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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Low Level Her2 Overexpression Is Associated with Rapid Tumor Cell Proliferation and Poor Prognosis in Prostate Cancer

Sarah Minner[1], Birte Jessen[1], Lars Stiedenroth[1], Eike Burandt[1], Jens Köllermann[1], Martina Mirlacher[1], Andreas Erbersdobler[1], Christian Eichelberg[2], Margit Fisch[2], Tim Henrik Brümmendorf[3], Carsten Bokemeyer[3], Ronald Simon[1], Thomas Steuber[4], Markus Graefen[4], Hartwig Huland[4], Guido Sauter[1], and Thorsten Schlomm[4]

Abstract

Purpose: The *HER2* oncogene is involved in the biology of many different tumor types and serves as a prognostic marker and a therapeutic target in breast cancer. In contrast to breast cancer, studies on Her2 overexpression and gene amplification in prostate cancer have yielded different results. The purpose of this study was to learn more on the prevalence and clinical significance of *HER2* amplification and overexpression in prostate cancer.

Experimental Design: A tissue microarray containing >2,000 prostate cancers with follow-up data was used. Tissue microarray sections were analyzed on protein and DNA level using two different antibodies (HercepTest, DAKO; Novocastra NCL-CB11) and fluorescence *in situ* hybridization.

Results: Immunohistochemical analyses showed highly similar results for both antibodies. Detectable Her2 immunostaining was observed in 17.2% for the HercepTest and in 22.5% for the Novocastra antibody with the vast majority of cases showing 1+ or 2+ staining. For both antibodies (HercepTest/Novocastra), significant associations were found between positive staining and high Gleason grade ($P < 0.0001$, both), advanced pT stage ($P < 0.0001/P = 0.0015$), rapid tumor cell proliferation ($P = 0.0004/P = 0.0071$), and tumor recurrence ($P < 0.0001$, both). *HER2* amplification was only found in 1 of 2,525 analyzable cases (0.04%).

Conclusions: Low-level Her2 overexpression occurs at relevant frequency in prostate cancer and in the absence of gene amplification. Increased Her2 expression may potentially lead to an aggressive behavior of tumor cells through the stimulation of tumor cell proliferation because Her2 staining was shown to be significantly associated with Ki67 labeling index. These data argue for reconsidering anti-Her2 therapy, possibly with modified approaches. *Clin Cancer Res; 16(5); 1553–60. ©2010 AACR.*

The Her2 protein, a transmembrane tyrosine kinase growth factor receptor, is found in normal and malignant epithelial cells and is involved in regulation of cell proliferation and differentiation (1). Her2 is well known as a strong prognostic factor and a successfully used therapeutic target in breast cancer (2). In 15% to 20% of breast cancer cases, Her2 is overexpressed as a consequence of gene amplification (2–4). Overexpressed Her2 protein serves as the therapeutic target for trastuzumab, a humanized monoclonal antibody (5, 6), which is now used both in a metastatic setting and as an adjuvant therapy of Her2-positive breast cancer.

Many other tumor types, including prostate cancer, express increased levels of Her2, and evidence accumulates that trastuzumab can also be effective in Her2-positive tumors other than breast cancer. In previous studies, widely divergent rates for Her2 expression and amplification in primary prostate cancers have been reported. Rates of positivity range from 0% to 100% in immunohistochemical studies (7–22) and from 0% to 53% in amplification analyses (18, 22–24). Most likely, these conflicting data are due to the use of different immunohistochemistry assays and definitions of gene amplification in fluorescence *in situ* hybridization (FISH) studies. Due to the fact that studies using a Food and Drug Administration–approved test kit for measuring Her2 expression have rarely yielded positive results, it must be assumed that Her2 expression occurs at significantly lower levels than in breast cancer for which these tests have been developed.

Authors' Affiliations: [1]Institute of Pathology, Departments of [2]Urology and [3]Oncology and Hematology, and [4]Martini-Clinic, Prostate Cancer Center University Medical Center, Hamburg-Eppendorf, Germany

Corresponding Author: Thorsten Schlomm, Martini-Clinic, Prostate Cancer Center, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. Phone: 049-40-7410-51314; Fax: 049-40-7410-51323; E-mail: tschlomm@uke.de.

