High B7-H3 expression is linked to increased risk of prostate cancer progression

Sarah Bonk1,2* | Pinar Tasdelen2* | Martina Kluth2 | Claudia Hube-Magg2 | Georgia Makrypidi-Fraune2 | Katharina Möller2 | Doris Höflmayer2 | Sebastian Dwertmann Rico2 | Franziska Büscheck2 | Sarah Minner2 | Hans Heinzer3 | Markus Graefen3 | Andrea Hinsch2 | Andreas M. Luebke2 | David Dum2 | Ria Uhlig2 | Thorsten Schlomm4 | Guido Sauter2 | Ronald Simon2 | Sören A. Weidemann2

1 Department of General, Visceral and Thoracic Surgery Department and Clinic, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
2 Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
3 Martini-Clinic, Prostate Cancer Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
4 Department of Urology, Charité - Universitätsmedizin Berlin, Berlin, Germany

Abbreviations:
ERG, V-ets avian erythroblastosis virus E26 oncogene homolog; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PD-L1, programmed cell death 1 ligand 1; PSA, prostate-specific antigen; PTEN, phosphatase and tensin homolog; TMA, tissue micro array; TMPRSS2, transmembrane protease, serine 2

B7-H3 is a member of the B7 superfamily of immune checkpoint molecules. B7-H3 up regulation has been linked to cancer development and progression in many tumors including prostate cancer. To clarify the potential utility of B7-H3 as a prognostic biomarker, B7-H3 expression was analyzed by immunohistochemistry in more than 17,000 prostate cancers. Normal prostatic glands were largely B7-H3 negative, while membranous B7-H3 immunostaining was seen in 47.0% of analyzed cancers. B7-H3 immunostaining was weak in 12.3%, moderate in 21.1% and strong in 13.5% of cases. High B7-H3 expression was associated with pT, Gleason score, lymph node metastasis, high Ki67 labeling index and early prostate-specific antigen recurrence (P < 0.0001 each). High B7-H3 expression was also linked to high androgen receptor expression and TMPRSS2:V-ets avian erythroblastosis virus E26 oncogene homolog (ERG) fusions (P < 0.0001 each). Multivariate analyses showed a strong independent prognostic impact of high B7-H3 expression in all cancers and in the ERG negative subgroup. Comparison with previously analyzed frequent chromosomal deletions revealed a close association with Phosphatase and Tensin Homolog deletions. Analysis of B7-H3, alone or in combination with other markers, might be of clinical utility, especially in the subgroup of ERG negative prostate cancers.

KEYWORDS
B7-H3, CD276, prognosis, prostate cancer, TMA

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*These authors contributed equally to this work

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INTRODUCTION

There were more than 1.3 million estimated new cases of prostate cancer in 2018 worldwide. Prostate cancer remains the most common cancer in males in over one half of the countries of the world and the third most common cause of cancer related death of men in Western countries. Its clinical course is highly variable. Many patients never need therapy. The currently available criteria used for the individual risk assessment of patients and consecutive treatment decisions include Gleason grade, tumor extent on biopsies, clinical stage and prostate-specific antigen (PSA) protein levels in the serum. Although these parameters are statistically powerful, they remain insufficient to safely predict cancer aggressiveness in all patients. Therefore, new and better-quality prognostic markers are needed.

B7-H3 (CD276) is a member of the B7 superfamily of immune checkpoint molecules. The extracellular domain of B7-H3 has strong similarities to PD-L1 – another member of the B7 family. However, the receptor of B7-H3 is still unknown. Its immunologic role is controversial since co-stimulatory and co-inhibitory effects have not been found. In addition to potential immunological effects on the tumor environment B7-H3 appears to exert non-immune mediated effects in tumor progression for example by inducing epithelial-to-mesenchymal transition or contributing to the Warburg effect. B7-H3 is known to be expressed in various immune cells like dendritic cells, macrophages, activated T-cells, B-cells and natural killer (NK) cells. Most other normal tissues show no or only low expression of B7-H3, while it is often overexpressed in tumor cells. The strong expression of B7-H3 in many cancer types makes it an attractive target for cancer immunotherapy – as are other B7 family members. Various anti-B7-H3 approaches have been studied in preclinical and clinical trials and identify B7-H3 as a powerful target in cancer immunotherapy. This includes therapies with anti-B7-H3 antibody (e.g., Enoblituzumab, 8H9), and antibody-drug conjugates (DX-8951 derivative, DXd), CAR T-cell therapies, and combined therapy forms. In addition, several authors found B7-H3 overexpression associated with advanced tumor stage, increased proliferation and poor patient outcome in breast, endometrial, pancreatic, cervical, ovarian, oral squamous cell, cholangiocellular carcinoma and lung cancer. Also nonpithelial tumors such as melanoma, osteosarcoma or gliomas show B7-H3 overexpression. Others described associations between high B7-H3 expression and favorable tumor course in pancreatic and gastric cancer.

