Prostate cancer is the second leading cause of cancer-related death. The androgen deprivation therapy is the standard treatment for advanced stages. Unfortunately, virtually all tumors become resistant to androgen withdrawal. The progression to castration-resistance is not fully understood, although a recent paper has suggested translationally controlled tumor protein to be implicated in the process. The present study was designed to investigate the role of this protein in prostate cancer, focusing on the correlation between its expression level with tumor differentiation and response to treatment. We retrieved 292 prostatic cancer specimens; of these 153 had been treated only by radical prostatectomy and 139 had undergone radical prostatectomy after neoadjuvant treatment with combined androgen blockade therapy. Non-neoplastic control were represented by 102 prostatic peripheral zone specimens. In untreated patients, the expression of the protein, evaluated by RT-qPCR and immunohistochemistry, was significantly higher in tumor specimens than in non-neoplastic control, increasing as Gleason pattern and score progressed. In treated prostates, the staining was correlated with the response to treatment. An association between protein expression and the main clinicopathological factors involved in prostate cancer aggressiveness was identified. These findings suggest that the protein may be a promising prognostic factor and a target for therapy.
fully understood [10]. It is well known that androgens are required for normal growth and functioning of the prostate gland by binding androgen receptors (AR) [11]. AR can modulate gene expression directly by interacting with specific elements in the regulatory regions of target genes or indirectly by activating various growth factor signaling pathways [12]. Most metastatic CRPC show mutations, amplifications, and deletions of the AR gene as well as conformational changes of the AR protein and sensitivity to growth factors and cytokines [13]. This leads to activation of AR without androgen [14]. An effective treatment for these patients is not yet available though a new drug (i.e., abiraterone acetate) seems to be promising [15]. Additional therapeutic strategies targeting molecular mechanisms-mediating resistance are required, either to delay or to prevent the emergence of the castration resistant phenotype [16]. The translationally controlled tumor protein (TCTP) is a protein highly expressed in mammals and in a wide range of other organisms. The conservation of TCTP-converging network through phylogeny underscores its relevance [17]. All the processes regulated by the protein, including promoting cell growth, activating components of the mTOR pathway, inhibiting BOX homodimerization and apoptosis, antagonizing p53, and being overexpressed in tumors in respect to the normal counterpart, may converge to a limited set of key events that control stemness pluripotency, tumor reversion, cell fate determination, and ultimately tumorigenesis [17]. Therefore, TCTP combines both a tumor suppressive and an oncogenic activity that results in a context-dependent cancer phenotype, representing a check point and a switch necessary for cellular reprogramming [17]. We have previously demonstrated the presence of both TCTP mRNA and protein in prostatic tissue, in prostate cancer cell lines and in the prostatic fluid [18], suggesting specific roles for the protein such as apoptosis and control of sperm functions. Recent studies have proposed TCTP as an androgen-regulated gene implicated in PC, providing preclinical proof-of-principle that combining antisense oligonucleotide-mediated TCTP knockdown with castration and/or docetaxel therapy could serve as a novel strategy to treat CRPC [9, 19]. However, it is not currently known whether there is a correlation between the different histological grades of PC and TCTP expression or whether the protein is expressed in preinvasive lesions as high-grade prostatic intraepithelial neoplasia (HGPIN). Therefore, the present study was designed to investigate TCTP expression in prostate carcinoma, focusing on the correlation between protein/gene expression level and tumor differentiation. Furthermore, TCTP expression was also studied in neoplastic tissue after androgen deprivation to obtain more information on the hormonal regulation of this protein.

2. Materials and Methods

2.1. Patients. Ethics approval for this study was obtained from the Institutional Review Board at the University of Siena (Italy). Informed written consent was gained from the patients and all specimens were handled and made anonymous. We retrieved from the archives of Siena University Hospital (Siena, Italy) prostate needle biopsies and the corresponding radical prostatectomy of 292 patients who had undergone surgery for PC between January 1999 and December 2003. Of these, 153 patients had been treated only by radical prostatectomy and 139 had undergone radical prostatectomy after neoadjuvant treatment with androgen-deprivation therapy. Non-neoplastic controls were represented by 102 prostatic peripheral zone specimens of patients who underwent cystoprostatectomy for bladder cancer but with no tumor in prostate gland. The mean age of the patients at the time of surgery was 69 years (range: 55 to 79 years). The following biochemical and pathological parameters were also recorded: total prostate specific antigen (tPSA), Gleason score (in both needle biopsies and surgical specimens for untreated patients, and only in needle biopsies for treated patients), surgical margins infiltration, extraprostatic extension, seminal vesicles invasion, lymph node metastasis, and TNM staging system (based on the AJCC Cancer Staging Manual, Seventh Edition, 2010, Springer New York, Inc.). The clinicopathological features of the 292 patients are summarized in Table 1.

