The Development and Validation of a 17-gene Prostate Cancer Assay: Summary of Current and Evolving Evidence

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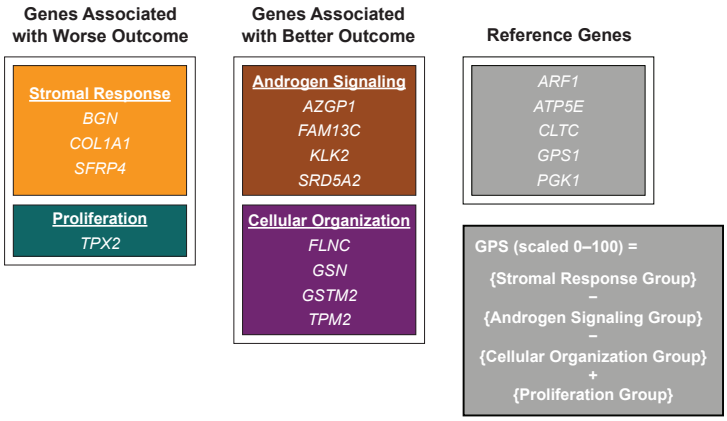
Genomic Prostate Score Development and Validation

- The development of a molecular biomarker into a diagnostic assay involves a series of feasibility and analytic studies, and adoption of that assay into clinical practice requires analytical and clinical validation (McShane JCO 2012; Simon JNCI 2009).[1-2]
- The 17-gene Onco*type* DX® Prostate Cancer Assay (Genomic Health, Inc., Redwood City, CA) is a commercially available biopsy-based RT-PCR assay that reports a Genomic Prostate Score (GPS, scaled 0–100) and provides a biologic measure of cancer aggressiveness.
- The GPS has been validated as a predictor of clinically relevant endpoints: 1) adverse pathology (AP) at surgery and 2) biochemical recurrence (BCR). GPS has also been shown to be significantly associated with metastasis.[3-4]
- The assay is indicated for men with NCCN® very low-, low-, and low-intermediate risk prostate cancer to inform decisions regarding immediate treatment or active surveillance.

Figure 1: 17-Gene Onco*type* DX Genomic Prostate Score

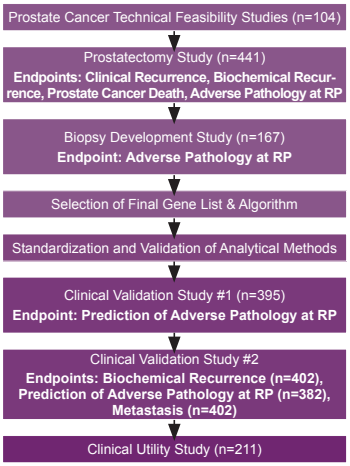


Each gene is individually weighted in the final algorithm

- The Genomic Prostate Score represents key genes and pathways and is calculated based on a validated algorithm of 12 cancer-related and 5 reference genes.

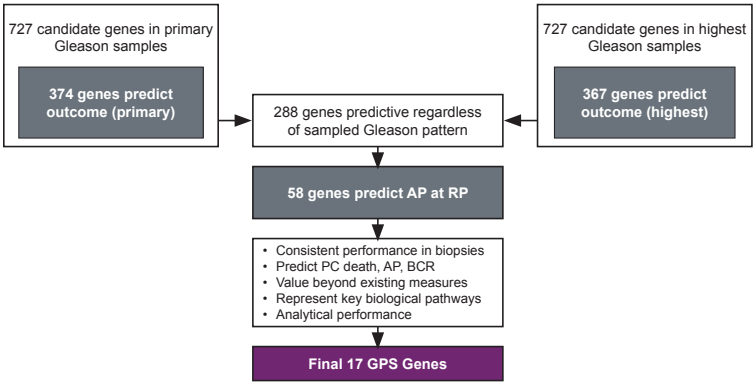
Figure 2: Development and Validation in >1,500 Patients

- **Feasibility studies** confirmed that gene expression could be measured in very small amounts of RNA obtained from fixed paraffin-embedded (FPE) needle biopsy specimens.
- **Development studies** identified genes whose expression was primarily predictive of clinical recurrence and also associated with prostate cancer death, BCR, and AP at surgery in the face of tumor heterogeneity and multifocality.
- **Analytic validation studies** demonstrated that the assay provided robust, reproducible results over a wide range of RNA inputs and analytical conditions.