Also, in prostate cancer, several studies on cohorts of 130–823 tumors have suggested an unfavorable prognostic role of high B7-H3 protein expression. In a RNA-based study on 2700 patients, Benzon et al. recently described significant associations of high B7-H3 expression with unfavorable patient outcome. To further investigate the potential prognostic utility of B7-H3 measurement in prostate cancer, a preexisting prostate cancer tissue microarray (TMA) consisting of more than 17,000 tumors with additional follow-up data was analyzed by immunohistochemistry (IHC) in this study.

MATERIALS AND METHODS

Patients

Histological specimens were available from 17,747 patients, who underwent radical prostatectomy between 1992 and 2015 at the Department of Urology and the Martini Clinic at the University Medical Center Hamburg-Eppendorf. Virtually all patients were of Caucasian ethnicity. Available data included pT, pN and the status of the resection margin. In addition to the classical Gleason categories, ‘quantitative’ Gleason grading was performed as described before. In brief, for every prostatectomy specimen, the percentages of Gleason 3, 4 and 5 patterns were recorded in cancerous tissues as part of the regular process of Gleason grading. Gleason 3+4 and 4+3 cancers were subdivided according to their percentage of Gleason 4. For practical use, we subdivided the 3+4 and 4+3 cancers in eight subgroups: 3+4 ≤5% Gleason 4, 3+4 >6–10%, 3+4 11–20%, 3+4 21–30%, 4+3 31–49%, 4+3 50–60%, 4+3 61–80% and 4+3 >80% Gleason 4. In addition, separate groups were defined by the presence of a tertiary Gleason 5 pattern, including 3+4 Tert.5 and 4+3 Tert.5. Follow-up data were available for 14,641 patients with a median follow-up of 48 months (range 1–275 months). PSA recurrence was defined as the time point when postoperative PSA was at least 0.2 ng/mL and increasing at subsequent measurements. Patient characteristics are summarized in Table 1.

TMA manufacturing and prognostic markers

A 0.6 mm diameter tissue core was taken from a tumor containing tissue block from each prostatectomy specimen and placed into TMA blocks. Molecular data were available from previous investigations on the Ki67 labeling index (Ki67 LI) for 5633 tumors, androgen receptor (AR) expression for 6188 tumors (expanded from), V-ets avian erythroblastosis virus E26 oncogene homolog (ERG) protein expression for 8110 and ERG rearrangement analysis by fluorescence in situ hybridization (FISH) for 5470 tumors (expanded from). Deletion data were available of 10q23 (PTEN) from 5241 tumors (expanded from), of 6q15 (MAP3K7) from 4722 tumors (expanded from), of 5q21 (CHD1) from 6031 tumors (expanded from), of 3p13 (FOXP1) from 5513 tumors (expanded from), of 8p21 from 5489 tumors, of 12p13 (CDKN1B) from 4887 tumors, of 12q24 from 5625 tumors, of 13q14 from 5915 tumors, of 16q24 from 4413 tumors, of 17p13 from 6249 tumors (expanded from), and of 18q21 from 5332 tumors.
Table 1  Study cohort

