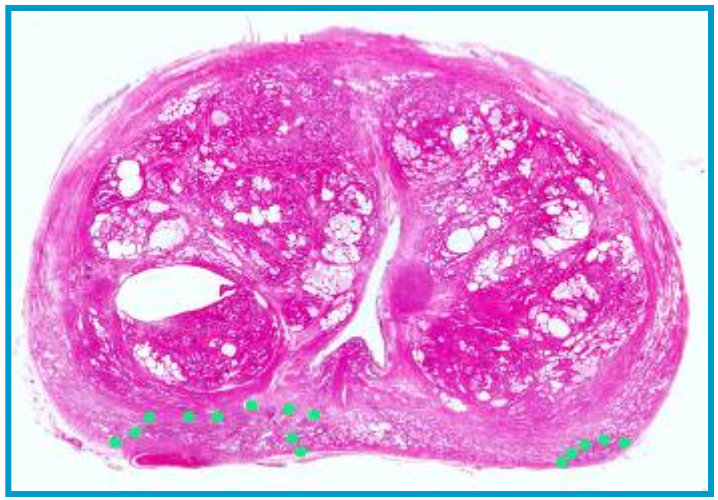
Pathologic Evaluation Challenges

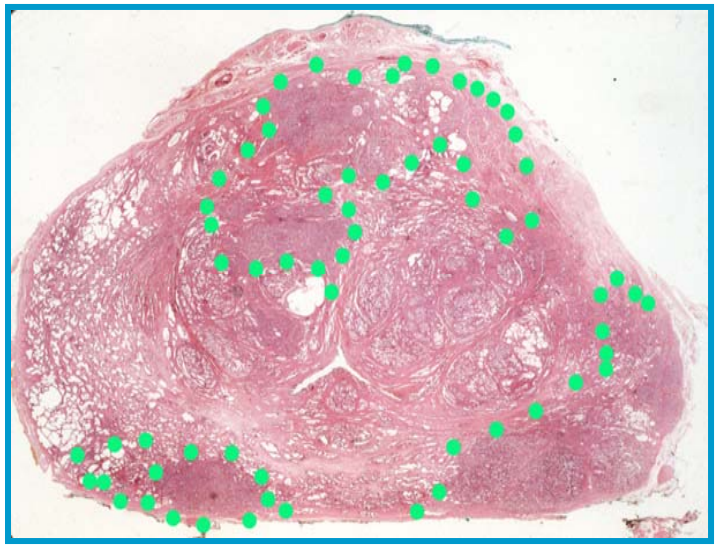


- **Tumors multifocal**
- **Tumors multizonal**
- **Tumor foci of different Gleason score and pathologic stage**

Kim J, 2008

Pathologic Evaluation Challenges

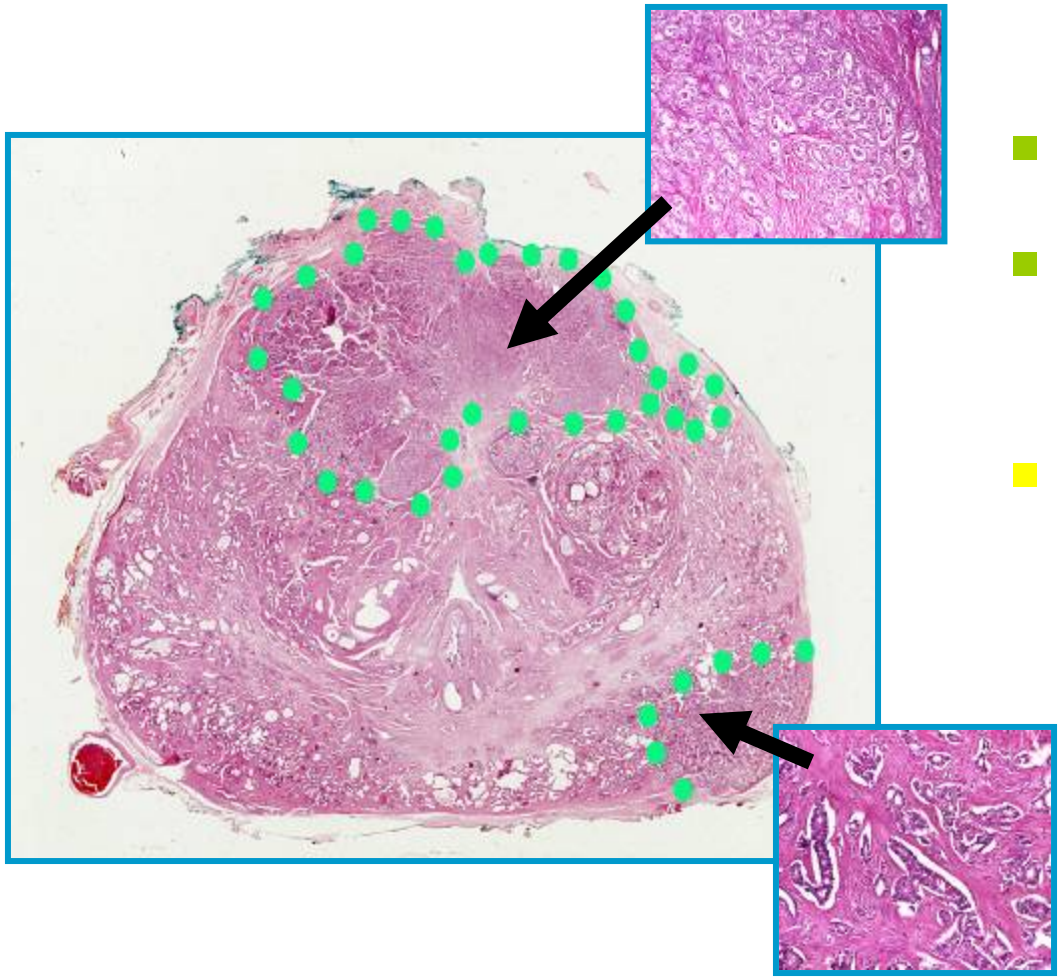




- **Tumors multifocal**
- **Tumors multizonal**
- **Tumor foci of different Gleason score and pathologic stage**

Kim J, 2008

Pathologic Evaluation Challenges



- **Tumors multifocal**
- **Tumors multizonal**
- **Tumor foci of different Gleason score and pathologic stage**

Kim J, 2008

PREOPERATIVE SELENIUM AND VITAMINE E

The University of Texas

M. D. Anderson Cancer Center

Eligibility

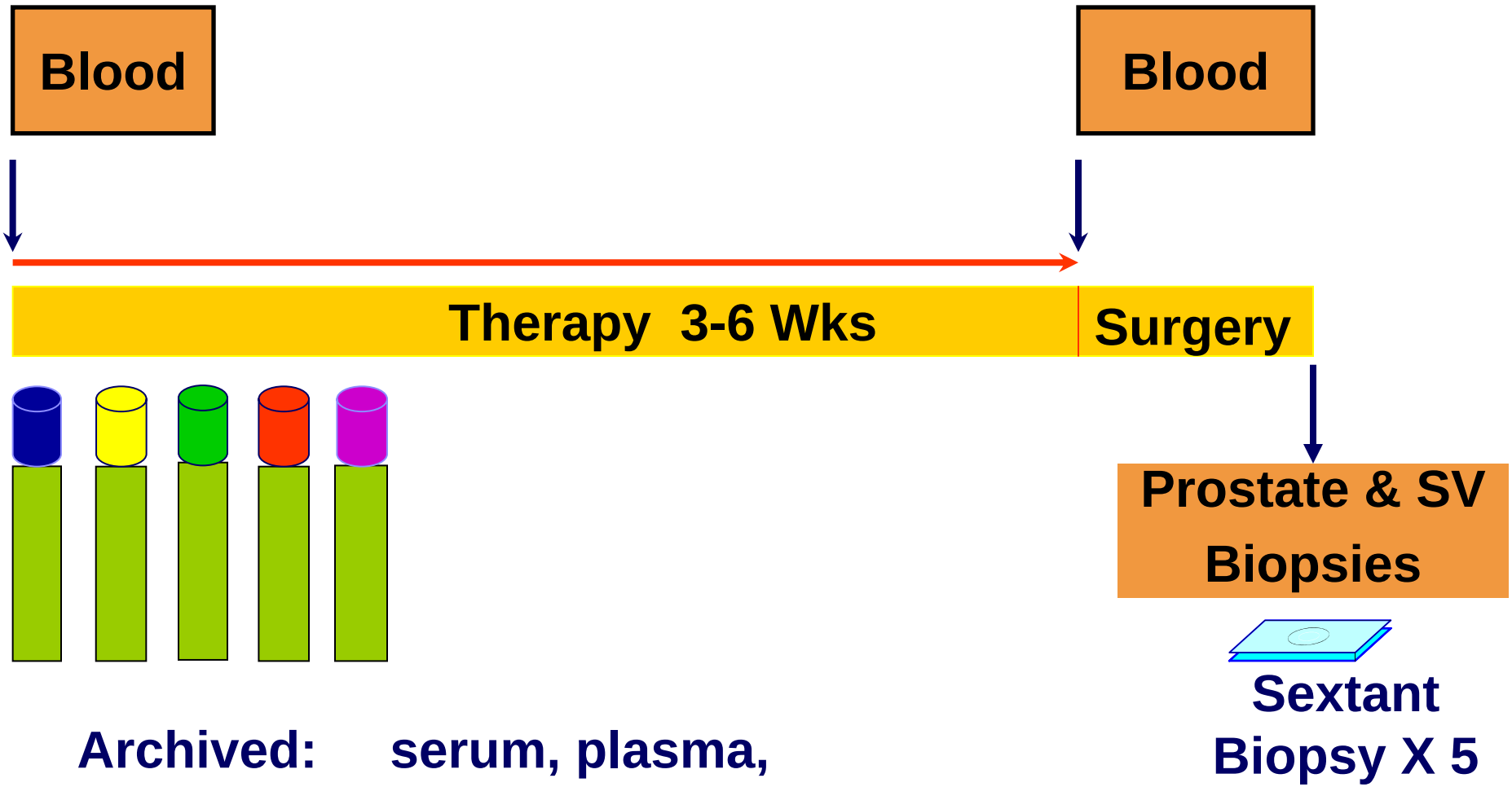
- Clinical T1c/T2
- GS ≤ 7
- PSA < 10
- Scheduled prostatectomy 3 - 6 wks from study entry
- Life expectancy ≥ 10 years
- PS 0,1
- In 1 mo. before study entry cumulative dose
  - selenium < 150 μg
  - vitamin E < 900 IU

Kim J, 2008

Summary

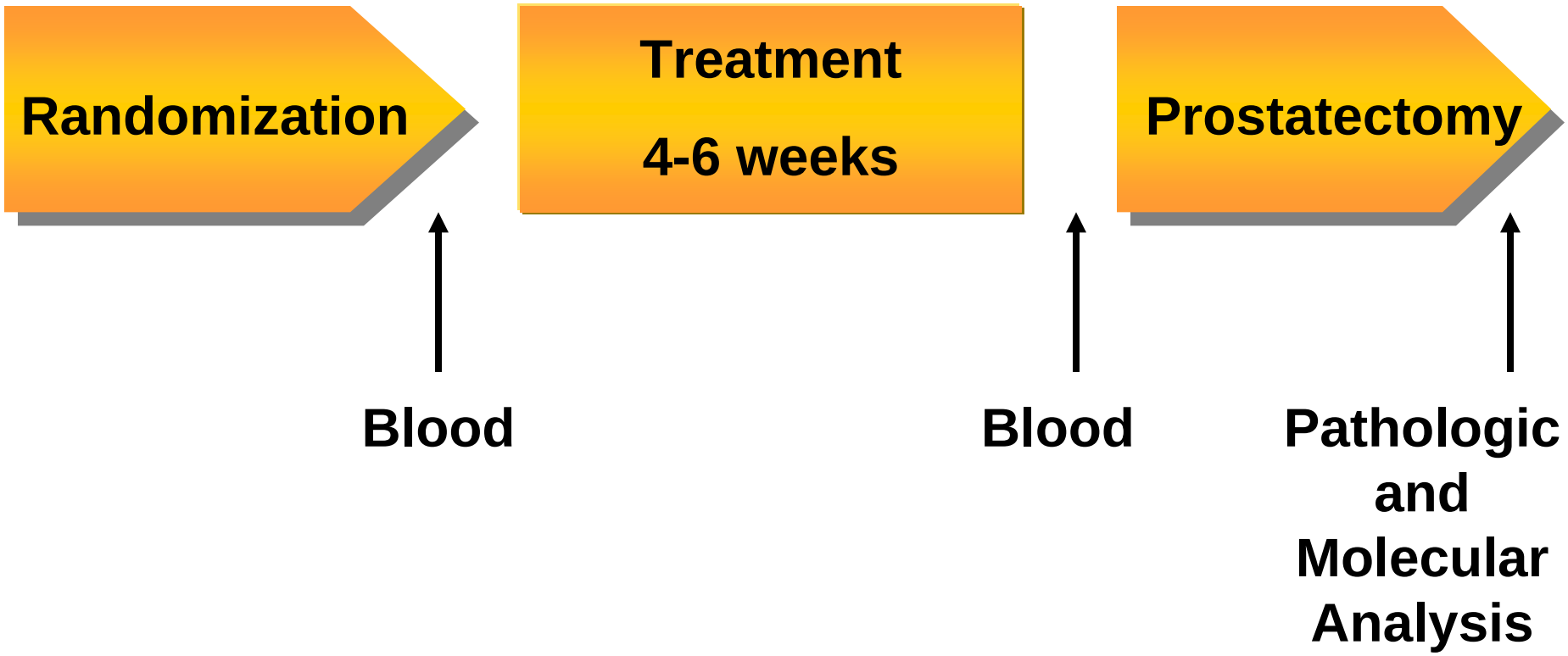
- Ex vivo core biopsies are a source of tissue for LCM and gene expression arrays
- The Pre-operative strategy can serve as investigational platform in *low volume cancer*
- Genes in the oxidative stress response and apoptosis pathways are differentially modulated by selenium, vitamin E, or selenium + vitamin E by cell compartment
- ***Link to biology will validate findings***

Biomarker Analyses



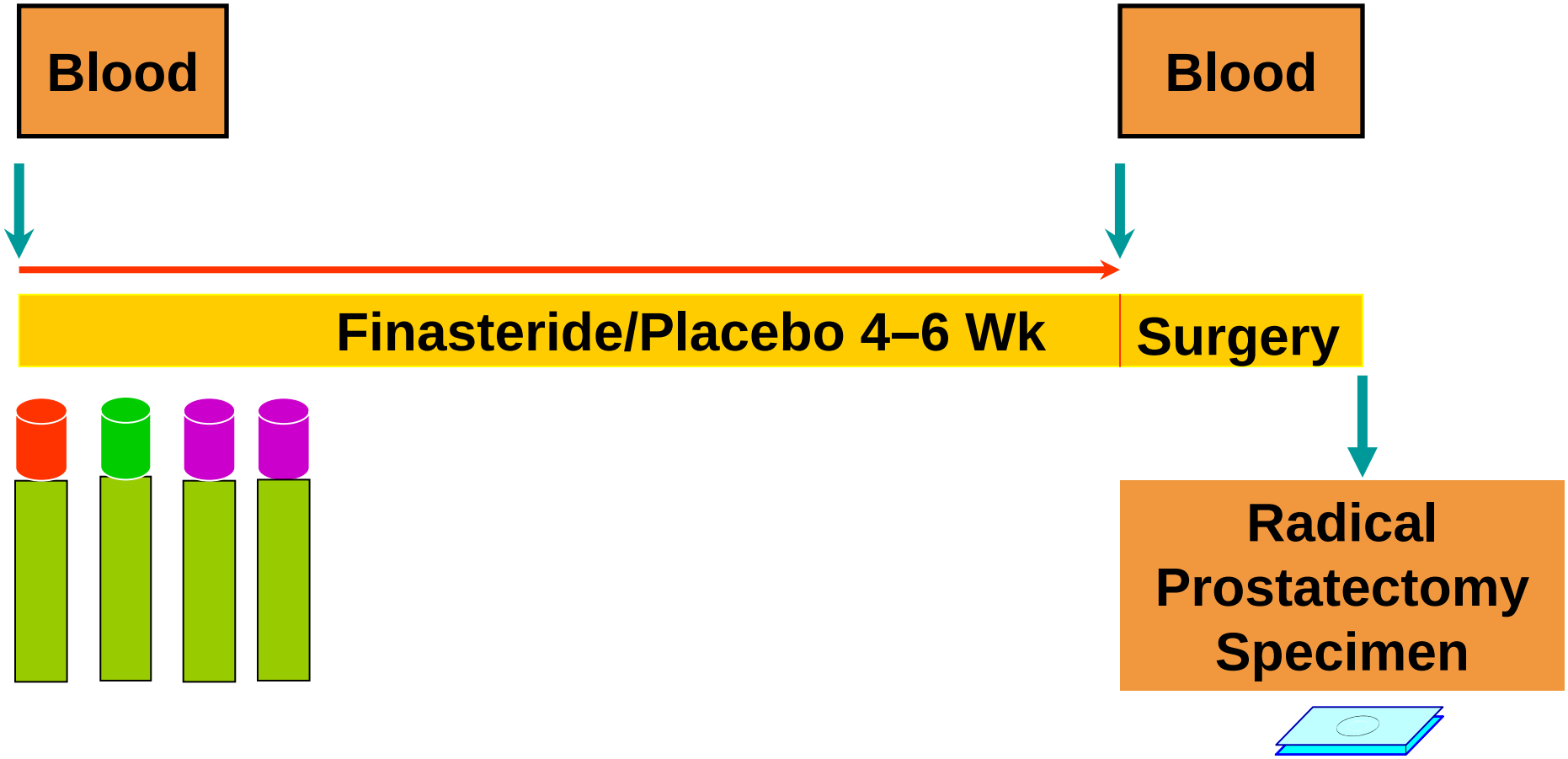
Archived: serum, plasma, RBCs, buffy coat

Study Schema



Kim J, 2008

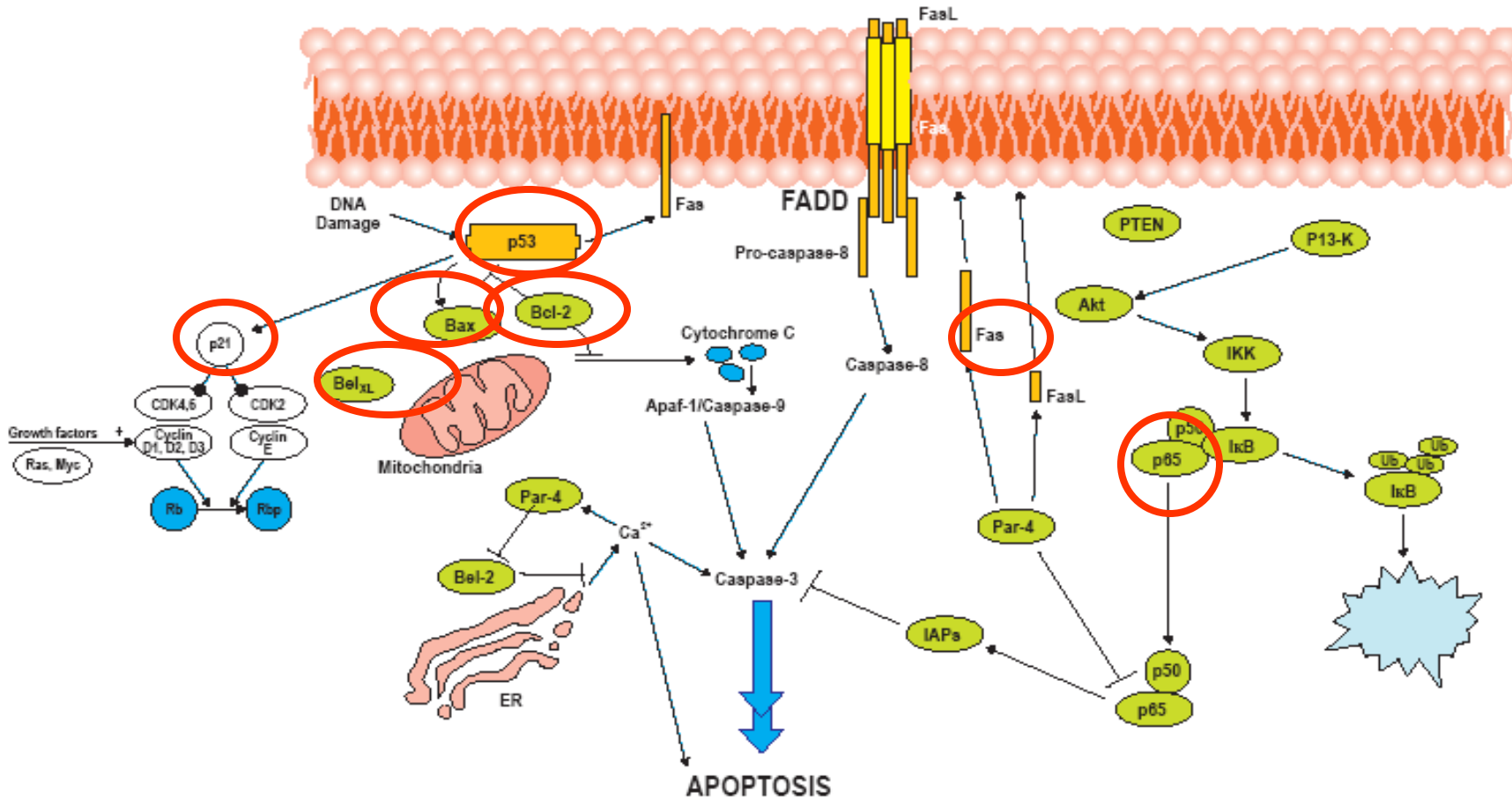
Blood and Tissue Archiving



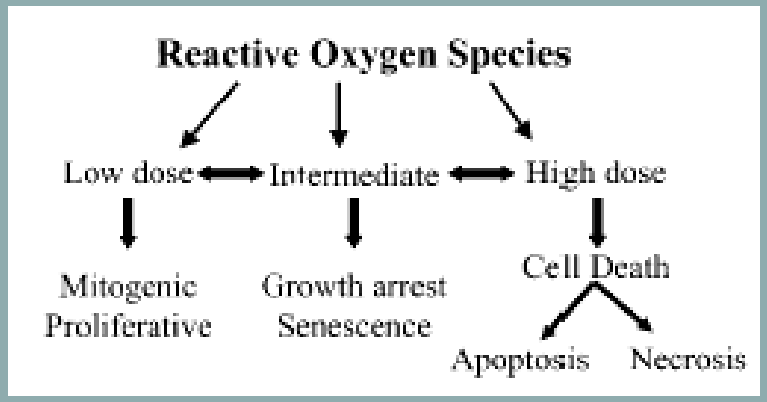
Blood components: **serum, plasma, whole blood**

