The prostate tissue-based telomere biomarker as a prognostic tool for metastasis and death from prostate cancer after prostatectomy

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Supplementary Materials and Methods

Health Professionals Follow-up Study (HPFS). The HPFS is an ongoing prospective cohort study on risk factors for cancer and other chronic diseases (https://www.hsph.harvard.edu/hpfs). In 1986, 51,529 men of ages 40–75 years were enrolled. On each follow-up questionnaire, we asked the men to report a diagnosis of prostate cancer, which are confirmed by medical records and pathology reports (for 94.5%). Tumor-node-metastasis (TNM) stage and PSA concentration at diagnosis were abstracted from these records. The men were followed from the date of their surgery to 2014. Diagnosis of recurrence and progression to distant metastasis (to bone or other organs) was collected by mailed questionnaire and then confirmed by the treating doctor. Investigators learned of a participant’s death from family members, the postal system, or by searches of the National Death Index. Men were classified as having died from their prostate cancer (underlying cause on the death certificate) if they also had documented extensive metastatic disease.

Investigators at Harvard previously developed a cohort study of prostate cancer cases as described [24]; prostatectomy tissue from these men were arrayed on five TMAs. The HPFS investigators extended follow-up of the men on the original 5 HPFS TMAs (596 of the 631 men were included in the original telomere biomarker paper after exclusions [7]) and added 2 newly constructed TMAs (159 men) to achieve 755 men surgically-treated for clinically organ-confined prostate cancer in the analysis. Of these men 227 recurred. Of these, 30 experienced distant metastases, and 68 (46 from original TMAs) men died of their prostate cancer. HPFS pathologists re-reviewed H&E-stained tissue sections containing prostate cancer and assigned
a standardized Gleason sum [25], which we used in the analyses. Supplementary material, Table S1 shows characteristics of the men in the HPFS who were included in the telomere biomarker analysis. PTEN was previously measured by immunohistochemistry (IHC) [26].

**Physicians’ Health Study.** The parent study, Physicians’ Health Study (PHS) was a large, randomized chemoprevention trials for cancer and cardiovascular disease [27]. The PHS included 29,067 male physicians who were 40–84 years old and who did not have a history of cancer at the time of randomization in 1983. Prostate cancer diagnoses were identified by self-report on a mailed questionnaire and were confirmed by medical record and pathology report review. Data abstracted from these records included Gleason sum, TNM staging, and PSA concentration at the time of diagnosis. The men were followed from the date of their surgery to 2014. Bony metastases were confirmed by the treating physician. Deaths were ascertained by a search of the National Death Index or by reports from the US Postal Service or next of kin. Men were classified as dying from their prostate cancer if they had evidence of extensive metastatic disease; the Endpoint Committee made this determination. Mortality follow-up is >99% complete for this cohort.

Investigators at Harvard University previously developed a cohort study of men diagnosed with prostate cancer in the PHS between 1983 and 2009; prostatectomy tissue from these men were arrayed on five TMAs. Of these men, 45 developed distant metastases or died of their prostate cancer over a mean of 12.9 years of follow-up [28]. This PHS sub-study was designed to identify tissue-based prognostic markers for lethal prostate cancer [24,28,29]. Two study pathologists re-reviewed all specimens to provide a standardized Gleason scoring, which was previously documented to improve prediction of death from prostate cancer [25]. PTEN was previously measured by IHC [26].

In the first run for this telomere biomarker study, less than half of the TMA spots were useable across the five TMAs. The PHS investigators subsequently provided sections from replica TMAs, which were available on four of the five TMAs; again, less than half of the spots were useable. Reasons included some the spots no longer contained tumor because the tissue fell out of the section matrix or was missing initially, or had high auto-fluorescent background (depending on the TMA, ~15–20% of spots) that prohibited determination of telomere length. Thus, we included in the analysis 151 men with evaluable spots in the analysis. Of these men 51 recurred, 5 experienced distant metastases, and 17 men died of their prostate cancer. Supplementary material, Table S2 shows characteristics of men in the PHS who were included in the telomere biomarker analysis.
Johns Hopkins Recurrence Nested Case–Control Study. At the Brady Urological Institute at Johns Hopkins, we previously developed a Recurrence Nested Case–Control Study to investigate tissue prognostic markers, such as PTEN by IHC [30] and intratumoral mast cells [31]. Cases and controls were drawn from men who had had a prostatectomy for clinically localized prostate cancer at Johns Hopkins between 1993 and 2001 and had not had hormonal or radiation therapy before surgery or adjuvant therapy before recurrence. Cases ($N = 524$) were men with biochemical recurrence (re-elevation of PSA $\geq 0.2$ ng/ml), metastasis, or prostate cancer death after surgery by 2004, whichever came first. For each case, a control ($N = 524$) was selected who had not recurred by the case’s date and was matched on age, race, pathological stage, and Gleason sum. Sixteen TMAs were constructed with matched case–control pairs spotted on the same TMA. Evaluable spots were available for 376 matched recurrence pairs. Supplementary material, Table S3 shows case and control characteristics for men included in the telomere biomarker analysis.