| Study cohort on TMA (n = 17 747) | Biochemical relapse among categories |
|---------------------------------|------------------------------------|
| No. of patients (%)             |                                    |
| **Follow-up (months)**          |                                    |
| n                               | 14 464 (81.5%)                     | 3612 (25%)                        |
| Mean                            | 56.3                               | -                                 |
| Median                          | 48                                 |                                    |
| Age (years)                     |                                    |
| <50                             | 433 (2.4%)                         | 66 (15.2%)                        |
| 51–59                           | 4341 (24.5%)                       | 839 (19.3%)                       |
| 60–69                           | 9977 (56.4%)                       | 2073 (20.8%)                      |
| ≥70                             | 2936 (16.6%)                       | 634 (21.6%)                       |
| **Pretreatment PSA (ng/mL)**    |                                    |
| <4                              | 2225 (12.6%)                       | 313 (14.1%)                       |
| 4–10                            | 10 520 (59.6%)                     | 1696 (16.1%)                      |
| 10–20                           | 3662 (20.8%)                       | 1043 (28.5%)                      |
| >20                             | 1231 (7%)                          | 545 (44.3%)                       |
| **pT stage (AJCC 2002)**        |                                    |
| pT2                             | 11 518 (65.2%)                     | 1212 (10.5%)                      |
| pT3a                            | 3842 (21.7%)                       | 1121 (29.2%)                      |
| pT3b                            | 2233 (12.6%)                       | 1213 (29.2%)                      |
| pT4                             | 85 (0.5%)                          | 63 (74.1%)                        |
| **Gleason grade**               |                                    |
| ≤3+3                            | 3570 (20.3%)                       | 264 (7.4%)                        |
| 3+4                             | 9336 (53%)                         | 1436 (15.4%)                      |
| 3+4 Tert.5                      | 798 (4.5%)                         | 165 (20.7%)                       |
| 4+3                             | 1733 (9.8%)                        | 683 (39.4%)                       |
| 4+3 Tert.5                      | 1187 (6.7%)                        | 487 (41%)                         |
| ≥4+4                            | 999 (5.7%)                         | 531 (53.2%)                       |
| **pN stage**                    |                                    |
| pN0                             | 10 636 (89.4%)                     | 2243 (21.1%)                      |
| pN+                             | 1255 (10.6%)                       | 700 (55.8%)                       |
| **Surgical margin**             |                                    |
| Negative                        | 14 297 (80.8%)                     | 2307 (16.1%)                      |
| Positive                        | 3388 (19.2%)                       | 1304 (38.5%)                      |

Percent in the column "Study cohort on TMA" refers to the fraction of samples across each category. Percent in column "Biochemical relapse among categories" refers to the fraction of samples with biochemical relapse within each parameter in the different categories. Numbers do not always add up to 17 747 in the different categories because of cases with missing data. Abbreviation: AJCC, American Joint Committee on Cancer.

The use of archived and diagnostic left-over tissues for manufacturing tissue microarrays and their analysis for research purposes as well as patient data analysis has been approved by local laws (HmbKHG, §12) and by the local ethics committee (Ethics commission Hamburg, WF-049/09). All work has been carried out in compliance with the Helsinki Declaration.

Immunohistochemistry

Freshly cut tissue microarray sections were stained the same day and in a single run. Slides were deparaffinized and exposed to proteinase K for 10 min at room temperature. Primary antibody against B7-H3 protein (rabbit monoclonal antibody, #M5650, dilution 1:1350; Zytomed, Bargtehaide, Germany) was applied at 37°C for 60 min. Bound antibody was visualized with the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer's instructions. B7-H3 typically shows membranous staining with a weak cytoplasmic background. The membranous B7-H3 staining was evaluated according to the following scoring system. The staining intensity (0, 1+, 2+, 3+) and the fraction of positive tumor cells were recorded for each tissue tumor spot and a final score was built of these two parameters: Negative, staining intensity of 0; weak, staining intensity of 1+ in ≤70% of tumor cells or staining intensity of 2+ in ≤30% of tumor cells; moderate, staining intensity of 1+ in more than 70% of tumor cells, staining intensity of 2+ in >30% but in ≤70% of tumor cells or staining intensity of 3+ in ≤30% of tumor cells; strong, staining intensity of 2+ in >70% of tumor cells or staining intensity of 3+ in >30% of tumor cells. Our scoring system is based on the assumption that the fraction of stained tumor cells is equally important as the staining intensity. Tumors with a high fraction of stained tumor cells (i.e., >70%) are, thus, upgraded to the next score level, whereas tumors with a low fraction (≤30%) will be downgraded. For example, a tumor with a ‘weak’ 1+ staining intensity but a high fraction of 80% of positive tumor cells will be upgraded to a moderate score, while a tumor with ‘strong’ 3+ staining but a low fraction of 10% positive tumor cells will be downgraded to a moderate score. The suitability of our scoring system for finding significant associations between molecular markers and clinico-pathological tumor features is largely independent of inter- or intra-observer differences (PMID: 20690048,21956230). We have regularly used this scoring system in our institute for almost 20 years, and reproduced all known clinically relevant associations with it.47