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Translational Relevance

Despite several previous studies on Her2 expression and gene amplification in prostate cancer, its clinical significance remains controversial. Here, we showed that low-level Her2 overexpression occurs in 17% to 20% of prostate cancer in the absence of gene amplification. The significant association of Her2 staining with Gleason grade, pT stage, increased cell proliferation, and poor prognosis argues for a biological relevance of this finding. It might therefore be considered to reevaluate Her2 as a possible drug target in prostate cancer, possibly with modified approaches because the mechanism and levels of Her2 overexpression evidently differ from breast cancer.

In this study, we took advantage of our existing large-scale prostate cancer TMA including >2,000 cancers with follow-up data (25–27). It was our aim to investigate a possible rate of low-level HER2 expression in prostate cancer. Two different antibodies were used to reduce the risk of staining artifacts.

Materials and Methods

Patients. Radical prostatectomy specimens were available from 3,261 patients, consecutively treated at the Department of Urology, University Medical Center Hamburg-Eppendorf between 1992 and 2005 (Table 1). The approval for the study was obtained from the local ethical committee. Follow-up data were available for 2,385 patients, ranging from 1 to 144 mo (mean, 34 mo). None of the patients received neoadjuvant or adjuvant therapy. Additional (salvage) therapy was only initiated in case of a biochemical relapse. In all patients, prostate-specific antigen (PSA) values were measured quarterly in the first year, followed by biannual measurements in the second and annual measurements after the third year following surgery. Recurrence was defined as a postoperative PSA of 0.1 ng/mL and increasing. The first PSA value above or equal to 0.1 ng/mL was used to define the time of recurrence. Patients without evidence of tumor recurrence were censored at last follow-up. All prostatectomy specimens were analyzed according to a standard procedure. All prostates were completely paraffin embedded, including whole-mount sections as previously described (28). All H&E-stained histologic sections from all prostatectomy specimens were reviewed, and the index tumors (within the prostate), as defined by the focus with the worst Gleason pattern or in the case of only one Gleason pattern by the largest tumor focus, were marked on the slides. One 0.6-mm tissue core was punched out from the index tumors of each case and transferred in a tissue microarray (TMA) format as described in ref. (24). The 3,261 cores were distributed among seven TMA blocks each containing 129 to 522 tumor samples. Each TMA block also contained various control tissues including normal prostate tissue, other normal tissues, and a set of tumor tissues including several HER2-positive breast cancers. In addition, 43 hormone refractory cancers, collected from palliative transurethral resections were analyzed on a separate TMA. Hormone refractory prostate cancer was defined as: serum castration levels of testosterone, three consecutive increases of PSA resulting in two 50% increases over the nadir, antiandrogen withdrawal for at least 4 wk, PSA progression despite secondary hormonal manipulations, and progression of osseous or soft tissue lesions (29).

Table 1. Descriptives of the entire study cohort of 3,261 patients

Characteristic	No. of patients (%)	
	Study cohort on TMA	Biochemical relapse among categories
	(*n* = 3,261)	(*n* = 2,385)
Follow-up (mo)		
Mean	34.9	—
Median	30.5	—
Age (y)		
<50	83	13 (15.7)
50-60	998	157 (15.7)
60-70	1,807	315 (17.4)
>70	175	46 (26.3)
Pretreatment PSA (ng/mL)		
<4	513	48 (9.4)
4-10	1,673	200 (12.0)
10-20	641	163 (25.4)
>20	225	113 (50.2)
pT category (AJCC 2002)		
pT2	2,080	129 (6.2)
pT3a	609	171 (28.1)
pT3b	372	200 (53.8)
pT4	42	38 (90.5)
Gleason grade		
≤3+3	1,426	66 (4.6)
3+4	1,311	263 (20.1)
4+3	313	172 (55.0)
≥4+4	55	37 (67.3)
pN category	1,156	
pN0	1,544	369 (23.9)
$pN^{3}1$	96	73 (76.0)
PNx	1,457	94 (6.5)
Surgical margin		
Negative	2,475	328 (13.3)
Positive	627	209 (33.3)

NOTE: Numbers do not always add up to 3,261 in the different categories because of cases with missing data.
Abbreviation: AJCC, American Joint Committee on Cancer.

