

## BACKGROUND

- A genomic test that distinguishes between clinically indolent and aggressive disease could help men decide between active surveillance and immediate therapy, or decide on the need for adjuvant therapy after immediate treatment.
- As a first step in the development of a biopsy-based genomic assay for localized prostate cancer, we conducted a large, well-designed study in radical prostatectomy (RP) tissue.
- This presentation describes the first results of our analyses that identify genes associated with clinical outcome.

## STUDY OBJECTIVES

- Primary:**
- To identify genes whose quantitative expression predicts Clinical Recurrence (cR) after RP.
- Secondary:**
- To determine whether those genes also predict outcome of other endpoints:
    - Biochemical Recurrence (PSA or salvage therapy)
    - Prostate Cancer-Specific Survival (PCSS)
- Exploratory:**
- To determine whether those genes also predict outcome of upgrading/upstaging from biopsy to RP

## STUDY DESIGN

- Database contained ~2600 patients who underwent RP at Cleveland Clinic (CC) between 1987 and 2004 for localized prostate cancer and met inclusion/exclusion criteria.
- A stratified cohort sampling design was used because of low (<5%) event rate. All patients with clinical recurrence were included, as were 3x as many patients without clinical recurrence.
- Patients without clinical recurrence were given additional weight, so that functionally, this study is representative of 2,600 patients.
- Each patient had two spatially distinct tumor specimens samples from the RP specimen that included the primary Gleason Pattern (GP) and the highest GP.
- Clinical data was centrally re-reviewed against source documentation as part of clinical database quality control.
- All pathology assessments on radical prostatectomy tissue were centrally re-reviewed by an expert genitourinary pathologist at CC.
- Original surgical pathology assessment of prostate biopsy was used for analysis of upgrading/upstaging.

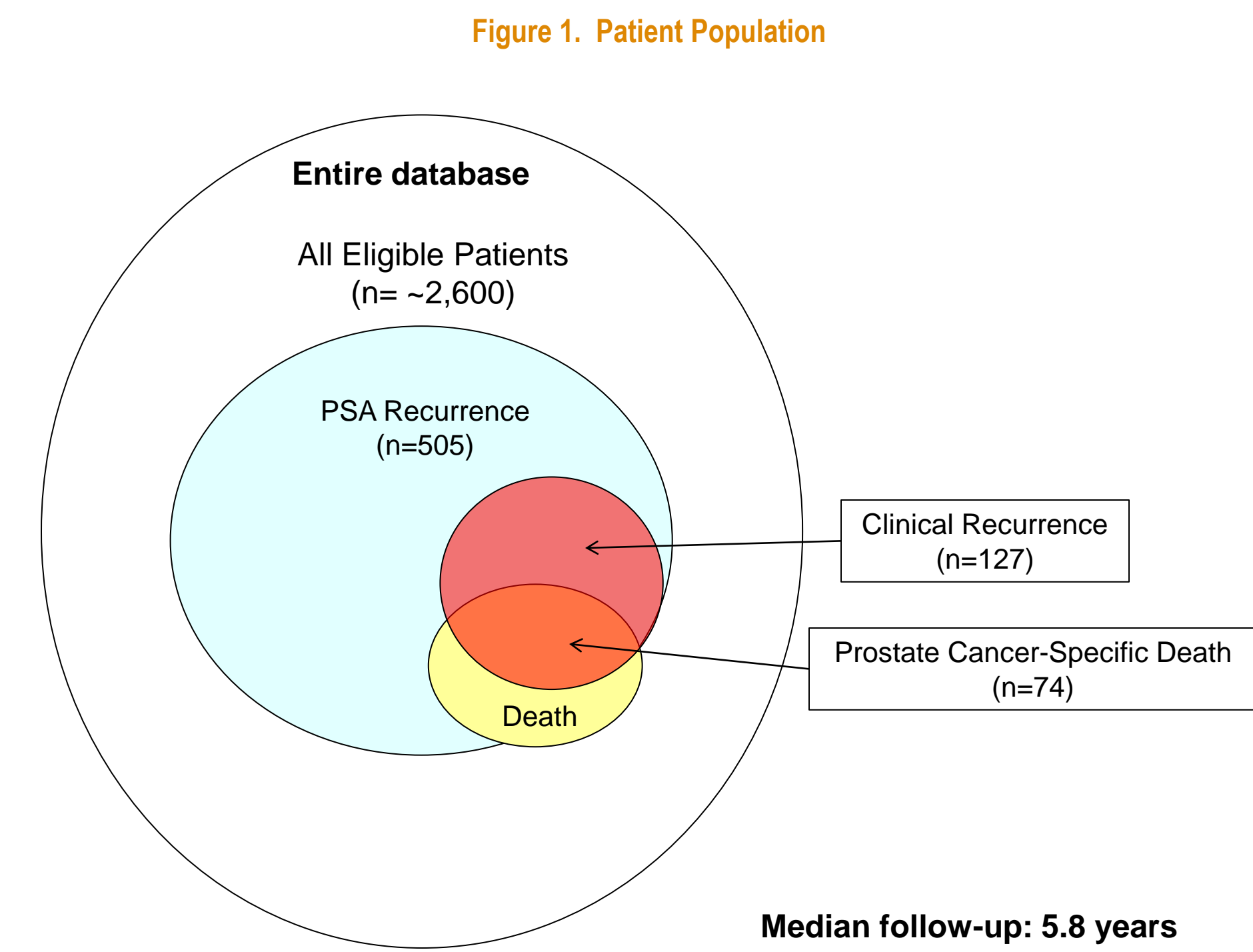
- Inclusion Criteria:**
- Patients who were diagnosed with early-stage prostate cancer and were treated with RP at the CC between the years of 1987 and 2004
  - Clinical Stage T1 or T2 prostate cancer
- Exclusion Criteria:**
- Neo-adjuvant and/or adjuvant therapy received
  - Missing pre-surgical PSA
  - No tumor block available from initial diagnosis in the CC archive

