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# Association of Molecular Subtypes With Differential Outcome to Apalutamide Treatment in Nonmetastatic Castration-Resistant Prostate Cancer

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**IMPORTANCE** There is a need to identify prognostic biomarkers to guide treatment intensification in patients with nonmetastatic castration-resistant prostate cancer (nmCRPC).

**OBJECTIVE** To examine whether molecular subtypes predict response to apalutamide, using archived primary tumor samples from the randomized, double-blind, phase 3 SPARTAN trial.

**DESIGN, SETTING, AND PARTICIPANTS** In this cohort study, gene expression data from 233 archived samples from patients with nmCRPC enrolled in the SPARTAN trial were generated using a human exon microarray. The present analysis was conducted from May 10, 2018, to October 15, 2020.

**INTERVENTIONS** Patients were randomized (2:1) to apalutamide, 240 mg/d, with androgen deprivation therapy (apalutamide+ADT) or placebo+ADT.

**MAIN OUTCOMES AND MEASURES** Patients were stratified into high-risk and low-risk categories for developing metastases based on genomic classifier (GC) scores for high (GC >0.6) and low to average (GC≤0.6) and into basal and luminal subtypes; associations between these molecular subtypes and metastasis-free survival (MFS), overall survival (OS), and progression-free survival 2 (PFS2) were evaluated using Cox proportional hazards regression and Kaplan-Meier analysis.

**RESULTS** Median age of the 233 included patients was 73 (range, 49-91) years. A total of 116 of 233 patients (50%) in the SPARTAN biomarker subset had high GC scores. Although all patients receiving apalutamide+ADT had improved outcomes, having high GC scores was associated with the greatest improvement in MFS (hazard ratio [HR], 0.21; 95% CI, 0.11-0.40; *P* < .001), OS (HR, 0.52; 95% CI, 0.29-0.94; *P* = .03), and PFS2 (HR, 0.39; 95% CI, 0.23-0.67; *P* = .001) vs placebo+ADT. In total, 152 of 233 patients (65%) had the basal molecular subtype. Although there were no significant differences in MFS, PFS2, or OS between patients with the luminal vs basal subtype in the placebo+ADT arm, patients with the luminal subtype in the apalutamide+ADT arm had a significantly longer MFS (apalutamide+ADT: HR, 0.40; 95% CI, 0.18-0.91; *P* = .03; placebo+ADT: HR, 0.66; 95% CI, 0.33-1.31; *P* = .23) compared with patients with basal subtype; similar trends were observed for OS (apalutamide+ADT: HR, 0.50; 95% CI, 0.25-0.98; *P* = .04; placebo+ADT: HR, 0.78; 95% CI, 0.38-1.60; *P* = .50), and PFS2 (apalutamide+ADT: HR, 0.71; 95% CI, 0.42-1.22; *P* = .22; placebo+ADT: HR, 0.72; 95% CI, 0.38-1.39; *P* = .33). In regression analysis, the luminal-basal subtype score was significantly associated with MFS in patients receiving apalutamide+ADT (HR, 2.65; 95% CI, 1.15-6.08; *P* = .02), whereas GC score was significantly associated with MFS in placebo+ADT recipients (HR, 2.09; 95% CI, 1.02-4.27; *P* = .04).

**CONCLUSIONS AND RELEVANCE** The findings of this study suggest that the GC score and basal-luminal subtype derived from archived tumor specimens may be biomarkers of response to apalutamide+ADT in the nmCRPC setting. Although overall, the addition of apalutamide to ADT was beneficial, higher-risk and luminal subtypes appeared to benefit most. Obtaining GC scores may be useful for identifying patients for early treatment intensification with apalutamide, and basal-luminal subtyping may be a beneficial approach for patient selection for further treatment intensification in trials combining novel therapies with apalutamide.

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Invited Commentary
Supplemental content

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Treatment with androgen deprivation therapy (ADT) represents a standard of care for patients with prostate cancer with recurrence following local therapy. However, most patients will develop resistance to ADT— termed castration-resistant prostate cancer (CRPC). One-third of patients with nonmetastatic CRPC (nmCRPC) will develop metastases or die within a median of 2.5 years.[1] In men with metastatic CRPC, the addition of second-generation androgen signaling inhibitors to ADT prolongs life.[2,3] The SPARTAN randomized, double-blind, phase 3 clinical trial demonstrated that the addition of the androgen-signaling inhibitor apalutamide to ongoing ADT in patients with nmCRPC significantly improved metastasis-free survival (MFS), time to symptomatic progression, time to second progression (PFS2), and overall survival (OS).[4,5] We sought to understand the molecular characteristics associated with these improved outcomes.

Genomic risk classifiers have been widely used in prostate cancer at initial diagnosis and in the adjuvant setting to determine risk for metastasis. The Decipher prostate test (Decipher Biosciences Inc) uses a clinical grade whole-transcriptome assay to report a 22-gene genomic classifier (GC) score that has been independently validated for predicting the risk of prostate cancer metastasis at initial diagnosis and after radical prostatectomy.[6,7] Other molecular subtyping approaches include the luminal and basal molecular classification. Approximately two-thirds of patients with localized prostate cancer have luminal-type and one-third have basal-like subtypes, with the suggestion that patients with luminal type may respond better to first-line ADT.[8] Prostate cancer with a luminal expression profile is associated with high androgen receptor signaling and steroid hormone receptor processing, and this profile is observed more frequently in indolent or hormone-sensitive disease settings.[9] Conversely, prostate cancer with basal expression profiles is associated with stem cell and epithelial-mesenchymal transition biology and MYC transcriptional programs, shown to be enriched in aggressive castration-resistant disease.[9]

We undertook gene expression profiling of archived radical prostatectomy or diagnostic biopsy specimens from patients enrolled in the SPARTAN trial to assess the utility of both of these molecular classifiers (Decipher GC and luminal and basal subtypes) in identifying patients who may benefit most from the subsequent addition of apalutamide to ADT in the nmCRPC setting.