- A **clinical validation study** confirmed that the GPS predicts AP at radical prostatectomy in men with NCCN clinical very-low, low-, and low-intermediate risk prostate cancer and provided independent predictive information beyond standard clinicopathologic measures.[3]
- A **second clinical validation study** showed, in a racially diverse population, that the GPS is 1) validated as a strong and independent predictor of BCR, 2) re-confirmed as a strong and independent predictor of AP at surgery, and 3) significantly associated with metastasis.[4]

Figure 3: Gene Identification and Refinement Process

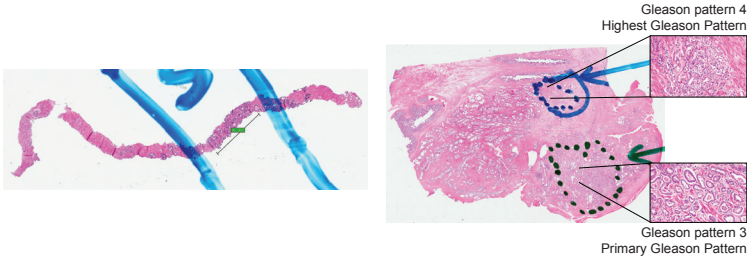


The multi-step process of gene identification and refinement identified genes that would be analytically robust and whose expression would be informative of outcome in both the primary and highest Gleason pattern.

Figure 4: Assay Designed to Work in Small Amount of Tissue Yields Consistent and Reproducible Results

A. H&E Image of a Representative Diagnostic Prostate Biopsy

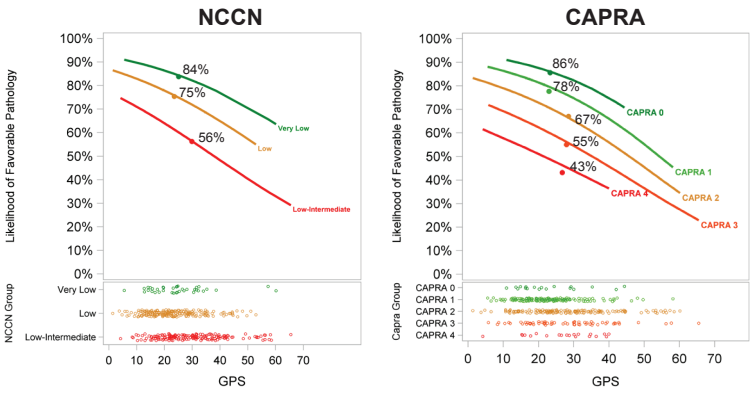
B. Gene Expression Represents Underlying Tumor Biology



- On average, 30-50 ng of RNA can be extracted from diagnostic biopsies containing 1 mm of tumor.
- The Onco*type* DX Prostate Cancer Assay was clinically validated for use with biopsies containing as little as 1 mm of tumor (Panel A).
- In the first validation study, 96% of biopsies yielded successful GPS results, and 99.5% of samples with >10 ng RNA yielded successful results.[3]
- The assay was specifically designed to address concerns of biopsy tumor heterogeneity. During assay development, two spatially distinct areas of tumor representing the primary and the highest Gleason pattern for each patient were analyzed to identify genes that are predictive regardless of sampled Gleason pattern (Panel B).
- Through this approach, genes were identified that were predictive of clinical outcome, independent of the area sampled.

Genomic Prostate Score Development and Validation

Figure 5: Clinical Validation #1: Improved Risk Discrimination with Addition of GPS



- The GPS, combined with NCCN risk group provides a continuous estimate of the likelihood of favorable pathology.
- Within each NCCN risk group, there is a wide spectrum of biologic risk.
  - Point estimates shown here are derived from the UCSF clinical validation cohort of n=395 patients.
- The addition of GPS allows for refinement of risk within each NCCN risk group.

Table 1: Clinical Validation #1: GPS Adds Independent Predictive Information Beyond Standard Criteria

Model	Variable	Odds ratio	95% CI	p value
1	GPS (per 20 units)	2.1	1.4–3.2	<0.001
	CAPRA (continuous)	1.6	1.2–2.0	<0.001
2	GPS (per 20 units)	1.9	1.3–2.8	0.001
	NCCN Low vs Very Low	1.8	0.7–4.6	0.201
	NCCN Intermediate vs Very Low	3.6	1.4–9.2	0.004
3	GPS (per 20 units)	1.9	1.2–2.8	0.003
	Age (continuous)	1.1	1.0–1.1	0.004
	PSA (continuous)	1.1	1.0–1.2	0.002
	Clinical stage T2 vs T1	1.6	1.0–2.5	0.059
	Biopsy Gleason Score (3+4 vs 3+3)	1.7	1.0–2.9	0.050

In separate multivariable analyses adjusting for significant clinical and pathology covariates, the GPS was a consistent predictor of high-grade and/or non–organ-confined disease.

