Advances in Brief

HER-2/*neu* (p185*neu*) Protein Expression in the Natural or Treated History of Prostate Cancer[1]

Iman Osman,[2] Howard I. Scher, Marija Drobnjak, David Verbel, Michael Morris, David Agus, Jeffrey S. Ross, and Carlos Cordon-Cardo[3]

Genitourinary Oncology Service, Departments of Medicine [I. O., H. I. S., M. M., D. A.], Pathology [M. D., C. C-C.], and Biostatistics and Epidemiology [D. V.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, New York 12208 [J. S. R.]

Abstract

Purpose: **Amplification of HER-2/*neu* gene and overexpression of its encoded product, the p185neu (HER-2/*neu*) tyrosine kinase membrane receptor, have been associated with tumor progression in certain neoplasms. We conducted this study to investigate patterns of HER-2/*neu* protein expression in prostate cancer, analyzing different points in the natural and treated history of the disease.**

Experimental Design: **Radical prostatectomy cases (83) and 20 metastatic lesions were studied for the association between HER-2/*neu* protein overexpression detected by immunohistochemistry and clinicopathological parameters, including time to prostate-specific antigen (PSA) relapse.**

Results: **HER-2/*neu* protein overexpression, defined as complete membrane staining in >10% of tumor cells using the Food and Drug Administration-approved Dako kit, was found in 9 of 45 (20%) of evaluable hormone naïve primary tumors and 23 of 34 (67%) primary tumors after androgen-deprivation therapy ($P = 0.0001$). Of the 20 metastatic lesions, positivity was noted in 16 (80%) of the cases. On univariate analysis, HER-2/*neu* overexpression was associated with pretreatment PSA ($P = 0.011$) and time to PSA relapse ($P = 0.02$). After controlling for pretreatment PSA, the association between hormone treatment and HER-2/*neu* was still observed. No association was found between HER-2/*neu* overexpression and Gleason score, capsular invasion, and tumor proliferative index determined by Ki67.**

Conclusions: **These data suggest that there is significant HER-2/*neu* overexpression in primary tumors that persist after androgen deprivation. It also emphasizes the importance of characterizing tumors at determined points in the natural or treated history of prostate cancer when targeting treatment to specific biological processes.**

Introduction

HER-2/*neu* protein is a transmembrane tyrosine kinase receptor with high homology to the epidermal growth factor receptor (1). Amplification of the HER-2/*neu* gene and overexpression of its encoded protein have been observed in certain tumor types, including breast, ovary, and lung carcinomas (2–4). In prostate cancer, several studies have reported HER-2/*neu* gene amplification or protein overexpression to variable degrees in cell lines (5) and xenografts (6), as well as in primary tumor samples (7, 8). Little consideration in the literature has been given to the point in the history of disease that the tumor sample being analyzed represents. The distinction between diagnostic biopsies and radical prostatectomy specimens, the specifics of prior therapy administered, and whether the tumor was obtained from the prostate or metastatic lesion are not reported consistently in different series. Disparate levels of reported HER-2/*neu* expression in prostate cancer may also be attributable to the lack of standardization of the immunohistochemical assays used, the antibody used to assess HER2 status, distinct antigen recovery strategies, and scoring methodologies with different definitions of "abnormal" (9–13).

The current study focused on the pattern of HER-2/*neu* protein expression in prostate cancers representing two distinct clinical states (14) assessed with a standardized immunohistochemical assay localized disease (pre and postandrogen ablation) and androgen-independent metastatic tumors. The associations between patterns of HER-2/*neu* protein expression and standard clinicopathological parameters of poor outcome were also examined.