Statistics

Statistical calculations were performed with JMP® 12.0 software (SAS Institute Inc., Cary, NC, USA). Contingency tables and the chi²-test were utilized to examine associations between molecular and histopathological tumor parameters. Survival curves were calculated according to Kaplan–Meier. The Log-Rank test was applied to detect significant differences between groups. Cox proportional hazards regression analysis was performed to test for statistical independence between pathological, molecular and clinical variables.

RESULTS

Technical issues and immunohistochemistry

A total of 12 808 (72.2%) of tumor samples were interpretable in the TMA analysis. Noninformativeness was attributed
to a lack of tissue or the absence of unambiguous cancer cells in a fraction of samples.

**B7-H3, tumor phenotype and PSA recurrence**

Nonneoplastic prostatic glands showed only faint cytoplasmic and no membranous staining in their luminal cells. Basal cells regularly showed moderate to strong cytoplasmic and membranous staining. Membranous B7-H3 immunostaining was seen in 6014 (47.0%) interpretable tumors and was considered weak in 12.3%, moderate in 21.1% and strong in 13.5%. Representative images of the immunostainings are given in Fig. 1. Strong B7-H3 immunostaining was significantly linked to advanced pathological tumor stage ($P < 0.0001$), high Gleason grade ($P < 0.0001$), presence of lymph node metastasis ($P < 0.0001$), positive surgical margins ($P = 0.0015$), and younger patient age ($<0.0001$; Table 2) as well as to early PSA recurrence ($P < 0.0001$; Fig. 2). Subset analyses of morphologically defined tumor subgroups showed a prognostic impact of B7-H3 expression only in Gleason 3+4 ($P = 0.0291$) and $\geq$8 cancers ($P = 0.0231$) but not in any subgroup defined by identical quantitative Gleason grade (Fig. S1).

**B7-H3 and TMPRSS2:ERG status**

Data on the TMPRSS2:ERG fusion status obtained by FISH were available from 5470 and by IHC from 8110 tumors with interpretable B7-H3 immunostaining. Data on both ERG FISH and IHC were concordant in 95.5% of the 4617 cancers with both FISH and IHC data. High B7-H3 expression was significantly associated with detectable ERG expression and TMPRSS2:ERG rearrangement ($P < 0.0001$, Fig. 3). Due to these marked differences, the relationship between B7-H3 expression data and tumor phenotype as well as patient outcome was separately analyzed in the ERG positive and ERG negative subgroups. This revealed that the relationship of high B7-H3 expression with the cancer phenotype was stronger in ERG negative cancers but still retained in ERG positive cancers (Table S1).

**B7-H3 and genomic deletions**

The relationship between B7-H3 immunostaining and 11 different chromosomal deletions is shown in Fig. S2. These analyses revealed a significant association of B7-H3 expression with 8 of 11 analyzed deletions. This was expected because both B7-H3 expression and most analyzed deletions are either linked to the subset of ERG negative (5q, 6q, 13q,
or ERG positive cancers (3p, 8p, PTEN, 12q, 16q, 17q, B7-H3). Separate analyses of ERG positive and negative cancers revealed significant association of B7-H3 expression with only four deletions in ERG negative (8p, PTEN, 12p, 17p) and five deletions in ERG positive cancers (3p, 8p, PTEN, 12p, 16q).

### B7-H3, androgen receptor and proliferative activity (Ki67 LI)

High AR expression was tightly linked to increased B7-H3 expression. This also hold true for the ERG positive and ERG negative subsets \( (P < 0.0001 \text{ each; Fig. 4}) \). Due to the strong association of B7-H3 and AR expression, the relationship between B7-H3 expression and PSA recurrence were analyzed in the prognostic relevant subgroups of tumors with AR low (negative to moderate) and AR high (strong) expression. This analysis revealed that AR has no impact on the prognostic relevance of B7-H3 \( (P = 0.0063 \text{ for AR low and } P = 0.0160 \text{ for AR high, Fig. 2}) \). Strong B7-H3 staining was also linked to accelerated tumor cell proliferation as measured by Ki67 LI \( (P < 0.0001, \text{ Table S2}) \). However, the absolute differences between cancers with weak and strong B7-H3 expression were small. In subgroups of tumors with identical Gleason grades, the differences in the Ki67 LI were not always significant between tumors defined by different B7-H3 expression levels.