- Stratified Sampling by:**
- T stage (T1 vs. T2)
  - Year of surgery (<1993 vs. ≥1993)
  - Gleason Score (≤7 vs. >7)
  - Clinical Recurrence (cR)

- Endpoints:**
- Primary:** Clinical Recurrence (cR)
  - Secondary:** Biochemical Recurrence (bR), Prostate Cancer-Specific Survival (PCSS), Overall Survival (OS)
  - Exploratory:** Upgrading/upstaging from biopsy to RP

1:3 Ratio of cR to No cR  
 Total Sample Size = 501

## METHODS



### Sample Preparation

- Six (6) 10 micron (µm) unstained sections, as well as a beginning and ending 5 µm H&E slide, from each fixed paraffin-embedded (FPE) tissue block were included in the study.
- Central pathology review by CC pathologists.
- Manually-macrodissected tissue enriched for tumor from each specimen was submitted into the analytical assay.
- Gleason Score (GS) was assessed according to the 2005 International Society of Urological Pathology Consensus Conference criteria.<sup>1</sup>

### Quantitative RT-PCR

- RNA was extracted from 60 µm of tissue per sample. Samples were treated with DNase I to remove genomic DNA and total RNA content was quantified.
- Gene-specific reverse transcription (RT) was used to convert RNA to complementary DNA prior to quantitative PCR (TaqMan®) with assays conducted in 384-well microtiter plate format. PCR reaction and detection was performed on the Roche Lightcycler480 instrument.
- Expression of each of 732 prostate cancer-related genes (chosen from previous prostate feasibility studies and literature including microarray experiments) was measured in single well qPCR reactions and normalized to the average of 6 reference genes. Reference gene expression was measured in triple well qPCR reactions.
- Reference-normalized gene expression measurements ranged from 2 to 15, where each 1-unit increase reflects about a two-fold increase in RNA.

