**Table 1S.** Determination of molecular characteristics, subtypes, and molecular patterns by focus and patient, presented by concordance pattern of all molecular characteristics in prostatic foci by patient

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concordant** | | | | | | | **Partially concordant** | | | | | | | **Discordant** | | | | | | |
| **P** | **Dx** | **F** | **Relative expression** | | | | **P** | **Dx** | **F** | **Relative expression** | | | | **P** | **Dx** | **F** | **Relative expression** | | | |
| **ERG** | **EZH2** | **NKX3.1** | **SPINK-1** | **ERG** | **EZH2** | **NKX3.1** | **SPINK-1** | **ERG** | **EZH2** | **NKX3.1** | **SPINK-1** |
| **1** | GL3 | **+** | 719.8 | 131.6 | 124.8 | 189.7 | 4 | GL3 | **-** | 7.50 | 3.95 | 7.38 | 0.01 | 10 | HGPIN | **+** | 25.67 | 0.25 | 2.45 | 5.87 |
| GL3 | **+** | 433.5 | 158.5 | 256.5 | 680.9 | GL4 | **-** | 13.06 | 3.23 | 7.05 | 0.01 | GL3 | **+** | 8.57 | 2.91 | 1.29 | 2.86 |
| GL4 | **+** | 17.54 | 1.70 | 2.72 | 9.19 | GL5 | **-** | 3.16 | 7.18 | 2.30 | 1.52 | GL4 | **+** | 25.13 | 0.27 | 2.28 | 6.34 |
| **PMP** |  | **+** | **+** | **-** | **+** | LN | **-** | 124.77 | 40.68 | 0.04 | 5.67 | GL4 | **-** | 0.64 | 0.07 | 2.74 | 0.71 |
| 2 | HGPIN | **-** | 0.03 | 3.46 | 21.55 | 0.18 | **PMP** |  | **+** | **+** | **+** | **+** | **PMP** |  | **+** | **+** | **-** | **+** |
| GL3 | **-** | 0.02 | 6.15 | 13.23 | 0.07 | 5 | HGPIN | **-** | 1.44 | 0.38 | 0.08 | 0.42 | 11 | HGPIN | **-** | 0.56 | 3.43 | 0.00 | 0.47 |
| GL3 | **-** | 0.01 | 2.57 | 6.76 | 0.02 | GL3 | **-** | 213.70 | 64.89 | 100.90 | 24.48 | GL3 | **+** | 15.41 | 8.25 | 1.84 | 1.89 |
| GL4 | **-** | 0.01 | 2.49 | 5.57 | 0.03 | GL4 | **-** | 16.63 | 12.33 | 11.96 | 58.20 | GL4 | **+** | 1.01 | 11.00 | 1.42 | 0.47 |
| **PMP** |  | **-** | **+** | **-** | **-** | LN | **-** | 134.16 | 26.75 | 0.01 | 0.17 | LN | **-** | 1.40 | 3.25 | 0.20 | 4.32 |
| 3 | HGPIN | **-** | 0.13 | 0.23 | 3.39 | 0.57 | **PMP** |  | **+** | **+** | **+** | **+** | **PMP** |  | **+** | **+** | **+** | **+** |
| GL3 | **-** | 0.03 | 0.19 | 1.90 | 0.52 | 6 | HGPIN | **-** | 9.47 | 0.10 | 0.17 | 0.10 | 12 | HGPIN | **-** | 6.60 | 1.22 | 0.67 | 1.02 |
| GL4 | **-** | 0.24 | 0.20 | 2.24 | 0.40 | GL3 | **+** | 14.56 | 0.02 | 0.15 | 0.02 | GL3 | **-** | 13.38 | 0.01 | 1.16 | 2.22 |
| LN | **-** | 3.33 | 1.74 | 0.00 | 0.43 | GL4 | **+** | 1.34 | 0.04 | 0.07 | 0.01 | GL4 | **-** | 6.16 | 1.91 | 1.73 | 1.13 |
| **PMP** |  | **+** | **+** | **+** | **-** | GL5 | **+** | 675.40 | 5.47 | 0.43 | 1.08 | LN | **+** | 12.95 | 2.13 | 0.16 | 1.06 |
|  |  |  |  |  |  |  | **PMP** |  | **+** | **+** | **+** | **-** | **PMP** |  | **+** | **+** | **+** | **+** |
|  |  |  |  |  |  | 7 | GL4 | **-** | 0.45 | 0.21 | 0.84 | 52.39 | 13 | HGPIN | **-** | 0.62 | 0.31 | 4.11 | 0.31 |
|  |  |  |  |  |  | GL4 | **-** | 0.70 | 0.17 | 0.65 | 12,78 | GL3 | **-** | 0.20 | 0.08 | 0.31 | 0.08 |