2.2. Histology. Core needle biopsies and surgical specimens had been fixed in 10% buffered formalin and embedded in paraffin. From each block, 4 μm thick sections had been cut and stained with haematoxylin and eosin (H&E). All the slides were reviewed by three expert uropathologists (MTdV, FC, and CC), who subsequently met to obtain a consensus diagnosis. Tumor grading was established according to the updated Gleason grading system in each sample [20, 21]. Representative tumor sections were then classified as low grade when the combined Gleason score (primary plus secondary pattern) was ≤7 and as high grade when the combined Gleason score was ≥8. Foci of HGPIN were also identified, when present in peritumoral areas. Treated adenocarcinoma patients were classified as good, moderate, or poor responders on the basis of the changes in the morphological parameters suggested by Montironi et al. (nuclear enlargement, prominent nucleoli, cell cytoplasm vacuolization, acinar shrinkage, isolated infiltrating tumor cells, difficulty in recognizing prostate cancer patterns, and amount of interstitial tissue stroma) [22].

2.3. RT-qPCR for TCTP Expression. The expression of TCTP was evaluated in non-neoplastic and neoplastic prostate cancers. In particular, neoplastic samples were representative of different Gleason pattern (Gleason patterns 3–5) and score and of different response to treatment (poor, moderate, and good responder patients). Tumor cells corresponding to different Gleason pattern were isolated by laser capture microdissection on H&E-stained sections (4-5 μm thick) from formalin-fixed paraffin-embedded tissues, using a laser capture microdissector (Arcturus, MWG, Florence, Italy). Microdissected cells were transferred to a Capsure transfer film, containing 200 μL of Trizol (Invitrogen, CA). RNA was then extracted following manufacturer’s instructions. Reverse transcription was carried out using the Quantitect Reverse transcription Kit (Qiagen, CA). For each RNA specimen, a negative control was prepared by omitting the reverse transcriptase. TCTP expression was analyzed both in
Table 1: Main characteristics of the patients included in this study. Univariate descriptive statistics (including frequencies and percentages) of each qualitative clinical parameter in relation to the two TCTP protein expression levels is shown with the relative \( P \) value. A statistically significant association between TCTP protein expression and the main prognostic factors involved in PC aggressiveness is detected.

| Variable                          | Group         | \( N \) | TCTP protein expression | \( P \) value |
|-----------------------------------|---------------|---------|-------------------------|---------------|
|                                   |               |         | High                    | Low           |               |
|                                   |               |         | 1 (0.8%)                | 5 (3.1%)      | <0.05         |
| Preoperative PSA                  | <4            | 6 (2%)  | 1 (0.8%)                | 5 (3.1%)      |               |
|                                   | 4–10          | 95 (32.5%) | 27 (20.6%)              | 68 (42.2%)    |               |
|                                   | >10           | 191 (65.5%) | 103 (76.6%)             | 88 (54.7%)    |               |
| Surgical margins infiltration     | Present       | 162 (55.5%) | 91 (69.5%)              | 71 (44.1%)    | <0.001        |
|                                   | Absent        | 130 (44.5%) | 40 (30.5%)              | 90 (55.9%)    |               |
| Extraprostatic extension          | Present       | 129 (44.2%) | 91 (69.5%)              | 38 (23.6%)    | <0.001        |
|                                   | Absent        | 163 (55.8%) | 40 (30.5%)              | 123 (76.4%)   |               |
| Seminal vesicles invasion         | Present       | 49 (16.8%)  | 40 (30.5%)              | 9 (5.6%)      | <0.001        |
|                                   | Absent        | 243 (83.2%) | 91 (69.5%)              | 152 (94.4%)   |               |
| Lymph node metastasis             | Present       | 34 (11.6%)  | 33 (25.2%)              | 1 (0.6%)      | <0.001        |
|                                   | Absent        | 258 (88.4%) | 98 (74.8%)              | 160 (99.4%)   |               |
| TNM stage                         | I             | 29 (9.9%)  | 0 (0%)                  | 29 (18%)      | <0.001        |
|                                   | II            | 128 (43.9%) | 37 (28.2%)              | 91 (56.5%)    |               |
|                                   | III           | 102 (34.9%) | 62 (47.4%)              | 40 (24.9%)    |               |
|                                   | IV            | 33 (11.3%)  | 32 (24.4%)              | 1 (0.6%)      |               |
| Recurrence                        | Present       | 120 (41.4%) | 114 (87%)               | 6 (3.7%)      | <0.001        |
|                                   | Absent        | 172 (58.6%) | 17 (13%)                | 155 (96.3%)   |               |