### Multivariate analysis

Four different multivariate analyses were performed to evaluate whether B7-H3 expression is a statistically independent prognostic marker (Table 3). Scenario 1 evaluated B7-H3 expression and all parameters available after surgery, including pT, pN, surgical margin status, preoperative PSA value and Gleason grade obtained after evaluation of the entire resected prostate. In scenario 2, B7-H3 expression and all postoperatively available parameters with the exception of nodal status were included. This was because the indication and extent of lymph node dissection is not standardized in the surgical therapy of prostate cancer, which may introduce a bias towards high grade cancers. Two additional scenarios had the purpose

### Table 2 B7-H3 expression and tumor phenotype

| Parameter                        | B7-H3                   |       |       |       |       | P value |
|----------------------------------|-------------------------|-------|-------|-------|-------|---------|
|                                 | n evaluable             | Negative (%) | Weak (%) | Moderate (%) | Strong (%) |
| All cancers                      | 12,808                  | 53.0  | 12.3  | 21.2  | 13.5  | <0.0001 |
| Tumor stage                      |                         |       |       |       |       |         |
| pT2                              |                         |       |       |       |       | <0.0001 |
| pT3a                             | 7925                    | 56.6  | 12.1  | 20.2  | 11.1  |         |
| pT3b-pT4                         | 3014                    | 51.5  | 13.4  | 21.3  | 13.8  |         |
| Gleason grade                    |                         |       |       |       |       | <0.0001 |
| ≤3+3                             | 2157                    | 52.5  | 13.6  | 21.7  | 12.2  |         |
| 3+4                              | 6873                    | 54.4  | 12.7  | 21.0  | 11.9  |         |
| 4+3                              | 625                     | 55.7  | 12.2  | 20.5  | 11.7  |         |
| 4+3-Tert.5                       | 1297                    | 49.4  | 10.3  | 23.0  | 17.3  |         |
| 4+3-Tert.5                       | 949                     | 49.1  | 11.8  | 21.3  | 17.8  |         |
| ≥4+4                             | 793                     | 51.3  | 10.2  | 17.0  | 21.4  |         |
| Lymph node metastasis            |                         |       |       |       |       | <0.0001 |
| N0                               | 7835                    | 54.3  | 11.9  | 20.8  | 13.0  |         |
| N+                               | 1022                    | 42.7  | 11.2  | 22.3  | 23.9  |         |
| Preop. PSA level (ng/mL)         |                         |       |       |       |       | 0.0437  |
| <4                               | 1444                    | 50.1  | 13.8  | 21.7  | 14.5  |         |
| 4–10                             | 7504                    | 53.4  | 12.4  | 21.2  | 12.9  |         |
| 10–20                            | 2782                    | 53.9  | 11.9  | 20.6  | 13.7  |         |
| >20                              | 1002                    | 53.3  | 10.9  | 19.9  | 16.1  |         |
| Surgical margin                  |                         |       |       |       |       | 0.0015  |
| Negative                         | 10,074                  | 53.8  | 12.3  | 20.9  | 13.0  |         |
| Positive                         | 2689                    | 50.4  | 12.6  | 21.3  | 15.6  |         |
| Patient age                      |                         |       |       |       |       | <0.0001 |
| <50                              | 305                     | 35.4  | 17.4  | 28.9  | 18.4  |         |
| 50–59                            | 3162                    | 47.9  | 14.1  | 22.4  | 15.7  |         |
| 60–70                            | 7247                    | 54.2  | 12.2  | 21.0  | 12.7  |         |
| >70                              | 2180                    | 59.0  | 10.5  | 18.9  | 11.7  |         |

Abbreviation: PSA, prostate-specific antigen.
to model the preoperative situation as much as possible. Scenario 3 included B7-H3 expression, preoperative PSA, clinical tumor stage, and Gleason grade obtained on the prostatectomy specimen. Since a postoperative determination of the Gleason grade is ‘better’ than the preoperatively determined Gleason grade (subjected to sampling errors and consequently under-grading in more than one third of cases⁹), this parameter was replaced by the original preoperative biopsy Gleason grade in Scenario 4. These analyses identified B7-H3 expression as an independent prognostic parameter in all tumors in the preoperative scenarios ($P < 0.0007$ each) and in the subgroup of ERG negative tumors for all four scenarios ($P < 0.0006$ each).
DISCUSSION

This study on a cohort of 17,747 patients identifies B7-H3 as a strong and independent prognostic marker in ERG negative prostate cancer.