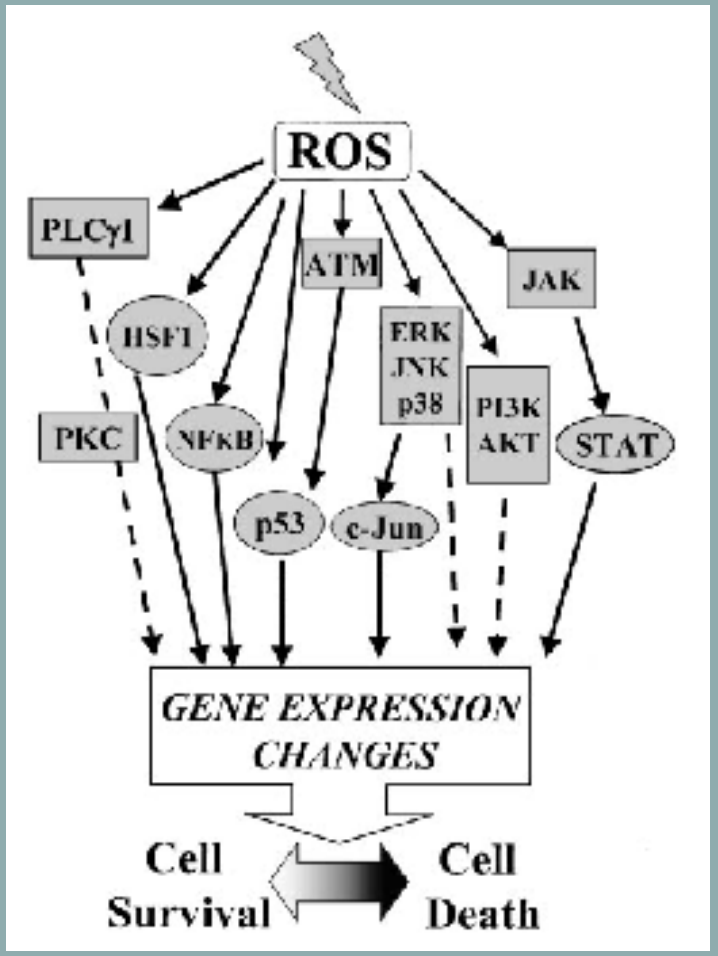
Frozen prostate tissue

Kim J, 2008

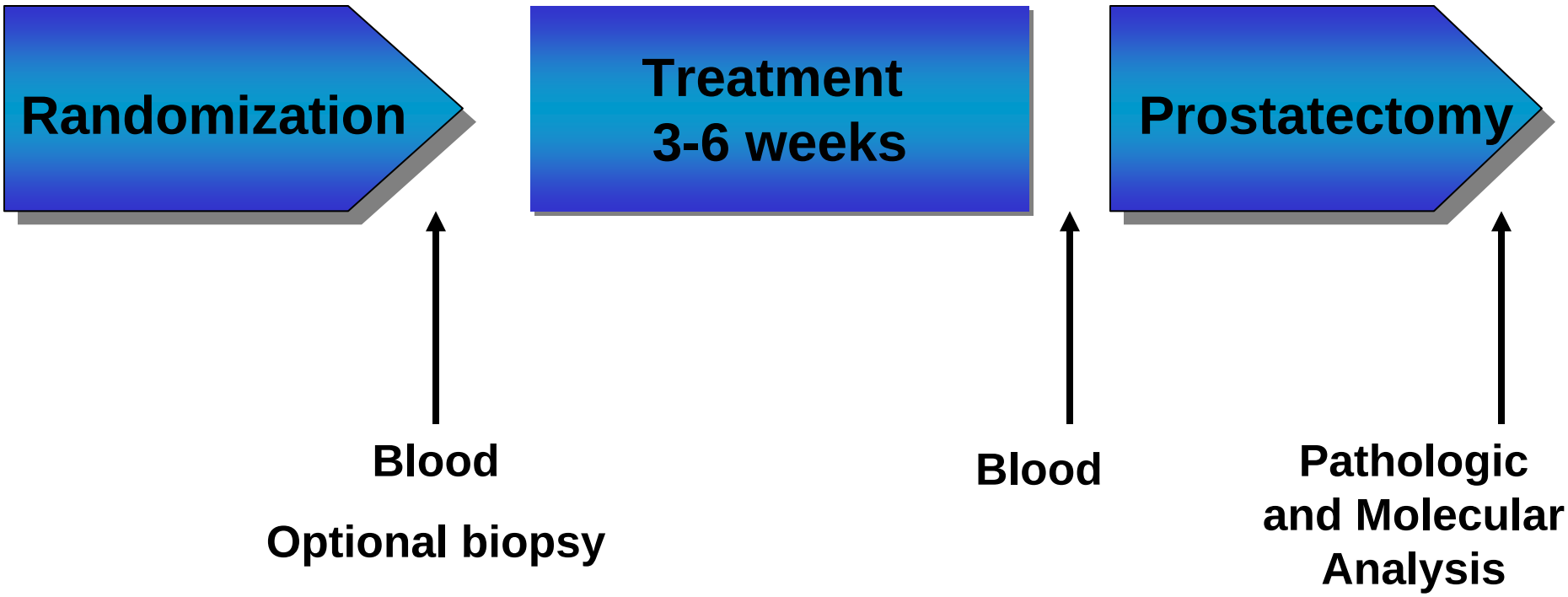

FasL
Fas
FADD
Pro-caspase-8
DNA Damage
p53
Fas
p21
Bax
Bcl-2
Bcl$_{XL}$
Cytochrome C
Apaf-1/Caspase-9
Caspase-8
Mitochondria
CDK4,6
CDK2
Cyclin D1, D2, D3
Cyclin E
Growth factors
Ras, Myc
Rb
Rbp
PTEN
P13-K
Akt
IKK
IκB
p65
Ub
Par-4
Ca^{2+}
Bcl-2
Caspase-3
IAPs
ER
p50
p65
APOPTOSIS

Response to Oxidative Stress Is Complex and Includes Changes in Gene Expression



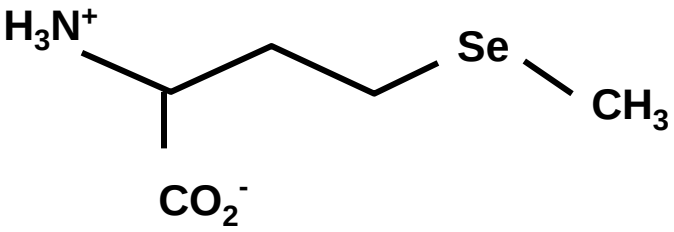


Study Schema



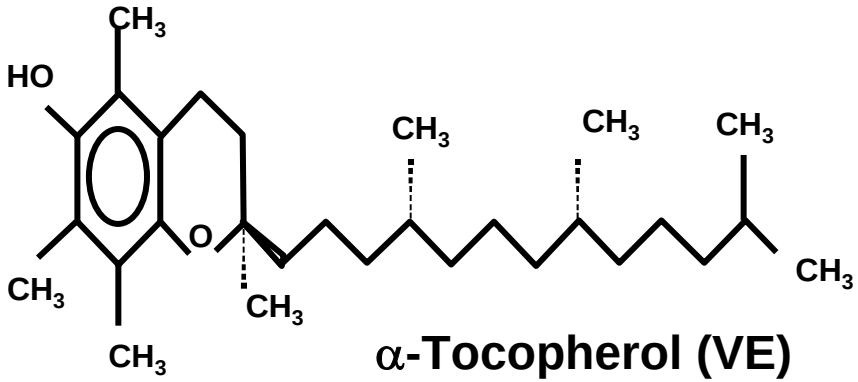
Kim J, 2008

Treatment Plan



l-Selenomethionine (SeMet)

200 µg



α-Tocopherol (VE)

400 IU

Kim J, 2008

Discriminating Molecular Signature

Table R3. Logistic analysis with response variable (Gleason score) and independent variables (molecular marker expressions) represented by involvement

Molecular Marker	GS[1]	Involvement 0,1,2	Involvement 3	Total	Univariable Analysis Odds Ratio	Univariable Analysis *P* value	Multivariable Model[2] Odds Ratio	Multivariable Model[2] *P* value
Bax	6	6 (40%)	9 (60%)	15	6	.179		
	7	1 (10%)	9 (90%)	10				
	Total	7	18	25				
Bcl-2	6	15 (100%)	0 (0%)	15				
	7	9 (90%)	1 (10%)	10				
	Total	24	1	30				
Bcl-XL	6	0 (0%)	15 (100%)	15				
	7	0 (0%)	10 (100%)	10				
	Total	0	25	25				
Bin1	6	9 (60%)	6 (40%)	15	6	.099		
	7	2 (20%)	8 (80%)	10				
	Total	11	14	25				
FAS	6	14 (93.3%)	1 (6.7%)	15	21	.007	16.14	.028
	7	4 (40%)	6 (60%)	10				
	Total	18	7	25				
MDM2	6	11 (73%)	4 (27%)	15	6.4	.049	4.46	.155
	7	3 (30%)	7 (70%)	10				
	Total	14	11	25				
p21	6	6 (40%)	9 (60%)	15	6	.179		
	7	1 (10%)	9 (90%)	10				
	Total	7	18	25				
p53	6	15 (100%)	0 (0%)	15	3.2*	.4		
	7	9 (90%)	1 (10%)	10				
	Total	24	1	25				
p65	6	9 (60%)	6 (40%)	15	13.5	.018		
	7	1 (10%)	9 (90%)	10				
	Total	10	15	25				
p27	6	7 (50%)	7 (50%)	14	2.3	.327		
	7	3 (30%)	7 (70%)	10				
	Total	10	14	24				

[1] GS is the response variable, and molecular marker expressions, represented by involvement, are predictors in the logistic model.

[2] GS is the response variable and the involvement measurements for *FAS* and *MDM2* are predictors.

Discriminating Molecular Signature—*Continued*

Table R4. Logistic analysis (GEE approach) based on the three highest values of molecular marker involvement for each patient using the GENMOD procedure in SAS

MOLECULAR MAKRER	GS[1]	INVOLVEMENT 0,1,2	INVOLVEMENT 3	TOTAL	UNIVARIABLE MODEL BY GEE[1] CHI-SQUARE	UNIVARIABLE MODEL BY GEE[1] *P* VALUE	MULTIVARIABLE MODEL BY GEE[1] CHI-SQUARE	MULTIVARIABLE MODEL BY GEE[1] *P* VALUE
Bax	6	21 (47%)	24 (53%)	45	2.55	.11		
	7	5 (17%)	25 (83%)	30				
	Total	26	49	75				
Bcl-2	6	45 (100%)	0 (0%)	45	not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
Bcl-XL	6	1 (2.2%)	44 (97.8%)	45	not converged	N/A		
	7	0 (0%)	30 (100%)	30				
	Total	1	74	75				
Bin1	6	31 (68.9%)	14 (31.1%)	45	3.81	.051		
	7	9 (30%)	21 (70%)	30				
	Total	40	35	75				
FAS	6	43 (95.6%)	2 (4.4%)	45	5.53	.019	2.98	.084
	7	17 (56.7%)	13 (43.3%)	30				
	Total	60	15	75				
MDM2	6	33 (73%)	12 (27%)	45	1.31	.2523		
	7	15 (50%)	15 (50%)	30				
	Total	48	27	75				
p21	6	20 (44.4%)	25 (55.6%)	45	3.73	.053		
	7	4 (13.3%)	26 (86.7%)	30				
	Total	24	51	75				
p53	6	45 (100%)	0 (0%)	45	not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
p65	6	32 (71%)	13 (29%)	45	6.17	.013	3.53	.06
	7	7 (23.3%)	23 (66.7%)	30				
	Total	39	36	75				
p27	6	21 (56.8%)	16 (43.2%)	37	1.13	.287		
	7	11 (36.7%)	19 (63.3%)	30				
	Total	32	35	67				

[1] Gleason score (GS) is response variable, and molecular marker involvement is predictor in the logistic model.

Discriminating Molecular Signature—*Continued*

Table R5. Logistic analysis (GEE approach) based on three random values of involvement for each patient using the GENMOD procedure in SAS

Molecular Marker	GS[1]	Involvement 0,1,2	Involvement 3	Total	Univariable Model by GEE[1] Chi-Square	Univariable Model by GEE[1] *P* Value	Multivariable Model by GEE[1] Chi-Square	Multivariable Model by GEE[1] *P* Value
Bax	6	21 (47%)	24 (53%)	45	.17	.676		
	7	10 (33%)	25 (67%)	30				
	Total	31	44	75				
Bcl-2	6	45 (100%)	0 (0%)	45	Not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
Bcl-XL	6	1 (2.2%)	44 (97.8%)	45	Not converged	N/A		
	7	0 (0%)	30 (100%)	30				
	Total	1	74	75				
Bin1	6	31 (68.9%)	14 (31.1%)	45	2.56	.109		
	7	12 (40%)	18 (60%)	30				
	Total	43	32	75				
FAS	6	43 (95.6%)	2 (4.4%)	45	4.95	.026	3.29	.07
	7	20 (66.7%)	10 (33.3%)	30				
	Total	63	12	75				
MDM2	6	33 (73%)	12 (27%)	45	1.29	.255		
	7	19 (63%)	11 (37%)	30				
	Total	52	23	75				
p21	6	20 (44.4%)	25 (55.6%)	45	.08	.774		
	7	12 (13.3%)	18 (86.7%)	30				
	Total	32	43	75				
p53	6	45 (100%)	0 (0%)	45	Not converged	N/A		
	7	28 (90%)	2 (10%)	30				
	Total	73	2	75				
p65	6	32 (71%)	13 (29%)	45	2.09	.148	1.1	.294
	7	16 (53%)	14 (47%)	30				
	Total	48	27	75				

[1] Gleason score (GS) is the response variable, and molecular marker involvement is the predictor in the logistic model.

Eligibility
Inclusion Criteria

- Clinical T1c/T2, GS $\leq$ 7, and PSA < 10
- Scheduled prostatectomy 4–6 weeks from study entry
- Life expectancy $\geq$ 10 years
- Performance status $\leq$ 2 (ECOG scale)

Kim J, 2008

Eligibility
Inclusion Criteria—*Continued*

- Agrees not to take DHEA, phytoestrogen supplements, antiandrogen agents, dutasteride, or finasteride while on study, independent of pill provided by MDACC
- Agrees to have tissue blocks of the prostatectomy specimen used for molecular marker studies
- Is scheduled for prostatectomy
- Agrees to use adequate contraception prior to study entry and for the duration of study participation
- Signs an informed consent

Kim J, 2008

Eligibility
Exclusion Criteria

- Active malignancy at any other site
- Prior radiation therapy for treatment of the primary tumor
- Participation in another investigational study within one month before enrollment
- History of allergic reactions attributed to compounds similar to finasteride in chemical or biological composition

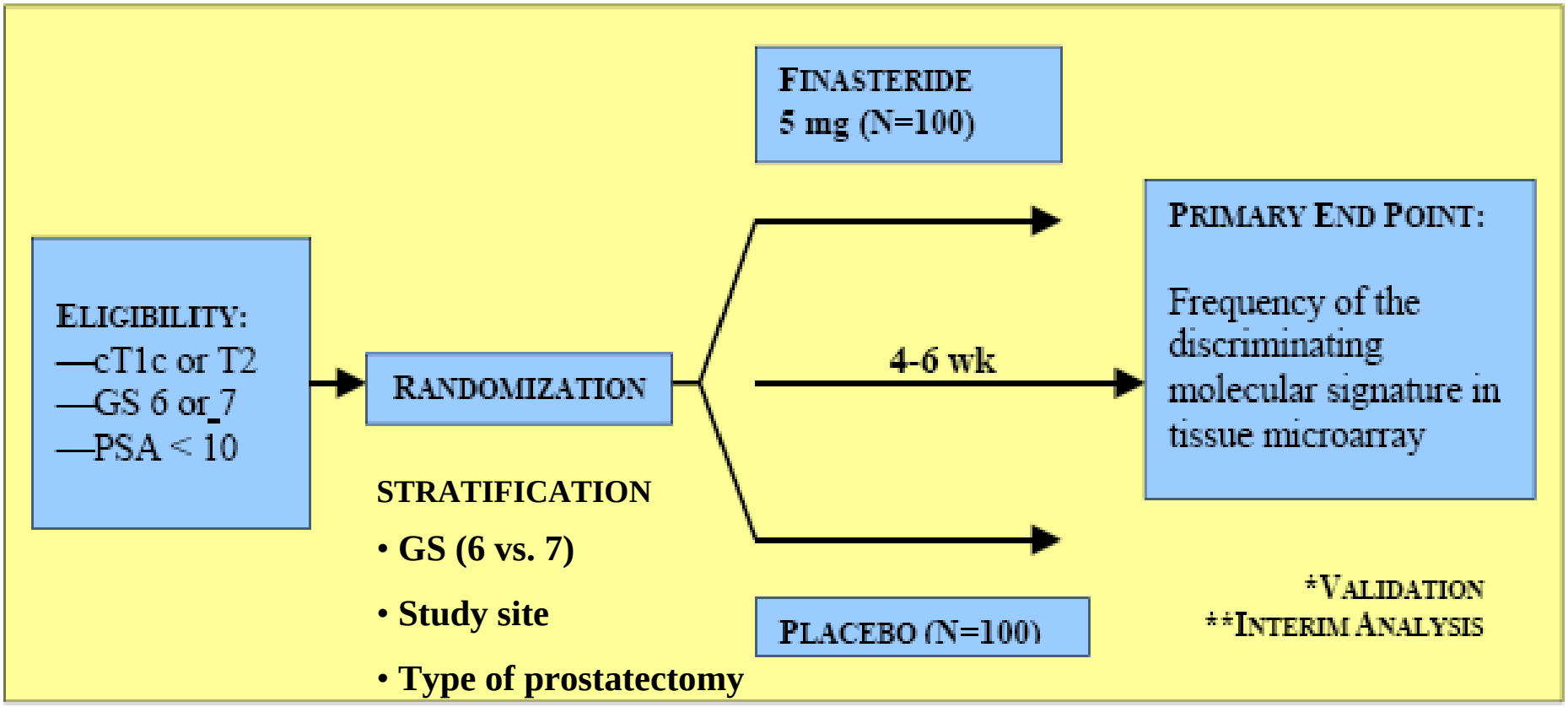
Kim J, 2008

Eligibility
Exclusion Criteria—*Continued*

- Uncontrolled intercurrent illness
- Use of any anticoagulation agents except daily aspirin (81–325 mg)
- Use of all hormonal agents, including dutasteride and finasteride, within 6 months of study entry
- Use of chemotherapy within 6 months of study entry