PC: prostatic cancer; \( N \): number of cases and percentage.

2.4. Immunohistochemistry. The most representative tumor blocks were selected on the basis of the morphological features, and the highest Gleason pattern and score were chosen both in needle biopsies and in radical prostatectomy for each nontreated patient. Since Gleason score is not applicable in treated patients because CAB determines a pronounced simplification of architectural pattern [22], each specimen was selected depending on the response to treatment (poor, moderate, and good responder patients). Immunohistochemical stainings were performed on 4 ± 0.5 \( \mu \)m thick sections of each block employing the Ultravision Detection System Antipolyvalent HRP (Ultra V Block) (LabVision, Fremont, CA, USA, Bio-Optica). All the procedures were carried out automatically by using the Bond-III machine. Slides were incubated with an anti-TCTP antibody (dilution: 1:25) and the reaction revealed using fucsin (Dako, Milan, Italy) as chromogen. Sections were weakly counterstained with Harris’ haematoxylin and examined under a light microscope. Nonimmune serum immunoglobulins were used as negative control, whereas the positive control was represented by placental tissue [24].

2.5. Staining Assessment. All of the samples were independently evaluated and scored by two investigators (MRA and BJR), who were blinded to the clinicopathological information of the patients. TCTP protein expression levels were classified semiquantitatively combining the proportion and intensity of positively stained cells [25–27]. The percentage of positive-staining tumor cells was scored as follows: 1 (<5% positive cells), 2 (5–50% positive cell), and 3 (>50% positive cells) [25–27]. Staining intensity was scored as follows: 1 (weak or not detectable staining), 2 (moderate staining), and 3 (strong staining) [25–27]. Three different fields (at least 100 cells/field) were evaluated at ×200 magnification. In PC samples TCTP protein expression level was evaluated and defined only in neoplastic cells. The sum of the staining intensity score and the percentage score was used to define the TCTP protein expression level, low: 0–2; high: 3–4. The agreement between the two pathologist was about 90%. Cases with discrepancies were reviewed and discussed to reach the 100% of concordance. In core needle biopsies and in untreated radical prostatectomies, staining assessment was performed separately in the two patterns (i.e., 3 and 4 in Gleason score 7, and 4 and 5 in Gleason score 9). In treated radical prostatectomies, TCTP expression was evaluated in comparison to the extent of histological response to treatment.

2.6. Statistical Analysis. Descriptive statistics was computed, including frequency count, minimum, maximum, mean, and standard deviation for quantitative variables and frequency count and percentage for qualitative variables.

The Kolmogorov-Smirnov test was applied to verify normal distribution of quantitative variables age and PSA. When normality was assessed, one-way analysis of variance (ANOVA) was used to compare the two TCTP levels, with post hoc test of Bonferroni for pairwise comparisons. For
non-neoplastic control in GR (b) (GR: good responders; MR: moderate responders; PR: poor responders) (Figure 1(b)) (GR: good responders; MR: moderate responders; PR: poor responders) (t-test, $P < 0.05$). The graphs are representative of three different experiments. Error bars indicate standard deviation.

The Kendall rank correlation coefficient, $r$, was computed to evaluate the correlation between TCTP levels and each qualitative variable. When computable, the Fisher exact test was applied to contingency tables to evaluate the association between the frequency distributions of TCTP levels and each qualitative variable. We alternatively used the classic $\chi^2$ test.