### Key Points

**Question** Are specific molecular features associated with benefit from the addition of apalutamide to androgen deprivation therapy in patients with nonmetastatic castration-resistant prostate cancer?

**Findings** In this cohort study examining data on 233 patients with nonmetastatic castration-resistant prostate cancer, patients with a high Decipher genomic classifier score demonstrated more sustained benefit with the addition of apalutamide compared with patients with a low genomic classifier score. Patients with luminal subtype tumors also showed more sustained benefit with the addition of apalutamide.

**Meaning** The molecular signatures examined in this study appear to have prognostic utility and can be useful in clinical decision-making regarding treatment intensification in patients with nonmetastatic castration-resistant prostate cancer at high risk for metastasis; larger studies are warranted for validation of these findings.

## Methods

### Study Design

Study design details for the SPARTAN trial (NCT01946204) have been reported.[4,10] The present study was conducted from May 10, 2018, to October 15, 2020. As previously reported, patients in the trial with nmCRPC were randomly assigned 2:1 to receive ongoing ADT with apalutamide, 240 mg/d (apalutamide+ADT), or placebo+ADT. On progression of the cancer, patients had the option of receiving subsequent therapy at the discretion of the treating physician, including open-label abiraterone acetate. Metastasis-free survival was defined as the time from randomization to first evidence of radiographically detectable bone or soft tissue distant metastasis (per central review by independent radiologists blinded to patient identifiers and treatment) or death from any cause, whichever occurred first. Second progression was defined as the time from randomization to investigator-assessed disease progression, including prostate-specific antigen progression, radiographically detected distant metastasis, symptomatic progression, or any combination during the first subsequent treatment for nmCRPC or death from any cause before the start of the second subsequent anticancer therapy, whichever occurred first. Overall survival was defined as the time from randomization to death. Metastasis-free survival was the primary end point, assessed at the initial analysis with a median follow-up of 20.3 months and a clinical cutoff date of May 19, 2017,[4] and PFS2 and OS were secondary end points assessed at the second interim analysis with a median follow-up of 42 months and clinical cutoff date of February 1, 2019.[5] Institutional review boards at all institutions approved the protocol; the study was conducted in accordance with the principles of the Declaration of Helsinki.[11] Participants provided informed consent.

### Clinical Samples and Microarray Processing

Tumor samples were collected from patients who consented for exploratory biomarker analysis. Formalin-fixed, paraffin-embedded tumor samples from the diagnostic biopsy or radical prostatectomy were processed for RNA extraction and complementary DNA generation. Samples lacking sufficient tumor content by pathological assessment or yielding less than 3 µg of complementary DNA after amplification were excluded from analysis. Gene expression data were generated using the Decipher Human Exon 1.0 ST microarray (ThermoFisher). Microarray processing was performed in a Clinical Laboratory Improvement Amendments-certified clinical operations laboratory. Quality control was performed using Affymetrix Power Tools and microarray data were normalized and summarized using the Single Channel Array Normal-

ization algorithm.[12] Only samples that passed all quality control criteria were included in the final analysis. Gene-level summarization was based on gene annotations from the Ensembl v79 human gene models and the hg38 human genome build.

### Decipher and Basal-Luminal Clustering

Normalized expression data were evaluated to generate the Decipher GC score and 2 different luminal vs basal molecular subtype signature scores based on the original algorithms reported by Erho and colleagues,[6] Zhao and colleagues,[8] and Zhang and colleagues.[9] Continuously distributed numeric scores were binarized into categorical variables using prespecified thresholds (GC score metastasis risk groups: low or intermediate risk [collectively termed as lower risk], ≤0.6 vs higher risk, >0.6; PAM50: luminal A, luminal B, and basal; Zhang et al[9]: luminal vs basal). Frequencies of GC risk groups, PAM50, and Zhang et al[9] luminal/basal subtypes in localized prostate cancer and molecular pathways enriched in the subtypes were evaluated from the Decipher Genomic Resource Informatics Database (GRID; n = 16 806), a prospective registry of tumors from clinical use of the Decipher test.[13] The Decipher test was conducted as standard of care for postoperative decision-making between December 2015 and September 2017 for tumor samples.[14]

### Statistical Analysis

Univariate and multivariable associations of molecular profiles with clinical outcome were evaluated using Cox proportional hazards regression model (included 3 terms: treatment, biomarker status, and treatment and biomarker status interaction) and Kaplan-Meier analysis. Genomic classifier and basal-luminal scores were evaluated in Cox proportional hazards regression models as binary variables, Eastern Cooperative Oncology Group performance status was entered as a categorical variable, and prostate-specific antigen level was considered a continuous variable. Evaluation of linearity assumption for prostate-specific antigen level at baseline and all end points showed no evidence of nonlinearity. The associations between the Decipher GC score and luminal-basal subtype scores were evaluated using Pearson correlation coefficient and Fisher exact tests. Significance testing was unpaired and 2-sided, with $P < .05$ required to claim statistical significance.