Kim J, 2008

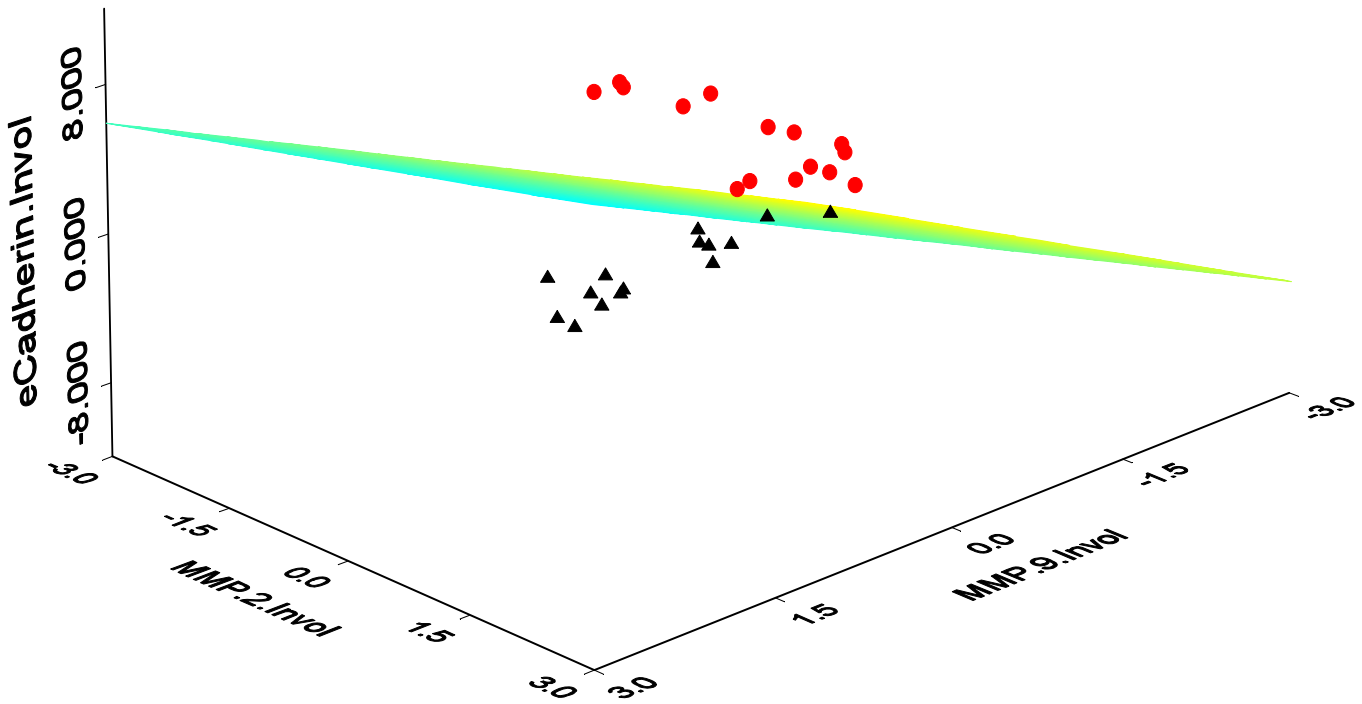
Study Schema—*Continued*



*VALIDATION (AFTER THE FIRST 52 PATIENTS [26 IN EACH TREATMENT ARM])
**INTERIM ANALYSIS (AFTER THE FIRST 100 PATIENTS [50 IN EACH TREATMENT ARM])

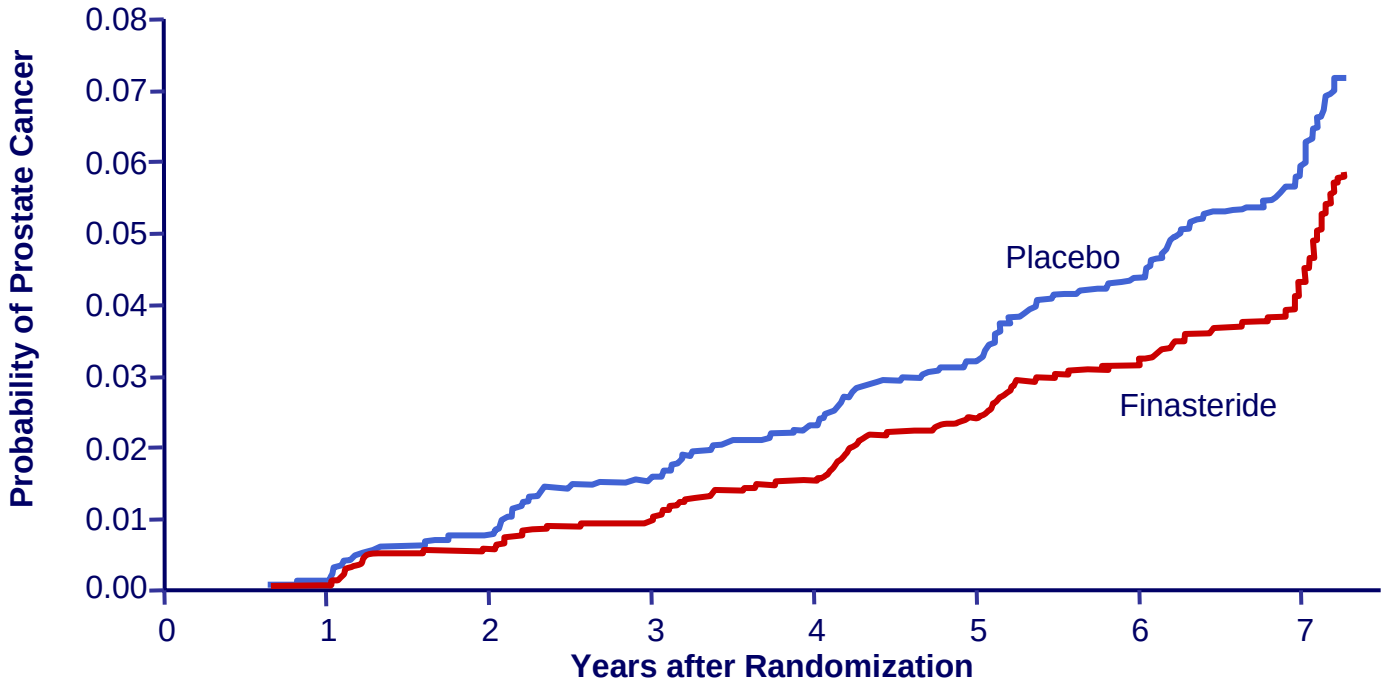
Kim J, 2008

3D plot of the relative expression of MMP2, MMP9 and e-cadherin the plane is determined by discriminant analysis R = Ecad/(3MMP9 +MMP2)



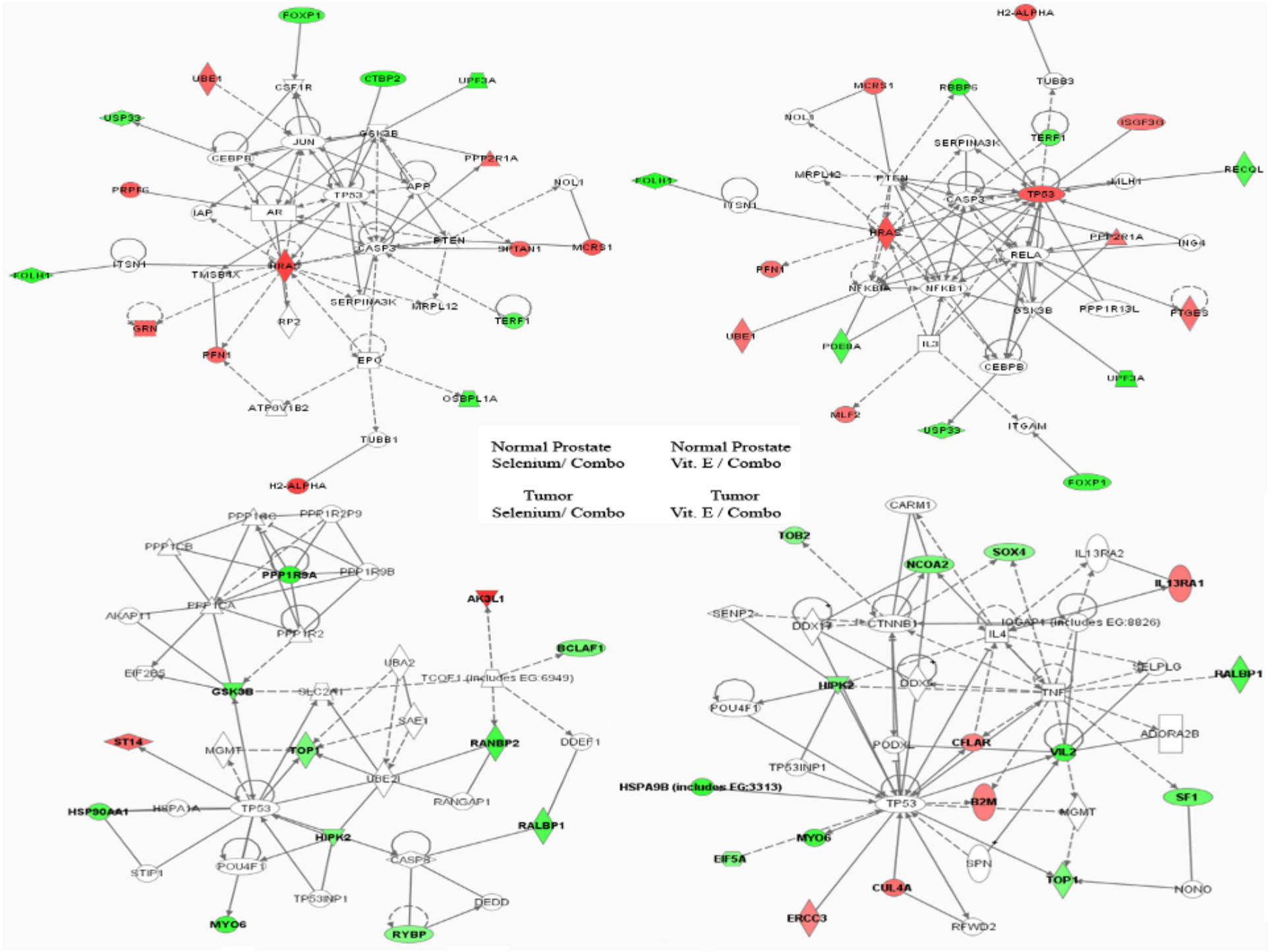
Efstathiou E, 2008

Prostate Cancer Prevention Trial



Placebo group							
Biopsy rate (%)	3.0	2.8	2.2	2.9	2.8	2.6	7.1
Total no. of cancers diagnosed	48	71	60	80	92	96	124
No. of grade 7-10 cancers	5	6	15	35	24	24	38
Finasteride group							
Biopsy rate (%)	3.3	2.0	2.1	2.5	2.1	2.2	7.0
Total no. of cancers diagnosed	42	35	39	68	78	51	122
No. of grade 7-10 cancers	11	11	17	31	28	26	64

Thompson et al., 2003


Normal Prostate
Selenium/ Combo
Normal Prostate
Vit. E / Combo
Tumor
Selenium/ Combo
Tumor
Vit. E / Combo

Figures From Manuscript

Slide 1 = Connectivity networks including p53. Interconnections of differentially expressed genes found uniquely in selenium or vitamin E treatments, and in common with combination treatment are shown. Networks that contain p53 are included in this figure. Red refers to up-regulation and green to down-regulation with respect to placebo

Slide 2 = Validation of 21 genes with quantitative PCR

Kim J, 2008

Coordinated Modulation of Sonic Hedgehog and Androgen Signaling in the Prostate Cancer Microenvironment by Chemo-Hormonal Therapy.

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Department of Genitourinary Medical Oncology[1], Pathology[2], Biostatistics[3] and Urology[4]

University of Texas MD Anderson Cancer Center,

Houston, Texas.

Background

Sonic hedgehog (Shh) and androgen signaling are implicated experimentally in prostate development, regeneration and neoplastic progression.

We assessed expression of components of these pathways in preoperatively treated and control high risk prostate cancers (PCa) in a hypothesis generating search for an association with resistance to therapy.

Efstathiou E, 2008

Methods I

Sixty-Four men with high risk PCa were randomized to receive either complete androgen ablation (AA) or AA and Ketoconazole, Adriamycin, Vinblastine and Estramustine (KAVE) for 16 weeks followed by radical prostatectomy (RP).

We constructed 3 Tissue Microarrays from RP specimens of:

a) 26 patients pretreated with AA,

b) 27 patients pretreated with AA + KAVE (CH)

c) 26 untreated patients (Control)

Patients were matched for clinical stage, biopsy Gleason Score and PSA at diagnosis.

Preoperative Characteristics		Control	Hormonally ablated	Chemo-hormonally treated
Clinical Stage at diagnosis	T2a	9	8	7
	T2b	14	17	14
	T3	3	1	6
Biopsy Gleason Score (GS)	7	10	9	10
	At least 1 biopsy GS≥8	16	17	17
Median PSA (ng/dl) (Range)		8 (2.2-38.6)	8 (2.2-130.8)	11 (0.7-205)
PSA>10ng/dl		10	10	12
PSA<10ng/dl		16	16	15

Table 1: Clinical characteristics used to match RPS across groups for TMA construction

Methods II

A total of 3178 cores were obtained [median cores: 36 (range 18-90), median blocks: 5 (range 3-9) per specimen].

Immunohistochemical expression of Shh pathway components (Shh ligand, smoothened and gli2) and Androgen Receptor (AR) were assessed in the tumor epithelium and stroma.

Scoring for involvement (percentage of cells exhibiting detectable staining of the marker of interest) was performed by a 11- point system:

0:0-1%, 10: 2-10%, 20: 11-20%, 30: 21-30%, 40: 31-40%, 50: 41-50%, 60: 51-60%, 70: 61-70%, 80:71-80%, 90:81-90%, 100: 91-100%.

Statistics: A mixed-effects model was used for fitting the correlated data with regard to involvement (multiple observations from a subject), and the between and within variations were estimated from the model. Pearson's correlations between biomarkers were calculated based on mean expression.

Results (I)

- **Hedgehog signaling** in the residual tumor epithelium and adjacent stroma of samples treated with androgen ablation or the combination of AA with chemotherapy was higher than in controls.

This was indicated by the difference in :

  - a. the mean nuclear expression of the transcription factor gli2, considered the main downstream effector of human hedgehog signaling,
  - b. the expression of the hedgehog signaling intermediate smoothened, considered the limiting factor of shh pathway activation in PCa and
  - c. the ligand Sonic Hedgehog.

	Mean control (sd)	Mean AA (sd)	Mean CH (sd)	P value Control vs AA	P value Control vs CH
Gli2 Epithelium	**39.6 (20.7)**	**79.6 (17.2)**	**85.5 (11.9)**	**<0.0001**	**<0.0001**
Gli2 Stroma	**22.9 (15.2)**	**43.3 (17.9)**	**54.7 (15.4)**	**<0.0001**	**<0.0001**
Smoothened Epithelium	**68.9 (18.2)**	**81.1 (14.2)**	**83.5 (8.9)**	**0.0047**	**0.0008**
Smoothened Stroma	**16.1 (11.2)**	**21.6 (10.7)**	**28.2 (13.9)**	**0.118**	**0.0011**
Shh Epithelium	**49.9 (24.8)**	**61.3 (19.2)**	**62.6 (22.4)**	**0.085**	**0.05**
Shh Stroma	**4.6 (6.5)**	**12.8 (13.5)**	**19.4 (13.9)**	**0.0203**	**0.0001**

Efstathiou E, 2008

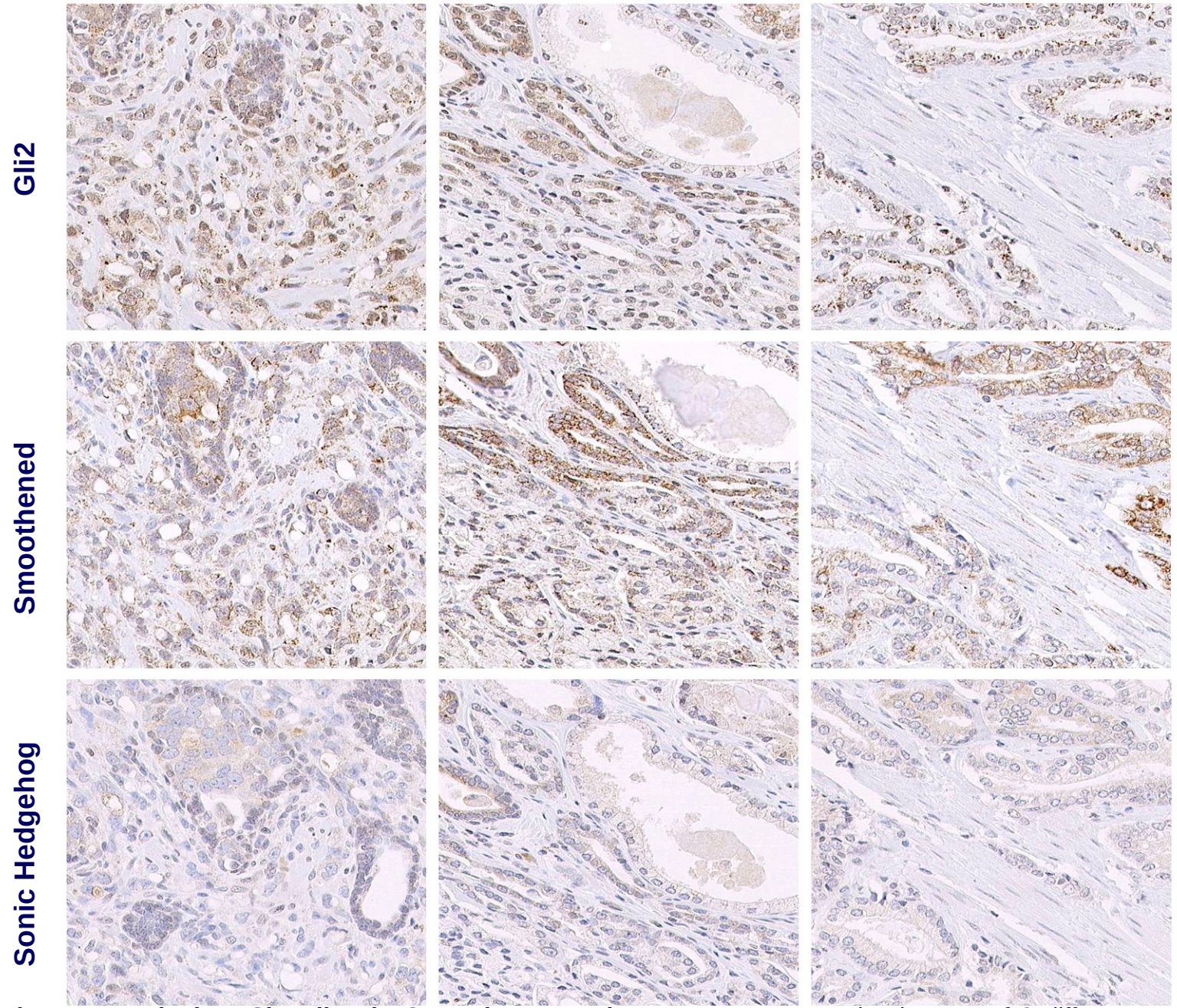


Figure 1: **Hedgehog Signaling in Control-Untreated tumors**. Representative images of 3 different untreated tumors. Active hedgehog signaling is heterogeneous and limited compared to that of treated tumors (figure 2,3) as illustrated by the expression of Gli2 and smoothened.

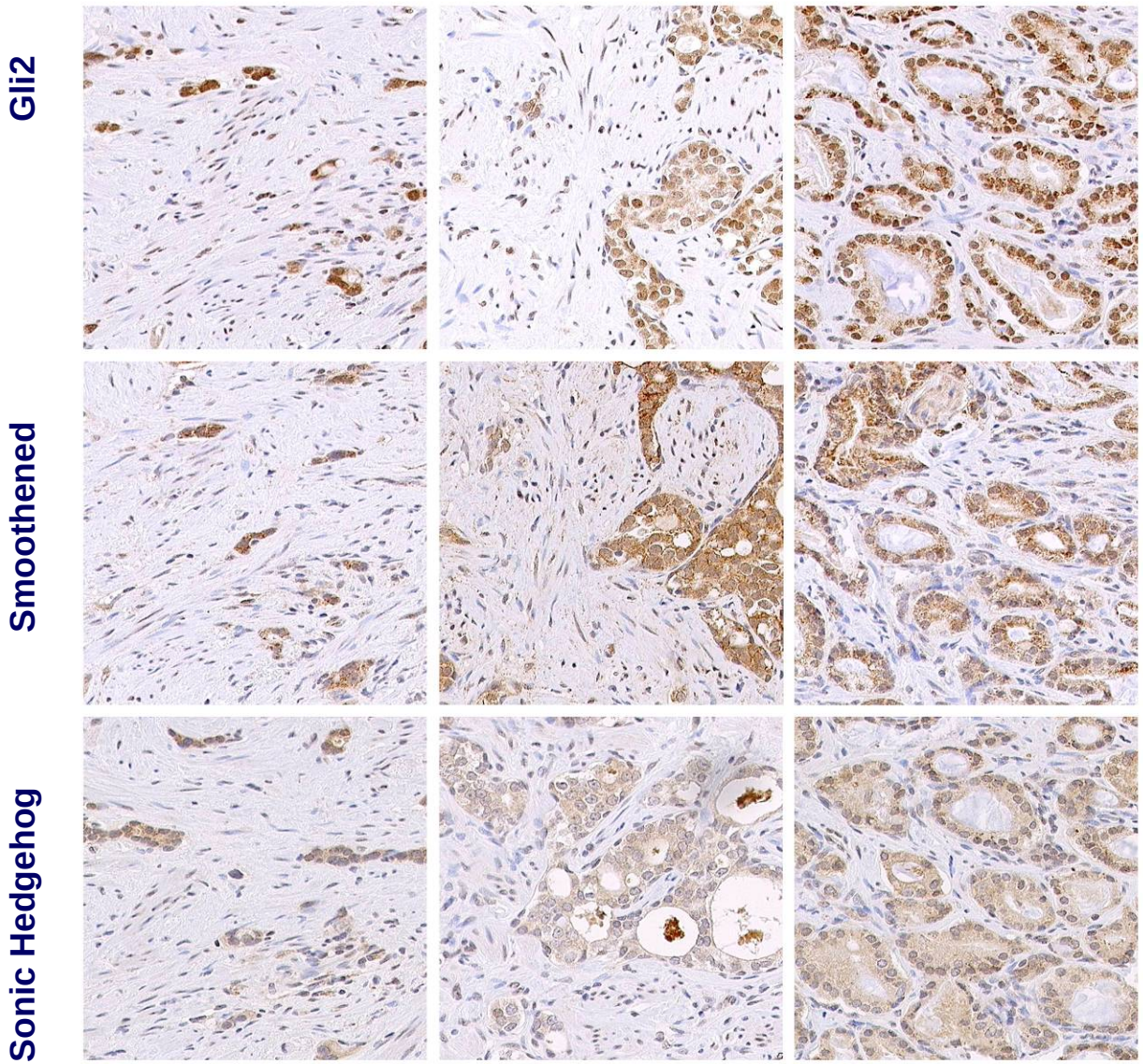


Figure 2: Increased Hedgehog Signaling in Residual tumors following androgen ablation. Representative images of 3 different radical prostatectomy specimens with varied extent of residual tumor. Expression of all components of hedgehog signaling assessed (Gli2, Smoothened and Sonic hedgehog) is higher than in untreated controls **(Figure 1)** Hedgehog signaling is active both in the residual tumor epithelium and stroma as indicated by the nuclear expression of the transcription factor gli2.