Intermediate-High Risk Case–Cohort Study – I. The Brady Urological Institute at Johns Hopkins developed an intermediate and high-risk case-cohort study and associated nine TMAs for use in identifying tissue markers of metastasis in men at higher risk for poor outcome [32–34]. Men were selected from the Johns Hopkins radical prostatectomy (RP) clinical research database (>20,000 patients, of which >13,000 have long term follow-up) of men who underwent RP at Johns Hopkins between 1992 and 2010 and who had intermediate or high-risk disease by the Cancer of the Prostate Risk Assessment (CAPRA)-S score $\geq 3$ [35]. Men with metastatic disease or positive lymph nodes detected by imaging before surgery, men who received neoadjuvant therapy, and men who did not have a PSA nadir of <0.2 ng/ml post-surgery were excluded. Men who received hormone, chemo-, or radiation therapy after surgery but before detection of metastasis by imaging were excluded to be able to address how biomarkers are associated with metastatic outcome without the interference of treatment during follow-up. The eligible cohort consisted of 745 men from which a ~35% sample was randomly selected. Men who did not develop metastases in the subcohort and in the eligible cohort were shown to be similar [32]. The study includes 267 men in the subcohort and 119 men (including 64 in the subcohort) who progressed to metastasis. PTEN was previously measured by IHC [36]. Included in the telomere analysis were 253 men from the subcohort (94.8%) and 115 of the metastatic cases (96.6%). Supplementary material, Table S4 shows characteristics of all of the men, men in the subcohort, and cases outside of the subcohort who were included in the
telomere biomarker analysis.

**Intermediate-High Risk Case–Cohort Study – II.** The Brady Urological Institute subsequently developed a second case–cohort study of men with intermediate and high-risk prostate cancer and associated nine TMAs also for use in identifying tissue markers of progression to disease with a lethal phenotype in men at higher-risk. Men with intermediate and high-risk disease who underwent RP between 2007 and 2015, had not had neoadjuvant therapy, and had data available on all required clinical and outcome variables in the Johns Hopkins radical prostatectomy database were selected. Risk was based on the D’Amico classification at the time of biopsy [37]: intermediate – stage T2b or Gleason 7 or PSA >10 and ≤20 ng/ml; and high – stage T2c or PSA >20 ng/ml or Gleason ≥8). This left 3762 eligible men in the source population, in whom 204 experienced metastases or rapidly rising PSA (PSA doubling time <10 months). For feasibility, 121 of the cases were sampled. Then, a random subcohort (N = 254, 6.8% of 3762) of size of double the number of cases was sampled. Of the 121 cases, 19 occurred in the subcohort. Median follow-up of the subcohort was 3.0 years. Median time to lethal progression in the cases was 3.5 years. Men who received chemo-, radiation, or hormone therapy between surgery and detection of metastasis were not excluded; any treatment subsequent to prostatectomy cannot affect the telomere biomarker in the primary tumor. Supplementary material, Table S5 shows characteristics of all of the men, men in the subcohort, and cases outside of the subcohort who were included in the telomere biomarker analysis.