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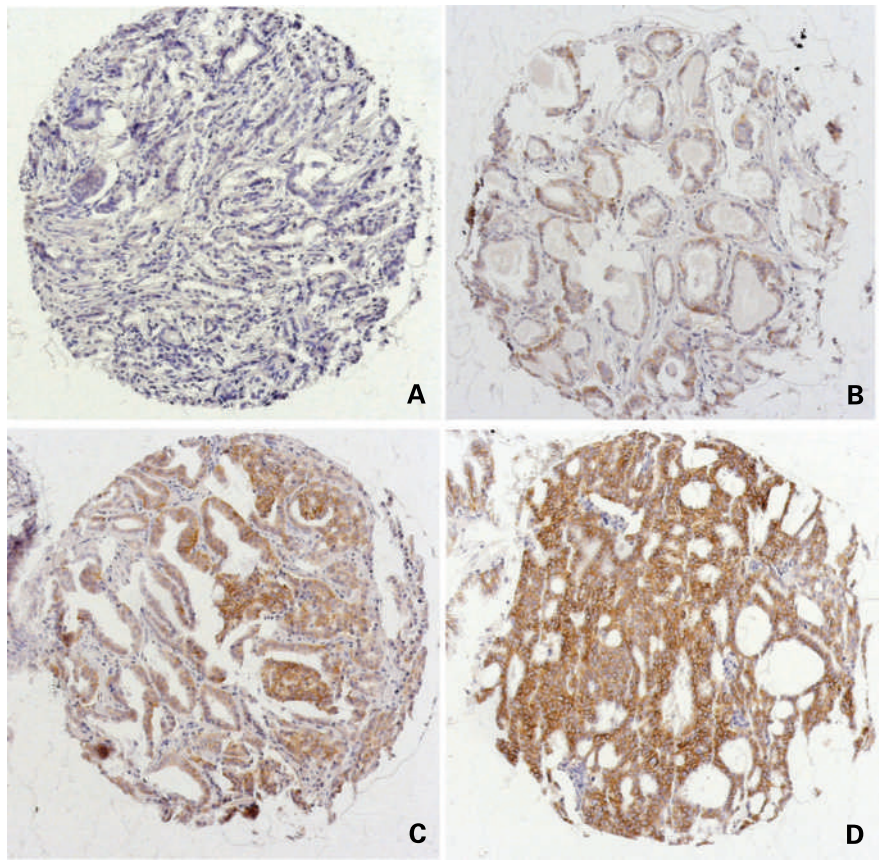

Fig. 1. Examples for Her2 immunohistochemistry. A, Her2-negative. B, Her2 (1+). C, Her2 (2+). D, Her2 (3+).

Immunohistochemistry. Freshly cut TMA sections were analyzed in 1 d in a single experiment for each antibody. Slides were also immunostained for high-molecular-weight cytokeratins to assure the presence of cancer cells in TMA spots. For this purpose, the antibody 34bE12 (clone MA903, DAKO; 1:12.5) was used for basal cell detection after boiling the sections in an autoclave in citrate buffer (pH 7.8). The Mib1 antibody (1:400) was used for Ki67 protein detection after steaming the sections at 98°C for 20 min in citrate buffer (pH 9.0). Her2 expression was detected with two different tests including the Food and Drug Administration–approved HercepTest kit (polyclonal rabbit antibody, DAKO) and the monoclonal antibody NCL-CB11 (Novocastra; Ventana; 1:450) after boiling the sections in an autoclave in citrate buffer (pH 7.8). The Envision system (DAKO) was used for both antibodies to visualize the immunostaining. Cancers with known Her2 positivity served as positive controls and normal prostate tissue served as negative controls on each TMA section. Only tissue samples with distinct loss of basal cells (proven prostate cancers) were used for Her2 analysis. In these samples, Her2 expression was scored exactly as described for the Food and Drug Administration–approved HercepTest in four categories: 0, 1+, 2+, and 3+ (30). The Ki67 labeling index (LI) was defined as the fraction of tumor cells showing any nuclear Ki67 immunoreactivity. For this purpose, 100 to 200 tumor cells were analyzed on each tissue spot to determine the Ki67 LI.