Materials and Methods

Clinical and Pathological Data. A cohort of 103 patients with prostate cancer was studied, including 83 cases with localized tumors and 20 different patients with metastatic lesions. The 83 localized tumors were obtained for the study by radical prostatectomy performed between 1990 and 1991 at Memorial Sloan-Kettering Cancer Center, and patients were followed up at the center. Metastatic lesions (20) from patients with progressive androgen-independent disease were also analyzed. Samples were formalin-fixed paraffin-embedded tissue specimens. Representative H&E stained sections of each paraffin block were examined microscopically to confirm the presence of tumor, as well as to evaluate the pathological grade and

Received 3/27/01; revised 6/7/01; accepted 6/7/01.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by NIH Grant CA-DK-47650 (to C. C-C.) and the PepsiCo Foundation (to M. D. and H. I. S.).
[2] Present address: Kaplan Comprehensive Cancer Center, New York University School of Medicine, 550 First Avenue, New York, NY 10016.
[3] To whom requests for reprints should be addressed, at Division of Molecular Pathology, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-7746; Fax: (212) 794-3186; E-mail: cordon-c@mskcc.org.

stage of the primary tumors analyzed. Thirty-four of the 83 patients with clinically localized tumors received preoperative neoadjuvant complete androgen ablation therapy, which composed of the combination of a luteinizing hormone-releasing hormone agonist and antiandrogen. The remaining 49 patients were hormone naïve. Forty-five of the 49 hormone-naïve primary lesions with sufficient tumor representation on tissue sections were assigned a histological grade. Cases were grouped as low Gleason score (<7, $n = 28$) and high Gleason score (≥ 7, $n = 17$). Postandrogen deprivation samples were not graded (15). According to pathological stage, cases were grouped into organ-confined tumors (pT2, $n = 50$) or tumors extending beyond prostatic capsule ($T \geq 3$, $n = 33$). The response variable time to PSA[4] relapse was defined as the time from radical prostatectomy to the time of the first detectable (nonzero) PSA. Three consecutive increases of PSA were required to confirm PSA relapse; however, the time of relapse was taken as the time of the first detectable PSA value. Three patients who did not achieve a nonmeasurable PSA after radical prostatectomy were excluded from the analysis. The 20 metastatic androgen-independent cases were all bone lesions.

Immunohistochemical Analysis of HER-2/*neu* Expression. The Dako Herceptest Immunohistochemical kit (Dako Corp., Carpenteria, CA) was used as described previously (16, 17). Membrane immunoreactivities for HER-2/*neu* protein were categorized as undetectable or "zero" to +3 category, as defined by the developers of the commercial kit and compared with the supplied positive and negative controls. Score "zero" is defined as undetectable staining or membrane staining in <10% of the tumor cells. Score +1 is defined as faint membrane staining detected in >10% of the tumor cells. Score +2 was considered as weak to moderate complete membrane staining observed in >10% of the tumor cells. Finally, score +3 was defined as a moderate to strong complete membrane staining observed in >10% of the tumor cells. HER-2/*neu* protein expression was classified into two categories defined as follows: negative (scores 0 and 1) and positive (scores 2 and 3). The cutoff point was used based on reported studies in breast cancer (16, 17) and as approved for use by the United States Food and Drug Administration.

Immunohistochemical Analysis of Ki67 Expression. Tumor proliferative activity was assessed by the anti-Ki67 monoclonal antibody MIB1 (1:50 dilution; Immunotech Corp., Luminy, France). Clone MIgs-KpI, a mouse monoclonal antibody of the same subclass as MIB1, was used as a negative control at similar working dilution (PharMingen Laboratories, San Diego, CA). Sections were immersed in boiling 0.01% citric acid (pH 6.0) for 15 min to enhance antigen retrieval, allowed to cool, and incubated with the primary antibody overnight at 4°C. Biotinylated horse antimouse IgG antibodies were applied for 1 h (1:500 dilution; Vector Laboratories, Burlingame, CA), followed by avidin-biotin peroxidase complexes for 30 min (1:25 dilution; Vector Laboratories). Diaminobenzidine was used as the final chromogen, and hematoxylin was used as the nuclear counterstain. Nuclear immunoreactivities were classified into two categories: negative (<20% of tumor cells displaying nuclear staining) and positive (≥20% tumor cells displaying nuclear staining). Ki67 proliferative index was considered high when ≥20% of tumor cells displayed a positive MIB1 nuclear staining pattern (18, 19).