Table 2: Clinical Validation #2: GPS is a Predictor of Multiple Clinical Endpoints

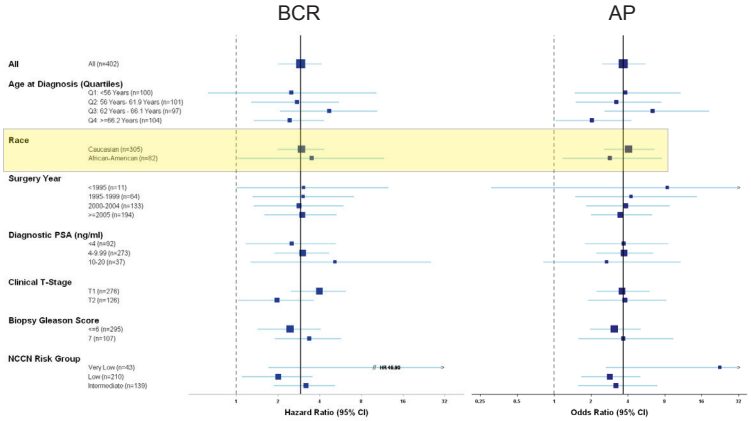
		Univariable			Multivariable		
	Variable	N (Events)	HR per 20 units (95% CI)	P-value	N (Events)	HR per 20 units (95% CI)	P-value
Biochemical Recurrence	GPS	402 (62)	2.9 (2.0–4.2)	<0.001	392** (60)	2.7 (1.8–4.0)	<0.001
	NCCN (L vs VL)					1.9 (0.6–11.7)	0.349
	NCCN (I vs VL)					2.2 (0.6–13.7)	0.249
Metastasis	GPS	402 (5)	3.8 (1.1–12.6)	0.032	small number of events (n=5) precludes multivariable analysis		

		Univariable			Multivariable		
	Variable	N (Events)	OR per 20 units (95% CI)	P-value	N (Events)	OR per 20 units (95% CI)	P-value
Adverse Pathology	GPS	382* (163)	3.2 (2.1–5.0)	<0.001	372** (159)	3.3 (2.1–5.1)	<0.001
	NCCN (L vs VL)					3.2 (1.3–8.8)	0.008
	NCCN (I vs VL)					4.5 (1.8–13.0)	<0.001

*20 patients were excluded due to unavailable RP specimens
**10 patients were missing NCCN risk classification

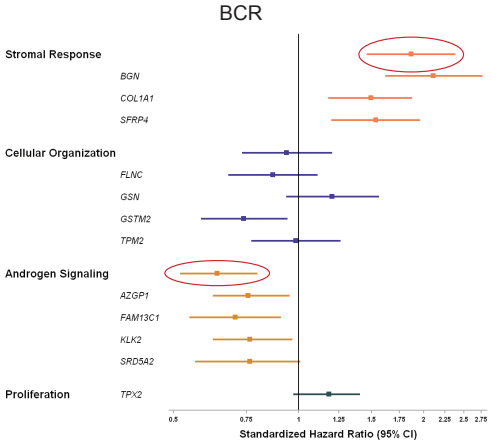
- In univariable analyses, GPS was strongly associated with three endpoints, validating it as a significant predictor of BCR, confirming it as a significant predictor of AP, and showing an association with metastasis.
- In multivariable analyses, GPS continued to be strongly associated with BCR and AP after adjustment for NCCN risk group, indicating that GPS adds value beyond standard clinicopathologic features.

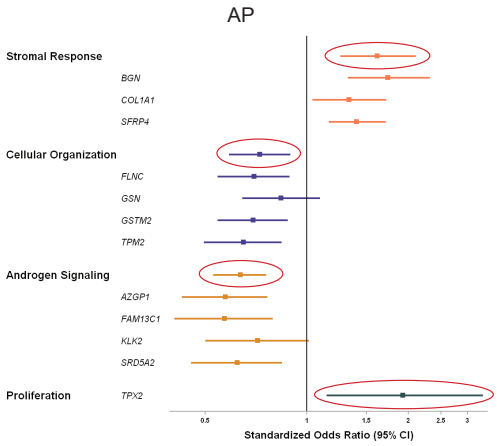
Figure 6: Clinical Validation #2: GPS Predicts BCR and AP Within Various Clinical Subsets, Including Different Racial Groups



- The GPS was a consistent predictor of time to BCR and AP within subgroups defined by baseline clinical and pathological factors.
- The association between GPS and outcome was similar in Caucasian and African American men.
- Race was not significantly associated with BCR (data not shown).