|  |  |  |  |  |  | GL5 | **-** | 0.26 | 0.26 | 0.48 | 11,39 | GL4 | **+** | 3.43 | 0.30 | 2.11 | 0.48 |
|  |  |  |  |  |  | GL5 | **-** | 0.63 | 0.09 | 0.15 | 14,64 | LN | **+** | 1.27 | 0.06 | 0.30 | 0.21 |
|  | | | | | | | GL5 | **+** | 1.40 | 0.08 | 0.18 | 39,82 | **PMP** |  | **+** | **-** | **+** | **-** |
| LN | **-** | 1.22 | 0.04 | 0.00 | 1.34 | 14 | HGPIN | **+** | 2.16 | 0.81 | 0.22 | 0.64 |
| **PMP** |  | **+** | **-** | **+** | **+** | GL3 | **+** | 5.78 | 0.50 | 0.28 | 0.50 |
| 8 | HGPIN | **-** | 0.35 | 191.10 | 0.40 | 3.40 | GL4 | **+** | 5.64 | 0.97 | 0.49 | 0.59 |
| GL3 | **-** | 0.04 | 74.14 | 1.08 | 2.31 | LN | **-** | 1.48 | 0.61 | 0.35 | 7.93 |
| GL3 | **-** | 0.68 | 37.90 | 2.73 | 0.51 | **PMP** |  | **+** | **-** | **+** | **+** |
| GL4 | **-** | 0.29 | 40.07 | 2.21 | 0.07 | 15 | HGPIN | **-** | 6.37 | 1.09 | 0.61 | 0.26 |
| GL4 | **-** | 0.26 | 27.84 | 2.70 | 0.02 | GL3 | **+** | 43.55 | 0.44 | 0.30 | 1.35 |
| **PMP** |  | **-** | **+** | **+** | **+** | GL4 | **+** | 37.50 | 2.08 | 0.74 | 0.73 |
| 9 | HGPIN | **-** | 0.84 | 48.16 | 0.99 | 55.39 | **PMP** |  | **+** | **+** | **+** | **+** |
| GL3 | **-** | 0.01 | 3.15 | 11.73 | 0.05 | 16 | HGPIN | **-** | 0.37 | 0.57 | 1.38 | 0.97 |
| GL3 | **-** | 0.04 | 2.65 | 2.38 | 0.07 | GL3 | **+** | 13.55 | 1.05 | 0.54 | 2.02 |
| GL4 | **-** | 0.14 | 13.43 | 3.84 | 0.58 | GL4 | **+** | 11.69 | 0.62 | 5.17 | 1.74 |
| **PMP** |  | **-** | **+** | **-** | **+** | GL5 | **+** | 111.74 | 1.44 | 1.18 | 2.10 |
|  |  |  |  |  |  |  | LN | **-** | 8.91 | 0.12 | 0.07 | 2.74 |
|  |  |  |  |  |  |  | **PMP** |  | **+** | **+** | **+** | **+** |
|  |  |  |  |  |  |  | 17 | HGPIN | **-** | 1.98 | 2.07 | 0.61 | 0.00 |
|  |  |  |  |  |  | GL3 | **-** | 0.00 | 0.06 | 0.29 | 0.04 |
|  | | | | | | | GL4 | **-** | 2.26 | 8.40 | 0.70 | 0.02 |
|  | | | | | | | GL5 | **-** | 0.27 | 0.70 | 0.35 | 0.00 |
| LN | **-** | 1.16 | 2.24 | 0.01 | 0.18 |
| **PMP** |  | **+** | **+** | **+** | **-** |
| 18 | HGPIN | **+** | 4.75 | 1.78 | 1.24 | 1.19 |
| GL3 | **+** | 3.88 | 0.92 | 1.27 | 13.57 |
| GL4 | **-** | 2.36 | 0.12 | 0.89 | 12.70 |
| LN | **-** | 1.27 | 1.97 | 0.85 | 1.17 |
| **PMP** |  | **+** | **+** | **-** | **+** |
|  | | | | | | |  | | | | | | | 19 | HGPIN | **-** | 0.38 | 0.11 | 1.14 | 0.48 |
|  | | | | | | |  | | | | | | | GL3 | **-** | 0.21 | 0.24 | 0.52 | 0.12 |
|  | | | | | | |  | | | | | | | GL4 | **-** | 0.91 | 2.71 | 2.33 | 0.65 |
|  | | | | | | |  | | | | | | | LN | **+** | 1.43 | 5.90 | 0.74 | 1.55 |
|  | | | | | | |  | | | | | | | **PMP** |  | **+** | **+** | **+** | **+** |
|  | | | | | | |  | | | | | | | 20 | HGPIN | **+** | 1.99 | 3.54 | 1.60 | 0.12 |
|  | | | | | | |  | | | | | | | GL3 | **-** | 0.84 | 3.34 | 0.11 | 1.60 |
|  | | | | | | |  | | | | | | | GL4 | **-** | 0.36 | 2.23 | 0.69 | 1.67 |
|  | | | | | | |  | | | | | | | LN | **+** | 1.30 | 0.91 | 0.11 | 1.93 |
|  | | | | | | |  | | | | | | | **PMP** |  | **+** | **+** | **+** | **+** |