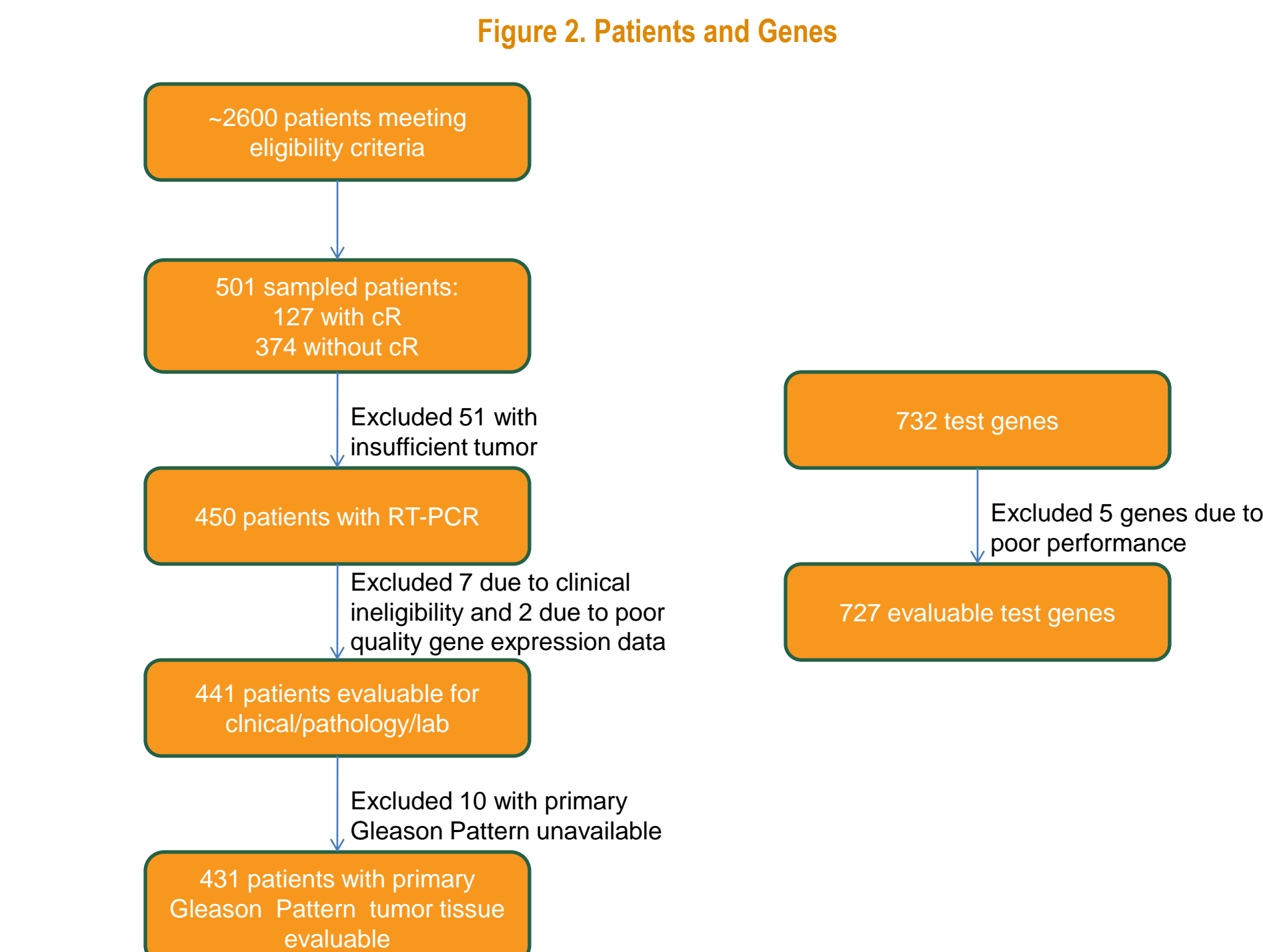
### Definition of endpoints

- Clinical recurrence (cR): local recurrence or distant metastases detected by imaging study or by biopsy.
- Clinical recurrence-free interval (cRFI): time (in months) from surgery to first clinical recurrence or death due to clinical recurrence of prostate cancer.
- Biochemical recurrence (bR): follow-up PSA ≥0.4 ng/mL or the initiation of salvage therapy as a result of a rising PSA.
- Biochemical recurrence-free interval (bRFI): time (in months) from surgery to first biochemical recurrence of prostate cancer.
- Prostate cancer-specific survival (PCSS): time (in months) from surgery to death from prostate cancer.
- Upgrading/Upstaging: change in Gleason grade from 3+3 at biopsy to 3+4 or greater at RP or 3+4 at biopsy to 4+3 or greater at RP, or seminal vesicle involvement (SVI), or extracapsular involvement (ECE) at RP.

### Statistical analyses

- Reference-normalized gene expression was standardized such that hazard ratios and odds ratios represent the association per standard deviation increase in gene expression.
- Univariate Cox Proportional Hazards (PH) Models using weighted pseudo partial-likelihood estimators were used to assess relationships between gene expression and recurrence or survival.<sup>2</sup>
- Univariate logistic regression models were used to assess relationships between gene expression and upgrading/upstaging.
- p-values using Wald's test of the null hypothesis that the hazard ratio (HR) or odds ratio (OR) is one are reported.
- Storey's method to control for false discoveries at 20% was used.<sup>3</sup>
- All statistical tests were two-sided and p<0.05 was considered statistically significant.