FISH. We conducted FISH analyses using a HER-2/*neu* gene copy number in 66 primary cases using a unique sequence HER-2/*neu* probe (Ventana Medical System, Tucson, AZ). The assay was considered to detect gene amplification if more than four copies of the HER-2/*neu* gene were identified in ≥40 tumor cells.

Statistical Analyses. The baseline variables examined were PSA (units) at time of diagnosis, Gleason score (divided into two mutually exclusive categories: <7 or ≥7), T stage of disease (pT2 or ≥pT3), and HER-2/*neu* membrane expression patterns (negative or positive, see above). Statistical analyses were conducted to explore: (*a*) the association between immunophenotypic variables and clinicopathological parameters, such as tumor grade, tumor stage, preoperative PSA, and hormonal status; and (*b*) the association between HER-2/*neu* phenotypes and PSA relapse free survival. The Fisher's exact test was used to assess the associations among the different variables, and results were considered significant if the P was <0.05 (20). The FREQ procedure in SAS was used for this study (21). The LOGISTIC procedure in SAS, using the Wald test, was used to assess the univariate association between preoperative PSA and HER-2/*neu* phenotypes considering PSA as a continuous variable (21). The univariate associations between preoperative PSA and hormonal status were also explored, whereas the univariate associations between time to PSA relapse and HER2 immunophenotype were evaluated using the Log-rank test (22). Survival distributions were generated using the Kaplan-Meier estimate (23). The Cox proportional hazards model was used to examine the relationship between time to PSA relapse and HER-2/*neu* protein overexpression after controlling for pretreatment PSA and hormone treatment (24). A Wald test was used to test for an association between hormonal status and HER-2/*neu* overexpression controlling for pretreatment PSA (21).

Results

Table 1 summarizes immunohistochemical data in relation to clinicopathological parameters. Fig. 1 illustrates the immunohistochemical patterns of HER-2/*neu* protein expression in representative tumors with different staining scores compared with the control. In normal prostate samples, as well as in normal and benign hyperplastic glands, we observed HER-2/*neu* expression in basal cells, which served as internal controls for the evaluation of HER-2/*neu* immunostaining. In contrast, luminal (or secretory) cells in the normal glands were unreactive to HER-2/*neu* antibodies.

HER-2/*neu* membrane overexpression was observed in 32 of 83 (38.5%) of the radical prostatectomy cases (Table 1). Twenty-two (26.5%) cases were scored as zero, 29 (35%) cases were scored as +1, and 32 cases (38.5%) were considered +2 (Fig. 1). This last value was used to define the cutoff point for

[4] The abbreviations used are: PSA, prostate-specific antigen; FISH, fluorescence *in situ* hybridization.

Table 1 Relationship of HER-2/*neu* overexpression and clinicopathological parameters in prostate cancer ($n = 83$) and hormone naïve prostate cancer ($n = 45$)

Parameter	HER-2-negative #	HER-2-negative %	HER-2-positive #	HER-2-positive %	Total	*P*
Prostate cancer						
Total	51	61.5	32	38.5	83	
Path stage						
pT2	33	66	17	34	50	
≥pT3	18	55	15	45	33	0.36
Pretreatment PSA						
<10	33	76	10	24	43	
≥10	18	45	22	55	40	0.011[a]
Ki index						
<20%	45	63	26	37	71	
≥20%	6	54	5	46	11	0.74
Hormone status						
Neoadjuvant	11	33	23	67	34	
Naïve	36	80	9	20	45	<0.0001
Hormone naïve prostate cancer						
Gleason score						
<7	23	82	5	18	28	
≥7	14	14	3	23	17	0.071
Path stage						
<T2	24	86	4	14	28	
≥T3	12	71	5	29	17	0.265
Pretreatment PSA						
<10	28	90	3	10	31	
≥10	8	57	6	43	14	0.003[a]
Ki index						
<20	32	82	7	18	39	
≥20	4	67	2	33	6	0.583

[a] *P* generated from the Wald test using SAS procedure LOGISTIC where PSA is a continuous variable.