## Results

Of the 1207 patients enrolled in the SPARTAN trial, tumor samples were collected from 340 individuals (eFigure 1 in the Supplement). Of the 340 samples, 280 had sufficient tumor tissue samples for gene expression microarray analysis and 233 samples passed microarray quality control: 154 from patients treated with apalutamide+ADT and 79 from patients who received placebo+ADT were enrolled. Notably, 15 patients in the placebo group within the biomarker subset crossed over to receive apalutamide as subsequent therapy. The mean time from obtaining the archived tissues subsequently used in the biomarker studies reported and randomization in the SPARTAN trial was 6.7 (range, 0.2-24.8) years.

The biomarker subset and overall intention-to-treat populations were comparable with respect to demographic and clinical characteristics (**Table 1**). Patients in the biomarker subset (n = 233) had a median age of 73 (range, 49-91) years (eTable 1 in the Supplement). Compared with the nonbiomarker subset, the biomarker subset had a shorter median time from diagnosis to randomization (6.66 vs 8.40 years) and had a different distribution of Gleason scores with fewer Gleason score less than 7 tumors and more Gleason score 7 or greater tumors (eTable 1 in the Supplement).

### Enrichment of High-Risk Basal Tumors

Gene expression profiles were assessed in archival samples of primary prostate tumors from patients who progressed to nmCRPC before enrollment in the SPARTAN trial. **Figure 1**A shows the distributions of GC and luminal vs basal scores in the biomarker subset. The PAM50 classifier was used to subtype the cohort and found few luminal A tumors compared with the basal and luminal B subtypes. We compared the proportions of patients in the SPARTAN biomarker subset in each class relative to the proportions observed in localized prostate cancer from the prospective Decipher GRID database (n = 16 806) (Figure 1B). The SPARTAN biomarker subset was enriched for patients with higher-risk GC scores (116 of 233 [50.0%]) relative to localized prostate cancer in the Decipher GRID database (6890 of 16 806 [41.0%]). Similarly, basal tumors were enriched in the SPARTAN data set relative to the Decipher GRID and retrospective cohorts (152 of 233 [65.2%] basal, 11 of 233 [4.7%] luminal A, and 70 of 233 [30.0%] luminal B in SPARTAN vs 6218 of 16 806 [37.0%] basal, 5714 of 16 806 [34.0%] luminal A, and 4705 of 16 806 [28.0%] luminal B in localized prostate cancer). A similar enrichment of basal tumors was observed with the Zhang et al[9] luminal vs basal signature score (152 of 233 [65.2%] basal and 81 of 233 [34.8%] luminal in SPARTAN vs 4425 of 16 806 [26.3%] basal and 12 381 of 16 806 [73.7%] luminal tumors in the Decipher GRID).

### Outcomes After Addition of Apalutamide to ADT in Patients With Higher- and Lower-Risk GC Scores

In the SPARTAN trial, apalutamide+ADT demonstrated MFS benefit with median MFS of 40.5 vs 16.2 months in the ADT-alone group (hazard ratio [HR], 0.28; 95% CI, 0.23-0.35; $P < .001$). Although in the biomarker subset, apalutamide+ADT was associated with a significantly longer MFS vs ADT alone in lower-risk patients with a GC score less than or equal to 0.6 (HR, 0.46; 95% CI, 0.23-0.95; $P = .04$) (**Figure 2**B), an even larger treatment effect of apalutamide+ADT was observed in patients at higher risk with a GC score greater than 0.6 (HR, 0.21; 95% CI, 0.11-0.40; $P < .001$) (Figure 2A). While the interaction between GC score and apalutamide treatment effect for MFS was not statistically significant (HR, 2.50; 95% CI, 0.98-6.38; $P = .055$), further research might find that a high GC-score may estimate the probability of the greatest response to apalutamide (eTable 2 and eFigure 3 in the Supplement).

Consistent with MFS, significant improvements in OS (HR, 0.52; 95% CI, 0.29-0.94; $P = .03$) (eFigure 2 in the Supple-