The prognostic power of TCTP, with relation to the disease-free survival (DFS) time, was investigated by using the Cox proportional hazards model. The multivariate model was designed in a stepwise manner, by including at any successive step only the prognostic factors (covariates) that could be associated with survival with a statistical significance greater than 95% ($P < 0.05$). The stepwise method allows univariate survival analysis to be also evaluated at step 0. The hazard ratio (HR) and its 95% confidence interval were computed for each prognostic factor, for both univariate and multivariate analyses. HR represents the odds that an individual in the group with the highest risk reaches the endpoint first. Finally, the Kaplan-Meier DFS curves for the two TCTP levels were drawn and statistically compared through the log rank test.

In each group, the difference in TCTP expression between needle biopsy and radical prostatectomy was evaluated. Results were considered statistically significant when they exceeded the 95% probability level ($P < 0.05$).

### 3. Results

#### 3.1. TCTP mRNA in Untreated and Treated PC

The expression of TCTP in non-neoplastic and neoplastic prostate samples was evaluated by RT-qPCR. Relative quantification indicated that the expression of TCTP is significantly higher in tumor specimens than in non-neoplastic control, increasing as Gleason pattern (Figure 1(a)) and Gleason score progressed (t-test, $P < 0.05$). In patients treated with CAB, TCTP expression is correlated to response to therapy, being higher in poor responders and lower in good responders (Figure 1(b)) (t-test, $P < 0.05$).

#### 3.2. TCTP Protein Expression in Untreated and Treated PC

Among the non-neoplastic specimens, 78 showed atrophy and 24 atrophy plus chronic inflammation. A low TCTP expression was detected in the cytoplasm of the epithelial cells, mainly located in the apical portion and blebs, whereas the basal layer cells showed a strong positivity. Among the 153 untreated PC, 57 were Gleason score 6, 52 Gleason score 7, 25 Gleason score 8, 17 Gleason score 9, and 2 Gleason score 10. For the Gleason scores 7 and 9, TCTP protein expression was also evaluated separately in the two patterns (i.e., 3 and 4 for Gleason score 7 and 4 and 5 for Gleason score 9). A correlation between TCTP immunostaining and Gleason pattern and score was observed. TCTP protein expression was higher in high Gleason pattern (i.e., 4 and 5) in comparison to low Gleason pattern (i.e., 3) ($P < 0.04$) (Figures 2(a)–2(c)). As far as Gleason score is concerned, TCTP immunostaining was significantly lower in Gleason score 6 PC ($P < 0.001$) and stronger in Gleason score 8 to 10 PC ($P < 0.001$) (Table 2(a)). In Gleason score 7 PC an intratumoral heterogeneity was observed, with a higher TCTP protein expression in Gleason pattern 4 than in Gleason pattern 3. The stromal cells both in non-neoplastic and in neoplastic specimens showed a mild staining. Strong staining was observed in cancer-associated HGPIN (Figure 2(d)). No differences in TCTP protein expression were observed between the needle biopsies and the corresponding radical prostatectomy of the same Gleason pattern. In treated prostates, 48 were good responders, 40 were moderate responders, and 51 were poor responders. The intensity of TCTP immunostaining was higher in poor responders and lower in good responders (Table 2(b) and Figures 3(a)-3(b)) (Kendall’s $r$: 0.773, $P < 0.001$).

#### 3.3. Association of TCTP Protein Expression with the Prognostic Factors of Prostatic Carcinoma

We investigated the
TCTP protein expression in untreated patients. TCTP staining increases as Gleason pattern progresses from Gleason pattern 3 (a) to Gleason pattern 4 (b) to 5 (c); in the latter isolated neoplastic cells strongly express the protein ($\chi^2$-test, $P < 0.001$). High positivity is also present in associated-cancer HGPIN ((d), arrow) in respect to the non-neoplastic tissue. (Original Magnification, O.M.: (a)–(d), 10x).