P: Patient. Dx: Diagnosis. F: TMPRSS2-ERG fusion. HGPIN: High-grade prostatic intraepithelial neoplasia (preneoplastic lesion). GL: Gleason pattern. LN: Lymph node (regional metastasis). Color pattern of gene expression or fusion: black: highly expressed (>1.20) or presence of fusion; medium gray: normally expressed (between 0.80-1.20); and white: lowly expressed (<0.80). The lower small table shows patient molecular patterns (PMP). These PMP were assigned considering whether any of the samples analyzed (prostatic or node samples) presented (+) the molecular subtype that favored progression (ERG(+), EZH2 high, NKX3.1 low, SPINK-1 high) or not (-). Fusion was not considered for PMP because ERG high include it. Color pattern of PMP: each color represents a different pattern. Eight different patterns were found: five ERG(+) (blue) and three ERG(-) (green). The most frequent patterns were ERG(+) in 17 patients (85%) and the most frequent pattern was the darkest blue pattern: ERG(+)/EZH2/NKX3.1/SPINK-1 (in 8 patients). The ERG(-) patterns were found in 3 patients (15%) and are colored in green; all of these are different.

**Table 2S.** Clinicopathological and molecular characteristics of the 20 patients analyzed by presence of the ERG subtype

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **ERG Subtype** | | **p-value** |
| **Positive**  **(n = 14)** | **Negative**  **(n = 6)** |
| **Age of diagnosis (years)** |  |  |  |
| Median [IQR] | 66.5 [6.50] | 56.0 [15.2] | 0.432 |
| **Prediagnosis\_PSA, ng/ml** |  |  |  |
| Median [IQR] | 15.0 [14.0] | 9.84 [8.33] | 0.629 |
| **Gleason score, n (%)** |  |  |  |
| 7 | 13 (68.4) | 6 (31.6) | 1.00 |
| 8 | 1 (100) | 0 (0.00) |  |
| **Gleason grade group, n (%)** |  |  |  |
| G2 | 3 (60.0) | 2 (40.0) | 1.00 |
| G3 | 10 (71.4) | 4 (28.6) |  |
| G4 | 1 (100) | 0 (0.00) |  |
| **Tumoral percentage (%)** |  |  |  |
| Mean ± SD | 39.6 ± 20.6 | 38.7 ± 17.3 | 0.926 |
| **Index diameter** |  |  |  |
| Mean ± SD | 2.04 ± 0.51 | 1.85 ± 0.41 | 0.443 |
| **Margin status in RP, n (%)** |  |  |  |
| Positive | 11 (68.8) | 5 (31.2) | 1.00 |
| Negative | 3 (75.0) | 1 (25.0) |  |
| **Perineural invasion, n (%)** |  |  |  |
| Intraprostatic | 5 (62.5) | 3 (37.5) | 0.642 |
| Intraprostatic + extraprostatic | 9 (75.0) | 3 (25.0) |  |
| **Lymphovascular invasion, n (%)** |  |  |  |
| Yes | 7 (70.0) | 3 (30.0) | 1.00 |
| No | 6 (66.7) | 3 (33.3) |  |
| Unknown | 1 (100) | 0 (0.00) |  |
| **Pathologic stage.pT, n (%)** |  |  |  |
| 2c-3a | 5 (71.5) | 2 (28.6) | 1.00 |
| 3b | 9 (69.2) | 4 (30.8) |  |
| **Pathologic stage.pN, n (%)** |  |  |  |
| 0 | 2 (50.0) | 2 (50.0) | 0.549 |
| 1 | 12 (75.0) | 4 (25.0) |  |