Table 1. Demographic and Baseline Characteristics

| Characteristic | Total (ITT population) | | Biomarker | |
|---|---|---|---|---|
| | Apalutamide + ADT (n = 806) | Placebo + ADT (n = 401) | Apalutamide + ADT (n = 154) | Placebo + ADT (n = 79) |
| Age, median (range), y | 74 (48-94) | 74 (52-97) | 73 (49-91) | 74 (52-90) |
| Median time from initial diagnosis to randomization, y | 7.95 | 7.85 | 6.67 | 6.55 |
| Tumor category at diagnosis, No. (%) | 794 | 394 | NA | NA |
| T1 | 141 (17.8) | 63 (16.0) | 21 (13.6) | 13 (16.5) |
| T2 | 265 (33.4) | 123 (31.2) | 56 (36.4) | 19 (24.1) |
| T3 | 296 (37.3) | 163 (41.4) | 59 (38.3) | 41 (51.9) |
| T4 | 32 (4.0) | 16 (4.1) | 4 (2.6) | 4 (5.1) |
| TX | 60 (7.6) | 29 (7.4) | 14 (9.1) | 2 (2.5) |
| Gleason score at initial diagnosis, No. (%) | 784 | 387 | NA | NA |
| <7 | 152 (19.4) | 72 (18.6) | 12 (7.8) | 10 (12.7) |
| 7 | 291 (37.1) | 146 (37.7) | 64 (41.6) | 35 (44.3) |
| >7 | 341 (43.5) | 169 (43.7) | 78 (50.6) | 34 (43.0) |
| Prostate-specific antigen (ng/mL), mean (SD) | 14.9 (22.53) | 15.9 (23.75) | 13.5 (25.6) | 17.5 (19.0) |
| Prostate-specific antigen doubling time | | | | |
| Median, mo | 4.40 | 4.50 | 4.15 | 4.60 |
| ≤6 mo, No. (%) | 576 (71.5) | 284 (70.8) | 115 (74.7) | 57 (72.2) |
| >6 mo, No. (%) | 230 (28.5) | 117 (29.2) | 39 (25.3) | 22 (27.8) |
| Use of bone-sparing agent, No. (%) | | | | |
| Yes | 82 (10.2) | 39 (9.7) | 13 (8.4) | 4 (5.1) |
| No | 724 (89.8) | 362 (90.3) | 141 (91.6) | 75 (94.9) |
| Classification of local or regional nodal disease, No. (%) | | | | |
| N0 | 673 (83.54) | 336 (83.8) | 122 (79.2) | 65 (82.3) |
| N1 | 133 (16.5) | 65 (16.2) | 32 (20.8) | 14 (17.7) |
| Previous prostate-cancer treatment, No. (%) | 803 | 401 | NA | NA |
| Prostatectomy or radiotherapy | 617 (76.6) | 307 (76.6) | 96 (62.3) | 48 (60.8) |
| Gonadotropin-releasing hormone analogue agonist | 780 (96.8) | 387 (96.5) | 151 (98.1) | 78 (98.7) |
| First-generation antiandrogen agent | 592 (73.4) | 290 (72.3) | 109 (70.8) | 60 (75.9) |
| Median metastasis-free survival (95% CI), mo | 40.5 (NR-NR) | 16.2 (14.6-18.4) | NR (25.9-NR) | 16.2 (14.5-22.1) |
| Median progression during subsequent therapy (95% CI), mo | 55.6 (53.0-61.2) | 41.2 (37.7-46.2) | 52.8 (48.4-NR) | 36.6 (28.8-49.3) |
| Median duration of subsequent therapy (95% CI), mo | 7.6 (6.5-9.5) | 18.4 (16.0-22.9) | 6.7 (5.1-11.1) | 17.6 (11.9-25.4) |
| Median overall survival (95% CI), mo | 73.9 (61.2-NR) | 59.9 (52.8-NR) | NR (59.8-NR) | 52.7 (45.3-NR) |

Abbreviations: ADT, androgen deprivation therapy; ITT, intention-to-treat; NA, not applicable; NR, not reached.

SI conversion: to convert prostate-specific antigen to micrograms per liter, multiply by 1.

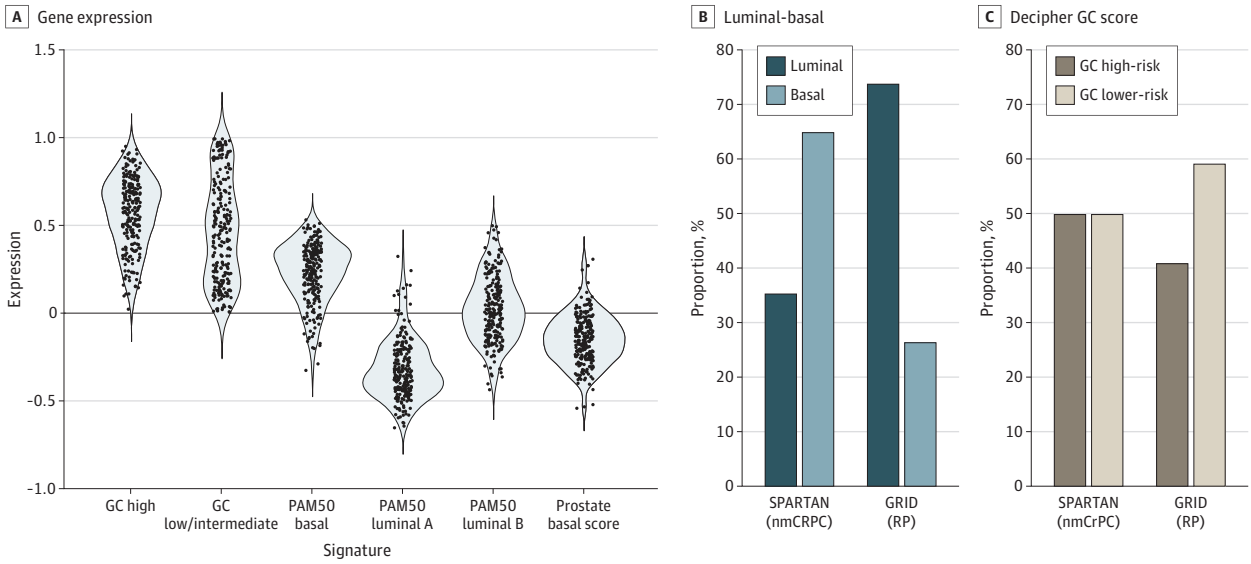
ment) and PFS2 (HR, 0.39; 95% CI, 0.23-0.67; $P = .001$) (eFigure 2 in the Supplement) were observed with apalutamide+ADT in patients with a higher-risk GC score. In patients with a lower-risk GC score, significant treatment effects on OS (eFigure 2 in the Supplement) and PFS2 (eFigure 2 in the Supplement) were not observed. There was no statistically significant interaction between GC score and apalutamide treatment effect for OS or PFS2 (eTable 2 and eFigure 3 in the Supplement).

In comparing clinical outcomes within the apalutamide+ADT and placebo+ADT groups, patients with higher-risk GC scores showed significantly shorter MFS vs those with lower-risk GC scores who received placebo+ADT (median MFS, 14.5 vs 22.1 months; HR, 0.43; 95% CI, 0.22-0.85; $P = .01$) (Figure 2D). Conversely, both GC higher- and lower-risk scores showed similar outcomes when patients received apalutamide+ADT (median MFS: not reached; HR, 1.11; 95% CI, 0.58-2.13; $P = .75$) (Figure 2C). These trends were consistently observed for OS (eFigure 2 in the Supplement) and PFS2 (eFigure 2 in the Supplement). Together, these findings suggest that the addition of apalutamide to ADT may overcome the poor prognosis associated with a higher-risk GC score.