Table 2: (a) Immunohistochemical results in untreated patients. The association between TCTP protein expression and Gleason score is shown, with higher TCTP protein expression in high Gleason score samples (Gleason score ≥8) ($P < 0.001$). (b) Immunohistochemical results in treated patients. The association between TCTP protein expression and response to treatment is shown, with lower TCTP protein expression in good responder patients ($P < 0.001$).

| Gleason score | $N$ | % | TCTP protein expression |
|---------------|-----|---|-------------------------|
|               |     |   | High | Low |
| 6             | 57  | 37.3 | 4 (7%) | 53 (93%) |
| 7             | 52  | 34  | 28 (53.8%) | 24 (46.1%) |
| 8             | 25  | 16.3 | 24 (96%) | 1 (4%) |
| 9             | 17  | 11.1 | 17 (100%) | 0 (0%) |
| 10            | 2   | 1.3  | 2 (100%) | 0 (0%) |

| Response to treatment | $N$ | % | TCTP protein expression |
|-----------------------|-----|---|-------------------------|
|                       |     |   | High | Low |
| Poor                  | 51  | 36.7 | 50 (98%) | 1 (2%) |
| Moderate              | 40  | 28.8 | 24 (60%) | 16 (40%) |
| Good                  | 48  | 34.5 | 4 (4.2%) | 46 (95.8%) |

Association between TCTP protein expression status and the well-recognized prognostic factors of PC. The intensity of TCTP was directly correlated with higher preoperative PSA ($P < 0.05$), stage ($P < 0.001$) and Gleason score ($P < 0.001$), surgical margins infiltration ($P < 0.001$), extraprostatic extension ($P < 0.001$), seminal vesicles invasion ($P < 0.001$), and lymph node metastasis ($P < 0.001$). On the contrary, no correlation has been identified between TCTP expression and the age of the patients ($P = 0.99$) (Figures 4(a)–4(f)).

3.4. Disease-Free Survival Analysis. The Kaplan–Meier curves of TCTP protein expression are shown in Figure 5. DFS was significantly different between the groups with high and low TCTP expression (log rank test, $P < 0.001$). In particular, the group with the higher TCTP protein expression had a shorter DFS (mean = 64 months; 95% CI = 57–70 months) when compared to the group showing the lower TCTP protein expression (mean = 178 months; 95% CI = 171–185 months). Univariate Cox analysis showed that all the prognostic factors known to be involved in PC are highly statistically correlated with DFS ($P < 0.001$) except for the age ($P = 0.73$). In addition, we found no differences in DFS between the treated and the untreated patients ($P = 0.32$), thus confirming that the neoadjuvant treatment does not affect patients’ prognosis. Stepwise multivariate analysis enrolling the above mentioned parameters and the TCTP protein staining demonstrated that TCTP was not an independent prognosticator but influences patients’ outcome (HR = 49.7, CI 95% = 19.4–127.1 for high TCTP staining).

4. Discussion

TCTP is a multifaceted protein which has been implicated in a number of key cellular processes, both physiologic
Figure 3: TCTP protein expression in treated patients. The intensity of immunostaining is absent or low in good responders (a) and high in poor responders (b) (Kendall’s $\tau$: 0.773, $P < 0.001$). (O.M., (a)-(b), 10x).

Figure 4: Association of TCTP protein expression with the main prognostic factors involved in PC aggressiveness. Univariate analysis by Fisher exact test and/or $\chi^2$-test shows that TCTP protein expression level is significantly associated with higher preoperative PSA (a) ($P < 0.05$), surgical margins infiltration (b) ($P < 0.001$), extraprostatic extension (c) ($P < 0.001$), seminal vesicles invasion (d) ($P < 0.001$), lymph node metastasis ($P < 0.001$) (e), and higher TNM stage (f).
Figure 5: Kaplan-Meier curves of TCTP protein expression levels. Disease free survival was significantly different between the groups with high and low TCTP protein expression (log rank test, \( P < 0.001 \)). The group with the higher TCTP protein expression has a shorter disease free survival (mean = 64 months) when compared to the group with the lower TCTP protein expression (mean = 178 months).