B7-H3 was virtually not seen in normal epithelial prostate cells but present at variable levels in prostate cancer cells of 47.0% of our patients. The staining level in normal gland basal cells was typically moderate to strong and less variable than in tumor cells. Higher B7-H3 expression in cancer as compared to normal tissue has earlier been described by several authors.28-30 For example, Roth et al.29 reported B7-H3 immunostaining in all of 338 examined prostate cancers and described lower B7-H3 staining in adjacent normal epithelium in 336 of these patients that had interpretable normal tissue available. Yuan et al.50 found higher B7-H3 levels in cancer as compared to normal prostate using an enzyme-linked immunosorbent assay. Overall, these data indicate, that B7-H3 expression increases in a high fraction of patients during prostate cancer development. The somewhat lower prevalence of detectable B7-H3 immunostaining in our study than seen in earlier reports might have first of all technical reasons such as different reagents, staining conditions and antibodies. In addition, our protocol was tailored to distinguish between low and high B7-H3 expressors and used a comparatively high antibody dilution (others, 1:80; we, 1:1350) which might have made it less sensitive as in other studies.28-30

The significant association with poor patient outcome found in our patients is consistent with several earlier reports. Analyzing 130 prostatectomy specimen Liu et al.27 found high B7-H3 expression to be linked to poor prognosis. In a study on 148 patients, who received salvage radiation therapy, Parker et al.28 observed that higher B7-H3 staining in primary prostate tumors was associated with increased risk of PSA recurrence. In a TMA study on 823 prostate cancers, Zang et al.30 reported a significant association of high B7-H3 expression levels with PSA recurrence. A recent study evaluating RNA expression on 2781 tumors also identified a significant association of B7-H3 expression with poor patient outcome.30 Altogether these findings overwhelmingly demonstrate that increased B7-H3 expression parallels prostate cancer progression. This also fits well with observations in a number of other cancer types where elevated B7-H3 expression was repeatedly described to be linked to poor prognosis.14-16

The unique aspect to the current study is the cohort size of more than 12,000 successfully analyzed cancers in combination with an extensive database that had been collected during various earlier studies. The analysis of molecularly defined tumor subgroups revealed, that the prognostic impact of B7-H3 protein expression was predominantly driven by the subgroup of ERG negative cancers. TMPRSS2:ERG fusions occur in about 50% of prostate cancers.35 They lead to a permanent overexpression of the transcription factor ERG.51 ERG expression by itself lacks prognostic relevance.35 However, ERG modulates the expression of more than 1600 genes in prostate epithelial cells.52 An interaction of B7-H3 with one or several ERG dependent genes may explain the higher number of B7-H3 expressing tumors within ERG positive subgroups. It is currently unknown, why the prognostic effect of B7-H3 was particularly relevant in ERG negative tumors. An ERG dependent prognostic role of biomarkers is not uncommon, however. For example, the prognostic significance of SOX9,53 SENP154 and mTOR expression55 was limited to ERG positive cancers, while expression of FOXA1,56 MTCO257 or FOXP258 were only prognostic in ERG negative cancers. A specific impact of the cellular microenvironment on the investigated pathways may explain the selective prognostic role of such markers in prostate cancer. A dependency of prognostic biomarkers on molecular cancer subtypes represents a challenge for...
regulation in case of PI3K/AKT/mTOR pathway activation. Deletions of PTEN blocks PI3K signaling by dephosphorylating PIP3 resulting in reduced AKT membrane binding ability. The strong association of high B7-H3 expression with 8p deletions raises the possibility of B7-H3 interacting with one of the genes that are targeted by the 8p deletion. However, functional studies would be necessary to prove this hypothesis. The significant association of B7-H3 upregulation with high AR expression fits well with recent findings of Benzon et al. who identified an AR-binding site upstream of B7-H3. The association of B7-H3 expression with increased cell proliferation seen in this study also fits to earlier observations. Liu et al. discussed the possibility of activation of the JAK2/STAT3-pathway as a possible mechanism. However, down-regulation of B7-H3 expression by small interfering RNA in PC-3 cells exerted no apparent impact on cell proliferation in one study.