## Basal and Luminal Tumor Responses to Apalutamide

Apalutamide+ADT was associated with a significantly longer MFS in both the basal (HR, 0.34; 95% CI, 0.20-0.58; $P < .001$) (**Figure 3**A) and luminal (HR, 0.22; 95% CI, 0.08-0.56; $P = .002$) (Figure 3B) tumor cohorts. Sustained long-term benefit with apalutamide+ADT compared with ADT alone was seen, with a trend toward longer OS in both the basal (HR, 0.67; 95% CI, 0.40-1.14; $P = .14$) (eFigure 4 in the Supplement) and luminal

Figure 1. Distribution of Decipher and Basal-Luminal Scores in the SPARTAN Study and the Decipher Genomic Resource Informatics Database (GRID)




A, Expression of genes in patients with high (>0.6) and low (≤0.6) genomic classifier (GC) scores and luminal and basal tumors. B, Proportion of patients with basal and luminal tumors. C, High-risk and lower-risk GC scores in the SPARTAN biomarker subset and the Decipher GRID database from patients treated with radical prostatectomy (RP). nmCRPC indicates nonmetastatic castration-resistant prostate cancer.

(HR, 0.43; 95% CI, 0.19-1.00; $P$ = .05) (eFigure 4 in the Supplement) cohorts, as well as significantly longer PFS2 in the basal (HR, 0.49; 95% CI, 0.30-0.81; $P$ = .01) (eFigure 4 in the Supplement) and luminal (HR, 0.47; 95% CI, 0.23-0.93; $P$ = .03) (eFigure 4 in the Supplement) cohorts. There was no significant interaction between basal-luminal subtyping and apalutamide treatment effect for MFS, OS, or PFS2 (eTable 3 and eFigure 5 in the Supplement).

In comparing clinical outcomes observed within treatment arms, there were no significant differences in MFS between patients with luminal vs basal subtypes who had received placebo+ADT (median MFS, 22.0 vs 14.6 months; HR, 0.66; 95% CI, 0.33-1.31; $P$ = .23) (Figure 3D). Conversely, patients with luminal subtypes had significantly longer MFS vs patients with basal subtypes who received apalutamide+ADT (median MFS: not reached; HR, 0.40; 95% CI, 0.18-0.91; $P$ = .03) (Figure 3C). Similar trends (luminal vs basal subtypes) were observed for long-term outcomes for apalutamide+ADT-treated patients, for both OS (apalutamide+ADT: HR, 0.50; 95% CI, 0.25-0.98; $P$ = .04; placebo+ADT: HR, 0.78; 95% CI, 0.38-1.60; $P$ = .50) (eFigure 4 in the Supplement) and PFS2 (apalutamide+ADT: HR, 0.71; 95% CI, 0.42-1.22; $P$ = .22; placebo+ADT: HR, 0.72; 95% CI, 0.38-1.39; $P$ = .33). (eFigure 4 in the Supplement). These results suggest that, although patients with basal or luminal tumors benefit from apalutamide, patients with luminal subtypes have better outcomes.

### Multivariable Analysis

Given the association of basal prostate cancer with aggressive disease and the enrichment of both patients with basal tumors and patients with higher-risk GC scores in the SPARTAN trial, we evaluated whether these 2 signatures provide independent information. A low level of correlation between the basal-luminal scores[9] and the GC scores[6] in the SPARTAN trial ($R^2$ = 0.2368) (eFigure 6 in the Supplement) was observed. Although patients with a higher-risk GC score were more likely to have basal vs luminal tumors, the difference in proportions was not statistically significant (eFigure 6 in the Supplement).