HER-2/*neu* protein overexpression. We did not observe staining score of +3 as per the Dako kit control in this cohort of patients.

Twenty-three of 34 (67%) patients who received neoadjuvant androgen ablation therapy were found to have HER-2/*neu* protein overexpression, *versus* 9 of 45 (20%) patients who were hormone naïve at the time of radical prostatectomy. This association was statistically significant ($P = 0.0001$). We also observed an association between baseline PSA and receipt of neoadjuvant hormones ($P = 0.0004$). This association, coupled with the observed association between pretreatment PSA and HER-2/*neu* protein overexpression ($P = 0.011$), suggests that pretreatment PSA may be a confounding factor in the relationship between hormone treatment and HER-2/*neu* expression. However, after controlling for pretreatment PSA, the association between hormone treatment and HER-2/*neu* expression still exists ($P = 0.0003$). We also found that 16 of the 20 (80%) androgen-independent metastatic cases displayed HER-2/*neu* protein overexpression. Three cases (15%) were scored as zero, 1 case (5%) was scored as +1, 10 cases (50%) were scored as +2, and 6 cases (30%) were considered +3.

No association was observed between HER-2/*neu* protein overexpression and tumor stage ($P = 0.36$) or Ki67 proliferative index ($P = 0.74$). The same observation was seen analyzing hormone-naïve group, as no association was observed between HER-2/*neu* protein overexpression and tumor stage ($P = 0.265$), Gleason score ($P = 0.071$), or Ki67 ($P = 0.583$). An association was found between HER-2/*neu* protein overexpression and time to PSA failure after radical prostatectomy ($P = 0.02$); however, after adjusting for pretreatment PSA and hormone treatment, the association between HER-2/*neu* protein overexpression and time to PSA relapse was not significant ($P = 0.94$).

FISH procedures revealed that only 2 of 66 cases analyzed had HER-2/*neu* gene amplification. The two cases displaying HER-2/*neu* gene amplification also showed a HER-2/*neu* protein overexpression phenotype.

Discussion

This study shows the importance of evaluating tumors representing specific points in the natural history of prostate cancer (14). In untreated hormone-naïve primary tumors, HER-2/*neu* expression was infrequent (20%). In contrast, overexpression was observed in 80% of metastatic cases and 67% of primary tumors surviving after androgen ablation. These patterns of HER-2/*neu* expression in prostate cancer should be regarded as "deregulated" rather than "ectopic," because HER-2/*neu* is detected in the basal cells of normal prostate glands.

Overall, HER-2/*neu* membrane overexpression was observed in 38% of the radical prostatectomy specimens. However, with reported rates of HER-2/*neu* overexpression in prostate cancer ranging from 0 to 94%, it is difficult to determine a precise estimate (25–30). An evaluation of methodological differences between the situations helps to clarify the significance of the outcomes. These include the nature of the material studied, as well as the method of assessment. In this regard, we used a standardized kit and established cutoff values, albeit validated for breast cancer. We observed a correlation between score +2 overexpression and PSA relapse. This is the same cutoff value used in the context of breast cancer studies (16, 17). We have also noted that HER-2/*neu* immunostaining in primary prostate cancer specimens is heterogeneous and focal. None of the primary tumors was scored as +3; however, 30% of metastatic lesions had a score of +3. In addition, we found that 80% (16 of 20) of tumors representing androgen-independent metastatic disease overexpressed HER-2/*neu* protein. This shows the contribution of prior therapy and clinical state to overall outcomes.

HER-2/*neu* protein overexpression in primary tumors was compared with gene amplification using FISH in 66 cases. None of the HER-2/*neu*-negative tumors showed amplification, whereas two of the HER-2/*neu*-positive cases revealed amplification signals. Similar observations were recently reported by Signoretti *et al.* (31). HER-2/*neu* overexpression in the absence of gene amplification may be attributable to transcriptional and posttranslational mechanisms (32), a phenomenon that is also clinically frequent for other oncogenes, such as cyclin D1 and mdm2 (33, 34).