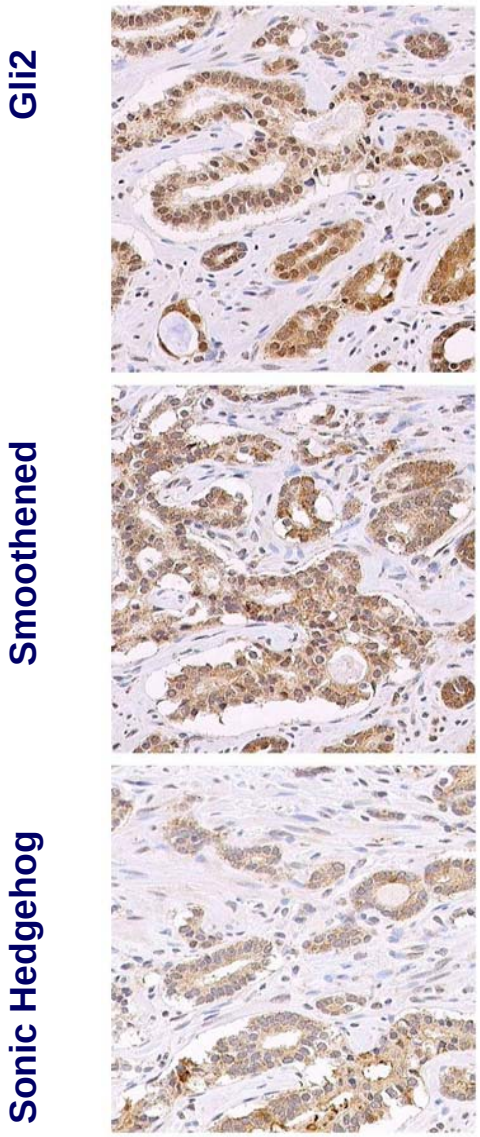


Figure 3: Increased Hedgehog Signaling in Residual tumor following androgen ablation and chemotherapy (KAVE)

Results (II)

- **Active hedgehog signaling** was parallel in the tumor epithelium and adjacent stroma as indicated by mean nuclear expression of gli2 (0.78 by Pearson’s correlation)

	Gli2. Epithelium	Gli2 Stroma	Smoothened Epithelium	Smoothened Stroma	Shh Epithelium	Shh Stroma
Gli2. Epithelium	1	**0.789**	0.516	0.359	0.349	0.545
Gli2 Stroma	**0.789**	1	0.443	0.601	0.305	0.731
Smoothened Epithelium	0.516	0.443	1	0.449	0.496	0.359
Smoothened Stroma	0.359	0.601	0.449	1	0.172	0.552
Shh Epithelium	0.349	0.305	0.496	0.172	1	0.333
Shh Stroma	0.545	0.731	0.352	0.552	0.333	1

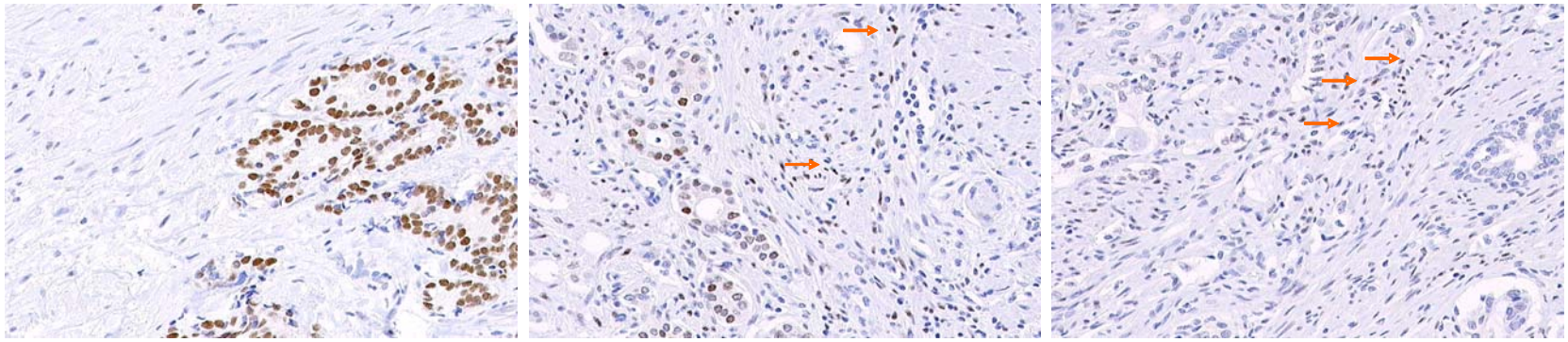


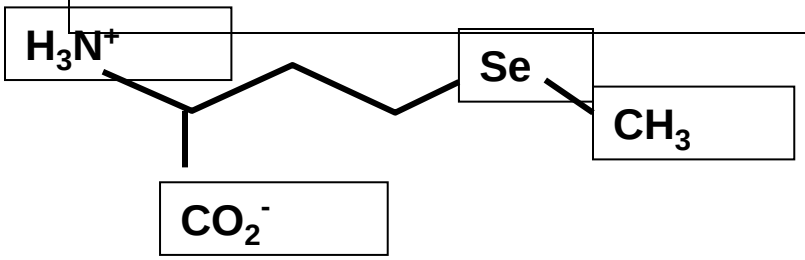
Figure 4: Androgen Receptor expression. There was a trend for lower androgen receptor expression in the tumor epithelium following AA (middle panel) and CH (right panel) for 16 weeks versus untreated control tumors (left panel). Interestingly when this occurred, adjacent stroma exhibited an increase in AR expression (arrows).

	Mean control (sd)	Mean AA (sd)	Mean CH (sd)	P value Control vs AA	P value Control vs CH
AR Epithelium	**59.4 (24.2)**	**47.4 (22.2)**	**47.8 (21.1)**	**0.077**	**0.087**
AR Stroma	**6.7 (11.6)**	**8.6 (10.2)**	**10.1 (8.2)**	**0.522**	**0.253**

Conclusions

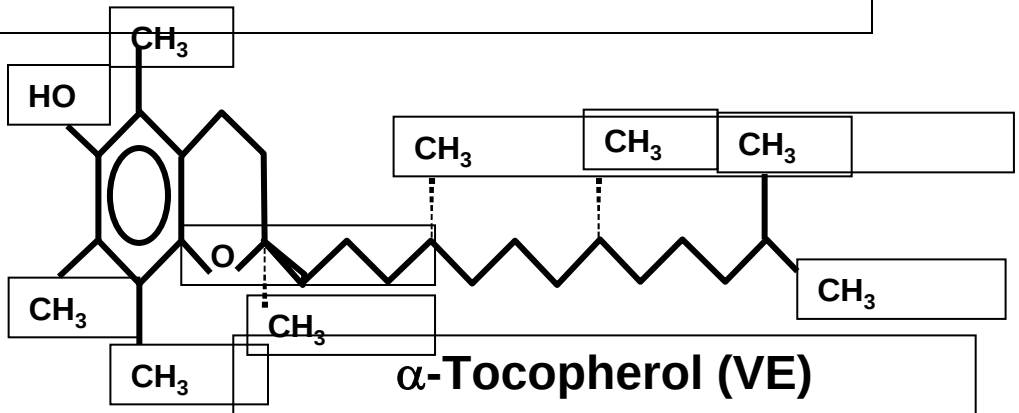
- Sonic Hedgehog and androgen pathways are modulated in a manner that suggests they behave in a compensatory fashion , and are determinants of therapy response. These data support the hypothesis that the tumor microenvironment is implicated in PCa therapy resistance.

Treatment Plan



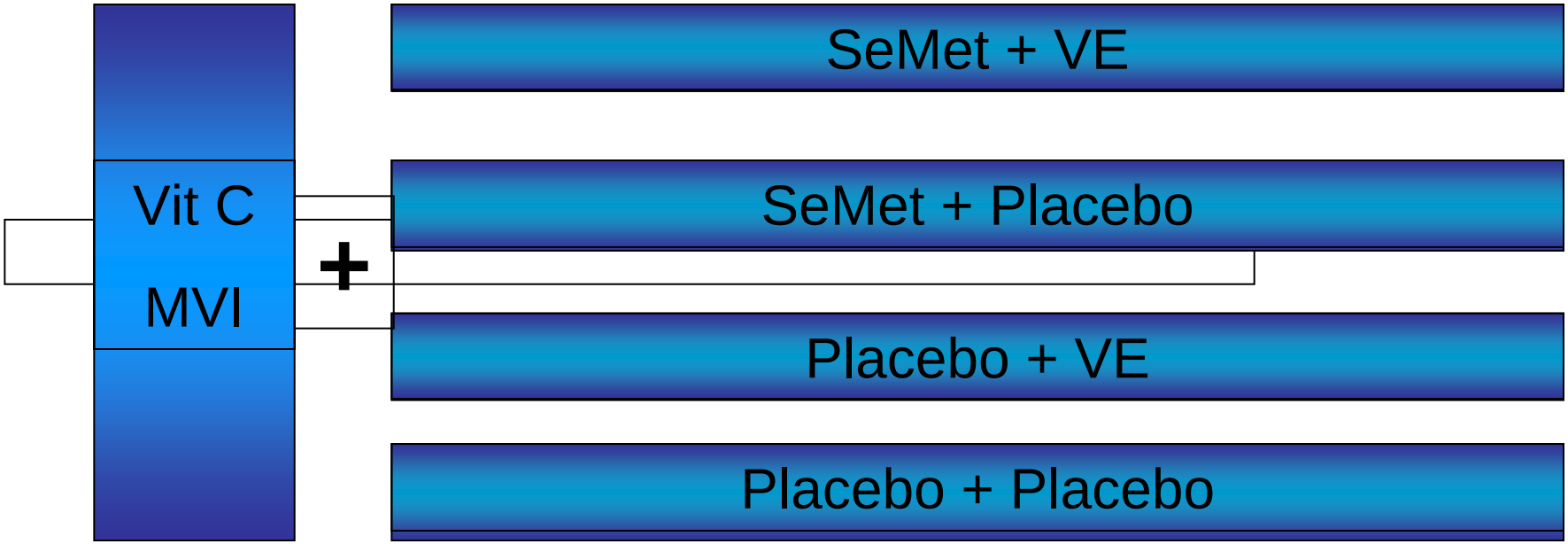
l-Selenomethionine (SeMet)

200 μg



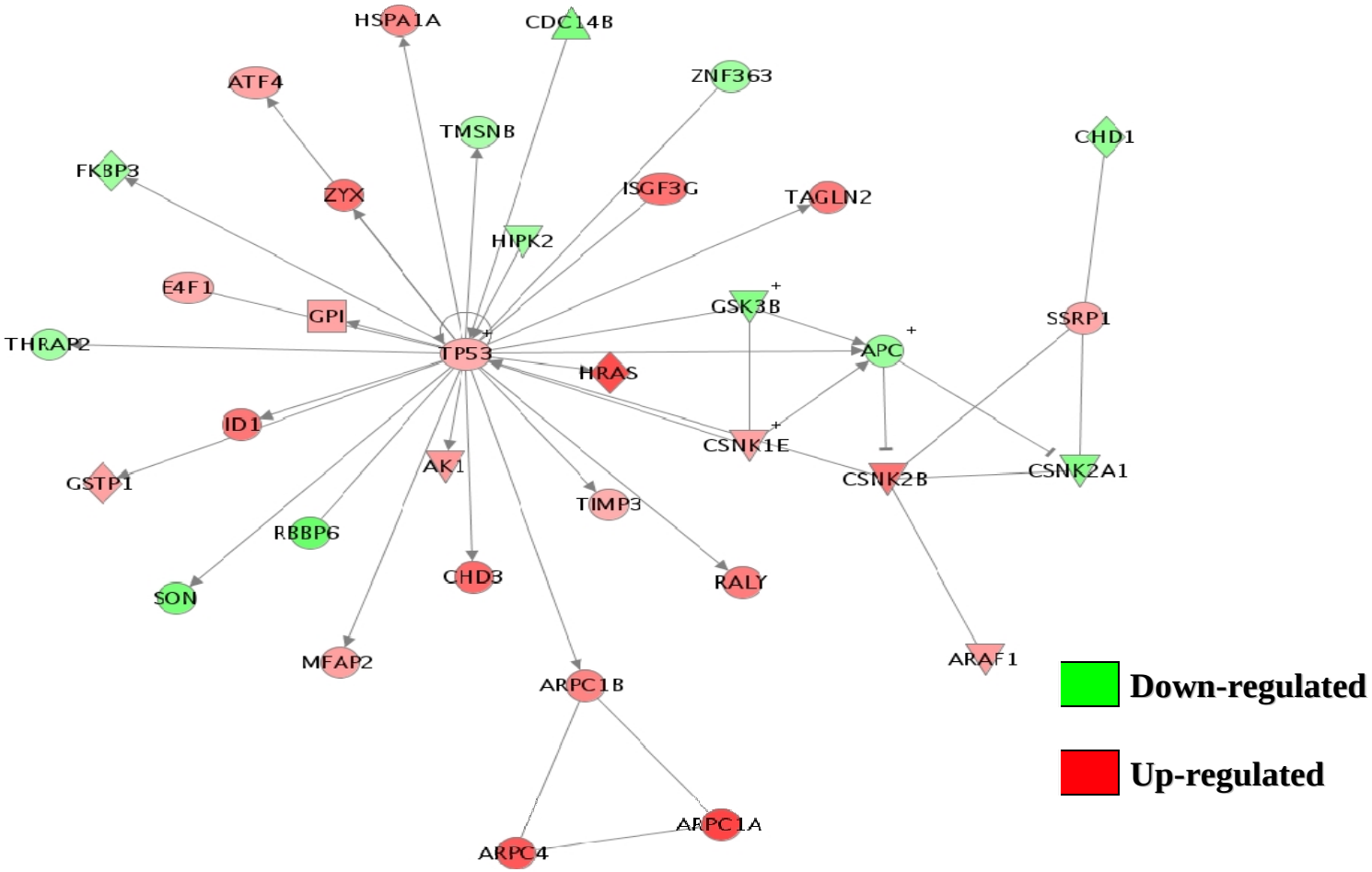
α-Tocopherol (VE)

400 IU

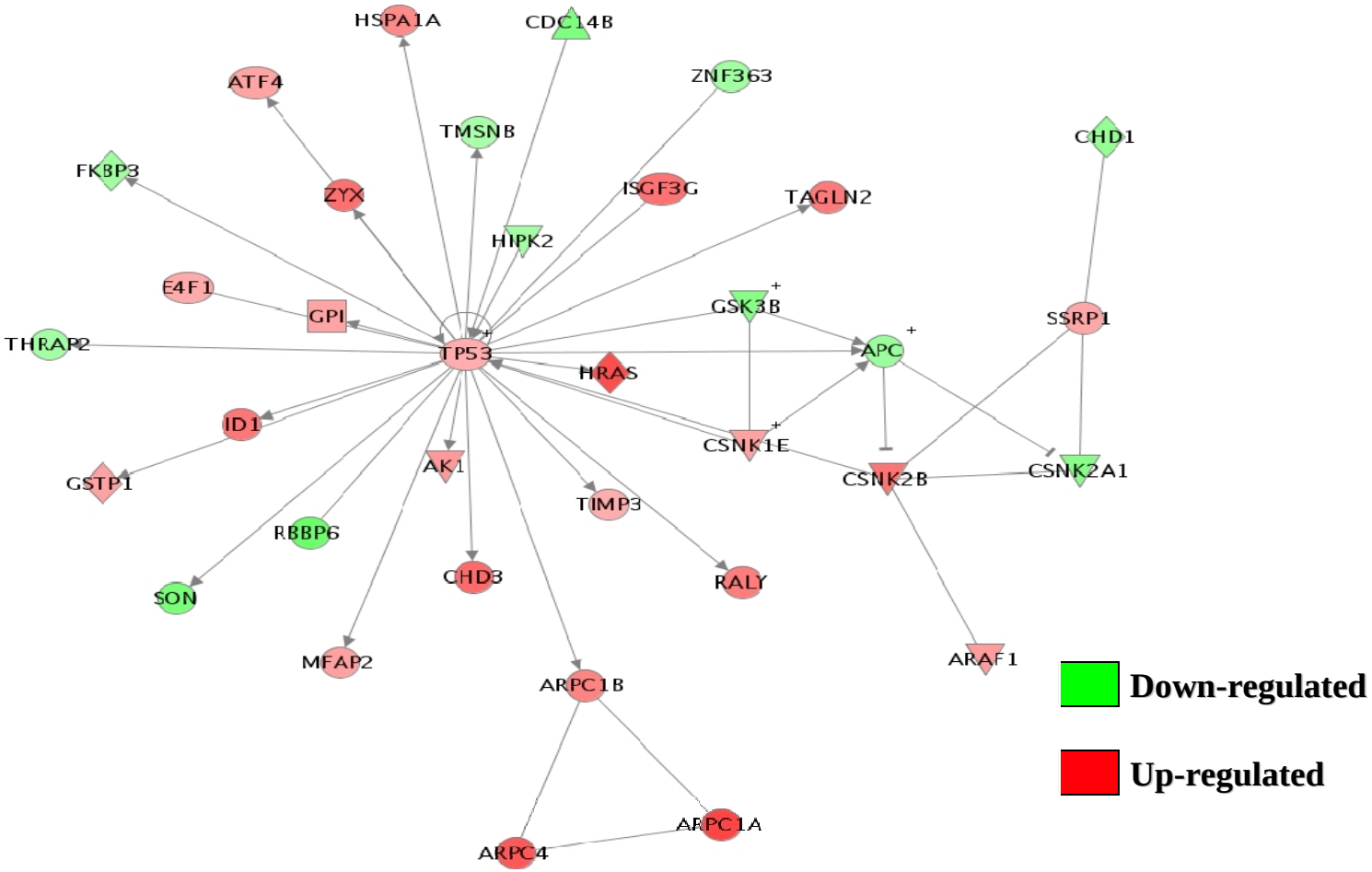


Kim J, 2008

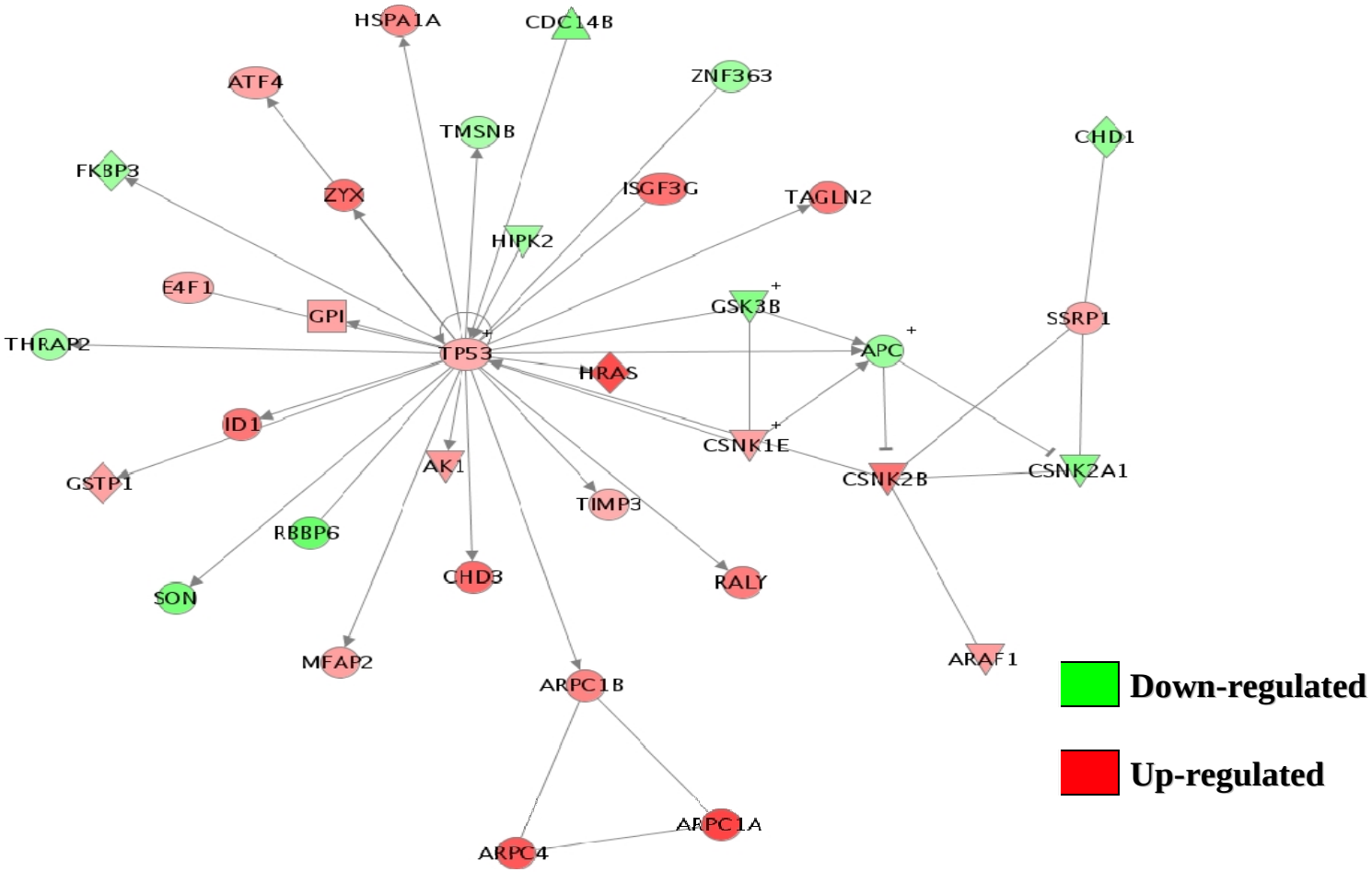
Selenium Treated Non-neoplastic Epithelium Network Analysis