Figure 7: Clinical Validation #2: GPS Predicts BCR and AP Within Various Clinical Subsets, Including Different Racial Groups





- Down-regulation of androgen signaling and up-regulation of stromal response gene groups were most strongly associated with time to BCR.
- All four gene groups within the GPS were significant predictors of AP.
- The inclusion of genes representing multiple biologic pathways contributes to the robust predictive capacity of the assay.

Genomic Prostate Score: Clinical Utility

- Previous studies have demonstrated both the readiness of physicians to incorporate GPS and the impact of the assay on treatment decisions for men with newly diagnosed, clinically localized prostate cancer.[6-7]
- This study aimed to assess:
  - rates of active surveillance/watchful waiting (AS/WW) as the single preferred treatment recommendation between GPS and Baseline patients, and
  - rates of AS/WW received between GPS and Baseline patients.

Study Design

- Physicians were identified who ordered at least four Onco*type* DX® prostate cancer tests between May 8, 2013, and February 1, 2014.
- Twenty-four physicians across 19 centers were identified for inclusion into the study, with a total of 228 GPS patients eligible for the study.
- To establish baseline treatment rates, each physician provided seven patients with similar clinical features to the GPS patients. Patients were chosen among those who were diagnosed with very low- to intermediate-risk prostate cancer in the year immediately before the introduction of GPS.
- Each physician completed an electronic study questionnaire, detailing the relevant clinical, pathologic, and treatment information from the medical records of qualifying patients for the GPS and Baseline groups.
- The proportions of patients recommended and receiving AS/WW were compared between GPS and Baseline groups using the Cochran-Mantel-Haenszel test, stratified by physician.

Results

- A total of 15 physicians agreed to participate and contributed 211 patients.
- Each urologist provided ≥4 GPS cases (range 4–13) and at least one Baseline case (range 1–7).
- A single preferred treatment recommendation was recorded in the patient chart for 174 (82%) patients (114 GPS and 60 Baseline).
- The treatment received was recorded for 200 (95%) patients (117 GPS and 83 Baseline).

Table 3: Physician Characteristics (N=15)

How many years practicing	N	%
≤10	3	20%
12–29	4	27%
20–24	4	27%
≥25	4	27%
Mean	20.4	
Median	15.4	
Std. Dev.	9.5	

Type of practice*	N	%
Large Urology Group Practice (LUGPA)	14	93%
Integrated health system	1	7%
Multispecialty group	2	13%
Solo private practice	1	7%
Hospital-employed	1	7%

*Three physicians practice in two settings; % may not sum to 100% because of multiple responses

Other tests used	N	%
Prolaris®	6	40%
Decipher®	2	13%
ConfirmMDx®	8	53%
Prostate Px (Aureon)	1	7%
None	5	33%

Geographic location: 1 California, 1 Colorado, 1 Georgia, 2 New Jersey, 1 New York, 2 Pennsylvania, 1 Texas, and 1 Virginia

- The physicians studied had an average of 20 years (range 8 to 41) of practice experience and were geographically spread across the country.
- Most participating physicians (93%) were in community-based LUGPAs.
- The majority of physicians (67%) had used at least one genomic or molecular test other than the Onco*type* DX GPS.

Table 4: Baseline Patient Characteristics and Risk Were Similar Between GPS and Baseline Patients

		Baseline		GPS		Total	
		N	%	N	%	N	%
		87		124		211	
Age	<50	2	2%	2	2%	4	2%
	50–65	46	53%	62	50%	108	51%
	>65	39	45%	60	48%	99	47%
	Mean/Median/SD	64.4/54.3/10.4		65.2/56.5/9.9		64.9/55.5/10.1	
Race	African American	16	18%	18	15%	34	16%
	White	65	75%	100	81%	165	78%
	Other	6	7%	6	5%	12	6%
Gleason Score	≤3+3	73	84%	107	86%	180	85%
	7	14	16%	17	14%	31	15%
Baseline PSA	0–4.09	23	26%	36	29%	59	28%
	4.1–10.99	63	72%	84	68%	147	70%
	11–20	1	1%	3	2%	4	2%
	>20	0		1	1%	1	
Clinical Stage	T1a/b	0		4	3%	4	2%
	T1c	80	92%	115	93%	195	92%
	T2a	4	5%	5	4%	9	4%
	T2b	3	3%	0		3	1%
NCCN (GPS n=109; Baseline n=87)*	very low	34	39%	46	42%		
	low	37	43%	44	40%		
	intermediate	16	18%	19	17%		

*NCCN as reported on the GPS requisition form for GPS patients and calculated for Baseline patients.
Some percentages may not sum to 100% because of rounding.