A recent study on prostate cancer reported that 8 of 86 (9%) cases displayed HER-2/*neu* gene amplification; however, only one of these eight tumors had a "moderate" amplification signal, whereas the remaining seven tumors were all described to have "low" amplification signals (35). It appears that, despite several earlier studies using a FISH-based assay that found higher gene amplification rates (28, 36), the frequency of HER-

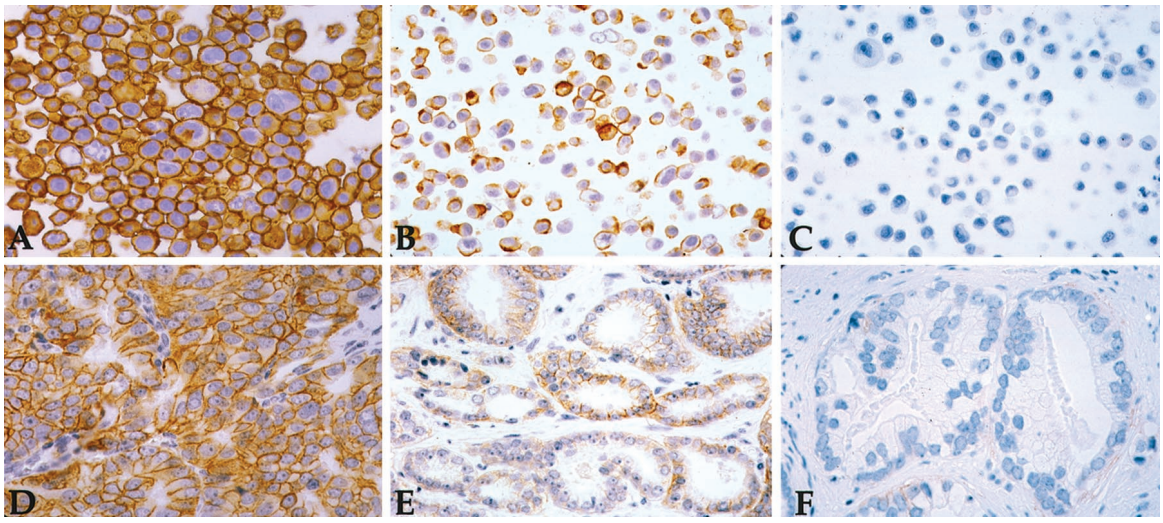

Fig. 1 Immunohistochemical staining of control cell lines provided by the standardized Dako kit and primary prostatic carcinomas with anti-HER-2/*neu* monoclonal antibody using the Dako kit. *A,* intense and homogeneous membrane staining in HER-2/*neu* overexpressing control cells. *B,* representative field of moderately intense control cells expressing low to intermediate HER-2/*neu* levels. *C,* representative area of HER-2/*neu* nonexpressing control cells, showing negative immunohistochemical staining profile. *D,* primary tumor displaying a strong membrane staining in tumor cells. Note the lack of immunoreactivities observed in the stroma elements. *E,* primary tumor showing low expression levels of HER-2/*neu*; however, still membrane staining can be visualized in most cells. *F,* primary tumor with undetectable levels of HER-2/*neu*, representative of the negative phenotype cases. Original magnifications: *A, B, C, D,* and *F,* ×400; *E,* ×200.

2/*neu* amplification in primary prostate carcinoma is generally lower than that reported in other tumor types, including ovary and larynx cancer (3, 37). The most significant association was observed between HER-2/*neu* overexpression and prior androgen deprivation, a factor often not considered as a prognostic factor. Even after controlling for the confounding effect of pretreatment PSA, this association was still observed.

Recent reports in which specific interactions between HER-2/*neu* and the androgen receptor have been described (38, 39). Forced overexpression of HER-2/*neu* in androgen-dependent prostate cancer cells allowed ligand-independent growth. In this setting, HER-2/*neu* was able to activate the androgen receptor signaling even in the absence of the ligand, namely androgens. This "cross-talk" is probably bi-directional, meaning that androgen ablation, known to down-regulate androgen receptors, could also up-regulate HER-2/*neu* in response to cellular stress.