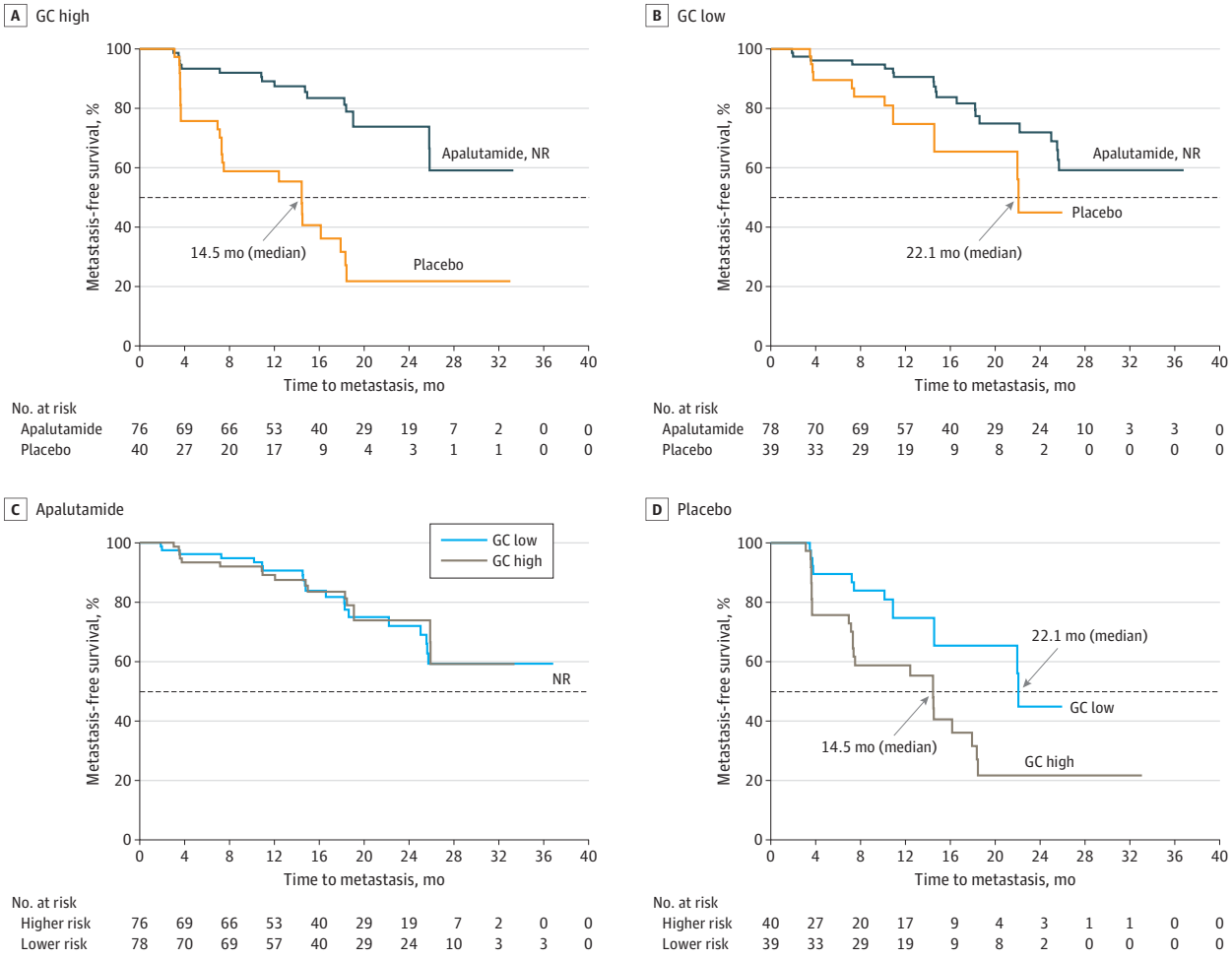
In multivariable analysis with clinicopathologic features and gene expression signature scores, the basal-luminal subtype score was the only variable significantly associated with MFS in patients who received apalutamide+ADT (HR, 2.65; 95% CI, 1.15-6.08; $P$ = .02), whereas the GC score was the only variable significantly associated with MFS in placebo+ADT recipients (HR, 2.09; 95% CI, 1.02-4.27; $P$ = .04) (**Table 2**).

## Discussion

This molecular analysis of tumor samples from a large phase 3 randomized clinical trial has identified possible genomic predictors of outcome in patients with nmCRPC.[4] Of note, molecular profiling was undertaken in archived tumor samples from ADT-naive patients, collected, on average, 6.7 years before their enrollment in the nmCRPC trial, suggesting that these molecular determinants of outcome were established at a much earlier clinical time.

All patients, regardless of GC risk group, experienced a longer MFS with apalutamide+ADT treatment; however, patients with high GC scores derived the greatest absolute benefit from treatment intensification with apalutamide. Although the Decipher GC score was specifically developed and vali-

Figure 2. Associations of Decipher Risk Scores With Metastasis-Free Survival (MFS)