FISH. A 4-μm TMA section was used for two-color FISH. For proteolytic slide pretreatment, a commercial kit was used (Paraffin pretreatment reagent kit, Vysis). A Spectrum-Orange–labeled HER2 probe was used together with a Spectrum-Green–labeled centromere 17 probe (PathVysion; Vysis). Before hybridization, TMA sections were deparaffinized, air dried, and dehydrated in 70%, 85%, and 100% ethanol followed by denaturation for 5 min at 74°C in 70% formamide-2 × SSC solution. Following overnight hybridization at 37°C in a humidified chamber, slides were washed and counterstained with 0.2 μmol/L 4′,6-diamidino-2-phenylindole, an antifade solution. The average gene and centromere copy numbers were estimated in each tumor. A tumor was considered amplified if the ratio of gene/centromere was ≥2.0.

Statistics. Statistical calculations were done with the PRISM 2.01 software (GraphPad). Contingency tables were calculated with the χ^2 test and Fisher's exact test. Survival curves were calculated by the Kaplan-Meier method and compared with the Log-rank test. Cox regression was

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Table 2. Her2 immunohistochemistry

	HER2 expression level							
	Novocastra CB11				DAKO HercepTest			
	Analyzable (*n*)	Weak (%)	Strong (%)	*P*	Analyzable (*n*)	Weak (%)	Strong (%)	*P*
All tumors	2,489	20.9	1.6		2,407	15.7	1.5	
Tumor stage								
pT2	1,508	20.1	0.9	0.0015	1,448	14.4	0.7	0.0001
pT3a	505	22.8	1.8		496	15.7	2.2	
pT3b	310	22.3	4.2		304	18.8	3.6	
pT4	38	13.2	5.3		38	31.6	0.0	
Gleason grade								
≤3+3	995	17.6	0.9	<0.0001	966	11.3	0.2	<0.0001
3+4	1,058	22.6	0.7		1,021	17.1	1.4	
4+3	264	25.4	5.7		254	23.6	5.5	
≥4+4	45	24.4	13.3		46	26.1	4.3	
Nodal stage								
pN0	1,268	18.9	2.2	0.075	1,268	15.4	1.6	0.0395
pN+	82	26.8	4.9		82	18.3	6.1	
PSA								
<4	364	20.9	1.4	0.9461	349	19.2	1.1	0.1571
4-10	1,241	20.9	1.3		1,194	13.7	1.5	
10-20	530	21.3	1.5		518	16.2	1.0	
>20	196	20.4	2.6		189	16.4	2.6	
Resection margin								
Positive	503	22.3	1.8	0.5705	489	17.4	6.5	0.1588
Negative	1,857	20.5	1.5		1,795	3.9	1.6	
Ki67 LI								
Low (<10%)	2,202	20.0	1.5	0.0071	2,129	14.5	1.3	0.0004
Moderate (10-20%)	196	27.6	3.1		186	24.7	2.2	
High (>20%)	40	35.0	0.0		39	28.2	5.1	

used to assess the independence of preoperative parameters and Her2 expression to predict PSA recurrence after radical prostatectomy. ANOVA was used to investigate the effect of HER2 and Gleason score on the Ki67 LI.

Results

Technical issues. A total of 736 tissue samples in TMA analysis were noninformative due to the complete lack of tissue samples or absence of unequivocal cancer tissue in the corresponding 34bE12-immunostained TMA section. In the remaining 2,525 cancers, the percentage of noninterpretable samples was 1.4% for Her2 immunohistochemistry using the NCL-CB11 antibody, 4.7% for Her2 immunohistochemistry using the HercepTest, and 10.6% for Ki67 LI.