## RESULTS



**Table 1. Baseline Characteristics**

Characteristic	Values	N (%)	Weighted %
Age	≤70	396 (90)	93
	>70	45 (10)	7
Race	Caucasian	391 (89)	83
	Black/Afro-Caribbean	36 (8)	12
	Other	14 (3)	5
Clinical Tumor Stage	T1	175 (40)	66
	T2	266 (60)	34
Pathologic Tumor Stage	T2	161 (37)	51
	T3	280 (64)	49
Baseline PSA	≤4	65 (15)	14
	>4-10	254 (58)	68
	>10-20	87 (20)	13
	>20	35 (8)	5
Surgical Gleason Score	≤6	61 (14)	25
	7	233 (53)	62
	≥8	147 (33)	13
AUA Risk Group	Low	163 (37)	55
	Intermediate	175 (40)	33
	High	103 (23)	12

Note: Due to rounding, percentages may not add up to 100%. Patients with no cR were given additional weight, so that functionally, this study is representative of 2,600 patients.

**Table 2. Outcome Distribution**

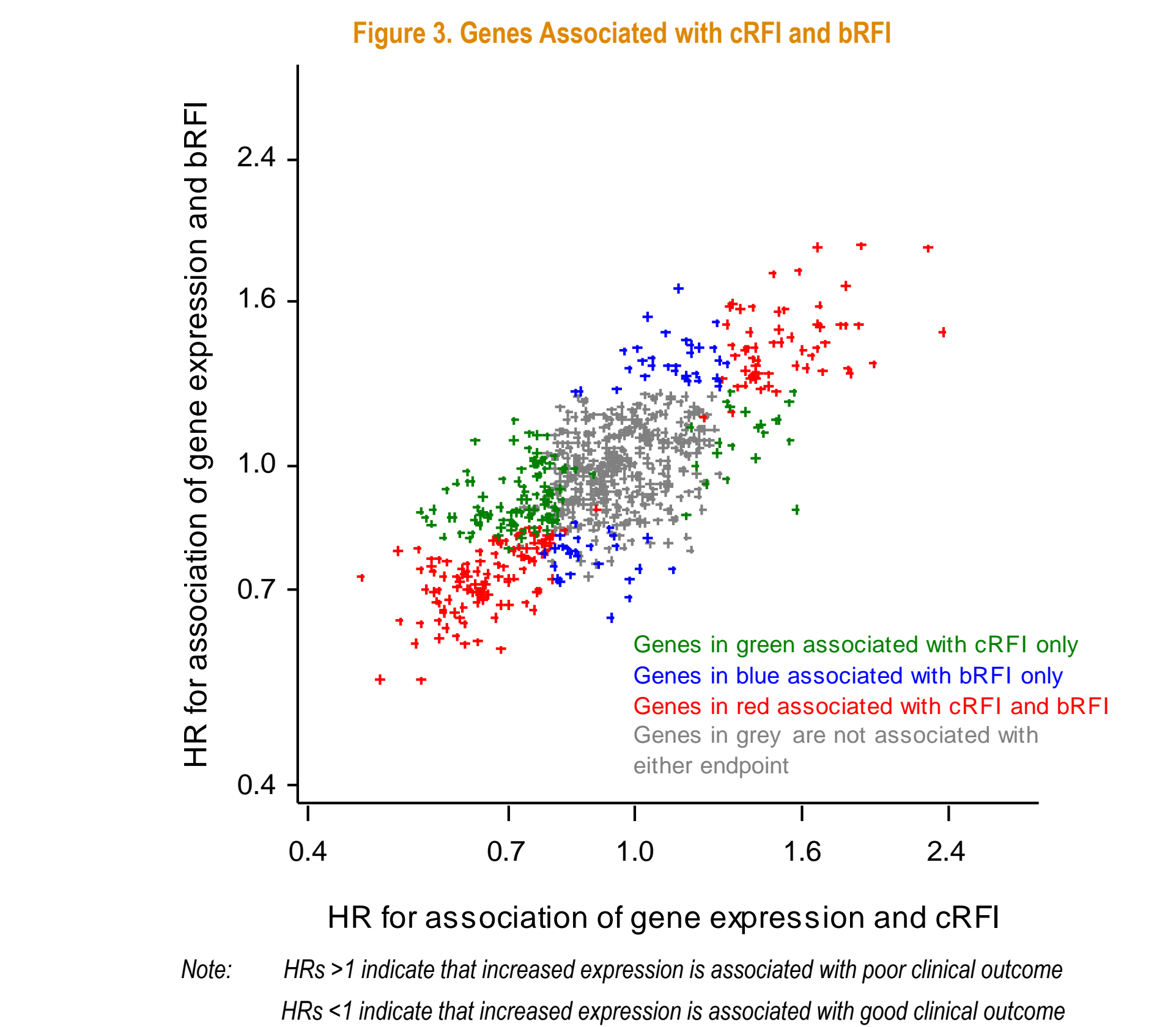
Characteristic	Values	N (%)	Weighted %
Clinical recurrence	Censored	330 (75)	95
	Local failure	24 (5)	1
	Distant Metastases	76 (17)	3
	Both	11 (3)	<1
Biochemical recurrence	Event	195 (44)	21
	Censored	246 (56)	79
Overall survival	Death	97 (22)	11
	Censored	344 (78)	89
Prostate cancer-specific survival	Death	39 (9)	2
	Censored	402 (91)	98