**Telomere-specific FISH and Immunostaining.** The staining methods are adapted from previously described studies [5,12,15], with the following modifications. Deparaffinized TMA slides were hydrated through a graded ethanol series, placed in deionized water, followed by deionized water plus 0.1% Tween-20. The TMA slides were steamed for 25 min in citrate buffer (catalog # H-3300; Vector Laboratories), removed, and allowed to cool at room temperature for 10 min. The TMA slides were washed in deionized water, dehydrated through a graded ethanol series, and then air-dried. Thirty-five microliters of the telomere-specific peptide nucleic acid (PNA) probe (0.33 μg/ml PNA [CCCTAACCTAACCTAA with the N-terminal covalently linked to Cy3; Panagene] in 70% formamide and 10 mmol/l Tris, pH 7.5) was applied, coverslipped, and denatured by incubation for 5 min at 84 °C. The TMA slides were then hybridized overnight in the dark in a humidified hybridization chamber. Next, the slides were washed in PNA wash buffer (70% formamide, 29% deionized distilled water; 1% 1 M Tris-Cl, pH 7.5) and then in PBST. Slides were incubated for 30 min at room temperature with serum-free protein block
(DAKO; catalog no.: X0909), washed in PBST, and then incubated for 2 h at room temperature with the following antibody cocktail diluted in antibody dilution buffer (Ventana; catalog no.: ADB250): basal-specific anti-cytokeratin primary antibody (34BE12, Enzo; 1:50 dilution), an anti-NKX3.1 primary antibody (Athena; 1:1000 dilution), an anti-FOXA1 primary antibody (Abcam; 1:500 dilution), an anti-CD3 primary antibody (DAKO; 1:200 dilution), and an anti-CD20 primary antibody (Abcam; 1:20 dilution). After incubation, the slides were washed in PBST and incubated for 30 min at room temperature with anti-rabbit IgG fraction Alexa Fluor 488 and anti-mouse IgG fraction Alexa Fluor 647 (secondary antibodies) in PBS at a 1:100 dilution. Following washes in PBST and deionized water, slides were stained with 4′-6-diamidino-2-phenylindole (DAPI) solution (500 ng/ml in deionized distilled water) for 10 min. The TMA slides were then mounted with Prolong antifade mounting medium (catalog no.: P-7481; Molecular Probes).
### Table S1. Characteristics of the Health Professionals Follow-up Study

|                          | Original cohort | Cohort after additional follow-up time and events and two additional TMAs |
|--------------------------|-----------------|---------------------------------------------------------------------------|
| N                        | 596             | 755                                                                       |
| Mean age at surgery (year) | 65.3            | 65.1                                                                      |
| Mean pre-operative PSA concentration (ng/ml) | 10.9            | 11.4                                                                      |
| Prostatectomy Gleason sum (%) |                |                                                                            |
| 4 to 6 (grade group 1)    | 21.3            | 16.7                                                                      |
| 3+4 (grade group 2)       | 35.7            | 36.2                                                                      |
| 4+3 (grade group 3)       | 24.8            | 25.8                                                                      |
| 8 to 10 (grade groups 4 and 5) | 18.1            | 21.3                                                                      |
| Pathologic stage (%)      |                 |                                                                            |
| T2                       | 68.0            | 71.7                                                                      |
| T3                       | 27.3            | 25.7                                                                      |
| T4/N1                    | 4.8             | 2.6                                                                       |
| PTEN status (%)           |                 |                                                                            |
| Intact                   | 56.2            | 62.5                                                                      |
| Null                     | 12.6            | 12.2                                                                      |
| Not assessed$^2$          | 31.2            | 25.3                                                                      |

$^1$Missing for 6 in the original cohort; missing for 12 in the cohort after additional follow-up time and events and 2 additional TMAs

$^2$Not assessed in prior work by the HPFS investigators
Table S2. Characteristics of the Physicians’ Health Study

|                                | Cohort spotted on TMAs | Cohort with useable TMA spots¹ |
|--------------------------------|------------------------|-------------------------------|
| N                              | 500                    | 151                           |
| Mean age at surgery (year)     | 65.1                   | 66.0                          |
| Mean pre-operative PSA concentration (ng/mL) | 12.8                  | 12.1                          |
| Prostatectomy Gleason sum (%)  |                        |                               |
| 4 to 6 (grade group 1)         | 44                     | 22                            |
| 7 (grade groups 2 and 3)       | 41                     | 53                            |
| 8 to 10 (grade groups 4 and 5) | 15                     | 22                            |
| Pathologic stage (%)           |                        |                               |
| T2                             | 72                     | 74                            |
| T3                             | 24                     | 21                            |
| T4/N1                          | 4                      | 5                             |
| PTEN status (%)                |                        |                               |
| Intact                         | 38.2                   | 42.4                          |
| Null                           | 11.0                   | 13.2                          |
| Not assessed                   | 50.8                   | 44.4                          |