Immunohistochemistry. Both antibodies used for Her2 analysis showed similar results. Her2 positivity was seen in 22.5% for the Novocastra antibody and 17.2% for the HercepTest. Representative images are given in Fig. 1. Most positive cancers showed 1+ or 2+ staining according to the HercepTest criteria. Only one tumor was 3+ with the NCL CB11 antibody and three tumors were 3+ with the HercepTest. For statistical analyses, 2+ and 3+ tumors were thus grouped together as strongly positive, whereas 1+ positive tumors were considered weakly positive. Her2

Table 3. Gleason grade–independent association between Her2 expression and cell proliferation as measured by Ki67 LI

Gleason	Her2 neg (*n*)	Ki67LI	Her2 pos (*n*)	Ki67LI	*P*
3+3	880	3.6	110	4.9	0.0004
3+4	850	4.4	191	5.5	0.0008
4+3	178	6.4	73	8.2	0.0613
4+4	32	9.2	14	11.3	0.6451

NOTE: The lack of a significant association in Gleason 4+4 samples is most likely due to the small number of samples in this group.

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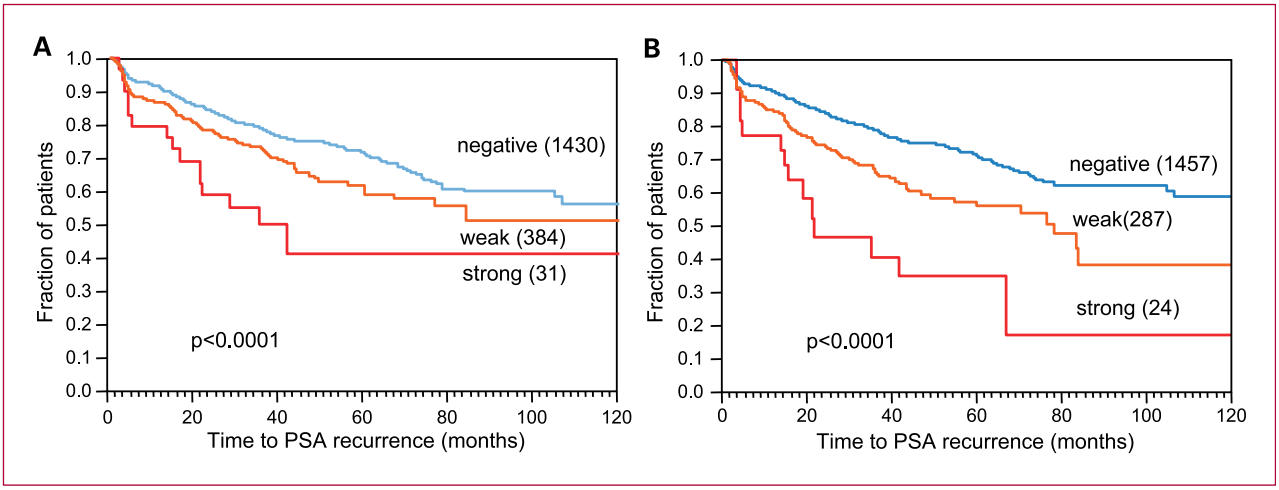

Fig. 2. Influence of Her2 immunhistochemistry on PSA recurrence. A, Novocastra; B, HercepTest.

immunostaining was strongly associated with high Gleason grade ($P < 0.0001$, each) and advanced pT stage ($P = 0.0015/<0.0001$) but unrelated to preoperative PSA, surgical margin, and nodal status (Table 2). Her2 expression was also associated with elevated Ki67 LI. The Ki67 LI was on average 6.1 in 388 Her2-positive cancers but only 4.3 in 1,940 Her2-negative carcinomas ($P < 0.0001$). In addition to Her2 expression, Ki67 LI was also linked to Gleason grade. An analysis of cancers of identical Gleason grade revealed at least in the large groups of Gleason 3+3 and Gleason 3+4 cancers that Her2 expression was still strongly linked to high Ki67 LI. In the smaller subsets of Gleason 4+3 and >4+4, the level of statistical significance was not reached despite of considerably higher average proliferation levels in the Her2-positive group. The results for the DAKO HercepTest are shown in Table 3.