Several authors had earlier suggested that B7-H3 expression may represent a clinically useful prognostic marker in prostate cancer. Even though the prognostic differences did not appear too striking in absolute numbers and were dependent on the ERG status, our data seem to support this notion. The prognostic impact of B7-H3 was independent of established prognostic parameters in the subgroup of ERG negative cancers – even if parameters were included that become available only after surgery. With respect to prognostic markers it is important to take into consideration that most clinically established, statistically strong prognostic features in prostate cancer suffer from substantial shortcomings in clinical practice. pN data are greatly influenced by the extent of surgery and the extent of pathological work-up of the removed materials. The Gleason grading system suffers from marked interobserver variability reaching close to 40%. This not only applies for nonspecialized pathologists but also for experts. Of note, this principal drawback of categorical Gleason grade groups has not changed with the recent update of the Gleason scoring system in 2016. That B7-H3 expression lacked prognostic impact in cancers defined by identical quantitative Gleason grade demonstrates the power of the quantitative Gleason grading system, which is, however, not universally applied and does not solve all issues of interobserver variability in prostate cancer grading. Biomarkers are thus needed that are not necessarily independent of established ones but better reproducible. For the future, we expect that panels of antibodies may assist the assessment of prostate cancer aggressiveness. Multicolor immunofluorescence has the potential to not only analyze multiple antibodies in parallel but also offers improved quantification. B7-H3 expression measurement may be necessary in a selected cohort was expected, as both B7-H3 expression and 8p deletions were associated with B7-H3-H3 expression in an unanalyzable prostate cancer. By small interfering RNA in PC-3 cells exerted no apparent impact on cell proliferation in one study.

Table 3 Cox proportional hazards regression analysis

| Tumor subset | Scenario | n analyzable | Preoperative PSA Level | pT stage | cT stage | Gleason grade prostatectomy | Gleason grade biopsy | pN stage | R stage | B7-H3-expression |
|--------------|----------|--------------|------------------------|----------|----------|-----------------------------|---------------------|-----------|---------|----------------|
| All cancers  | 1        | 6786         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | <0.0001 0.2898 |
|              | 2        | 10 177       | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | <0.0001 0.3514 |
|              | 3        | 10 018       | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | 0.0007   |
|              | 4        | 8679         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | <0.0001 |
| ERG negative cancers | 1        | 2671         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | 0.0005 0.1581 0.0004 |
|              | 2        | 4120         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | 0.0022 0.0006   |
|              | 3        | 4078         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | 0.0001   |
|              | 4        | 4023         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | 0.0001   |
| ERG positive cancers | 1        | 2116         | 0.0004                 | 0.0001   | 0.0001   | -                           | -                   | 0.0307    | 0.0019 0.5254   |
|              | 2        | 3289         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | <0.0001 | 0.7006   |
|              | 3        | 3235         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | -        | 0.4385   |
|              | 4        | 3181         | 0.0001                 | 0.0001   | 0.0001   | -                           | -                   | -         | -        | 0.0903   |

Abbreviations: ERG, V-ets avian erythroblastosis virus E26 oncogene homolog; PSA, prostate-specific antigen.
thus have the potential to become an element in a future multiparametric prognostic test.

In summary, this study identifies B7-H3 expression as a potential prognostic marker in prostate cancer. Expression analysis of B7-H3, alone or in combination with other markers, might be of clinical utility, mostly in the subgroup of ERG negative prostate cancers.

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DISCLOSURE STATEMENT

None declared

AUTHOR CONTRIBUTIONS

SB, PT, RS, SW and GS designed the study, and drafted the manuscript. HHe, MG and TS have a part in study design. SB, PT, FB, MCT and SW performed IHC analysis and scoring. SDR, DH, AL and SM participated in pathology data analysis. KM, CHM, GMF and RS performed statistical analysis. KM, DD, RU and AH participated in data interpretation, and helped to draft the manuscript. All authors read and approved the final manuscript.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.