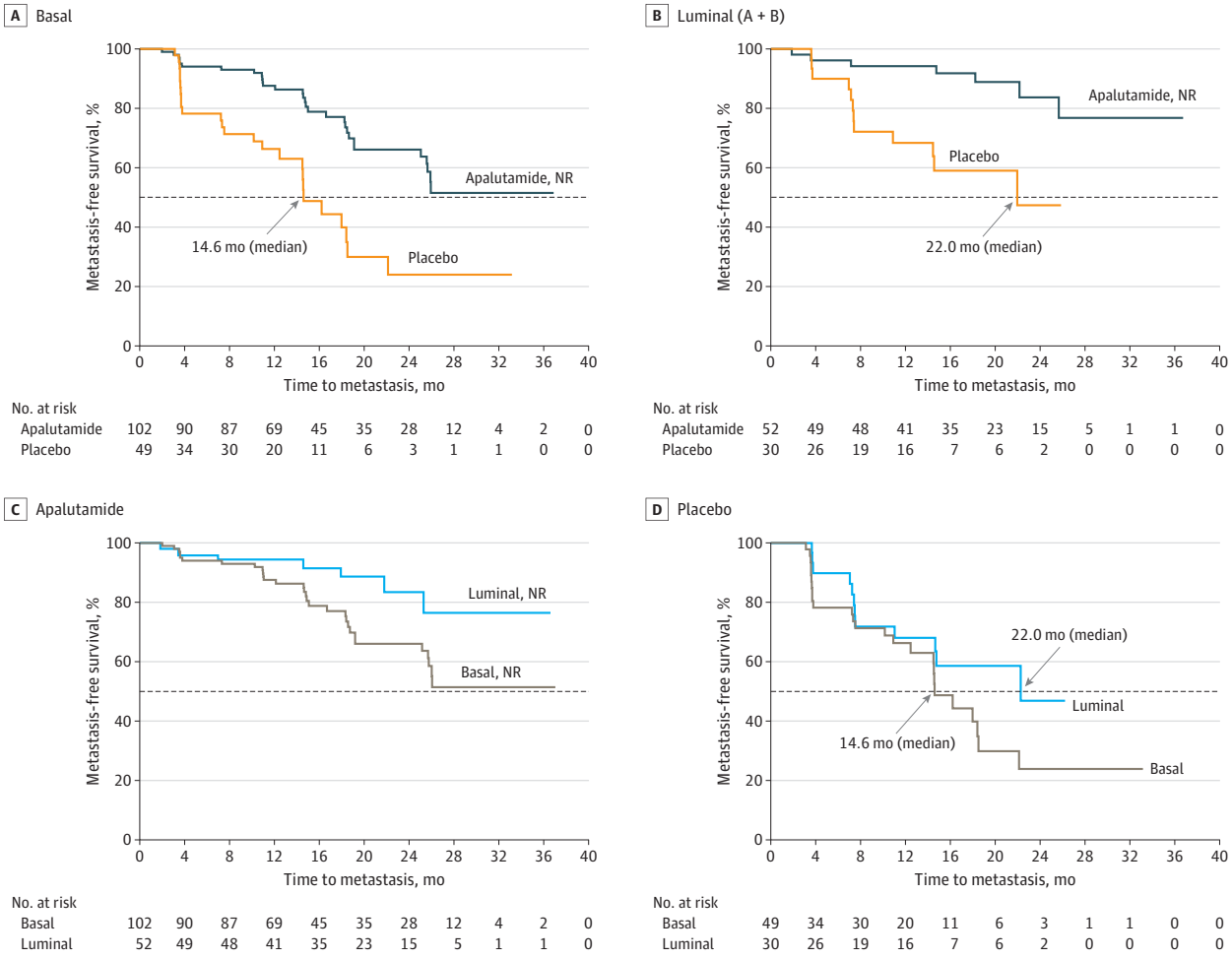



A, MFS by treatment arm in patients with high (>0.6) Decipher genomic classifier (GC) scores (hazard ratio [HR], 0.21; 95% CI, 0.11-0.40; $P < .001$). B, MFS by treatment arm in patients with low (≤0.6) GC scores (HR, 0.46; 95% CI, 0.23-0.95; $P = .04$). C, MFS in patients with high and low GC scores in the group receiving apalutamide, 240 mg/d, with androgen deprivation therapy (apalutamide+ADT) (HR, 1.11; 95% CI, 0.58-2.13; $P = .75$). D, MFS in patients with high and low GC scores within the placebo+ADT group (HR, 0.43; 95% CI, 0.22-0.85; $P = .01$). NR indicates not reached.

dated to predict a metastasis outcome, but not OS or PFS2, patients with a higher-risk GC score also had significantly longer OS and PFS2 with apalutamide+ADT vs ADT alone. By contrast, patients with a lower-risk GC score showed only marginal incremental benefit with apalutamide+ADT over ADT alone. Although the study was underpowered to measure biomarker:treatment interaction effects (based on an ad hoc power analysis), future studies may find that a high GC-score may estimate the probability of greatest benefit for MFS from the addition of apalutamide. Moreover, molecular subtyping of tumors revealed that both patients with luminal and basal tumors had significantly longer MFS when treated with apalutamide+ADT; however, patients with luminal tumors treated with apalutamide+ADT showed more sustained benefit and had better long-term outcomes (OS and PFS2). Notably, results for long-term outcomes (OS and PFS2) in the GC lower-risk score group and patients with basal tumors may have been confounded by the broad use of subsequent therapies and the need for large sample sizes to demonstrate treatment effects.

The molecular profiling we report adds prognostic information to previous reports of positive phase 3 trials in patients with CRPC.[2,15-22] One of the perceived barriers to identifying prognostic or predictive biomarkers of outcome in the CRPC setting is the lack of availability of a recent tissue sample from patients. Traditionally, it has been assumed that, given the amount of time elapsed between diagnosis and primary treatment and development of CRPC, archived samples from the primary tumor will not reflect the biological behavior of CRPC in the same patient.[23,24] However, in this study, despite the long interval between collection of the primary tumor sample and subsequent enrollment in the SPARTAN trial, molecular signatures from the primary tumor predicted outcome of treatment in the CRPC state. Compared with molecular profiles reported in patients with localized primary prostate cancer, we observed a significant enrichment of higher-risk and

Figure 3. Associations of Basal and Luminal Subtypes With Metastasis-Free Survival (MFS)




A, MFS by treatment arm in patients with basal tumors (hazard ratio [HR], 0.34 (95% CI, 0.20-0.58; $P < .001$). B, MFS by treatment arm in patients with luminal tumors (HR, 0.22; 95% CI, 0.08-0.56; $P = .002$). C, MFS in patients with basal and luminal tumors within the group receiving apalutamide, 240 mg/d, with androgen deprivation therapy (apalutamide+ADT) (HR, 0.40; 95% CI, 0.18-0.91; $P = .03$). D, MFS in patients with basal and luminal tumors within the placebo+ADT group (HR, 0.66; 95% CI, 0.33-1.31; $P = .23$). NR indicates not reached.

basal molecular subtypes, consistent with the aggressive nature of cancers that progress to the castration-resistant state. Overall, our finding that analysis of primary tumor specimens can inform prognosis and prediction of treatment response in the subsequent CRPC state has significant implications for future CRPC studies and may motivate the retrieval and analysis of primary tumors from previously conducted CRPC studies.

Previous reports using samples from randomized trials have reported that the Decipher GC is prognostic in 2 castration-sensitive settings: patients with prostate-specific antigen recurrences after surgery and patients with metastatic castration-sensitive disease.[25-27] Our study supports the use of GC as a prognostic indicator in the nmCRPC setting as well.

We report an interaction with a $P = .055$, which suggests a possibility of future benefit, pending future research into high-risk GC status and apalutamide-treatment effect. Although a high GC score was associated with significantly worse prognosis compared with lower GC scores in patients treated with ADT alone, the outcomes of patients with high GC scores improved so substantially with apalutamide that there were no significant differences between outcomes in patients with high vs lower GC scores in the apalutamide+ADT arm, suggesting that apalutamide overcame the negative prognosis associated with high GC scores.