A separate analysis of 25 interpretable hormone-refractory prostate cancers revealed a slightly higher percentage of Her2-positive tumors (28%) but not a significant difference compared with hormone-sensitive primary tumors ($P = 0.1834$). Her 2 was analyzed by the HercepTest in this group and showed 1+ staining in five tumors and 2+ staining in two tumors.

The concordance between both antibodies was very high ($P < 0.0001$). Of 2,373 interpretable samples, 297 were positive (+1, +2, +3) for both antibodies and 1,732 were negative for both antibodies. A total of 236 tumors, which were positive for the Novocastra antibody, were negative for the HercepTest. Conversely, 108 tumors, which were positive for the HercepTest, were negative for the Novocastra antibody (concordance rate, 85.5%).

HER FISH. A total of 2,525 cancers were successfully analyzed by FISH. Only one single case was *HER2* amplified. Forty tumor cells were scored in this case. The *HER2* count ranged from 4 to 12 per cell (average 4.3). In the same cells, two to four centromere 17 signals were detected (average, 1.96). This resulted in a ratio of 2.2. No clear-cut *HER2* gene clusters were detectable in this tumor. According to the HercepTest criteria, this tumor showed a 2+ staining. All other tumors had equal *HER2* and centromere 17 counts or only slightly elevated *HER2* numbers not coming close to the 2.0 ratio.

Relationship with PSA recurrence. Follow-up data were available for 2,385 patients, ranging from 1 to 144 months (mean, 34 months). As expected, Gleason grade, pT stage, preoperative PSA, presence of positive surgical margins, and Ki67 LI were strongly linked to PSA recurrence in this patient subset ($P < 0.0001$, both). Her2 expression was also associated with prognosis. For both antibodies, the likelihood of a biochemical relapse–free survival decreased in a dose-dependent manner with increasing HER2 expression level ($P < 0.0001$; Fig. 2A and B). However, in a multivariate analysis including preoperative PSA, pT stage, and Gleason score, HER2 expression was not an independent predictor of PSA recurrence (Table 4).

Table 4. Multivariate analysis (Cox regression model)

Parameter	Variable	RR	95% CI	*P*
pT stage	pT2 vs pT3	1.07	0.92-1.24	<0.0001
	pT2 vs pT4	2.71	2.08-3.46	
Gleason grade	≤3+3 vs 3+4	0.79	0.68-0.93	<0.0001
	≤3+3 vs 4+3	1.73	1.45-2.05	
	≤3+3 vs ≥4+4	2.14	1.61-2.79	
PSA level	<4 vs 4-10	0.89	0.77-1.04	0.001
	<4 vs 10-20	1.11	0.95-1.30	
	<4 vs >20	1.29	1.07-1.54	
HER2	Pos vs neg	0.91	0.81-1.01	0.099

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Discussion

The results of this study show that the level of Her2 expression is variable in prostate cancer and that an increased Her2 expression level is associated with unfavorable tumor phenotype, rapid tumor cell proliferation, and poor prognosis.

In this study, a TMA containing one 0.6-mm tissue core per cancer was used. The ability to independently detect significant associations with tumor phenotype and prognosis with two different immunohistochemistry protocols highlights the power of this TMA approach for identification of clinically relevant associations. It seems that the high number of cases analyzed in combination with high level of standardization of both immunostaining and analysis compensates for the low amount of tissue analyzed per patient. Literally, all previous studies using large TMAs had succeeded to identify previously well-established associations between molecular findings and tumor phenotype and prognosis (31–33).