Note: Due to rounding, percentages may not add up to 100%. Patients with no cR were given additional weight, so that functionally, this study is representative of 2,600 patients.

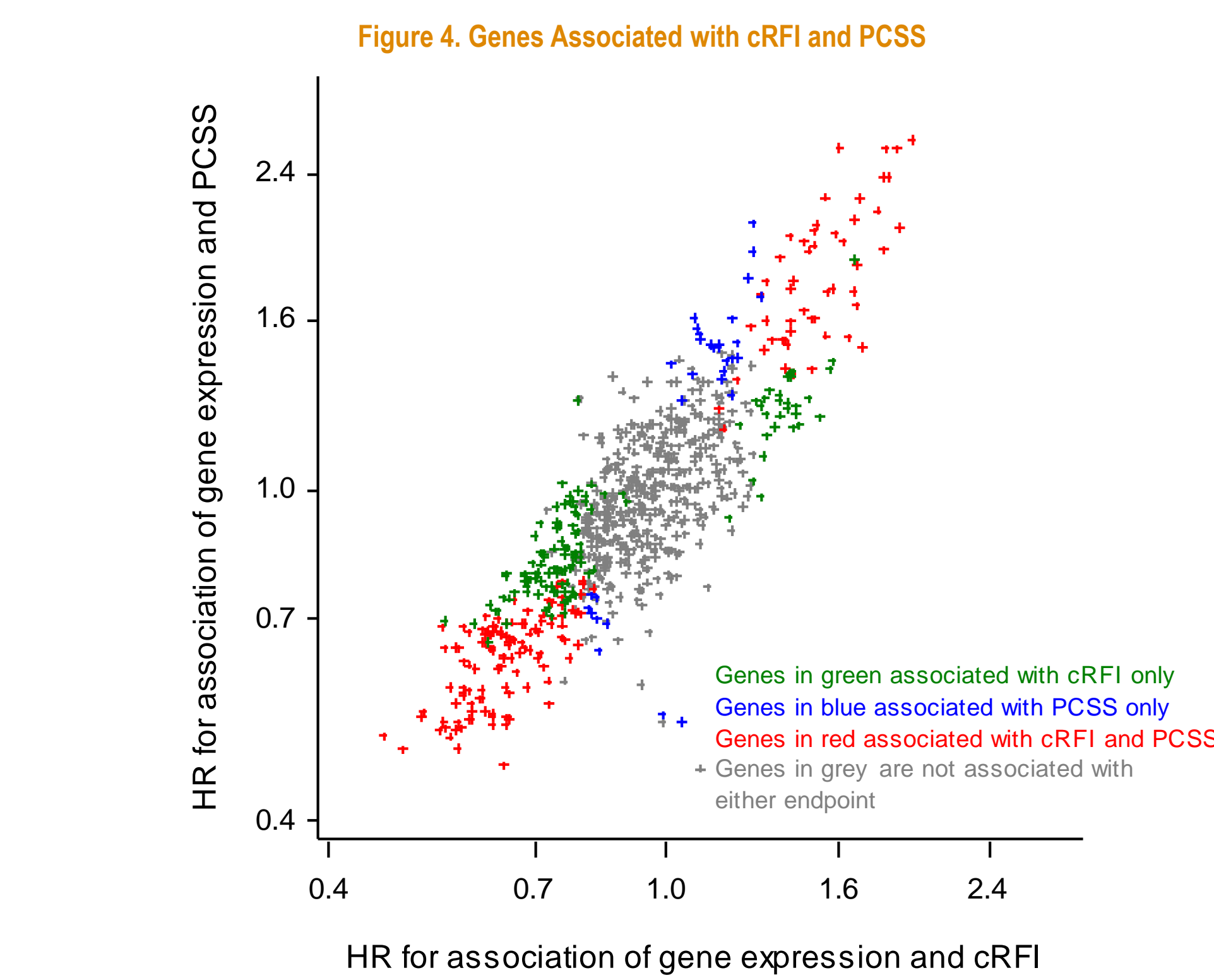
**Table 3. Number of Genes Associated with Each Endpoint**