- The majority (82%) presented with NCCN very low- or low-risk disease.
- Both groups had similar clinical risk and demographic features, including age, race and ethnicity, Gleason score, PSA, and clinical stage.

Treatment: Recommended and Received

Table 5: Single Treatment Recommended

Treatment recommended	Baseline N=60		GPS N=114		Absolute difference	Relative difference
	N	%*	N	%*		
Active surveillance/watchful waiting	30	50%	69	61%	**11%**	**22%**
Immediate Treatment	30	50%	45	39%		
Radical prostatectomy only	19	32%	19	17%		
External beam radiation therapy (EBRT), including IMRT, only	6	10%	14	12%		
Brachytherapy only	2	3%	4	4%		
Multimodality therapy	1	2%	1	1%		
Other monotherapy (e.g. cryotherapy, HIFU, etc.)	2	3%	7	6%		

*Column percentages were calculated.
37 (18%) of patients did not have a treatment recommendation documented in the charts.
p=0.110 for difference between Baseline and GPS rates of AS, using Cochran-Mantel-Haenszel test, stratified by physician.

Table 6: Treatment Received

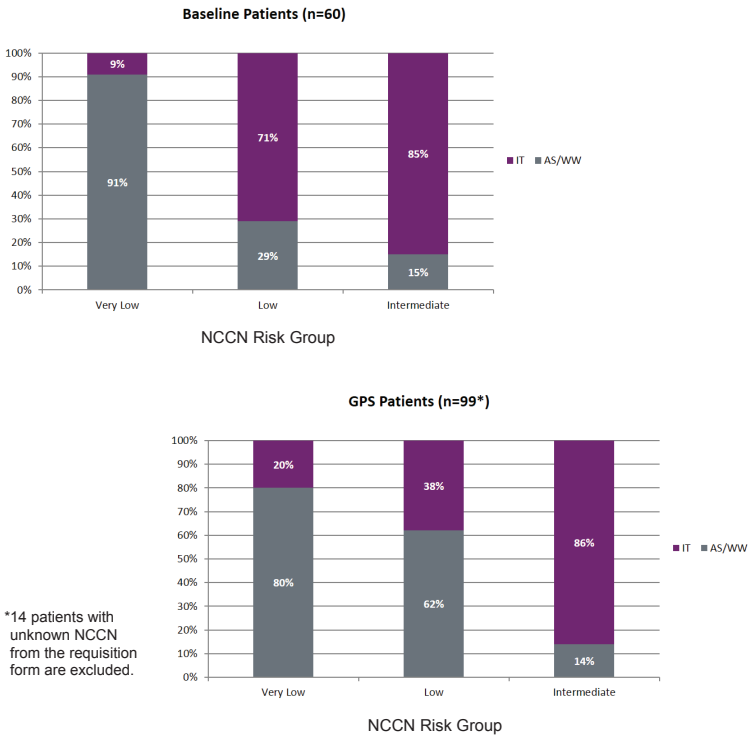
Treatment recommended	Baseline N=83		GPS N=117		Absolute difference	Relative difference
	N	%*	N	%*		
Active surveillance/watchful waiting	36	43%	78	67%	**24%**	**56%**
Immediate Treatment	47	57%	39	33%		
Radical prostatectomy only	22	27%	16	14%		
External beam radiation therapy (EBRT), including IMRT, only	12	14%	7	6%		
Brachytherapy only	5	6%	5	4%		
Multimodality therapy	4	5%	4	3%		
Other monotherapy (e.g. cryotherapy, HIFU, etc.)	4	5%	7	6%		

*Column percentages were calculated
11 (5%) of patients did not have their received treatment documented in the charts.
p=<0.001 for difference between Baseline and GPS rates of AS, using Cochran-Mantel-Haenszel test, stratified by physician.

- In the primary analysis, treatment recommendations for AS/WW were higher for GPS patients compared with Baseline patients (GPS=61%, Baseline= 50%, absolute increase of 11% and relative increase of 22%).
- AS/WW was the treatment received for 67% of GPS and 43% of Baseline patients, which was an absolute increase of 24% and relative increase of 56%.