An alternative explanation is that the higher frequency of positivity in neoadjuvant-treated tumors is simply a function of disease extent. The association observed between HER-2/*neu* overexpression and higher pretreatment PSA is consistent with this postulate. Similarly, the association between HER-2/*neu* overexpression and time to PSA relapse in this cohort may also reflect the more advanced nature of the tumor treated in this cohort. Against this is the fact that the association between HER2 status and prior hormone exposure persisted after controlling for baseline PSA.

The present study emphasizes the importance of characterizing the clinical state of the patient from which the tumor is studied. On the basis of our observation, we believe that detailed information regarding prior hormone exposure is essential when designing clinical trials targeting HER2 and other signaling molecules associated with prostate cancer progression. In that regard, xenograft studies with targeted therapy to HER-2/*neu* demonstrated disparate responses dependent on the androgen status of the prostate cancers (40).

References

1. Aguilar, Z., Akita, R. W., Finn, R. S., Ramos, B. L., Pegram, M. D., Kabbinavar, F. F., Pietras, R. J., Pisacane, P., Sliwkowski, M. X., and Slamon, D. J. Biologic effects of heregulin/*neu* differentiation factor on normal and malignant human breast and ovarian epithelial cells. Oncogene, *18:* 6050–6062, 1999.

2. Klijanienko, J., Couturier, J., Galut, M., El-Naggar, A. K., Maciorowski, Z., Padoy, E., Mosseri, V., and Vielh, P. Detection and quantitation by fluorescence *in situ* hybridization (FISH) and image analysis of HER-2/*neu* gene amplification in breast cancer fine-needle samples. Cancer (Phila.), *87:* 312–318, 1999.

3. Ross, J. S., Yang, F., Kallakury, B. V., Sheehan, C. E., Ambros, R. A., and Muraca, P. J. HER-2/*neu* oncogene amplification by fluorescence *in situ* hybridization in epithelial tumors of the ovary. Am. J. Clin. Pathol., *111:* 311–316, 1999.

4. Shackney, S. E., Smith, C. A., Pollice, A., Levitt, M., Magovern, J. A., Wiechmann, R. J., Silverman, J., Sweeney, L., and Landreneau, R. J. Genetic evolutionary staging of early non-small cell lung cancer: the P53 HER-2/*NEU ras* sequence. J. Thorac. Cardiovasc. Surg., *118:* 259–267, 1999.

5. Lyne, J. C., Melhem, M. F., Finley, G. G., Wen, D., Liu, N., Deng, D. H., and Salup, R. Tissue expression of *neu* differentiation factor/heregulin and its receptor complex in prostate cancer and its biologic effects on prostate cancer cells *in vitro*. Cancer J. Sci Am., *3:* 21–30, 1997.

6. Skrepnik, N., Zieske, A. W., Bravo, J. C., Gillespie, A. T., and Hunt, J. D. Recombinant oncotoxin AR209 (anti-p185/erbB-2) diminishes human prostate cancer xenografts. J. Urol., *161:* 984–989, 1999.

7. McCann, A., Dervan, P. A., Johnston, P. A., Gullick, W. J., and Carney, D. N. c-erbB-2 oncoprotein expression in primary human tumors. Cancer (Phila.), *65:* 88–92, 1990.

8. Ware, J. L., Maygarden, S. J., Koontz, W. W., and Strom, S. C. Immunohistochemical detection of c-erbB2 protein in human benign and neoplastic prostate. Hum. Pathol., *22:* 254–258, 1991.

9. Mellon, K., Thompson, S., Charlton, R. G., Marsh, C., Robinson, M., Lane, D. P., Harris, A. L., Horne, C. H., and Neal, D. E. P53, c-*erb*B-2 and the epidermal growth factor receptor in the benign and malignant prostate. J. Urol., *147:* 496–499, 1992.