¹Reasons that spots were not useable included some the spots no longer contained tumor (due to the repeated sectioning for multiple prior studies), the tissue fell out of the section matrix, or was missing initially, had high auto-fluorescent background (depending on the TMA, ~15–20% of spots) that prohibited determination of telomere length.
Table S3. Characteristics of 376 prostate cancer biochemical recurrence cases and 376 matched controls in the Johns Hopkins Recurrence Nested Case-Control Study\(^1\)

|                                | Cases      | Controls   | \(p\)     |
|--------------------------------|------------|------------|-----------|
| Mean age at surgery (year) ± standard deviation | 58.9 ± 6.2 | 59.0 ± 5.9 | Matched   |
| Race (%)                        |            |            |           |
| White                           | 86.4       | 89.6       |           |
| Black                           | 8.8        | 7.2        |           |
| Other race/ethnicity            | 4.8        | 3.2        |           |
| Follow-up time (yr), median, IQR | 2 (1–3)    | 6 (3–8)    | <0.0001   |
| Pre-operative PSA concentration (ng/mL), median (IQR) | 12.2 (6.0–14.3) | 8.8 (6.1–14.3) | 0.1       |
| Prostatectomy Gleason sum (%)   |            |            |           |
| \(\leq 6\) (grade group 1)     | 15.1       | 15.4       |           |
| 3+4 (grade group 2)             | 36.7       | 47.6       |           |
| 4+3 (grade group 3)             | 21.8       | 12.8       |           |
| >7 (grade groups 4 and 5)       | 26.3       | 24.2       |           |
| Pathologic stage (%)            |            |            |           |
| T2                              | 12.8       | 13.0       |           |
| T3a                             | 10.1       | 17.6       | Matched   |
| T3b or N1                       | 77.1       | 69.4       |           |
| Positive surgical margins (%)   | 34.8       | 24.7       | 0.001     |
| PTEN status (%)                 |            |            |           |
| Intact                          | 47.9       | 57.5       | 0.001     |
| Null                            | 39.6       | 37.0       |           |
| Not assessed                    | 12.5       | 5.6        |           |

\(^1\)Controls were sampled using incidence density sampling; the number of unique men is 549.

\(^2\)IQR – Interquartile range
Table S4. Characteristics of men in the Johns Hopkins Intermediate-High Risk Case-Cohort Study - I

| Characteristic                                      | All men in sample | Subcohort | Cases outside of the subcohort |
|-----------------------------------------------------|-------------------|-----------|--------------------------------|
| Median age at surgery, year (IQR<sup>3</sup>)       | 306               | 253<sup>2</sup> | 53                             |
| White (%)                                           | 90.2              | 89.3      | 94.3                           |
| Prostatectomy Gleason sum (%)                       |                   |           |                                |
| ≤7 (grade groups 1, 2, and 3)                       | 55.6              | 62.4      | 22.6                           |
| 8 (grade group 4)                                   | 11.8              | 11.5      | 13.2                           |
| 9 (grade group 5)                                   | 31.7              | 24.9      | 64.2                           |
| Missing                                             | 1.0               | 1.2       | 0                              |
| Pathologic stage (%)                                |                   |           |                                |
| T2                                                  | 24.5              | 28.5      | 5.7                            |
| T3a                                                 | 47.1              | 49.4      | 35.8                           |
| T3b                                                 | 27.5              | 20.9      | 58.5                           |
| Missing                                             | 1.0               | 1.2       | 0                              |
| Positive surgical margins (%)                       | 27.5              | 24.9      | 39.6                           |
| Median preoperative PSA concentration, ng/mL (IQR<sup>3</sup>) | 10.2 (6.4–15.1)   | 10.1 (6.3–15.0) | 10.8 (6.7–15.1) |
| PTEN status (%)                                     |                   |           |                                |
| Intact                                              | 63.7              | 67.2      | 47.2                           |
| Null                                                | 36.3              | 32.8      | 52.8                           |