Our findings also suggest that basal-luminal subgrouping provides information independent of the GC score. In a multivariable analysis, basal-luminal subtype was the only variable significantly associated with MFS in apalutamide-treated patients.[5] Other studies[8,9] have used molecular profiling to identify luminal and basal subtypes of prostate cancer associated with disease biologic factors, androgen receptor activity, and disease progression. Basal and luminal cells include self-sustaining lineages that can give rise to prostate cancer,[28] and previous studies[8,9] have demonstrated that luminal prostate cancers may respond better than basal sub-

Table 2. Multivariable Analysis of Metastasis-Free Survival With Clinicopathologic Factors

| Treatment and variable | Median (95% CI)[a] | *P* value[b] | HR (95% CI) |
|---|---|---|---|
| Apalutamide+ADT[c,d] | | | |
| Decipher GC score | NR (25.9-NR) | .62 | 0.84 (0.43-1.64) |
| ECOGBL | NA | .23 | 0.52 (0.18-1.51) |
| PSABL | NA | .32 | 1.18 (0.85-1.64) |
| PSADTGR1 | NA | .73 | 0.87 (0.41-1.87) |
| Luminal vs basal score | NA | .02 | 2.65 (1.15-6.08) |
| Placebo+ADT[e] | | | |
| Decipher GC score | 16.2 (14.5-22.1) | .04 | 2.09 (1.02-4.27) |
| ECOGBL | NA | .87 | 0.93 (0.40-2.18) |
| PSABL | NA | .42 | 1.13 (0.84-1.53) |
| PSADTGR1 | NA | .53 | 0.77 (0.34-1.73) |
| Luminal vs basal score | NA | .35 | 1.40 (0.69-2.82) |
| All patients[f] | | | |
| Decipher GC score | NR (25.6-NR) | .36 | 1.24 (0.78-1.98) |
| ECOGBL | NA | .24 | 0.68 (0.35-1.29) |
| PSABL | NA | .13 | 1.19 (0.95-1.49) |
| PSADTGR1 | NA | .53 | 0.84 (0.48-1.45) |
| Luminal vs basal score[g] | NA | .03 | 1.79 (1.06-3.04) |

Abbreviations: ADT, androgen deprivation therapy; ECOGBL, baseline Eastern Cooperative Oncology Group performance status; GC, genomic classifier; HR, hazard ratio; NA, not applicable; NR, not reached; PSABL, baseline prostate-specific antigen; PSADT, prostate-specific antigen doubling time.

[a] The median time to MFS was not reached because half of the patients did not progress to MFS. In this is a multivariable model, there was only 1 median time to event in each treatment arm. Median time to event was associated with the treatment arm itself and not dependent on any variable.

[b] Determined using Cox proportional hazards regression analysis.

[c] Apalutamide, 240 mg/d.

[d] The events/total was 37 of 154 (24.0%).

[e] The events/total was 37 of 79 (46.8%).

[f] The events/total was 74 of 233 (31.8%).

[g] Basal and luminal scores were derived using the Zhang et al[9] 2016 basal signature. Higher scores indicate basal tumor biology; lower scores, luminal tumor biology.

type disease to first-line ADT in the castration-sensitive setting. Basal tumors are associated with androgen receptor independence, epithelial-mesenchymal transition biologic factors, and dedifferentiation that confers low sensitivity to ADT and high risk for metastasis (eFigure 7 in the Supplement). A recent report also described the presence of basal lineage in nonresponders to enzalutamide in the metastatic CRPC setting.[29] Our results suggest that these characteristics continue to have significant clinical implications even relatively late in the course of disease progression, and that the increased responsiveness of luminal-subtype cancers to first-line ADT translates to increased responsiveness of these same cancers to apalutamide in the nmCRPC setting. Our findings reinforce the need to identify better therapies for patients with basal nmCRPC disease; although these patients derive benefit from apalutamide, their worse outcomes suggest the need for future studies to improve on this therapy.

### Limitations

Although the clinical data reported in this analysis are from a prospective, double-blind, randomized trial, there are limitations inherent to the retrospective analyses of samples. In addition, although the demographic and clinical characteristics of the subset of patients who had samples available for analysis were similar to those of the overall SPARTAN population, the potential for selection bias remains. Furthermore, this study did not address whether the outcome of subsequent treatment with androgen-signaling inhibitors may be sufficient to overcome poor prognosis in patients with higher-risk GC scores or those with basal tumors receiving placebo because of the relatively small sample size; the observed outcome during primary treatment may have been diluted by heterogeneous secondary treatments. Nevertheless, these results have useful implications in both clinical research and practice settings.

## Conclusions

Overall, given that these molecular signatures have been previously validated in multiple larger cohorts and represent inherent biologic features of prostate cancer, stratifying patients with nmCRPC using these signatures is likely to be of high utility. Further studies to elucidate molecular changes and identify mechanisms of castration resistance and further progression may provide additional insights on factors that regulate higher-risk GC scores and basal-luminal lineage and differential response to ADT. Although logistically challenging, serial biopsies could provide more accurate real-time reflection of tumor biologic features. The findings reported herein on the addition of apalutamide to ADT may help in making an informed clinical decision regarding appropriate treatment options for management of advanced prostate cancer. Our study results suggest that, although all patients with nmCRPC may benefit from the addition of apalutamide to ADT, those with high Decipher GC scores and those with the luminal subtype of prostate cancer may derive the greatest sustained benefit from apalutamide therapy.

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