Selenium Treated Non-neoplastic Epithelium Network Analysis



Selenium Treated Non-neoplastic Epithelium Network Analysis



Discriminating Molecular Signature

Table R3. Logistic analysis with response variable (Gleason score) and independent variables (molecular marker expressions) represented by involvement

Molecular Marker	GS[1]	Involvement 0,1,2	Involvement 3	Total	Univariable Analysis Odds Ratio	Univariable Analysis *P* value	Multivariable Model[2] Odds Ratio	Multivariable Model[2] *P* value
Bax	6	6 (40%)	9 (60%)	15	6	.179		
	7	1 (10%)	9 (90%)	10				
	Total	7	18	25				
Bcl-2	6	15 (100%)	0 (0%)	15				
	7	9 (90%)	1 (10%)	10				
	Total	24	1	30				
Bcl-XL	6	0 (0%)	15 (100%)	15				
	7	0 (0%)	10 (100%)	10				
	Total	0	25	25				
Bin1	6	9 (60%)	6 (40%)	15	6	.099		
	7	2 (20%)	8 (80%)	10				
	Total	11	14	25				
FAS	6	14 (93.3%)	1 (6.7%)	15	21	.007	16.14	.028
	7	4 (40%)	6 (60%)	10				
	Total	18	7	25				
MDM2	6	11 (73%)	4 (27%)	15	6.4	.049	4.46	.155
	7	3 (30%)	7 (70%)	10				
	Total	14	11	25				
p21	6	6 (40%)	9 (60%)	15	6	.179		
	7	1 (10%)	9 (90%)	10				
	Total	7	18	25				
p53	6	15 (100%)	0 (0%)	15	3.2*	.4		
	7	9 (90%)	1 (10%)	10				
	Total	24	1	25				
p65	6	9 (60%)	6 (40%)	15	13.5	.018		
	7	1 (10%)	9 (90%)	10				
	Total	10	15	25				
p27	6	7 (50%)	7 (50%)	14	2.3	.327		
	7	3 (30%)	7 (70%)	10				
	Total	10	14	24				

[1] GS is the response variable, and molecular marker expressions, represented by involvement, are predictors in the logistic model.

[2] GS is the response variable and the involvement measurements for *FAS* and *MDM2* are predictors.

Discriminating Molecular Signature—*Continued*

Table R4. Logistic analysis (GEE approach) based on the three highest values of molecular marker involvement for each patient using the GENMOD procedure in SAS

MOLECULAR MAKRER	GS[1]	INVOLVEMENT 0,1,2	INVOLVEMENT 3	TOTAL	UNIVARIABLE MODEL BY GEE[1] CHI-SQUARE	UNIVARIABLE MODEL BY GEE[1] *P* VALUE	MULTIVARIABLE MODEL BY GEE[1] CHI-SQUARE	MULTIVARIABLE MODEL BY GEE[1] *P* VALUE
Bax	6	21 (47%)	24 (53%)	45	2.55	.11		
	7	5 (17%)	25 (83%)	30				
	Total	26	49	75				
Bcl-2	6	45 (100%)	0 (0%)	45	not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
Bcl-XL	6	1 (2.2%)	44 (97.8%)	45	not converged	N/A		
	7	0 (0%)	30 (100%)	30				
	Total	1	74	75				
Bin1	6	31 (68.9%)	14 (31.1%)	45	3.81	.051		
	7	9 (30%)	21 (70%)	30				
	Total	40	35	75				
FAS	6	43 (95.6%)	2 (4.4%)	45	5.53	.019	2.98	.084
	7	17 (56.7%)	13 (43.3%)	30				
	Total	60	15	75				
MDM2	6	33 (73%)	12 (27%)	45	1.31	.2523		
	7	15 (50%)	15 (50%)	30				
	Total	48	27	75				
p21	6	20 (44.4%)	25 (55.6%)	45	3.73	.053		
	7	4 (13.3%)	26 (86.7%)	30				
	Total	24	51	75				
p53	6	45 (100%)	0 (0%)	45	not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
p65	6	32 (71%)	13 (29%)	45	6.17	.013	3.53	.06
	7	7 (23.3%)	23 (66.7%)	30				
	Total	39	36	75				
p27	6	21 (56.8%)	16 (43.2%)	37	1.13	.287		
	7	11 (36.7%)	19 (63.3%)	30				
	Total	32	35	67				

[1] Gleason score (GS) is response variable, and molecular marker involvement is predictor in the logistic model.

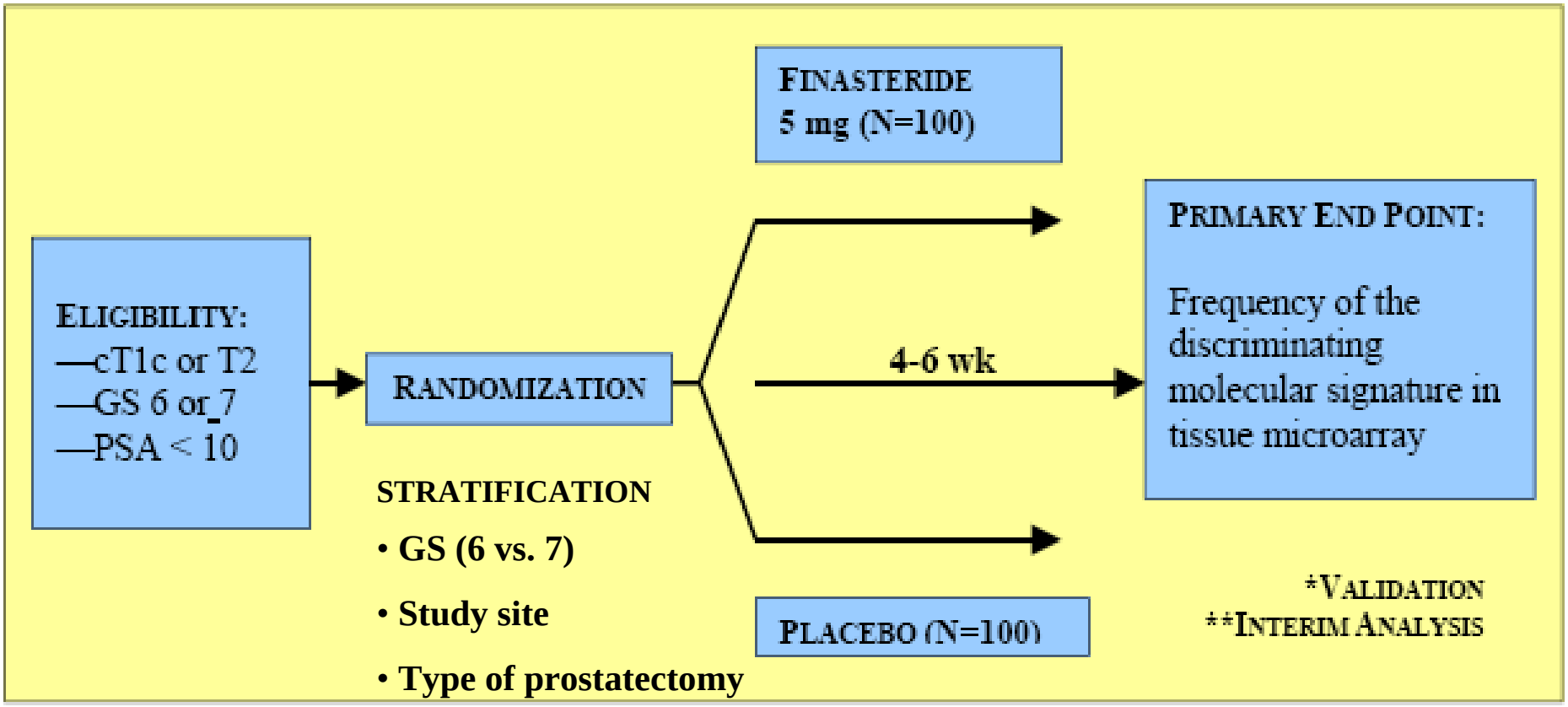
Discriminating Molecular Signature—*Continued*

Table R5. Logistic analysis (GEE approach) based on three random values of involvement for each patient using the GENMOD procedure in SAS

Molecular Marker	GS[1]	Involvement 0,1,2	Involvement 3	Total	Univariable Model by GEE[1] Chi-Square	Univariable Model by GEE[1] *P* Value	Multivariable Model by GEE[1] Chi-Square	Multivariable Model by GEE[1] *P* Value
Bax	6	21 (47%)	24 (53%)	45	.17	.676		
	7	10 (33%)	25 (67%)	30				
	Total	31	44	75				
Bcl-2	6	45 (100%)	0 (0%)	45	Not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
Bcl-XL	6	1 (2.2%)	44 (97.8%)	45	Not converged	N/A		
	7	0 (0%)	30 (100%)	30				
	Total	1	74	75				
Bin1	6	31 (68.9%)	14 (31.1%)	45	2.56	.109		
	7	12 (40%)	18 (60%)	30				
	Total	43	32	75				
FAS	6	43 (95.6%)	2 (4.4%)	45	4.95	.026	3.29	.07
	7	20 (66.7%)	10 (33.3%)	30				
	Total	63	12	75				
MDM2	6	33 (73%)	12 (27%)	45	1.29	.255		
	7	19 (63%)	11 (37%)	30				
	Total	52	23	75				
p21	6	20 (44.4%)	25 (55.6%)	45	.08	.774		
	7	12 (13.3%)	18 (86.7%)	30				
	Total	32	43	75				
p53	6	45 (100%)	0 (0%)	45	Not converged	N/A		
	7	28 (90%)	2 (10%)	30				
	Total	73	2	75				
p65	6	32 (71%)	13 (29%)	45	2.09	.148	1.1	.294
	7	16 (53%)	14 (47%)	30				
	Total	48	27	75				

[1] Gleason score (GS) is the response variable, and molecular marker involvement is the predictor in the logistic model.

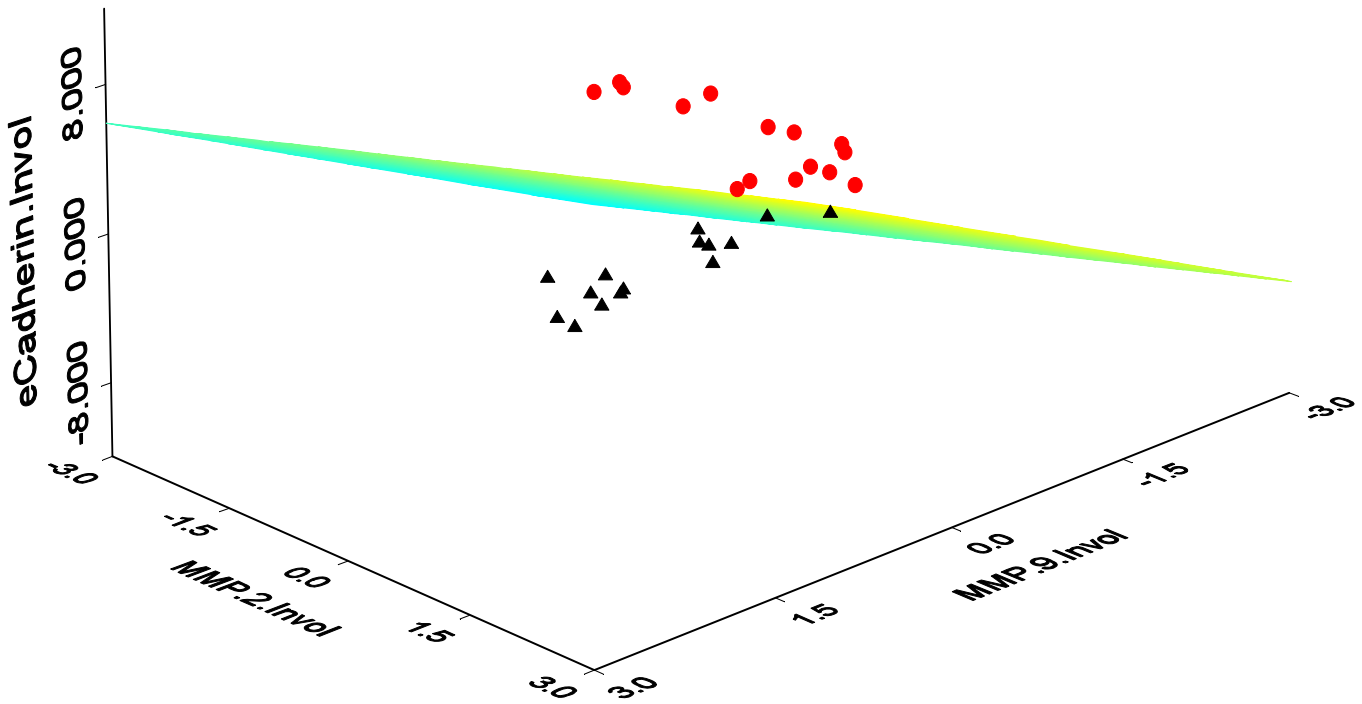
Study Schema—*Continued*



*VALIDATION (AFTER THE FIRST 52 PATIENTS [26 IN EACH TREATMENT ARM])
**INTERIM ANALYSIS (AFTER THE FIRST 100 PATIENTS [50 IN EACH TREATMENT ARM])

Kim J, 2008

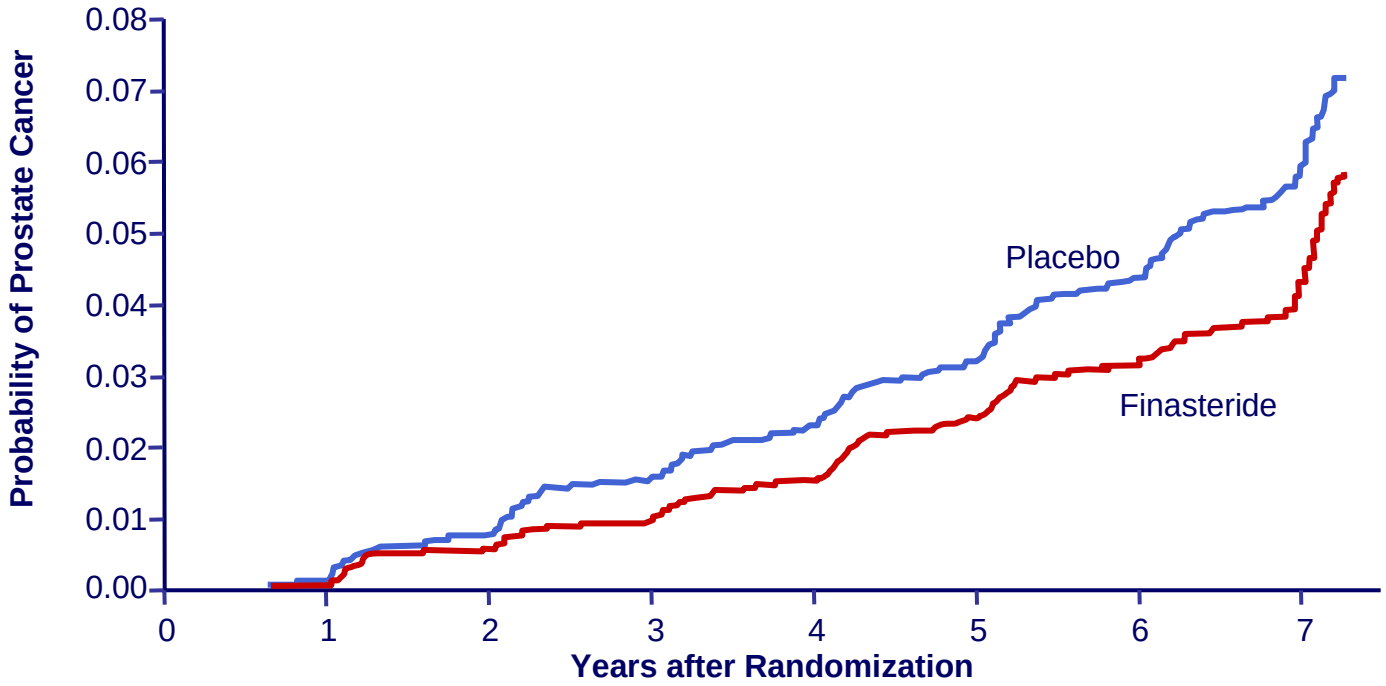
3D plot of the relative expression of MMP2, MMP9 and e-cadherin the plane is determined by discriminant analysis R = Ecad/(3MMP9 +MMP2)



Efstathiou E, 2008

RESULTS

Prostate Cancer Prevention Trial



Placebo group							
Biopsy rate (%)	3.0	2.8	2.2	2.9	2.8	2.6	7.1
Total no. of cancers diagnosed	48	71	60	80	92	96	124
No. of grade 7-10 cancers	5	6	15	35	24	24	38
Finasteride group							
Biopsy rate (%)	3.3	2.0	2.1	2.5	2.1	2.2	7.0
Total no. of cancers diagnosed	42	35	39	68	78	51	122
No. of grade 7-10 cancers	11	11	17	31	28	26	64

Thompson et al., 2003

Methods I

Sixty-Four men with high risk PCa were randomized to receive either complete androgen ablation (AA) or AA and Ketoconazole, Adriamycin, Vinblastine and Estramustine (KAVE) for 16 weeks followed by radical prostatectomy (RP).

We constructed 3 Tissue Microarrays from RP specimens of:

a) 26 patients pretreated with AA,

b) 27 patients pretreated with AA + KAVE (CH)

c) 26 untreated patients (Control)

Patients were matched for clinical stage, biopsy Gleason Score and PSA at diagnosis.

Preoperative Characteristics		Control	Hormonally ablated	Chemo-hormonally treated
Clinical Stage at diagnosis	T2a	9	8	7
	T2b	14	17	14
	T3	3	1	6
Biopsy Gleason Score (GS)	7	10	9	10
	At least 1 biopsy GS≥8	16	17	17
Median PSA (ng/dl) (Range)		8 (2.2-38.6)	8 (2.2-130.8)	11 (0.7-205)
PSA>10ng/dl		10	10	12
PSA<10ng/dl		16	16	15

Table 1: Clinical characteristics used to match RPS across groups for TMA construction

Methods II

A total of 3178 cores were obtained [median cores: 36 (range 18-90), median blocks: 5 (range 3-9) per specimen].

Immunohistochemical expression of Shh pathway components (Shh ligand, smoothened and gli2) and Androgen Receptor (AR) were assessed in the tumor epithelium and stroma.

Scoring for involvement (percentage of cells exhibiting detectable staining of the marker of interest) was performed by a 11- point system:

0:0-1%, 10: 2-10%, 20: 11-20%, 30: 21-30%, 40: 31-40%, 50: 41-50%, 60: 51-60%, 70: 61-70%, 80:71-80%, 90:81-90%, 100: 91-100%.

Statistics: A mixed-effects model was used for fitting the correlated data with regard to involvement (multiple observations from a subject), and the between and within variations were estimated from the model. Pearson's correlations between biomarkers were calculated based on mean expression.

Results (I)

- **Hedgehog signaling** in the residual tumor epithelium and adjacent stroma of samples treated with androgen ablation or the combination of AA with chemotherapy was higher than in controls.

This was indicated by the difference in :

  - a. the mean nuclear expression of the transcription factor gli2, considered the main downstream effector of human hedgehog signaling,
  - b. the expression of the hedgehog signaling intermediate smoothened, considered the limiting factor of shh pathway activation in PCa and
  - c. the ligand Sonic Hedgehog.