<sup>1</sup>With useable TMA spots.
<sup>2</sup>Includes 62 men who later become cases.
<sup>3</sup>IQR – interquartile range
### Table S5. Characteristics of men in the Johns Hopkins Intermediate-High Risk Case-Cohort Study – II

|                                      | All men in sample | Subcohort\(^2\) | Cases outside of the subcohort |
|--------------------------------------|-------------------|------------------|--------------------------------|
| Median age at surgery, year (IQR)\(^3\) | 59 (55–65)        | 59 (55–65)       | 60 (55–66)                     |
| White (%)                            | 81.4              | 80.7             | 82.8                           |
| Prostatectomy Gleason sum (%)        |                   |                  |                                |
| ≤7 (grade groups 1, 2, and 3)        | 75.6              | 90.1             | 47.5                           |
| 8 (grade group 4)                    | 9.3               | 6.3              | 15.2                           |
| 9 (grade group 5)                    | 15.1              | 3.7              | 37.4                           |
| Pathologic stage (%)                 |                   |                  |                                |
| T1c                                  | 59.8              | 65.6             | 48.5                           |
| T2a                                  | 21.0              | 21.9             | 19.2                           |
| T2b                                  | 15.8              | 10.9             | 25.3                           |
| T2c                                  | 2.1               | 1.0              | 4.0                            |
| T3                                   | 1.0               | 0.5              | 2.0                            |
| Missing                              | 0.3               | —                | 1.0                            |
| Positive surgical margins (%)        | 21.8              | 16.2             | 33.0                           |
| Median preoperative PSA concentration, ng/mL (IQR) | 6.0 (4.5–9.0) | 5.5 (4.3–8.6) | 7.1 (5.2–12.9) |
| PTEN status (%)                      | Not assessed      | Not assessed     | Not assessed                   |

\(^1\)With useable TMA spots.  
\(^2\)Includes 62 men who later become cases.  
\(^3\)IQR – interquartile range.
Table S6. Associations\(^1\) of more variable telomere length among prostate cancer cells and shorter telomere length in prostate cancer-associated stromal cells with risk of recurrence, metastasis, and prostate cancer death after prostatectomy in 5 cohorts

| Prostate cancer outcome | N Person-years | More variable length among cancer cells | Shorter length in stromal cells | N Person-years | More variable length among cancer cells | Shorter length in stromal cells | Cases | Controls | More variable length among cancer cells | Shorter length in stromal cells | Cases | Sub-cohort | More variable length among cancer cells | Shorter length in stromal cells | Cases | Sub-cohort | More variable length among cancer cells | Shorter length in stromal cells |
|------------------------|---------------|----------------------------------------|-------------------------------|---------------|----------------------------------------|-------------------------------|-------|----------|----------------------------------------|-------------------------------|-------|------------|----------------------------------------|-------------------------------|-------|------------|----------------------------------------|-------------------------------|
| Recurrence             | 747           | 9102                                   | 1.28                          | 0.97–1.68     | 1.05                                   | 0.79–1.39                      | 149   | 1668     | 1.35                                  | 0.74–2.47                     | 0.57  | 0.31–1.03  | 376                                    | 1.18–2.83                     | 0.95  | —          | —                                    | —                             |
| Metastasis             | 747           | 11291                                  | 1.23                          | 0.59–2.60     | 1.26                                   | 0.57–2.79                      | 149   | 2067     | 10.93                                 | 0.40–268                      | 0.15  | 0.01–2.01  | —                                    | —                             | —    | 115        | 1.26                                  | 0.76–2.07                     | 1.18  | 161        | 1.38\(^5\)                             | 0.66–2.81                     |
| Prostate cancer death  | 755           | 12305                                  | 1.29                          | 0.78–2.15     | 2.55                                   | 1.36–4.78                      | 151   | 2092     | 2.02                                  | 0.55–7.46                     | 0.58  | 0.18–1.91  | —                                    | —                             | —    | —          | —                                    | —                             |
Table S7. Meta-analytic summary relative risks (RR) and 95% confidence intervals (CI) of more variable telomere length among prostate cancer cells and shorter telomere length in prostate cancer-associated stromal cells with risk of recurrence, metastasis, and prostate cancer death after prostatectomy in 5 cohorts excluding 50 prostate cancer deaths and 596 men from the original study in which we described the telomere biomarker