Genomic Prostate Score: Clinical Utility

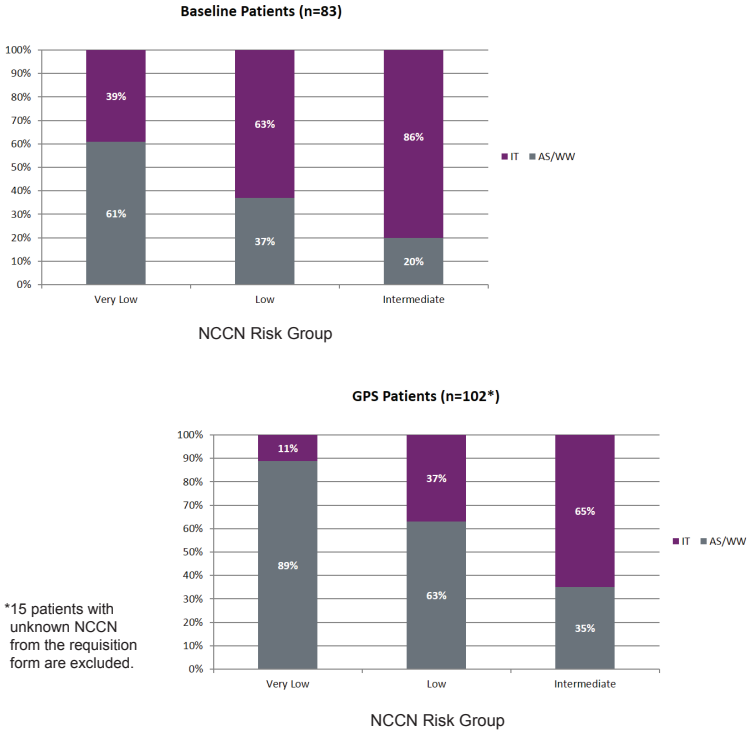
Figure 8: Treatment Recommendations by NCCN Risk Group



*14 patients with unknown NCCN from the requisition form are excluded.

- The rate of AS/WW recommendations was consistent with NCCN clinical risk among both Baseline and GPS patients.
- Among GPS patients, AS/WW was most often recommended (80%) in the NCCN very low-risk group.

Figure 9: Treatment Received by NCCN Risk Group



*15 patients with unknown NCCN from the requisition form are excluded.

- Higher rates of AS/WW were aligned with a lower NCCN risk group.
- AS/WW treatment received was higher for GPS patients compared with Baseline patients within each NCCN risk group.

Figure 10: Proportion of GPS Patients Recommended and Receiving AS/WW

	<60%	60-<70%	70-<80%	80-<90%	>=90%
Evaluable for recommendation (n=99)	12	13	24	46	4
Evaluable for received (n=102)	12	15	27	44	4

- A higher predicted likelihood of favorable pathology was associated with a higher proportion of recommendations for and receipt of AS/WW.

Summary/Conclusions

Development and Validation

- The development program addressed several challenges, including tumor heterogeneity, multifocality, and biopsy under-sampling.
- Clinical validation of the 17-gene assay used archived tissue and a prospectively specified statistical plan to validate the ability of the GPS to predict AP in a large, contemporary cohort of men with prostate cancer.
- A second clinical validation study in a racially diverse group confirmed the validation of the GPS with AP, validated GPS as a predictor of BCR following RP, and demonstrated an association with MR.
- **The development and validation of the assay meet the established criteria for Level 1B evidence.**

Clinical Utility

- In this real-world chart review study, despite higher than expected baseline rates of AS/WW, the proportion of men in the GPS group who were recommended and chose AS/WW as initial disease management was higher than in the Baseline group.
  - In the Baseline group, fewer men (43%) initiated AS than recommended (50%), suggesting that uncertainty may drive some men to choose immediate therapy despite a recommendation of AS from their physicians.
- Recommendations for and choice of AS/WW were directionally aligned with the GPS assay-predicted likelihood of favorable pathology and were consistent across all clinical risk categories.
- Physician recommendation for and use of active surveillance as initial management of men with very low- to intermediate-risk prostate cancer were higher in men receiving GPS compared to the baseline group.
- **This clinical utility study suggests that the assay provides actionable information to patients and physicians to individualize treatment recommendations and decisions.**

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This study was sponsored by Genomic Health, Inc.

The authors thank Kevin Chew for his contributions.

GHI40118