	Mean control (sd)	Mean AA (sd)	Mean CH (sd)	P value Control vs AA	P value Control vs CH
Gli2 Epithelium	39.6 (20.7)	79.6 (17.2)	85.5 (11.9)	<0.0001	<0.0001
Gli2 Stroma	22.9 (15.2)	43.3 (17.9)	54.7 (15.4)	<0.0001	<0.0001
Smoothened Epithelium	68.9 (18.2)	81.1 (14.2)	83.5 (8.9)	0.0047	0.0008
Smoothened Stroma	16.1 (11.2)	21.6 (10.7)	28.2 (13.9)	0.118	0.0011
Shh Epithelium	49.9 (24.8)	61.3 (19.2)	62.6 (22.4)	0.085	0.05
Shh Stroma	4.6 (6.5)	12.8 (13.5)	19.4 (13.9)	0.0203	0.0001

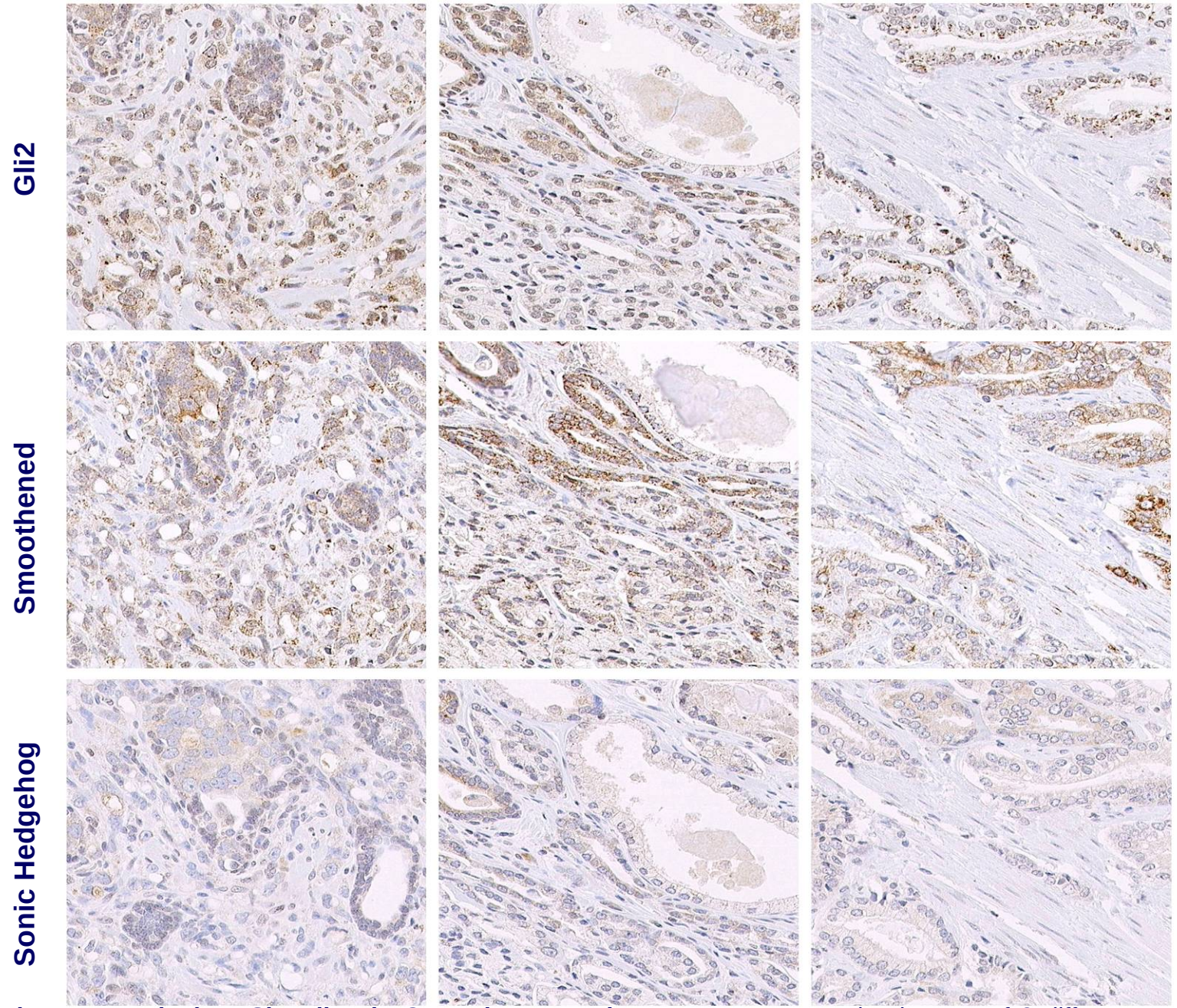


Figure 1: **Hedgehog Signaling in Control-Untreated tumors**. Representative images of 3 different untreated tumors. Active hedgehog signaling is heterogeneous and limited compared to that of treated tumors (figure 2,3) as illustrated by the expression of Gli2 and smoothened.

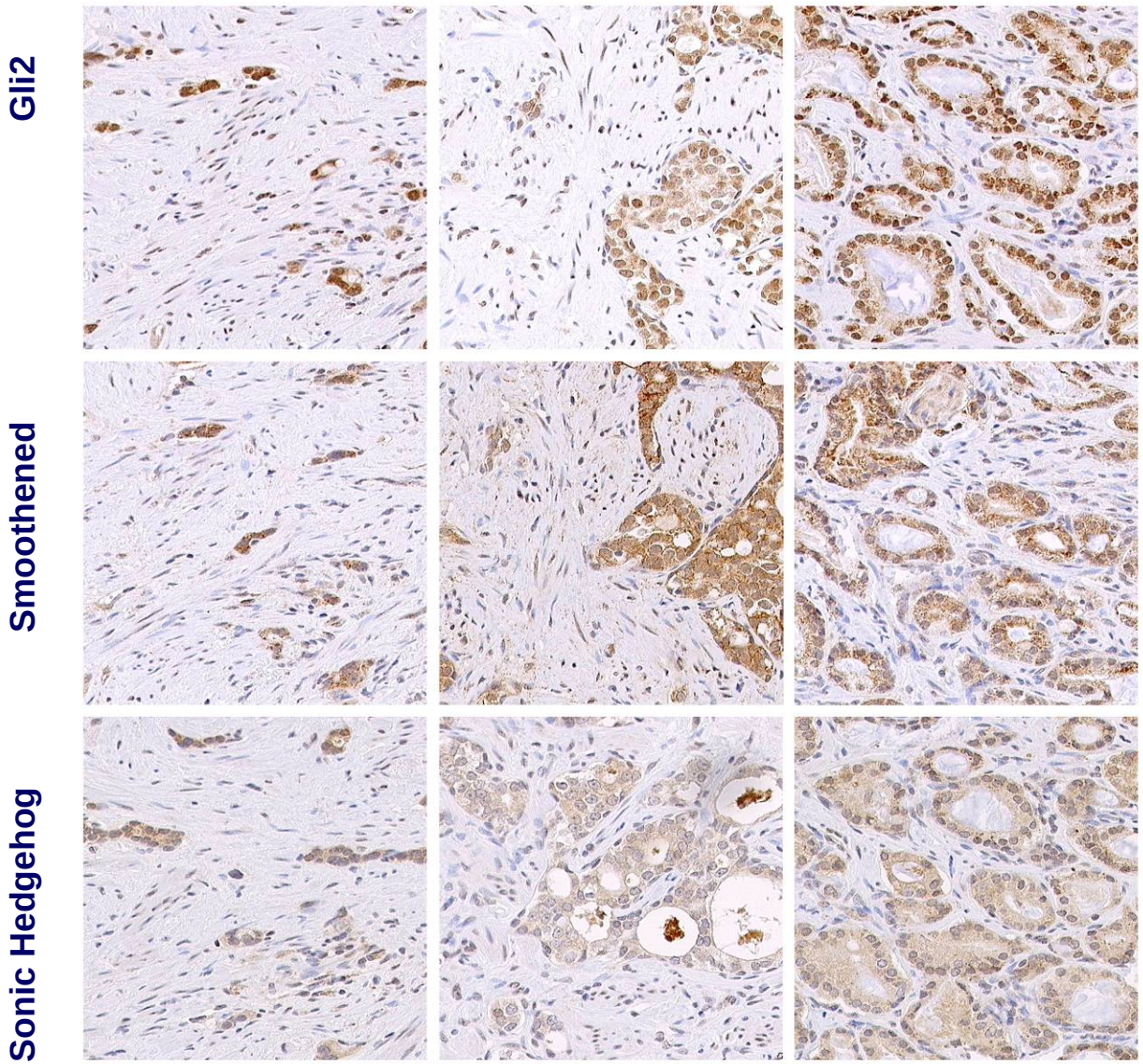


Figure 2: Increased Hedgehog Signaling in Residual tumors following androgen ablation. Representative images of 3 different radical prostatectomy specimens with varied extent of residual tumor. Expression of all components of hedgehog signaling assessed (Gli2, Smoothened and Sonic hedgehog) is higher than in untreated controls **(Figure 1)** Hedgehog signaling is active both in the residual tumor epithelium and stroma as indicated by the nuclear expression of the transcription factor gli2.

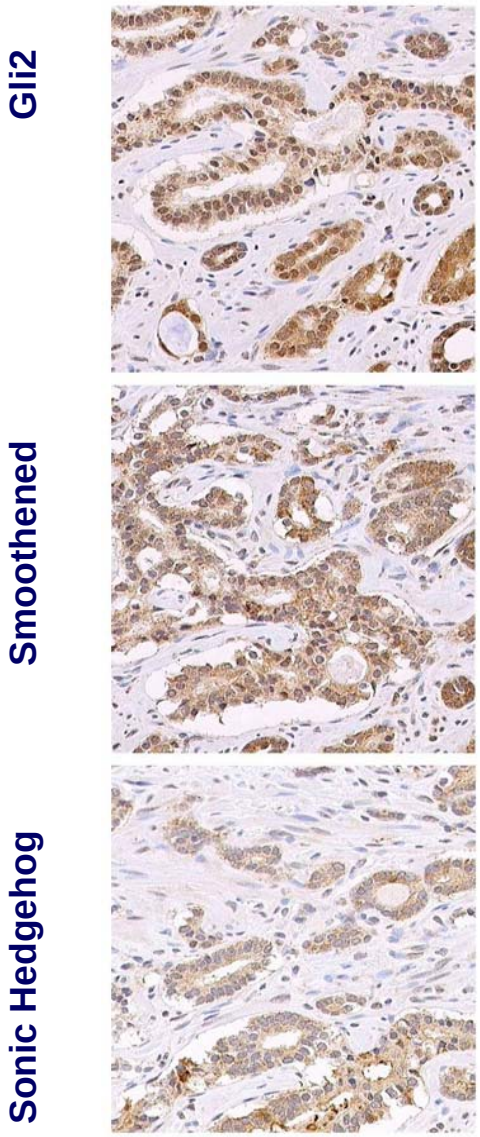


Figure 3: Increased Hedgehog Signaling in Residual tumor following androgen ablation and chemotherapy (KAVE)

Results (II)

- **Active hedgehog signaling** was parallel in the tumor epithelium and adjacent stroma as indicated by mean nuclear expression of gli2 (0.78 by Pearson’s correlation)

	Gli2. Epithelium	Gli2 Stroma	Smoothened Epithelium	Smoothened Stroma	Shh Epithelium	Shh Stroma
Gli2. Epithelium	1	**0.789**	0.516	0.359	0.349	0.545
Gli2 Stroma	**0.789**	1	0.443	0.601	0.305	0.731
Smoothened Epithelium	0.516	0.443	1	0.449	0.496	0.359
Smoothened Stroma	0.359	0.601	0.449	1	0.172	0.552
Shh Epithelium	0.349	0.305	0.496	0.172	1	0.333
Shh Stroma	0.545	0.731	0.352	0.552	0.333	1

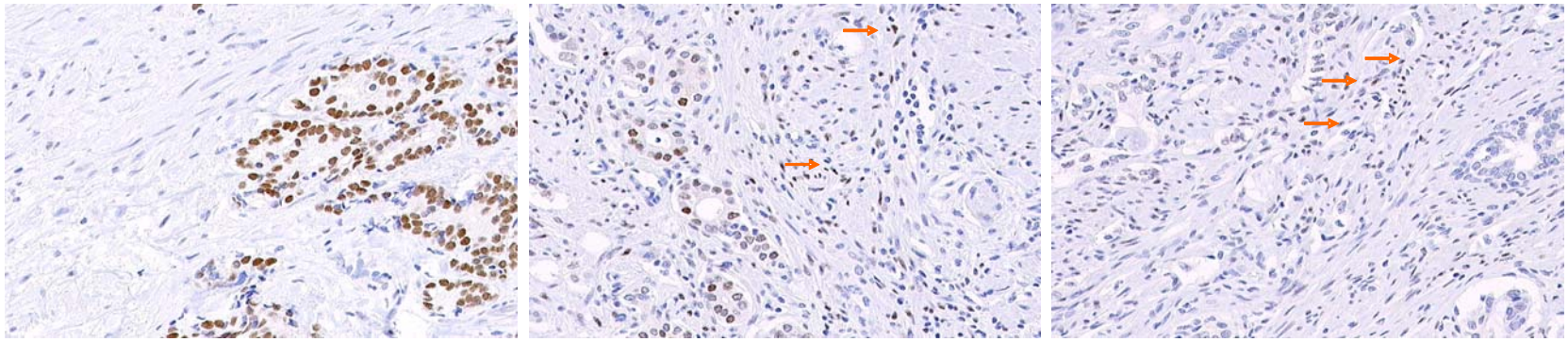


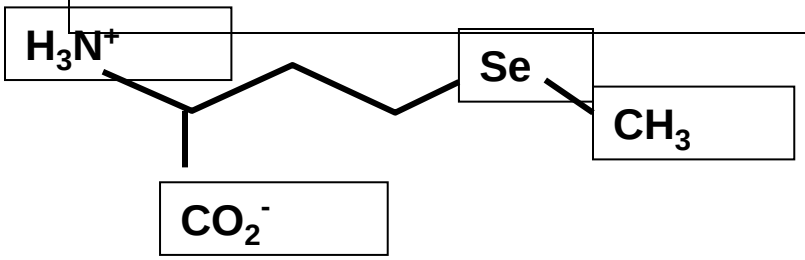
Figure 4: Androgen Receptor expression. There was a trend for lower androgen receptor expression in the tumor epithelium following AA (middle panel) and CH (right panel) for 16 weeks versus untreated control tumors (left panel). Interestingly when this occurred, adjacent stroma exhibited an increase in AR expression (arrows).

	Mean control (sd)	Mean AA (sd)	Mean CH (sd)	P value Control vs AA	P value Control vs CH
AR Epithelium	59.4 (24.2)	47.4 (22.2)	47.8 (21.1)	0.077	0.087
AR Stroma	6.7 (11.6)	8.6 (10.2)	10.1 (8.2)	0.522	0.253

Conclusions

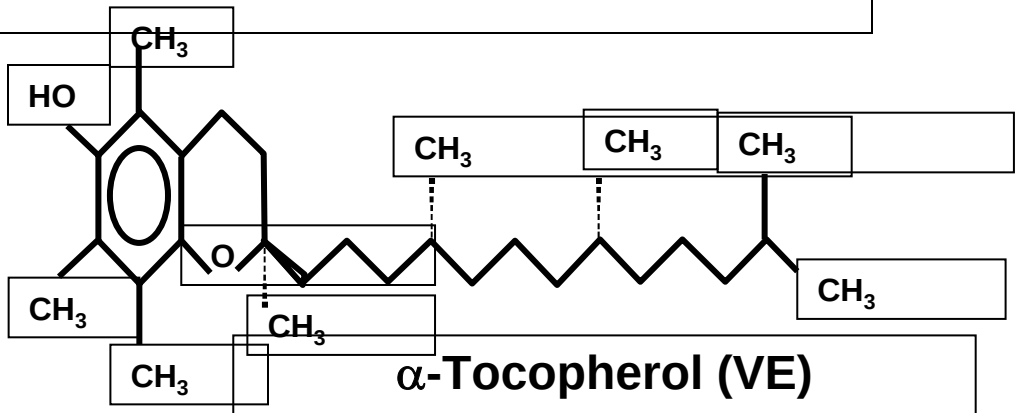
- Sonic Hedgehog and androgen pathways are modulated in a manner that suggests they behave in a compensatory fashion , and are determinants of therapy response. These data support the hypothesis that the tumor microenvironment is implicated in PCa therapy resistance.

Treatment Plan



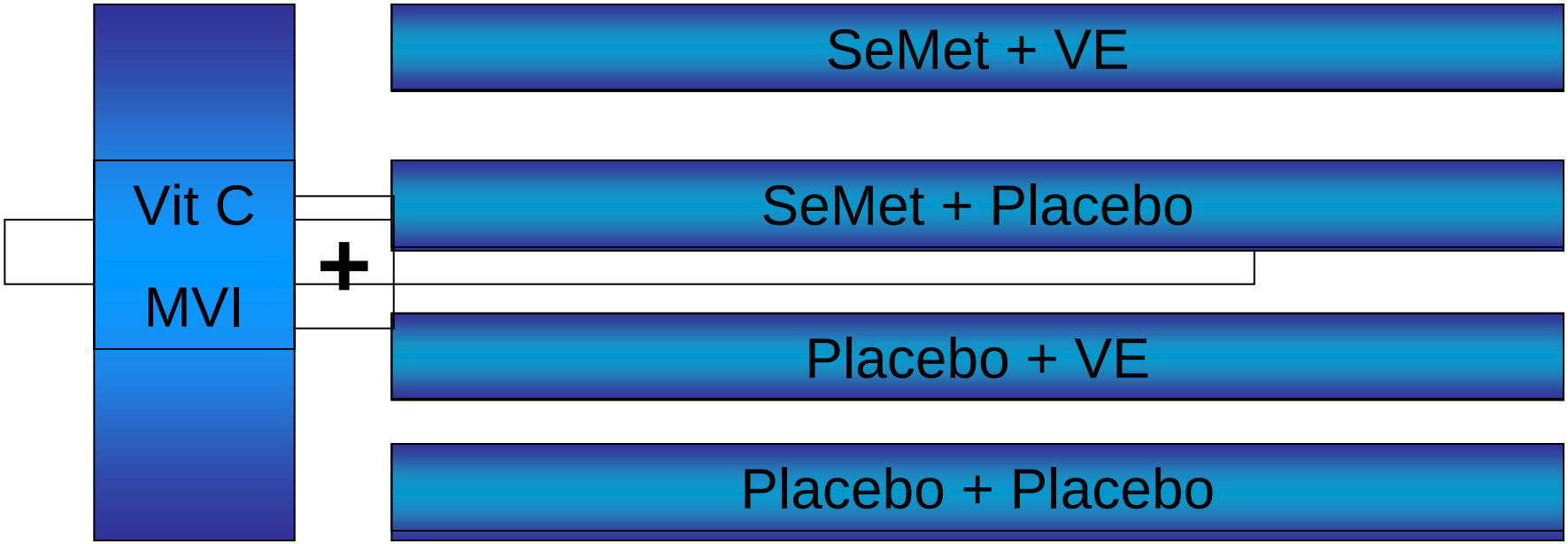
l-Selenomethionine (SeMet)

200 μg



α-Tocopherol (VE)

400 IU



Kim J, 2008

Table R3. Logistic analysis with response variable (Gleason score) and independent variables (molecular marker expressions) represented by involvement

Molecular Marker	GS[1]	Involvement		Total	Univariable Analysis		Multivariable Model[2]	
		0,1,2	3		Odds Ratio	*P* value	Odds Ratio	*P* value
Bax	6	6 (40%)	9 (60%)	15	6	.179		
	7	1 (10%)	9 (90%)	10				
	Total	7	18	25				
Bcl-2	6	15 (100%)	0 (0%)	15				
	7	9 (90%)	1 (10%)	10				
	Total	24	1	30				
Bcl-XL	6	0 (0%)	15 (100%)	15				
	7	0 (0%)	10 (100%)	10				
	Total	0	25	25				
Bin1	6	9 (60%)	6 (40%)	15	6	.099		
	7	2 (20%)	8 (80%)	10				
	Total	11	14	25				
FAS	6	14 (93.3%)	1 (6.7%)	15	21	.007	16.14	.028
	7	4 (40%)	6 (60%)	10				
	Total	18	7	25				
MDM2	6	11 (73%)	4 (27%)	15	6.4	.049	4.46	.155
	7	3 (30%)	7 (70%)	10				
	Total	14	11	25				
p21	6	6 (40%)	9 (60%)	15	6	.179		
	7	1 (10%)	9 (90%)	10				
	Total	7	18	25				
p53	6	15 (100%)	0 (0%)	15	3.2*	.4		
	7	9 (90%)	1 (10%)	10				
	Total	24	1	25				
p65	6	9 (60%)	6 (40%)	15	13.5	.018		
	7	1 (10%)	9 (90%)	10				
	Total	10	15	25				
p27	6	7 (50%)	7 (50%)	14	2.3	.327		
	7	3 (30%)	7 (70%)	10				
	Total	10	14	24				

[1] GS is the response variable, and molecular marker expressions, represented by involvement, are predictors in the logistic model.

[2] GS is the response variable and the involvement measurements for *FAS* and *MDM2* are predictors.