Endpoint	Number of significant genes (p<0.05)	Proportion of significant genes (p<0.05) also associated with cRFI (%)	Number of Genes allowing for a 10% false discovery rate (FDR) <sup>4</sup>
cRFI	295		374
bRFI	235	75	247
PCSS	203	84	194
Upgrading/Upstaging	200	68	176

- <sup>4</sup>The method of Storey et al. was used to control the false discovery rate<sup>3</sup>
- Quantitative gene expression was strongly predictive of recurrence, prostate cancer-specific survival, and upgrading/upstaging, with standardized hazard ratios as high as 2.4 for cRFI.
  - The number of genes predicting outcome were well in excess of that expected by chance.
  - Approximately 50% of significant genes have hazard ratios >1.4, similar to ER and HER2 in breast cancer.
  - Observed p-values as low as 4 × 10<sup>-13</sup>
  - 28% of p-values <0.0001 for cRFI

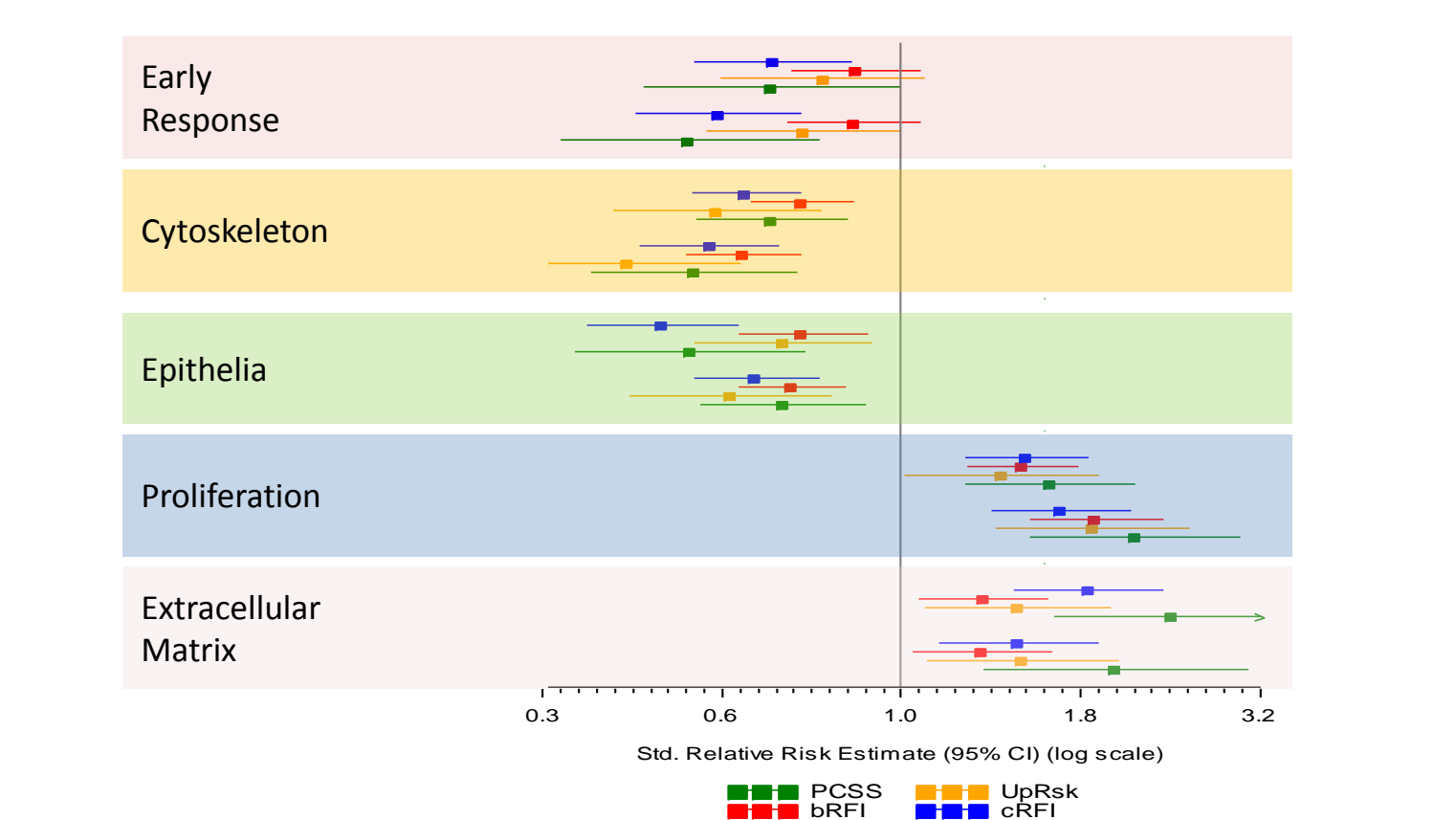


- These results show that some genes are associated with cRFI only, some with bRFI only, some with neither, and a large number of genes were associated with cRFI and bRFI.



- These results show that some genes are associated with cRFI only, some with PCSS only, some with neither, and a large number of genes are associated with cRFI and PCSS.

Figure 5. Associations Across Endpoints for Several Biological Pathways



- Results are shown for two representative genes from each gene family.
- For cRFI, bRFI, and PCSS, the relative risk estimate is the standardized hazard ratio, that is the hazard ratio per 1 standard deviation increase in gene expression.
- For UpRsk (upgrading or upstaging), the relative risk estimate is the standardized odds ratio, that is the odds ratio per 1 standard deviation increase in gene expression.
- Preliminary analysis indicates that combinations of pathways provide greater power in predicting clinical recurrence than any single pathway.

## STRENGTHS & LIMITATIONS

### Strengths

- Large number of clinical recurrences
- Multiple endpoints evaluated
- Central pathology review of RP specimens
- Clinical data review as part of data quality control