Two different antibodies were used in this study to minimize the effect of antibody and protocol selection on the study outcome. Remarkably, both protocols yielded highly similar results and showed detectable low level Her2 overexpression in 17% to 20% of prostate cancers. These figures are well in the range of previous studies (20, 21), although other studies showed positive immunostaining in up to 100% of prostate cancers (16). The significant associations that were found by both methods with various pathologic and clinical parameters argue for a marked biological relevance of Her2 protein for prostate cancer biology. Other studies that had found significant fractions of Her2-expressing prostate cancers had also described associations with tumor phenotype. Shi et al. (34) had found an association of Her2 expression with Gleason grade. Sanchez et al. (7) had described associations with tumor stage. However, other authors found no associations between Her2 expression and grade, stage, or prognosis (35).

Using biochemical (PSA) recurrence as a clinical end point, a dose-dependent association was found between Her2 expression levels and patient prognosis. This is consistent with several previous studies by other groups (36–38). Our data suggest that increased cell proliferation in Her2-expressing cells may represent one mechanism by which aggressive behavior is conferred to tumor cells. Even in the large homogeneous groups of Gleason 3+3 and Gleason 3+4 cancers, the Ki67 LI was significantly higher in Her2-positive compared with Her2-negative cancers. Associations between Her2 overexpression and accelerated tumor cell proliferation have been described for instance in breast cancer (39) and endometrial carcinoma (40).

HER2 amplification was almost nonexistent in our cancers. Only 1 of 2,525 cancers showed *HER2* amplification according to the generally accepted definition using a ratio of at least two *HER2* copies per centromere 17 to define amplification. Furthermore, this case did not show classic high-level amplification with typically 20 to 60 of clustered *HER2* signals per cell but only a borderline result with 2 to 4 centromere copies and 4 to 12 *HER2* copies resulting in a ratio of 2.2. Our finding is coherent with several reports finding either no or only very rare and low-level *HER2* amplifications (24, 41). Early studies describing *HER2* amplification in up to 53% of prostate cancers used different definitions of gene amplification and, due to the lack of a chromosome-specific reference probe, did not exclude the influence of aneuploidy (18, 23). It is therefore not surprising that no association between *HER2* amplification and Her2 expression could be observed in this study (23). In most other Her2-expressing cancers, gene amplification was found to represent the only mechanism for high-level protein expression (42). Her2 overexpression in prostate cancer may result from transcriptional and posttranslational mechanisms (42). The absence of high-level amplifications fits, therefore, well with the only low to moderate expression levels of our HER2-positive prostate cancers.

In hormone-refractory prostate cancers, Her2 expression is reported from relatively uncommon (19, 35) up to about 50% to 80% of the cases (43, 44). Several studies have raised the possibility that Her2 overexpression provides an alternative mechanism for the activation of androgen receptor signaling pathways in the setting of castrate levels of testosterone, thereby contributing to androgen independence (45). However, our data did not show a significant difference in Her2 expression in hormone-refractory tumors compared with hormone-sensitive prostate cancers.

The question whether prostate cancer patients could benefit from anti-Her2 therapy is unresolved. The observation of a therapeutic effect of herceptin on human prostate cancer cells in animal models supports the potential utility of such a treatment (46). The unequivocal association of low-level Her2 overexpression with poor prognosis and high tumor cell proliferation argue in favor of using Her2 as a drug target in prostate cancer. However, trastuzumab showed little efficacy in Her2-expressing breast cancers without amplification. Moreover, the effect of trastuzumab was limited in few clinical prostate cancer trials (47–49). It could be argued, nevertheless, that different dosages of trastuzumab or combination therapy (50) would be required to be effective in prostate cancers with relatively low levels of overexpression. Independent of this, it might be interesting to include prostate cancer patients in clinical studies using new generations of more efficient anti-Her2 drugs such as the anti-Her2 recombinant humanized monoclonal antibody 2C4 or antisense approaches (51, 52).

In summary, the results of this study show that low-level overexpression of Her2 has biological significance in prostate cancer with effect on tumor cell proliferation and prognosis. In contrast to most other Her2-positive tumors, Her2 overexpression in prostate cancer occurs in the

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absence of gene amplification. These data argue for reconsidering anti-Her2 therapy, possibly with modified approaches.

Disclosure of Potential Conflicts of Interest

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