| **EZH2, n (%)** |  |  |  |
| Positive | 10 (66.7) | 5 (33.3) | 1.00 |
| Negative | 4 (80.0) | 1 (20.0) |  |
| **NKX3.1, n (%)** |  |  |  |
| Positive | 7 (77.8) | 2 (22.2) | 0.642 |
| Negative | 7 (63.6) | 4 (36.4) |  |
| **SPINK-1, n (%)** |  |  |  |
| Positive | 10 (83.3) | 2 (16.7) | 0.161 |
| Negative | 4 (50.0) | 4 (50.0) |  |
| **Molecular\_concordance, n (%)** |  |  |  |
| Concordant | 1 (33.3) | 2 (66.7) | 0.216 |
| Partially concordant | 4 (66.7) | 2 (33.3) |  |
| Discordant | 9 (81.8) | 2 (18.2) |  |

IQR: Interquartile range; SD: Standard deviation, RP: Radical prostatectomy.

**Table 3S.** Clinicopathological and molecular characteristics of the 20 patients analyzed by presence of the EZH2 subtype

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **EZH2** | | **p-value** |
| **Positive**  **(n = 15)** | **Negative**  **(n = 5)** |
| **Age of diagnosis (years)** |  |  |  |
| Median [IQR] | 66.0 [16.5] | 66.0 [3.00] | 0.484 |
| **Prediagnosis\_PSA, ng/ml** |  |  |  |
| Median [IQR] | 15.6 [12.9] | 8.00 [5.90] | 0.179 |
| **Gleason score, n (%)** |  |  |  |
| 7 | 15 (78.9) | 4 (21.1) | 0.250 |
| 8 | 0 (0.00) | 1 (100) |  |
| **Gleason grade group, n (%)** |  |  |  |
| G2 | 4 (80.0) | 1 (20.0) | 0.311 |
| G3 | 11 (78.6) | 3 (21.4) |  |
| G4 | 0 (0.00) | 1 (100) |  |
| **Tumoral percentage (%)** |  |  |  |
| Mean ± SD | 39.3 ± 18.8 | 39.4 ± 22.7 | 0.989 |
| **Index diameter** |  |  |  |
| Mean ± SD | 1.99 ± 0.46 | 1.94 ± 0.58 | 0.836 |
| **Margin status in RP, n (%)** |  |  |  |
| Positive | 12 (75.0) | 4 (25.0) | 1.00 |
| Negative | 3 (75.0) | 1 (25.0) |  |
| **Perineural invasion, n (%)** |  |  |  |
| Intraprostatic | 6 (75.0) | 2 (25.0) | 1.00 |
| Intraprostatic + extraprostatic | 9 (75.0) | 3 (25.0) |  |
| **Lymphovascular invasion, n (%)** |  |  |  |
| Yes | 8 (80.0) | 2 (20.0) | 1.00 |
| No | 7 (77.8) | 2 (22.2) |  |
| Unknown | 0 (0.00) | 1 (100) |  |
| **Pathologic\_stage.pT, n (%)** |  |  |  |
| 2c-3a | 6 (85.7) | 1 (14.3) | 0.613 |
| 3b | 9 (69.2) | 4 (30.8) |  |
| **Pathologic\_stage.pN, n (%)** |  |  |  |
| 0 | 3 (75.0) | 1 (25.0) | 1.00 |
| 1 | 12 (75.0) | 4 (25.0) |  |
| **ERG, n (%)** |  |  |  |
| Positive | 10 (71.4) | 4 (28.6) | 1.00 |
| Negative | 5 (83.3) | 1 (16.7) |  |
| **NKX3.1, n (%)** |  |  |  |
| Positive | 6 (66.7) | 3 (33.3) | 0.617 |
| Negative | 9 (81.8) | 2 (18.2) |  |
| **SPINK-1, n (%)** |  |  |  |
| Positive | 10 (83.3) | 2 (16.7) | 0.347 |
| Negative | 5 (62.5) | 3 (37.5) |  |
| **Molecular\_concordance, n (%)** |  |  |  |
| Concordant | 2 (66.7) | 1 (33.3) | 1.00 |
| Partially concordant | 5 (83.3) | 1 (16.7) |  |
| Discordant | 8 (72.7) | 3 (27.3) |  |