| Event                        | Number of contributing cohorts | More variable \(^2\) telomere length among prostate cancer cells (vs less variable) | Shorter \(^3\) telomere length in cancer-associated stromal cells (vs longer) |
|------------------------------|--------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Recurrence                   | 3                              | 1.49 (1.07–2.06; \(p = 0.02\))                                                | 0.82 (0.60–1.13; \(p = 0.22\))                                                |
| Metastasis                   | 4\(^4\)                        | 1.34 (0.90–2.01; \(p = 0.15\))                                               | 1.08 (0.70–1.68; \(p = 0.73\))                                               |
| Prostate cancer death        | 2                              | 2.25 (0.74–6.82; \(p = 0.15\))                                               | 0.52 (0.18–1.48; \(p = 0.22\))                                               |

Bold denotes statistically significant at \(p < 0.05\) for a two-sided test.

\(^1\) Each contributing RR and 95% CI was adjusted for currently used prognostic pathologic factors. RRs were summarized using inverse variance weights.

\(^2\) More variable was defined as the top tertile of variability in telomere length among prostate cancer cells. Less variable was defined as the bottom and middle tertiles.

\(^3\) Shorter was defined as the shortest and middle tertiles of the median telomere length in cancer-associated stromal cells. Longer was defined as the longest tertile.

\(^4\) Includes all events (\(N = 161\)) from the Johns Hopkins Intermediate-High Risk – II. Including only the 35 metastatic events: more variable length among cancer cells HR = 1.33, 95% CI 0.81–2.16 (\(p = 0.26\)), shorter length among stromal cells HR = 0.99, 95% CI 0.58–1.69 (\(p = 0.96\)).
biomarker categories to obtain a stable estimate.

5
4
3
2
1
NE
cancer death
Metastasis
Recurrence
prostate cancer death after prostatectomy in 5 cohorts

Table S8. Associations between the telomere biomarker – the combination of variability in telomere length among cancer cells and telomere length in cancer-associated stromal cells – and risk of recurrence, metastasis, and prostate cancer death after prostatectomy in 5 cohorts

| Telomere biomarker | HPFS Prostate Cancer Study | PHS | Johns Hopkins Recurrence | Johns Hopkins Intermediate-High Risk - I | Johns Hopkins Intermediate-High Risk - II |
|--------------------|---------------------------|-----|--------------------------|----------------------------------------|----------------------------------------|
|                    | No. | PY  | HR, 95% CI               | No. | PY  | HR, 95% CI | Cases | Controls | OR, 95% CI | Cases | Subcohort | HR, 95% CI | Cases | Subcohort | HR, 95% CI |
| Recurrence         |     |     |                         |     |     |                         |       |          |            |       |           |            |       |           |            |
| Less variable/longer| 31  | 1502| 1.00 Reference          | 6   | 217 | 1.00 Reference          | 43   | 50       | 1.00 Reference | —     | —          | —            | —     | —          | —            |
| More variable/longer| 40  | 1318| 1.18 0.73–1.90          | 13  | 357 | 2.21 0.76–6.45          | 115  | 76       | 2.21 1.06–4.62 | —     | —          | —            | —     | —          | —            |
| Less variable/shorter| 103 | 4620| 1.05 0.70–1.58          | 23  | 874 | 1.07 0.41–2.80          | 157  | 202      | 1.38 0.71–2.64 | —     | —          | —            | —     | —          | —            |
| More variable/shorter| 48  | 1229| 1.48 0.52–4.37          | 9   | 219 | 1.51 0.52–4.37          | 61   | 48       | 2.65 1.17–5.97 | —     | —          | —            | —     | —          | —            |
| Metastasis         |     |     |                         |     |     |                         |       |          |            |       |           |            |       |           |            |
| Less variable/longer| 3   | 1842| 1.00 Reference          | 1   | 280 | NE                      | —    | —        | —            | 15    | 29        | 1.00 Reference | 6     | 22        | 1.00 Reference |
| More variable/longer| 6   | 1729| 1.69 0.42–6.89          | 2   | 475 | NE                      | —    | —        | —            | 17    | 41        | 0.38–3.55 | 0.17 1.17    | 0.41–14.3    | 30    | 41        | 2.26          |
| Less variable/shorter| 15  | 5582| 1.64 0.47–5.70          | 0   | 1018| NE                      | —    | —        | —            | 64    | 99        | 0.44–3.25 | 1.20 | 1.0        | 0.40–12.7    | 72    | 93        | 0.88          |
| More variable/shorter| 6   | 1642| 1.98 0.49–8.02          | 2   | 294 | NE                      | —    | —        | —            | 19    | 22        | 0.62–5.59 | 1.87 | 1.0        | 0.66–88.1    | 17    | 10        | 7.62          |
| Prostate cancer death |    |     |                         |     |     |                         |       |          |            |       |           |            |       |           |            |
| Less variable/longer| 4   | 1982| 1.00 Reference          | 1   | 282 | 1.00 Reference          | —    | —        | —            | —     | —          | —            | —     | —          | —            |
| More variable/longer| 7   | 1898| 1.19 0.34–4.17          | 3   | 483 | 1.96 0.11–33.9          | —    | —        | —            | —     | —          | —            | —     | —          | —            |
| Less variable/shorter| 32  | 6084| 2.50 0.88–7.10          | 11  | 1029| 0.38 0.02–6.47          | —    | —        | —            | —     | —          | —            | —     | —          | —            |
| More variable/shorter| 24  | 1812| 0.62–20.6              | 2   | 298 | 1.04 0.05–20.6          | —    | —        | —            | —     | —          | —            | —     | —          | —            |