10. Visakorpi, T., Kallioniemi, O-P., Koivula, T., Harvey, J., and Isola, J. Expression of epidermal growth factor receptor and c-*erb*B-2 (HER-2/*neu*) oncoprotein in prostatic carcinomas. Mod. Pathol., *5:* 643–648, 1992.

11. Ibrahim, G. K., MacDonald, J. A., Kerns, B. J., Ibrahim, S. N., Humphrey, P. A., and Robertson, C. N. Differential immunoreactivity of HER-2/*neu* oncoprotein in prostatic tissues. Surg. Oncol., *1:* 151–155, 1992.

12. Giri, D. K., Wadhwa, S. N., Upadhaya, S. N., and Talwar, G. P. Expression of NEU/HER-2 oncoprotein (p185neu) in prostate tumors: an immunohistochemical study. Prostate, *23:* 329–336, 1993.

13. Kuhn, E. J., Kurnot, R. A., Sesterhenn, I. A., Chang, E. H., and Moul, J. Expression of the c-*erb*B-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. J. Urol., *150:* 1427–1433, 1993.

14. Scher, H., and Heller, G. Clinical states in prostate cancer. Urology, *55:* 323–327, 2000.

15. Bostwick, D. G. Prostatic adenocarcinoma following androgen deprivation therapy. The new difficulty in histologic interpretation. Anat. Pathol., *3:* 1–16, 1998.

16. Edorh, A., Parache, R. M., Migeon, C., N′Sossani, B., and Rihn, B. Expression of the c-erbB-2 oncoprotein in mammary Paget's disease. Immunohistochemical study by using 3 antibodies. Pathol. Biol., *43:* 584–589,1995.

17. Jacobs, T. W., Gown, A. M., Yaziji, H., Barnes, M. J., and Schnitt, S. J. Specificity of HercepTest in determining HER-2/*neu* status of breast cancers using the United States Food and Drug Administration-Approved Scoring System. J. Clin. Oncol., *17:* 1983–1988, 1998.

18. Osman, I., Drobnjak, M., Fazzari, M., Ferrara, J., Scher, H. I., and Cordon-Cardo, C. Inactivation of the p53 pathway in prostate cancer: impact on tumor progression. Clin. Cancer Res., *5:* 2082–2088, 1999.

19. Osman, I., Scher, H., Zhang, Z. F., Soos, T. J., Hamza, R., Eissa, S., Khaled, H., Koff, A., and Cordon-Cardo, C. Expression of cyclin D1, but not cyclins E and A, is related to progression in bilharzial bladder cancer. Clin. Cancer Res., *3:* 2247–2251, 1997.

20. Agresti, A. (ed.). Categorical Data Analysis, pp.39–44. New York: Wiley, 1996.

21. SAS/STAT User Guide, Version 6. Cary, SAS Institute Inc., 1990.

22. Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Rep., *50:* 163–170, 1996.

23. Kaplan, E. L., amd Meier, P. Nonparametric estimation from incomplete observation. J. Am. Stat. Assoc., *53:* 457–481, 1958.

24. Cox, D. R. Regression models and life tables. J. Roy. Statist. Soc., *34:* 187–220, 1972.

25. Stokes, M. E., Davis, C. S., and Kouch, G. G. Categorial data analysis using the SAS system, SAS Institute Inc., 1995.

26. Fox, S. B., Persad, R. A., Coleman, N., Day, C. A., Silcocks, P. B., and Collins, C. C. Prognostic value of c-erbB-2 and epidermal growth factor receptor in stage A1 (T1a) prostatic adenocarcinoma. Br. J. Urol., *2:* 214–220, 1994.

27. Sadasivan, R., Morgan, R., Jennings, S., Austenfeld, M., Van Veldhuizen, P., Stephens, R., and Noble, M. Overexpression of Her-2/*neu* may be an indicator of poor prognosis in prostate cancer. J. Urol., *150:* 126–131, 1993.

28. Myers, R. B., Srivastava, S., Oelschlager, D. K., and Grizzle, W. E. Expression of p160erbB-3 and p185erbB-2 in prostatic intraepithelial neoplasia and prostatic adenocarcinoma. J. Natl. Cancer Inst. (Bethesda), *86:* 1140–1145, 1994.