IQR: Interquartile range; SD: Standard deviation; RP: Radical prostatectomy.

**Table 4S.** Clinicopathological and molecular characteristics of the 20 patients analyzed by presence of the NKX3.1 subtype

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **NKX3.1** | | **p-value** |
| **Positive**  **(n = 9)** | **Negative**  **(n = 11)** |
| **Age of diagnosis (years)** |  |  |  |
| Median [IQR] | 67.0 [3.00] | 60.0 [17.0] | 0.269 |
| **Prediagnosis\_PSA, ng/ml** |  |  |  |
| Median [IQR] | 19.7 [11.9] | 11.5 [7.85] | 0.160 |
| **Gleason score, n (%)** |  |  |  |
| 7 | 8 (42.1) | 11 (57.9) | 0.450 |
| 8 | 1 (100) | 0 (0.00) |  |
| **Gleason grade group, n (%)** |  |  |  |
| G2 | 2 (40.0) | 3 (60.0) | 0.804 |
| G3 | 6 (42.8) | 8 (57.2) |  |
| G4 | 1 (100) | 0 (0.00) |  |
| **Tumoral percentage (%)** |  |  |  |
| Mean ± SD | 36.4 ± 20.7 | 41.6 ± 18.6 | 0.562 |
| **Index diameter** |  |  |  |
| Mean ± SD | 1.82 ± 0.52 | 2.11 ± 0.43 | 0.192 |
| **Margin status in RP, n (%)** |  |  |  |
| Positive | 6 (37.5) | 10 (62.5) | 0.284 |
| Negative | 3 (75.0) | 1 (25.0) |  |
| **Perineural invasion, n (%)** |  |  |  |
| Intraprostatic | 6 (75.0) | 2 (25.0) | 0.064 |
| Intraprostatic + extraprostatic | 3 (25.0) | 9 (75.0) |  |
| **Lymphovascular invasion, n (%)** |  |  |  |
| Yes | 4 (40.0) | 6 (60.0) | 1.00 |
| No | 4 (44.4) | 5 (55.6) |  |
| Unknown | 1 (100) | 0 (0.00) |  |
| **Node involvement, n (%)** |  |  |  |
| Yes | 6 (40.0) | 9 (60.0) | 0.617 |
| No | 3 (60.0) | 2 (40.0) |  |
| **Pathologic\_stage.pT, n (%)** |  |  |  |
| 2c-3a | 3 (42.8) | 4 (57.2) | 1.00 |
| 3b | 6 (46.2) | 7 (53.8) |  |
| **Pathologic\_stage.pN, n (%)** |  |  |  |
| 0 | 2 (50.0) | 2 (50.0) | 1.00 |
| 1 | 7 (43.7) | 9 (56.2) |  |
| **ERG, n (%)** |  |  |  |
| Positive | 7 (50.0) | 7 (50.0) | 0.642 |
| Negative | 2 (33.3) | 4 (66.7) |  |
| **EZH2, n (%)** |  |  |  |
| Positive | 6 (40.0) | 9 (60.0) | 0.617 |
| Negative | 3 (60.0) | 2 (40.0) |  |
| **SPINK-1, n (%)** |  |  |  |
| Positive | 4 (33.3) | 8 (66.7) | 0.362 |
| Negative | 5 (62.5) | 3 (37.5) |  |
| **Molecular\_concordance, n (%)** |  |  |  |
| Concordant | 0 (0.00) | 3 (100) | 0.168 |
| Partially concordant | 2 (33.3) | 4 (66.7) |  |
| Discordant | 7 (63.6) | 4 (36.4) |  |