Table R4. Logistic analysis (GEE approach) based on the three highest values of molecular marker involvement for each patient using the GENMOD procedure in SAS

MOLECULAR MAKRER	GS[1]	INVOLVEMENT 0,1,2	INVOLVEMENT 3	TOTAL	UNIVARIABLE MODEL BY GEE[1] CHI-SQUARE	UNIVARIABLE MODEL BY GEE[1] *P* VALUE	MULTIVARIABLE MODEL BY GEE[1] CHI-SQUARE	MULTIVARIABLE MODEL BY GEE[1] *P* VALUE
Bax	6	21 (47%)	24 (53%)	45	2.55	.11		
	7	5 (17%)	25 (83%)	30				
	Total	26	49	75				
Bcl-2	6	45 (100%)	0 (0%)	45	not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
Bcl-XL	6	1 (2.2%)	44 (97.8%)	45	not converged	N/A		
	7	0 (0%)	30 (100%)	30				
	Total	1	74	75				
Bin1	6	31 (68.9%)	14 (31.1%)	45	3.81	.051		
	7	9 (30%)	21 (70%)	30				
	Total	40	35	75				
FAS	6	43 (95.6%)	2 (4.4%)	45	5.53	.019	2.98	.084
	7	17 (56.7%)	13 (43.3%)	30				
	Total	60	15	75				
MDM2	6	33 (73%)	12 (27%)	45	1.31	.2523		
	7	15 (50%)	15 (50%)	30				
	Total	48	27	75				
p21	6	20 (44.4%)	25 (55.6%)	45	3.73	.053		
	7	4 (13.3%)	26 (86.7%)	30				
	Total	24	51	75				
p53	6	45 (100%)	0 (0%)	45	not converged	N/A		
	7	27 (90%)	3 (10%)	30				
	Total	72	3	75				
p65	6	32 (71%)	13 (29%)	45	6.17	.013	3.53	.06
	7	7 (23.3%)	23 (66.7%)	30				
	Total	39	36	75				
p27	6	21 (56.8%)	16 (43.2%)	37	1.13	.287		
	7	11 (36.7%)	19 (63.3%)	30				
	Total	32	35	67				

[1] Gleason score (GS) is response variable, and molecular marker involvement is predictor in the logistic model.

Table R5. Logistic analysis (GEE approach) based on three random values of involvement for each patient using the GENMOD procedure in SAS

MOLECULAR MARKER	GS[1]	INVOLVEMENT 0,1,2	INVOLVEMENT 3	TOTAL	UNIVARIABLE MODEL BY GEE[1] CHI-SQUARE	UNIVARIABLE MODEL BY GEE[1] *P* VALUE	MULTIVARIABLE MODEL BY GEE[1] CHI-SQUARE	MULTIVARIABLE MODEL BY GEE[1] *P* VALUE
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	Total	31	44	75				
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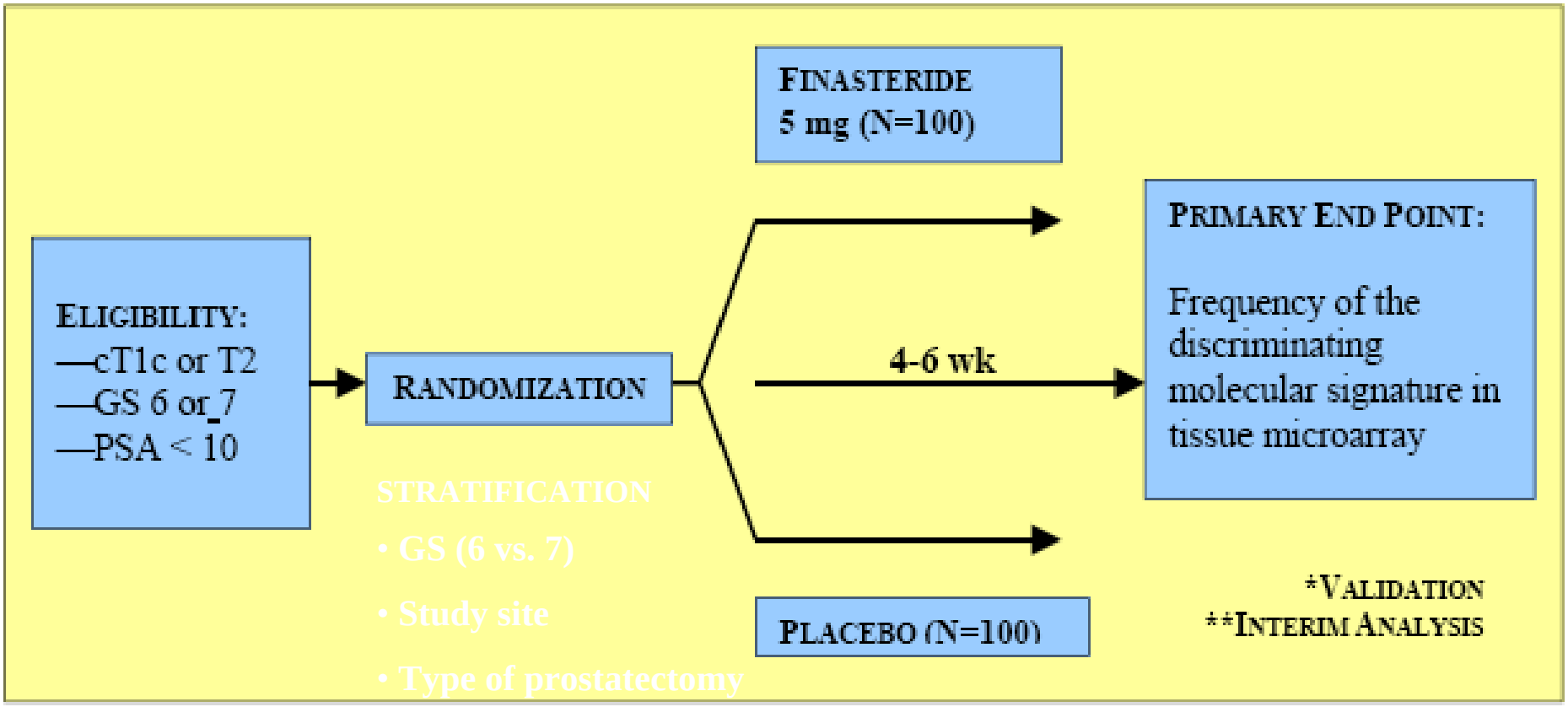

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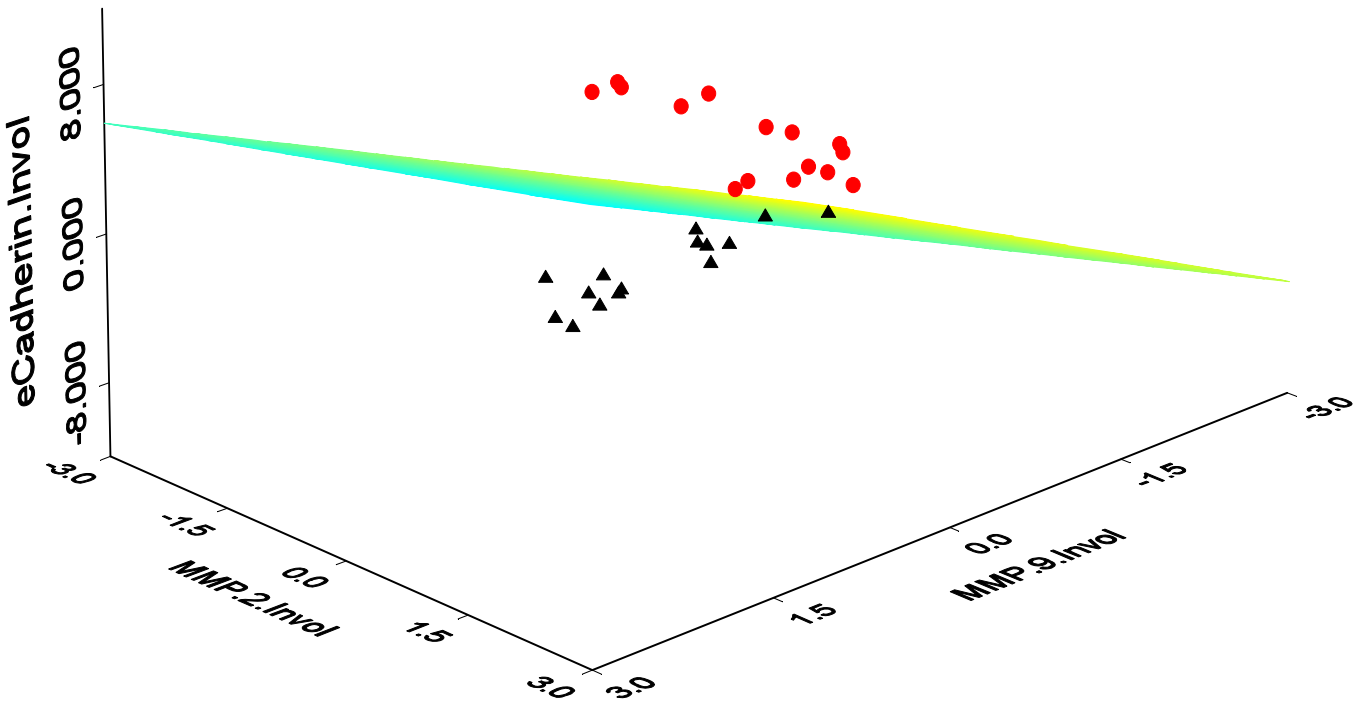
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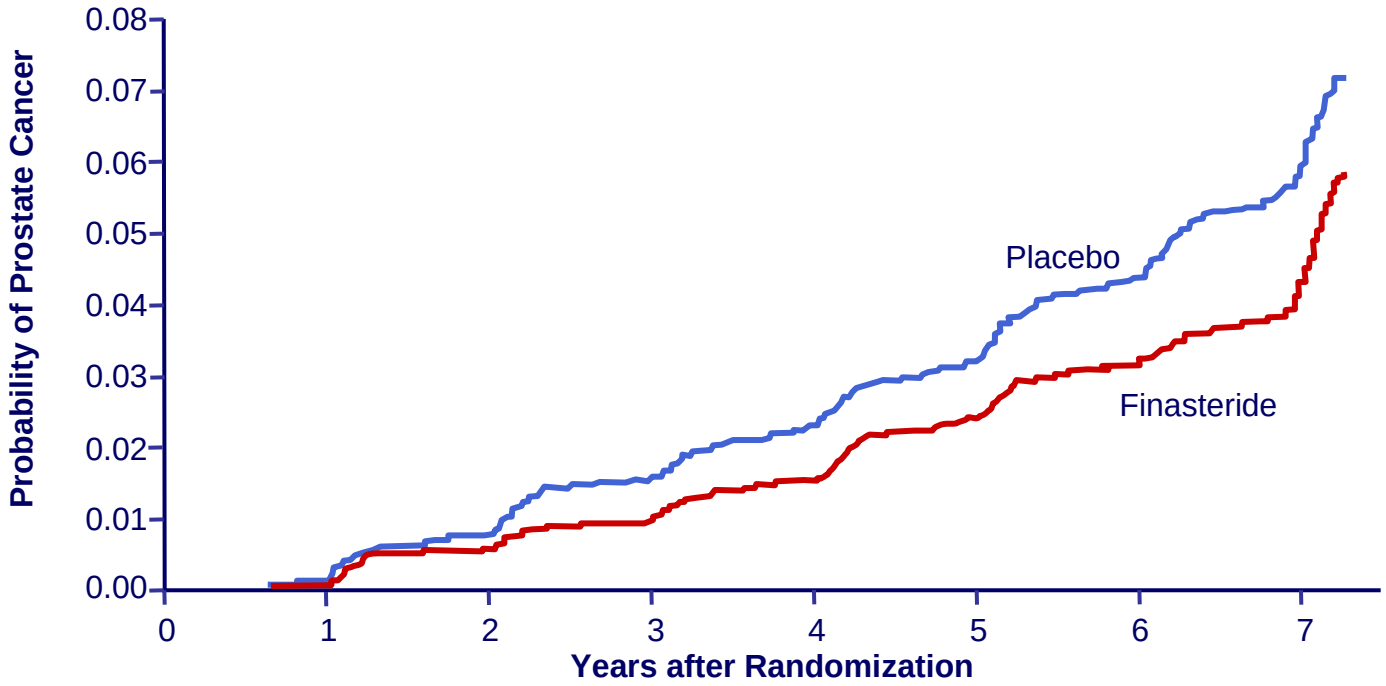
3.0
MMP.9.Invol

Prostate Cancer Prevention Trial



Placebo group							
Biopsy rate (%)	3.0	2.8	2.2	2.9	2.8	2.6	7.1
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No. of grade 7-10 cancers	5	6	15	35	24	24	38
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	T3	3	1	6
Biopsy Gleason Score (GS)	7	10	9	10
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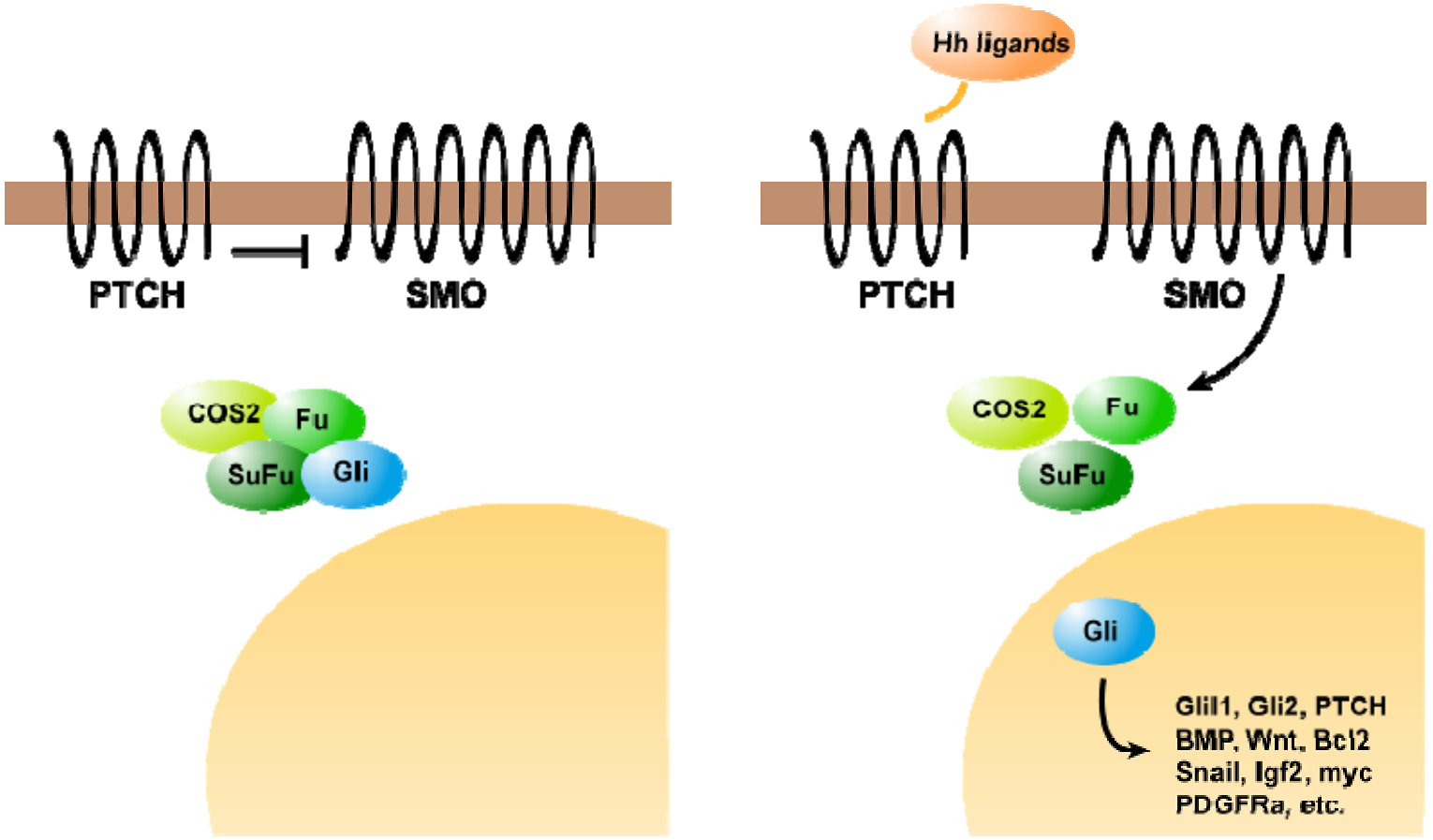
  - b. the expression of the hedgehog signaling intermediate smoothened, considered the limiting factor of shh pathway activation in PCa and
  - c. the ligand Sonic Hedgehog.

Future Directions

- Confirm findings in larger patient cohorts
- Elucidate the mechanism by which stromal epithelial interaction affects phenotype
- Determine if a mechanistic link between Androgen and Hedgehog signaling is a determinant of therapy response

Overview of Hedgehog Signaling (II)



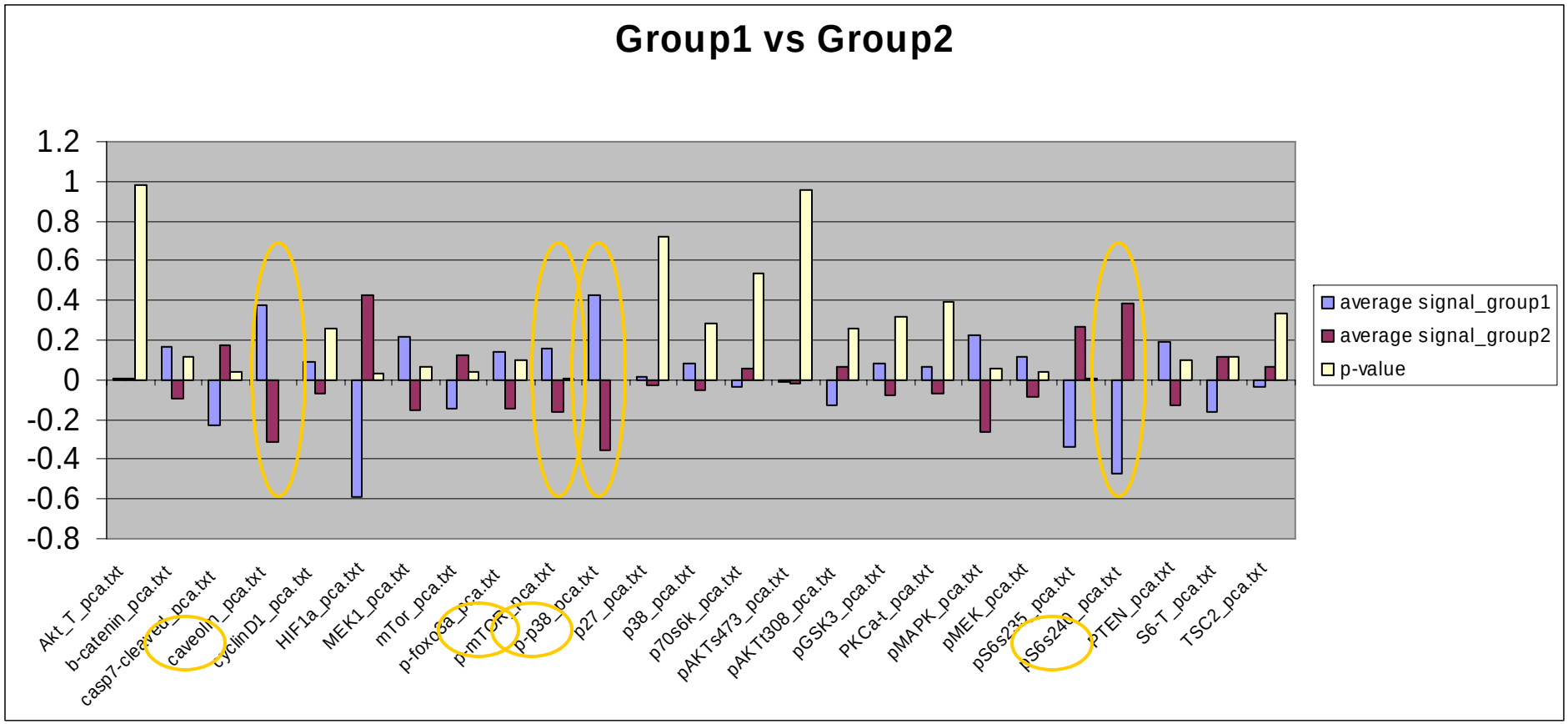
Inactive Signaling

Active Signaling

Implications for Hedgehog – Androgen Signaling Associations in Prostate Development/Regeneration

- *Shh* mutant fetuses display abnormal urogenital development and fail to form prostate buds. This prostate defect can be rescued by explant culture in the presence of androgens, and administration of dihydrotestosterone (DHT) to pregnant mice

- Hh pathway blockade blocks epithelial regeneration in androgen –ablated rodent ventral prostate upon androgen supplementation