- Long follow-up (5.8 years)
- Highly quantitative assay, similar platform as used for Oncotype DX® Breast and Colon assays

### Limitations

- Characteristics associated with the type of data collected in an observational cohort vs. randomized trial
- Examines clinical recurrences despite subsequent treatment in many cases
- Lack of central review of biopsy GS for upgrading/upstaging analysis

## SUMMARY

- Quantitative gene expression using RT-PCR identified a large number of genes strongly associated with clinical recurrence and consistent across multiple endpoints: PSA recurrence, prostate cancer-specific survival and upgrading/upstaging.
- Identified a number of genes across several biological pathways that predict prostate cancer aggressiveness:
  - Early Response
  - Cytoskeleton (e.g. FLNA)
  - Epithelia (e.g. FRT5)
  - Proliferation
  - Extracellular Matrix (e.g. COL3A1)
- Clinical validation of these results are required.

## CONCLUSIONS

- A genomic test that distinguishes between clinically indolent and aggressive disease could help men and their doctors decide between active surveillance and immediate therapy, or decide on the need for adjuvant therapy after immediate treatment.
- This study utilizing RP tumor tissue is the foundation for a biopsy-based prostate cancer diagnostic tool to address this unmet clinical need.
- Additional analyses will be performed on this study to address the following questions:
  - Do genes add value beyond traditional clinical and pathologic measures?
  - Do genes overcome multifocality and tumor heterogeneity?
  - What is the role of TMPRSS2 fusions and are they alone a sufficient stratifier of risk?

## REFERENCES

- Epstein JI, Allsbrook WC, Amin MB, Egevad LL. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. *Am J Surg Pathol.* 2005;29(9):1228-42.
- Cox DR. Regression models and life tables (with discussion). *JR Stat Soc B.* 1972;34(2):187-220.
- Storey JD, Tibshirani R. Estimating the Positive False Discovery Rate Under Dependence, with Applications to DNA Microarrays. Dept. of Statistics, Stanford University, 2001.