29. Gu, K., Mes-Masson, A. M., Gauthier, J., and Saad, F. Overexpression of her-2/*neu* in human prostate cancer and benign hyperplasia. Cancer Lett., *99:* 185–189, 1996.

30. Ross, J. S., Sheehan, C. E., Hayner-Buchan, A. M., Ambros, R. A., Kallakury, B. V., Kaufman, R. P., Jr., Fisher, H. A., Rifkin, M. D., and Muraca, P. J. Prognostic significance of HER-2/*neu* gene amplification status by fluorescence *in situ* hybridization of prostate carcinoma. Cancer (Phila.), *79:* 2162–2170, 1997.

31. Signoretti, S., Montironi, R., Manola, J., Altimari, A., Tam, C., Bubley, G., Balk, S., Thomas, G., Kaplan, I., Hlatky, L., Hahnfeldt, P., Kantoff, P., and Loda, M. Her-2-*neu* expression and progression toward androgen independence in human prostate cancer. J. Natl. Cancer Inst. (Bethesda), *92:* 1918–1925, 2000.

32. Child, S. J., Miller, M. K., and Geballe, A. P. Cell type-dependent and -independent control of HER-2/*neu* translation. Int. J. Biochem. Cell Biol., *31:* 201–213, 1999.

33. Kim, S. H., Lewis, J. J., Brennan, M. F., Woodruff, J. M., Dudas, M., and Cordon-Cardo, C. Overexpression of cyclin D1 is associated with poor prognosis in extremity soft-tissue sarcomas. Clin. Cancer Res., *4:* 2377–2382, 1998.

34. Osman, I., Scher, H. I., Zhang, Z. F., Pellicer, I., Hamza, R., Eissa, S., Khaled, H., and Cordon-Cardo, C. Alterations affecting the p53 control pathway in bilharzial-related bladder cancer. Clin. Cancer Res., *3:* 531–536, 1997.

35. Mark, H. F., Feldman, D., Das, S., Kye, H., Mark, S., Sun, C. L., and Samy, M. Fluorescence *in situ* hybridization study of HER-2/*neu* oncogene amplification in prostate cancer. Exp. Mol. Pathol., *66:* 170–178, 1999.

36. Ross, J. S., Sheehan, C., Hayner-Buchan A., Ambros, R. A., Kallakury, B. V. S., Kaufman, R., Fisher, H. A. G., and Muraca, P. J. HER-2/*neu* gene amplification status in prostate cancer by fluorescence *in situ*-hybridization. Hum. Pathol., *28:* 827–833, 1997.

37. Tantawy, A., Youins, L., and Hamza, M. Expression of c-erb B-2 oncoprotein in cancer of the larynx in relation to invasion of the cartilagenous framework and prognosis. Eur. Arch. Otorhinolaryngol., *256:* 72–77, 1999.

38. Craft, N., Shostak, Y., Carey, M., and Sawyers, C. L. A. mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/*neu* tyrosine kinase. Nat. Med., *5:* 280–285, 1999.

39. Mydlo, J. H., Kral, J. G., Volpe, M., Axotis, C., Macchia, R. J., and Pertschuk, L. P. An analysis of microvessel density, androgen receptor, p53 and HER-2/*ne*u expression and Gleason score in prostate cancer. Preliminary results and therapeutic implications. Eur. Urol., *34:* 426–432, 1998.

40. Agus, D. B., Scher, H. I., Higgins, B., Fox, W. D., Heller, G., Fazzari, M., Cordon-Cardo, C., and Golde, D. W. Response of prostate cancer to anti-Her-2/*neu* antibody in androgen dependent and independent human xenograft models. Clin. Cancer Res., *59:* 4761–4764, 1999.

Clinical Cancer Research

HER-2/*neu* (p185*neu*) Protein Expression in the Natural or Treated History of Prostate Cancer

Iman Osman, Howard I. Scher, Marija Drobnjak, et al.

Clin Cancer Res 2001;7:2643-2647.