IQR: Interquartile range; SD: Standard deviation; RP: Radical prostatectomy.

**Table 5S.** Clinicopathological and molecular characteristics of the 20 patients analyzed by presence of the SPINK-1 subtype

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **SPINK-1** | | **p-value** |
| **Positive**  **(n = 12)** | **Negative**  **(n = 8)** |
| **Age of diagnosis (years)** |  |  |  |
| Median [IQR] | 63.0 [21.0] | 66.5 [4.25] | 0.486 |
| **Prediagnosis\_PSA, ng/ml** |  |  |  |
| Median [IQR] | 8.25 [8.10] | 17.0 [10.6] | 0.160 |
| **Gleason score, n (%)** |  |  |  |
| 7 | 11 (57.8) | 8 (42.2) | 1.00 |
| 8 | 1 (100) | 0 (0.00) |  |
| **Gleason grade group, n (%)** |  |  |  |
| G2 | 3 (60.0) | 2 (40.0) | 1.00 |
| G3 | 8 (57.2) | 6 (42.8) |  |
| G4 | 1 (100) | 0 (0.00) |  |
| **Tumor percentage (%)** |  |  |  |
| Mean ± SD | 42.9 ± 20.5 | 33.9 ± 16.9 | 0.315 |
| **Index diameter** |  |  |  |
| Mean ± SD | 2.04 ± 0.52 | 1.89 ± 0.43 | 0.497 |
| **Margin status in RP, n (%)** |  |  |  |
| Positive | 9 (56.3) | 7 (43.7) | 0.618 |
| Negative | 3 (75.0) | 1 (25.0) |  |
| **Perineural invasion, n (%)** |  |  |  |
| Intraprostatic | 4 (50.0) | 4 (50.0) | 0.648 |
| Intraprostatic + extraprostatic | 8 (66.7) | 4 (33.3) |  |
| **Lymphovascular invasion, n (%)** | | | |
| Yes | 5 (50.0) | 5 (50.0) | 0.349 |
| No | 7 (77.8) | 2 (22.2) |  |
| Unknown | 0 (0.00) | 1 (100) |  |
| **Pathologic \_stage.pT, n (%)** |  |  |  |
| 2c-3a | 6 (85.7) | 1 (14.3) | 0.157 |
| 3b | 6 (46.2) | 7 (53.8) |  |
| **Pathologic\_stage.pN, n (%)** |  |  |  |
| 0 | 4 (100) | 0 (0.00) | 0.116 |
| 1 | 8 (50.0) | 8 (50.0) |  |
| **ERG, n (%)** |  |  |  |
| Positive | 10 (71.4) | 4 (28.6) | 0.161 |
| Negative | 2 (33.3) | 4 (66.7) |  |
| **EZH2, n (%)** |  |  |  |
| Positive | 10 (66.7) | 5 (33.3) | 0.347 |
| Negative | 2 (40.0) | 3 (60.0) |  |
| **NKX3.1, n (%)** |  |  |  |
| Positive | 4 (44.4) | 5 (55.6) | 0.361 |
| Negative | 8 (72.7) | 3 (27.3) |  |
| **Molecular\_concordance, n (%)** |  |  |  |
| Concordant | 1 (33.3) | 2 (66.7) | 0.686 |
| Partially concordant | 4 (66.7) | 2 (33.3) |  |
| Discordant | 7 (63.6) | 4 (36.4) |  |

IQR: Interquartile range; SD: Standard deviation; RP: Radical prostatectomy.