5. Conclusions

Collectively, our data demonstrate that, in PC, TCTP expression is directly correlated with tumor differentiation and with the main clinicopathological factors involved in PC aggressiveness. The assessment of TCTP staining in needle biopsies from PC patients may be of help in evaluating the more appropriate therapeutic strategies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, "Global cancer statistics," CA Cancer Journal for Clinicians, vol. 61, no. 2, pp. 69–90, 2011.
[2] E. Mazaris and A. Tsiotras, "Molecular pathways in prostate cancer," Nephro-Urology Monthly, vol. 5, no. 3, pp. 792–800, 2013.
[3] K. D. Sørensen and T. F. Ørntoft, "Discovery of prostate cancer biomarkers by microarray gene expression profiling," Expert Review of Molecular Diagnostics, vol. 10, no. 1, pp. 49–64, 2010.
[4] F. Kunath, B. Keck, G. Rücker et al., "Early versus deferred androgen suppression therapy for patients with lymph nodal-positive prostate cancer after local therapy with curative intent: a systematic review," BMC Cancer, vol. 13, article 131, 2013.
[5] D. P. Petrylak, C. M. Tangen, M. H. A. Hussain et al., “Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer,” The New England Journal of Medicine, vol. 351, no. 15, pp. 1513–1520, 2004.

[6] I. F. Tannock, R. de Wit, W. R. Berry et al., “Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer,” The New England Journal of Medicine, vol. 351, no. 15, pp. 1502–1512, 2004.

[7] N. Mottett, J. Bellmunt, M. Bolla et al., “EAU guidelines on prostate cancer. Part II: treatment of advanced, relapsing, and castration-resistant prostate cancer,” European Urology, vol. 59, pp. 572–583, 2011.

[8] A. Heidenreich, G. Aus, M. Bolla et al., “EAU guidelines on prostate cancer,” European Urology, vol. 53, no. 1, pp. 68–80, 2008.

[9] V. Baylot, M. Katsogiannou, C. Andrieu et al., “Targeting TCTP as a new therapeutic strategy in castration-resistant prostate cancer,” Molecular Therapy, vol. 20, no. 12, pp. 2244–2256, 2012.

[10] R. Ottman, C. Nguyen, R. Lorch, and R. Chakrabarti, “MicroRNA expressions associated with progression of prostate cancer cells to androgen therapy resistance,” Molecular Cancer, vol. 13, article 1, 2014.

[11] J. Javidan, A. D. Deitch, X. B. Shi, and R. W. de Vere White, “The androgen receptor and mechanisms for androgen independence in prostate cancer,” Cancer Investigation, vol. 23, no. 6, pp. 520–528, 2005.

[12] B. Seruga, A. Ocan, and I. F. Tannock, “Drug resistance in metastatic castration-resistant prostate cancer,” Nature Reviews Clinical Oncology, vol. 8, no. 1, pp. 12–23, 2011.

[13] B. J. Feldman and D. Feldman, “The development of androgen-independent prostate cancer,” Nature Reviews Cancer, vol. 1, pp. 34–45, 2001.

[14] H. I. Scher, G. Buchanan, W. Gerald, L. M. Butler, and W. D. Tilley, “Targeting the androgen receptor: improving outcomes for castration-resistant prostate cancer,” Endocrine-Related Cancer, vol. 11, no. 3, pp. 459–476, 2004.

[15] A. J. Armstrong and D. J. George, “New drug development in metastatic prostate cancer,” Urologic Oncology, vol. 26, no. 4, pp. 430–437, 2008.

[16] D. R. Ciocca and S. K. Calderwood, “Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications,” Cell Stress and Chaperones, vol. 10, no. 2, pp. 86–103, 2005.

[17] R. Armon, S. Pece, J. C. Marine, P. P. di Fiore, and A. Tellerman, “TPT1/TCTP-regulated pathways in phenotypic reprogramming,” Trends in Cell Biology, vol. 23, no. 1, pp. 37–46, 2013.

[18] F. Arcuri, A. Carducci et al., “Translational control of the human prostate and prostate cancer cells: expression, distribution, and calcium binding activity,” Prostate, vol. 60, no. 2, pp. 130–140, 2004.

[19] M. Kaarbo, M. L. Storm, S. Qu et al., “TCTP is an androgen-regulated gene implicated in prostate cancer,” PLoS ONE, vol. 8, no. 7, Article ID e69398, 2013.

[20] P. M. Pierorazio, P. C. Walsh, A. W. Partin, and J. I. Epstein, “Prognostic Gleason grade grouping: data based on the modified Gleason scoring system,” The British Journal of Urology International, vol. 110, no. 5, pp. 753–760, 2013.

[21] L. Egevad, A. S. Ahmad, F. Algaba et al., “Standardization of Gleason grading among 337 European pathologists,” Histopathology, vol. 62, no. 2, pp. 247–256, 2013.