NE = Not estimable. Bold denotes statistically significant at $p < 0.05$ for a 2-sided test.

1Of these, 50 prostate cancer deaths and 596 men were included in the original study in which we described the telomere biomarker. Adjusted for age, year of surgery, prostatectomy Gleason sum, pathologic stage, pre-operative serum PSA.

2Adjusted for age, year of surgery, prostatectomy Gleason sum, pathologic stage, and pre-operative serum PSA concentration.

3Adjusted for year of surgery, primary and secondary Gleason pattern, pre-operative PSA, and surgical margins (cases and controls were matched on age, race, prostatectomy Gleason sum, and pathologic stage).

4Adjusted for age, race, pathologic stage, prostatectomy Gleason sum, year of surgery, pre-operative PSA, and surgical margins.

5Outcome is the combination of rapidly rising PSA, which is indicative of likely progression to metastasis, and metastases. When including only the 35 metastatic events, the number was too small distributed across the 4 telomere biomarker categories to obtain a stable estimates.
Table S9. Meta-analytic summary RRs and 95% CIs for the associations\(^1\) between the telomere biomarker (combination of variability in telomere length among prostate cancer cells and telomere length in prostate cancer-associated stromal cells) and risk of recurrence, metastasis, and prostate cancer death after prostatectomy in the 5 cohorts excluding 50 prostate cancer deaths and 596 men from the original study in which we described the telomere biomarker

| Number of contributing cohorts | Less variable/ longer | More variable/ longer | Less variable/ shorter | More variable/ shorter |
|-------------------------------|-----------------------|-----------------------|------------------------|------------------------|
| Recurrence                    | 1.00                  | 1.57                  | 1.01                   | 1.70                   |
|                               | Reference             | 0.91–2.73             | 0.63–1.63              | 0.96–3.01              |
|                               |                       |                       | \(p = 0.11\)           | \(p = 0.97\)           |
|                               |                       |                       |                       | \(p = 0.07\)           |
| Metastasis                    | 1.00                  | 1.43                  | 1.24                   | 2.11                   |
|                               | Reference             | 0.56–3.68             | 0.53–2.89              | 0.82–5.41              |
|                               |                       |                       | \(p = 0.46\)           | \(p = 0.62\)           |
|                               |                       |                       |                       | \(p = 0.12\)           |
| Prostate cancer death         | 1.00                  | 1.02                  | 0.21                   | 0.88                   |
|                               | Reference             | 0.14–7.55             | 0.02–1.78              | 0.12–6.70              |
|                               |                       |                       | \(p = 0.93\)           | \(p = 0.15\)           |
|                               |                       |                       |                       | \(p = 0.90\)           |

Bold denotes statistically significant at \(p < 0.05\) for a two-sided test.

\(^{1}\)Each contributing RR was adjusted for prognostic pathologic factors. RRs were summarized using inverse variance weights.

\(^{2}\)Includes all events (\(N = 161\)) from the Johns Hopkins Intermediate-High Risk – II. When including only metastatic events (\(N = 35\)), associations were not estimable.