Group1 vs Group2
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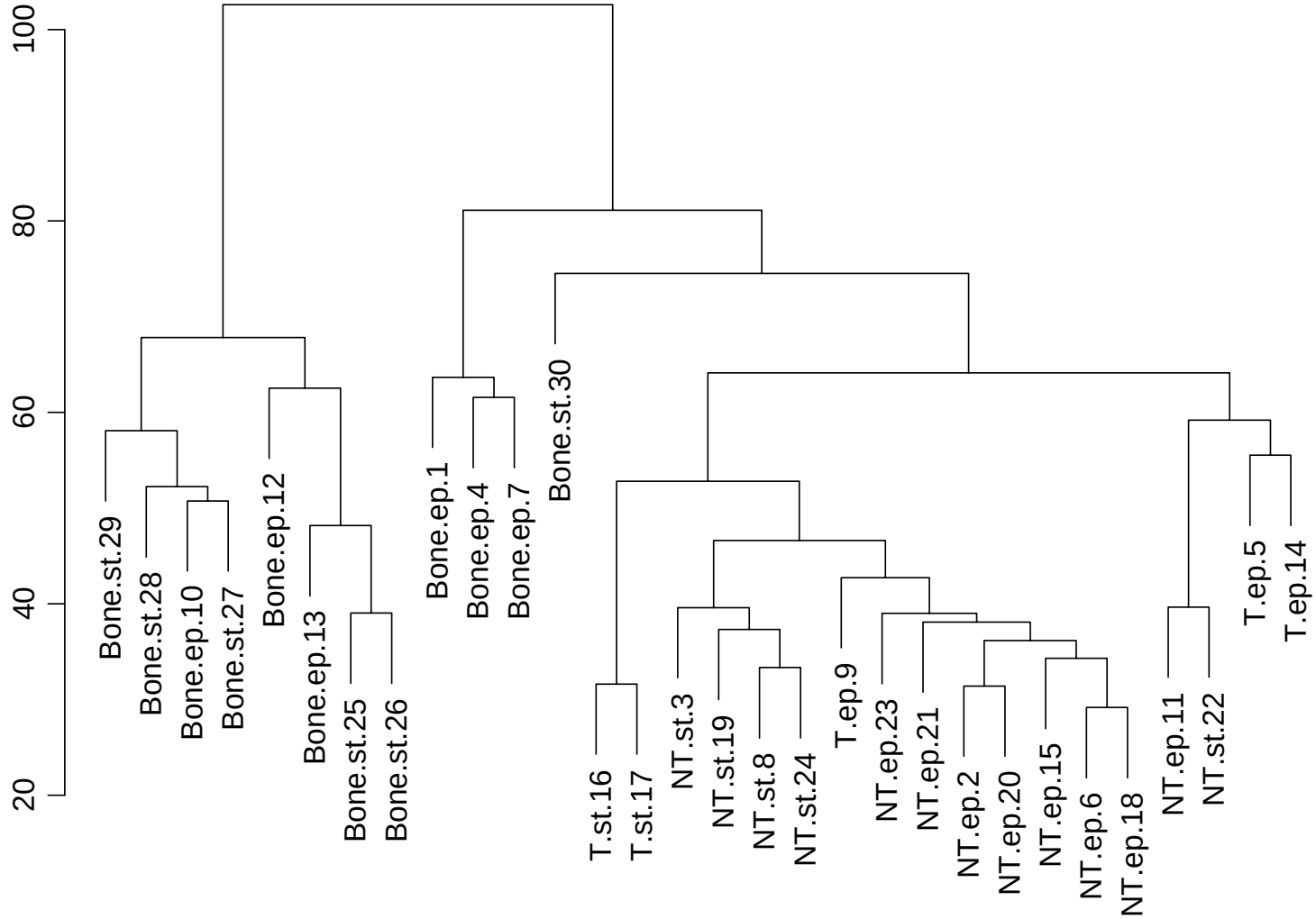
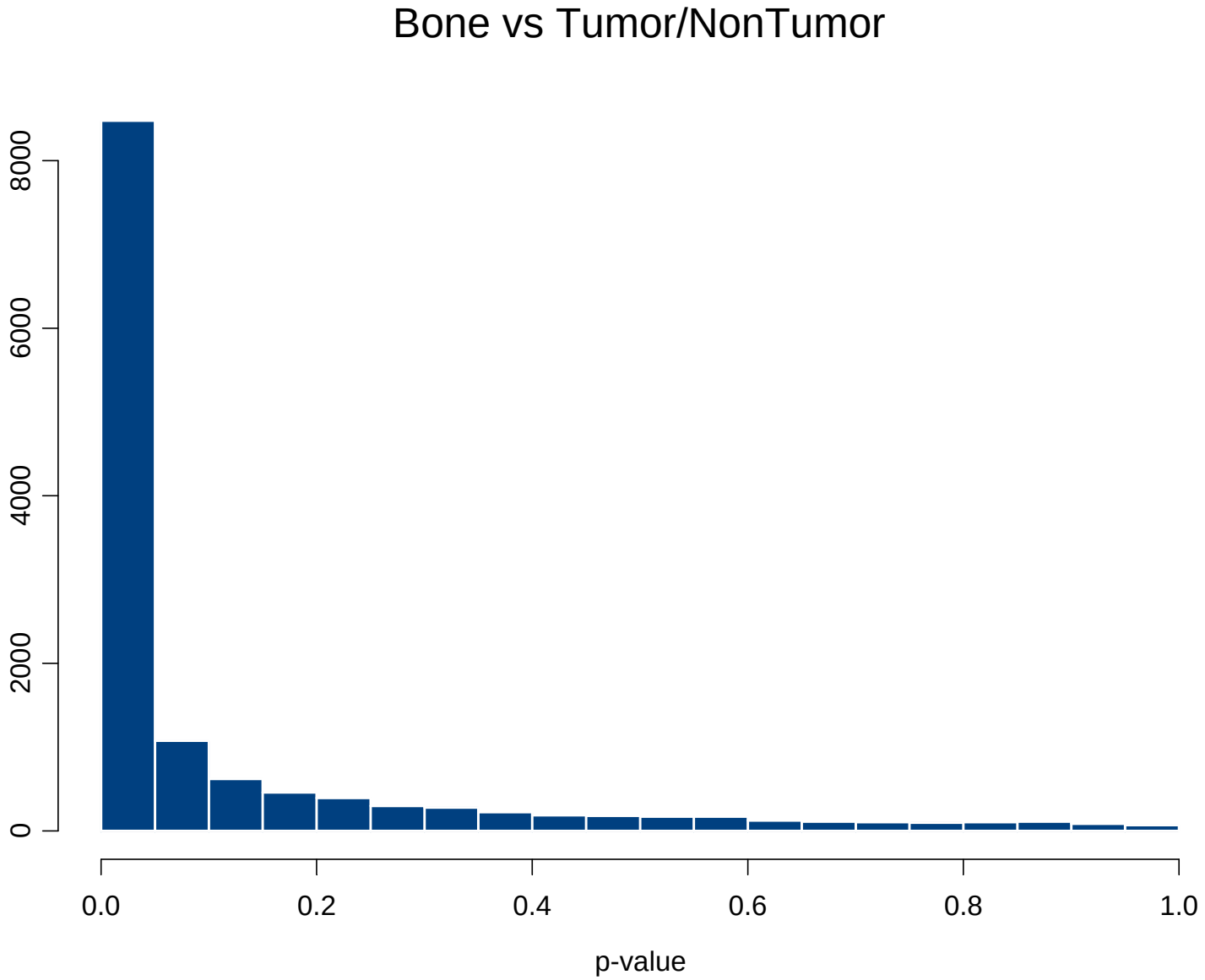
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
average signal_group1
average signal_group2
p-value
Akt_T_pca.txt
b-catenin_pca.txt
caveolin_pca.txt
cyclinD1_pca.txt
HIF1a_pca.txt
MEK1_pca.txt
mTor_pca.txt
p-p38_pca.txt
p27_pca.txt
p38_pca.txt
p70s6k_pca.txt
pAKTs473_pca.txt
pAKTt308_pca.txt
pGSK3_pca.txt
PKCa-t_pca.txt
pMAPK_pca.txt
pMEK_pca.txt
S6-T_pca.txt
TSC2_pca.txt

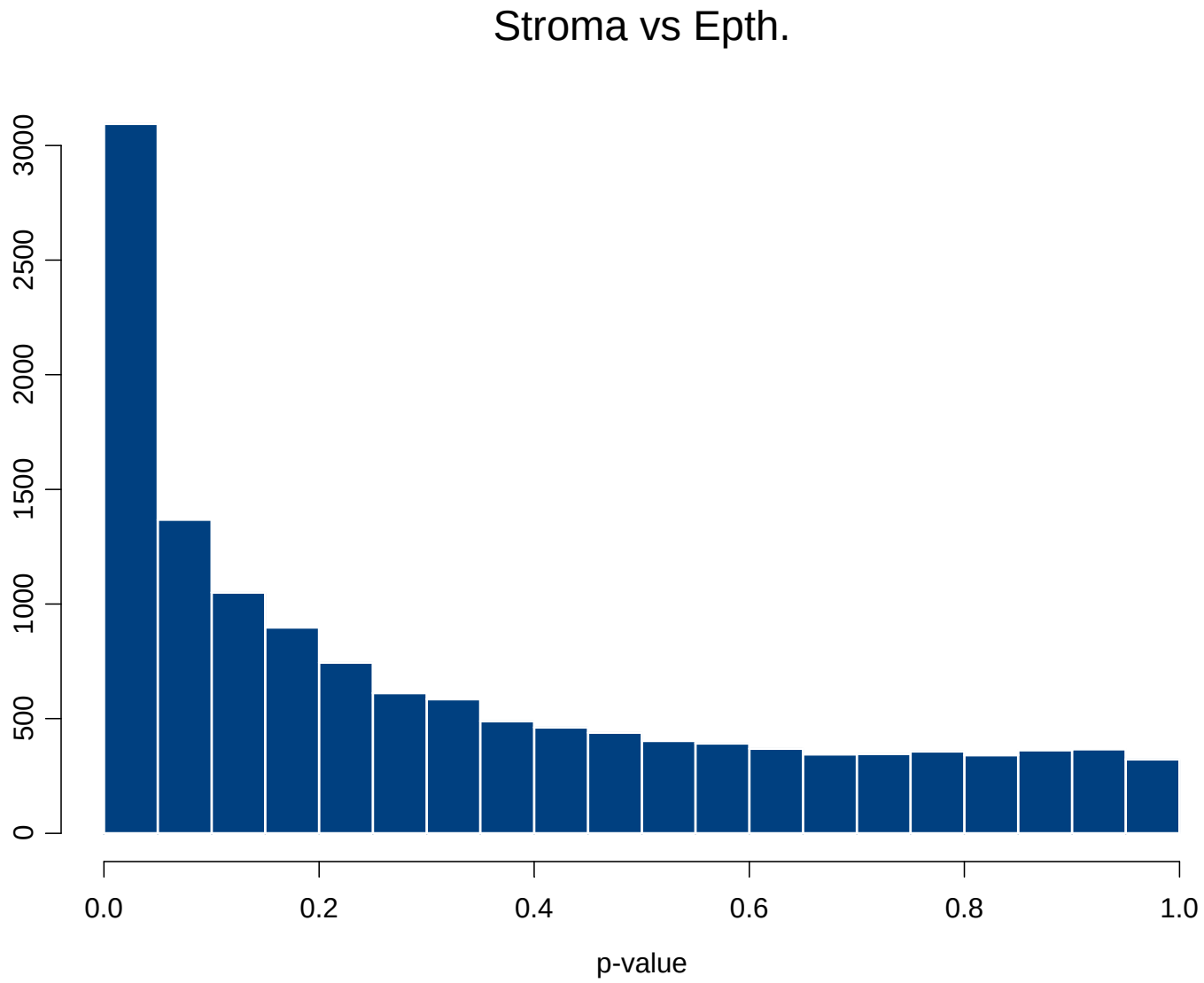
Hierarchical clustering based on all genes (n=13346)



FDR table for Bone analysis (mixed model)

Bone vs Tumor/Non-Tumor	FDR	p-value	number of genes
	0.01	0.021	7169
	0.001	0.001	3413
	0.0001	0.00006	1077
	0.00001	<0.00001	167
Eps vs stroma			
	0.05	0.012	1451
	0.01	0.0005	262
	0.001	<0.00001	14


Bone vs Tumor/NonTumor
8000
6000
4000
2000
0
0.0
0.2
0.4
0.6
0.8
1.0
p-value


Stroma vs Epth.
0
500
1000
1500
2000
2500
3000
0.0
0.2
0.4
0.6
0.8
1.0
p-value

Pre-Operative High Risk Prostate Cancer Protocols

Preoperative Protocols	Years	RXPX	ACCRUAL	EXPECTED
KAVE & Horm.abl.*	97-98	30	33	30
p53*	98-99	26	29	30
TNP*	98-00	24	27	27
KAVE vs Horm. abl.*	99-03	58	64	64
Thalidomide*	01-02	15	18	40
Docetaxel & Imatinib, & Horm. Abl.*	03-05	37	37	36
CCI-779 (UTMDACC & UCLA)	04-05	18	18	40
Total		208	226	267

* Completed

7/13/05

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CANCER CENTER

Laparoscopic Radical Prostatectomy (LRP) or Robotic-Assisted Laparoscopic Prostatectomy (RALP)

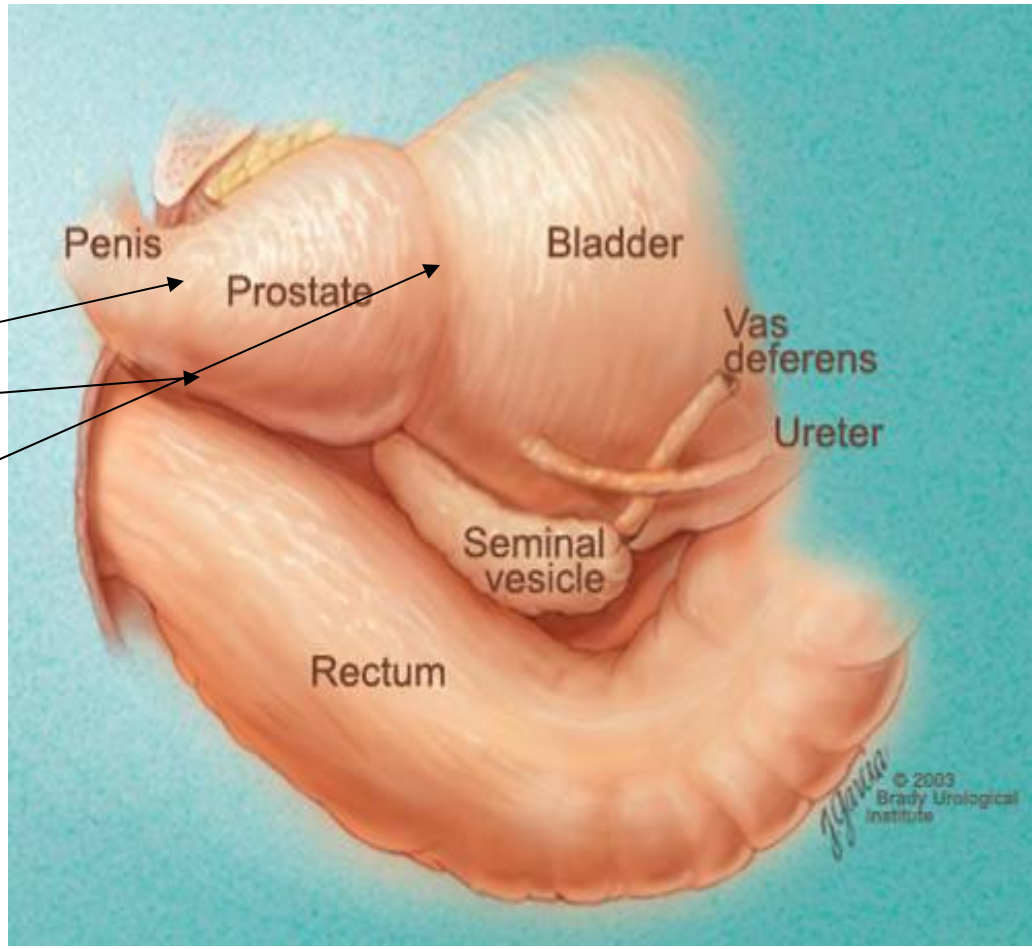
- Techniques employ multiple port access to the surgical field
- Dissection is antegrade—starts at the bladder neck, then pedicles, then urethra
- Pneumoperitoneum with 15 mmHg C02 required
- *Typically entire case finished before organ extracted to maintain pneumoperitoneum*

Davis J, 2008

Tissue Ischemia and Laparoscopy: Potentially increased time compared to open

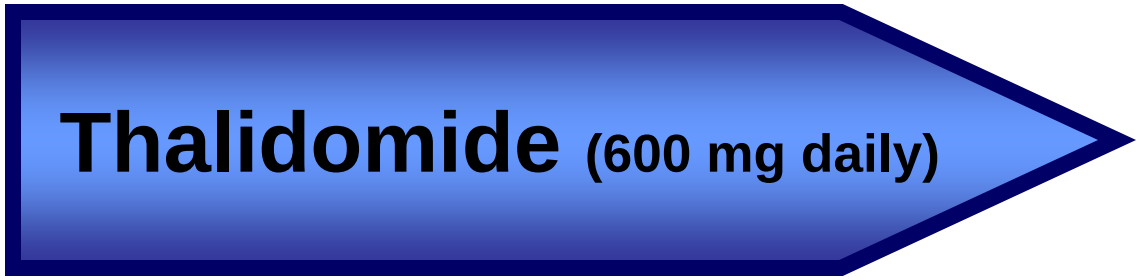
- **Open RP: organ immediately available to place on ice**
- **Retrograde dissection starts with the urethra, then pedicles and ends with the bladder neck**
- **? Differential ischemia**

  > at apex than base

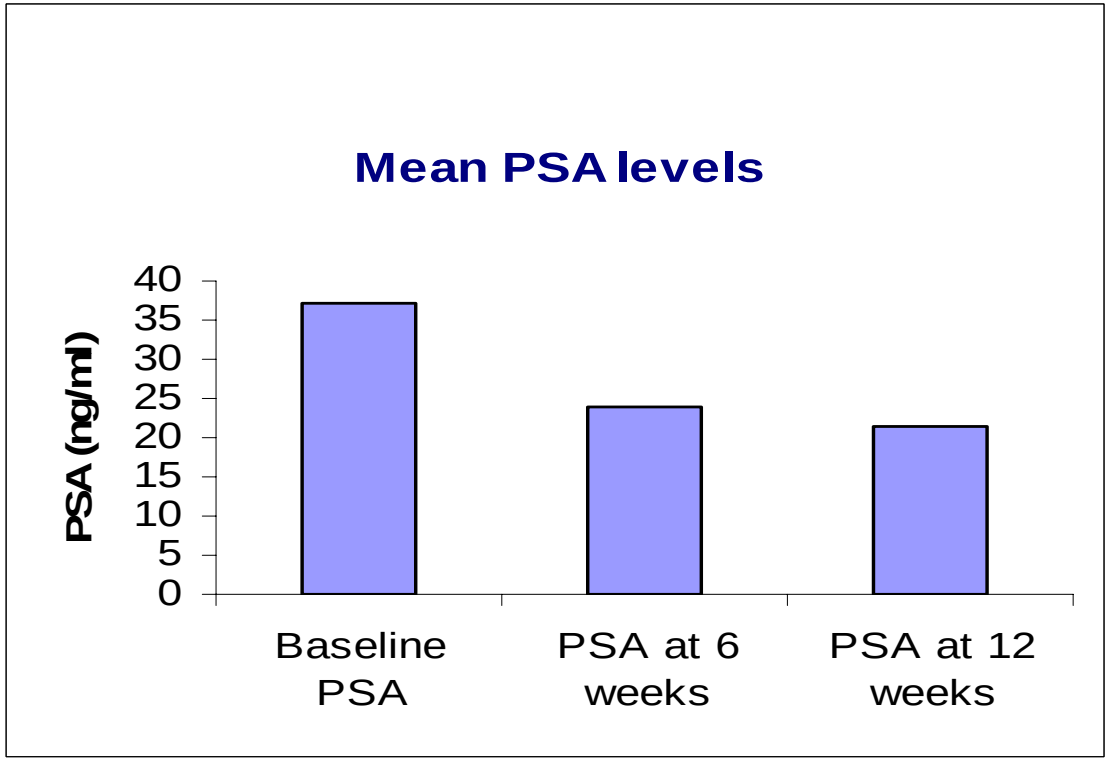


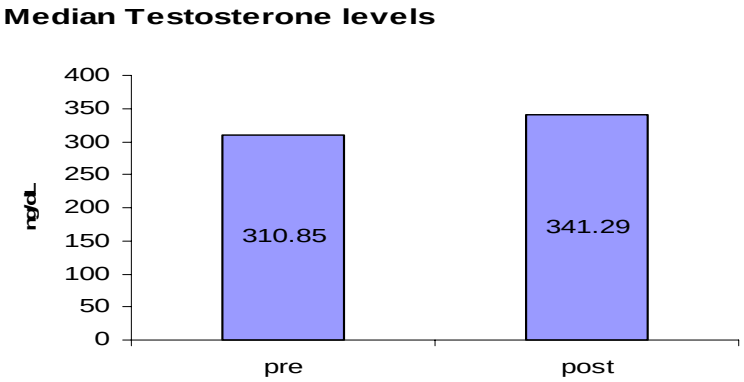
Davis J, 2008

Study Design



Change in PSA Levels





PSA drop > 50% = 33%
Median PSA reduction at 6 weeks 38.28%
Median PSA reduction at 12weeks 41.82%
No effect on testosterone

Materials/Methods

- Tissue microarrays of **15 treated cancer & 15 matched controls**: Median cores per case 30
- Protein expression by IHC
- Statistical Analysis: 3 methods on raw data (hierarchical clustering, standard t-test, mixed model incorporating sample variation)